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To the Graduate Council:

I am submitting herewith a dissertation written by Robin Ann Ruark Brothers entitled "Effect of Carnitine on the Disposition of Alcohols." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Human Ecology.

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Vice Provost and
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EFFECT OF CARNITINE ON THE DISPOSITION OF ALCOHOLS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Robin Ann Ruark Brothers

December 1989

DEDICATION

I dedicate this dissertation and the completion of this degree to the glory of God the Father, the name of Jesus Christ and the power of the Holy Spirit.

ACKNOWLEDGMENTS

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Finally, I am grateful to the University of Tennessee for financial assistance and the people of Tennessee for the opportunity to pursue this degree.

ABSTRACT

Male Sprague-Dawley rats, 300-500g, were fed Purina rodent chow with or without a supplement of 0.5 % L-carnitine (CS or NS groups) for 5-7 days, after which they were given a single oral dose of methanol (2.25 g/kg), isopropanol (2.0 g/kg), ethylene glycol (5.56 g/kg), or ethylene glycol (5.56 g/kg) followed by ethanol (3.0 g/kg) 1 hour later in aqueous solutions. Serial blood and urine samples were collected over time and analyzed for the respective alcohols by gas chromatography. The data were subjected to pharmacokinetic analysis and statistical analysis.

There was significant reduction in blood methanol concentration in the CS group at 2 and 4 hours which was supported by the kinetic analysis: a lower area under the blood methanol curve, a lower absorption slope to the peak blood concentration and longer time to reach the peak concentration. The urinary excretion of methanol was significantly increased in the CS group at 2 and 4 hours post dosing.

There were no significant differences between the NS and CS groups for blood isopropanol concentrations at all time points. Following isopropanol administration the urine output was significantly higher in the CS than the NS animals which was reflected in higher total isopropanol excretion in the urine of the CS group . Urinary clearance of isopropanol was significantly higher in the CS than the NS group. Acetone appearance in blood was not significantly affected by carnitine, however, the excretion of acetone in the urine was enhanced due to higher urine volume of the CS group.

Blood ethylene glycol concentration curves were not significantly different between the NS and CS groups. Carnitine supplementation did not increase the urinary excretion of ethylene glycol or the urinary volume following ethylene glycol gavage.

Ethanol administration following a dose of ethylene glycol resulted in a higher blood ethylene glycol concentration and more rapid elimination of ethylene glycol from the blood

of the CS animals compared to that from the NS animals. There were no differences in the urinary volumes or concentrations of ethylene glycol between the NS and CS animals. However, there was a marked rise in the urinary clearance of ethylene glycol in the CS group compared to the NS group. There were no differences in the blood ethanol concentration curves or urinary ethanol excretion of the NS and CS animals when ethanol was given after ethylene glycol.

It is concluded that supplementary L-carnitine offered protection against accumulation of methanol, isopropanol and ethylene glycol with ethanol in the early phase of their appearance in blood via increased urinary clearance of these alcohols.

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CHAPTER 1

INTRODUCTION

The future of research in nutrition and toxicology is leading towards the study of the interactions of naturally occurring agents and toxicants. These naturally occurring agents may be so called inert substances such as fibers in plant foods or the products of fermentation as are found in dairy products or spirit beverages. The toxicants may be pesticides, environmental pollutants, voluntarily ingested legal or illegal drugs or even naturally occurring toxicants such as solanine, an alkaloid found in potatoes.

In this light, research into the interactions of carnitine, a naturally occurring vitamin B type compound, and aliphatic alcohols was undertaken. Aliphatic alcohols are common industrial solvents, heat exchange fluids and anti-freezes and starting frameworks for complex organic synthesis reactions. These alcohols are unknowingly consumed in wines and liquors. Rum, brandy and whisky may contain 100-400 mg/dl of aliphatic alcohols other than ethanol (1). These alcohols are the result of alcoholic fermentations and add the characteristic flavors and bouquets to wines and brandies. Methanol is also present in amounts up to 0.36 mg/dl in brandy as a result of the fermentation of pectin (1).

The higher alcohols and methanol are also consumed knowingly by ethanol abusers as cheap or readily available substitutes. Isopropyl alcohol (2-propanol) rubs are common as a medicinal treatment for fever conditions and thus the alcohol must be metabolized by an already compromised body after absorption through the skin. Ethylene glycol (1,2,-ethanediol), a common anti-freeze, is used in space modules and automobiles. Vapors and liquid ethylene glycol are potential hazards for domestic animals and humans.

These alcohols as well as ethanol (ethyl alcohol) have become common environmental pollutants in the industrialized world. Ethanol has been extensively studied for its interactions with nutrients including carnitine. It has been reported that supplementary carnitine lessened fatty infiltration of the liver following ethanol intoxication in the rat model (2). This effect of carnitine was dose dependent (3) and appeared to be mediated by an attenuation of ethanol metabolism (4). In light of these observations it was hypothesized that supplemental carnitine may also protect against the toxic effects of other aliphatic alcohols.

Therefore the objectives of this timely study were:

1. to determine the effects of supplemental carnitine on the metabolism and disposition of methanol, isopropanol and ethylene glycol.
2. to assess from these data the nature of the interaction between carnitine and alcohols.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Toxicology of Alcohols

2.1.1 Methanol

Wimer et al (5) outlined the major symptoms and pathologies of methanol poisoning shown in Table 2.1. There are differences between the species in the severity of the intoxication and nature of the symptoms. These are directly related to the metabolism of methanol.

2.1.2 Isopropanol

Isopropanol toxicity is marked by many symptoms in various animals and they vary with the route of administration. In chickens and rabbits inertia and collapse resulted and cats immediately entered stupor. The effect of isopropanol on dogs and monkeys was not as severe with drowsiness, nausea and vomiting being the primary symptoms. Chronic inhalation in rats produced symptoms of hemorrhage in the sinuses and hyperplasia of the spleen with some liver damage (6). Nordmann et al (7,8) also showed an increase in hepatic triglycerides with acute isopropanol administration.

2.1.3 Ethylene Glycol

Ethylene glycol is widely used as an anti-freeze and heat exchange fluid. Its use in automobiles, machinery and space modules makes the possibility for human and animal intoxication great. Ethylene glycol produces a central nervous system (CNS) depression and induces severe renal injury and renal failure. The ethylene glycol itself produces the

Table 2.1 Methanol Poisoning in Humans and Nonprimates

	Humans	Nonprimates
Lethal Dose	0.85-1.4g/kg	6-10 times the equivalent human dose in rodents and dogs.
Symptoms Intoxication	Less early intoxication followed by symptomless latent period,then sickness and death.	Severe and early intoxication with narcosis, lasting until death.Narcosis is predominant symptom.
Eye Changes	In many cases, partial or complete blindness accompanied by eyeground changes such as hyperemia of the optic discs and venous engorgement.	Early pupillary changes and corneal opacification following exposure and keratitis Eyeground changes not seen in nonprimates.
Acidosis	Often severe acidosis (CO_2-combining capacity less than 20 %)	Rarely occurring and only at near lethal or lethal doses.

Source: Wimer et al (1983) Alcohols Toxicology. Noyes Data Corporation, Park Ridge, New Jersey (ref. 5)

CNS depression but metabolites of ethylene glycol, glycolic and glyoxylic acid, produce CNS damage and nephrotoxicity. Oxalic acid crystals , also formed from ethylene glycol have been found in brain tissue and kidneys following intoxication in humans and animals (10). Increased lactic acid levels caused by the disruption in cellular metabolism and formic acid, another metabolite, contributes to metabolic acidosis (9). Treatment of ethylene glycol poisoning is multifaceted. Ethanol is administered as a competitive inhibitor of alcohol dehydrogenase to decrease the formation of metabolites of ethylene glycol. Sodium bicarbonate is used to treat the acidosis and calcium is administered to treat the muscle spasms caused by chelation of calcium with oxalate. Dialysis is effective in removing more unchanged ethylene glycol as is the induction of diuresis (9,10).

2.2 The Metabolism of Alcohols

The metabolism of aliphatic alcohols has been investigated for well over a century. Lutwak-Mann (11) studied the early preparation techniques for alcohol and aldehyde mutase (dehydrogenase) using horse liver. These studies first investigated the requirements of cofactors, coenzymes and methods of protein precipitation. The horse liver enzyme system was also compared to the yeast system. The ability of the enzyme preparations to metabolize various alcohols was tested by the reduction of methylene blue. The results are summarized in Table 2.2.

Since that time Forsander (12) found that horse liver alcohol dehydrogenase (ADH) oxidized 1-butanol faster than ethanol but did not utilize methanol or 2-propanol. The slow reaction of methanol with ADH was first thought to be due to poisoning by formaldehyde but only large quantities of acetaldehyde and formaldehyde could inhibit the enzyme (11). Methanol did not inhibit ethanol metabolism except in the yeast ADH (11).

Table 2.2 Reduction of Methylene Blue as a Result of the Oxidation of Alcohols in Horse Liver Preparations

Alcohol	Reduction Time
Ethyl Alcohol	1 min 35 sec
Propyl Alcohol	2 min
Amyl Alcohol	5 min 35 sec
Methyl Alcohol	11 min
Saligenin*	no reaction
Glycerol	no reaction
α-Glycerophosphate	no reaction

* $C_6H_4(OH)CH_2OH$ ortho hydroxy benzyl alcohol

Source: Lutwak-Mann, C. (1938) Alcohol dehydrogenase of animal tissues. Biochem. J. 32: 1364-1374. (ref.11)

Catalase, microsomal oxidizing systems and glucuronidation are all non-ADH pathways for alcohol metabolism but vary in importance with the alcohol and the animal. Figure 2.1 shows the metabolic scheme of ethanol in the hepatocyte as an illustration of the linked nature of ADH and non-ADH pathways for alcohol metabolism. Catalase and oxidases capable of generating hydrogen peroxide exist in both the soluble and particulate fraction of the hepatic cell. Within the particulate fraction catalase and several oxidases reside in the microbodies or peroxisomes (13, 14).

Excretion of unchanged alcohol in the expired air or urine is also considered to be a relatively minor means of disposition. Slight changes in excretion of unchanged alcohols have been considered heretofore clinically insignificant. Depending on the body load of the alcohol, the toxicity of the alcohol itself and the toxicity of metabolites, mechanisms for increasing the excretion of unchanged alcohol must be given some consideration.

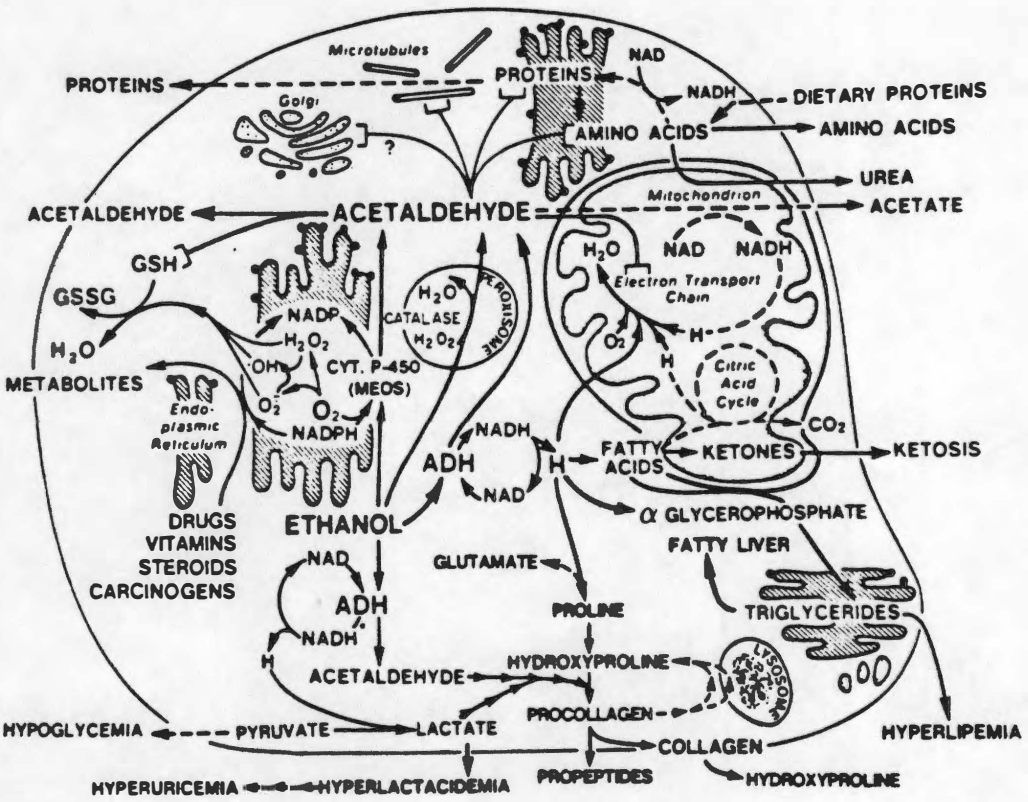


Figure 2.1. Oxidation of ethanol in the hepatocyte. The broken lines indicates pathways depressed by ethanol, brackets denote interference and multi-arrows indicate stimulation or activation.

Source: Lieber, C.S. et al (1987) Metabolism of and metabolic effects of ethanol, including interaction with drugs, carcinogens and nutrition. Mutation Res. 186: 201-223 (ref.15)

2.2.1 Methanol Metabolism

As was shown in Table 2.1 there is a difference in the metabolism of methanol between primates and nonprimates. Humans and other primates utilize ADH for oxidation in a reaction requiring oxidized nicotinamide adenine dinucleotide. Nonprimates utilize a catalase peroxidase system (Figure 2.2)

The catalase reaction takes place in the following stages.

$$\underset{\textit{very rapid reaction}}{\text{Catalase} + H_2O_2} \longrightarrow \underset{\textit{Complex I}}{\text{Catalase}\cdot H_2O_2} + \underset{\textit{rapid second order reaction}}{RCH_2OH} \longrightarrow$$

$$\underset{\textit{relatively unstable}}{\text{Catalase}\cdot H_2O_2\cdot RCH_2OH} \longrightarrow \text{Catalase} + 2\,H_2O + \underset{\textit{aldehyde}}{RCHO}$$

Ethanol and methanol have equal reactivity with Complex I (16, 17). The metabolic scheme of methanol was developed with the use of 3- amino 1,2,4-triazole (AT) which is known to bind irreversibly to Complex I and thus inhibit catalase. This binding caused a 90% reduction in hepatic and renal catalase activity and reduced the methanol oxidizing capacity in rats by 70% (16). In monkeys, AT also reduced the catalase activity but methanol metabolism was not significantly effected because primates do not utilize catalase to metabolize methanol (18) (see Table 2.1). The H_2O_2 needed for methanol metabolism in the rat is contributed primarily by urate oxidase (14).

The rate of metabolism of a 1g/kg intraperitoneal (i.p.) dose of methanol in rats was constant during the first 28 hours at 24 mg/kg·hr (16). By 36 hours 77% of the dose had been recovered as CO_2 and 24% had been excreted unchanged, divided equally between pulmonary and renal plus fecal routes. An oral dose of 3 g/kg resulted in 2/3 of the dose

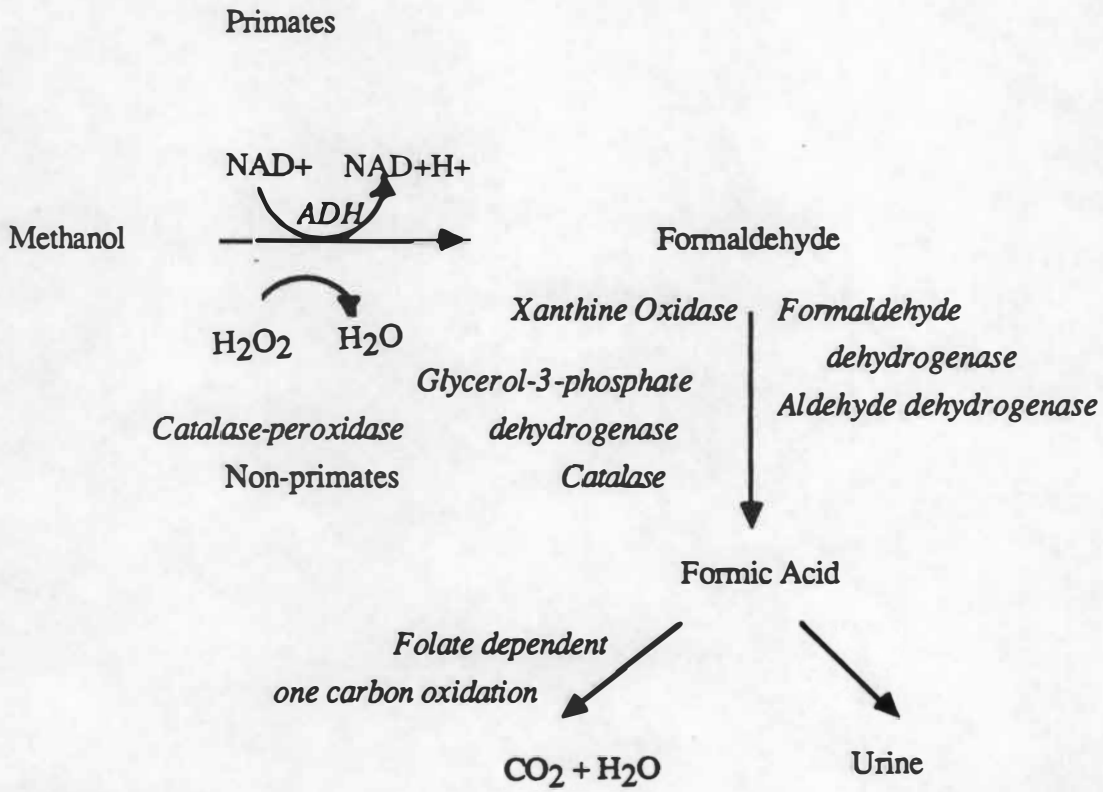


Figure 2.2. Oxidation of methanol in primates and non-primates.

After Wimer et al (1983) Alcohols Toxicology. Noyes Data Corporation, Park Ridge, New Jersey (ref.5)

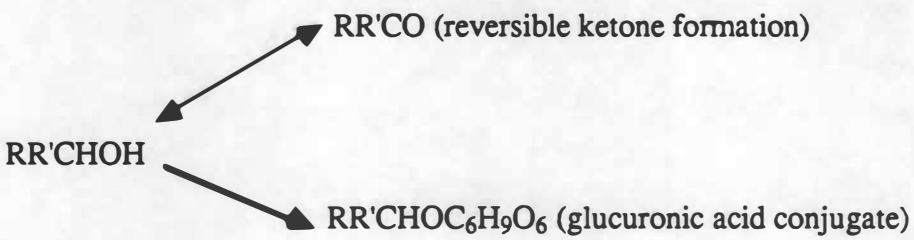
being excreted by renal and pulmonary routes and 1/3 being oxidized. The oxidation of methanol follows first order kinetics when the dose is less that 1g/kg (16).

2.2.2 Metabolism of Isopropanol

Isopropanol is metabolized and excreted by the following mechanisms (1).

1. Oxidation to acids, aldehydes, ketones and CO_2 and their elimination in urine and expired air.
2. Conjugation with glucuronic acid and excretion in the urine.
3. Excretion of unchanged isopropanol in air and urine.

Kamil (1) defines a simple scheme for the metabolism of a branched chain alcohol .



Conjugation reaction gain importance with the branching of the alcohol and therefore conjugation reactions proceed as tertiary alcohols > secondary alcohols > primary alcohols while oxidation reactions proceed in the reverse order (1). In rabbits given a 50 mmol/kg oral dose of isopropanol, 10.2 % of the dose was excreted as the glucuronic acid conjugate (1).

The exact metabolic pathway for isopropanol has been difficult to deduce. No qualitative relationship between isopropanol and acetone production have been established since the ketone to alcohol reaction is readily reversible (6,1)

Nordmann et al (7) investigated the role of catalase and ADH in the metabolism of isopropanol in the rat. Pyrazole, an inhibitor of ADH and of catalase in high doses and AT were used. The control blood isopropanol levels for 1g/kg, i.p., dose reached 140 mg/dl

in 30 minutes. Acetone reached 70 mg/dl in 6 hours. With a 6 mg/kg oral dose the peak blood isopropanol level of about 550 mg/dl was reached in 8 hours by which time acetone had reached 150 mg/dl. The effect of AT was to produce only a non-significant reduction of less than 10% in the maximum peak blood isopropanol level and acetone when given with the 1g/kg, i.p., dose which showed little or no involvement of catalase in isopropanol metabolism in the rat.

Pyrazole treatment (300 mg/kg, orally, 23 hours prior to isopropanol, 1 g/kg, i.p.) increased blood isopropanol concentrations above the control and decreased the rate of oxidation of isopropanol. The pyrazole treated animals had blood isopropanol levels of greater than 120 mg/dl for 6 hours and the blood acetone level was less than 20 mg/dl, while the control animals blood isopropanol and acetone levels were about 60 mg/dl and 80 mg/dl, respectively. This established the role of ADH in the metabolism of isopropanol in the rat (7).

The lactate/pyruvate ratio, a measure of the extramitochondrial redox state, was not effected by oral or i.p. isopropanol (7) which is contrary to the effects of ethanol (12). The production of ß-hydoxybutyrate was slightly increased causing a slightly increased ß-hydroxybutyrate/acetoacetate ratio. The authors (7) speculated that the extramitochondrial reduction state may be maintained by the metabolism of acetone which proceeds: acetone → acetol (or acetol phosphate) ⟶ 1,2-propanediol (or 1,2-propanediol phosphate) and requires NADH for reduction (4).

Forsander (12) found a low lactate to pyruvate ratio for isopropanol which was similar to the effects of methanol but unlike the effect of ethanol. The lactate/pyruvate ratio is inversely related to the respiratory quotient (R.Q.), the ratio of CO_2 produced to O_2 uptake. The effect of isopropanol (2-propanol) and other alcohols on the respiratory quotients is

shown in Table 2.3. The cause of lower respiratory quotients following ethanol and isopropanol administration was due to depressed CO_2 production rather than diminished O_2 uptake.

Table 2.3 Respiration of Liver Slices in Kreb's Ringer Bicarbonate Buffer and 25 µl of Alcohol

Alcohol	O_2 Uptake	CO_2 Produced	Acid Produced	R.Q.
	----------(µmoles/g liver·min)--------			
none	1.25	0.94	0.36	0. 75
methanol	1.18	1.36	0.25	1. 15
ethanol	1.30	0.20	1.02	0. 15
2-propanol	1.04	0.52	0.46	0.50

Source: Forsander, O.A. (1967) Influence of some aliphatic alcohols on the metabolism of rat liver slices. Biochem. J. 105: 93-97. (ref.12)

Fatty acids are the main substrates for liver and the main route of CO_2 production is the tricarboxylic acid cycle (TCA). Ethanol reduces the functioning of the TCA cycle and acetate and organic acids accumulate (12). Table 2.3 shows this acid accumulation with ethanol. Nordmann et al (8) concluded that the bulk of the metabolic effects of isopropanol metabolism are apparent in microsomes and not in mitochondria as they would have been in the case of ethanol.

Fat accumulation in the liver followed isopropanol administration. The fat accumulation in the liver of isopropanol treated animals was related to an increased mobilization of adipose tissue triglycerides and fatty acids and not the result of reduced fatty acid oxidation to CO_2. The authors (8) suggested that the increased free fatty acid

release from adipose tissue was a non-specific response to the acute isopropanol dose and not due to a direct effect of isopropanol or acetone.

2.2.3 Metabolism of Ethylene Glycol

Ethylene glycol is metabolized primarily in the liver according to the scheme shown in Figure 2.3. The initial steps in the oxidation of ethylene glycol to glycol aldehyde and glyoxylic acid seem to be mediated by alcohol dehydrogenase. McChesney et al (19) gave an intravenous (i.v.) dose of 139 mg/kg ^{14}C labelled ethylene glycol to four rats. After four hours 37.7% of the radioactivity had been excreted in urine and 5.8% in air. By 24 hours 46.5% of ^{14}C had been excreted in urine and 14.4% in air. The excretion rate of 1.5% of the dose/hour was noted for 4 hours. A slower rate of 0.4% of the dose/hour was observed over the next 20 hours. The ethylene glycol had fairly evenly distributed throughout all tissues by one hour with the total amount in tissues decreasing over time. The liver concentration of ^{14}C did not diminish due to its central role in the metabolism of ethylene glycol and its metabolites.

In rats given an oral dose of 1 ml/kg of ethylene glycol and a dose of water (25 ml/kg) 55.7% of the ^{14}C was excreted in 24 hours , over half of this being unchanged ethylene glycol. After 8 hours 27.8% of the ethylene glycol was excreted unchanged with a total of 45.9% of the dose excreted. Eighty percent of the ^{14}C of ethylene glycol was excreted in the first 8 hours of the period. The tissue half life was on the order of 2.0 to 2.5 hours (19).

The excreted metabolites in the urine are the stable compounds glycollic acid, oxalic acid and in expired air, CO_2. Glycol aldehyde and glyoxylic acid are very reactive and have a short half life (19). Parry and Wallach (10) mention the possibility of damage to the body caused by glycol aldehyde but little evidence exists to support the claim that glycol

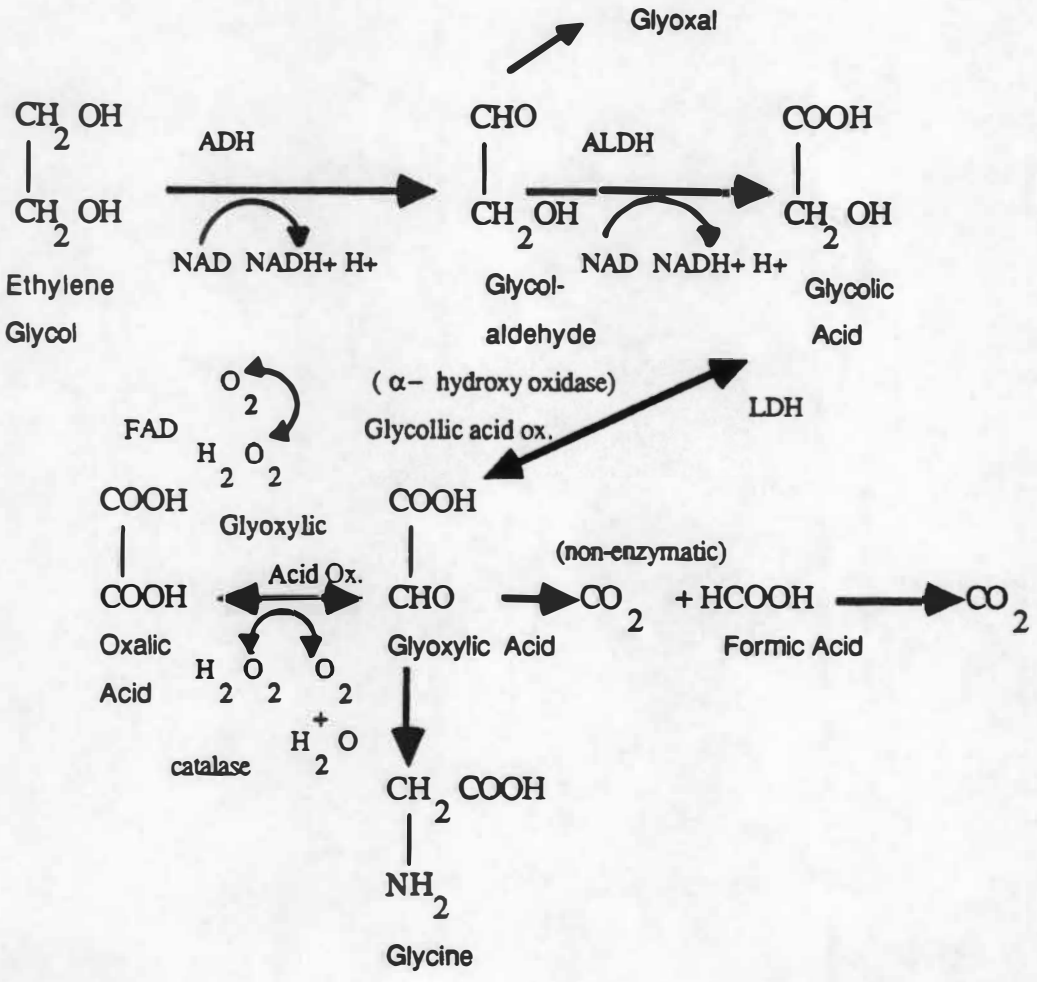


Figure 2.3. Metabolic pathways for ethylene glycol.

Source: Van Harken, D.R. (1965) Role of the hepatic catalase-peroxidase system in the metabolism of methanol by the rat. Doctoral Dissertation, University of Minnesota. (ref.17)

aldehyde is transported throughout the body before immediate metabolism. Pooled urine samples from rats for 0-8 hours contained glycolic acid and oxalic acid in a ratio of 13:1 but 8-24 hour sample had a ratio of 8:3 showing an increase in the production of oxalic acid after a rise in the blood glycolic acid level. This ratio in monkeys was 80:3 in the 8-24 hour sample . This reflects the fact that the monkey converts less ethylene glycol to oxalate than rats (19).

In isolated liver mitochondria from female rats neither ethylene glycol, glycolic acid nor propylene glycol at concentrations up to 10 mM, had any effect on the rates of mitochondrial respiration using succinate, α-ketoglutarate or β-hydroxybutyrate as substrates (20). Glyoxylic acid and oxalic acid did decrease respiration with all substrates. Oxidative phosphorylation was not affected except when α-ketoglutarate was used with higher levels of glyoxylic acid. Mitochondria from male rats responded similarly to female rats except that oxidative phosphorylation was inhibited with succinate and glyoxylic acid also (20).

Liver mitochondria from monkeys exposed to ethylene glycol by inhalation for 5 months showed a reduced rate of respiration and uncoupling of oxidative phosphorylation (reduced P/O or ADP/O ratios). Glyoxylic acid, however, may be more involved in this response as the addition of ethylene glycol to control mitochondria showed no effect (20). Glyoxylic acid may condense with oxaloacetate to form oxalomalate which is a potent inhibitor of the citric acid cycle enzymes (20).

2.3 Nutritional Influences on Alcohol Metabolism

2.3.1 Methanol

The rate of methanol metabolism in rats is dependent upon enzymes that generate H_2O_2. Reduction of the level of H_2O_2 in the catalase complex reduces the methanol oxidizing capacity. Peroxide generating systems such as glucose:glucose oxidase, urate: uric acid oxidase and xanthine: xanthine oxidase are required for optimal rates of methanol oxidation (21).

Treatment of rats or liver homogenates with sodium tungstate results in a marked reduction of xanthine oxidase activity as well as methanol metabolizing capacity by reducing the activity of the molybdoflavoprotein moiety of the xanthine oxidase system. The addition of purified xanthine oxidase to tungstate treated rat liver homogenates restored the methanol oxidizing ability. Molybdenum supplementation of the tungstate treated animals restored the xanthine oxidase activity but only partially restored the methanol oxidation capacity (21, 17).

2.3.2 Ethylene Glycol

Ethylene glycol metabolism like many other alcohols requires NAD, therefore niacin (nicotinamide) for its initial dehydrogenations. Glycolate oxidation for glyoxalate requires either LDH (lactic dehydrogenase) or the flavin requiring glycolic acid oxidase (10). Glyoxalate can be metabolized via pyridoxal phosphate dependent transaminases with several different amino acid donors to yield glycine. Thiamine pyrophosphate and magnesium are necessary for the conjugation to α-hydroxy-ß-ketoadipate. Deficiencies of any of these cofactors then could have a profound influence on ethylene glycol metabolism. Formation of conjugates which inhibit citric acid cycle enzymes will reduce available

Formation of conjugates which inhibit citric acid cycle enzymes will reduce available energy, O_2 and oxidized cofactors needed for continued metabolism (10).

Deficiency of vitamin B_6 (pyridoxine) in rats resulted in the increased deposition of oxalate crystals in the kidney which was prevented by feeding supplemental magnesium oxide. Magnesium did not effect ethylene glycol oxidation which led to the hypothesis that magnesium protects against the renal deposition of oxalate by altering the solvent characteristics of the urine (23). It is interesting to note that male rats were more susceptible to ethylene glycol than female rats. If the female rats were made deficient in pyridoxine they too were just as susceptible as male rats. (24).

2.4 Interactions of Alcohols

2.4.1 Methanol and Ethanol

Ethanol is a competitive inhibitor of the peroxidative pathway for methanol metabolism in rats. In perfused rat livers ethanol given in equimolar concentrations with methanol resulted in a 58% inhibition of methanol oxidation during a 3 hour period. There was a 70% inhibition in the first hour. Methanol, however, had no discernible effect on ethanol metabolism even at molar ratios of 50:1 (25).

2.4.2 Methanol and Ethylene Glycol

The effect of ethylene glycol on the metabolism of methanol in the perfused rat liver was studied using ^{14}C-methanol (17). Ethylene glycol doubled the rate of methanol oxidation down to molar ratios of 1 methanol: 0.125 ethylene glycol. The same results were seen in vivo with i.p. doses of ethylene glycol and methanol. This effect was not expected as ethylene glycol is a substrate for ADH. If methanol were oxidized by ADH

then ethylene glycol should have depressed the rate of methanol oxidation. The H_2O_2 produced by the FAD catalyzed oxidation of glycolic and glyoxylic acids, metabolites of ethylene glycol, was responsible for the increase in the rate of methanol metabolism (17).

2.4.3 Methanol and other Alcohols

Other alcohols antagonize the oxidation of methanol in the rat at equimolar concentrations in the following order: (alcohol, % inhibition) ethanol, 55% > propanol and isopropanol, 35% > n-butanol, 30% > n-pentanol, 20% (21, 17).

2.4.3 Ethylene Glycol and Ethanol

Peterson et al (26) calculated the effective dose of ethanol for use in human ethylene glycol poisoning. The optimal blood level of ethanol to displace ethylene glycol at ADH and increase ethylene glycol excretion in urine are between 100-200 mg/dl. These levels were achieved by repeated oral dosing with 0.109 g/kg ethanol per hour given as a 20% solution after loading with 0.6 g/kg ethanol in a 50 % solution. In experiments with male rats receiving 10 ml/kg of ethylene glycol, Borden and Bidwell (27) found an increase in the 96 hour survival from 14% of controls to 73 % of the animals that received 0.2 g ethanol 15 minutes after ethylene glycol and continuing 6 times at 6 hour intervals following the ethylene glycol treatment. Survival was increased further by the combination of ethanol and sodium bicarbonate. Examination of the kidneys of ethylene glycol treated animals found that 94% of the controls showed oxalate crystals while only 55% of the ethanol treated group showed crystal development. None of the bicarbonate and ethanol treated group showed crystal development. In this study the ethylene glycol was given orally and the ethanol was administered intraperitoneally (27).

2.5 The Physiological Role of Carnitine

Carnitine, 3-hydroxy-4-N-trimethylammonium butyrate is a polar compound that is widely distributed in nature. The role of carnitine as a vitamin or growth factor was established when Fraenkel and others demonstrated the essentiality of carnitine for the mealworm, *Tennebrio molitor* (28). The physiological role of carnitine was later established to be in the ß-oxidation of long chain fatty acids (29, 30).

The dietary essentiality of carnitine for humans has yet to be established although deficiencies of carnitine have been reported in infants fed formulas, renal patients and in patients with lipid storage disorders (31). Carnitine participates in two separate transferase systems in the mitochondrial transport of long chain fatty acids; carnitine palmitoyltransferase I (CPT-I) and carnitine palmitoyltransferase II (CPT-II). The outer mitochondrial membrane is the site of CPT-I and the inner mitochondrial membrane is the site of CPT-II. The activity of CPT-I is a rate controlling factor in the transport of fatty acids across the mitochondrial membrane (32). The location of CPT-II is in intimate proximity with the enzymes of ß-oxidation. Figure 2.4 depicts the role of carnitine and carnitine palmitoyltransferase in the oxidation of fatty acids.

Carnitine and carnitine acyltransferases are important in several organelles of mammalian organs. These enzyme systems are involved in the transport of acyl groups of various sizes across biological membranes. The liver contains at least three enzymes showing carnitine acetyltransferase activity, one is associated with peroxisomes, another with the endoplasmic reticulum and the third with the mitochondria (33). The roles of carnitine and carnitine acyltransferases are not elucidated in their entirety.

Carnitine is found primarily in foods of animal origin, sheep muscle meats being the richest source (208 mg/100g) with beef muscle meats next (67mg/100g) (34). Chicken and eggs were very poor sources of carnitine as were most plant foods. Avocado, is the

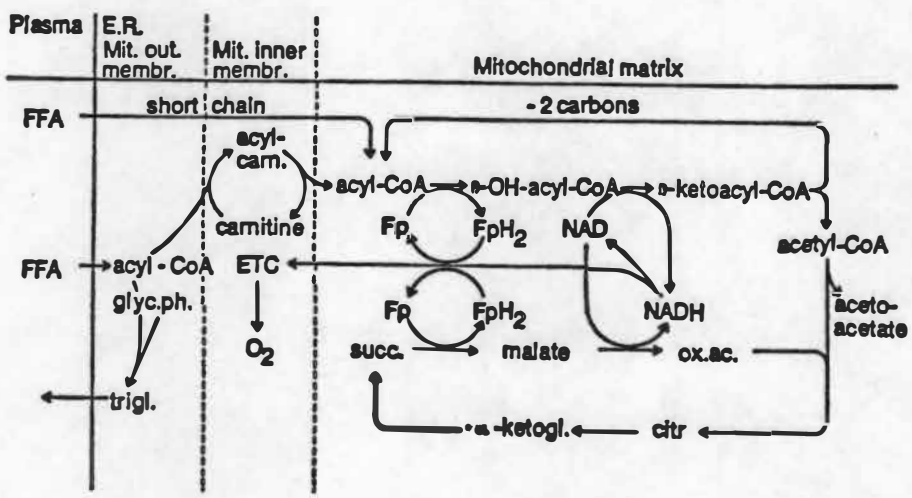


Figure 2.4. The role of carnitine in fatty acid metabolism in the liver.

Source: Bremer et al (1974) Factors controlling the metabolism of fatty acids in the liver. In: Regulation of Hepatic Metabolism, Proceedings of the Alfred Benzoin Symposium VI. (Lundquist, F. and Tygstrup, N., eds.) pp.159-179, Academic Press, New York. (ref.32)

richest (1.25 mg/100mg) source of carnitine among the foods of plant origin. In general carnitine excretion is related to dietary intake. Low intakes in human volunteers resulted in reduced urinary carnitine excretion. (34). Carnitine is rapidly absorbed from the upper jejunum (35, 36, 37). The active isomer, L-carnitine, is taken up by the intestine twice as fast as the D isomer (35). Nearly 50% of non-esterified carnitine is acetylated in the small intestine and is stored temporarily in the liver (37). The majority of an absorbed dose of carnitine will be found in the muscle tissue (36).

Carnitine is also synthesized in the human from two essential amino acids, lysine and methionine. The synthesis in humans begins with protein bound lysine which is methylated by S-adenosylmethionine and lysine methyltransferase to form 6-N-trimethyllysine. Trimethyllysine is then hydroxylated by dioxygenases which utilizes α-ketoglutarate as a co-substrate and requires ascorbic acid, ferrous iron and molecular oxygen (33, 34) . The 3-hydroxy-6-N-timethyllysine is released into the cytosol and the cleavage of the glycine moiety yields γ-butyrobetaine aldehyde. The aldehyde is oxidized to γ-butyrobetaine and then a final hydroxylation similar to that with trimethyllysine forms carnitine, $+N(CH_3)_3\ CH_2\ CHOH\ CH_2\ COOH$ (33, 34).

2.6 The Interaction of Carnitine and Alcohol

Carnitine supplementation has been shown to ameliorate the hepatic steatosis induced by long term ethanol feeding in animals (38, 2). This supplementation also lowered plasma lipids associated with chronic alcoholism and was shown to be a dose dependent effect (3). Observation of the animals used in these chronic alcohol feeding studies led to the investigation of the blood ethanol levels as the animals appeared more intoxicated to the researchers (39). Blood ethanol levels 2 hours after dosing with 3g/kg of ethanol in rats

fed over a long term with liquid ethanol diets supplemented with 0.8 or 1.0% DL-carnitine were significantly elevated over the control levels. In experiments with rats fed carnitine supplemented diets for 5 or 6 days prior to a single ethanol dose the 2 hour blood ethanol level was also significantly elevated above the control (77 mg/dl,control vs. 213 mg/dl,carnitine). The effect of carnitine on blood ethanol levels was also dose dependent with the higher carnitine supplementation yielding the highest 2 hour blood ethanol values (39).

Sachan and Berger (4) also established that the effect of carnitine supplementation was not found in the absorption phase of the blood alcohol curve but was sustained over the 8 hours of their study indicating a possible effect on ethanol metabolism or disposition (Figure 2.5). They also established that 1.0 % DL-carnitine supplementation attained blood carnitine equilibrium in 3 days and that longer than 5 days of supplemental feeding may be necessary for lower carnitine levels of supplementation to reach an effective blood level.

Co-administration of carnitine and ethanol, i.p., has also been shown to be effective in reducing the blood triglycerides, and accumulations of liver triglycerides and total liver lipids as seen in ethanol administration alone (40). Carnitine also lowered the SGOT levels after ethanol administration(40). The effects on lowering the blood triglycerides and SGOT were thought to be independent of any alcohol effects as they occurred in non-ethanol treated animals as well. Carnitine was shown to overcome the ethanol induced mitochondrial membrane derangement which produced impermeability to NADH and long chain acylcarnitines.

Kondrup and Grunnet (41) found that after an acute ethanol dose the non-esterified carnitine and acetylcarnitine in the liver increased by 50% and long-chain acylcarnitine was decreased by 50 %. The total carnitine was increased with a higher proportion of the carnitine being non-esterified. The ratio of acylated carnitine to free reflects the direction

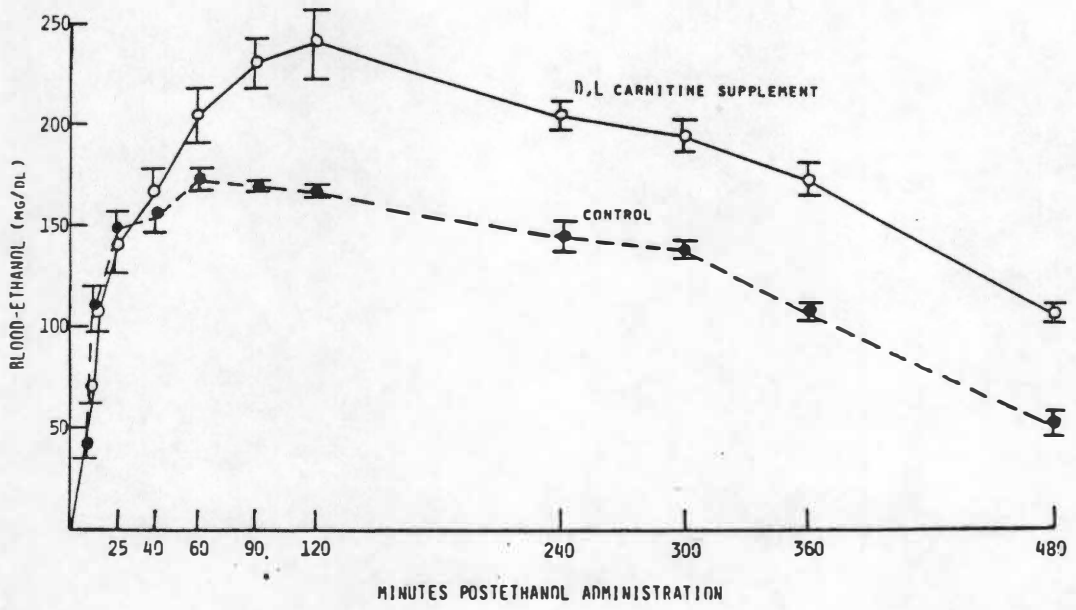


Figure 2.5. Time course of blood ethanol concentration after 5 days of feeding a 1% D,L-carnitine supplemented chow diet.

Source: Sachan, D.S. and Berger, R. (1987) Attenuation of ethanol metabolism by supplementary carnitine in rats. Alcohol 4: 31-35. (ref. 4)

of hepatic fat metabolism. For example in starved animals the ratio is increased and after an acute ethanol treatment where fat oxidation is decreased, the ratio is decreased (41).

In an experiment with human volunteers, Adamo and co-workers (42) administered a dose of 0.5g/kg ethanol in the form of white wine. They also co-administered a saline solution or a 3 gram intravenous dose of L-carnitine. There were no differences between the saline group and the carnitine group blood ethanol curves. Differences were found in the kinetics of blood acetate with the saline group having a higher mean blood concentration and area under the curve. The urinary acetylcarnitine excretion was significantly increased only when ethanol and carnitine were simultaneously administered. The single carnitine dose alone did not significantly raise urinary acetylcarnitine excretion. (42)

Ethanol was shown to directly inhibit carnitine palmitoyltransferase I, (CPT-I), the rate limiting step in the transport of fatty acids in to the mitochondrial matrix of liver (43). This ethanol induced modulation of CPT-I activity is very difficult to detect as normal cell disruption and isolation of mitochondria techniques caused the effect to disappear. The authors also state that it is the presence of active ethanol metabolism that is inhibitory to CPT-I. The state of active ethanol metabolism resulted in an increased intracellular malonyl-CoA concentration. Malonyl-CoA is a potent inhibitor of CPT-I. Keeping in mind the role of carnitine in the acyl group metabolism and stated effects on the metabolism of ethanol, the role of carnitine in the metabolism and disposition of methanol, isopropanol and ethylene glycol was examined.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Plan

Male rats were divided into two groups, the non-supplemented chow fed group (NS) and the group fed chow supplemented with 0.5% L-carnitine (CS). These animals were then used to test the effects of carnitine on the metabolism and disposition of methanol, isopropanol and ethylene glycol. Table 3.1 depicts the experimental plans.

3.2 Animals, Diets and Housing

Male Sprague-Dawley rats were used in all experiments. Table 3.1 lists the animals' suppliers, weight ranges and other experimental specifications. All animals were housed in an AALAC accredited animal facility. The light cycle was from 6:00 a.m.until 6:00 p.m.. The temperature of the facility was 72 $\pm$ 2° F and the relative humidity was 40-50%. The animals received food and water ad libitum. Animals were individually housed in stainless steel suspended wire cages and were transferred to stainless steel metabolic cages after dosing with the alcohol.

The diet used in these experiments was ground rodent chow (#5001, Raltson Purina, St. Louis, MO.) as such (NS group) or supplemented with 0.5% L-carnitine HCl (CS group) (Sigma Chemicals, St. Louis, MO.) or the equivalent dose of L-carnitine (gift of

Table 3.1. Summary of Experimental Plans.

Alcohol Treatment	Dietary Treatment NS[1]	Dietary Treatment CS[2]
1. Methanol[3]		
No. of animals[4]	14	14
Body weight (g)[5]	507.0 ± 12.0	480.0 ± 11.0
Dose per kg (g) / (moles)	2.25 / 0.07	2.25 / 0.07
2. Isopropanol[6]		
No. of animals[7]	5	5
Body weight (g)[8]	409.2 ± 7.9	438.4 ± 6.5
Dose per kg (g) / (moles)	2.0 / 0.03	2.0 / 0.03
3. Ethylene Glycol[9]		
No. of animals[7]	6	6
Body weight (g)	402.0 ± 3.0	401.0 ± 12.0
Dose per kg (g) / (moles)	5.56 / 0.089	5.56 / 0.089
4. Ethylene Glycol +Ethanol[10]		
No. of animals[7]	4	4
Body weight (g)	288.0 ± 9.8	297.0 ± 12.8
Dose per kg-ethylene glycol (g) / (moles)	5.56 / 0.089	5.56 / 0.089
Dose per kg-ethanol (g) / (moles)	3.0 /.07	3.0 / 0.07

1. Non-supplemented ground chow, Purina #5001, Ralston Purina, St. Louis, Mo. fed for 7 days in methanol experiment, 5 days in all other experiments.
2. Ground chow, supplemented with 0.5% L-carnitine and fed for 7 days in methanol experiment and 5 days in all other experiments.
3. Methanol (Fisher Scientific, Fair Lawn, NJ) administered in 13% aqueous solution by oral gavage.
4. Tac:N(SD)fBR ,Taconic Farms, Inc.,Germantown, NY.
5. Values are means ± SEM.
6. Isopropanol (Fisher Scientific,Fair Lawn, NJ) administered in 13% aqueous solution by oral gavage.
7. Hsd: Sprague-Dawley®SD™, Harlan Sprague Dawley, Indianapolis, IN.
8. Significantly different by t test, $P>|t|=0.05$.
9. Ethylene glycol (J.T. Baker, Phillipsburg, NJ) administered in a 1:1 aqueous solution by oral gavage.
10. Ethylene glycol was administered first and one hour later ethanol dose was administered. Ethanol (Warner Graham Co., Cockeysville, MD, 200 proof anhydrous) administered in 13% aqueous solution by oral gavage.

Sigma Tau, Italy) . Purina Chow #5001 provides 4.25 kcal/g and is a mixed diet from vegetable matter and fish meal. Similar diets have an estimated total carnitine content of 64.2 nmoles/g (38). The animals were fed the supplemented diet for 5 or 7 days as shown in Table 3.1.

3.3 Dosing Procedure

The animals were given a dose of methanol, isopropanol (2-propanol), ethylene glycol (1,2-ethandiol) or ethylene glycol and ethanol at the end of the supplementation period (Table 3.1). No animals were fasted before alcohol dosing and doses were administered between 10:00 a.m. and noon. The animals were given the diluted alcohol by means of a stainless steel stomach tube attached to a syringe. The proper placement of the tube in the stomach was insured by monitoring respiratory distress and the dose was steadily infused. The experimental protocol was approved by the Animal Research Committee of the Department of Nutrition . All animals were given a single dose of the alcohol except for animals used to evaluate methanol urinary clearance who were dosed first to measure urine methanol excretion then allowed to recover and dosed a second time for the evaluation of blood methanol. This repetition of the dosing was necessary as the animals often urinated while having blood samples taken. Proficiency gained in the methanol experiment in handling the animals made this practice unnecessary in later experiments.

3.4 Sampling Procedure

3.4.1 Blood Samples

Blood samples of 20 µl were taken from a freely bleeding cut in the tail vein which was prepared by wiping with a saline moistened gauze. The blood was collected in heparinized microcapillary tubes and transferred to a microcentrifuge tube containing 80 µl of 0.9% NaCl solution. The diluted blood samples were kept on ice until they were mixed and centrifuged in a Brinkmann microcentrifuge for 2 minutes. The supernatant was then analyzed for the alcohol by gas chromatography. Mild pressure was applied to the tail after the cut was made to seal the wound and successive cuts for each sample were made proximally (towards the base of the tail) to avoid excessive leakage of tissue fluids into the sample.

3.4.2 Urine Samples

Urine collections were made every two hours after dosing and with the methanol treatment the urine collection continued for the rest of the 24 hour period. The urine samples were collected in stainless steel metabolic cages with stainless steel collection funnels. A 50 ml Erlenmeyer flask which was bathed in ice water contained the urine sample. This style of metabolic cage is not always 100% efficient in separating feces from urine. Any fecal pellets which fell into the urine were removed at the end of the collection period and the urine was mixed and centrifuged in a refrigerated centrifuge (Beckman TJ-6R) at 1500 X g for 10 minutes.

3.5 Analysis of Samples

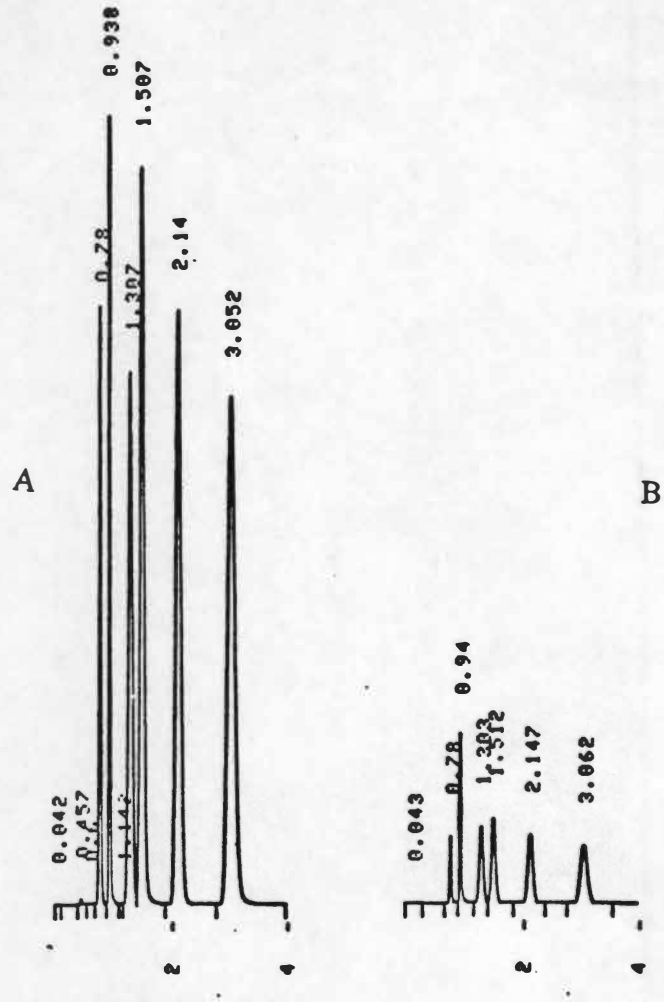
3.5.1 Blood Samples

3.5.1.1 Analysis for blood acetone, methanol, isopropanol and ethanol

Analysis of blood for acetone, methanol, isopropanol and ethanol was performed by gas chromatography using a Shimadzu GC-9AM (Shimadzu Scientific Instruments, Columbia, Md.) The GC was equipped with a clinical alcohol column (2.1 meters x 2.6 mm i.d., packing, 5% Carbowax 20M on 60 to 80 mesh Carbopack B, Supelco, Bellefonte, Pa.).Sensitivity range of the GC was set for 1x 10^{-11} amps full scale deflection. The output from the GC was analyzed by an integration unit-microprocessor (C-R3A, Shimadzu Scientific Instruments, Columbia, Md.) using a two point external standardization program. The attenuation setting was 64 millivolts full scale deflection. Using helium as a carrier gas with a flow rate of 28 ml/min, isothermal temperature program of 100° C and flame ionization detector good separation was achieved between the alcohols. Standards of the alcohols (Anhydrous Alcohols Standards, Polyscience Corp., Nile, Il.) were prepared in cold glass distilled water (GDW) to yield the µg/µl concentrations in the range needed, about 0.08 to 0.8 µg/µl . Sample standard chromatograms are shown in Figure 3.1. (Acetaldehyde, 99%, Aldrich Chemicals, Milwaukee, WI, and n-propanol are also shown but were not internal or external standards in these experiments.) One to two µl injections were used of the standards or blood supernatant. And all samples were analyzed within 24 hours after collection.

3.5.1.2 . Analysis of blood ethylene glycol

Ethylene glycol (Baker Reagent Grade, J.T. Baker, Phillipsburg, N.J.) was analyzed on the same Shimadzu equipment described above. The column used was a glass column 2.1 meters x 2.6 mm i.d., packed with GP 80/100 Carbopack C/ 0.8% THEED,



CALIBRATION MADE IN IDENTIFICATION FILE 2
MODES 127

IDNO	NAME	TIME	BAND	FACTOR1/2	CONC1/2
1	ACETALD	0.79	0.04	8.23112E-6	0.788
				-0.0337655	0.0788
2	MEOH	0.94	0.03	3.55538E-6	0.7915
				-0.0168516	0.07915
3	ACETONE	1.29	0.04	3.73878E-6	0.788
				-0.0329965	0.0788
4	ETOH	1.52	0.05	2.59636E-6	0.789
				-0.00730316	0.0789
5	ISOPROP	2.13	0.04	2.55545E-6	0.785
				-0.00489585	0.0785
6	NPROP	3.05	0.3	2.15239E-6	0.885
				-0.00499262	0.0885

Figure 3.1. Separation of alcohols by gas chromatography. Part A is a high mixed standard. Part B is one-tenth of A. The actual µg/µl concentrations are listed in the CONC 1/2 column.

(Supelco, Bellefonte, Pa..). The output from the GC was processed by the C-R3A using area normalization and a standard curve was used to manually calculate the concentration of ethylene glycol. The attenuation setting was 8 millivolts full scale deflection. Helium was the carrier gas with a flow rate of about 50 ml/min and an isothermal temperature program of 112° C. One to 2 µl injections of appropriately diluted standards (0.5 to 3.0 µg/µl) or blood supernatant were either manually injected or injected by the auto sampler of the Shimadzu GC within 72 hours after collection. Samples not analyzed immediately were stored in the cold. When the auto sampler was used no attempt was made to keep the samples in the sample queue above room temperature.The ethylene glycol was eluted in about 28 minutes. A sample chromatogram for ethylene glycol is shown in Figure 3.2 and standard curve for ethylene glycol is shown in Figure 3.3.

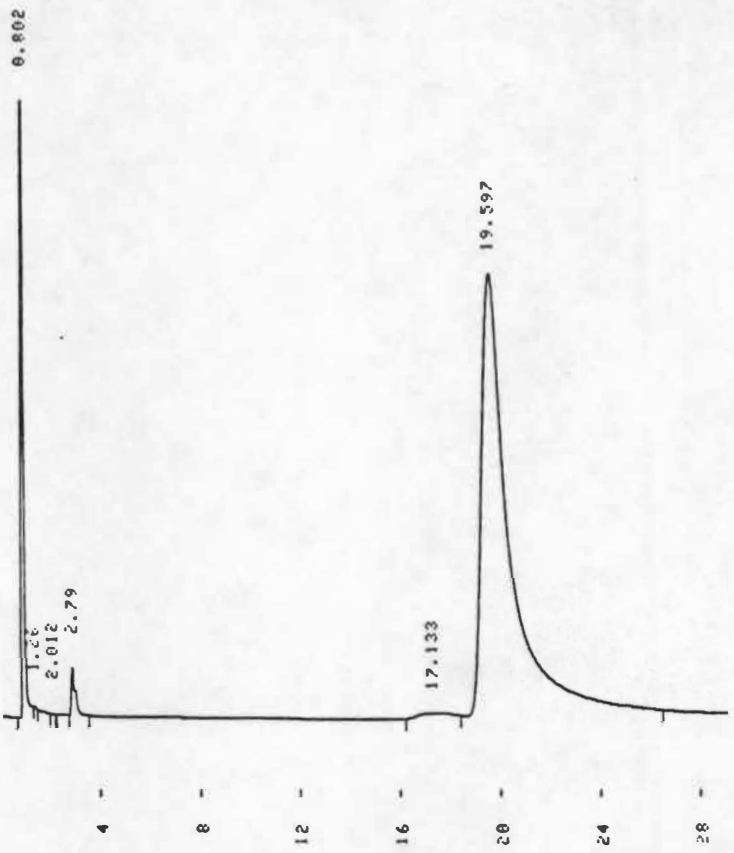
3.5.2 Urine Analysis

3.5.2.1 Analysis of urine for acetone, methanol,isopropanol and ethanol

Acetone, methanol, isopropanol and ethanol in the urine were analyzed according to the scheme outlined in Section 3.5.1.1. The centrifuged urine was diluted three to five fold with cold glass distilled water (GDW) and the samples were stored on ice until they were manually injected into the GC. All samples were analyzed within 24 hours.

3.5.2.2 Analysis of urine for ethylene glycol

Ethylene glycol in the urine was analyzed according to the scheme outlined in Section 3.5.1.2. The centrifuged was diluted three to five fold with cold GDW and injected manually by the auto sampler into the GC within 72 hours after cold storage.



CHROMATOPAC C-R3A FILE 3
SAMPLE NO 0 METHOD 2461
REPORT NO 800

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.802	56024	S		12.0509	
		7.6sec				
2	1.26	135	T		0.029	
		3.9sec				
3	2.012	16	T		0.0035	
		6.8sec				
4	2.79	6008			1.2923	
		10.5sec				
5	17.133	4236			0.9112	
		71 sec				
6	19.597	398477	V		85.7131	
		75.9sec				
	TOTAL	464896			100	

Figure 3.2. Quantitation of ethylene glycol by gas chromatography.

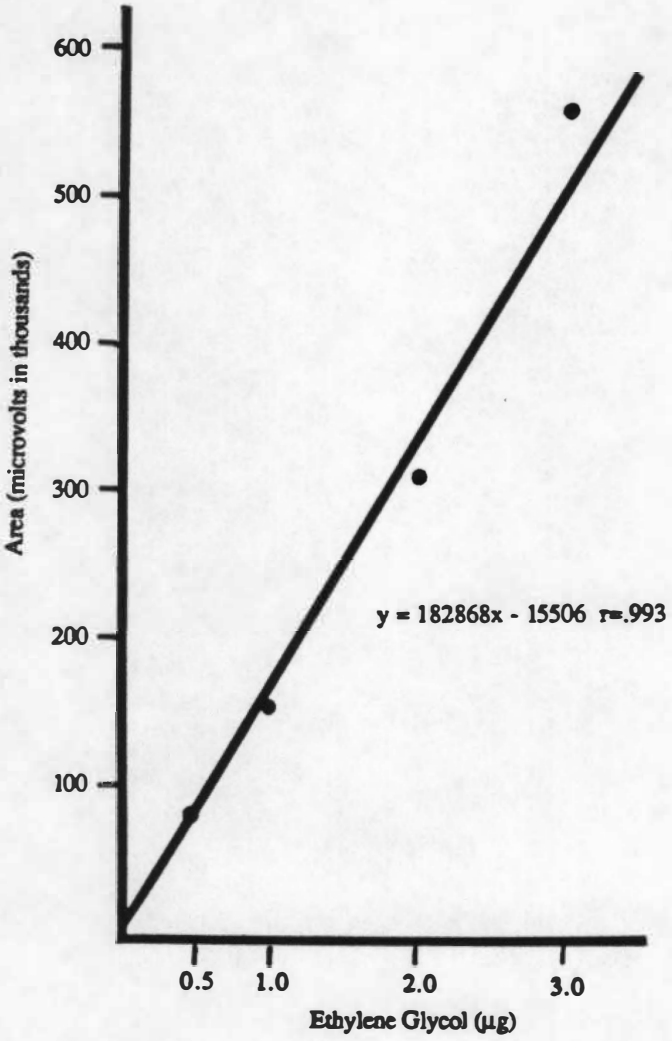


Figure 3.3. Standard curve for ethylene glycol.

3.5.3 Data Analysis

3.5.3.1 Kinetic Analysis

The following kinetic analyses based on Baggot (44) were performed on the individual animals' blood alcohol concentration data and urinary alcohol excretion data without any logarithmic transformations.

Absorption Slope (initial): Simple linear regression analysis was used to determine the slope of the blood alcohol concentration from time zero (concentration zero) to 0.66 hours (40 minutes).

Absorption Slope (to peak): The slope of the blood alcohol concentration from time zero (concentration zero) to the point of maximum value over time was calculated by simple linear regression.

Peak Blood Value: The highest blood alcohol concentration value reached at any time following dosing.

Peak Blood Time: The time at which the peak blood alcohol concentration was reached.

Area Under the Curve (AUC): The AUC was calculated manually by the method of trapezoidal approximation (Figure 3.4). The areas of triangles and trapezoids included in the blood time course curve were summed and the hypothetical end tails lying outside of the designated time segment were not included.

Systemic Clearance: This represents the sum of all clearance processes in the body. It is the volume of blood cleared per hour per kg of body weight and was calculated as dose/ AUC.

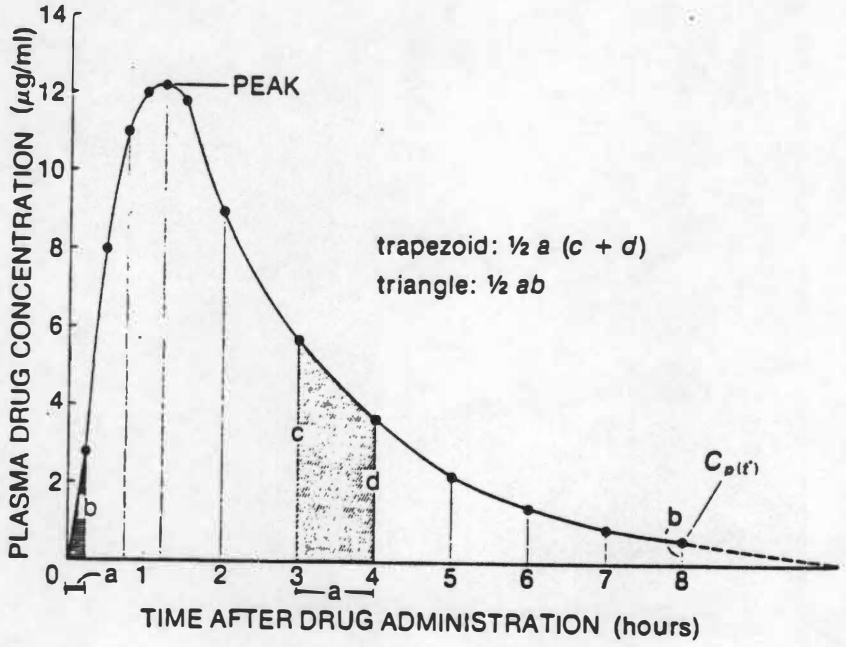


Figure 3.4. Trapezoidal method for estimating the area under the curve. The total area under a curve may be estimated by adding areas of the trapezoids and triangles at each end of the curve.

Source: Baggot, J.D. (1977) Principles of Drug Disposition in Domestic Animals, W.B. Saunders Company, Philadelphia, p. 34. (ref.44)

Elimination Slope (K_{el}): This is the elimination rate constant for the disappearance of the drug from the blood. The blood alcohol concentration curve from the peak to the last point on the time course within the time band of interest were used to calculate a line by simple regression. Since no transformations were used the slope is the rate constant.

Theoretical Instantaneous Distribution Concentration (C_o): This is the theoretical blood alcohol concentration assigned had the drug been given as a bolus intravenous dose or been entirely and immediately absorbed from the gastro-intestinal system. This was calculated as the y intercept of the elimination slope line at time zero.

Volume of Distribution (V_d): The volume of distribution is a proportionality constant relating plasma concentrations to the total amount of the drug in the body. It was calculated as C_o/dose. The V_d is not a true physiologic space. Baggot (44) states the following:

> This parameter which is derived from plasma (or serum) concentrations of the drug, provides some idea on or the extent or magnitude of distribution. A large value for the apparent volume of distribution can be interpreted to mean that the drug is widely distributed in body fluids and tissues, or that the drug is avidly bound to tissue constituents (and perhaps localized), or extensively bound to plasma proteins (if volume of distribution is derived from free drug levels in plasma). A small volume of distribution could mean that the drug is restricted to certain fluid compartments of the body (e.g., extracellular fluid) or is extensively bound to plasma proteins, when volume of distribution is based on total drug concentration in plasma, or both....[D]espite the limitations... estimates of the magnitude of distribution provided by this kinetic parameter can be helpful in describing the disposition of drugs in the body. (p.54)

Urinary Clearance: The urinary clearance is a ratio of the blood and urine concentrations of compound based on the flow rate of the urine and the body weight of the animal. Baggot (44) defines urinary (renal) clearance as " that fraction of the body clearance attributable to excretion of unchanged drug by the kidney". It was calculated as follows.

$$\frac{\text{[concentration in urine (mg/ml)][ml of urine for time period]}}{\text{[concentration in blood (mg/ml at mid point of time period)][body weight (kg)]}}$$

The midpoint blood was either the actual value for the sample taken at the true midpoint for the urine collection or was calculated as an average from the beginning and ending blood levels or calculated based on the slope of the blood curve when the end point was missing.

3.5.3.2 Statistical Analysis

Statistical analysis of the data was performed by a Statistical Analysis Systems program (SAS Institute Inc., Cary, N.C., USA, CMS SAS Release 5.18, University of Tennessee). The Student's t test was used for two point comparisons. Data from time curves were analyzed as kinetic transformations and therefore did not violate rules for use of the t test repeatedly over time. The level of significance was 0.05 but the borderline significances were also shown to validate trends.

CHAPTER 4

RESULTS

4.1 Methanol

The blood methanol curves obtained from a 2.25 g/kg dose in male rats are depicted in Figure 4.1. The solid line represents the NS animals and the dotted line the CS animals. This figure represents each time point equally and not in an exact time scale. The NS curve is higher in the early stages of the intoxication and appears to peak at about 4 hours compared to 8 hours for the CS group. The elimination side of the curves appear similar for both groups. The numerical data are shown in Table 4.1. The 2 and 4 hour blood methanol concentrations (BMC) are significantly lower in the CS group than the NS group.

The blood kinetic analysis is shown in Table 4.2. There were no differences in the initial absorption slope (40 minutes) between the two groups but there was a significant reduction in the absorption slope to the peak in the CS group. The time to reach the peak blood concentration was almost doubled by carnitine supplementation but there was no difference in the peak BMC. The total area under the BMC curve for 8 hours was smaller in the CS group than the NS group. The area under the 24 hour BMC curve showed no significant differences between groups. Systemic clearances, which relate area under the curve to dose, were not significantly different for the two groups at 8 or 24 hours. There were no significant differences between the groups in the elimination rate constants, theoretical instantaneous distributions or volumes of distribution.

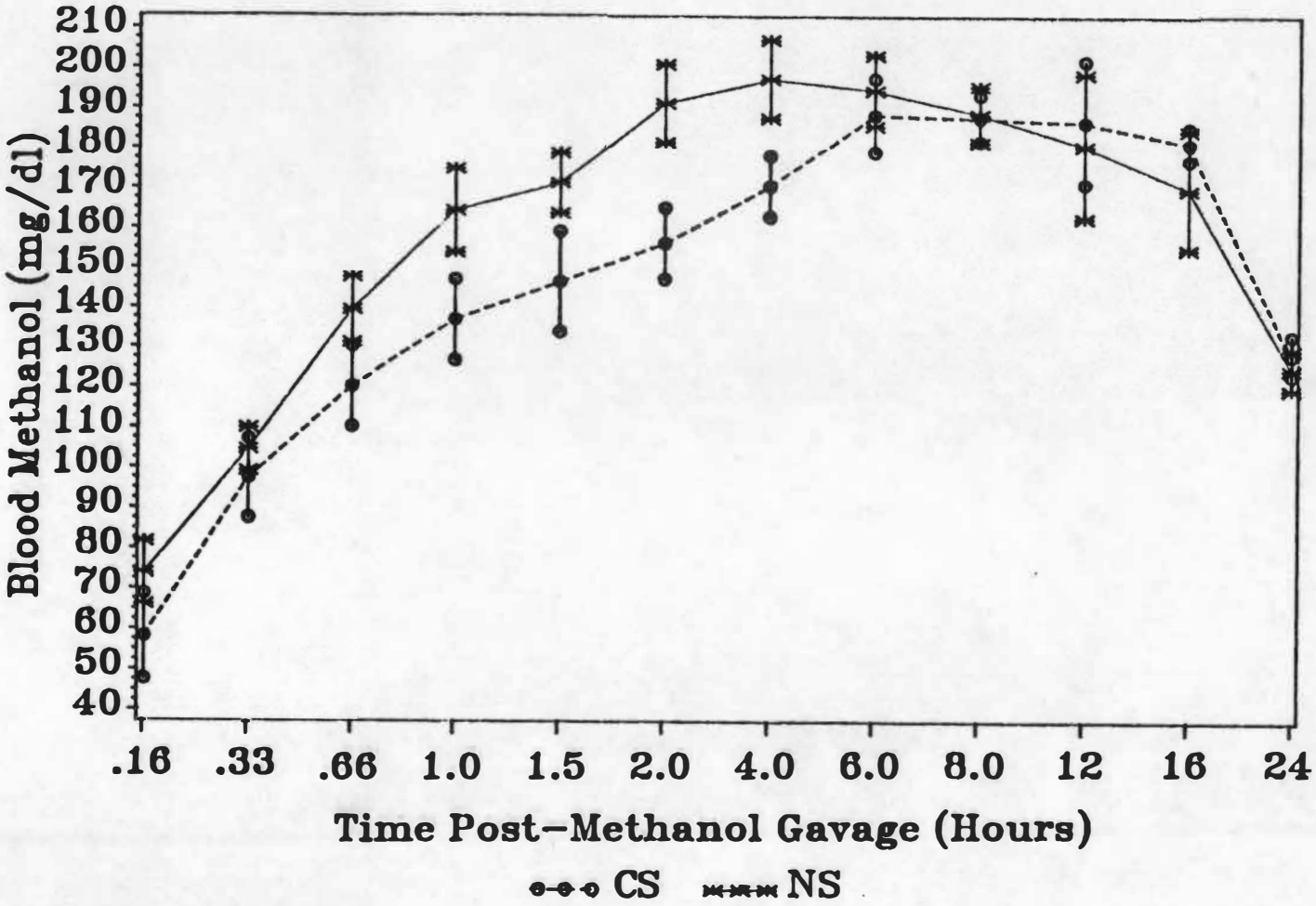


Figure 4.1. Blood methanol concentrations in male rats. Time scale is expanded.

Table 4.1. Blood Methanol Concentrations.

Hours Post Methanol Administration	Blood Methanol (mg/dl)		
	NS	(Diet)	CS
0.16	73.87 ± 7.79 (8)	58.17 ± 10.50 (8)	
0.33	104.56 ± 5.37 (10)	97.41 ± 9.79 (11)	
0.66	139.47 ± 8.24 (10)	120.25 ± 9.94 (11)	
1.0	164.19 ± 10.52 (10)	136.84 ± 10.16 (11)	
1.5	171.06 ± 7.57 (10)	146.40 ± 12.57 (10)	
2.0	191.25 ± 9.72 (10)	156.00 ± 8.89 (11)	
4.0	197.31 ± 9.74 (10)	170.36 ± 7.62 (11)	
6.0	194.48 ± 8.82 (10)	188.37 ± 9.10 (11)	
8.0	188.68 ± 6.96 (9)	187.64 ± 5.71 (11)	
12.0	180.78 ± 17.90 (3)	186.67 ± 15.29 (3)	
16.0	169.91 ± 14.74 (3)	181.35 ± 4.07 (3)	
24.0	125.14 ± 4.95 (5)	128.61 ± 5.00 (5)	

Values are means ± SEM. Numbers in parentheses are numbers of animals in groups.

Table 4.2. Blood Methanol Kinetics.

Parameter	Diet NS	Diet CS	P>|t| =0.05
Absorption Slope (0.666 hours) (mg/dl/hr)	197.50 ± 11.23 (10)	174.02 ± 14.05 (11)	NS
Peak Blood Level Time (hour)	4.50 ± 0.78 (10)	8.18 ± 0.99 (11)	0.05
Concentration (mg/dl)	218.35 ± 6.25 (10)	205.70 ± 5.36 (11)	NS
Absorption Slope (to peak) (mg/dl/hr)	52.58 ± 9.90 (10)	18.12 ± 2.85 (11)	0.05
Area Under Curve 8 hours (mg.hrs/dl)	1430 ± 67 (10)	1271 ± 51 (11)	0.07
Area Under Curve 24 hours (mg.hrs/dl)	3893 ± 189 (10)	3847 ± 116 (11)	NS
Systemic Clearance 8 hours (L/hr/kg)	0.161 ± 0.010 (10)	0.180 ± 0.001 (11)	NS
(ml/min/kg)	9.66 ± 0.61	10.80 ± 0.44	NS
Systemic Clearance 24 hours (L/hr/kg)	0.058 ± 0.003 (10)	0.059 ± 0.002 (11)	NS
(ml/min/kg)	3.51 ± 0.03	3.52 ± 0.10	NS
Elimination Slope K_{el} (mg/dl/hr)	-8.24 ± 2.66 (10)	-7.61 ± 3.12 (11)	NS
C_o (g/ L)	2.46 ± 0.24 (10)	2.68 ± 0.20 (11)	NS
Volume of Distribution (L/kg)	0.96 ± 0.04 (10)	0.87 ± 0.06 (11)	NS

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups. C_o is the theoretical instantaneous distribution concentration.

Elimination of methanol in the urine is shown in Table 4.3. There was a significant increase in the urinary methanol concentration of the CS animals at 2 hours and in the total for 24 hours. Carnitine supplementation almost doubled the volume of urine output in the 2 hour collection, however, there were no significant differences in the 24 hour collection volume. The total methanol excreted was doubled at 2 hours and 4 hours in the CS group but there were no significant differences in the total for 24 hours. The carnitine supplementation resulted in a significantly higher percentage of the methanol dose being excreted in the first 2 hours after dosing, but the percentage of the dose excreted is still less than 10%. The hourly methanol excretion rate shows a significant doubling in the excretion of unchanged methanol in the urine in the CS group at 2 and 4 hours.

The urinary clearances (Table 4.4) are doubled at 2 hours in the CS animals above the NS animals which nears statistical significance. The carnitine induced increase in the urinary clearance was evident at 4 hours also, however, there were no significant differences in the urinary clearance of methanol for the 6-24 hour collection period or the total 24 hour test period.

The effects of carnitine supplementation on methanol distribution in the blood and urine are summarized in Figure 4.2. The decreased BMC in the CS group is accompanied by an increased urinary methanol excretion in the first 4 hours.

4.2 Isopropanol

The blood isopropanol concentration (BIC) curves for a 2.0 g/kg dose in male rats are depicted in Figure 4.3. which is on an expanded time scale. The data for the curves are shown in Table 4.5 There are no significant differences in the blood isopropanol concentrations between NS and CS groups with the blood levels of neither group exceeding 125 mg/dl.

Table 4.3. Excretion of Unchanged Methanol in Urine.

Parameter	Collection Times (Hours Post Methanol Administration) 0-2	2-4	4-6	6-24	24 hour total
Concentration					
NS (mg/ml)	2.28 ± 0.14(4)	2.60 ± 0.26(4)	2.27 ± 0.14(4)	1.84 ± 0.17(4)	2.11 ± 0.08(4)
CS	2.82 ± 0.12(3)	2.83 ± 0.02(2)	2.49 ± 0.02(3)	1.59 ± 0.14(3)	2.35 ± 0.04(3)
NS (μmole/ml)	71.41 ± 4.36(4)	81.25 ± 8.16(4)	71.17 ± 5.16(4)	57.50 ± 5.34(4)	66.02 ± 2.59(4)
CS	88.27 ± 3.76(3) [.04]	88.43 ± 0.63(2)	77.71 ± 0.55(3)	49.67 ± 4.38(3)	73.54 ± 1.27(3) [.067]
Urine Output (ml)					
NS	6.75 ± 0.32(4)	1.95 ± 0.43(4)	2.12 ± 0.72(4)	13.80 ± 2.39(4)	24.43 ± 2.91(4)
CS	11.06 ± 2.22(3) [.070]	2.73 ± 1.83(3)	1.70 ± 0.17(3)	10.17 ± 2.12(3)	25.66 ± 4.55(3)
Total Methanol Excretion					
NS (mg)	15.40 ± 1.03(4)	5.17 ± 1.44(4)	4.98 ± 1.71(4)	26.35 ± 6.22(4)	51.94 ± 7.05(4)
CS	31.82 ± 7.92(3)	11.55 ± 5.85(2)	4.22 ± 0.42(3)	16.61 ± 4.51(3)	60.35 ± 11.98(3)
NS (μmole)	481.48 ± 32.35(4)	161.64 ± 44.95(4)	155.70 ± 53.46(4)	823.59 ± 194.60(4)	1622.50 ± 220.38(4)
CS	994.48 ± 247.53(3) [.059]	360.94 ± 182.81(2) [.202]	131.88 ± 13.02(3)	518.96 ± 140.97(3) [.292]	1885.94 ± 374.38(3)
Percent of Dose Excreted					
NS	1.35 ± 0.12(4)	0.46 ± 0.14(4)	0.41 ± 0.12(4)	2.31 ± 0.58(4)	4.55 ± 0.69(4)
CS	3.02 ± 0.71(3) [.050]	1.00 ± 0.48(2)	0.39 ± 0.03(3)	1.52 ± 0.32(3)	5.61 ± 0.84(3)
Hourly Methanol Excretion					
NS(mg/g BW·hour)	0.016 ± .001(4)	0.005 ± .001(4)	0.005 ± .002(4)	0.003 ± .001(4)	0.004 ± .001(4)
CS	0.035 ± .008(3)	0.012 ± .006(2)	0.005 ± .000(3)	0.002 ± .000(3)	0.005 ± .001(3)
NS (μmole/g BW·hour)	0.497 ± .046(4)	0.169 ± .051(4)	0.154 ± .047(4)	0.94 ± .023(4)	0.139 ± .020(4)
CS	1.091 ± .245(3) [.038]	0.364 ± .178(2) [.216]	0.145 ± .008(3)	0.61 ± .013(3)	0.169 ± .024(3)

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

Table 4.4. Urinary Clearance of Methanol

Urine Collection (Blood Sample)	Diet				P>|t| =0.05
	NS		CS		
	ml/period·kg ml/min·kg		ml/period·kg ml/min·kg		
0-2 Hours (1 Hour)	19.14 ±	1.92 (4)	39.59 ±	10.81 (3)	0.08
	0.159 ±	0.016	0.329 ±	0.090	
2-4 Hours (3 Hours)	5.83 ±	1.78 (4)	12.10 ±	6.08 (2)	NS
	0.049 ±	0.015	0.101 ±	0.051	
4-6 Hours (5 Hours)	5.25 ±	1.63 (4)	4.73 ±	0.15 (3)	NS
	0.044 ±	0.014	0.039 ±	0.001	
6-24 Hours (15 Hours)	33.53 ±	9.65 (4)	20.46 ±	4.35 (3)	NS
	0.031 ±	0.009	0.019 ±	0.004	
24 Hour Total (12 Hours)	61.32 ±	11.41 (4)	70.67 ±	7.43 (3)	NS
	0.043 ±	0.008	0.049 ±	0.005	

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups.

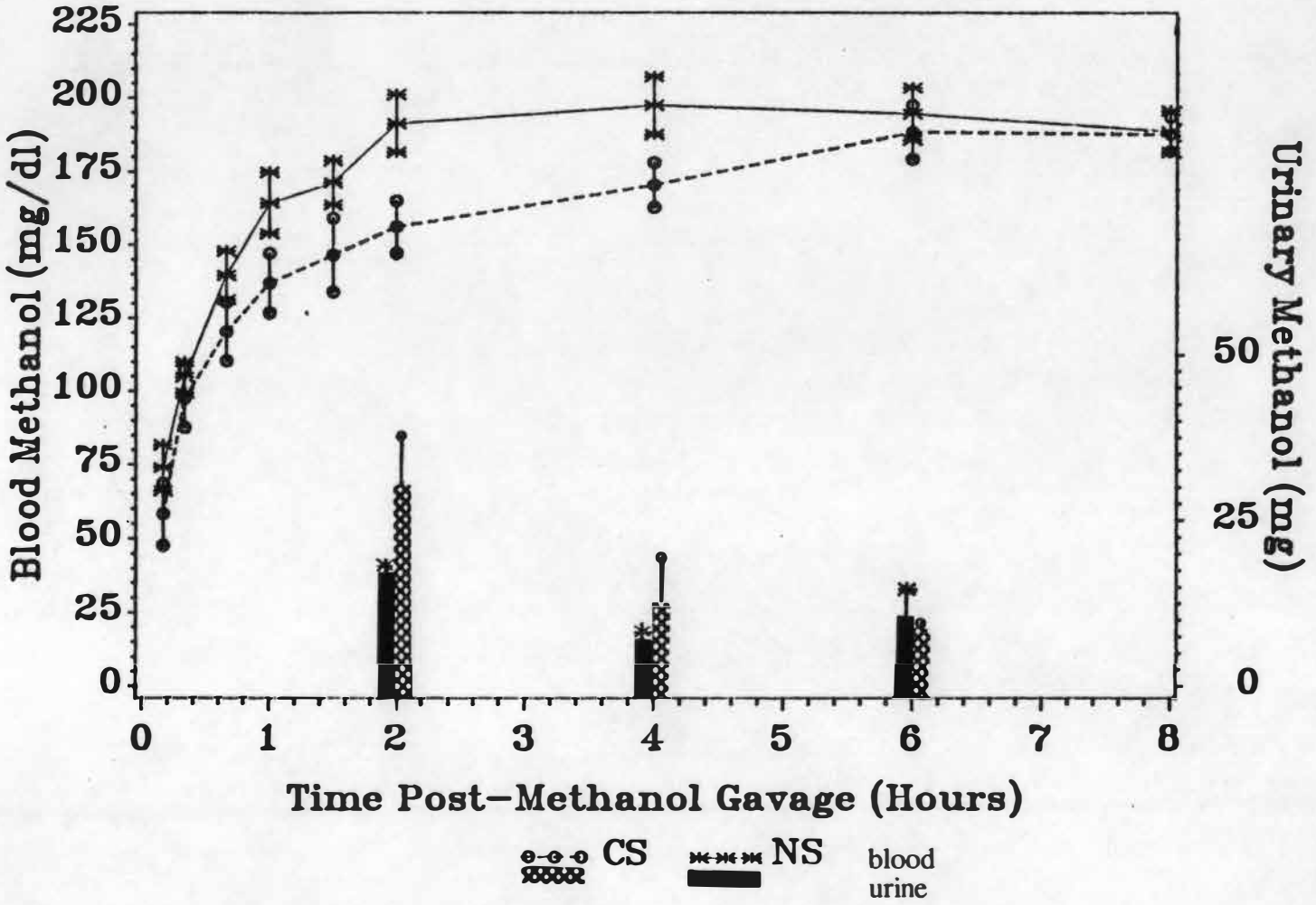


Figure 4.2. Blood methanol concentrations and total urinary methanol excretion.

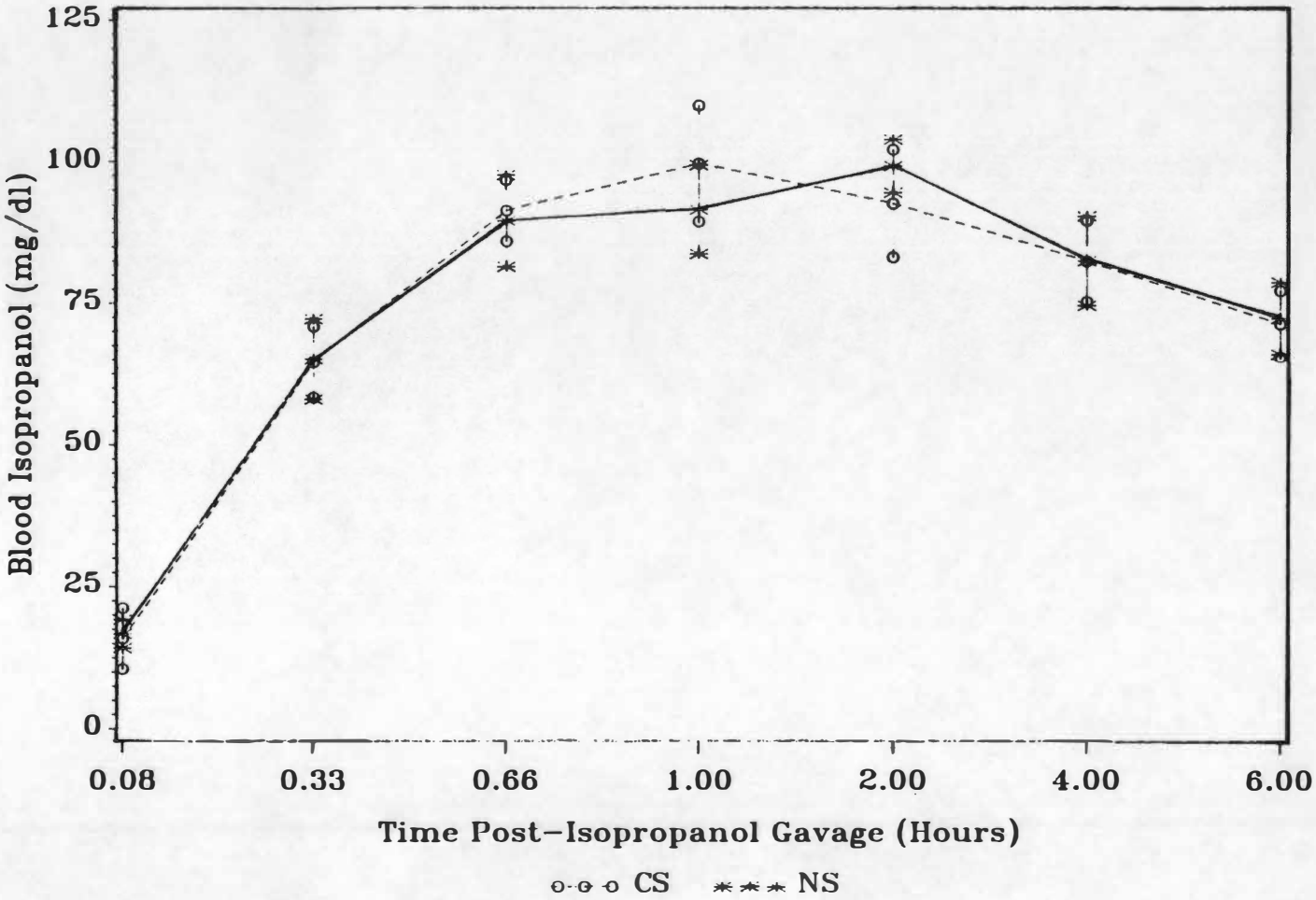


Figure 4.3. Blood isopropanol concentrations. Time scale is expanded.

Table 4.5. Blood Isopropanol Concentrations.

Hours Post Isopropanol Administration	Blood Isopropanol (mg/dl)		
	NS	(Diet)	CS
0.08	16.67 ± 2.49 (5)	15.85 ± 5.33 (5)	
0.33	65.11 ± 7.11 (5)	64.63 ± 6.26 (5)	
0.66	89.59 ± 8.09 (5)	91.42 ± 5.42 (5)	
1.0	91.71 ± 7.81 (5)	99.83 ± 10.24 (5)	
2.0	99.44 ± 4.68 (5)	92.89 ± 9.45 (5)	
4.0	82.78 ± 7.89 (5)	82.75 ± 7.15 (5)	
6.0	72.66 ± 6.37 (5)	71.66 ± 5.88 (5)	

Values are means ± SEM. Numbers in parentheses are numbers of animals in groups.

The kinetic analysis of the BIC curves is given in Table 4.6. There were no significant differences in absorption slopes, peak blood levels or areas under the curves, although the absorption slope to the peak was non-significantly elevated and the time to reach the peak BIC was shortened in the CS animals. Higher numbers of animals and reduced sample deviations may prove these to be significant differences. There were no significant differences in systemic clearances, elimination slopes, theoretical instantaneous distribution or volumes of distribution between the groups.

The effect of carnitine supplementation on isopropanol excretion in urine is shown in Table 4.7. Carnitine had no significant effect on the concentration of isopropanol in the urine at any time period. There was an increase in urine volume in the CS group which is similar to that seen in the methanol experiment. The net result was a near doubling of the total urine output with carnitine supplementation for the 6 hours of the experiment. The total isopropanol excretion was significantly increased in the CS animals at 2 hours and in the total for 6 hours. The percentage of the isopropanol dose excreted in 6 hours was also significantly increased by carnitine supplementation. The hourly excretion rate of isopropanol in the CS group shows a near doubling at 2 hours but only a slight increase in the total for 6 hours. Urinary clearance of isopropanol (Table 4.8) was significantly increased with carnitine supplementation which is related to the differences in the early BICs and urine volumes of this group.

Acetone was measured as a metabolite of isopropanol. The blood acetone concentration (BAcC) curves are depicted in Figure 4.4 with an expanded time scale and data are shown in Table 4.9 There are no significant differences in the appearance of acetone in the blood. The peak BAcC was less than 100 mg/dl and was not reached until nearly 6 hours in both groups. There were no significant differences in any blood acetone appearance kinetic parameters; appearance slopes, peak BAcC, peak time or area under the curve (Table 4.10).

Table 4.6. Blood Isopropanol Kinetics.

Parameter	Diet						P>|t| =0.05
	NS			CS			
Absorption Slope (0.666 hours) (mg/dl·hr)	136.42	±	14.04 (5)	139.62	±	7.39 (5)	NS
Peak Blood Level Time (hour)	2.20	±	0.49 (5)	1.40	±	0.24 (5)	NS
Concentration (mg/dl)	102.32	±	4.93 (5)	103.29	±	9.02 (5)	NS
Absorption Slope (to peak) (mg/dl·hr)	52.42	±	14.16 (5)	83.34	±	19.14 (5)	NS
Area Under Curve 6 hours (mg/dl·hrs)	509.22	±	23.85 (5)	480.90	±	43.73 (5)	NS
Systemic Clearance 6 hours (L/hr·kg)	0.396	±	0.019 (5)	0.428	±	0.033 (5)	NS
(ml/min·kg)	6.60	±	0.32	7.13	±	0.55	NS
Elimination Slope K_{el} (mg/dl·hr)	-7.58	±	0.94 (5)	-6.45	±	1.31 (5)	NS
C_o (mg/dl)	116.52	±	5.40 (5)	109.27	±	10.27 (5)	NS
Volume of Distribution (L/kg)	1.73	±	0.08 (5)	1.89	±	0.18 (5)	NS

Values are means ±SEM. C_o is the theoretical instantaneous distribution concentration at time of dosing. Values in parentheses are numbers of animals in groups.

Table 4.7. Excretion of Unchanged Isopropanol in Urine.

Parameter	Collection Times (Hours Post Isopropanol Administration) 0-2	2-4	4-6	6 hour total
Concentration				
NS (mg/ml)	1.05 ± 0.21(5)	1.39 ± 0.11(3)	1.05 ± 0.11(5)	1.11 ± 0.10(4)
CS	1.11 ± 0.16(5)	1.18 ± 0.11(5)	0.99 ± 0.05(5)	1.03 ± 0.09(4)
NS (µmole/ml)	17.47 ± 3.48(5)	23.17 ± 1.95(3)	17.47 ± 1.81(5)	18.53 ± 1.62(4)
CS	18.47 ± 2.76(5)	19.73 ± 1.91(5) [.283]	16.43 ± 0.81(5)	17.13 ± 1.50(4)
Urine Output (ml)				
NS	3.36 ± 0.63(5)	1.08 ± 0.63(4)	2.25 ± 0.26(4)	7.05 ± 0.75(4)
CS	5.26 ± 0.71(5) [.080]	2.38 ± 0.33(5) [.090]	2.38 ± 0.10(4)	10.47 ± 0.88(4) [.030]
Total Isopropanol Excretion				
NS (mg)	3.43 ± 0.77(5)	3.18 ± 0.70(2)	2.60 ± 0.38(4)	7.05 ± 0.60(4)
CS	5.51 ± 0.62(5)	2.92 ± 0.52(5)	2.40 ± 0.23(4)	10.68 ± 0.60(4)
NS (µmole)	57.20 ± 12.92(5)	53.00 ± 11.67(2)	43.25 ± 6.28(4)	117.55 ± 12.51(4)
CS	91.90 ± 10.28(5) [.069]	48.60 ± 8.67(5)	39.96 ± 3.89(4)	177.93 ± 17.62(4) [.032]
Percent of Dose Excreted				
NS	0.42 ± 0.09(5)	0.39 ± 0.08(4)	0.32 ± 0.04(4)	0.88 ± 0.10(4)
CS	0.62 ± 0.06(5)	0.33 ± 0.06(5)	0.28 ± 0.03(4)	1.21 ± 0.10(4) [.056]
Hourly Isopropanol Excretion				
NS (mg/g BW·hour)	0.004 ± .001(5)	0.004 ± .001(2)	0.003 ± .0004(4)	0.003 ± .0002(4)
CS	0.006 ± .001(5)	0.003 ± .001(5)	0.003 ± .0003(4)	0.004 ± .0004(4)
NS (µmole/gBW·hour)	0.069 ± .015(5)	0.066 ± .013(2)	0.054 ± .007(4)	0.050 ± .004(4)
CS	0.104 ± .010(5)	0.055 ± .010(5)	0.046 ± .005(4)	0.067 ± .006(4)

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

Table 4.8. Urinary Clearance of Isopropanol

Urine Collection (Blood Sample)	Diet				P>|t| =0.05
	NS		CS		
	ml/period·kg ml/min·kg		ml/period·kg ml/min·kg		
0-2 Hours (1 Hour)	8.79 ±	2.22 (5)	12.95 ±	1.72 (5)	NS
	0.073 ±	0.019	0.108 ±	0.014	
2-4 Hours (3 Hours)	8.71 ±	1.03 (4)	7.32 ±	1.14 (5)	NS
	0.073 ±	0.009	0.061 ±	0.010	
4-6 Hours (5 Hours)	8.03 ±	1.43 (4)	6.99 ±	0.89 (4)	NS
	0.067 ±	0.012	0.058 ±	0.007	
6 Hour Total (3 Hours)	19.37 ±	2.39 (4)	27.67 ±	2.32 (4)	0.047
	0.054 ±	0.007	0.077 ±	0.006	

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups.

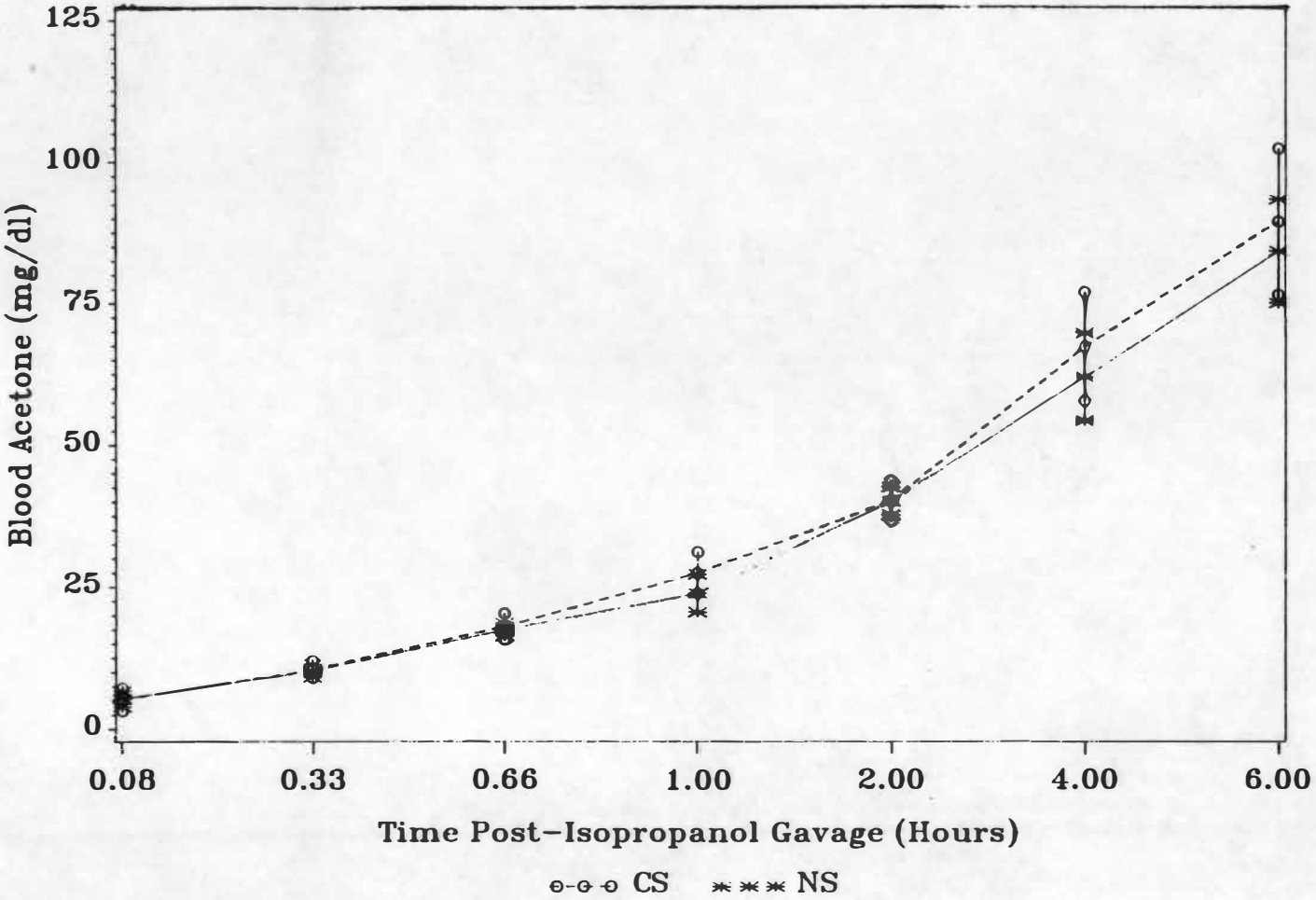


Figure 4.4. Blood acetone concentrations following isopropanol treatment. Time scale is expanded.

Table 4.9. Blood Acetone Concentrations Post Isopropanol Administration

Hours Post Isopropanol Administration	Blood Acetone (mg/dl)		
	NS	(Diet)	CS
0.08	5.31 ± 1.40 (5)	5.22 ± 1.98 (4)	
0.33	10.33 ± 1.14 (5)	10.55 ± 1.49 (5)	
0.66	17.62 ± 1.13 (5)	18.15 ± 2.29 (5)	
1.0	24.00 ± 3.32 (5)	27.56 ± 3.56 (5)	
2.0	40.17 ± 2.46 (5)	40.22 ± 3.51 (5)	
4.0	62.10 ± 7.70 (5)	67.43 ± 9.56 (5)	
6.0	84.29 ± 9.14 (5)	89.48 ± 12.87 (5)	

Values are means ± SEM. Numbers in parentheses are numbers of animals in groups.

Table 4.10. Blood Acetone Kinetics.

Parameter	Diet						
	NS			CS			P>|t| =0.05
Appearance Slope (0.666 hours) (mg/dl·hr)	24.80	±	1.53 (5)	26.15	±	2.68 (5)	NS
Peak Blood Level Time (hour)	6.00	±	0.00 (5)	5.60	±	0.40 (5)	NS
Concentration (mg/dl)	84.29	±	9.14 (5)	89.82	±	12.71 (5)	NS
Appearance Slope (to peak) (mg/dl·hr)	13.69	±	1.60 (5)	15.38	±	1.87 (5)	NS
Area Under Curve 6 hours (mg/dl·hrs)	294.38	±	29.40 (5)	312.92	±	38.65 (5)	NS

Values are means ±SEM. Values in parentheses are numbers of animals in groups.

The excretion of acetone in the urine is shown in Table 4.11. Carnitine supplementation did not significantly increase the urine acetone concentration. The carnitine induced increase in urine volume resulted in increased total acetone excretion and increased hourly rate of acetone excretion in urine. The urinary clearance of acetone in the CS group (Table 4.12) followed a pattern similar to that of isopropanol with a 40% increase above the NS group (13.53, NS vs. 19.29,CS) in the total for 6 hours. Figures 4.5 and 4.6 graphically depict the blood and urine concentration curves for both isopropanol and acetone. Contrary to the observations with methanol, carnitine's effect on urine output and hence on isopropanol or acetone excretion was not sufficient to alter blood isopropanol or acetone levels as less than 2% of the dose was excreted unchanged.

4.3 Ethylene Glycol

Blood ethylene glycol concentrations (BEGC) following a 5.56 g/kg dose of ethylene glycol in male rats (Figure 4.7, Table 4.13) were 22% lower at 1 hour with carnitine supplementation. This apparent depression in the BEGC of the CS group did not result in any statistically significant differences of absorption slopes even though they were lower in the CS group (Table 4.14). The peak BEGCs were not significantly different in the CS group and the NS group and the time to reach the peak BEGC was slightly lengthened with carnitine supplementation in the time span of this observation period. The CS group BEGC curve may still be increasing and this may not be the true peak. The areas under the curves of the two groups were not significantly different between the groups, nor were systemic clearances. Only two CS animals showed declines in their BEGC curves by 6 hours, whereas all six of the NS animals had entered the elimination phase, but there were no statistical differences in the calculated elimination slopes. The theoretical instantaneous

Table 4.11. Excretion of Acetone in Urine Post Isopropanol Administration.

Parameter	Collection Times (Hours Post Isopropanol Administration) 0-2	2-4	4-6	6 hour total
Concentration				
NS (mg/ml)	0.27 ± 0.07(5)	0.51 ± 0.03(3)	0.58 ± 0.10(5)	0.42 ± 0.08(4)
CS	0.33 ± 0.05(5)	0.58 ± 0.13(5)	0.69 ± 0.15(5)	0.43 ± 0.04(4)
NS (µmole/ml)	4.69 ± 1.19(5)	8.85 ± 0.57(3)	9.93 ± 1.73(5)	7.22 ± 0.72(4)
CS	5.69 ± 0.91(5)	10.00 ± 2.19(5)	11.93 ± 2.53(5)	7.48 ± 1.30(4)
Urine Output (ml)				
NS	3.36 ± 0.63(5)	1.08 ± 0.63(4)	2.25 ± 0.26(4)	6.63 ± 0.52(4)
CS	5.26 ± 0.71(5) [.08]	2.38 ± 0.33(5) [.09]	2.38 ± 0.10(4)	10.47 ± 0.88(4) [.009]
Total Acetone Excretion				
NS (mg)	0.93 ± 0.25(5)	1.09 ± 0.23(2)	1.50 ± 0.32(4)	2.79 ± 0.37(4)
CS	1.66 ± 0.25(5)	1.49 ± 0.35(5)	1.60 ± 0.49(4)	4.62 ± 0.98(4)
NS (µmole)	16.03 ± 4.37(5)	18.88 ± 4.05(2)	25.82 ± 5.58(4)	48.01 ± 6.43(4)
CS	28.59 ± 4.26(5) [.074]	25.65 ± 5.98(5)	27.58 ± 8.49(4)	79.69 ± 16.85(4) [.129]
Hourly Acetone Excretion				
NS (mg/g BW·hour)	0.001 ± .0003(5)	0.001 ± .0002(2)	0.002 ± .0004(4)	0.001 ± .0001(4)
CS	0.002 ± .0003(5)	0.002 ± .0004(5)	0.002 ± .001(4)	0.002 ± .0004(4)
NS (µmole/g BW·hour)	0.019 ± .005(5)	0.024 ± .004(2)	0.032 ± .007(4)	0.020 ± .003(4)
CS	0.032 ± .004(5) [.089]	0.029 ± .007(5)	0.032 ± .010(4)	0.030 ± .007(4) [.188]

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

Table 4.12. Urinary Clearance of Acetone

Urine Collection (Blood Sample)	Diet				P>|t| =0.05
	NS		CS		
	ml/period·kg ml/min·kg		ml/period·kg ml/min·kg		
0-2 Hours (1 Hour)	10.28 ±	3.00 (5)	15.92 ±	4.05 (5)	NS
	0.086 ±	0.025	0.133 ±	0.034	
2-4 Hours (3 Hours)	5.78 ±	0.57 (2)	7.05 ±	1.87 (5)	NS
	0.048 ±	0.005	0.059 ±	0.016	
4-6 Hours (5 Hours)	5.88 ±	2.00 (4)	4.63 ±	1.44 (4)	NS
	0.049 ±	0.017	0.039 ±	0.012	
6 Hour Total (3 Hours)	13.53 ±	2.52 (4)	19.29 ±	3.32 (4)	0.217
	0.037 ±	0.007	0.054 ±	0.009	

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups.

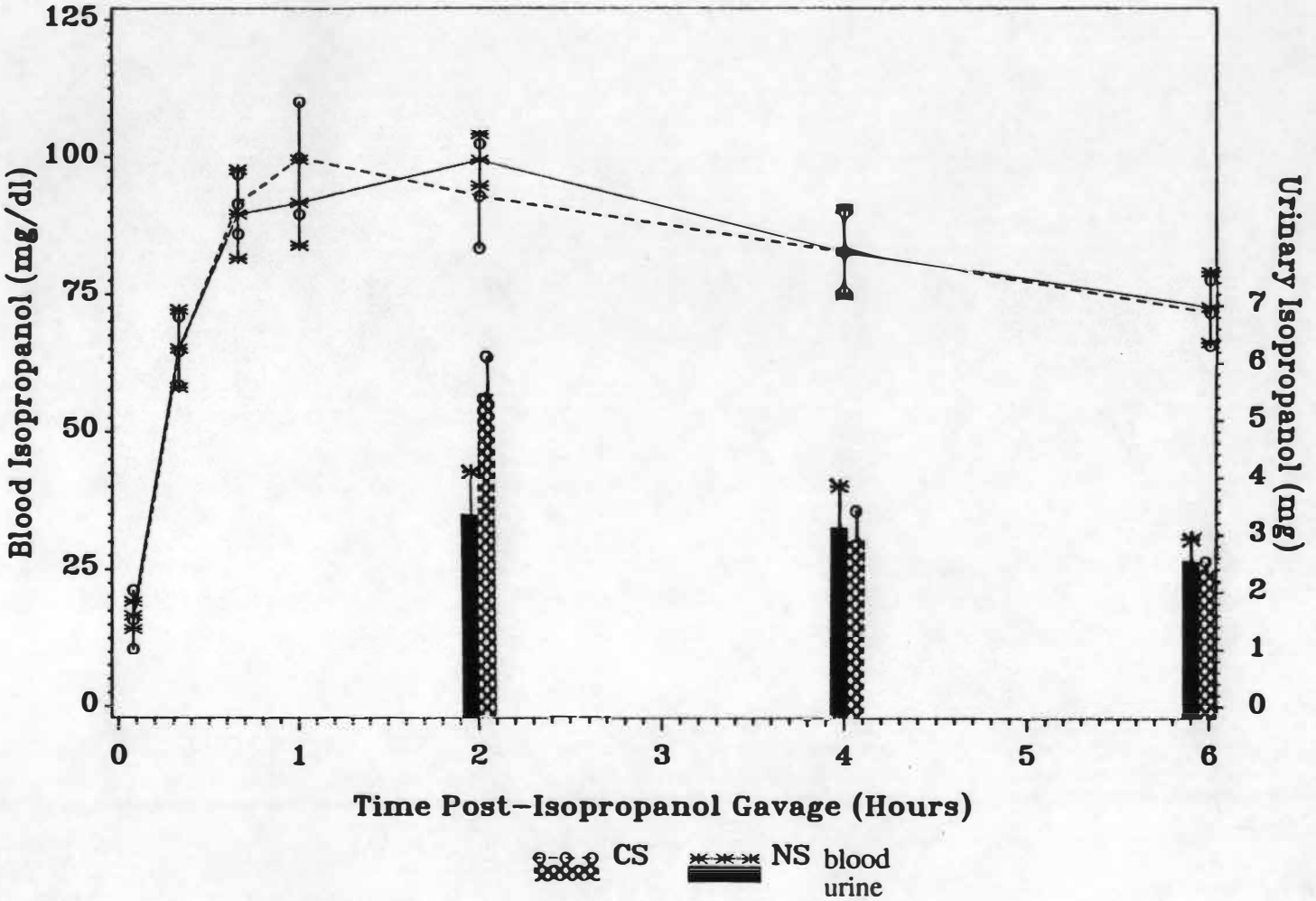


Figure 4.5. Blood isopropanol concentrations and total urinary isopropanol excretion.

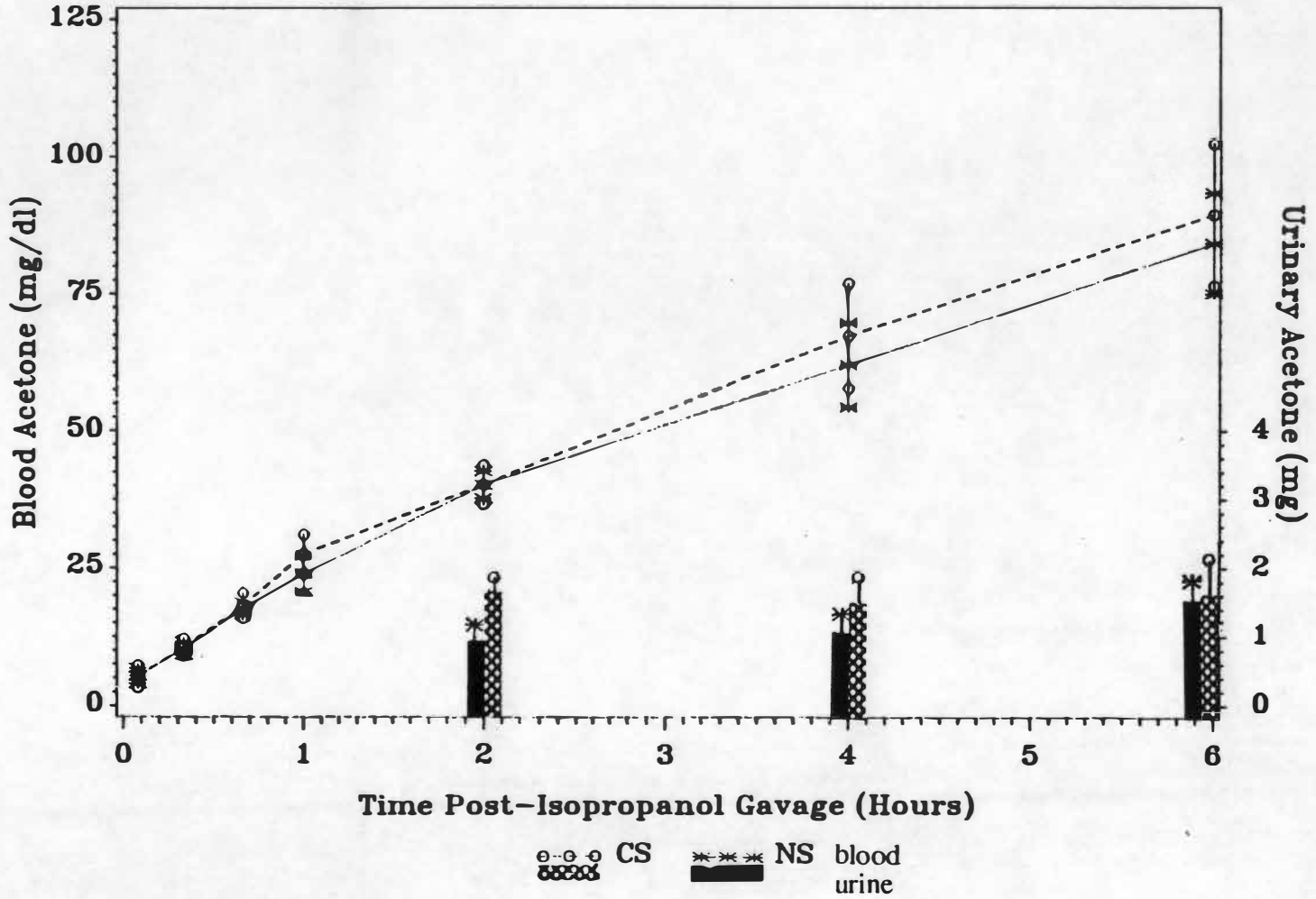


Figure 4.6. Blood acetone concentrations and total urinary acetone excretion following isopropanol administration.

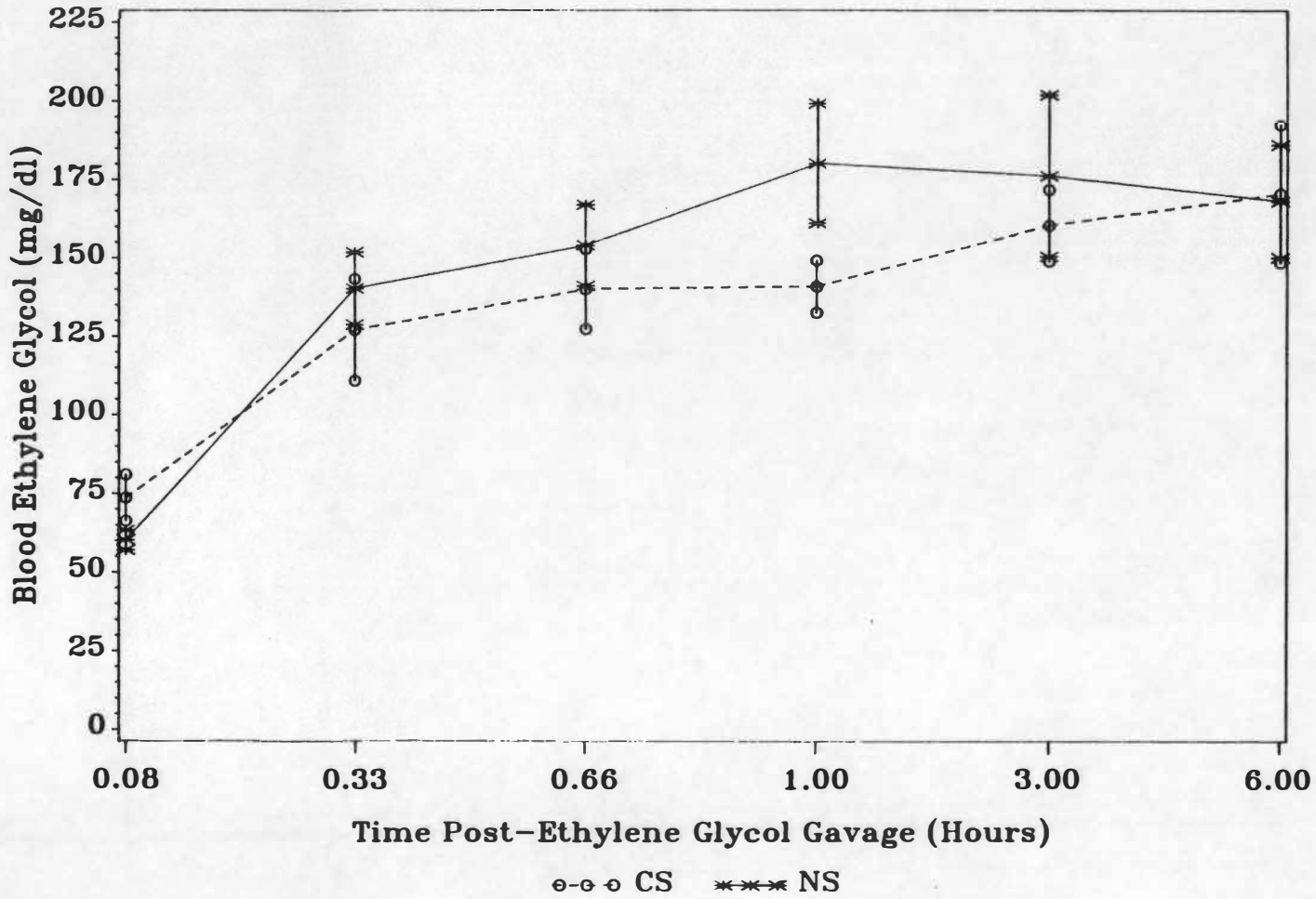


Figure 4.7. Blood ethylene glycol concentrations. Time scale is expanded.

Table 4.13. Blood Ethylene Glycol Concentrations.

Hours Post Ethylene Glycol Administration	Blood Ethylene Glycol (mg/dl)		
	NS	(Diet)	CS
0.08	60.16 ± 3.17 (5)	73.53 ± 7.34 (6)	
0.33	140.18 ± 11.49 (5)	127.02 ± 16.18 (5)	
0.66	153.92 ± 12.92 (5)	139.98 ± 12.74 (6)	
1.0	180.00 ± 19.05 (5)	140.85 ± 8.29 (6)	
3.0	176.12 ± 27.51 (5)	160.35 ± 11.39 (6)	
6.0	167.95 ± 17.81 (6)	170.33 ± 21.84 (6)	

Values are means ± SEM. Number in parentheses is number of animals in groups.

Table 4.14. Blood Ethylene Glycol Kinetics

Parameter	Diet NS	Diet CS	P>|t| =0.05
Absorption Slope (0.666 hours) (mg/dl·hr)	263.68 ± 37.33 (6)	182.95 ± 17.83 (6)	NS
Peak Blood Level Time (hour)	2.83 ± 0.75 (6)	3.77 ± 1.04 (6)	NS
Concentration (mg/dl)	206.38 ± 16.19 (6)	186.18 ± 13.91 (6)	NS
Absorption Slope (to peak) (mg/dl·hr)	93.61 ± 32.00 (6)	74.08 ± 33.16 (6)	NS
Area Under Curve 6 hours (mg/dl·hrs)	1015 ± 86 (6)	915 ± 58 (6)	NS
Systemic Clearance 6 hours (L/hr·kg)	0.561 ± 0.044 (6)	0.620 ± 0.040 (6)	NS
(ml/min·kg)	9.35 ± 0.73	10.33 ± 0.67	NS
Elimination Slope K_{el} (mg/dl·hr)	-16.52 ± 4.75 (6)	-10.78 ± 4.41 (2)	NS
C_o (mg/dl)	245.15 ± 37.18 (6)	175.75 ± 13.05 (2)	NS
Volume of Distribution (L/kg)	2.46 ± 0.38 (6)	3.20 ± 0.23 (6)	NS

Values are means ±SEM. Data for Elimination Slope and C_0 for CS group are taken from 2 points only as all others in group reached a plateau at 6 hours and did not show elimination. K_{el} is the elimination rate constant. C_0 is the theoretical instantaneous distribution concentration at time of dosing. Values in parentheses are numbers of animals in groups.

distribution was lower in the CS animals than the in the NS animals which corresponds to the higher volume of distribution in CS animals .

Unlike the isopropanol and methanol experiments, carnitine supplementation induced only a slight non-significant increase in the concentration of ethylene glycol in the urine in the 0-2 hour collection and had no effect on urine volume in any collection period (Table 4.15). Carnitine supplementation did not result in a statistically significant increase in total ethylene glycol excretion, percentage of the dose excreted, or rate of hourly ethylene glycol excretion. The percentage of the ethylene glycol dose excreted is considerably higher than for methanol or isopropanol. The urinary clearance of ethylene glycol from the blood (Table 4.16) showed no statistically significant increases in the CS group but a slight increase in the 0-2 hour clearance is noteworthy. The blood and urine ethylene glycol concentration curves are summarized in Figure 4.8.

4.4 Ethylene Glycol and Ethanol

The effect of a 3.0 g/kg dose of ethanol on ethylene glycol metabolism was studied in the CS rats because ethanol is frequently administered as an antidote for ethylene glycol poisoning. The BEGC curves show that the BEGC curve of the CS group enters the elimination phase within an hour after the ethanol administration which was actually 2 hours post-ethylene glycol gavage. The BEGC curve of the NS group plateaued and quickly dropped off 3 hours post ethanol or 4 hours post-ethylene glycol gavage (Figure 4.9, Table 4.17). The BEGCs are much higher in this study than in the study with ethylene glycol alone even though the dose of ethylene glycol was the same (see Table 4.13). For example, the peak BEGC for the NS group is 257 mg/dl vs. 206 mg/dl and the peak BEGC for the CS group is 220 mg/dl vs. 170 mg/dl. This may be an artifact of the differences in sizes of rats used for the two studies or may be due to the effect of the

Table 4.15. Excretion of Unchanged Ethylene Glycol in the Urine

Parameter	Collection Times (Hours Post Ethylene Glycol Administration) 0-2	2-4	4-6	6 hour total
Concentration				
NS (mg/ml)	17.82 ± 1.93(6)	31.14 ± 3.21(6)	25.88 ± 3.08(6)	26.66 ± 2.61(6)
CS	21.14 ± 2.59(6)	30.69 ± 4.69(6)	24.68 ± 3.18(6)	26.59 ± 3.44(6)
NS (µmole/ml)	287.44 ± 31.22(6)	502.31 ± 51.74(6)	417.42 ± 49.73(6)	429.97 ± 42.16(6)
CS	340.91 ± 41.88(6)	495.03 ± 75.69(6)	398.09 ± 51.26(6)	428.84 ± 35.52(6)
Urine Output (ml)				
NS	3.23 ± 0.83(6)	7.23 ± 0.92(6)	7.43 ± 0.99(6)	17.90 ± 2.50(6)
CS	3.40 ± 0.54(6)	6.66 ± 0.89(6)	7.91 ± 0.48(6)	17.98 ± 1.27(6)
Total Ethylene Glycol Excretion				
NS (mg)	55.58 ± 12.28(6)	220.90 ± 36.12(6)	190.10 ± 33.79(6)	466.76 ± 75.18(6)
CS	71.74 ± 13.05(6)	219.23 ± 54.81(6)	192.80 ± 25.14(6)	483.78 ± 79.46(6)
NS (µmole)	896.59 ± 198.06(6)	3562.90 ± 582.55(6)	3066.13 ± 545.09(6)	7528.33 ± 1212.56(6)
CS	1157.15 ± 210.59(6)	3536.02 ± 884.09(6)	3109.68 ± 405.53(6)	7802.96 ± 1281.70(6)
Percent of Dose Excreted				
NS	2.46 ± 0.54(6)	9.87 ± 1.64(6)	8.48 ± 1.49(6)	20.83 ± 3.36(6)
CS	3.27 ± 0.64(6)	9.83 ± 2.40(6)	8.63 ± 1.12(6)	21.76 ± 3.56(6)
Hourly Ethylene Glycol Excretion				
NS(mg/g BW·hour)	0.069 ± .015(6)	0.275 ± .046(6)	0.236 ± .041(6)	0.193 ± .031(6)
CS	0.091 ± .018(6)	0.274 ± .067(6)	0.240 ± .031(6)	0.202 ± .033(6)
NS (µmole/g BW·hour)	1.11 ± 0.24(6)	4.43 ± 0.73(6)	3.80 ± 0.67(6)	3.12 ± 0.50(6)
CS	1.47 ± 0.29(6)	4.42 ± 1.08(6)	3.88 ± 0.51(6)	3.26 ± 0.53(6)

Values are means ± SEM. Values in parentheses are numbers of animals in groups. There were no significant differences by t test at P>|t| = 0.05.

Table 4.16. Urinary Clearance of Ethylene Glycol

Urine Collection (Blood Sample)	Diet	
	NS	CS
	ml/period·kg ml/min·kg	ml/period·kg ml/min·kg
0-2 Hours (1 Hour)	90.36 ± 29.02 (5) 0.753 ± 0.242	133.05 ± 26.11 (6) 1.108 ± 0.217
2-4 Hours (3 Hours)	324.50 ± 82.67 (5) 2.704 ± 0.689	333.35 ± 68.36 (6) 2.778 ± 0.569
4-6 Hours (5 Hours)	326.25 ± 53.76 (5) 2.718 ± 0.448	291.46 ± 39.21 (6) 2.492 ± 0.327
6 Hour Total (3 Hours)	790.36 ± 139.86 (5) 2.195 ± 0.389	758.33 ± 112.62 (6) 2.106 ± 0.313

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups. There were no significant differences by t test at P>|t |=0.05

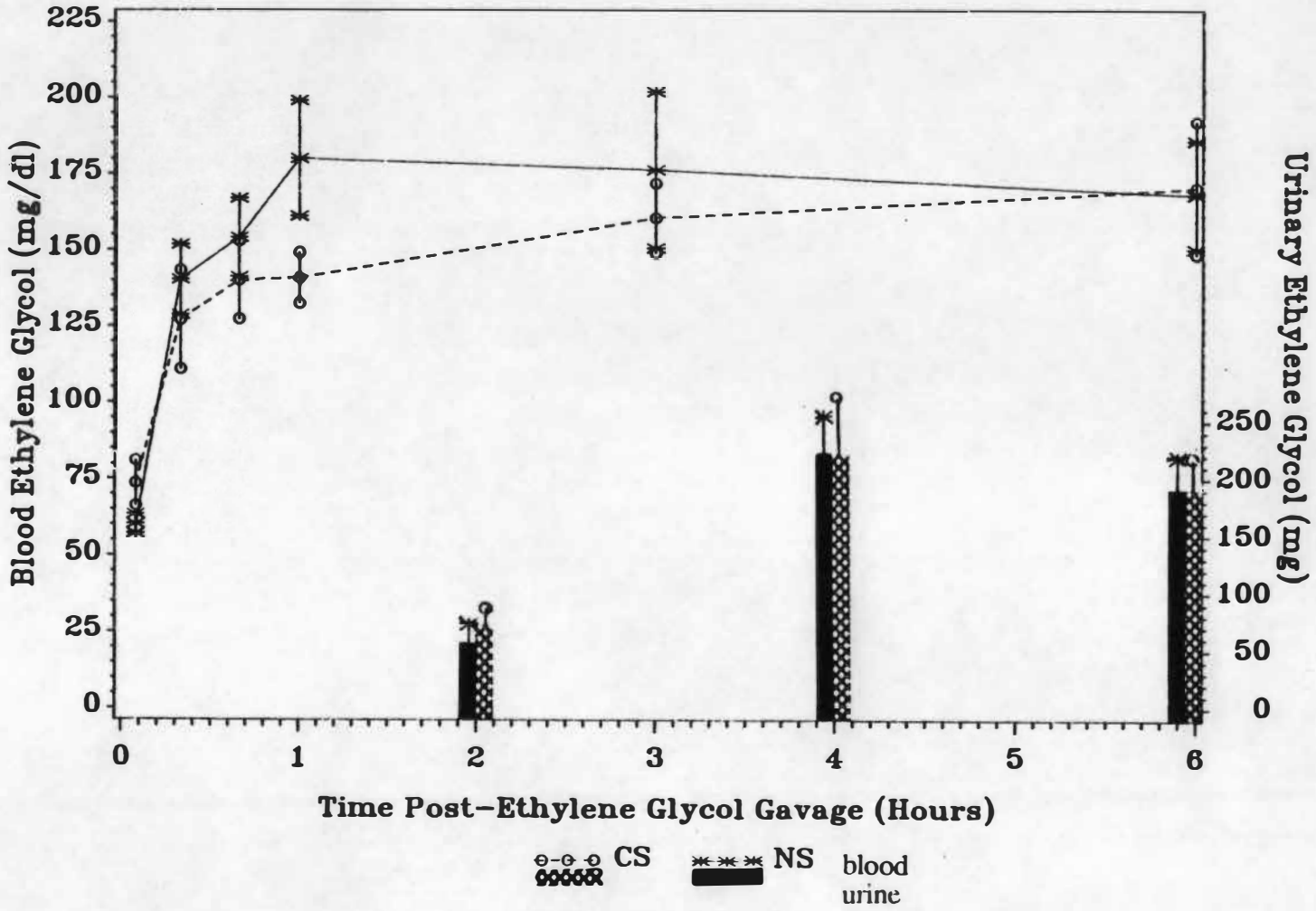


Figure 4.8. Blood ethylene glycol concentrations and total urinary ethylene glycol excretion.

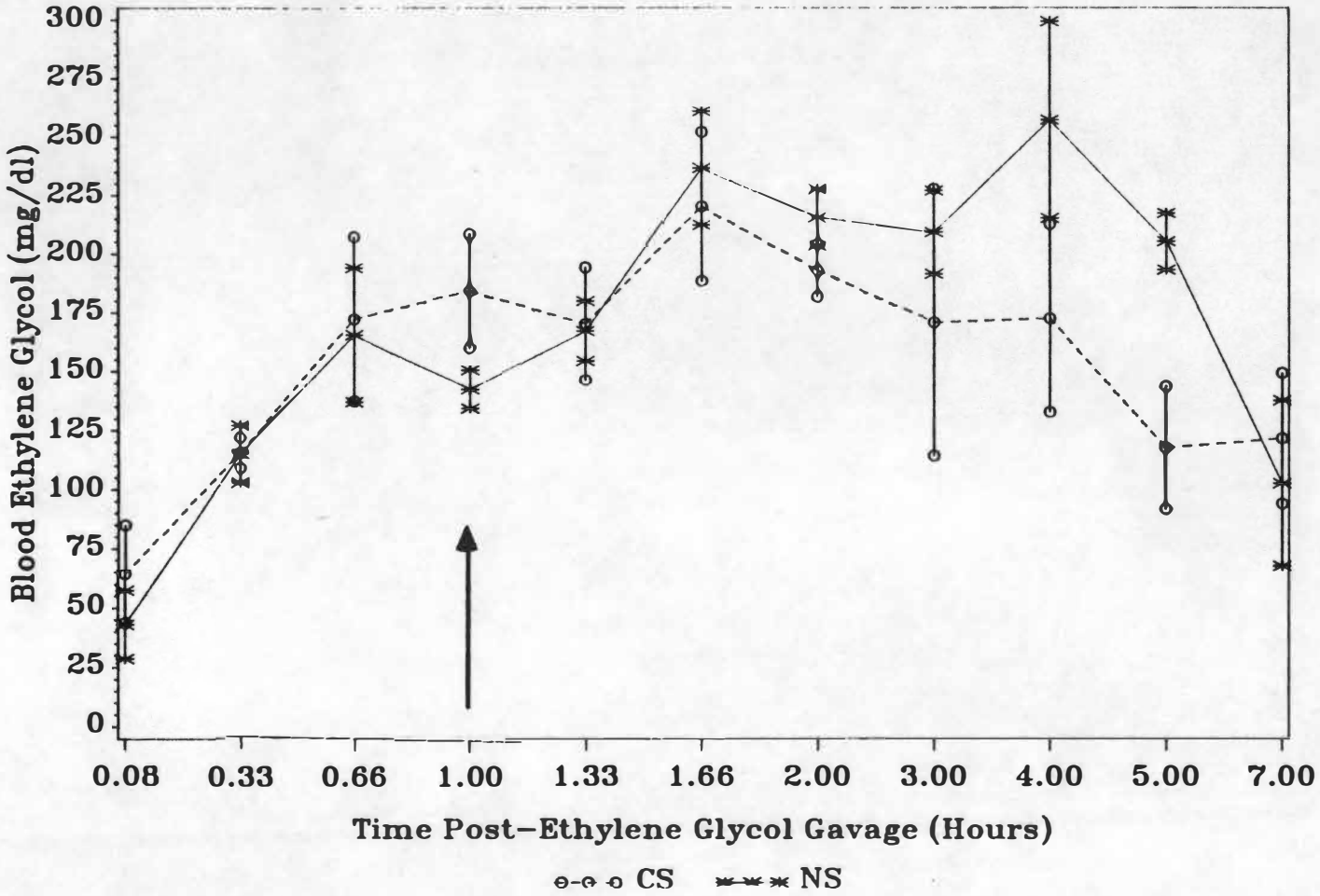


Figure 4.9. Blood ethylene glycol concentrations with ethanol treatment. Time scale is expanded. Arrow indicates time at which ethanol was administered.

Table 4.17. Blood Ethylene Glycol Concentrations with Ethanol Treatment

Hours Post Ethylene Glycol Administration	Blood Ethylene Glycol (mg/dl)		
	NS	(Diet)	CS
0.08	43.08 ± 14.43 (4)	64.33 ± 20.61 (4)	
0.33	115.42 ± 12.09 (4)	115.88 ± 6.45 (4)	
0.66	165.82 ± 28.38 (4)	172.48 ± 34.72 (4)	
1.0 (Ethanol Treatment)	142.83 ± 8.14 (4)	184.63 ± 24.19 (4)	
1.33	167.70 ± 12.69 (4)	170.70 ± 23.95 (3)	
1.66	236.85 ± 24.22 (4)	220.55 ± 31.69 (4)	
2.0	215.85 ± 12.10 (4)	193.37 ± 11.14 (3)	
3.0	209.35 ± 17.69 (4)	171.10 ± 56.74 (3)	
4.0	257.25 ± 41.92 (4)	172.95 ± 39.88 (4)	
5.0	205.50 ± 12.05 (4)	118.07 ± 25.94 (4)	
7.0	103.05 ± 35.00 (4)	122.00 ± 27.61 (4)	

Values are means ± SEM. Numbers in parentheses are numbers of animals in groups.

ethanol treatment. It may be pointed out that all the CS animals of this study entered the elimination phase by 5 hours post ethylene glycol gavage compared to only 2 of 6 CS animals given ethylene glycol alone (see Section 4.3).

The kinetic analysis of the BEGC curve in the presence of ethanol is shown in Table 4.18. The initial absorption slopes prior to ethanol treatment revealed no significant differences between the two diet groups. The absorption slope taken after ethanol treatment reveals a 50 % reduction in the rate of absorption in the CS group though wide deviations and small sample size prevent this from being a statistically significant effect. The peak BEGC and peak time indicate that the elimination of ethylene glycol began sooner after ethanol administration in the CS group than in the NS group. There were no significant differences in areas under the curve though the increased systemic clearance in the CS group approached a significant difference above the NS group. The elimination slope of ethylene glycol was not as steep in the CS group as it was in the NS group but commenced earlier. Carnitine supplementation lowered the theoretical instantaneous distribution which resulted in a higher volume of distribution. This effect was also noted in Table 4.14 for ethylene glycol alone.

As was the case with the ethylene glycol alone there were no significant effects of carnitine supplementation on the excretion of ethylene glycol in the urine even after the ethanol treatment (Table 4.19). There were no significant differences between the NS and CS groups in the concentration of ethylene glycol in the urine, urine volume, total ethylene glycol excretion, percent of the dose excreted or the hourly excretion rate. The percent of the dose of ethylene glycol excreted was 75% higher in this experiment than with ethylene glycol alone (Table 4.15). The urinary clearance showed a non-significant but noticeable trend, however, the CS group was more effective in ridding the body of ethylene glycol by renal excretion (Table 4.20). This was not seen with the ethylene glycol alone. Larger sample sizes in future studies may reveal the significance of this effect.

Table 4.18. Blood Ethylene Glycol Kinetics with Ethanol Treatment.

Parameter	Diet NS	Diet CS	P>|t| =0.05
Absorption Slope (0.666 hours) (mg/dl·hr)	242.67 ± 38.59 (4)	237.44 ± 44.64 (4)	NS
Peak Blood Level Time (hour)	2.83 ± 0.67 (4)	1.91 ± 0.41 (4)	.292
Concentration (mg/dl)	286.72 ± 31.26 (4)	245.92 ± 15.00 (4)	.284
Absorption Slope (post ethanol 0.666 hours) (mg/dl·hr)	142.43 ± 34.98 (4)	74.20 ± 50.13 (4)	.307
Area Under Curve 7 hours (mg/dl·hrs)	1297.37 ± 32.28 (4)	1075.83 ± 144.94 (4)	.186
Systemic Clearance 7 hours (L/hr·kg)	0.429 ± 0.011 (4)	0.541 ± 0.058 (4)	.109
(ml/min·kg)	7.15 ± 0.18	9.02 ± 0.97	
Elimination Slope K_{el} (mg/dl·hr)	-41.88 ± 21.49 (4)	-23.61 ± 6.23 (4)	NS
C_o (mg/dl)	406.14 ± 118.69 (4)	265.85 ± 45.83 (4)	.312
Volume of Distribution (L/kg)	1.64 ± 0.33 (4)	2.31 ± 0.44 (4)	.273

Values are means ±SEM. Values in parentheses are numbers of animals in groups. C_o is the theoretical instantaneous distribution concentration at time of dosing.

Table 4.19. Excretion of Unchanged Ethylene Glycol in Urine Following Ethanol Treatment.

Parameter	Collection Times (Hours Post Ethylene Glycol Administration)					
	0-1Pre ETOH	1-2 Post ETOH	2-3	3-5	5-7	7 hour total
Concentration						
NS (mg/ml)	24.43 ± 3.55(4)	24.73 ± 1.42(4)	32.89 ± 5.08(4)	44.19 ± 2.73(4)	43.93 ± 3.70(4)	35.39 ± 2.35(4)
CS	29.19 ± 7.48(4)	26.22 ± 3.19(4)	40.70 ± 5.10(4)	42.27 ± 5.73(4)	49.61 ± 3.14(4)	39.17 ± 1.59(4)
NS (µmole/ml)	394.11 ± 57.27(4)	398.87 ± 22.90(4)	530.48 ± 81.95(4)	712.74 ± 44.14(4)	708.51 ± 59.68(4)	570.85 ± 37.87(4)
CS	470.81 ± 120.68(4)	422.94 ± 51.48(4)	656.57 ± 82.31(4)	681.69 ± 92.46(4)	800.12 ± 50.65(4)	631.81 ± 25.77(4)
Urine Output (ml)						
NS	1.98 ± 0.19(4)	2.35 ± 0.55(4)	4.28 ± 0.19(4)	4.75 ± 0.58(4)	2.87 ± 0.71(4)	16.22 ± 1.51(4)
CS	1.48 ± 0.54(4)	3.40 ± 0.62(4)	3.60 ± 0.34(4)	4.72 ± 0.46(4)	2.70 ± 0.43(4)	15.90 ± 1.03(4)
Total Ethylene Glycol Excretion						
NS (mg)	46.72 ± 3.91(4)	60.43 ± 17.07(4)	139.86 ± 20.56(4)	214.71 ± 35.66(4)	119.01 ± 17.47(4)	580.98 ± 79.04(4)
CS	52.90 ± 4.41(3)	88.06 ± 17.22(4)	149.64 ± 28.38(4)	205.98 ± 45.03(4)	130.80 ± 13.84(4)	614.16 ± 49.28(4)
NS (µmole)	753.5 ± 63.0(4)	974.7 ± 275.4(4)	2255.8 ± 331.7(4)	3463.1 ± 575.2(4)	1919.6 ± 281.8(4)	9370.7 ± 1274.9(4)
CS	853.2 ± 71.2(3)	1420.4 ± 277.7(4)	2413.6 ± 457.7(4)	3322.2 ± 726.3(4)	2109.7 ± 223.2(4)	10035.2 ± 758.3(4)
Percent of Dose Excreted						
NS	2.91 ± 0.24(4)	3.69 ± 0.98(4)	8.64 ± 1.08(4)	13.29 ± 2.07(4)	7.34 ± 0.79(4)	35.87 ± 3.96(4)
CS	3.19 ± 0.42(3)	5.40 ± 1.17(4)	9.24 ± 1.94(4)	12.53 ± 2.92(4)	7.87 ± 0.63(4)	37.44 ± 3.87(4)
Hourly Ethylene Glycol Excretion						
(mg/g BW·hour)						
NS	0.162 ± .013(4)	0.206 ± .054(4)	0.481 ± .060(4)	0.367 ± .061(4)	0.205 ± .022(4)	0.285 ± .032(4)
CS	0.178 ± .023(3)	0.300 ± .065(4)	0.515 ± .108(4)	0.348 ± .081(4)	0.219 ± .018(4)	0.301 ± .029(4)
(µmole/g BW·hour)						
NS	2.62 ± 0.21(4)	3.32 ± 0.87(4)	7.76 ± 0.97(4)	5.92 ± 0.98(4)	3.30 ± 0.36(4)	4.61 ± 0.51(4)
CS	2.87 ± 0.37(3)	4.85 ± 1.05(4)	8.31 ± 1.74(4)	5.62 ± 1.31(4)	3.53 ± 0.28(4)	4.86 ± 0.47(4)

Values are means ± SEM. Values in parentheses are numbers of animals in groups. There were no significant differences by t test at P>|t| = 0.05.

Table 4.20. Urinary Clearance of Ethylene Glycol with Ethanol Treatment

Urine Collection (Blood Sample)	Diet NS	Diet CS	P>|t| =0.05
	ml/period·kg ml/min·kg	ml/period·kg ml/min·kg	
Pre Ethanol Treatment			
0-1 Hours (0.5 Hours)	125.10 ± 16.92 (4) 2.085 ± 0.282	122.45 ± 12.97 (3) 2.041 ± 0.216	NS
Post Ethanol Treatment			
1-2 Hours (1.5 Hours)	102.42 ± 26.34 (4) 1.707 ± 0.439	195.68 ± 81.31 (4) 3.261 ± 1.355	NS
2-3 Hours (2.5 Hours)	230.48 ± 41.89 (4) 3.841 ± 0.698	307.51 ± 93.92 (3) 5.125 ± 1.565	NS
3-5 Hours (4 Hours)	293.42 ± 42.57 (4) 2.444 ± 0.354	751.92 ± 326.72 (4) 6.265 ± 2.722	NS
5-7 Hours (6 Hours)	279.52 ± 48.40(4) 2.329 ± 0.403	408.45 ± 149.27 (4) 3.404 ± 1.244	NS
7 Hour Total (3.5 Hours)	715.03 ± 100.72 (4) 1.702 ± 0.239	1235.66 ± 350.59 (4) 2.942 ± 0.834	0.161

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups.

The blood ethanol concentration (BEC) curves for ethylene glycol-ethanol treated animals are shown in Figure 4.10 and data are given in Table 4.21. The only visual effect noted was that the BEC curve of the NS animals appears to be consistently lower than the CS BEC curve, but the shape of the two curves was similar. The kinetic analysis (Table 4.22) show no significant differences between the NS and CS groups in the initial absorption slope, the peak blood levels or time to reach the peak. The absorption slope to the peak was slightly lower in the CS group than the NS group. The area under the BEC curve was higher in the CS group than in the NS group but not significantly so. Systemic clearance, elimination slope and C_o and volume of distribution were not significantly different between the two groups.

The urinary excretion of ethanol (Table 4.23) was not significantly different between the two groups. There was a slight increase in the total ethanol excretion, percent of the dose excreted, and the hourly excretion rate of ethanol in the CS group and these increases were independent of urine volumes which were not different between groups. Wide deviations make these effects statistically non-significant. The urinary clearance of ethanol with the ethylene glycol treatment in the CS group shows higher clearances than the NS group in the 3-5 hour collection (2-4 hours post ethanol) and in the total for the period (Table 4.24). This was seen in the urinary ethylene glycol clearance of this experiment (Table 4.20) and shows the effect of the carnitine supplement and ethanol on the ethylene glycol excretion patterns. The blood and urine concentration curves of ethylene glycol with ethanol treatment are shown in Figure 4.11 and the blood and urine levels of ethanol with ethylene glycol are shown in Figure 4.12.

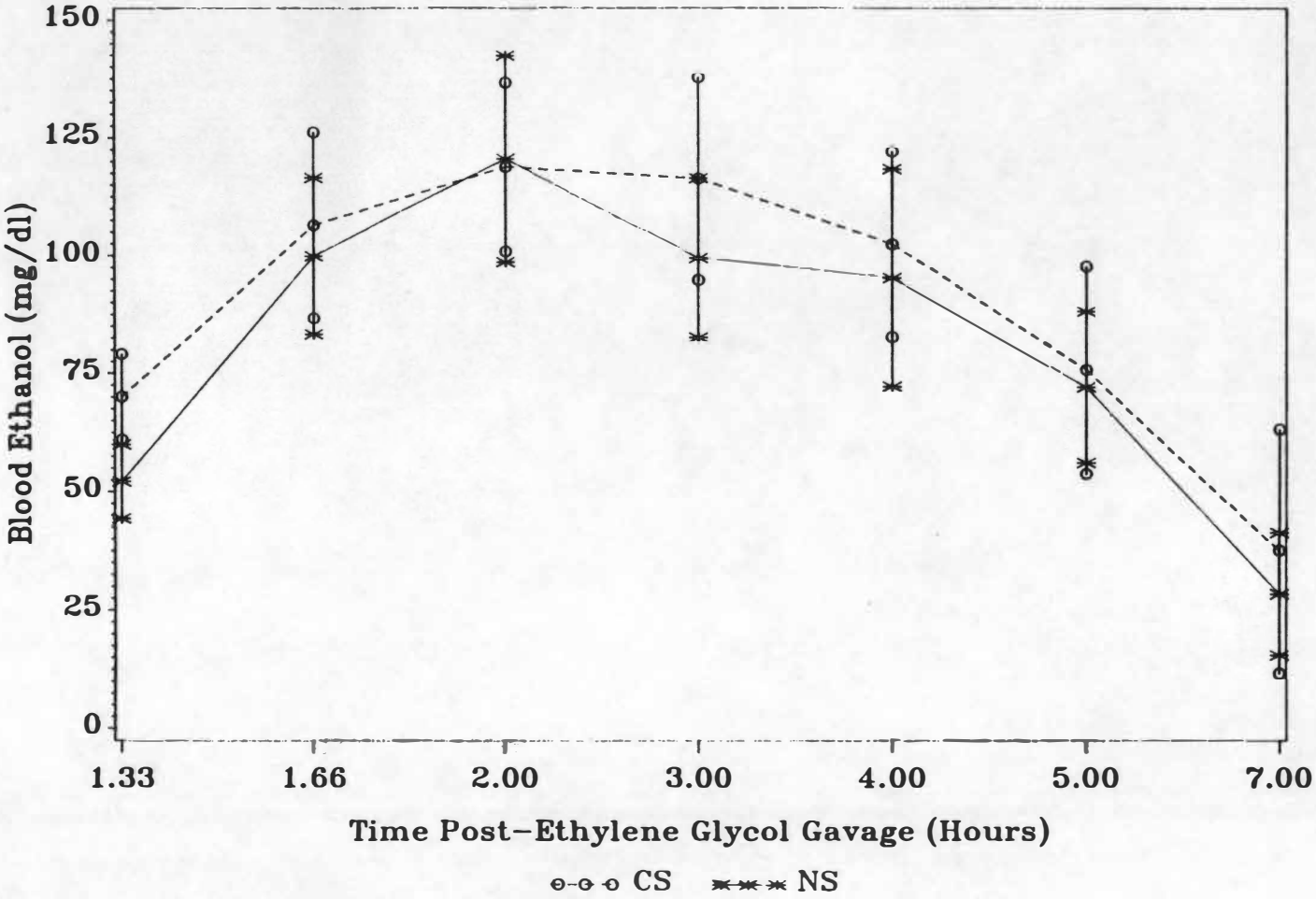


Figure 4.10. Blood ethanol concentrations of rats receiving ethylene glycol treatment. Time scale is expanded.

Table 4.21. Blood Ethanol Concentrations Post Ethylene Glycol Treatment

Hours Post Ethylene Glycol Administration	Blood Ethanol (mg/dl)		
	NS	(Diet)	CS
1.33	52.15 ± 7.82 (4)	70.04 ± 9.06 (4)	
1.66	99.86 ± 16.59 (4)	106.43 ± 19.73 (4)	
2.0	120.44 ± 21.90 (4)	118.73 ± 17.91 (4)	
3.0	99.54 ± 16.82 (4)	116.34 ± 21.41 (4)	
4.0	95.30 ± 23.04 (4)	102.45 ± 19.64 (4)	
5.0	72.11 ± 15.94 (4)	75.76 ± 21.94 (4)	
7.0	28.37 ± 12.81 (4)	37.46 ± 25.72 (4)	

Values are means ± SEM. Numbers in parentheses are numbers of animals in groups.

Table 4.22. Blood Ethanol Kinetics with Ethylene Glycol Treatment

Parameter	Diet						P>|t| =0.05
	NS			CS			
Absorption Slope (0.666 hours) (mg/dl·hr)	151.30	±	25.13 (4)	161.26	±	29.89 (4)	NS
Peak Blood Level Time (hour)	2.00	±	0.00 (4)	2.25	±	0.25 (4)	NS
Concentration (mg/dl)	120.44	±	21.89 (4)	120.77	±	19.81 (4)	NS
Absorption Slope (to peak) (mg/dl·hr)	128.95	±	16.91 (4)	93.39	±	9.84 (4)	.119
Area Under Curve 6 hours (mg/dl·hrs)	461.77	±	90.28 (4)	507.07	±	118.39 (4)	NS
Systemic Clearance 6 hours (L/hr·kg)	0.750	±	0.177 (4)	0.682	±	0.134 (4)	NS
(ml/min·kg)	12.50	±	2.95	11.37	±	2.23	NS
Elimination Slope K_{el} (mg/dl/hr)	-17.99	±	3.52 (4)	-17.93	±	1.00 (4)	NS
C_o (mg/dl)	158.77	±	26.67 (4)	166.33	±	19.07 (4)	NS
Volume of Distribution (L/kg)	2.15	±	0.52 (4)	1.87	±	0.19 (4)	NS

Values are means ±SEM. Values in parentheses are the numbers of animals in groups. C_o is the theoretical instantaneous distribution concentration at time of dosing.

Table 4.23. Excretion of Unchanged Ethanol in Urine with Ethylene Glycol Treatment.

Parameter	Collection Times (Hours Post Ethylene Glycol Administration) 1-2	2-3	3-5	5-7	6 hour total
Concentration					
NS (mg/ml)	1.36 ± 0.15(4)	1.15 ± 0.19(4)	1.13 ± 0.22(4)	0.72 ± 0.27(4)	1.01 ± 0.18(4)
CS	1.27 ± 0.39(4)	1.56 ± 0.31(4)	1.28 ± 0.33(4)	0.47 ± 0.15(4)	1.21 ± 0.31(4)
NS (µmole/ml)	29.45 ± 3.20(4)	25.00 ± 4.24(4)	24.56 ± 4.74(4)	15.49 ± 5.88(4)	23.96 ± 3.95(4)
CS	27.60 ± 8.56(4)	33.97 ± 6.72(4)	27.77 ± 7.22(4)	10.27 ± 3.27(4)	26.47 ± 6.74(4)
Urine Output (ml)					
NS	2.35 ± 0.55(4)	4.28 ± 0.19(4)	4.75 ± 0.58(4)	2.87 ± 0.71(4)	14.25 ± 1.64(4)
CS	3.40 ± 0.62(4)	3.60 ± 0.34(4)	4.72 ± 0.46(4)	2.70 ± 0.43(4)	14.42 ± 0.88(4)
Total Ethanol Excretion					
NS (mg)	3.06 ± 0.69(4)	4.98 ± 1.00(4)	5.45 ± 1.41(4)	1.82 ± 0.59(4)	15.31 ± 2.64(4)
CS	4.93 ± 1.93(3)	5.80 ± 1.48(4)	6.34 ± 2.37(4)	1.13 ± 0.23(4)	18.21 ± 5.73(4)
NS (µmole)	66.63 ± 14.98(4)	108.26 ± 21.80(4)	118.48 ± 30.64(4)	39.51 ± 12.97(4)	332.88 ± 57.39(4)
CS	107.17 ± 41.89(3)	126.19 ± 32.25(4)	137.83 ± 51.66(4)	24.67 ± 5.13(4)	395.92 ± 124.71(4)
Percent of Dose Excreted					
NS	0.35 ± 0.08(4)	0.59± 0.12(4)	0.64 ± 0.17(4)	0.21 ± 0.07(4)	1.78 ± 0.32(4)
CS	0.56 ± 0.23(4)	0.67 ± 0.19(4)	0.73 ± 0.29(4)	0.13 ± 0.03(4)	2.09 ± 0.71(4)
Hourly Ethanol Excretion (mg/g BW·hour)					
NS	0.011 ± .002(4)	0.018 ± .004(4)	0.010 ± .003(4)	0.003 ± .0010(4)	0.009 ± .001(4)
CS	0.017 ± .007(3)	0.020 ± .005(4)	0.011 ± .004(4)	0.002 ± .0004(4)	0.010 ± .003(4)
(µmole/g BW·hour)					
NS	0.231 ± .052(4)	0.381 ± .082(4)	0.207 ± .055(4)	0.068 ± .023(4)	0.194 ± .035(4)
CS	0.368 ± .152(3)	0.437 ± .122(4)	0.238 ± .096(4)	0.042 ± .010(4)	0.227 ± .077(4)

Values are means ± SEM. Values in parentheses are numbers of animals in groups. There were no significant differences by t test at P>|t| = 0.05.

Table 4.24. Urinary Clearance of Ethanol in Ethylene Glycol Treated Animals

Urine Collection (Blood Sample)	Diet	
	NS	CS
	ml/period·kg ml/min·kg	ml/period·kg ml/min·kg
Ethanol Treatment (1.00 Hours)		
1-2 Hours (1.5 Hours)	14.41 ± 2.87 (4) 0.240 ± 0.048	12.50 ± 4.76 (4) 0.208 ± 0.079
2-3 Hours (2.5 Hours)	10.77 ± 4.26 (4) 0.180 ± 0.071	13.77 ± 2.40 (4) 0.230 ± 0.040
3-5 Hours (4 Hours)	20.13 ± 2.32 (4) 0.168 ± 0.019	39.69 ± 19.75 (4) 0.331 ± 0.165
5-7 Hours (6 Hours)	11.99 ± 3.04 (4) 0.100 ± 0.025	8.03 ± 1.10(4) 0.067 ± 0.009
7 Hour Total (3.5 Hours)	57.62 ± 7.36 (4) 0.160 ± 0.020	62.68 ± 20.88 (4) 0.174 ± 0.058

Values are means ±SEM. Numbers in parentheses are numbers of animals in groups. There were no significant differences by t test at P>|t |=0.05.

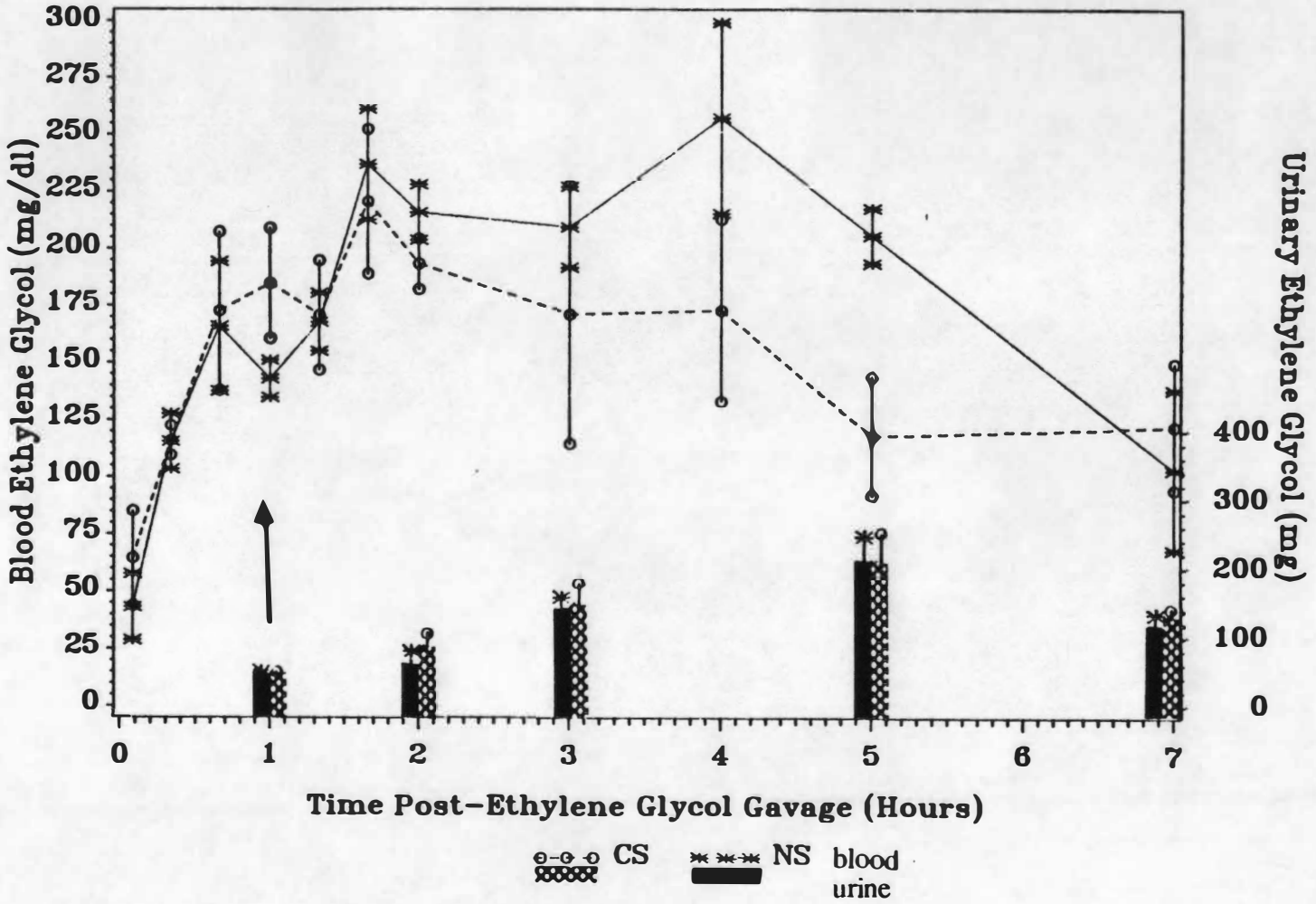


Figure 4.11. Blood ethylene glycol concentrations and total urinary ethylene glycol excretion following ethanol treatment. Arrow indicates time of ethanol administration

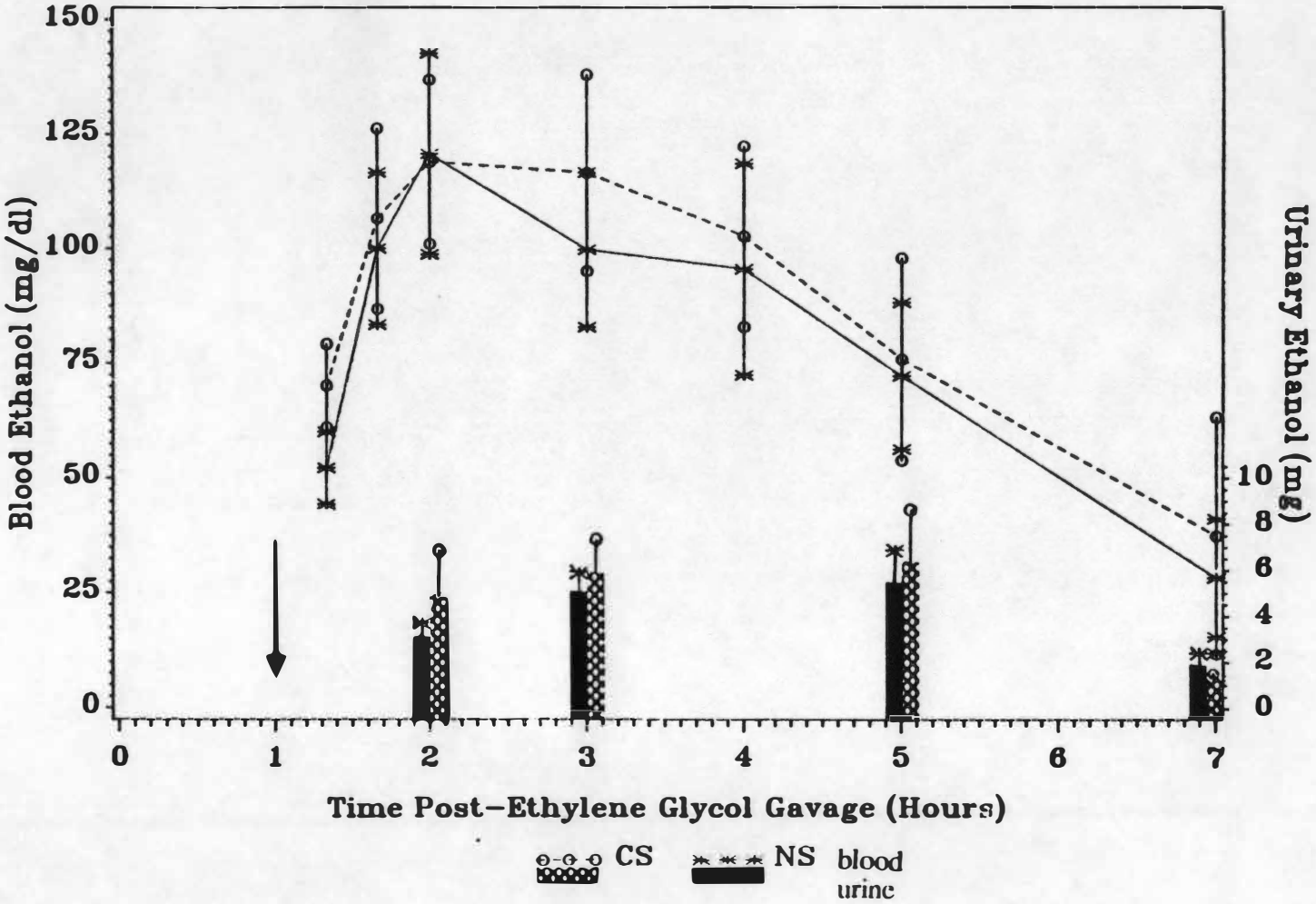


Figure 4.12. Blood ethanol concentrations and total urinary ethanol excretion with ethylene glycol treatment. Arrow indicates time of ethanol administration.

CHAPTER 5

DISCUSSION

5.1 Factors Governing Metabolism and Excretion of the Alcohols in General

A general scheme of the factors influencing drug disposition is shown in Figure 5.1. The concentration of the drug in the central compartment (blood or extracellular fluid) is related to the rate of absorption. The rate of removal from the central compartment is related to the activity of enzymes in the pathways of biotransformation and also to the routes of excretion. It must be noted that metabolism and excretion can and do begin before absorption is complete (44).

Baggot (44) makes the following general comments about drug elimination by excretion. "The degree of lipid-solubility and extent of ionization in biological fluids are the main factors that determine the proportion of the therapeutic dose of any drug eliminated by excretion. Polar drugs and the majority of drug metabolites are excreted in the urine." Excretion usually follows first order kinetics except when special transport mechanisms are used and overwhelmed then it become zero order (44).

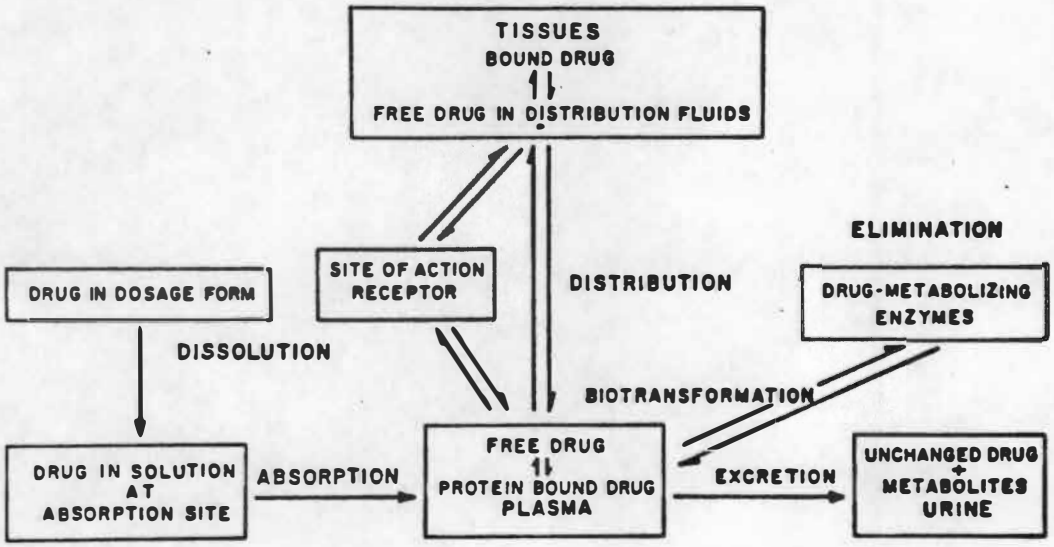


Figure 5.1. Schematic representation of the processes that influence uptake, access to site of action, and removal of a drug from the body.

Source: Baggot, J.D. (1977) Principles of Drug Disposition in Domestic Animals, W.B. Saunders Company, Philadelphia, p. 4. (ref.44)

5.2 Effects of Carnitine on the Metabolism of Alcohols

5.2.1 Methanol

The effect of carnitine on blood methanol was to decrease the blood methanol concentrations especially at time points less than 4 hours. This may be only due to the effect of increased urinary excretion of methanol. The actual percent of the methanol dose excreted in the urine is so low that increased urinary methanol excretion alone cannot account for this decline. Increased loss to expired air could also be occurring. Tephly et al (16) found an equal division of excreted methanol between the renal and pulmonary routes. If the distribution remained equal then an increase methanol excretion in the expired air also occurred. The absorption slopes of methanol in the CS group were lower than the NS group which may be due to an actual decrease in absorption due to complex formation (See Section on Micelles 5.3.1) or due to rapid initiation of elimination before absorption was complete.

Carnitine may induce the peroxisomal methanol metabolizing enzyme system. Carnitine is found in the peroxisomes (33) and may enhance the action of the catalase-peroxidase system by reducing membrane disorder as was seen by Guzman and Geleen (43) with ethanol and carnitine. Carnitine supplementation and the concomitant rise in long-chain acylcarnitines may be creating the proper intracellular conditions for catalase and peroxide to combine. It may be that the acylcarnitines are acting as detergents to an extent and promoting the release of catalase from the peroxisome. Van Harken (17) found that treatment of peroxisomes with deoxycholate, a detergent, increased the catalase activity . This follows in accord with deDuve who stated that catalase and the peroxide generating systems reside together in the peroxisomes for maximum effectiveness (13).

Hertz and Bar-Tana (45) postulated a role for carnitine acyltransferase in the inductions of peroxisomal enzymes of fatty acid ß-oxidation. Natural proliferators of the peroxisomal ß-oxidation system are long chain fatty acids. Xenobiotics such as benzafibrate and clofibrate, hyperlipidemic agents, are also shown to induce the peroxisomal ß-oxidation system (45,46). Clofibrate feeding stimulated both ethanol and methanol oxidation in the Sprague Dawley rat. This stimulation was sensitive to 3-amino-1,2,4 triazole, the catalase inhibitor. This study showed that stimulation of one peroxisomal enzyme system (ß-oxidation of fatty acids) could result in the stimulation of another enzyme system (catalase). Carnitine feeding may increase the activity of peroxisomal enzymes by increasing the number of binding sites for long-chain fatty acids in the peroxisomes of various tissues, a sort of simulated state of starvation which has been shown to enhance the benzafibrate mediated induction of enzyme systems (45). Catalase activity in the Wistar rats used by Hertz and Bar-Tana was not increased by starvation which was contrary to the effect observed in Sprague-Dawley rats (45). Mynatt (47) measured the catalase activity in liver fractions from non-supplemented and carnitine supplemented Sprague-Dawley rats. He reported no differences in the catalase activity as measured by the reduction of H_2O_2.

Van Harken (17) discussed the need for experiments designed for measurement of catalase with "nascent peroxide". The direct addition of H_2O_2 to reaction mixtures yields erroneous measurements of catalase mediated conversion of alcohol to aldehyde. Hydrogen peroxide must be added, according to Van Harken by adding an entire enzyme system (e.g. glucose:glucose oxidase) or by diffusing H_2O_2 vapor or mist into the mixture or by the dropwise addition of minute amounts of H_2O_2 solution (17). The apparent lack of any significant differences in the catalase activity of carnitine supplemented animals has been reported (47) but the conclusions of Van Harken (17) leave room for the speculation

that it is not necessarily the activity of catalase alone that is of interest in the metabolism of methanol. The placement of catalase within the context of a peroxide generating system must be considered as a whole and the activities of the entire system must be assessed.

5.2.2. Isopropanol

Carnitine supplementation resulted in no differences in the blood isopropanol or blood acetone concentration curves. It is therefore inferred that there were no differences in the metabolism of isopropanol and acetone between the CS and NS animals. This lack of difference could be due to the nature of isopropanol metabolism which is not as highly reactive with ADH as ethanol and has alternate means of disposition via conjugation (1). The fact that alterations in the redox state do not occur with isopropanol (7,8) also may limit the influence of carnitine on metabolism. The increased reduction state of the extra-mitochondrial enzyme systems as well as the mitochondrial enzyme systems with ethanol administration precipitates many of the metabolic alterations seen with ethanol administration such as decreasing the activity of the tricarboxylic acid cycle (12). The rate of ß-oxidation of fatty acids is not regulated by the NAD+H+/NAD ratio (32) and therefore may continue in the presence of factors increasing fat mobilization to the liver. Carnitine supplementation of ethanol fed animals resulted in a reduced fat accumulation in the liver and also reduced blood triglycerides (3). If the action of carnitine in alcohol metabolism is dependent upon the redox state of the cell then differences in the isopropanol metabolism of NS and CS animals cannot be expected. If the role of carnitine as a lipotropic agent is also dependent upon the redox state of the cell then there should be no reduction in the fat accumulation in the liver as a result of isopropanol.

5.2.3 Ethylene Glycol

There was only a slight lowering of the blood ethylene glycol levels in the CS group which was reflected in non-significant reductions in absorption slopes and areas under the curve. Any direct action of carnitine on ADH would have been manifested in altered blood ethylene glycol concentration curves similar to those seen with ethanol administration to carnitine supplemented animals (4).

Van Harken noted that the metabolism of ethylene glycol to glycolate and glyoxalate leads to an increase in the catalase activity (17). Although catalase is thought to be a minor contributor to ethanol metabolism increases of catalase activity do result in increased rates of ethanol oxidation (46). Therefore, ethylene glycol may be of importance in studying the role of carnitine in catalase activity.

The interaction of carnitine, ethylene glycol and ethanol proved to be an enlightening and complex study. The blood ethylene glycol concentrations in the CS group were slightly lower than the NS group especially after the ethanol treatment. The peak blood ethylene glycol values and time to reach the peak were also slightly lower in the CS group than the NS group, but not significantly so. The slightly lower theoretical instantaneous distribution concentration of ethylene glycol in the CS group indicates that elimination of ethylene glycol commenced rapidly after the administration of ethanol, before absorption was completed. It was shown that the absorption of ethylene glycol alone in the rat required at least 6 hours (Table 4.14). This study allowed for the confirmation of the role of catalase in the attenuation of alcohol metabolism by carnitine. If the ethylene glycol stimulation of catalase was affected by carnitine then the blood ethanol levels of the CS group should have been affected. The blood ethanol levels after ethylene glycol administration to the CS animals were slightly elevated over the NS animals and the peak blood ethanol levels and times to reach the peak level were not different between the groups. A larger population and a reduced deviation in the ethylene glycol measurements

may have revealed a difference in the displacement of ethylene glycol by ethanol at ADH. It seems evident that the effect of carnitine on ethanol metabolism is not due to action at ADH. The 3 g/kg dose of ethanol is not a therapeutic dose according to the literature. This is considerably higher than the 0.6 g/kg dose suggested by Peterson et al (26) for treatment of ethylene glycol intoxication in humans. The blood ethanol concentrations were maintained between 100-200 mg/dl for the entire length of this study which is the range suggested as the optimal blood levels for displacing ethylene glycol at ADH in humans (26). In this study the total percent of the ethylene glycol dose excreted in rat urine was increased from about 20% with ethylene glycol alone to 35% with the ethanol treatment regardless of dietary treatment.

5.3 Effects on the Absorption of Alcohols

5.3.1 The Formation of Micelles

In aqueous solution species that are dominated by polar groups, such as methanol, exist as single molecules. When there is a balance between polar and non-polar groups a dynamic equilibrium exists between single molecules and micelles (48). The formation of micelles of acylcarnitines is a definite possibility in the carnitine supplemented animals. Yalkowsky and Zografi (49) estimated the critical micelle concentration (CMC), the point at which micelle formation commences, of the cationic and zwitterionic forms of acylcarnitines in water or 0.20 M KCl solution. The CMC results ranged from 1.0×10^{-1} moles/liter for octylcarnitine to 8.5×10^{-6} moles/liter for palmitylcarnitine. The ionization constants and surface potentials of the C12-16 acylcarnitine micelles are independent of chain length but highly dependent on ionic strength of the milieu.

The role of acylcarnitines as surface active agents has not been extensively investigated yet, however it must be thoughtfully considered. Danielli (48) lists the results

of micelle formation as follows: 1. If a physiological action is based on concentration of single molecules in solution then action may be independent of concentration. 2. As the number of carbons in the aliphatic chain increases, the physiologic activity tends to increase. A point is reached when micelle formation occurs before the concentration for maximal physiologic activity is attained. 3. Substances can be inactivated by micelles. The example given from Danielli (Figure 5.2) shows the uptake of hexyl resorcinol retarded by complexation with micelles.

Alcohols are known to react with micellar systems. Zana et al (50) found that the addition of alcohols to micellar systems decreased the CMC and increased the micelle ionization. The surfactant aggregation number increased with increasing surfactant concentrations. The alcohols were found to be solubilized in the palisade layer of the micelle, that is, the layer of the micelle below the ionic head group and above the core. The result of the addition of alcohols to micelles was to increase ionization, increase repulsive action and increase dispersion into smaller micelles.

The presence of surface active agents at less than the CMC may result in enhanced drug absorption across a number of membranes due to a direct action on the biologic membrane and not an interaction with the drug (51). Some basic assumptions about micellar systems in gastrointestinal absorption studies were outlined by Hem (51) who stated that, " 1) The micellar solution consists of two phases. 2) The partition ratio of drug between the micellar phase and the aqueous phase is constant, independent of drug concentration; and 3) absorption of drug incorporated in the micelle is negligible."

Carnitine supplementation has been shown to increase the concentration of all carnitine fractions in the intestinal fluids of chow fed rats (52). Depending upon the amount of fluid in the gut lumen and the concentration of long-chain acylcarnitines (AIAC) in localized micro-environments of the intestine, the 153 nmoles of AIAC reported may be sufficient to generate micelles especially if alcohols lower the CMC. The study by Sachan and Berger

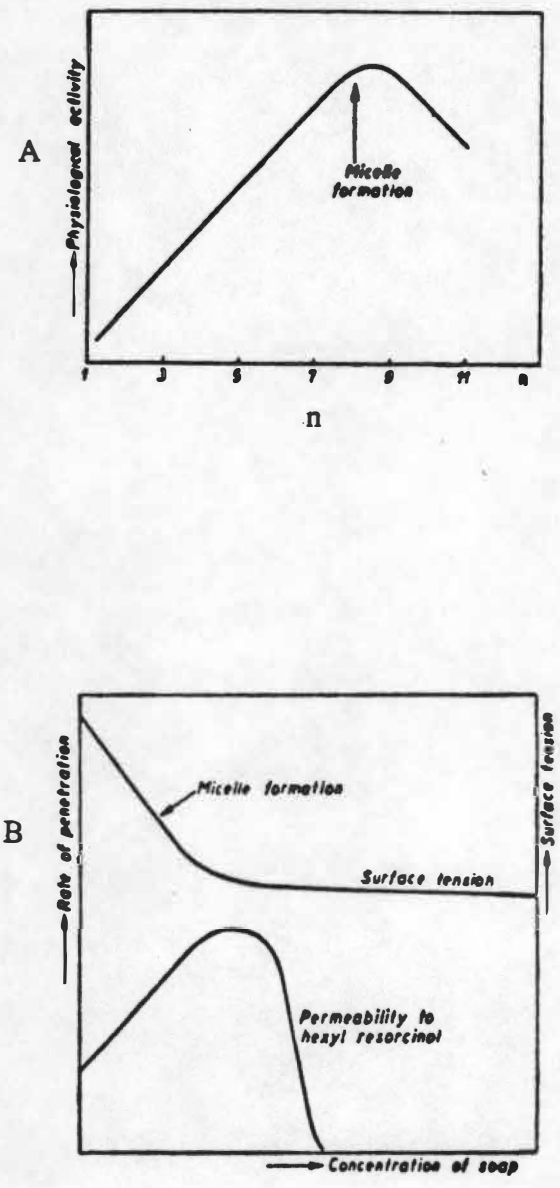


Figure 5.2. Physiological activity and micelle formation. Part A shows the relationship between physiological activity and n, in a series $CH_3(CH_2)_nX$, where the physiological activity is a function of the concentration of single molecules. Part B shows the prevention of permeation of hexyl resorcinol into *Ascaris* by micelle formation in a soap solution.

Source: Danielli, J.F. (1968) Cell Physiology and Pharmacology, Hafner Publishing Company, New York, pp. 42-43. (ref.48)

(4) found, however no difference in the rate of ethanol absorption in the first 60 minutes. This would possibly indicate that sub-CMC levels of acylcarnitines were not enhancing the absorption of ethanol and levels above the CMC were not retarding ethanol absorption by complex formation in the gut.

5.3.2 Viscosity and Blood Flow

The question of whether or not the physical properties of the alcohol played a role in the absorption and the effects of carnitine must be addressed. Ethylene glycol is a viscous solution. A high viscosity solution may reduce absorption by increasing gastric emptying time, increase intestinal transit rate, reduce the movement to the absorptive surface or reduce the ability of the drug to come into intimate contact with microvilli (51). The effect of a viscous solution such as ethylene glycol on ethancl absorption might show similar results to that with methyl cellulose as shown by Hem (51). Ethanol absorption was decreased with increasing viscosity.

Carnitine supplementation could have effected regional differences in blood flow to the intestines and that was the cause of differences in blood levels of the alcohol . Figure 5.3 clearly shows that blood flow increases should have influenced all the alcohols used in this study as the absorption of all the alcohols used in this study were increased by increased blood flow.

5.4 The Disposition of Alcohols in the Urine

The effect of carnitine supplementation on the excretion of alcohols in the urine was fairly consistent with all the alcohols used in this study. The urinary alcohol concentrations in the CS animals were increased especially in the sample collected in the first two hours after the gavage. The volume of urine was significantly increased in the 2 and 4 hour

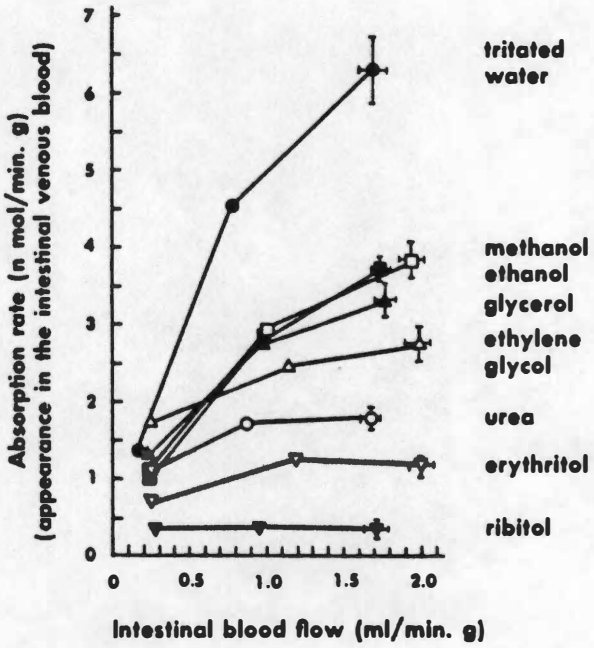


Figure 5.3. Influence of intestinal blood flow on absorption.

Source: Rowland, M. (1973) Effect of some physiological factor in bioavailability of oral dosage form. In: Current Concepts in the Pharmaceutical Sciences, Dosage Form Design and Bioavailability (Swarbrick, J., ed.) Lea and Febiger, Philadelphia, pp. 181-222.

collections of the CS group with methanol and isopropanol but not in either of the ethylene glycol studies. The total excretion of alcohol in CS animals was significantly increased at 2 and 4 hours post gavage with methanol and isopropanol but only sightly increased at 2 hours post gavage with ethylene glycol. The percentage of the dose excreted by the CS groups was increased above the NS group with all alcohols studied and was significantly elevated with methanol and isopropanol. The urinary clearance was nearly doubled in the CS animals treated with methanol, isopropanol or ethylene glycol and ethanol. The urinary clearance of ethylene glycol alone was only slightly increased by carnitine supplementation.

The fact that carnitine stimulated increases in urine volume were seen with methanol and isopropanol but not with ethylene glycol points to other factors that need to be investigated. One such factor in this regard is the total fluid load given to the animal with the alcohol dose. The methanol, isopropanol and ethanol were given as 13% (v/v) solution which amounted to an average gavage volume of 10 ml. The ethylene glycol was given as a 1:1 diluted solution and the gavage was usually less than 3 ml. The fluid load of the ethylene glycol test animals was much lower than the fluid load of the other alcohols.

In a study shown in the appendix, the hypothesis that fluid load of the animal was influencing the carnitine mediated increased alcohol excretion in the urine was investigated. Animals were fed either a chow diet or a nutritionally adequate liquid diet with or without carnitine supplementation. Animals fed the liquid diet consumed more than twice the fluid of chow fed animals (Table A-1.1) and the urine output was increased 5 to 6 fold.

The effect of a gavage of water equal to a dose of 3 g/kg of ethanol on urine output and creatinine concentration is shown in the lower halves of Tables A-1.2 and A-1.3. The water or ethanol gavage given to carnitine supplemented chow fed animals increased the urine volume in the first 4 hours after gavage resulting in a lower creatinine excretion. However, the water or ethanol gavage given to carnitine supplemented animals fed the

liquid diet resulted in a reduced urine output at all collection times up to 4 hours and increased the creatinine concentrations in the urine. In this study (Appendix), the excretion of ethanol in the urine of CS animals was non-significantly increased in the chow diets. Carnitine supplemented animals fed liquid diets showed a significant decrease in the excretion of unchanged ethanol in urine (Tables A-1.4, A-1.5) compared to NS liquid diet controls. It can therefore be recommended that further studies of the influence of carnitine on alcohol metabolism must include a careful consideration of the fluid load given with the alcohol dose.

It has been suggested in informal discussions with other researchers that the effect of carnitine on increasing urinary excretion of alcohols is a general non-specific effect due to changes in solute load or some other factor. Sachan and Berger (54) reported the results of a study with control, carnitine supplemented and choline supplemented rats. In this study they found no differences between the control and the choline supplemented animals in clearance of ethanol. Clearance of ethanol in the CS group was increased. The urinary concentrations of ethanol were elevated in the CS group and this effect was not reproducible by choline. Choline supplemented animals did not show the same effects on blood ethanol concentrations and thus the effects of carnitine were deemed to be specific.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The influence of carnitine supplementation on the disposition of selected alcohols was studied. Supplementary carnitine decreased the blood methanol concentrations and the blood ethylene glycol concentrations when followed by an ethanol treatment. The role of carnitine in the metabolism of methanol, isopropanol and ethylene glycol cannot be directly stated from this study. The urinary concentration of unchanged methanol and isopropanol was increased by the carnitine supplementation . Urine volumes of CS animals were also significantly increased with methanol and isopropanol resulting in a near doubling in the amount of alcohol excreted unchanged in the urine. This is of limited clinical significance. The fluid load given at the time of dosing appears to have greatly complicated interpretations of the effect of carnitine on the disposition of alcohols.

6.2 Conclusions

1. Carnitine supplementation resulted in significant reductions in the blood methanol concentrations and blood ethylene glycol concentrations after ethanol treatment.

2. Methanol and ethylene glycol utilize to a certain extent catalase/peroxidase pathways for primary or secondary metabolism. The extent of the involvement of catalase cannot be fully deduced at this time.

3. The action of carnitine to attenuate blood ethanol concentrations does not appear to be in limiting the action of alcohol dehydrogenase (ADH) as blood ethylene glycol concentrations were not similarly effected.

4. Carnitine does influence the disposition of alcohols because alterations in urinary volume and urinary concentrations of methanol and isopropanol were seen during the first 2 to 4 hours after dosing.

5. The effect of carnitine on the urine volume is variable with the fluid load given to the animal at the time of dosing and the state of hydration of the animal.

6.2 Recommendations for Future Research

Future studies are needed in the area of carnitine and the metabolism of alcohols and other environmental toxicants. An example of a research area is the potentiation of carbon tetrachloride poisoning by previous exposure to isopropanol or acetone. Cote et al (55) found that exposure to a 2.5 ml/kg single oral dose of isopropanol 18 hours before a dose of 0.1 ml/kg of carbon tetrachloride resulted in alterations in liver structures comparable to a 1.0 ml/kg carbon tetrachloride dose. The possibility that carnitine provides a protective effect to this damage is of great interest and brings to the forefront the role of carnitine nutriture in health and wellness.

Kamil et al (1) discussed the conjugation (esterification) of alcohols with each other during alcoholic fermentation. Carnitine may also conjugate (esterify) directly to alcohols as a metabolic or detoxication pathway. Bieber (33) mentions the presence of unusual acylcarnitines but their functions or sources are not yet known. Carnitine also functions in the metabolism of ketones and this may play a direct or indirect role in the metabolism of higher alcohols.

The role of carnitine in the induction of the peroxisomes needs more investigation. This investigation must include the measurement of the activities of hydrogen peroxide generating systems as they function together with catalase. The use of inhibitors of enzyme systems such as 3-amino-1,2,4-triazole may lead to precise definition of the site of carnitine action.

The role of carnitine in the prevention of isopropanol induced fatty liver is a high priority for future research. Since the mechanism of isopropanol induced fatty liver is different from ethanol induced fatty liver the elucidation of this difference may indicate new metabolic roles of carnitine in the body.

The effect of simultaneous administration and post-dosage administration of carnitine in alcohol intoxication needs additional studies to elucidate a possible role of carnitine as an antidote to overdoses and accidental exposure to environmental alcohols. The potential for increased excretion of unchanged alcohols is shown by the methanol study and holds certain promise of reward.

The state of hydration of the animals in future experiments involving carnitine and the urinary excretion of toxicants or endogenous substances must be carefully controlled to avoid confounding the data. The nature of the effect of carnitine on the kidney function, especially in the excretion of alcohols, is an important subject for further investigation.

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APPENDIX

Table A-1.1. Urine and Diet Parameters from Rats Fed Chow or Liquid Diets.

Parameter		Days on Diet 1	2	3	4	5
Urine Output (ml)						
Liquid	NS	57.4 ± 1.66(5)	68.4 ± 1.94(5)	...	...	...
	CS	69.6 ± 2.08(5) [.0018]	59.8 ± 3.44(5) [.061]	59.6 ± 1.88(5)	54.0 ± 2.92(5)	56.60 ± 2.64(5)
Chow	NS	13.9 ± 0.71(9)	12.6 ± 1.22(4)	12.8 ± 1.79(4)	...	...
	CS	10.8 ± 0.97(5) [.023]	11.8 ± 1.16(5)	9.1 ± 0.84(5)	8.82 ± 0.97(5) [.091]	8.24 ± 1.12(5)
Creatinine Excretion (μmole/g BW·hour)						
Liquid	NS	0.012 ± .000(5)	0.012 ± .000(5)	...	...	...
	CS	0.014 ± .000(5)	0.011 ± .001(5) [.002]	0.012 ± .000(5)	.013 ± .000(5)	0.014 ± .000(5)
Chow	NS	0.018 ± .001(9)	0.017 ± .001(4)	0.019 ± .001(4)	...	...
	CS	0.022 ± .001(5) [.038]	0.022 ± .002(5) [.105]	0.019 ± .001(5)	0.019 ± .001(5)	0.017 ± .001(5)
Body Weight (g)						
Liquid	NS	289.2 ± 5.52(5)	289.4 ± 10.31(5)	...	...	...
	CS	305.8 ± 3.24(5) [.032]	305.8 ± 3.24(5) [.032]	305.8 ± 3.24(5)	305.8 ± 3.24(5)	305.8 ± 3.24(5)
Chow	NS	303.8 ± 9.53(9)	325.0 ± 15.4(4)	315.2 ± 15.4(4)	...	...
	CS	290.0 ± 4.48(5) [.325]	290.0 ± 4.48(5) [.046]	290.0 ± 4.48(5) [.046]	290.0 ± 4.48(5)	300.0 ± 6.20(5)
Fluid Intake (ml)						
Liquid	NS	85.6 ± 0.40(5)	104.8 ± 1.65(5)	...	...	...
	CS	102.0 ± 3.00(5) [.0006]	99.8 ± 3.31(5) [.213]	96.4 ± 2.43(5)	91.8 ± 4.09(5)	94.2 ± 2.75(5)
Chow	NS	34.1 ± 1.64(9)	38.2 ± 1.79(4)	44.5 ± 2.72(4)	...	...
	CS	40.2 ± 2.18(5) [.046]	37.4 ± 1.72(5)	41.0 ± 2.59(5)	37.8 ± 1.98(5).	33.4 ± 1.54(5)
Energy Intake (kcal)						
Liquid	NS	85.60 ± 0.40(5)	104.80 ± 1.65(5)	...	...	...
	CS	102.00 ± 3.00(5) [.0006]	99.80± 3.31(5) [.213]	96.40 ± 2.43(5)	91.80 ± 4.09(5)	94.20 ± 2.75(5)
Chow	NS	76.50 ± 4.49(9)	81.60 ± 6.33(4)	88.51 ± 4.09(4)	...	...
	CS	103.70 ± 4.57(5) [.009]	101.15 ± 4.07(5) [.029]	99.45 ± 3.29(5) [.073]	93.16 ± 4.01(5)	90.78 ± 3.49(5)

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

Table A-1.2. Excretion of Carnitine in Urine from Rats Fed Chow Diets Following Gavage .

Parameter	Baseline	Collection Times (Hours Post Gavage) 0-2	2-4	4-6	6-24
Carnitine Fraction (nmoles/ml)					
Non-Esterified					
Water Gavage					
NS	412.16 ± 76.21(4)	...	76.57 ± 24.64(4)*	...	624.20 ± 86.04(4)
CS	18766.00 ± 2293.0(5) [.0002]	...	1879.68 ± 317.8(5)* [.002]	...	10212.70 ± 867.6(5) [.002]
Ethanol Gavage					
NS	...	58.93 ± 15.71(3)	79.69 ± 30.91(3)	...	547.96 ± 97.07(3)
CS	...	2892.20 ± 573.6(5) [.011]	1432.44 ± 331.2(5) [.02]	...	10253.20 ± 779.7(5) [.0001]
Acid Soluble Acyl					
Water Gavage					
NS	18.76 ± 4.08(4)	...	29.39 ± 16.57(4)*	...	123.15 ± 24.71(4)
CS	405.72 ± 120.2(2) [.0064]	...	632.50 ± 213.1(3)* [.020]	...	414.00 ± 9.00(2) [.012]
Ethanol Gavage					
NS	...	9.03 ± 3.24(2)	8.78 ± 2.96(3)	...	46.69 ± 29.60(3)
CS	...	295.98 ± 44.24(5) [.010]	431.14 ± 90.01(5) [.012]	...	2921.50 ± 908.21(5) [.055]
Acid Insoluble Acyl					
Water Gavage					
NS	3.52 ± 0.70(4)	...	0.86 ± 0.13(4)*	...	1.58 ± 0.80(3)
CS	94.41 ± 14.60(4) [.0009]	...	7.83 ± 1.87(5)* [.014]	...	46.71 ± 11.38(5) [.025]
Ethanol Gavage					
NS	...	1.97 ± 0.01(3)	...	...	1.52 ± 0.47(3)
CS	...	8.07 ± 1.70(5) [.030]	3.69 ± 1.81(5)	...	22.59 ± 1.41(5) [.0001]

Table A-1.2. Excretion of Carnitine in Urine from Rats Fed Chow Diets Following Gavage (continued).

Parameter	Collection Times (Hours Post Gavage)				
	Baseline	0-2	2-4	4-6	6-24
Carnitine Fraction (nmoles/ml)					
Total Carnitine					
Water Gavage					
NS	434.42 ± 73.00(4	...	106.87 ± 15.53(4)*	...	748.53 ± 107.25(4)
CS	19022.20 ± 2368.5(5) [.0002]	...	2267.01 ± 283.6(5)* [.0003]	...	10359.46 ± 1888.2(5) [.003]
Ethanol Gavage					
NS	...	66.93 ± 19.90(3)	88.47 ± 30.20(5)	...	1.52 ± 125.58(3)
CS	...	3077.46 ± 562.2(5) [.007]	1867.27 ± 420.0(5) [.019]	...	13196.40 ± 1601.5(5) [.001]
Urine Output (ml)					
Water Gavage					
NS	12.75 ± 1.79(4)	...	5.20 ± 0.68(4)*	1.38 ± 0.49(4)	7.70 ± 1.89(4)
CS	8.24 ± 1.12(5) [.061]	...	6.36 ± 0.59(5)* [.223]	1.16 ± 0.39(5)	10.90 ± 0.89(5) [.145]
Ethanol Gavage					
NS	...	6.20 ± 0.20(3)	1.66 ± 0.48(3)	0.00 ± 0.00(3)	7.33 ± 1.28(3)
CS	...	6.80 ± 0.52(5)	2.56 ± 0.53(5) [.298]	0.52 ± 0.32(5) [.268]	9.28 ± 0.66(5) [.198]
Creatinine Excretion (umole/ml)					
Water Gavage					
NS	11.78 ± 1.61(4)	...	3.99 ± 0.60(4)*	6.41 ± 1.51(4)	13.55 ± 1.89(4)
CS	15.20 ± 1.29(5)	...	2.32 ± 0.25(5)* [.026]	4.92 ± 0.27(4) [.370]	9.53 ± 0.95(5) [.080]
Ethanol Gavage					
NS	...	2.98 ± 0.37(3)	4.91 ± 0.94(3)	...	14.44 ± 1.71(3)
CS	...	1.46 ± 0.28(5) [.017]	1.82 ± 0.33(5) [.013]	5.82 ± 1.37(2)	9.56 ± 0.49(5) [.013]

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups. Asterisk indicates combined 2 and 4 hour sample. Baseline is a 24 hour collection made prior to any gavage tests.

Table A-1.3. Excretion of Carnitine in Urine from Rats Fed Liquid Diets Following Gavage.

Parameter	Collection Times (Hours Post Gavage)				
	Baseline	0-2	2-4	4-6	6-24
Carnitine Fraction (nmoles/ml)					
Non-Esterified					
Water Gavage					
NS	13.39 ± 4.28(5)	3.04 ± 0.61(5)	6.97 ± 1.64(5)	...	13.53 ± 1.80(5)
CS	1951.26 ± 385.4(5)	3389.80 ± 561.7(5)	3149.45 ± 1985.1(2)	...	1084.74 ± 110.4(5)
	[.001]		[.030]		[.00]
Ethanol Gavage					
NS	...	2.58 ± 0.54(5)	2.00 ± 0.46(5)	...	2.89 ± 0.36(5)
CS	...	2632.60 ± 236.3(5)	1883.80 ± 339.7(4)	...	2433.12 ± 573.3(5)
	[.0001]	[.0004]	[.007]		[]
Acid Soluble Acyl					
Water Gavage					
NS	1.62 ± 0.32(5)	2.40 ± 0.35(5)	9.00 ± 1.42(5)	...	1.67 ± 0.48(5)
CS	64.87 ± 23.15(3)	207.08 ± 80.1(5)	743.75 ± 429.9(2)	...	20.80 ± 0.00(1)
	[.009]	[.022]	[.0]		[.001]
Ethanol Gavage					
NS	...	0.64 ± 0.41(4)	2.38 ± 0.40(5)	...	3.71 ± 0.78(5)
CS	...	204.90 ± 53.94(3)	128.20 ± 0.00(1)	...	154.92 ± 57.0(5)
		[.006]	[.001]		[.029]
Acid Insoluble Acyl					
Water Gavage					
NS	1.62 ± 0.22(5)	1.41 ± 0.18(5)	...	...	...
CS	3.89 ± 1.39(5)	4.88 ± 1.29(5)	6.75 ± 4.36(2)	...	14.30 ± 0.30(5)
	[.159]	[.029]			
Ethanol Gavage					
NS	...	1.64 ± 0.15(5)	...	...	...
CS	...	6.97 ± 1.40(5)	2.33 ± 0.48(4)	...	8.06 ± 2.12(5)
		[.006]			[.0001]

Table A-1.3. Excretion of Carnitine in Urine from Rats Fed Liquid Diets Following Gavage (continued).

Parameter	Collection Times (Hours Post Gavage)				
	Baseline	0-2	2-4	4-6	6-24
Carnitine Fraction (nmoles/ml)					
Total Carnitine					
Water Gavage					
NS	16.30 ± 4.27(5)	6.84 ± 0.45(5)	15.97 ± 2.95(5)	...	15.20 ± 1.83(5)
CS	2120.02 ± 317.9(5)	3560.48 ± 629.6(5) [.0005]	3899.95 ± 1559.6(2) [.0053]	...	1103.20 ± 115.9(5) [.007]
Ethanol Gavage					
NS	...	4.73 ± 0.52(5)	4.38 ± 0.69(5)	...	6.02 ± 0.61(3)
CS	...	2762.44 ± 240.2(5) [.0001]	1918.18 ± 357.2(4) [.0]	...	2596.00 ± 625.3(5) [.003]
Urine Output (ml)					
Water Gavage					
NS	68.40 ± 1.94(5)	8.00 ± 0.44(5)	3.36 ± 0.57(5)	1.64 ± 0.71(5)	58.80 ± 2.27(5)
CS	53.80 ± 2.71(5) [.002]	7.96 ± 0.54(5)	1.16 ± 0.79(5) [.050]	1.56 ± 0.24(5)	46.20 ± 2.27(5) [.0044]
Ethanol Gavage					
NS	65.00 ± 2.45(5)	8.16 ± 0.80(5)	4.08 ± 0.52(5)	1.52 ± 0.44(5)	45.60 ± 3.37(5)
CS	51.40 ± 3.41(5) [.012]	7.24 ± 0.33(5)	2.36 ± 0.76(5)	2.80 ± 0.42(5)	38.80 ± 7.01(5)
Creatinine Excretion (µmole/ml)					
Water Gavage					
NS	1.22 ± 0.04(5)	0.79 ± 0.06(5)	2.17 ± 0.42(5)	2.16 ± 0.52(3)	1.04 ± 0.03(5)
CS	1.75 ± 0.14(5)	1.21 ± 0.18(5) [.052]	2.04 ± 0.94(2)	4.58 ± 0.47(5)	1.59 ± 0.10(5) [.0008]
Ethanol Gavage					
NS	1.38 ± 0.02(5)	0.81 ± 0.03(5)	1.26 ± 0.06(5)	3.77 ± 0.55(4)	1.21 ± 0.09(5)
CS	1.87 ± 0.20(5) [.044]	1.26 ± 0.11(5) [.005]	1.64 ± 0.21(4) [.098]	2.56 ± 0.26(5) [.069]	2.14 ± 0.39(5) [.052]

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups. Baseline is a 24 hour collection made prior to any tests or gavages.

Table A-1.4. Excretion of Unchanged Ethanol in Urine from Rats Fed Chow Diets.

Parameter	Collection Times (Hours Post Ethanol Administration) 0-2	2-4	4-6	6-24	24 hour total
Concentration					
NS (mg/ml)	1.82 ± 0.24(5)	1.93 ± 0.07(3)	...	0.13 ± 0.068(3)	1.04 ± 0.06(3)
CS	2.27 ± 0.24(5)	2.21 ± 0.24(5)	2.13 ± 0.42(2)	0.15 ± 0.068(5)	1.24 ± 0.17(5)
NS(µmole/ml)	40.44 ± 5.33(5)	42.96 ± 1.48(3)	...	2.96 ± 1.52(3)	23.16 ± 1.35(3)
CS	50.48 ± 5.33(5) [.261]	49.07 ± 5.25(5)	47.44 ± 9.44(2)	3.38 ± 1.51(5)	27.59 ± 3.85(5)
Urine Output (ml)					
NS	6.20 ± 0.20(3)	1.66 ± 0.48(3)	0.00 ± 0.00(3)	7.33 ± 1.28(3)	15.20 ± 1.38(3)
CS	6.80 ± 0.52(5) [.0]	2.56 ± 0.53(5)	0.52 ± 0.32(5)	9.28 ± 0.66(5)	19.08 ± 0.71(5) [.031]
Total Ethanol Excretion					
NS(mg)	11.27 ± 1.42(3)	3.26 ± 1.01(3)	...	1.09 ± 0.67(3)	15.67 ± 0.55(3)
CS	15.64 ± 2.27(5)	5.81 ± 1.43(5)	2.82 ± 0.77(2)	1.26 ± 0.45(5)	23.86 ± 3.74(5)
NS(µmole)	250.40 ± 31.65(3)	72.59 ± 22.41(3)	...	24.38 ± 14.92(3)	348.30 ± 12.21(3)
CS	347.60 ± 50.47(5) [.220]	129.08 ± 31.84(5) [.261]	62.62 ± 17.02(2)	27.91 ± 10.02(5)	530.28 ± 83.07(5) [.152]
Percent of Dose Excreted					
NS	1.22 ± 0.17(3)	0.35 ± 0.09(3)	...	0.11 ± 0.07(3)	1.68 ± 0.01(3)
CS	1.75 ± 0.26(5) [.207]	0.66 ± 0.18(5) [.255]	0.13 ± 0.08(2) [.297]	0.15 ± 0.06(5)	2.68 ± 0.45(5) [.150]
Hourly Ethanol Excretion					
NS(mg/g BW·hour)	0.018 ± .002(3)	0.005 ± .001(3)	...	0.004 ± .002(3)	0.002 ± .0001(3)
CS	0.026 ± .004(5)	0.009 ± .002(5)	0.005 ± .001(2)	0.005 ± .002(5)	0.003 ± .0005(5)
NS(µmole/g BW·hour)	0.405 ± 0.055(3)	0.232 ± 0.067(3)	...	0.0002 ± .0001(3)	0.047 ± .003(3)
CS	0.579 ± 0.088(5) [.210]	0.435 ± 0.114(5) [.251]	0.105 ± 0.031(2)	0.0002 ± .0001(5)	0.074 ± .013(5) [.151]

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

Table A-1.5. Excretion of Unchanged Ethanol in Urine from Rats Fed Liquid Diets.

Parameter	Collection Times (Hours Post Ethanol Administration) 0-2	2-4	4-6	6-24	24 hour total
Concentration					
NS (mg/ml)	1.73 ± 0.13(5)	1.93 ± 0.10(5)	1.57 ± 0.10(4)	0.031 ± 0.019(5)	0.44 ± 0.03(5)
CS	1.34 ± 0.12(5)	1.39 ± 0.03(4)	1.04 ± 0.05(5)	0.030 ± 0.004(5)	0.37 ± 0.06(5)
NS(µmole/ml)	38.44 ± 2.71(5)	42.84 ± 2.33(5)	35.00 ± 2.20(4)	0.76 ± 0.21(5)	9.86 ± 0.76(5)
CS	29.78 ± 2.47(5) [.045]	31.05 ± 0.72(4) [.003]	23.07 ± 2.20(5)	0.67 ± 0.10(5)	8.23 ± 1.40(5)
Urine Output (ml)					
NS	8.16 ± 0.80(5)	4.08 ± 0.52(5)	1.52 ± 0.44(5)	45.60 ± 3.37(5)	59.36 ± 3.22(5)
CS	7.24 ± 0.33(5) [.0]	2.36 ± 0.76(4)	2.80 ± 0.42(5)	38.80 ± 7.01(5)	51.20 ± 7.53(5)
Total Ethanol Excretion					
NS(mg)	14.18 ± 1.84(5)	7.80 ± 1.03(5)	3.06 ± 0.64(4)	1.63 ± 0.53(5)	25.98 ± 1.24(5)
CS	9.70 ± 0.97(5)	4.07 ± 0.84(4)	2.90 ± 0.44(5)	1.07 ± 0.16(5)	17.15 ± 0.59(5)
NS(µmole)	315.26 ± 40.84(5)	173.35 ± 22.82(5)	68.04 ± 14.24(4)	36.22 ± 11.89(5)	577.42 ± 27.64(5)
CS	215.52 ± 21.63(5)	90.53 ± 18.79(4)	64.47 ± 9.75(5)	23.69 ± 3.66(5)	381.03 ± 13.24(5)
Percent of Dose Excreted					
NS	1.64 ± 0.20(5)	0.91 ± 0.13(5)	0.29 ± 0.10(5)	0.18 ± 0.06(5)	3.02 ± 0.16(5)
CS	0.95 ± 0.09(5) [.01]	0.32 ± 0.10(5) [.007]	0.28 ± 0.04(5)	0.12 ± 0.03(5)	1.67 ± 0.04(5) [.0001]
Hourly Ethanol Excretion					
NS(mg/g BW·hour)	0.023 ± .003(5)	0.013 ± .001(5)	0.005 ± .001(4)	0.0003 ± .0001(5)	0.004 ± .0002(5)
CS	0.014 ± .001(5)	0.006 ± .001(4)	0.004 ± .001(5)	0.0001 ± .0000(5)	0.002 ± .0001(5)
NS(µmole/g BW·hour)	0.52 ± 0.06(5)	0.29 ± 0.04(5)	0.23 ± 0.05(4)	0.006 ± .002(5)	0.084 ± .004(5)
CS	0.31 ± 0.03(5) [.018]	0.13 ± 0.03(4) [.018]	0.19 ± 0.03(5)	0.004 ± .001(5) [.253]	0.047 ± .001(5) [.001]

Values are means ± SEM. Levels of significance for differences compared by t test at P>|t| are shown in brackets. Values in parentheses are numbers of animals in groups.

VITA

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