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Carnitine Mediated Alterations of Ethanol and Fatty Acid Metabolism

Randall L. Mynatt
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To the Graduate Council:

I am submitting herewith a dissertation written by Randall L. Mynatt entitled "Carnitine Mediated Alterations of Ethanol and Fatty Acid Metabolism." I have examined the final electronic 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.

Dileep S. Sachan, Major Professor

We have read this dissertation and recommend its acceptance:

Roy E. Beauchene, Richard N. Heitman, John W. Koontz

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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CARNITINE MEDIATED ALTERATIONS OF ETHANOL AND FATTY ACID METABOLISM

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Randall L. Mynatt

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ABSTRACT

The purpose of this dissertation was to examine the effect of dietary carnitine supplementation on ethanol and fatty acid metabolism. Rats were fed purina chow as such (non-supplemented, NS) or supplemented with 0.5% L-carnitine (carnitine supplemented, CS). Carnitine supplementation for 7d resulted in decreased oxidation of an oral dose of [1-^{14}C]-ethanol. Expired $^{14}CO_2$ was significantly reduced at hours 4-12 in the CS group. There were no significant differences in urinary excretion of the ^{14}C-label between the two groups which accounted for only about 3% of the total dose. The ^{14}C-label remaining in tissues after 12h was also not significantly affected by carnitine supplementation. Blood-ethanol concentrations (BEC) were 88.5 and 125.9 mg/dl in the NS and CS groups, respectively, 3h post-ethanol administration (PEA). Liver-ethanol concentrations (LEC) were 1.70 and 0.85 µg/mg in the NS and CS groups, respectively 3h PEA. Examination of liver concentrations of pyruvate, lactate, acetoacetate, and 3-hydroxybutyrate revealed no significant differences between the NS and CS groups. Plasma concentrations of lactate were 2-fold higher in the CS group, but pyruvate, acetoacetate and 3-hydroxybutyrate were not significantly different between the two groups. Plasma and liver non-esterified carnitine (NEC), acid soluble acylcarnitine (ASAC) and acid insoluble acylcarnitine (AIAC) concentrations were all significantly higher in the CS group 3h after ethanol administration. The effect of carnitine supplementation on the urinary excretion of ethanol, its metabolites and carnitine was determined by feeding rats the NS and CS diets for 7d. The rats were then given an oral dose of [1-^{14}C]-ethanol and urine was collected for the next twenty

four hours. Food intake, water intake and urine volume were not significantly different between the NS & CS groups over the 24h period. Urinary excretion of ethanol and the ^{14}C-label were not significantly affected by carnitine supplementation. Urinary excretion non-esterified carnitine (NEC), acid-soluble acyl-carnitine (ASAC) and acid-insoluble acyl-carnitine (AIAC) were significantly elevated in the CS group over the 24h period. Supplementary carnitine did not significantly affect the oxidation of [$^{14}C(U)$]-palmitate. There were no significant differences between the NS and CS groups with regard to the rates of $^{14}CO_2$ appearance or percent of the dose following 5, 10, 20, 30 & 40 days of feeding CS diet. Ethanol administration resulted in decreased palmitate oxidation, at hours 4-24, but dietary carnitine supplementation did not affect the decreased oxidation due to ethanol.

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CHAPTER I

INTRODUCTION

One of the most prominent metabolic effects of ethanol consumption is the disturbance in lipid metabolism leading to hepatic steatosis. As with most clinical disorders the actual mechanism(s) leading to a fatty liver are not well understood and are probably the result of a combination of factors. Several potential mechanisms responsible for the accumulation of lipids in the ethanol-compromised liver have been discussed by Baraona and Lieber (1); for example, increased supply of lipids from the small intestine, increased uptake of fatty acids by the liver, increased mobilization of fatty acids from adipose tissue, increased synthesis of fatty acids in the liver, increased esterification of fatty acids in the liver, decreased oxidation of fatty acids in the liver, decreased hydrolysis of fatty acid esters in the liver, decreased excretion of liver fat into the bile, and decreased release of serum lipoproteins. It is obvious there is no clear solution to the steatogenic effect of ethanol on the liver, but the most probable explanation is that ethanol inhibits fatty acid oxidation and promotes fatty acid synthesis and elongation resulting in an increased supply of long-chain fatty acids available for esterification to glycerol.

Ethanol oxidation occurs primarily in the liver (2-4). It is first oxidized to acetaldehyde by one of three enzyme systems; alcohol dehydrogenase (ADH), catalase or the microsomal ethanol oxidizing system (MEOS) (5-7). ADH is located in the cytosol and catalyzes the major pathway of ethanol oxidation. However, MEOS is

known to contribute significantly to ethanol oxidation following chronic or high doses of ethanol (8). Acetaldehyde is subsequently oxidized to acetate in the mitochondrial matrix by aldehyde dehydrogenase

(5, 7, 9). Both dehydrogenase-catalyzed reactions are coupled to the reduction of NAD^+ to NADH. This results in an increase in the NADH/NAD^+ ratio in both the cytosol and the mitochondrial matrix of the hepatocyte (10, 11). The excess in reducing equivalents will result in inhibition of glycolysis, the TCA cycle and fatty acid oxidation and promote fatty acid synthesis and esterification (individual reactions will be discussed in the review of literature). The acetate generated from ethanol oxidation has the potential to be incorporated into fatty acids, which would further promote the lipogenic effect of ethanol. Thus ethanol oxidation has a possible three-fold effect on lipid metabolism in the liver by (1) decreasing fatty acid oxidation, (2) enhancing the synthesis of fatty acids and (3) increasing fatty acid incorporation into triglycerides.

Alcoholic fatty liver and hyperlipidemia are often associated with more serious diseases such as hepatitis, liver cirrhosis, and atherosclerosis (1). This has prompted attempts to ameliorate ethanol-induced hepatic steatosis. Supplementation of a liquid ethanol diet with D,L-carnitine results in decreased concentrations of lipids, especially triglycerides, in the liver (12, 13). There are several possible mechanisms by which carnitine may have a lipid lowering effect in the liver. All of these mechanisms involve the increased availability of carnitine, due to supplementation, to serve as a substrate for carnitine acyl-transferase. The possible mechanisms are:

1. Increased levels of free carnitine could accommodate the influx of fatty acids by serving as a co-substrate for carnitine acyl-transferase. This would result in higher concentrations of acyl-carnitines which may enhance the activity of carnitine acylcarnitine translocase and facilitate the transport of acyl-carnitine into the mitochondrial matrix. The higher concentrations of fatty acids inside the matrix may then promote β-oxidation.
2. The carnitine-induced increase in carnitine acyl-transferase activity would compete with glycerol-phosphate acyl-transferase for acyl-CoA's. This could result in decreased formation of phosphatidate, a substrate for synthesis of triglycerides and phosphoglycerides. Carnitine acyl-transferase would also compete for acyl-CoA's needed to synthesize triglycerides from diglycerides. The net effect would be a decrease in triglyceride synthesis resulting from the esterification of acyl groups to carnitine rather than to the glycerol.
3. There may also be increased activity of carnitine acetyl-transferase which would compete with acetyl-CoA carboxylase for acetyl CoA units. This may reduce the amount of malonyl CoA available for fatty acid synthesis.
4. In each of the above situations there is increased formation of acyl-carnitine. The acyl-carnitines can enter the blood stream and be distributed in the extra-hepatic organs or excreted in the urine. Thus, removal of the fatty acids as acyl-carnitines from the liver pool should result in less of a burden on the liver due to alcohol.

Another related effect of dietary carnitine supplementation is the elevated blood ethanol concentrations following both acute and chronic doses of ethanol (14, 15). It is generally agreed that the redox state of the hepatocyte is rate controlling for ethanol oxidation (2-4). Excess cytosolic NADH generated via ADH will inhibit the dissociation of the ADH-NADH complex, which is the rate limiting step in ethanol oxidation (4-6). If carnitine does increased β-oxidation, it would provide reducing equivalents and produce acetyl-CoA. Both of which would have the potential to inhibit ethanol oxidation and result in higher blood ethanol concentrations.

The objective of this dissertation was to examine the possible mechanisms responsible for the lipotropic effect of carnitine on ethanol-induced fatty liver, its effect on ethanol oxidation and the possible relationship of the two effects. The mechanisms were investigated by the following courses of action.

1. The effect of dietary carnitine on ethanol oxidation was determined by measuring the oxidation of [1-^{14}C]-ethanol to $^{14}CO_2$ along with the effect of carnitine on the redox state which was determined by measuring lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios in the liver and blood.
2. The effect of dietary carnitine supplementation on β-oxidation was determined by measuring the oxidation of [^{14}C(U)]-palmitate to $^{14}CO_2$ with and without ethanol administration.
3. The effect of dietary carnitine on urinary excretion of ethanol, its metabolites, and acyl-carnitines was determined by measuring the concentration of ethanol, its

metabolites and acyl-carnitines in the urine following [1-^{14}C]-ethanol administration.

CHAPTER II

REVIEW OF LITERATURE

ETHANOL METABOLISM

Ethanol is readily absorbed from the gastro-intestinal tract (3). Gastric absorption accounts for about 20% of ethanol absorption with the remainder being absorbed by the small intestine (16). After absorption ethanol is evenly distributed in body water (16) and accumulates in tissues with the highest water content (17). There is a general consensus that the liver is responsible for the majority of ethanol metabolism. Hepatic oxidation accounts for around 75% of the metabolism of ethanol as demonstrated by liver perfusion (18, 19), hepatic vein catherization (20), ethanol infusions (21), partial (22) and complete hepatectomy (23), and evisceration (23). The remainder of ethanol is metabolized extra hepatically or eliminated through the kidneys and lungs (2-10%) (3).

Ethanol is first oxidized to acetaldehyde via either alcohol dehydrogenase (ADH, reaction a), the microsomal ethanol oxidizing (MEOS, reaction b) or catalase (reaction c).

(a) $CH_3CH_2OH + NAD^+ \xrightarrow{ADH} CH_3CHO + NADH + H^+$

(b) $CH_3CH_2OH + NADPH + H^+ + O_2 \xrightarrow{MEOS} CH_3CHO + NADP^+$

(c) $CH_3CH_2OH + H_2O_2 \xrightarrow{CATALASE} CH_3CHO + 2H_2O$

ADH catalyzes the oxidation of ethanol to acetaldehyde coupled to the reduction of NAD^+ to NADH. ADH occurs at highest concentrations (> 90% of total ADH

activity) in the liver where it is found in the cytosol (2-7). Mammalian alcohol dehydrogenases are polymorphic and the isozymes can be classified based on their function and physicochemical properties (24-26). The enzyme responsible for ethanol oxidation in rat liver is isozyme ADH III. It has a Km of 1.4 mM for ethanol and Ki of 10.4 mM for pyrazole (24). All evidence indicates that ADH is the principle enzyme responsible for the oxidation of ethanol (2-5). Studies using the ADH inhibitors pyrazole and 4-methylpyrazole, result in a 85-90% inhibition of ethanol metabolism in vivo (27). The rate limiting step, in vitro, in the ADH catalyzed reaction is the dissociation of the ADH-NADH complex (4, 10). This most likely is responsible for the rate limiting step in ethanol oxidation, in vivo, which is the re-oxidation of NADH (which will be discussed later) (10).

The observance that chronic alcohol administration results in the proliferation of hepatic smooth endoplasmic reticulum led to the speculation that it may be involved in ethanol oxidation (28). It was later demonstrated than an ethanol-oxidizing system could be reconstituted with cytochrome P-450, NADPH-Cytochrome C-reductase, and phospholipids (29). Most recently, a 52,000 dalton polypeptide, termed P450IIEI (also P-450), has been purified by immunoelectrophoresis and shown to be inducible by ethanol. Like other cytochrome P-450 - dependent monooxygenases, MEOS requires oxygen and NADPH. The number one carbon of ethanol is hydroxylated in a cytochrome P-450 dependent manner with the concomitant oxidation of NADPH. The doubly hydroxylated carbon is unstable and water splits-out leaving acetaldehyde. Historically, the role of MEOS in ethanol metabolism has been controversial. The

contribution of MEOS to total ethanol oxidation increases with chronic abuse and high doses of ethanol (8, 31). Since MEOS utilizes NADPH and the NADP/NADPH and NAD/NADH systems are linked, ethanol oxidation via MEOS could result not only in accelerated ethanol metabolism because of MEOS activity, but it may also accelerate the ADH pathway by favoring the re-oxidation of NADH (11). The theory for the increased MEOS activity at higher ethanol concentrations is that the Km for ethanol by ADH is around 2 mM and the Km for ethanol by the MEOS is around 10 mM. So at low concentrations of ethanol (10 mM) ADH is already saturated and at higher ethanol concentrations the MEOS has the ability to oxidize more ethanol.

Catalase is a peroxisomal enzyme that can catalyze the peroxidative cleavage of ethanol to acetaldehyde in the presence of a H_2O_2 generating system. It is generally accepted that the H_2O_2 - mediated ethanol peroxidation by catalase is limited by the rate of H_2O_2 generation. Since the physiological rate of H_2O_2 production is rather low, the contribution of catalase to in vivo ethanol elimination is not quantitatively significant (2, 3, 26).

Regardless of the pathway of the oxidation of ethanol to acetaldehyde the next step in the complete oxidation of ethanol is the oxidation of acetaldehyde to acetate catalyzed by aldehyde dehydrogenase (ALDH). The overall reaction is:

$$CH_3CH_2O + NAD^+ + H_2O \xrightarrow{ALDH} CH_3CHOOH + NADH + H^+$$

The reaction is essentially non-reversible with a ΔG = -13 kcal/mole and is responsible for the rapid removal of acetaldehyde (4). Rat liver contains several ALDH isozymes (9). The isozyme responsible for acetaldehyde oxidation is localized

in the mitochondrial matrix as a soluble protein and has a Km of 0.7 mM for acetaldehyde (9).

Most reviews state that ethanol and acetaldehyde are metabolized primarily in the liver and acetate is disposed of in the extrahepatic tissues (1-4). This could be misleading and downplay the role of the liver in acetate metabolism. Studies using liver perfusion, liver slices and hepatocytes find that ^{14}C-ethanol is partially recovered as $^{14}CO_2$ and TCA cycle intermediates (32-39), indicating further oxidation of acetate in the liver. The incorporation of ^{14}C-ethanol into acetoacetate, β-hydroxybutyrate, triglycerides and cholesterol indicates that the liver is involved in the utilization of acetate for the synthesis of these compounds (35-38). Also, in vivo, the acetate released into the blood no doubt is re-circulated through the liver and would be available for re-entry into the hepatocyte.

When ethanol is oxidized to acetaldehyde in the cytoplasm via ADH, there is a stoichiometric reduction of NAD^+. The mitochondrial membrane is impermeable to NADH. So reducing equivalents must be transferred into the mitochondria via the α-glycerophosphate or malate shuttle (4). NAD^+ may also be regenerated in the cytosol by coupling NADH oxidation to reduction reactions (4). The oxidation of ethanol exceeds the hepatocyte's ability to utilize the reducing equivalents resulting in an increased NADH\NAD$^+$ ratio (10, 11). It is this decreased ratio which is responsible for many of the deleterious effects of ethanol.

DEVELOPMENT OF FATTY LIVER

The accumulation of lipids in the ethanol compromised liver can originate from three main sources: dietary lipids, adipose tissue lipids and endogenous synthesis by the liver. The source of the lipids varies with the type of ethanol administration. The administration of a single large dose of ethanol leads to hepatic accumulation of fatty acids resembling those of adipose tissue. In contrast, prolonged ethanol ingestion produces fatty livers consisting of endogenously synthesized fatty acids and dietary fatty acids (40, 43). The proposed theory for the difference is that the large acute dose represents a stressful situation to the rat causing the release of catecholamines. So through β-adrenergic stimulation, lipase is activated resulting in the hydrolysis of triglycerides and the release of fatty acids from adipose tissue (1). Chronic administration, as the liquid ethanol diet, represents a less stressful situation and fatty acid turnover is more representative of "normal" metabolism. It has also been demonstrated that the degree of triglyceride accumulation in the liver varies with increasing amounts of fat in the diet and with increasing chain length of fatty acids in the diet (44, 45).

Although there are several potential mechanisms for the accumulation of fat in the ethanol-compromised liver the most likely candidates are decreased β-oxidation, increased synthesis of triglycerides and increased synthesis and elongation of fatty acids. All three can be at least partially explained by the change in the redox state and excess acetate formed during ethanol oxidation.

There is considerable evidence that ethanol does inhibit the oxidation of fatty acids. In humans, the formation of $^{14}CO_2$ from ^{14}C-oleic acid was reduced and the effect was partially abolished by 4-methylpyrazole (46). The addition of ethanol to human and rat liver slices reduces $^{14}CO_2$ from ^{14}C-labeled oleic acid, palmitate and linoleic acid and the addition of 4-methylpyrazole inhibited the effect (47). The above results do not prove that β-oxidation is inhibited since the inhibition of ^{14}C-fatty acid to $^{14}CO_2$ could result from TCA cycle inhibition. However, ethanol was later shown to decrease formation of ketone bodies (48), acetyl-CoA (49) and CO_2 (48, 49) from labeled fatty acids, thus confirming the decrease in β-oxidation due to ethanol.

The common feature to all the above experiments is that the effects of ethanol on fatty acid oxidation were decreased considerably when ethanol metabolism via ADH was inhibited by 4-methylpyrazole. In fact, pyrazole titration experiments demonstrate a correlation between changes in the mitochondrial NADH/NAD ratio and both inhibition of the TCA cycle and inhibition of the β-oxidation pathway (49). The TCA cycle is sensitive to inhibition by NADH at isocitrate dehydrogenase and at ketoglutarate dehydrogenase steps (49). β-oxidation would be restricted at β-hydroxyacyl-COA dehydrogenase and acyl-CoA dehydrogenase (1). Ethanol also influences the activity of carnitine palmitoyltransferase I (CPT-I). In hepatocytes isolated from rats fed a liquid ethanol diet CPT-I activity was markedly decreased and its sensitivity to inhibition by exogenously added malonyl-CoA was increased (50). Pre-incubation of hepatocytes from rats fed control diets with ethanol or acetaldehyde also decreased CPT-I activity and increased sensitivity to malonyl-CoA (50, 51).

CPT-I is generally considered to catalyze the rate limiting step in the transport of long chain fatty acids into the mitochondrial matrix (50).

The effect of ethanol on fatty acid synthesis remains unsettled. The incubation of hepatocytes, isolated from rats given an acute dose of ethanol, with either ^{14}C-ethanol or ^{14}C-acetate resulted in increased incorporation of the label into fatty acids (52). Perfusion of isolated rat liver demonstrated that ethanol directly enhanced the synthesis of fatty acid from labeled acetate (53). However, rats fed the liquid ethanol diet failed to show an increase in the incorporation of $^{3}H_2O$ into fatty acids but did show an increase in triglycerides in the liver (55-57). Although it seems probable that the liver would increase fatty acid synthesis in order to cope with the excess NADH and acetate; conflicting data do not entirely support the theory.

The increase in lipid content in the ethanol-induced liver is mostly due to the increase in triglyceride content. Ethanol administration results in enhanced incorporation of ^{14}C-palmitate into triglycerides in hepatocytes (47, 48). Rats fed a liquid ethanol diet incorporate more ^{14}C-glycerol into triglycerides than control animals (54). Isolated perfused livers incorporate more ^{14}C-acetate into triglycerides in the presence of ethanol (53). There is a rapid increase of the α-glycerophosphate content of the liver after ethanol administration (58). The proposed mechanism is the increased concentration of NADH will increase the production of α-glycerophosphate from dihydroxyacetone phosphate (58). Thus with increased levels of fatty acids (from decreased oxidation and possibly increased synthesis) and higher concentrations of α-glycerophosphate, conditions are favorable for triglyceride synthesis.

CARNITINE AND ETHANOL METABOLISM

Carnitine serves as an essential carrier for long chain acyl groups across the mitochondrial membrane to sites of oxidation. It was this role of carnitine that first prompted investigators to examine the possibility that carnitine might help prevent the alcohol-induced fatty liver. The addition of carnitine to the ethanol solution in rats orally receiving a dose of 6g ethanol/kg b.w. resulted in significantly lower concentrations of triglycerides in the liver and blood 24h after ethanol administration (59). The addition of carnitine to a diet (containing 36% calories as ethanol) fed to rats for 56d resulted in significantly lowered levels of total lipids, free and esterified cholesterol, triglycerides and phospholipids in the liver (12). The effect was later found to be dose dependent up to 0.8% DL-carnitine (13). Dietary carnitine supplementation of the liquid ethanol diet results in elevated blood-ethanol concentrations (BEC) (14). Carnitine supplementation has also resulted in elevated BEC under acute ethanol administration (14, 15). Under both acute and chronic conditions carnitine administration has resulted in both decreased lipids in the liver and higher BEC. One possible explanation for the observations is that carnitine is causing increased β-oxidation of fatty acids which results in lower lipid levels. The increase in β-oxidation will result in an increased intramitochondrial redox potential ($E = Eo-2.3RT/nF \log [RED]/[OX]$) (61) which would inhibit regeneration of NAD^+ from NADH and elevate BEC. Another possible mechanism for the lipid lowering effect of carnitine is that carnitine traps excess acetyl groups derived both from free

acetate, formed by ethanol oxidation, and from acetyl-CoA, accumulated as a result of the ethanol-induced decrease in the TCA cycle (61).

CHAPTER III

MATERIALS AND METHODS

ANIMALS, HOUSING AND DIETS

Animals used in these studies were mature male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN). They were kept in an ALAS accredited facility and individually housed in 7" X 10" X 7" wire mesh, stainless steel cages and kept in ventilated cubicles with glass sealing doors. The temperature in each cubicle was maintained at 72 $\pm$ 2°C, relative humidity between 40-50% with a cycle of 12 hours light and 12 hours dark. The rats were fed ground Purina Rat Cow #5001 (Ralston Purina, St. Louis, MO). The diets were fed as such (non-supplemented, NS) or supplemented with 0.5% (w/w) L-Carnitine (carnitine supplemented, CS). Carnitine was supplemented as a hydro-chloride salt (Sigma, St. Louis, MO). All diets and water were supplied ad libitum.

EXPERIMENTAL PROCEDURES

Experiment 1 [1-^{14}C]-Ethanol Oxidation

The objective of this experiment was to determine the effect of dietary carnitine supplementation on [1-^{14}C]- ethanol oxidation, distribution and urinary excretion. Ten rats (300-350g) were divided into 2 groups of 5 and fed Purina rat chow as such (non-supplemented, NS) or supplemented with 0.5% (w/w) L-carnitine (carnitine supplemented, CS) for 7d.

[1-^{14}C]-ethanol (New England Nuclear, Boston, MA) was mixed with absolute ethanol and double distilled water to yield a 13% (v/v) solution containing

1 x 10^7dpm/g ethanol. The rats orally received 3g ethanol/kg bwt and were placed in respiratory chambers. $^{14}CO_2$ was collected as described later. After 12 h the animals were anesthetized with Metofane (Pitman-Moore, Washington Crossing, NJ) blood was collected by cardiac puncture; liver, kidney, heart, semitendinusus leg muscle, epididymal fat pad, and brain were removed and stored at -20°C.

Experiment 2 Blood and Liver Ethanol Concentrations and Redox State

The objective of this experiment was to determine the effect of dietary carnitine supplementation on the redox state of the liver following ethanol administration. Ten rats (300-350g) were divided into 2 groups of 5 and fed Purina rat chow as such (NS) or supplemented with 0.5% L-carnitine (CS) for 7d.

[1-^{14}C]-ethanol (New England Nuclear, Boston, MA) was mixed with absolute ethanol and glass distilled water to yield a 13% v/v solution containing 1 X 10^7 dpm/g ethanol. The rats orally received 3g ethanol/kg bwt. Three hours later the rats were decapitated, blood was collected from the neck and liver was removed within 45 sec and dropped in liquid nitrogen. Plasma and livers were stored at -70°C.

Experiment 3 Urinary Excretion of [1-^{14}C] Ethanol

The objective of this experiment was to determine the effect of dietary carnitine supplementation on urinary excretion of ethanol, its metabolites and carnitine following ethanol administration. Ten rats (300-350g) were divided into 2 groups of 5 and fed Purina rodent chow as such (NS) or supplemented with 0.5% L-carnitine (CS) for 10d. [1-^{14}C]-ethanol (New England Nuclear, Boston, MA) was mixed with absolute ethanol and double glass distilled water to yield a 13% (v/v) solution

containing 1 x 10^7dpm/g ethanol. The rats orally received 3 g ethanol/Kg bwt and were placed in metabolic cages. Urine was collected for the next 24 hr.

Experiment 4 [^{14}C(U)]-Palmitate Oxidation Time Course

The objective of this experiment was to determine the effect of dietary carnitine supplementation on [^{14}C(U)]-palmitate oxidation. Thirty rats (300-350 g) were divided into 5 groups of 6. One-half the rats from each group received Purina rodent chow as such (NS) or supplemented with 0.5% L-carnitine (CS). The groups were fed their respective diets for 5, 10, 20, 30 and 40d.

A 2.5% palmitate solution was prepared by adding 2.5 g palmitic acid to a mixture of 50 ml double distilled water and 0.25 ml of 4.0 N KOH. The mixture was then sonicated until homogeneous. The pH was adjusted to 7.5 by the addition of concentrated HCl. It was necessary to slowly add HCl then sonicated in order to obtain accurate pH readings and to ensure homogeneity. Total volume was then brought to 100 ml with double distilled water. The final solution had the consistency of coconut milk. [^{14}C(U)]-palmitate (New England Nuclear, Boston, MA) was mixed with freshly sonicated 2.5% palmitate to yield a solution containing 2 x 10^7 dpm/g palmitate. The rats orally received 0.5 g palmitate/kg bwt (this would amount to about one-half of the normal daily intake of palmitate) and were placed in respiratory chambers. $^4CO_2$ was collected as described later. After 24 h the animals were anesthetized with Metofane (Pitman-Moore, Washington Crossing, NJ) blood was collected by cardiac puncture; liver, kidney, heart, semitendinusus leg muscle, epididymal fat pad, and brain were removed and stored at -20°C.

Experiment 5 [^{14}C(U)]-Palmitate Oxidation With Ethanol

The objective of this experiment was to determine the effect of dietary carnitine supplementation superimposed with a single oral dose of ethanol on [^{14}C(U)]-palmitate oxidation. Ten rats (300-350g) were divided into 2 groups of 5 and fed Purina rodent chow as such (NS) or chow supplemented with 0.5% L-carnitine (CS) for 10d.

[^{14}C(U)]-palmitate was administered as in experiment 3. The rats were then placed in a respiratory chamber for 2 h. Air inside the chamber was evacuated by blocking influent air and allowing a vacuum to form. This should have prevented any loss of expired air. The rats were then removed and given an oral dose (3g ethanol/kg bwt) of 13% ethanol and returned to the respiratory chambers. $^{14}CO_2$ was determined as described later. After 24 h the animals were anesthetized with Metofane (Pitman-Moore, Washington Crossing, NJ) blood was collected by cardiac puncture; liver, kidney, heart, semitendinosus leg muscle, epididymal fat pad, and brain were removed and stored at -20°C.

ANALYTICAL PROCEDURES

$^{14}CO_2$ Analysis

After receiving the labelled dose of either palmitate of ethanol the rats were placed into a respiratory chamber (Fig. 1). Air flowing into the chamber at 1.0L/min (monitored by flow meter), first passed through a CO_2 trap (Baralyme, Allied Healthcare Products, St. Louis, MO) and a moisture trap (Molecular sieve 5A with Indicating Drierite, American Scientific Products, McGraw Park, IL). Effluent air from the metabolic chamber entered another moisture trap and was bubbled through

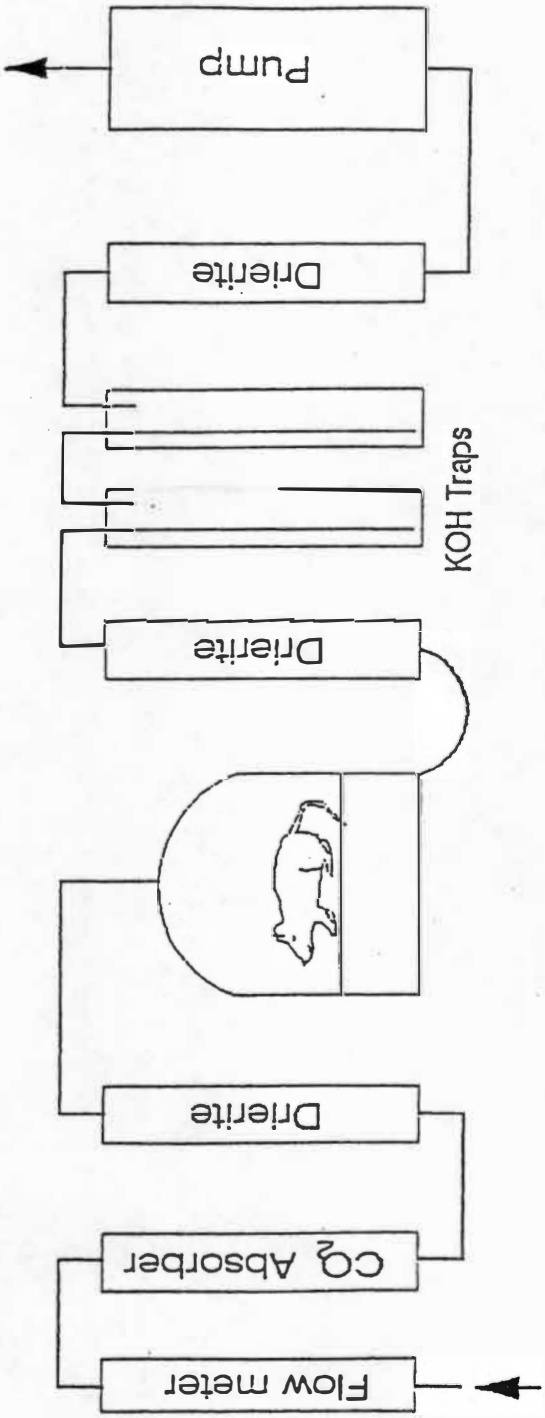

Figure 1. Respiratory CO_2 Analysis

800 ml of 1.0 M KOH, then into a second CO_2 trap containing 350 ml of 1.0 M KOH (virtually all the radioactivity was found in the first trap). 1.0 ml samples of the KOH solution were removed and mixed with 2.0 ml water and 15 ml of Aquasol II LSC cocktail (New England Nuclear, Boston, MA) in a scintillation vial; these were allowed to sit overnight, then counted in a Beckman LS 3801 Liquid Scintillation Counter.

^{14}C Determination in Tissues

Approximately 100 mg of tissue was added to 1.0 ml 2.0 M Methanolic-KOH and digested at 60°C for 1 hr in a tightly capped glass scintillation vial. The samples were then cooled to -20°C for 20 min. and 0.4 ml of glacial acetic acid was added followed by 15 ml of Aquasol II (New England Nuclear, Boston, MA). The vials were then placed in the dark overnight to reduce additional background counts due to chemilumesence. The next morning the vials were shaken, and counted in a Beckman Model 13801 Liquid Scintillation Counter. A blank for each specific tissue from rats not receiving labelled compounds was ran to determine the contribution of color to background DPM and was found to be negligible. Plasma and urine samples (0.2 to 1.0 ml) were counted with or without KOH digestion and the KOH digestion did not alter the DPM.

Sampling of Tail Vein Blood

The dorsal tail vein was cut by making an incision directly over the vein and blood was collected in a 20 µl capacity, heparinized micro-hematocrit capillary tube. The contents of the tube were immediately delivered into a 1.5 ml capacity microfuge

tube containing 980 µl of cold normal saline, capped and mixed well. The tube was then centrifuged at 1500 x g for 10 min at 2°C and kept on ice until ethanol concentration was determined within 24 hours (usually on the day of collection).

Tissue Homogenation for Total Lipids, Triglycerides, and Carnitine

Approximately 1.0 g of tissue was added to 2 volumes of 1.15% KCl containing 0.01 M Na_2HPO_4/KH_2PO_4 buffer (pH 7.4) and homogenized by four strokes in teflon/glass homogenizer. The top of the teflon plunger was then rinsed with 1 volume of KCl solution and homogenize further by 2 more strokes. The homogenate was then poured into a graduated conical tube and the homogenizer was rinsed with 1 volume of KCl solution and added to the homogenate. This yielded a w/v homogenate. The homogenate was stored at
-70°C in 1 ml portions to avoid repeated thawings of the sample.

Tissue Homogenation for Pyruvate, Lactate, Acetoacetate, 3-Hydroxybutyrate and Ethanol

Frozen livers were pulverized into a fine power by mortar and pestle. Liquid nitrogen was constantly poured into the mortar to prevent the liver from thawing. Approximately 100 mg of powdered liver, 200 µl plasma or 100 µl urine + 100 µl 10% BSA were added to pre-weighed conical tubes containing 300 µl of 3.0 M $HClO_4$ and 1.25 ml of double distilled water. The tubes were vortexed vigorously for 30 seconds and allowed to sit in an ice water bath for 5 minutes, vortexed again and centrifuged for 10 minutes at 1500 x g at 2°C. 1.0 ml of the supernatant was removed and added to another conical tube on ice. The original tube was then allowed to warm

to room temperature and weighed. 200 µl of 3 M $KHCO_3$ was added and allowed to sit for 15 min., then centrifuged for 10 min at 1500 x g at 2°C. The supernatant was then poured off. Acetoacetate, pyruvate, and ethanol were assayed immediately. Lactate and 3-Hydroxybutyrate were assayed within 2 hours.

Pyruvate Determination

Pyruvate was determined fluorimetrically by the method of Passonneau & Lowry (62). The decrease in NADH concentration was measured by the decrease in florescence as pyruvate was reduced to lactate by bovine heart lactate dehydrogenase.

Reagents

1. 1.0 M K_2HPO_4/NaH_2PO_4 (pH 7.0):

   Dissolve 21.8g K_2HPO_4 and 19.0g NaH_2PO_4 $2H_2O$ in 200 ml of double distilled water adjust pH to 7.0 as necessary with 4.0 M KOH and bring up to 250 ml with double distilled water. Store at 4°C.

2. 5mM NADH:

   Dissolve 3.5 mg B-NADH-Na_2, Grade III from yeast (Sigma, St. Louis, MO) per ml carbonate buffer. Heat to 60°C for 15 min and store at -20°C. Make fresh weekly.

3. 0.1M $Na_2CO_3/NaHCO_3$ (pH10.6):

   Dissolve 0.85g Na_2CO_3 and 0.17 g $NaHCO_3$ in 7.5 ml double distilled water adjust pH as necessary with 4.0 M KOH and bring up to 100 ml with double distilled water. Store at -20°C.

4. 20 mM Tris Buffer (pH 8.0):

   Dissolve 0.24 g Tris (Sigma, St. Louis, MO) in 75 ml of double distilled water, adjust pH to 8.0 with 1.0 M HCl, add 200 µl 10% BSA and bring to 100 ml. Store at -20°C

5. Lactate Dehydrogenase (LDH):

   Dilute stock LDH, Bovine Heart Type III in 2.1 M $(NH_4)^2SO_4$ (Sigma, St. Louis, MO) with 20mM Tris to yield 500 u/ml. Make fresh daily.

6. 1.0 mM Pyruvate:

   Dissolve 18.3 mg Pyruvic Acid-potassium salt Type III (Sigma, ST. Louis, MO) per ml double distilled water. This yields a 0.1 M solution. Dilute to 1.0 mM by adding 100 µl to 9.9 ml double distilled water. Make fresh daily.

7. 3.0 M $HClO_4$:

   Dilute 25 ml 70% $HClO_4$ to 100 ml with double distilled water. Store at 4°C.

8. Reagent Mixture:

   Add 10 ml 1.0 M phosphate buffer and 100 µl of 5mM NADH to 90 ml double distilled water. Prepare fresh daily.

9. 10% BSA:

   Dissolve 10g BSA in 75 ml double distilled water and bring to 100 ml with double distilled water.

10. 3.0 M $KHCO_3$:

    Dissolve 30 g $KHCO_3$ in double distilled water and make up to 100 ml. Store at -20°C.

Procedure

A standard curve was prepared by adding 0, 10, 20, 40, 60, 80 and 100 µl (Fig. 2) of pyruvate standard to tubes containing 100 µl 10% BSA, 300 µl of 3.0 M $HClO_4$ and double distilled water to a total volume of 1.65 ml. The tubes were vortexed vigorously for 30 sec and allowed to sit in an ice water bath for 5 min, vortexed again and centrifuged for 10 min at 1500 x g at 2°C. 1.0 ml of the supernatant was removed and added to another conical tube on ice. 200 µl of 3M $KHCO_3$ were added and allowed to sit for 15 min, then centrifuged for 10 min at 1500 x g at 2°C. The supernatant was then poured off into another tube on ice. A 100 µl aliquot of the neutralized standard or tissue supernatant was added to 1.9 ml of reaction mixture in a 4.5 ml polystyrene Ultra-Vu disposable cuvette (Baxter Scientific Products, Atlanta, GA) and mixed by inversion. Baseline florescence was determined in a Hitachi F-2000 Florescence Spectrophotometer (Exitation 360 and Emission 460 um). 20 µl of LDH (approx. 10U) was then added. The cuvettes were mixed by inversion and let sit at room temperature for 30 min in the dark. After 30 min florescence was determined again. The decrease in florescence was proportional to the concentration of pyruvate.

Calculations

Florescence X 1/slope X 5 = nmoles/ml plasma or urine

Florescence X 1/slope ÷ mg liver = nmoles/mg liver

Florescence X 1/slope ÷ 10 = nmoles/ml/nmoles/ml urine

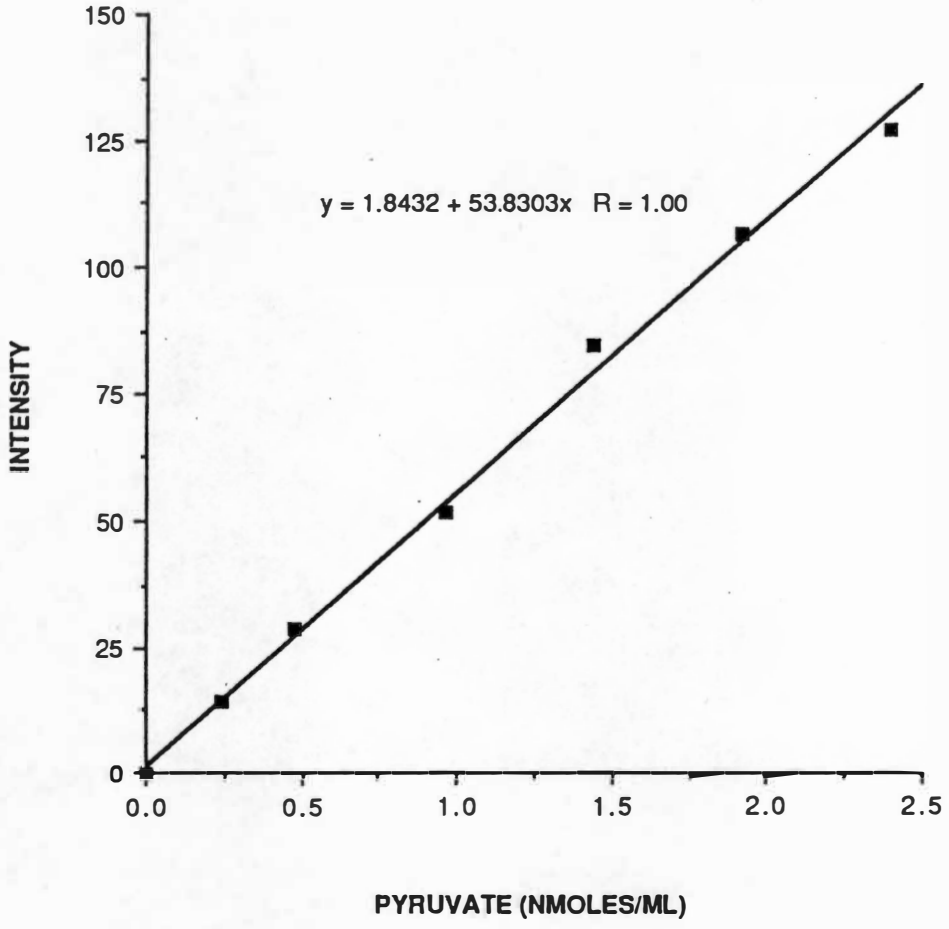

Figure 2. Pyruvate Standard Curve

Lactate Determination

Lactate was determined by the method of Passonneau (63). The increase in NADH concentration was measured by the increase in fluorescence as lactate is oxidized to pyruvate by bovine heart lactate dehydrogenase.

Reagents

1. 2-Amino-2-methyl-1-propanol buffer (1 M; pH 10.0):

   Dissolve 8.9 g 2-amino-2-methyl-1-propanol (9.5 ml if liquid) in double distilled water, adjust pH to 10.0 with 12 M HCl and dilute to 100 ml. with double distilled water. Store at -20°C.

2. Tris buffer (20 mM; pH 8.0):

   Dissolve 0.24 g Tris (Sigma, St. Louis, MO) in double distilled water adjust pH to 8 with 1.0 m HCL add 200 µl 10% BSA and dilute with double distilled water to 100 ml. Store at -20°C.

3. Hydrazine Hydrate, (Sigma, St. Louis, MO)

4. Nicotinamide-adenine dinucleotide (0.1 M B-NAD Grade III) from yeast (Sigma, St. Louis, MO).

   Dissolve 70 mg. NAD in 1 ml double distilled water. Store frozen.

5. Lactate dehydrogenase, LDH

   Dilute stock LDH, Bovine heart Type III in 2.1 M $(NH_4)_2SO_4$ (Sigma, St. Louis, MO) with 20 mm Tris to yield 500 u/ml.

6. 1.0 mM Lactate

   Dissolve 10.3 mg L (+)-Lactic Acid-Lithium salt Grade L-X (Sigma, St. Louis, MO) per ml double distilled water this yields a 0.1 M solution. Dilute to 1.0 mM by adding 100 µl to 9.9 ml double distilled water. Make fresh daily.

7. Perchloric acid (3 M):

   Dilute 25 ml. 70% $HClO_4$ with double distilled water to 100 ml.

8. Potassium hydrogen carbonate (3 M):

   Dissolve 30 g. $KHCO_3$ with double distilled water and make up to 100 ml.

9. Reagent mixture

   Mix 10.0 ml buffer solution (1), 250 µl Hydrazine Hydrate and 50 µl NAD and bring to 100 ml with double distilled water.

Procedure

A standard curve was prepared by adding 0, 10, 20, 40, 60, 80 and 100 µl (Fig. 3) of lactate standard to tubes containing 100 µl 3.0 M $HClO_4$ and double distilled water to a total volume of 1.65 ml. The tubes were vortexed vigorously for 30 sec and allowed to sit in an ice water bath for 5 min vortexed again and centrifuged for 10 min at 1500 X g at 2°C. 1.0 ml of the supernatant was removed and added to another conical tube on ice. 200 µl of 3 M $KHCO_3$ were added and allowed to sit for 15 min, then centrifuged for 10 min at 1500 x g at 2°C. The supernatant was then poured off into another tube on ice. A 100 µl aliquot of the neutralized standard or tissue supernatant was added to 1.9 ml of reaction mixture in a 4.5 ml polystyrene Ultra-Vu disposable cuvette (Baxter Scientific Products, Atlanta, GA) and mixed by inversion.

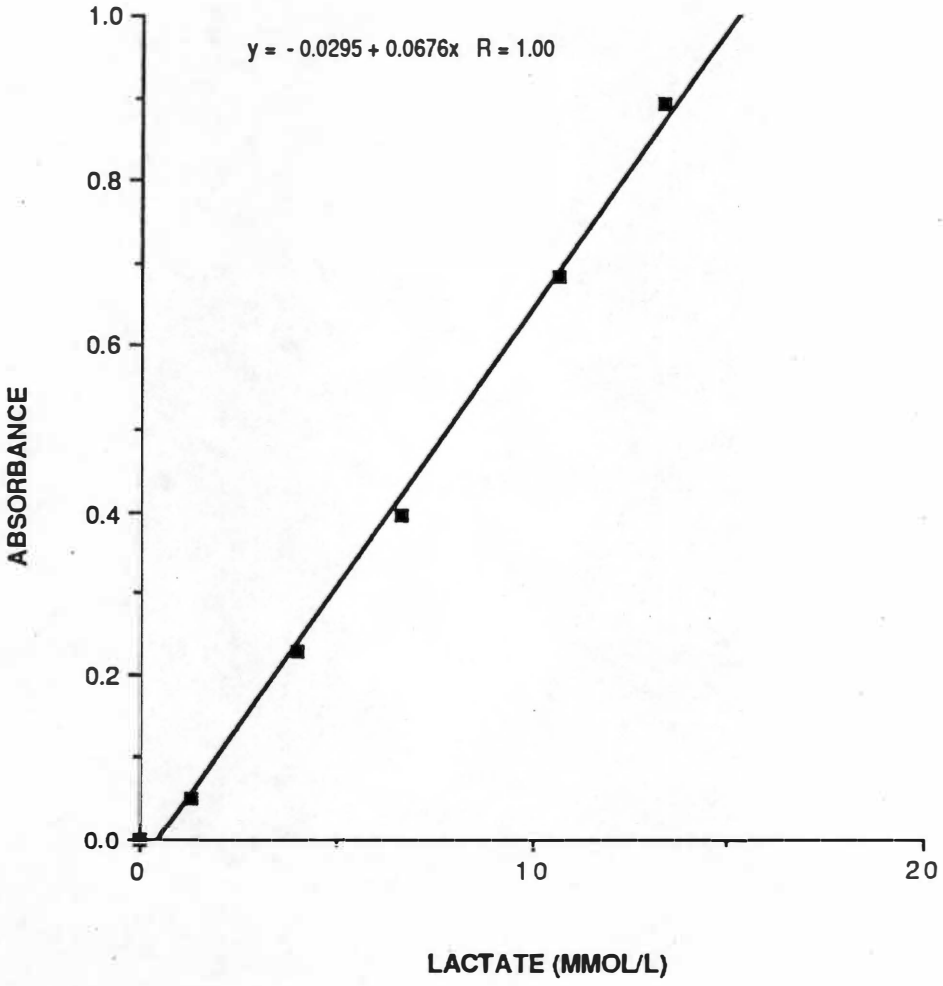

Figure 3. Lactate Standard Curve

Baseline florescence was determined in a Hitachi F-2000 Fluorescence Spectrophotometer (Exitation 360 and Emission 460nm). 10 µl of LDH was then added, the cuvettes were mixed by inversion and let sit at room temperature for 30 min in the dark. After 30 min fluorescence was determined again. The increase in florescence was proportional to the concentration of lactate

Calculations

Fluorescence X 1/slope X 5 = nmoles/ml plasma

Fluorescence X 1/slope ÷ mg liver = nmoles/mg liver

Fluorescence X 1/slope x 10 = nmoles/ml urine

Acetoacetate Determination

Acetoacetate was determined by the method of Mellanby and Williamson (64) adapted from spectrophotometry to fluorescent spectrophotometry. The decrease in NADH concentration was measured by the decrease in fluorescence as acetoacetate was reduced to 3-Hydroxybutyrate.

Reagents

1. 1.0M K_2HPO_4/NaH_2PO_4 (pH 7.0):

   Dissolve 21.8 g K_2HPO_4 and 19.0g NaH_2PO_4 $2H_2O$ in 200 ml of double distilled water adjust pH to 7.0 as necessary with 4.0MKOH and bring up to 250 ml with double distilled water. Store at 4°C.

2. 5mM NADH:

   Dissolve 3.5 mg ß-NADH-Na_2, Grade III from yeast (Sigma, St. louis, MO) per ml of carbonate buffer. Heat to 60°C for 15 min and store at -20°C. Make fresh weekly.

3. 0.1 M Na_2CO_3/$NaHCO_3$ (pH 10.6):

   Dissolve 0.85g Na_2CO_3 and 0.17g $NaHCO_3$ in 75 ml double distilled water adjust pH as necessary with 4.0 MKOH and bring up to 100 ml with double distilled water. Store at -20°C.

4. 20mM Tris Buffer (pH 8.0):

   Dissolve 0.24g Tris (Sigma, St. Louis, MO) in 75 ml of double distilled water adjust pH to 8.0 with 1.0M HCl add 200 µl 10% BSA and bring to 100 ml. Store at -20°C.

5. 3-Hydroxybutyrate Dehydrogenase. Type II from Rhodopseudomonas spheroides in 3.2M $(NH_4)_2So_4$ (Sigma, St. Louis, MO).

6. 1.0mM Acetoacetate

   Dissolve 11.5 mg Acetoacetic Acid-Lithium Salt (Sigma, St. Louis, MO) per ml double distilled water. This yields a 0l1M solution. Dilute to 1.0mM by adding 100 µl to 9.9 ml double distilled water.

7. 3.0M $HClO_4$:

   Dilute 25 ml 70% $HClO_4$ to 100 ml with double distilled water. Store at 4°C.

8. 10% BSA:

   Dissolve 10g BSA in 75 ml double distilled water bring to 100 ml with double distilled water.

9. 3.0M $KHCO_3$:

   Dissolve 30g of $KHCO_3$ in double distilled water and make up to 100 ml. Store at -20°C.

10. Reagent Mixture:

    Add 10 ml 1.0M phosphate buffer and 100 µl of 5mM nadh to 90 ml double distilled water. Prepare fresh daily.

Procedure

A standard curve was prepared by adding 0, 10, 20, 40, 60, 80 and 100 µl (Fig. 4) of acetoacetate standard to tubes containing 100 µl 10% BSA, 300 µl 3.0M $HClO_4$ and double distilled water to a total volume of 1.65 ml. The tubes were vortexed vigorously for 30 sec and allowed to sit in an ice water bath for 5 min vortexed again and centrifuged for 10 min at 1500 X g at 2°C. The supernatant was then poured off into another tube on ice. A 100 µl aliquot of the neutralized standard or tissue supernatant was added to 1.9 ml of reaction mixture in a 4.5 ml polystyrene Ultra-Vu disposable cuvette (Baxter Scientific Products, Atlanta, GA) and mixed by inversion. Baseline fluorescence was determined in a Hitachi F-2000 Fluorescence Spectrophotometer (Exitation 360 and Emission 400 nm) 10 µl of BHBDH was then added. The cuvettes were mixed by inversion and let sit at room temperature for 60

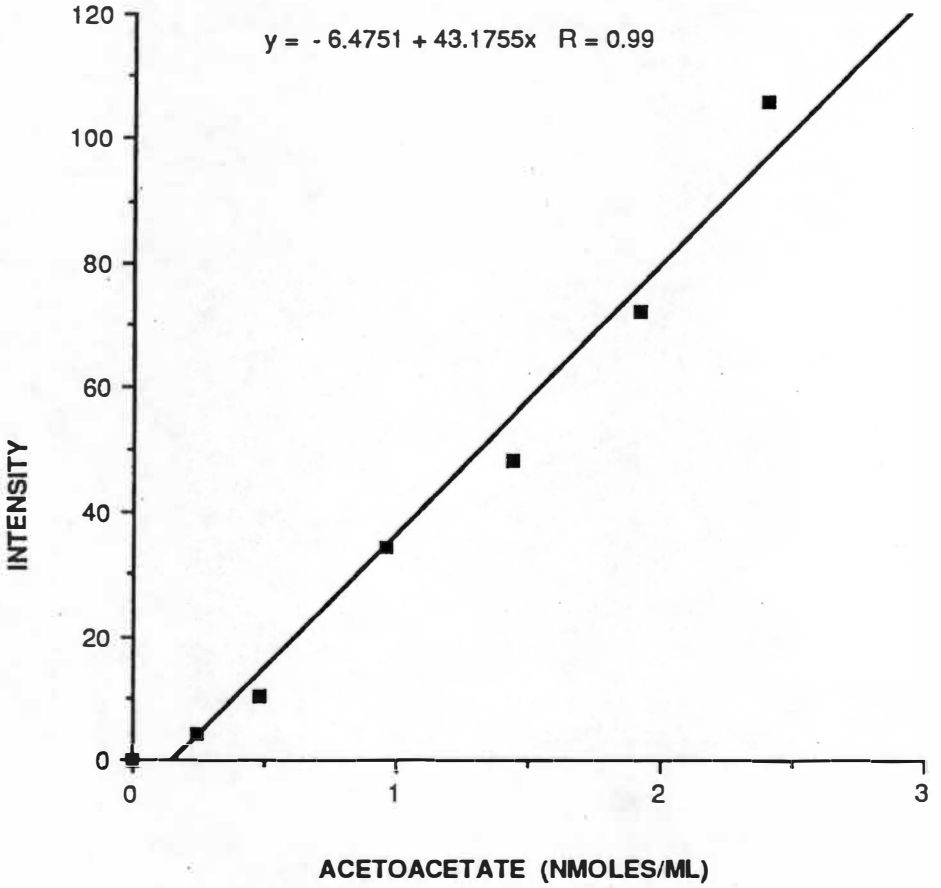

Figure 4. Acetoacetate Standard Curve

min in the dark. After 60 min fluorescence was determined again. The decrease in fluorescence was proportional to the concentration of acetoacetate.

Calculations

Fluorescence X 1/slope X 5 = nmoles/ml plasma

Fluorescence X 1/slope ÷ mg liver = nmoles/mg liver

Fluorescence X 1/slope X 10 = nmoles/ml urine

3-Hydroxybutyric Acid Determination

3 Hydroxybutyric Acid was determined according to the procedure of Williamson and Mellanby (65) adapted for fluorescence. The concentration of NADH is measured by an increase in fluorescence as 3-Hydroxylbutyrate is oxidized to acetoacetate.

Reagents

1. 2-Amino-2-methyl-1-propanol buffer (1 M; pH 10.0):

   Dissolve 8.9 g 2-amino-2-methyl-1-propanol (9.5 ml if liquid) in double distilled water, adjust pH to 10.0 with 12 M HCl and dilute to 100 ml. wit double distilled water. Store at -20°C.

2. Tris buffer (20 mM; pH 8.0):

   Dissolve 0.24 g Tris (Sigma, St. Louis, MO) in double distilled water adjust pH to 8 with 1.0 m HCL add 200 µl 10% BSA and dilute with double distilled water to 100 ml. Store at -20°C.

3. Hydrazine Hydrate (20 m) (Sigma, St. Louis, MO)

4. Nicotinamide-adenine dinucleotide (0.1 M B-NAD Grade III) from yeast (Sigma, St. Louis, MO).

   Dissolve 70 mg. NAD in 1 ml double distilled water. Store at -20°C .

5. Lactate dehydrogenase, LDH

   Dilute stock LDH, Bovine heart Type III in 2.1 M $(NH_4)_2SO_4$ (Sigma, St. Louis, MO) with 20 mm Tris

6. 1.0 mM 3-Hydroxybutyrate

   Dissolve 15.4 mg DL-3Hydroxybutyric Acid-Sodium salt (Sigma, St. Louis, MO) per ml double distilled water this yields a 0.1 M solution. Dilute to 2.0 mM by adding 200 µl to 9.8 ml double distilled water. Make fresh daily.

7. Perchloric acid (3 M):

   Dilute 25 ml. 70% $HClO_4$ with double distilled water to 100 ml.

8. Potassium hydrogen carbonate (3 M):

   Dissolve 30 g. $KHCO_3$ with double distilled water and make up to 100 ml.

9. Reagent mixture

   Mix 10 ml buffer (1), 250 µl Hydrazine Hydrate and 50 µl NAD and bring to 100 ml with double distilled water. Make fresh daily

Procedure

A standard curve was prepared by adding 0, 10, 20, 40, 60, 80 and 100 µl (Fig. 5) of 3-Hydroxybutyrate standard to tubes containing 100 µl 10% BSA, 300 µl 3.0 M $HClO_4$ and double distilled water to a total volume of 1.65 ml. The tubes were vortexed vigorously for 30 sec and allowed to sit in an ice water bath for 5 min,

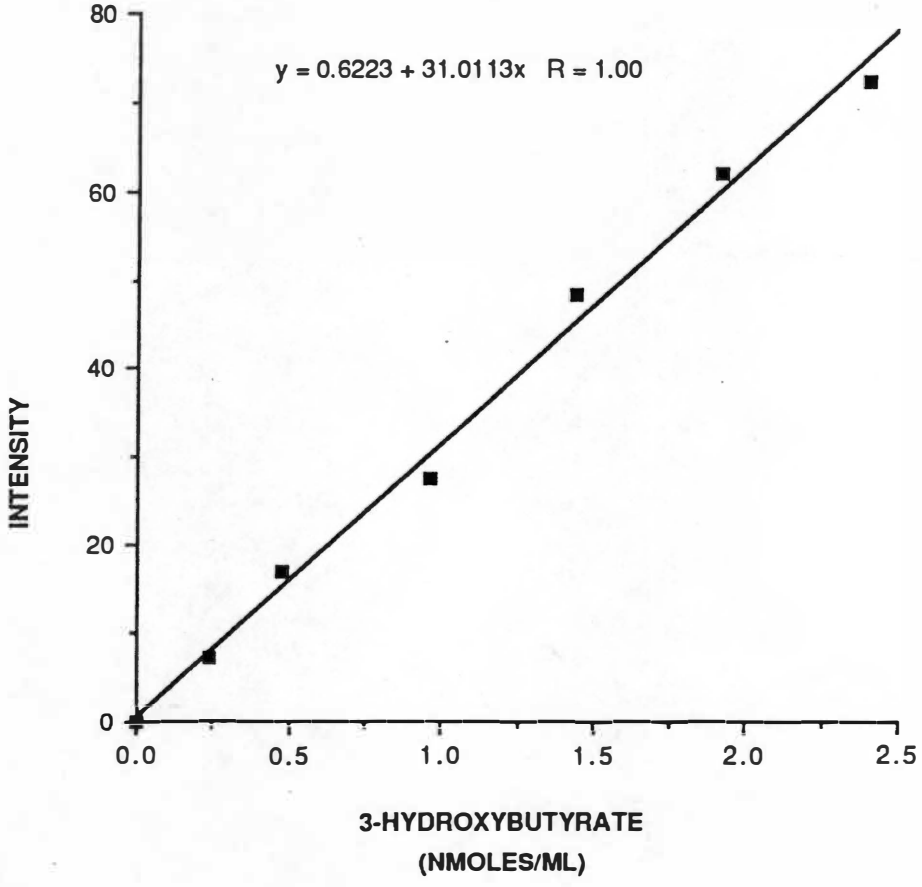

Figure 5. 3-Hydroxybutyrate Standard Curve

vortexed again and centrifuged for 10 min at 1500 x g at 2°C. 1:0 ml of the supernatant was removed and added to another conical tube on ice. 200 µl of 3 M $KHCO_3$ were added and allowed to sit for 15 min, then centrifuged for 10 min at 1500 x g at 2°C. The supernatant was then poured off into another tube on ice. A 100 µl aliquot of the neutralized standard or tissue supernatant was added to 1.9 ml of reaction mixture in a 4.5 ml polystyrene Ultra-Vu disposable cuvette (Baxter Scientific Products, Atlanta, GA) and mixed by inversion. Baseline fluorescence was determined in a Hitachi F-2000 Fluorescence Spectrophotometer (Exitation 360 and Emission 460 nm). 10 µl of BHBDH were then added, the cuvettes were mixed by inversion and let sit at room temperature for 60 min in the dark. After 60 min fluorescence was determined again. The decrease in fluorescence was proportional to the concentration of 3-Hydroxybutyric Acid.

Calculations

Fluorescence X 1/slope X 5 = nmoles/ml plasma, urine

Fluorescence X 1/slope ÷ mg liver = nmoles/mg liver

Fluorescence X 1/slope x 10 = nmoles/mg liver

Total Lipids Determination

Total lipids were determined according to the method of Ellenston and Caraway (66).

Reagents

1. Concentrated H_2SO_4

2. Phosphoric Acid-Vanillin Reagent

   Dissolve 1.0g vanillin in 160 ml double distilled water. Bring to 500 ml with concentrated phosphoric acid. Store at room temperature.

3. Olive Oil standard (1%)

   Mix 1.0g of olive oil with 7.5 ml chloroform and bring to 100 ml with chloroform. Store at -20°C.

4. Working standard (0.1%)

   Mix 0.5 ml of 1% standard with 4.5 ml chloroform. Store at -20°C.

Procedure

A standard curve was prepared by adding 0, 50, 100, 200, and 400 µl (Fig. 6) of working standard to 16 X 150 mm glass tube. The chloroform was then evaporated off under a stream of nitrogen and 200 µl of double distilled water were added to each tube. 200 µl of plasma or 100 µl liver homogenate plus 100 µl double distilled water were added to 16 X 150 mm glass tubes while the chloroform was evaporating off. 5 ml of H_2SO_4 were added to all tubes, vortexed and placed in a heating block at 100^C for 10 min, then allowed to cool to room temperature. 200 µl from each tube were added to 3.0 ml of phosphoric acid-vanillin reagent, vortexed and placed in the dark for 1 hour. Absorbance was determined in a Beckman Model 34 Dual-Wavelength Spectrophotometer at 520 nm.

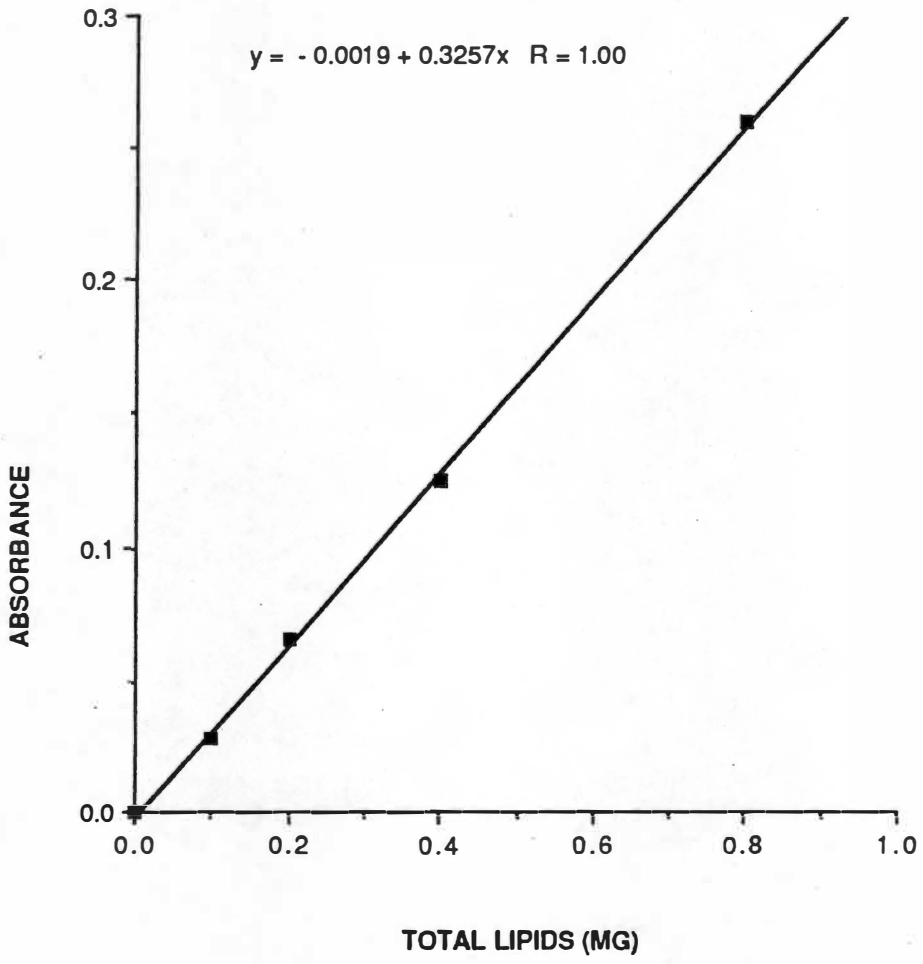

Figure 6. Total Lipids Standard Curve

Calculation

Abs X 1/slope X 5 = mg lipid/ml plasma

Abs X 1/slope X 10/mg liver/ml homogenate = mg lipid/mg liver

Triglyceride Determination

Triglycerides were determined according to the method Geigel et al (67). Lipids are partitioned between water/isopropanol and nonane phases with the triglyceride extracted into the nonane. Glycerol is liberated from the triglyceride by sodium hydroxide and oxidized to formaldehyde by periodate. The formaldehyde reacts with 2, 4-pentanedione to form 3,5-diacetyl-1,3-dihydrolutidine, the color compound measured.

Reagents

1. Extraction reagent: n-nonane/isopropanol (2.0/3.5 v/v). Caution: flammable. Not necessary to redistill.
2. Triolein standard: triolein (Sigma Chem. Col, St. Louis, MO) 100 mg/dl in extraction reagent (1.13 nmol/liter).
3. Dilute sulfuric acid, 40 nmol/liter: 1.10 ml conc. H_2So_4 diluted in small amount of distilled water and diluted to 500 ml.
4. Transesterifying reagent: NaOH in isopropanol, 100 mmol/liter. 1 g NaOH is dissolved in small amount of isopropanol and diluted to 250 ml. A fine precipitate may be present in the reagent, primarily as a result of carbonate formation. It will settle on standing, or it can be removed by filtration. A slight yellow color may develop with age but will not affect test results.

5. Oxidizing reagent: sodium periodate ($NaIO_4$), 18 mmol/liter, in 2.0 mol/liter acetic acid (CH_3COOH). 0.962 g of $NaIO_4$ is dissolved in 2 M acetic acid and diluted to 250 ml. 2 M acetic acid is prepared by adding 51 ml of glacial acetic acid to some distilled water and dilute to 500 ml.
6. Color buffer: Ammonium acetate (CH_2COOHN_4) (6.0 mol/liter, pH 6.0 at 25°C). 462g CH_2COONH_4 is dissolved in approximately 600 ml distilled water. The pH is adjusted to 6.0 with glacial acetic acid (H). The solution is diluted to one liter.
7. Acetylacetone ($C_5H_8O_2$) (2,4-pentanedione), reagent grade.
8. n-Nonane (Phillips "pure" grade.)

   The above reagents all are stable for at least a year at room temperature. Store them in tightly stoppered vessels.
9. Working color reagent: Add 4 ml of acetylacetone to 100 ml of the color buffer. Shake vigorously and allow to stand for at least 15 min. before use. This solution is stable for 8 hours at room temperature.

Procedure

A standard curve was prepared by adding 0, 50, 100, 200, 400 µl (Fig. 7) to 16 X 100 mm glass screw-top tubes. Total volume was brought to 5.0 ml with extracting reagent. 200 µl of double distilled water were than added to each standard. 200 µl of liver homogenate or plasma was then added to tubes containing 5.0 ml extracting reagent. The tubes were vortexed and centrifuged at 1500 x g at 2°C. 500 µl of the upper phase was added to an equal volume of transesterifying reagent, vortexed and

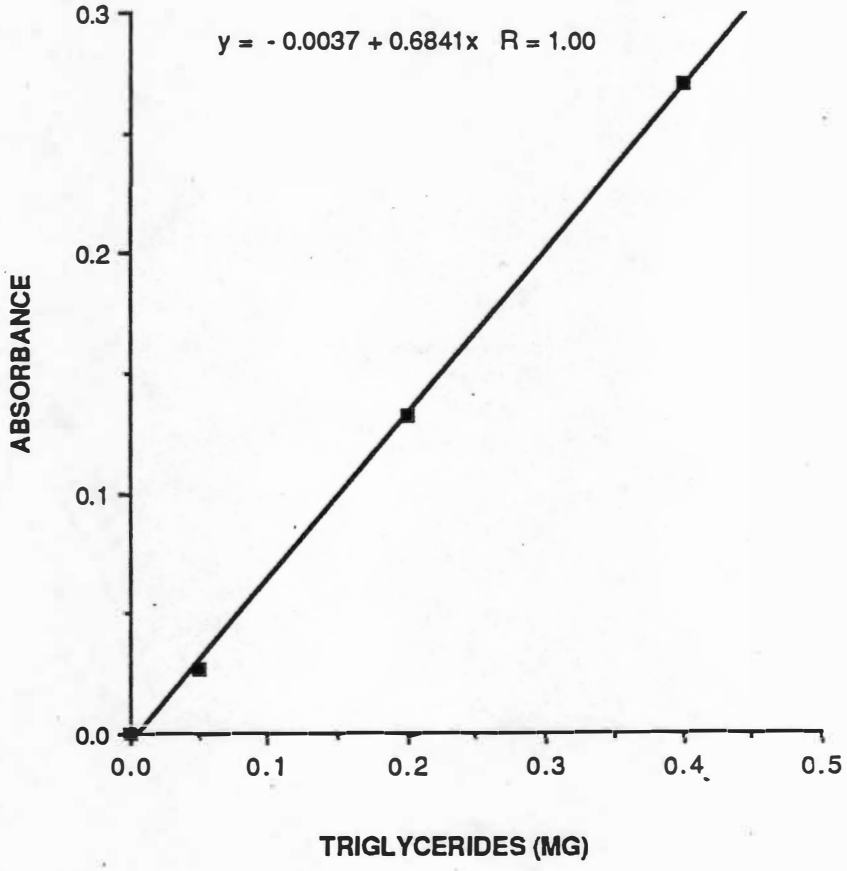

Figure 7. Triglyceride Standard Curve

held at room temperature for five min. 500 µl of oxidizing reagent was added, vortexed and held at room temperature for five min. 6.0 ml of working color reagent was added and incubated at 60°C for ten min. The tubes were cooled to room temperature and absorbances was determined at 415 nm.

Calculation

Abs X 1/slope X 5 = mg Triglyceride/ml plasma

Abs X 1/slope X 5 = mg liver/ ml homogenate = mg Triglyceride/ml plasma

Carnitine Determination

Carnitine was determined by the method of Cederblad & Lindstedt (68) with modifications (69-71). Total carnitine is fractionated by PCA into non-esterified carnitine (NEC), acid soluble acylcarnitine (ASAC) and acid insoluble acylcarnitine (AIAC). The acylcarnitine fractions are first hydrolyzed with KOH then assayed as NEC. Labelled ([1-^{14}C]AcetylCoA or [CH_3-H]Acetyl CoA) acetyl CoA is then esterified to NEC by carnitine acetyl transferase (CAT). Excess acetyl-CoA is removed by anion exchange chromotography. Radioactivity in the eluent which would be proportional to carnitine concentration, is determined by liquid scintillation.

Procedure

A standard curve was prepared by adding 0, 10, 20, 40, 60, 80, 100 µl (Fig. 8-10) to plastic conical tubes and total volume was brought to 100 µl with double distilled water. 100 µl BSA and 200 µl PCA were added to each tube. Plasma was assayed by adding 100 µl plasma to 100 µl double distilled water and 200 ml PCA. Liver carnitine was determined by mixing 200 µl liver homogenate with 200 µl PCA.

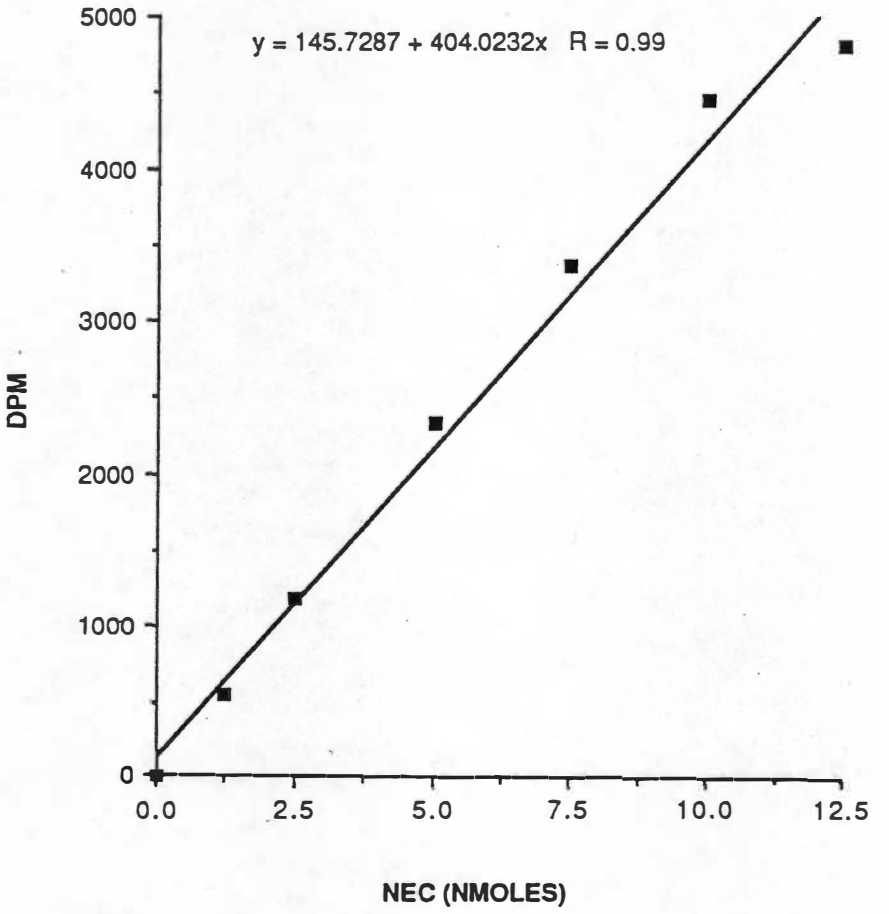

Figure 8. Non-Esterified Carnitine (NEC) Standard Curve

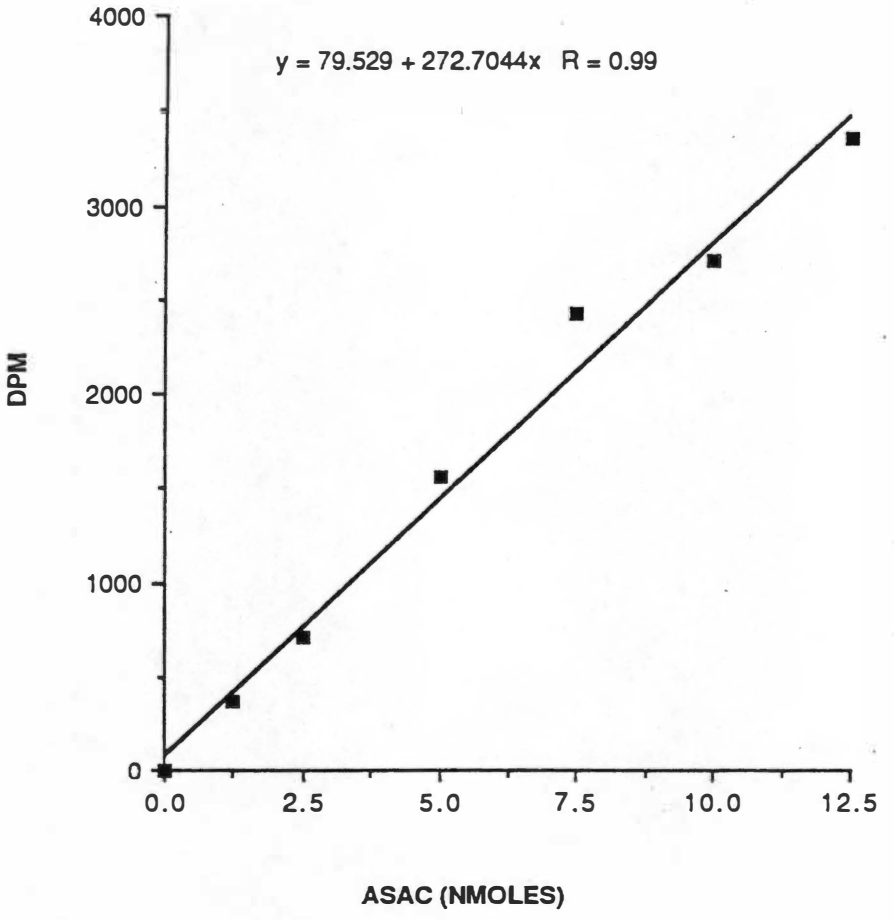

Figure 9. Acid-Soluble Acyl-Carnitine (ASAC) Standard Curve

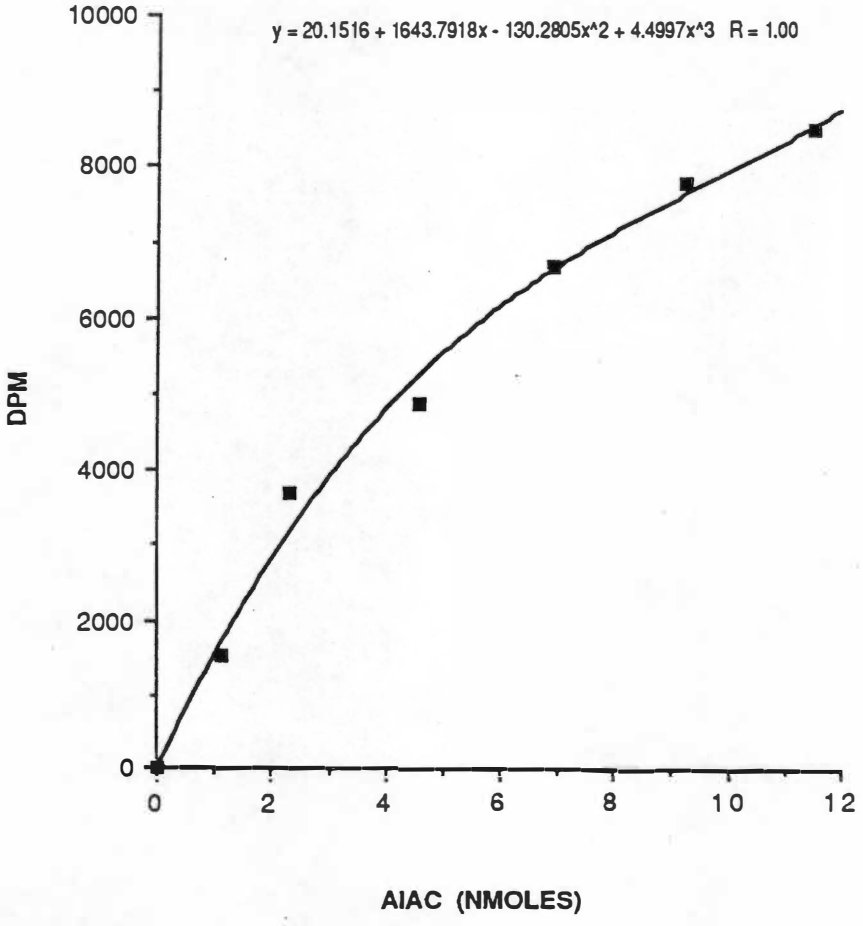

Figure 10. Acid-Insoluble Acyl-Carnitine (AIAC) Standard Curve

100 µl of urine sample were mixed with 100 µl BSA before the addition of PCA. (It is necessary to dilute urine (with normal saline) from carnitine supplemented rats 20-30 fold in order to determine NEC and ASAC and 10 fold for AIAC). The tubes were vortexed, centrifuged at 1500 X g for 10 min at 4°C (Model TJ-6, Beckman Instruments, Palo Alto, CA) and put on ice.

Reagents

1. 0.5 mM carnitine standard solution (stock): 9.88 mg L-carnitine HCL (sigma Chemical Co., St. Louis, MO) was dissolved in cold glass distilled water (GDW) and diluted to 100 ml in a volumetric flask. This stock standard was diluted 1:2 to give a working standard concentration of 0.25 mM.
2. 22.90 mM L-palmitoylcarnitine standard (stock): 1.0 ml of GDW was added to a vial containing 10 ml of L-palmityl carnitine (Sigma Chemical Co., St. Louis, MO). This stock standard was diluted 1:100 to give a working standard concentration of 0.229 mM.
3. Carnitine standard mixture: Working standard were prepared by mixing equal volumes of L-carnitine and L-palmitoyl carnitine.
4. 0.5 M Potassium hydroxide: 28.05 g of KOH was dissolved in some GDW and diluted to a volume of 1 liter.
5. 0.5 M Potassium hydroxide: 56.11 g of KOH was dissolved in some GDW and diluted to a volume of 250 ml.
6. 0.6 M Perchloric acid (PCA): 51.26 ml of 70% PCA was added in some GDW and diluted to a volume of 1 liter.

7. 0.1 M Sodium tetrathionate: 0.6756 g of anhydrous sodium tetrathionate (J.T. Baker Chem. Co., Phillipsburg, NJ) was dissolved in GDW and diluted to a volume of 25 ml.

8. 0.1% Phenol red: 100 mg of phenol red (Sigma Chemical Co., st. Louis, MO) was dissolved in absolute ethanol and made to a volume of 100 ml. Phenol red tubes were prepared by adding one drop of phenol red to 12 x 75 mm Pyrex glass tubes and allowed to dry.

9. [1-^{14}C] acetyl Coenzyme A: 10 uCi of [^{3}H] acetyl Coenzyme A (New England Nuclear, Boston, MA) was dissolved in cold GDW and made to a volume of 300 ml. 5.0 ml aliquots were dispensed into plastic vials and frozen at -70°C.

10. 1M Potassium bicarbonate: 1 g potassium bicarbonate (Certified ASC, Fisher Scientific Co., Fair Lawn, NJ) was dissolved and made to a volume of 10 ml with GDW. Solution was stored at room temperature and made daily.

11. 0.1 M acetic anhydride: 0.5 ml of acetic anhydride (Fisher Scientific Co., Fair Lawn, NJ) was added to 4.95 ml of cold GDW and used immediately for acetyl CoA solution.

12. 0.1 mM acetyl Coenzyme A: 10 mg of acetyl Coenzyme A (Sigma Chemical Co., St. Louis, MO) was added to 0.5 ml cold GDW and mixed. Then, 100 µl of 1 M potassium bicarbonate was added and mixed, and followed by the addition of 200 µl of 0.1 M acetic anhydride. The solution was made up to 80 ml with cold GDW, mixed thoroughly, and dispensed into plastic vials and frozen at -70°C.

13. Carnitine acetyltransferase (CAT): CAT from pigeon breast muscle (Sigma Chemical Co., St. Louis, MO) was diluted with GDW to give an enzyme activity of 50 units/ml.

14. 1 M [3-(4-morpholino)propanesulfonic acid]MOPS: 20.92 g of MOPS (Sigma Chemical Co., St. Louis, MO) was added to about 80 ml of GDW. The pH was adjusted 7.4 with 4 M potassium hydroxide and was diluted to a volume of 100 ml with GDW.

15. PCA/MOPS-I: 20.9 g of MOPS was added to 50 ml of 0.6 M PCA and brought up to a volume of 100 ml with GDW. The solution was stored at 4°C.

16. PCA/MOPS-II: 20.9 g of MPOS was added to 20 ml of 0.6 M PCA and brought up to a volume of 100 ml with GDW. The solution was stored at 4°C.

17. 0.1 M ethylene glycol-bis (beta-amino-ethylether) N, N-tetraacetic (EGTA), pH 7: 1.902 g EGTA (Sigma Chemical co., St. Louis, MO) was added in 30 ml GDW, adjusted to pH 7.0 with 4 M potassium hydroxide, and diluted to 50 ml with GDW.

18. 0.1 mM [^{3}H] acetyl Coenzyme A solution: 2:1 volumes of [1-^{14}C]acetyl CoA and 0.1 mM acetyl CoA were mixed and stored at 4°C.

19. Reagent mixture for one assay: 1 M MOPS, pH 7.4, 120 µl; 0.1 M EGTA, pH 7.0, 20 µl; 0.1 M sodium tetrathionate, 20 µl; 0.1 mM [1-14C]-acetyl CoA solution, 200 µl; GDW, 40 µl; to a volume of 400 µl.

20. 8% bovine serum albumin (BSA): 4 g of BSA (Fatty acid poor, Fraction V, ICN Pharmaceuticals Inc., Life Sci. Group, Cleveland, OH) was dusted into about 20 ml of GDW, stirred gently, and diluted to 50 ml.
21. Scintillation fluid; Ready-Solv®CP (Beckman Instruments, Palo Alto, CA).
22. Mini Columns: The mini columns were made by stuffing the area above 5-3/4" Pasteur pipettes (Fisher Scientific Co., Fair Lawn, NJ) with glass wool. Anion Exchange Resin, AG I-X8, 200-400 mesh, CL-form (Bio-Rad Laboratories, Richmond CA) was added up to the 9 cm mark measured from the tip of the pipette.

Nonesterified Carnitine Determination

35 µl of 1 M potassium bicarbonate was added to the 150 µl of the supernatant in a phenol red test tube and vortexed. The contents turned a golden yellow color. The mixtures were held for 30 min on ice and centrifuged at 1500 x g for 10 min.

Acid Soluble Acylcarnitine Determination

75 µl of 0.5 M potassium hydroxide was added to the 100 µl of the supernatant in the phenol red test tube and vortexed. The contents turned a purple-red color indicating alkalinity for hydrolysis. The contents were incubated in a 37°C rotating water bath (100 shakes/min) for 30 min for complete hydrolysis. The mixtures were then neutralized by adding 30 µl of PCA/MOPS-II, and vortexed. They were held on ice for 30 in and centrifuged at 1500 x g for 10 min.

Acid Insoluble Acylcarnitine Determination

The pellet was resuspended in 200 µl of 0.6 M PCA and centrifuged at 1500 x g for 10 min, drained by inverting the tube and repeated. One drop of phenol red indicator and 200 µl of 0.5 M potassium hydroxide were added to each tube and the pellets were resuspended. The contents turned a purple-red color and were hydrolyzed in a 65°C shaking water bath (100 spm) for 60 min.

The contents were neutralized by adding 100 µl of PCA/MOPS-I. The tubes were vortexed, held on ice for 30 min and centrifuged at 1500 x g for 10 min at 4°C.

Assay for Carnitine

A 100 µl aliquot was transferred from each of the fractions to 1.5 ml microfuge tubes separately. Four hundred µl of reagent mixture were added followed by 20 µl of carnitine acetyltransferase (CAT). The tubes were capped, mixed by gentled tapping, incubated in a 37°C shaking water bath for 30 min. Later, 200 µl of the incubated mixture was transferred onto a mini column, and the eluant was collected into plastic mini scintillation vial. When the 100 µl aliquot was fully absorbed, the column was washed with 2 portions of 500 µl GDW, and the washes were also collected into the same respective scintillation vial. Then, 5 ml of scintillation fluid was added to each vial, capped, shaken, wiped-off with ethanol, counted for 10 min/vial in a scintillation counter (LS-3801 Beckman Instruments, Irving, CA).

Calculation

DPM x 1/slope x 10 - nmoles/ml plasma

DPM x 1/slope x 5 ÷ mg Liver/ml homogenate = mg liver

Ethanol Determination

Ethanol was determined by the procedure of Bernt and Gutmann (72). As ethanol is oxidized to acetate by alcohol dehydrogenase the reduction of NAD to NADH is followed spectrophotometrically.

Reagents

1. 0.5% (v/v) Ethanol:

   Add 500 µl absolute ethanol to 100 ml volumetric flask containing 75 ml of cold double distilled water and bring up to mark with cold double distilled water. Dilute the 0.5% ethanol 50 fold to yield working standard 0.01% ethanol.

2. Sodium pyrophosphate buffer:

   (75 mM pyrophosphate buffer, pH 8.7, 75 mM semicarbazide, 21 mM glycine). Dissolve 33.3 g $Na_4P_2O_7$ X 10 H_2O + 8.25 g semicarbazide HCl + 1.65 g glycine in 800 ml distilled H_2O, adjust to pH 8.7 with about 16.7 ml 4N KOH and dilute to 1 liter with double distilled water. Stable for two weeks at 4°C.

3. Nicotinamide-adenine dinucleotide:

   (24mM ß-NAD Type III from yeast (Sigma, ST, Louis, MO). Dissolve 83.3 mg NAD in 5.0 ml double distilled water. Make fresh.

4. Alcohol dehydrogenase:

   Dissolve 10.0 mg of lyophilized enzyme (Sigma, St. Louis, MO) in 1.0 ml GDW (10 mg/ml). Make fresh.

5. Reagent Mixture:

   For one assay, 2.38 ml buffer, 100 µl NAD and 20 µl ADH.

Procedure

A standard curve was prepared by adding 0, 25, 50, 75 and 100 µl (Fig. 11) of working standard to 16 X 125 plastic tubes. Total volume was brought to 100 µl with double distilled water. Then, 2.5 ml of reagent mixture were added to each tube. A 100 µl aliquot of 50 fold diluted blood or 1:3 fold diluted neutralized PCA supernatant of liver sample was added to tubes containing 2.5 ml reagent mixture. The tubes were very gently vortexed and incubated at 37°C for 25 min in a shaking water bath. After cooling to room temperature absorbance was determined at 340 nm with a Beckman Model 34 Dual-Wavelength Spectrophotometer.

Calculations

Abs X 1/slope x 50 = ug ethanol/ml blood

Abs x 1/slope x 3 ÷ mg liver/ml PCA sn

STATISTICS

All data are presented as group means ± the standard error of the mean. The paired t-test was used to analyze the data from $^{14}CO_2$ analysis. The students t-test was used to analyze data between 2 groups (91) and Duncan's Multiple Range Test was employed if there were more than 2 groups (98).

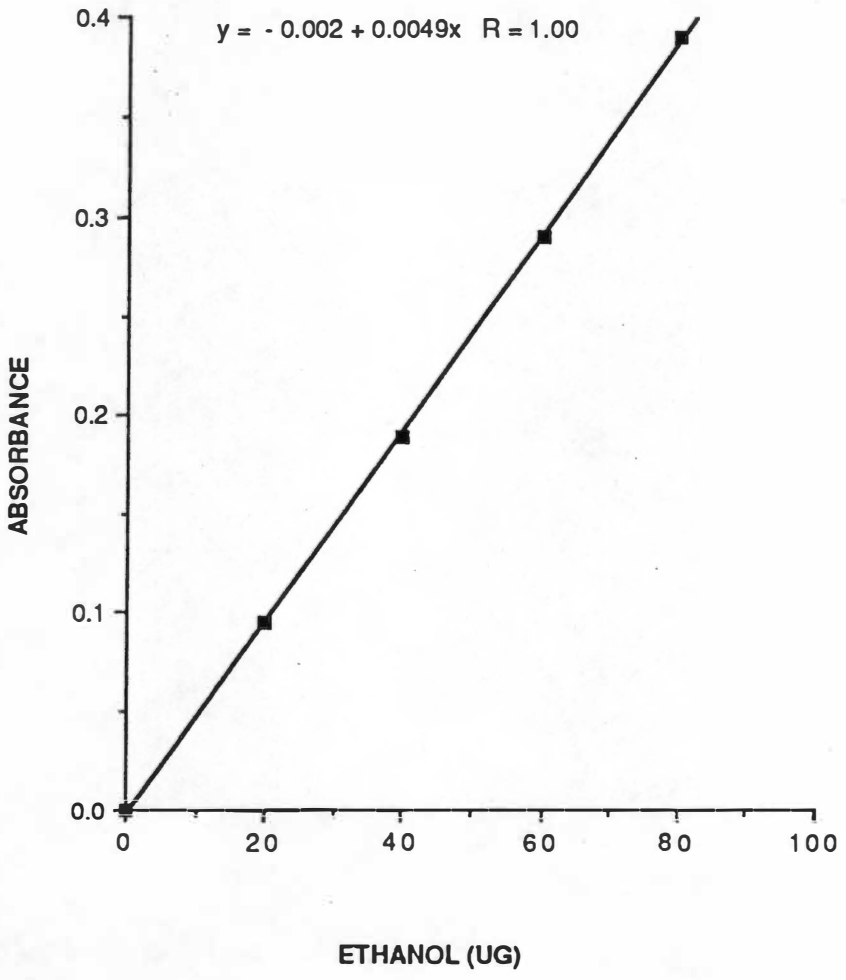

Figure 11. Ethanol Standard Curve

CHAPTER IV

RESULTS

Experiment 1 [1-^{14}C]-Ethanol Oxidation

Dietary carnitine supplementation for 7d resulted in a decrease in the oxidation of [1-^{14}C]-ethanol to $^{14}CO_2$ (Table 1). The amount of $^{14}CO_2$ present in the KOH traps is lower at all time points and significantly reduced at hours 4-12 in the CS group. The CS group also expired less $^{14}CO_2$ as a percentage of the total dose. The decrease in the percentage of total expired $^{14}CO_2$ in the CS group gradually increases from 0.5% at 1 h to 10.2% by 12 h (Table 1). By 24 h the NS and CS groups had expired 85.4% and 75.2% of the total dose as CO_2. In terms of both the total expired $^{14}CO_2$ and percentage of the total dose, dietary carnitine supplementation results in the inhibition of ethanol oxidation. This is consistent with earlier observations reporting elevated blood-ethanol concentrations with the CS diet (14, 15).

The urinary excretion of the ^{14}C-label, which theoretically could have been ethanol, acetaldehyde, acetate, or any metabolite that acetate could be incorporated into, is shown in Table 2. There is no significant difference in the 12 h urine volume between the NS (20.4ml) and CS (22.3 ml) groups. The total DPM in the urine after 12 h was also not significantly different between the NS (4.86×10^5) and CS (5.05×10^8) groups. The percentage of the total dose excreted in the urine after 12 h was 2.95% in the NS group and 2.91% in the CS group. Thus, carnitine is not affecting blood-ethanol concentrations by inhibiting ethanol or its metabolites excretion by the kidneys.

TABLE 1

EFFECT OF L-CARNITINE SUPPLEMENTED DIET ON [1-^{14}C]-ETHANOL OXIDATION.[1]

Hr. Post-Ethanol Administration	Groups				
	NS		CS		% of Dose NS - % of Dose CS
	DPMs x 10^{-6}	% of Dose	DPMs x 10^{-6}	% of Dose	% NS-CS
1	0.54	3.1	0.48	2.6	0.5
2	1.21	6.9	1.02	5.6	1.3
3	1.90	10.8	1.60	8.8	2.0
4	2.82	16.1	2.34*	12.8	3.3
5	3.60	20.5	3.12**	17.1	3.4
6	4.82	27.4	4.35**	23.8	3.6
7	6.12	34.8	5.46**	29.9	4.9
8	7.97	45.4	7.27**	39.8	5.0
10	11.31	64.4	10.40*	57.0	7.4
12	15.00	85.4	13.73**	75.2	10.2

1. Oxidation of ethanol is represented as expired $^{14}CO_2$.
   All values are group means ± SEM (n=3)
   NS = nonsupplemented. CS = L-carnitine supplemented.
   * $p \leq 0.05$
   **$p \leq 0.01$

TABLE 2

URINARY EXCRETION OF ^{14}C-LABEL FOR TWELVE HOURS AFTER ETHANOL DOSE[1]

	Groups	
Parameter	NS	CS
12 hr Urine Volume (ml)	20.4 ± 3.0^a	22.3 ± 3.9^a
Total DPM	$4.86 \times 10^5 \pm 3.7 \times 10^{4a}$	$5.05 \times 10^5 \pm 3.3 \times 10^{4a}$
% Dose	2.95 ± 0.36^a	2.91 ± 0.28^a

1. All values are group means ± SEM (n=5).
   Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented
   CS = L-carnitine supplemented

The distribution of the ^{14}C-label remaining in the body 12 h after ethanol administration was measured by determining the DPM in several tissues (Table 3). There were no significant differences in the DPM in plasma, liver, kidney, heart, brain, skeletal muscle, and the epididymal fat pad between the NS and CS groups, respectively. The highest concentration of radioactivity was in the plasma followed by the liver, kidney, heart, brain, skeletal muscle, and the epididymal fat pad for both the NS and CS groups. Assuming skeletal muscle, fat and blood represent 50%, 20%, and 6%, respectively, of total body mass, and using the actual weights of other organs. It was possible to account for approximately 95% of the total dose being expired as CO_2, excreted in the urine or remaining in the body.

In summary, carnitine supplementation reduced the amount of ethanol oxidized to CO_2 over a 12 h period with 85% (NS) and 75% (CS) of the total dose being expired as CO_2. There was no effect of carnitine supplementation on urine volume, the excretion of the label, or tissue distribution of the label 12 h after ethanol administration.

Experiment 2 Blood and Liver-Ethanol Concentrations and Redox State

Carnitine supplementation for 7d resulted in 30% higher blood-ethanol concentrations, which were 88.52 and 125.99 mg/dl in the NS and CS groups, respectively (Table 4). Since plasma-ethanol concentrations are 1.12 times higher than whole blood-ethanol concentrations (94), it would be expected that plasma DPM concentrations would also be higher in the carnitine supplemented group. Three hour plasma-DPM concentrations were 31,800 and 33,800 DPM/ml for the NS and CS

TABLE 3

EFFECT OF SUPPLEMENTARY CARNITINE ON ^{14}C TISSUE DISTRIBUTION TWELVE HOURS AFTER [1-^{14}C]-ETHANOL ADMINISTRATION.

	Groups	
Tissue	NS	CS
Plasma	80.78 ± 5.38[a]	75.17 ± 2.99[a]
Liver	22.10 ± 3.19[a]	28.54 ± 5.44[a]
Kidney	20.20 ± 1.90[a]	21.40 ± 1.48[a]
Heart	9.10 ± 0.78[a]	9.24 ± 0.97[a]
Brain	4.56 ± 0.82[a]	4.18 ± 0.21[a]
Skeletal Muscle	4.28 ± 0.82[a]	4.24 ± 0.65[a]
Epididymal Fat Pad	1.93 ± 0.29[a]	3.72 ± 2.35[a]

1. ^{14}C distribution is expressed as DPM/mg tissue or ml plasma. All values are group means (n=5), those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented
   CS = Carnitine supplemented

TABLE 4

BLOOD AND LIVER ETHANOL CONCENTRATIONS AND ^{14}C CONTENT THREE HOURS AFTER ETHANOL ADMINISTRATION[1]

Tissue	GROUPS NS	CS	% NS/CS
Blood Ethanol (mg/dl)	88.52 ± 5.78[a]	125.99 ± 1.57[b]	70%
Plasma DPM (DPM/ml)	31,800 ± 3600[a]	33,800 ± 3000[a]	94%
Liver Ethanol (µg/mg)	1.70 ± 0.21[a]	0.85 ± 0.08[b]	200%
Liver DPM (DPM/mg)	34.90 ± 1.60[a]	36.31 ± 1.90[a]	96%

1. All values are group means ± SEM (n=5). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
NS = nonsupplemented, CS = L-carnitine supplemented

groups, respectively (Table 4). Carnitine supplementation resulted in elevated blood-ethanol concentrations without the expected rise in plasma-DPM concentrations. The effect of carnitine supplementation on liver-ethanol concentrations is opposite of the blood-ethanol concentrations. Liver-ethanol concentrations were 1.70 and 0.85 µg/mg in the NS and CS groups, respectively. However, as in the plasma, there were no significant differences with respect to liver-DPM concentrations between the NS (34.9 DPM/mg) and CS (36.3 DPM/mg) groups.

The oxidation of ethanol leads to an excess of NADH and a shift in the redox potential of the liver to a more reduced state (10, 11, 94). It is possible to access the cytosolic redox state by measuring the lactate/pyruvate ratio and the inner-mitochondrial redox state by measuring 3-hydroxybutyrate/acetoacetate ratio. Carnitine supplementation did not significantly affect liver pyruvate and lactate concentrations and there were no significant differences in the lactate/pyruvate ratio between the NS (22.17) and CS (19.98) groups (Table 5). Ethanol administration lowered liver pyruvate concentrations, raised liver lactate concentration and doubled the lactate/pyruvate ratio when compared to non-ethanol treated rats (Table 5). Acetoacetate and 3-Hydroxybutyrate concentrations in the liver were not significantly different between the NS and CS groups (Table 5). There were also no significant differences in the 3-hydroxybutyrate/acetoacetate ratio between the NS (54.57) and the CS (51.29) groups (Table 5). Ethanol administration lowered liver acetoacetate concentrations, raised liver 3-hydroxybutyrate concentrations and increased the 3-

TABLE 5

LIVER REDOX PAIR CONCENTRATIONS THREE HOURS AFTER ETHANOL ADMINISTRATION[2]

Metabolite (nmoles/g liver)	Groups NS	CS	%NS/CS
Pyruvate (130)[2]	68.65 ± 9.96[a]	88.72 ± 17.13[a]	77%
Lactate (1620)[2]	2441.7 ± 426.9[a]	2490.5 ± 257.5[a]	98%
L/P	22.17 ± 4.00[a]	19.98 ± 0.70[a]	111%
Acetoacetate (55)[2]	14.00 ± 2.41[a]	13.20 ± 2.80[a]	06%
3-Hydroxybutyrate (144)[2]	728.25 ± 15.59[a]	622.0 ± 65.17[a]	117%
H/A	54.57 ± 3.87[a]	51.29 ± 4.31[a]	106%

1. All values are group means ± SEM (n=5). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented
   CS = L-carnitine supplemented
   L/P = Lactate/Pyruvate
   H/A = 3-Hydroxybutyrate/Acetoacetate
2. Values in parenthesis are from fed control rats (93).

Hydroxybutyrate/acetotate ratio from about 3 to 50 when compared to non-ethanol treated rats (Table 5). Plasma pyruvate concentrations were lowered by ethanol administration but were not significantly different between the NS (81.6 nmoles/ml) and CS (75.6 nmoles/ml) groups (Table 6). Ethanol administration elevated plasma lactate concentrations when compared to non-ethanol treated rats which is consistent with earlier reports of lactic acidosis following ethanol administration (39.96). Carnitine supplementation significantly increased plasma lactate concentrations from 3553 (NS) to 6849 (CS) nmole/ml (Table 6). The lactate/pyruvate ratio was also increased from 44 (NS) to 74 (CS) by carnitine supplementation (Table 6). Plasma acetoacetate concentrations were not significantly affected by either ethanol administration or carnitine supplementation (Table 6). Plasma 3-hydroxybutyrate concentrations and 3-hydroxybutyrate/acetoacetate were elevated by ethanol administration but not significantly affected by carnitine supplementation (Table 6)

Carnitine supplementation had a dramatic effect on plasma and liver carnitine concentrations 3h after ethanol administration (Table 7). Plasma concentrations of NEC were significantly increased from 33 (NS) to 54 (CS) nmoles/ml. Plasma concentrations of ASAC were significantly increased from 6.94 (NS) to 16.48 (CS) nmoles/ml of AIAC were significantly increased from 13.94 (NS) to 23.96 (CS) nmoles/ml. Liver NEC concentrations were 1.3 times in the CS group (199 vs 265 nmoles/g). Liver ASAC was 2.3 times higher (27 vs 63 nmoles/g) and AIAC was 2.7 times higher (8.7 vs 23.6 nmoles/g) in the CS group (Table 7).

TABLE 6

PLASMA REDOX PAIR CONCENTRATIONS THREE HOURS AFTER ETHANOL ADMINISTRATION[2]

Metabolite (nmoles/ml)	Groups NS	CS	% NS/CS
Pyruvate (234)[2]	81.6 ± 6.0[a]	75.6 ± 4.8[a]	108%
Lactate (2960)[2]	3553.0 ± 331.0[a]	6849.0 ± 1388.0[b]	52%
L/P	43.53 ± 2.43[a]	73.92 ± 8.79[b]	59%
Acetoacetate (76)[2]	116.0 ± 21.2[a]	68.4 ± 16.4[a]	170%
3-Hydroxybutyrate (82)[2]	395.2 ± 106.0[a]	260.8 ± 50.86[a]	152%
H/A	3.51 ± 0.68[a]	3.19 ± 0.98[a]	110%

1. All values are group means ± SEM (n=5). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented
   CS = L-carnitine supplemented
   L/P = Lactate/Pyruvate
   H/A = 3-Hydroxybutyrate/Acetoacetate
2. Values in parenthesis are from fed control rats (93).

TABLE 7

PLASMA AND LIVER CARNITINE CONCENTRATION THREE HOURS AFTER ETHANOL ADMINISTRATION[1,2]

Carnitine Fraction	Groups NS	CS	% NS/CS
Plasma (nmoles/ml)			
NEC	33.32 ± 1.88[a]	54.08 ± 2.31[b]	62%
ASAC	6.97 ± 1.73[a]	16.48 ± 1.73[b]	42%
AIAC	13.94 ± 1.91[a]	23.96 ± 7.43[b]	58%
Total	54.23 ± 5.52[a]	94.52 ± 7.43[b]	57%
Liver (nmoles/g)			
NEC	199.64 ± 11.74[a]	264.64 ± 20.08[b]	75%
ASAC	27.44 ± 4.60[a]	63.41 ± 5.02[b]	43%
AIAC	8.67 ± 3.37[a]	23.61 ± 2.62[b]	37%
Total	235.75 ± 19.71[a]	351.66 ± 27.75[b]	67%

1. All values are group means ± SEM (N=5). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = nonsupplemented, CS = L-carnitine supplemented
2. All NEC and ASAC values should be multiplied by a factor of 2.

Experiment 3 Urinary Excretion of [1-^{14}C]-Ethanol

Urine volume over 24h period following ethanol administration was not significantly different between the NS and CS groups (Table 8, Figure 12). Total 24h urine volume for the NS and CS groups was 26.8 and 27.9 ml, respectively. The largest volume was excreted in the first 3h and accounted for about 50% of the total 24h volume. The 3h urine volume is approximately equal to the volume of the gavage. From 3-6 h urine volume drops sharply followed by a linear increase over the next 18h (Figure 12). Food intake over the 24h period was not significantly different between the NS (18.4g) and CS (18.4g) groups (Table 8).Water intake over the 24h period was not significantly different between the NS (31.2 ml) and CS (32.4 ml) groups (Table 8)

The excretion of ethanol in the urine for 24h was not significantly affected by carnitine supplementation (Table 9, Figure 12). Total 24h ethanol for the NS and CS groups was 29.35 and 26.36 mg, respectively. This accounted for 2.9% of the total ethanol dose in the NS group and 2.6% in the CS group. The greatest amount of ethanol excreted in the urine was in the first 3h following ethanol administration. By 3h the NS group excreted 77% of the total 24h ethanol and the CS group 84% of the total 24h ethanol. After the initial 3h period ethanol excretion drops sharply and remains low for the remainder of the 24h period.

The total amount of radioactivity excreted over the 24h period was not significantly affected by carnitine supplementation (Table 9, Figure 12). Total 24h excretion of the ^{14}C-label was not significantly different between the NS (1.28 X 10^6

TABLE 8

TWENTY FOUR HOUR FOOD AND WATER INTAKE AND URINARY OUTPUT FOLLOWING ETHANOL GAVAGE IN RATS FED NS AND CS DIETS FOR SEVEN DAYS[2]

Parameter	Groups	
	NS	CS
24 hr Food (g) intake	18.4 ± 1.0[a]	18.4 ± 0.9[a]
24 hr Water (ml) intake	31.2 ± 3.0[a]	32.4 ± 4.1[a]
	Urine Volume (ml)	
0-3 hr	11.52 ± 1.13[a]	14.04 ± 1.24[a]
3-6 hr	1.66 ± 0.27[a]	2.24 ± 0.90[a]
6-12 hr	4.46 ± 0.70[a]	3.38 ± 0.74[a]
12-24 hr	9.16 ± 1.01[a]	8.28 ± 0.39[a]
Total	26.80 ± 3.11[a]	27.94 ± 3.27[a]

1. All values are group means ± SEM (n=5). Those bearing the same superscript within a row are not significantly different ($p > 0.05$.
NS = Nonsupplemented, CS = L-carnitine supplemented

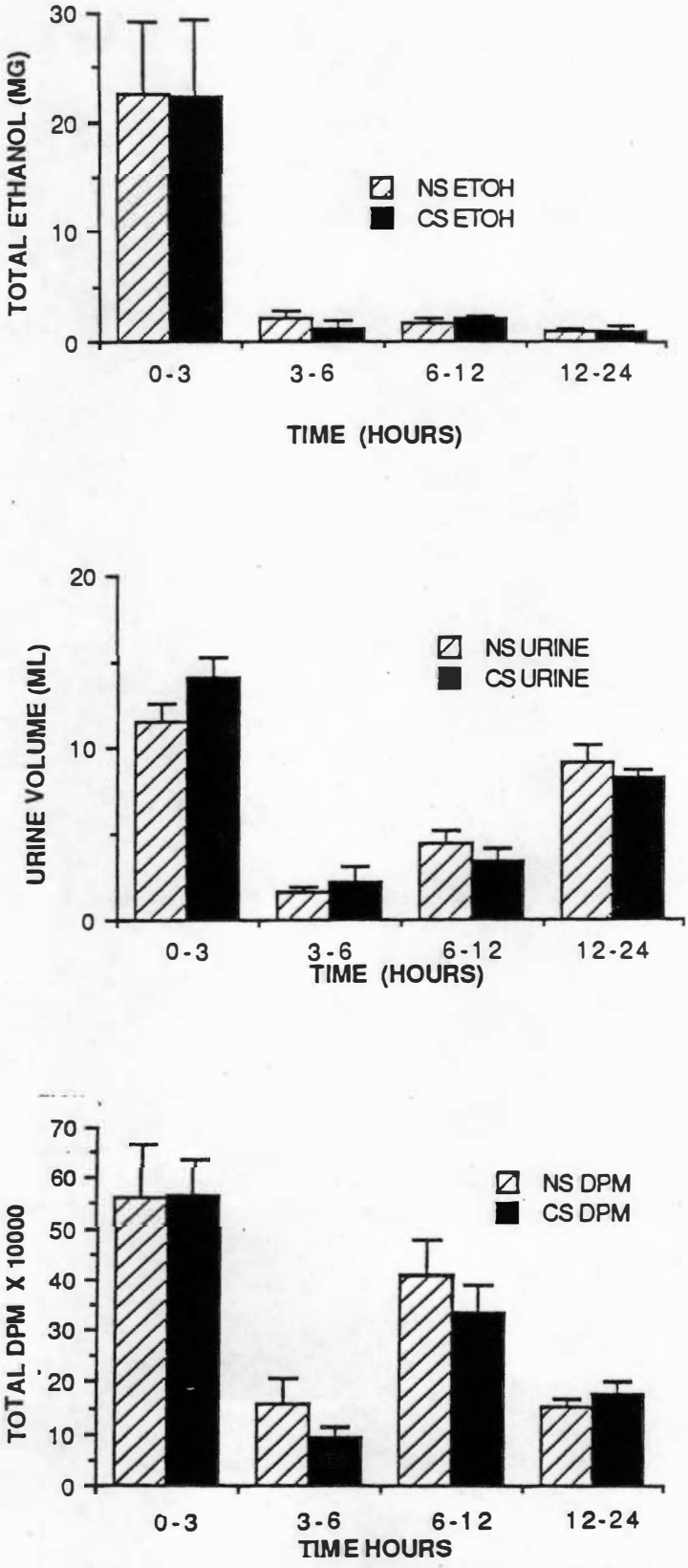

Figure 12. Urinary Volume, Ethanol, DPM

TABLE 9

TWENTY FOUR HOUR URINARY ETHANOL EXCRETION IN RATS FED NS AND CS DIETS FOR SEVEN DAYS[2]

Parameter	Groups NS	CS
Urine Ethanol (mg)		
0-3 hr	22.60 ± 66.50[a]	22.17 ± 7.42[a]
3-6 hr	2.02 ± 0.90[a]	1.24 ± 0.52[a]
6-12 hr	1.66 ± 0.46[a]	2.02 ± 0.36[a]
12-24 hr	1.00 ± 0.10[a]	0.90 ± 0.40[a]
Total	29.35 ± 9.31[a]	26.36 ± 8.72[a]
% Total Dose	2.90 ± 0.45[a]	2.60 ± 0.53[a]
Urine DPM (total)		
0-3 hr	$5.63 \times 10^5 \pm 1.01 \times 10^5$[a]	$5.68 \times 10^5 \pm 6.81 \times 10^4$[a]
3-6 hr	$1.57 \times 10^5 \pm 5.1 \times 10^4$[a]	$9.42 \times 10^4 \pm 2.16 \times 10^3$[a]
6-12 hr	$4.11 \times 10^5 \pm 6.5 \times 10^4$[a]	$3.36 \times 10^5 \pm 5.31 \times 10^3$[a]
2-24 hr	$1.52 \times 10^5 \pm 1.6 \times 10^4$[a]	$1.77 \times 10^5 \pm 2.29 \times 10^3$[a]
Total	$1.28 \times 10^6 \pm 2.36 \times 10^4$[a]	$1.15 \times 10^6 \pm 1.69 \times 10^5$[a]
% Total Dose	6.35 ± 0.82[a]	5.72 ± 0.73[a]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different
($p > 0.05$.
NS = Nonsupplemented, CS = L-carnitine supplemented
NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol

DPM) and CS (1.16 X 10^6 DPM) groups. Over 24h the NS group had excreted 6.35% of the total dose and the CS group had excreted 5.72% of the total dose. The greatest amount of ^{14}C-label was excreted in the first 3h and accounted for 44% (NS) and 49%(CS) of the total 24h excretion. Over the next 3h there was a sharp drop in urinary radioactivity, followed by an increase from 6-12h (Figure 12). In the final 12h only a small amount of the ^{14}C-label was excreted.

Dietary carnitine supplementation resulted in dramatic increases in the concentration of carnitine in the urine (Table 10). CS rats had a 156 fold increase in NEC concentrations, a 62 fold increase in ASAC concentrations and an 11 fold increase in AIAC concentrations for the first 3h (Table 10). Over the next 3h period CS rats had a 144 fold increase in NEC concentrations, a 23 fold increase in ASAC concentrations and a 86 fold increase in AIAC concentrations (Table 10). From 6-12h the CS rats had a 52 fold increase in NEC concentrations, a 3 fold increase in ASAC concentrations and a 16 fold increase in AIAC concentrations (Table 10). In the final 12h period the CS rats had a 42 fold increase in NEC concentrations, a 83 fold increase in ASAC concentrations and a 43 fold increase in AIAC concentrations (Table 10).

Urinary excretion of the total amount of carnitine (nmoles/ml X urine volume) was also significantly increased in the CS group (Table 11). In the first 3h the CS rats had 162 fold increase in NEC, a 58 fold increase in ASAC and a 27 fold increase in AIAC, (Table 11). From 3-6h the CS rats had a 97 fold increase in NEC, a 13 fold increase in ASAC and a 46 fold increase in AIAC (Table 11). From 6-12h the CS rats had a 39 fold increase in NEC, a 5 fold increase in ASAC, a 22 fold increase in AIAC (Table 11). In

TABLE 10

TWENTY FOUR HOUR URINARY CARNITINE CONCENTRATIONS FOLLOWING ETHANOL GAVAGE IN RATS FED NS AND CS DIETS FOR SEVEN DAYS[1,2]

Carnitine Fraction (nmoles/ml)	Groups NS	CS
0-3 hr		
NEC	4.33 ± 1.08[a]	674.5 ± 93.1[b]
ASAC	5.78 ± 0.58[a]	359.3 ± 38.2[b]
AIAC	0.58 ± 0.12[a]	6.47 ± 0.46[b]
3-6 hr		
NEC	7.76 ± 1.48[a]	1122.2 ± 127.2[b]
ASAC	8.85 ± 2.52[a]	207.5 ± 66.10[b]
AIAC	0.19 ± 0.04[a]	16.31 ± 3.16[b]
6-12 hr		
NEC	34.26 ± 9.01[a]	1788.3 ± 263.7[b]
ASAC	25.87 ± 12.13[a]	86.79 ± 23.21[b]
AIAC	1.36 ± 0.67[a]	22.14 ± 5.43[b]
12-24 hr		
NEC	49.89 ± 12.98[a]	2069.3 ± 203.4[b]
ASAC	12.65 ± 3.66[a]	1048.9 ± 57.09[b]
AIAC	0.38 ± 0.12[a]	16.26 ± 1.01[b]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different
   ($p > 0.05$.
   NS = Nonsupplemented, CS = L-carnitine supplemented
   NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol
2. All NEC and ASAC values should be multiplied by a factor of 2

TABLE 11

TWENTY FOUR HOUR URINARY TOTAL CARNITINE EXCRETION FOLLOWING ETHANOL GAVAGE IN RATS FED NS AND CS DIETS FOR SEVEN DAYS[1,2]

Carnitine Fraction	Groups NS	CS
	nmoles/time period	
0-3 hr		
NEC	50.77 ± 8.14[a]	8,253 ± 878[b]
ASAC	78.88 ± 13.40[a]	4,624 ± 329[b]
AIAC	3.02 ± 0.73[a]	81.7 ± 7.2[b]
Total	293.53 ± 93.19[a]	12,959 ± 1214[b]
3-6 hr		
NEC	17.72 ± 3.20[a]	1,739 ± 254[b]
ASAC	22.31 ± 10.59[a]	289 ± 66[b]
AIAC	0.56 ± 0.26[a]	26.1 ± 6.14[b]
Total	40.59 ± 14.05[a]	2,054 ± 326[b]
6-12 hr		
NEC	145.3 ± 45.6[a]	5,604 ± 962[b]
ASAC	74.6 ± 20.7[a]	381.8 ± 90[b]
AIAC	3.07 ± 0.78[a]	67.3 ± 13.8[b]
Total	222.9 ± 67.1[a]	6,053 ± 1066[b]
12-24 hr		
NEC	429.4 ± 86.60[a]	17,137 ± 1352[b]
ASAC	110.8 ± 28.90[a]	8,833 ± 8200[b]
AIAC	3.02 ± 0.73[a]	124.3 ± 4[b]
Total	543.20 ± 116.20[a]	26,094 ± 9556[b]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different
   ($p > 0.05$.
   NS = Nonsupplemented, CS = L-carnitine supplemented
   NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol
2. All NEC and ASAC values should be multiplied by a factor of 2

the final 12h the CS rats had a 40 fold increase in NEC, a 80 fold increase in ASAC, and a 41 fold increase in AIAC (Table 11.)

Experiment 4 [^{14}C(U)] Palmitate Oxidation Time Course

Supplementation of the diet with carnitine did not increase the oxidation of [^{14}C(U)]-palmitate to CO_2 (Table 12). There was about a 4h lag between any detectable $^{14}CO_2$ and the palmitate gavage. From 4-24h the increase in $^{14}CO_2$ in the KOH traps was close to linear. There was no effect of the length of time on the diets on palmitate oxidation with the total 24h $^{14}CO_2$ being essentially the same for 5, 10, 20, 30 and 40d. Both groups expired about 25% of the total dose as $^{14}CO_2$ by 24h.

Since there were no significant differences in palmitate oxidation over the forty day period, ten and forty days were chosen to represent short and long term carnitine supplementation. Plasma NEC concentrations were increased from 20.33 (NS) to 39.76 (CS) nmoles/ml in rats fed the diet for ten days (Table 13). Plasma ASAC concentrations were not significantly affected by ten days of supplementation. Plasma AIAC concentrations increased from 2.81 (NS) to 6.30 (CS) but were not significantly at the 95% level of confidence. Forty days of supplementation also resulted in a significant increase of the NEC fraction 30.67 (NS) vs. 47.06 (CS) nmoles/ml. Forty day ASAC concentrations were almost doubled by carnitine supplementation 6.81 (NS) vs 11.51 (CS), but owing to a large standard deviation were not significantly different at the 95% level of confidence (Table 13). Forty day AIAC concentrations were significantly elevated, 2.39 (NS) and 4.46 (CS) by carnitine supplementation

TABLE 12

EFFECT OF L-CARNITINE SUPPLEMENTED DIET ON [^{14}C(U)]-PALMITATE OXIDATION[1]

		Days on Diet				
Diet[2]	Time[3]	5	10	20	30	40
NS	4	129 ± 58	59 ± 5	31 ± 17	88 ± 20	68 ± 29
CS	4	155 ± 68	74 ± 38	30 ± 18	118 ± 33	70 ± 13
NS	8	422 ± 144	262 ± 84	249 ± 47	421 ± 125	291 ± 62
CS	8	385 ± 113	240 ± 13	342 ± 76	440 ± 123	258 ± 38
NS	12	1540 ± 314	827 ± 65	879 ± 100	992 ± 434	972 ± 183
CS	12	1155 ± 204	790 ± 114	818 ± 225	1039 ± 156	753 ± 100
NS	24	1667 ± 354	1412 ± 196	1219 ± 181	1862 ± 289	1577 ± 369
CS	24	1529 ± 80	1428 ± 134	1228 ± 84	1775 ± 167	1544 ± 121

1. Oxidation of palmitate is represented as expired $^{14}CO_2$ (DPM/ml). All values are group means ± SEM (N=3).
2. NS = Nonsupplemented. CS = L-carnitine supplemented.
3. Hours after palmitate gavage.

TABLE 13

PLASMA CARNITINE CONCENTRATIONS IN RATS FED NS AND CS DIETS FOR TEN AND FORTY DAYS[1,2]

Carnitine Fraction (nmoles/ml)	Groups		%NS/CS
	NS	CS	
10d			
NEC	20.33 ± 1.65[a]	39.67 ± 0.55[b]	51%
ASAC	8.07 ± 1.69[a]	7.38 ± 1.41[a]	91%
AIAC	2.81 ± 4.19[a]	6.30 ± 1.39[a]	45%
Total	31.81 ± 4.19[a]	53.44 ± 3.35[b]	59%
40d			
NEC	30.67 ± 1.97[a]	47.06 ± 5.53[b]	65%
ASAC	6.81 ± 1.18[a]	11.51 ± 3.29[a]	59%
AIAC	2.39 ± 0.09[a]	4.46 ± 0.61[b]	53%
Total	39.87 ± 3.24[a]	63.03 ± 9.43[b]	63%

1. All values are group means ± SEM (N=3 for NS and CS groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = nonsupplemented, CS = L-carnitine supplemented
2. All NEC and ASAC values should be multiplied by a factor of 2

(Table 13). In general, dietary supplementation for forty days was superior to ten days in elevating carnitine concentrations in the plasma.

Liver carnitine concentrations are presented in Table 14. There were no significant differences in NEC, ASAC, and AIAC concentrations between the NS and CS groups after 10d of carnitine supplementation. Thus the increased plasma concentrations of NEC and AIAC observed after 10d of carnitine supplementation (Table 13) are not reflected in the liver (Table 14). However, by 40d liver NEC is 25% higher, ASAC is 43% higher and AIAC is 55% higher in the CS group (Table 14). These differences would possibly be significant if the number of rats per group were increased. CS groups (Table 15) at either ten or forty days. There were also no significant differences between 10d and 40d of supplementation with respect to total lipids in the plasma and liver. Plasma and liver triglycerides were not significantly different between the NS and CS groups (Table 16) at either 10d or 40d. There was a significant decrease in plasma triglycerides in both the NS and CS groups at 40d. However, since these values are not from the same rats at both 10d and again at 40d it is difficult to draw any conclusion due to the individual variability of triglycerides in the plasma.

Experiment 5 [^{14}C(U)]-Palmitate Oxidation with Ethanol

The administration of ethanol 2h after [^{14}C(U)]-palmitate gavage significantly reduced the amount of palmitate oxidized to CO_2 for the next 22 hours (Table 17, Figure 13). However, supplementation of the diet with carnitine did not significantly

TABLE 14

LIVER CARNITINE CONCENTRATIONS IN RATS FED NS AND CS DIETS FOR TEN AND FORTY DAYS[1,2]

Carnitine Fraction (nmoles/g)	Groups		
	NS	CS	%NS/CS
10d			
NEC	154.31 ± 24.83[a]	163.40 ± 10.18[a]	94%
ASAC	74.60 ± 36.79[a]	77.68 ± 12.94[a]	96%
AIAC	2.91 ± 0.30[a]	3.12 ± 1.20[a]	93%
Total	231.83 ± 61.93[a]	244.12 ± 24.32[a]	94%
40d			
NEC	206.46 ± 3.60[a]	273.43 ± 36.04[a]	75%
ASAC	144.58 ± 6.71[a]	213.78 ± 50.03[a]	57%
AIAC	4.96 ± 1.13[a]	8.87 ± 1.25[a]	55%
Total	356.00 ± 11.44[a]	490.08 ± 87.32[a]	73%

1. All values are group means ± SEM (n=3 for NS and CS groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$). NS = Nonsupplemented, CS = L-carnitine supplemented
2. All NEC and ASAC values should be multiplied by a factor of 2

TABLE 15

PLASMA AND LIVER TOTAL LIPIDS IN RATS FED NS AND CS DIETS FOR TEN AND FORTY DAYS[1]

	Groups		
	NS	CS	% NS/CS
Plasma (mg/dl)			
10d	285.96 ± 30.98[a]	264.10 ± 16.48[a]	108%
40d	316.06 ± 30.10[a]	329.73 ± 23.94[a]	96%
Liver (mg/g)			
10d	32.16 ± 1.74[a]	29.46 ± 0.44[a]	109%
40d	30.15 ± 1.82[a]	33.02 ± 0.40[a]	91%

1. All values are group means ± SEM (n=3 for NS and CS groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
NS = Nonsupplemented, CS = 0.5% L-carnitine supplemented

TABLE 16

PLASMA AND LIVER TRIGLYCERIDES IN RATS FED NS AND CS DIETS FOR TEN AND FORTY DAYS[1]

	Groups		
	NS	CS	% NS/CS
Plasma (mg/dl)			
10d	77.90 ± 1.72[a]	80.01 ± 0.84[a]	97%
40d	56.83 ± 1.05[a]	53.41 ± 2.08[a]	106%
Liver (mg/g)			
10d	1.78 ± 0.09[a]	1.96 ± 0.12[a]	91%
40d	1.37 ± 0.19[a]	1.68 ± 0.13[a]	82%

1. All values are group means ± SEM (n=3 for NS and CS groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
NS = Nonsupplemented, CS = 0.5% L-carnitine supplemented

TABLE 17

[^{14}C(U)]-PALMITATE OXIDATION WITH AND WITHOUT ETHANOL ADMINISTRATION IN RATS FED NS AND CS DIETS FOR TEN DAYS.[1]

Hours After Palmitate Gavage	Groups			
	NS	CS	NSE	CSE
4	59 ± 5[a]	74 ± 38[a]	22 ± 17[b]	19 ± 12[b]
6	ND	ND	87 ± 19[b]	90 ± 17[b]
8	262 ± 84[a]	240 ± 13[a]	164 ± 22[b]	103 ± 33[b]
10	ND	ND	241 ± 25[b]	240 ± 50[b]
12	827 ± 65[a]	790 ± 114[a]	313 ± 36[b]	306 ± 66[b]
24	1412 ± 196[a]	1428 ± 134[a]	923 ± 112[b]	944 ± 171[b]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented, CS = L-carnitine supplemented
   NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol
   ND = Not determined

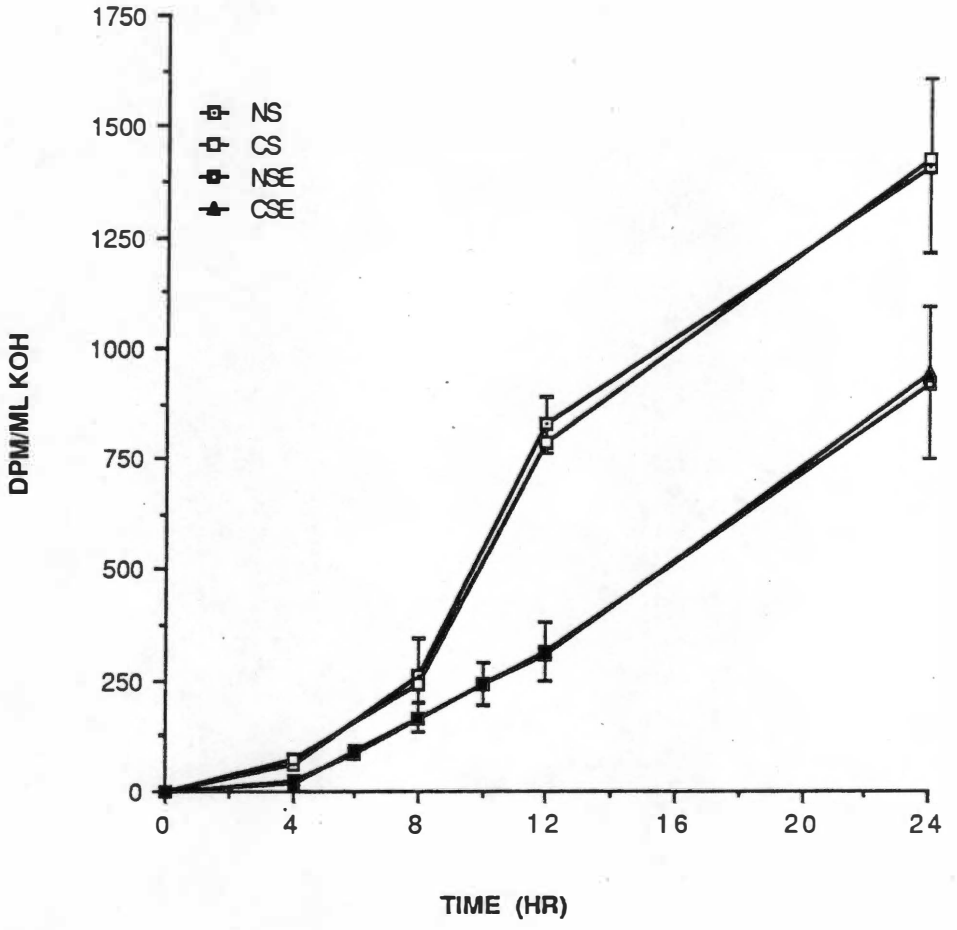

Figure 13. Palmitate Oxidation With and Without Ethanol Administration

affect palmitate oxidation with or without ethanol administration (Table 17). The ethanol treated rats oxidized 69%, 35%, 62% and 35% less palmitate to CO_2 at 4h, 8h, 12h, and 24h, respectively.

Plasma NEC concentrations were significantly higher in carnitine supplemented rats 20.33 (NS), 39.76 (CS), 24.82 (NSE) and 43.06 nmoles/ml (CSE) (Table 18), but there was no significant effect of ethanol on NEC values. Plasma ASAC concentration was significantly reduced in the NSE group, but there were no significant differences between the NS, CS, and CSE groups. AIAC concentrations were not significantly different between the NS, CS, NSE, and CSE groups (Table 18). There were no significant differences in NEC and ASAC concentrations in the liver between NS, CS, NSE, and CSE groups (Table 19). Ethanol administration resulted in significantly elevated 2.92 (NS) and 3.12 (CS) vs 7.29 (NSE) and 12.16 (CSE) AIAC concentrations in the liver (Table 19). There was a 2.4 fold increase in the NSE group over the NS group and a 3.9 fold increase in the CSE group over the CS group. However, comparing NS to CS and NSE to CSE groups revealed no significant differences in liver AIAC values.

Plasma and liver total lipids were not significantly different between the NS, CS, NSE and CSE groups (Table 20). Plasma triglycerides were also not significantly different between the four groups (Table 21). Ethanol administration resulted in a 3 fold increase in liver triglyceride concentrations 1.78 (NS) & 1.96 (CS) vs 5.20 (NSE) and 5.37 (CSE); but carnitine supplementation was without significant effect (Table 21).

TABLE 18

PLASMA CARNITINE CONCENTRATIONS WITH AND WITHOUT ETHANOL ADMINISTRATION IN RATS FED NS AND CS DIETS FOR TEN DAYS[1,2]

Carnitine Fraction (nmoles/ml)	Groups			
	NS	CS	NSE	CSE
NEC	20.33 ± 1.65[a]	39.76 ± 0.55[b]	24.82 ± 0.97[a]	43.06 ± 4.19[b]
ASAC	8.07 ± 1.70[a]	7.58 ± 1.41[a]	3.14 ± 0.64[b]	11.64 ± 1.36[a]
AIAC	2.81 ± 1.10[a]	6.30 ± 1.39[a]	3.25 ± 0.36[a]	5.48 ± 0.59[a]
Total	31.81 ± 4.45[a]	53.44 ± 3.35[b]	31.21 ± 1.97[a]	60.18 ± 6.14[b]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$).
   NS = Nonsupplemented, CS = L-carnitine supplemented
   NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol
2. All NEC & ASAC values should be multiplied by a factor of 2

TABLE 19

LIVER CARNITINE CONCENTRATIONS WITH AND WITHOUT ETHANOL ADMINISTRATION IN RATS FED NS AND CS DIETS FOR TEN DAYS[1,2]

Carnitine Fraction (nmoles/g)	Groups			
	NS	CS	NSE	CSE
NEC	154.31 ± 24.83[a]	163.40 ± 10.18[a]	197.25 ± 16.56[a]	144.95 ± 16.56[a]
ASAC	74.60 ± 36.79[a]	77.60 ± 12.94[a]	58.05 ± 18.73[a]	78.43 ± 16.78[a]
AIAC	2.92 ± 0.33[a]	3.12 ± 1.20[a]	7.29 ± 1.30[b]	12.16 ± 1.61[b]
Total	231.83 ± 61.95[a]	244.12 ± 24.32[a]	262.59 ± 36.06[a]	235.54 ± 34.95[a]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different
   ($p > 0.05$).
   NS = Nonsupplemented, CS = L-carnitine supplemented
   NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol
2. All NEC and ASAC values should be multiplied by a factor of 2

TABLE 20

PLASMA AND LIVER TOTAL LIPIDS WITH AND WITHOUT ETHANOL ADMINISTRATION IN RATS FED NS AND CS DIETS FOR TEN DAYS[1]

	Groups			
Tissue	NS	CS	NSE	CSE
Plasma (mg Lipid/dl)	285.96 ± 39.98[a]	264.10 ± 21.28[a]	254.01 ± 15.92[a]	254.90 ± 20.01[a]
Liver (mg Lipid/g)	32.16 ± 1.73[a]	29.46 ± 0.57[a]	30.58 ± 1.61[a]	32.72 ± 1.37[a]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different
($p > 0.05$.
NS = Nonsupplemented, CS = L-carnitine supplemented
NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol

TABLE 21

PLASMA AND LIVER TRIGLYCERIDES WITH AND WITHOUT ETHANOL ADMINISTRATION IN RATS FED NS AND CS DIETS FOR TEN DAYS[1]

Tissue	Groups			
	NS	CS	NSE	CSE
Plasma (mg Lipid/dl)	77.90 ± 1.72[a]	80.01 ± 0.84[a]	74.03 ± 6.20[a]	75.90 ± 5.84[a]
Liver (mg Triglyceride/g)	1.78 ± 0.09[a]	1.96 ± 0.12[a]	5.20 ± 0.27[b]	5.37 ± 0.64[b]

1. All values are group means ± SEM (n=3 for NS and CS groups, n=5 for NSE and CSE groups). Those bearing the same superscript within a row are not significantly different ($p > 0.05$.
NS = Nonsupplemented, CS = L-carnitine supplemented
NSE = Nonsupplemented + ethanol, CSE = L-carnitine supplemented + ethanol

In summary, ethanol administration resulted in decreased palmitate oxidation, but dietary carnitine supplementation did not affect palmitate oxidation with or without ethanol administration (Table 17). Carnitine supplementation significantly increased NEC levels but did not change ASAC and AIAC concentration in the plasma (Table 18). Liver AIAC concentrations were elevated by ethanol administration but not affected by carnitine supplementation. Liver NEC and ASAC levels were not changed by either carnitine supplementation or ethanol administration (Table 19). Ethanol administration also resulted in elevated triglyceride concentrations in the liver (Table 21).

CHAPTER V

DISCUSSION

Experiment 1 [1-^{14}C]-Ethanol Oxidation

The oxidation of [1-^{14}C]-ethanol to $^{14}CO_2$ was reduced by feeding the 0.5% L-carnitine diet for seven days (Table 1). This could offer a partial explanation for the earlier observations of elevated blood-ethanol concentrations following carnitine supplementation (14,15). It would be expected that blood and hepatocyte ethanol should be at an equilibrium. Ethanol oxidation within the hepatocyte would result in an influx of ethanol from the blood in order to maintain the equilibrium. The entry of ethanol into the hepatocyte would lower blood-ethanol concentrations. So, by reducing ethanol oxidation there should be an increase in blood-ethanol concentrations. This point has been demonstrated in several studies involving the use of ethanol oxidation inhibitors (73-76).

In the earlier studies (14,15), carnitine supplementation resulted in at least a 30% increase in blood-ethanol concentrations at hours 1 through 6. When ethanol oxidation was expressed as a percentage of the total dose expired as $^{14}CO_2$, it was possible to show a linear difference between the NS and CS groups (Table 1). At one h the difference is only 0.5% but by 12h the difference is 10%. There seems to be a discrepancy between ethanol oxidation to CO_2 and blood-ethanol concentrations (i.e. there is only a 2% reduction in ethanol oxidation to CO_2 at 3h but a 30% increase in blood-ethanol concentrations). The explanation probably lies in the accumulation of acetate following ethanol administration. Oxidation of ethanol to acetate will lower

blood-ethanol concentrations, but the acetate is not oxidized to CO_2 immediately, since the majority of ethanol is recovered in the blood as free acetate (39, 97). This is evident 8h after ethanol because administration by 8h blood-ethanol concentrations have almost returned to zero, but only a 45% (NS) and 39% (CS) of the ethanol dose has been oxidized to CO_2. As the excess reducing equivalents generated from ethanol are depleted, acetate is then oxidized to CO_2 and by 12 hr 85% (NS) and 75% (CS) of the total dose has been oxidized to CO_2.

By determining the amount of the ^{14}C-label remaining in the body or excreted in the urine it was possible to assess the fate of ethanol not expired as CO_2. Urinary excretion of [1-^{14}C]-ethanol or its metabolites was not significantly effected by carnitine supplementation and represented only 3% of the total dose (Table 2). It should be noted that evaporation or decomposition of components in the urine may have resulted in an underestimation in urinary excretion of the label. Tissue distribution of the ^{14}C-label was also not significantly affected by carnitine supplementation (Table 3).

Experiment 2 - Blood and Liver Ethanol Concentrations and Redox State

Dietary carnitine supplementation for 7d resulted in a 30% increase in blood-ethanol concentrations 3h after ethanol administration (Table 4) which is comparable to earlier data (14,15). At the same time liver-ethanol concentrations were twice as high in the NS group compared to CS rats (Table 4). In contrast, it would be expected that the number of DPM would be higher in the NS liver due to elevated ethanol concentrations, but there is no significant difference in the number of DPM

between the two groups (Table 4). The CS group would also be expected to have a higher number of DPM in the plasma, but there were no significant differences in plasma DPM between the NS and CS groups (Table 4).

One possible mechanism to explain the above situation is that carnitine could be inhibiting the entry of ethanol into the hepatocyte. This would explain the increased blood and decreased liver concentrations of ethanol in the CS group. The lower concentration of ethanol in the hepatocyte would present less of a "redox burden" on the liver and result in quicker oxidation of ethanol to acetaldehyde and acetate. Acetate would then accumulate within the hepatocyte because of the inhibition of the TCA cycle by ethanol (49). Normally most of the acetate would be activated to acetyl-CoA and transported out of the mitochondrial matrix as citrate and appear in the blood as free acetate (39, 19). However, Brass and Hoppel have shown that exogenous carnitine increased acetyl-carnitine concentrations in the liver and the effect was greater when the rats were starved (i.e. high acetate levels) and suggested that the hepatic acetyl-carnitine pool reflects changes in the hepatic acetyl-CoA pool (99). Table 7 clearly shows the increase in NEC, ASAC, and AIAC concentrations in the liver of CS rats. Thus, it is probable that the acetate formed from ethanol is being esterified to carnitine and remaining in the hepatocyte rather than entering the blood as free acetate. This would explain why there are lower liver-ethanol concentrations but no difference in the number of DPM in the CS group. In short, in NS rats the ethanol/metabolite ratio would be higher in the liver and lower in the blood than in CS rats.

It was originally suspected that carnitine supplementation was increasing ß-oxidation resulting in a more reduced state in the liver which would inhibit ethanol oxidation. However, the data from the above experiment would suggest that carnitine supplementation might result in a more oxidized state in the CS liver due to lower ethanol concentrations in the liver. Examination of liver revealed no significant differences in pyruvate, lactate, 3-hydroxybutyrate and acetoacetate between the NS and CS groups (Table 5). In both groups there were decreased liver concentrations of pyruvate and acetoacetate and increased concentrations of lactate and 3-hydroxybutyrate. The shift in redox pairs to a more reduced state is characteristic of ethanol metabolism and is the major metabolic consequence of ethanol oxidation (10, 11, 94).

Ethanol administration caused nearly a 3-fold decrease in plasma pyruvate concentrations, but there were no significant differences between the NS and CS groups (Table 6). Plasma lactate concentrations were increased by ethanol administration. Moreover, CS rats had almost a 2-fold increase in plasma lactate compared to NS rats. Himwich, et al (95) stated that ethanol acts as a glycogenolytic agent in the muscle and the glycogen is oxidized to lactate in the muscle and released into the blood. However, the role of carnitine in this process is not clear at this point in time. Plasma 3-hydroxybutyrate concentrations and 3-hydroxybutyrate/acetoacetate were elevated by ethanol administration but not significantly affected by carnitine supplementation (Table 6).

Earlier studies in this laboratory have demonstrated that plasma NEC concentrations are elevated by carnitine supplementation (for 7 days) without ethanol administration (15,77) and after 45 days on a liquid ethanol diet containing 36% of the calories as ethanol (13). However, in these studies plasma ASAC and AIAC fractions were not significantly increased. In this experiment all three plasma carnitine fractions were about 2-fold higher in the CS group 3h after ethanol administration (Table 7). Dietary carnitine supplementation (0.5% w/w) for 6 wk resulted in increased liver NEC but failed to increase ASAC & AIAC concentrations (96). But after 45d of the liquid ethanol diet NEC, ASAC, and AIAC concentrations were elevated in the CS group (13). Kondrup and Grunett (99) found that a single dose of ethanol had no effect of liver CoA content, but acetyl-CoA, acetylcarnitine and free carnitine concentrations were increased and concentrations of long-chain acyl-CoA and long-chain acylcarnitines were decreased. In this study NEC, ASAC and AIAC content of the liver were significantly higher in the CS group (Table 7). Ethanol administration results in an increased amount of acyl groups in the liver. Short-chain acyl groups are probably in the form of acetate which would be the result of ethanol oxidation. Long-chain acyl groups are probably the result of mobilization from adipose tissue through the action of ethanol on lipases (1). Increasing the concentrations of NEC in the liver through dietary carnitine supplementation should favor the formation of acyl-carnitines. In short, ethanol is increasing one substrate (acyl groups) and supplementation is increasing the other substrate (carnitine) for carnitine acyl transferases which results in increased concentrations of the product (acyl carnitines)

(Table 7). Three hours after an acute dose of ethanol all three carnitine fractions were higher in the CS group (Table 7). Liver carnitine concentrations have been shown not to be altered by carnitine supplementation without ethanol administration (96), but after 45 days on the liquid ethanol diet NEC, ASAC, and AIAC concentrations were elevated in the CS groups (13). Three hours after an acute dose of ethanol all three carnitine fractions were higher in the CS group (Table 7).

Experiment 3 Urinary Excretion of [1-^{14}C]-Ethanol

Carnitine is known to enhance the excretion of organic acids associated with metabolic disorders such as methylmalonic and propionic aciduria (89-91). Ethanol oxidation creates an abundance of acetate in the liver and blood. Ethanol also causes an influx of fatty acids into the liver. Fatty acids have the potential to become esterified to carnitine and excreted in the urine. Therefore, it was decided to determine if carnitine supplementation would increase the excretion of acyl-carnitines following ethanol administration.

Urinary volume was not significantly affected by carnitine supplementation for 24h following ethanol administration (Table 8, Figure 12). The largest volume was excreted in the first 3h following ethanol administration. This volume is approximately equal to the volume of the gavage. For the next 9h urine volume drops sharply followed by an increase over the next twelve hours. Food and water intakes were not significantly different between the NS and CS groups (Table 8).

Twenty four hour excretion of ethanol in the urine was not significantly different between the NS and CS groups (Table 9, Figure 12). The highest amount of ethanol

in the urine was for the first 3h with the NS group excreting 77% of the total 24h ethanol and the CS group excreting 84%. Ethanol excretion declined steadily for the next 21h. This coincides with blood-ethanol concentrations peaking at three hours and sharply declining to near zero by 8h.

The total amount of radioactivity excreted over the 24h period was not significantly affected by carnitine supplementation (Table 9, Figure 12). The greatest amount was excreted in the first three hours. Over the next 3h there was a sharp drop in radioactivity; followed by an increase from 6 to 12 h. In the final 12h only a small amount of the ^{14}C-label is excreted.

A possible scenario to explain the above situation is as follows. The gavage results in a large increase in blood volume and blood-ethanol concentrations. The body reacts by increasing urinary excretion and ethanol is excreted along with water. This would account for the increased urine volume, total ethanol and DPM for the first 3h. Over the next 3h urine output drops sharply and blood-ethanol concentrations decline; explaining decreased total ethanol and DPM. From 6 to 12 h blood-ethanol concentrations virtually disappear, the amount of ethanol in the urine drops, but radioactivity in the urine rises sharply. It would seem reasonable to assume that the ethanol has been metabolized to acetate by the liver, transported out of the mitochondria, into the blood and excreted in the urine. By 12h hours most of the acetate derived from ethanol has been further oxidized to CO_2 or excreted in the urine and the radioactivity in the urine drops.

Urinary excretion of NEC, ASAC, and AIAC is dramatically increased by carnitine supplementation (Table 10, 11). The high NEC values in the urine are expected since excess carnitine is excreted in the urine unchanged. Of particular interest are the ASAC and AIAC fractions. In the first 3h following the ethanol gavage there is a 60 fold increase in ASAC and a 27 fold increase in AIAC in the CS group. The total ASAC and AIAC is 4,706 nmoles. During this same time period there were no significant differences in ethanol excretion or total DPM (Table 9). In other words, the increases in ASAC and AIAC are not reflected in DPM or ethanol excretion. Over the next 3h total ASAC and AIAC drops to 315 nmoles, but there still is a 13-fold increase in ASAC and a 46-fold increase in AIAC in the CS group. From 6 to 12 h there is a slight increase (≈100 nmoles) in total ASAC and AIAC. However, the number of DPM in the urine increases dramatically (Table 19). The largest amount of total ASAC and AIAC, 8957 nmoles, are excreted from twelve to twenty four hours. There is an 80-fold increase in ASAC and a 41-fold increase in AIAC in the CS group. These increases are not reflected in DPM excreted which actually drop (Table 9).

Originally it was presumed that the excess acetate generated from ethanol oxidation would be esterified to carnitine and excreted in the urine. This was observed in humans given IV carnitine after drinking wine (61). It is definitely clear that the excretion of both short and long chain acyl-carnitines is enhanced by carnitine supplementation, but it is not clear if the acyl-units are derived from ethanol. The number of DPM would be expected to increase and follow the same pattern of

excretion as ASAC and AIAC if the acyl-units were derived from ethanol, but there are no significant differences in DPM between the NS and CS groups. Furthermore, DPM peaked from 0-3 hrs and 6-12 hrs while carnitine peaked from 0-3 hr and 12-24 hr. So, it appears that the acyl-units being excreted in the urine are not entirely derived from ethanol.

Experiment 4 (^{14}C(U)] - Palmitate Oxidation Time Course

A number of early in vitro studies demonstrated that the addition of carnitine to preparations of rat liver slices (78), isolated mitochondria (79), hepatocytes (80) or the perfusing media of livers (81) could stimulate fatty acid oxidation and ketogenesis. However, the conclusions of several (82-87) in vivo studies were contradictory with regards to the stimulation of fatty acid oxidation and were performed under a variety of conditions. One of the original theories for elevated blood-ethanol concentrations and the decrease in hepatic steatosis was that dietary carnitine supplementation is able to stimulate fatty acid oxidation. Therefore, it was decided to determine if fatty acid oxidation was stimulated by dietary carnitine supplementation in rats under control conditions. Dietary carnitine supplementation over a 40d period did not significantly affect the oxidation of [^{14}C(μ)]-palmitate to $^{14}CO_2$ over a 24h period (Table 12). The amount of palmitate oxidized to CO_2 in 24h was only 25% of the total dose; compared to ethanol where 80% of the dose was oxidized to CO_2 in 12h. This demonstrates the urgency that the body places on the metabolism of ethanol compared to a natural dietary component.

Carnitine supplementation for ten days resulted in significantly higher plasma NEC and unchanged ASAC and AIAC (Table 13). Which is in agreement with earlier data (15, 77), but contrasts with the elevated ASAC and AIAC concentrations seen three hours after ethanol (Table 7). Carnitine supplementation for forty days resulted in significantly higher plasma NEC and AIAC concentrations. ASAC concentrations were almost doubled in the CS group, but not significantly different. It appears that long term carnitine supplementation has an inductive effect on the synthesis of acyl-carnitines.

Examination of liver carnitine revealed no significant differences in the NEC, ASAC, and AIAC fractions between the NS and CS groups after ten days of feeding the CS diet (Table 14). There was a substantial increase in NEC, ASAC and AIAC concentrations in the 40d livers. However, the increase was not significant because of large standard deviation. This may prove to be significant if larger numbers of rats are used. It should also be pointed out the plasma increases in the NEC fraction are not present in the liver after ten days, but after forty days liver carnitine content is reflecting plasma carnitine values in terms of % NS/CS.

Plasma and liver total lipids and triglycerides were not significantly different between the NS and CS groups at 10 or 40 d of carnitine supplementation (Tables 15, 16). No decrease would be expected since there were no differences in palmitate oxidation.

Experiment 5 [^{14}C(U)]Palmitate Oxidation with Ethanol

The inhibition of fatty acid oxidation by ethanol administration is one of the primary causes of hepatic steatosis (46-49). It was originally speculated that carnitine supplementation would partially prevent the decrease in fatty acid oxidation by ethanol. Experiment IV, Table 12, demonstrated that carnitine supplementation did not stimulate fatty acid oxidation under normal circumstances, but it was not clear whether or not carnitine supplementation would help alleviate the acyl burden imposed upon the liver by ethanol.

Ethanol was orally administered two hours after a [^{14}C(U)]-palmitate gavage. The oxidation of palmitate to CO_2 was significantly reduced for the next twenty two hours (Table 17, Figure 13). However, carnitine supplementation did not significantly affect $^{14}CO_2$ expiration with or without ethanol. On the average there was about a 50% reduction in palmitate oxidation with ethanol treatment.

As demonstrated earlier (13, 15, 77, 96), there was an increase in NEC concentrations in plasma in the CS and CSE groups, but ethanol administration did not significantly change NEC concentrations (Table 18). AIAC concentrations were significantly reduced in the NS group which may indicate an increased need for carnitine (that can't be met by endogenous synthesis or the chow diet) following ethanol administration. There were no significant differences in AIAC concentrations between the NS, CS, NSE, and CSE groups. Thus the increased plasma carnitine values observed in the CS group at three hours after ethanol administration (Table 7) have returned to the pre-ethanol status (pattern) after 24h.

There were no significant differences in liver NEC and ASAC concentrations between the NS, CS, NSE and CSE groups (Table 19). AIAC concentrations were significantly higher in the NSE and CSE groups, but carnitine supplementation did significantly change AIAC concentrations. The increased AIAC concentration is probably the result of increased concentrations of long chain fatty acids available for esterification to carnitine. Again, the increased NEC, ASAC, and AIAC concentrations observed three hours (Table 7) after ethanol administration are not evident at twenty four hours.

Unlike what occurs with chronic ethanol administration (12, 13) plasma and liver total lipids were not significantly increased by a single acute dose of ethanol in the NS, CS, NSE, and CSE groups (Table 20). The acute dose did increase liver triglycerides, but the increase was not affected by carnitine. Plasma triglycerides were not significantly different between the NS, CS, NSE, and CSE groups (Table 21).

CHAPTER VI

SUMMARY

The purpose of this dissertation was to explore the potential mechanisms by which dietary carnitine supplementation causes elevated blood-ethanol concentrations and decreased hepatic lipids following ethanol administration. The hypothesis was that dietary carnitine supplementation would result in higher than normal concentrations of carnitine and acyl-carnitines and alter the oxidation of ethanol and its metabolic consequences. Ethanol administration results in an increase in the hepatic pool of acyl-CoA's. Two fates of hepatic acyl-CoA's is esterification to glycerol or carnitine. Esterification to glycerol would lead to the storage of the acyl groups as triglycerides and cause hepatic steatosis. Esterification to carnitine would lead to an increase in the oxidation of the acyl groups or their transport out of the hepatocyte since acyl-CoA is impermeable to cell membranes. By increasing the availability of carnitine, through dietary carnitine supplementation, it seemed possible to increase the quantity of acyl moities being esterified to carnitine. The excess carnitine would also be available for esterification to the excess acetylmoities generated from ethanol oxidation. Thus, by binding Acyl and Acetyl moities, carnitine would be shuttling these groups away from their normal routes following ethanol oxidation.

The effect of carnitine supplementation on ethanol oxidation was examined by feeding rats purina chow as such (non-supplemented, NS) or supplemented with 0.5% L-carnitine (carnitine supplemented, CS) for 7d. The rats were then given an oral dose of [1-^{14}C]-ethanol and placed in respiratory chambers. Expired $^{14}CO_2$ was

collected for the next 12h at which time the rats were killed. Ethanol oxidation to $^{14}CO_2$ was significantly reduced at hours 4-12 in the CS group. At the end of 12h the NS group had expired 85% of the total dose as CO_2 and the CS group 75%. There were no significant differences in urinary excretion of the ^{14}C-label between the two groups which accounted for only about 3% of the total dose. The ^{14}C-label remaining in tissues after 12h was also not significantly affected by carnitine supplementation. Thus a partial explanation for carnitine supplementation resulting in increased blood-ethanol concentrations is a reduction in ethanol oxidation. The effect of carnitine supplementation on blood and liver-ethanol concentrations and the redox state was examined by feeding the rats NS and CS diets for 7d. The rats were then given an oral dose of [1-^{14}C]-ethanol and killed 3h later. Blood-ethanol concentrations were 88.5 mg/dl in the NS group and 125.9 mg/dl in the CS group at 3h. Liver-ethanol concentrations were 1.70 µg/mg in the NS group and 0.85 µg/mg in the CS group. However, there were no significant differences in the number of DPM in the plasma or liver between the two groups. Measurement of liver concentrations of pyruvate, lactate, acetoacetate, and 3-hydroxybutyrate revealed no significant differences between the NS and CS groups. Plasma concentrations of lactate were 2-fold higher in the CS group, but pyruvate, acetoacetate and 3-hydroxybutyrate concentrations were not significantly different between the two groups. Plasma and liver non-esterified carnitine (NEC), acid soluble acylcarnitine (ASAC) and acid insoluble acylcarnitine (AIAC) concentrations were all significantly higher in the CS group 3h after ethanol administration which would support the hypothesis of increased acyl-carnitines after

ethanol administration. It is concluded that dietary carnitine supplementation results in increased blood-ethanol concentrations and decreased liver-ethanol concentrations possibly by inhibiting the entry of ethanol into the hepatocyte. Additionally, carnitine supplementation had no effect on the redox state of the liver but may effect muscle metabolism due to elevated plasma lactate concentration. Finally, both of the above effects may be related to the elevated carnitine concentrations observed in the CS group.

The effect of carnitine supplementation on the urinary excretion of ethanol, its metabolites and carnitine was determined by feeding rats the NS and CS diets for 7d. The rats were then given an oral dose of [1-^{14}C]-ethanol and urine was collected for the next 24h. Food intake, water intake and urine volume were not significantly different between the NS & CS groups over the 24h period. Urinary excretion of ethanol and the ^{14}C-label were not significantly affected by carnitine supplementation. Urinary excretion of NEC, ASAC, and AIAC was dramatically increased by carnitine supplementation. From 0-3h there was a 165, a 60, and a 27-fold increase in NEC, ASAC & AIAC, respectively in the CS group. From 3-6h there was a 100, a 13, and a 46-fold increase in NEC, ASAC & AIAC, respectively in the CS group. From 6-12h there was a 40, a 5, and a 20-fold increase in NEC, ASAC & AIAC, respectively in the CS group. From 12-24h there was a 40-fold (NEC), a 80-fold (ASAC), and a 40-fold (AIAC) increase in the CS group. It is concluded that dietary carnitary supplementation enhances the excretion of acyl groups in the urine following ethanol administration without increasing excretion of ethanol or its metabolites. The effects

of supplementary carnitine on the oxidation of palmitate was determined by feeding the NS and CS diets for 5, 10, 20, 30, and 40 days. A single oral dose of [^{14}C(U)]-palmitate was given and the rats were placed in a respiratory chamber. Expired CO_2 was trapped in KOH solution which was counted to determine amount of radioactivity at 4, 8, 12 & 24 h post-gavage. There were no significant differences between the NS and CS groups with regard to the rates of $^{14}CO_2$ appearance or percent of the dose following 5, 10, 20, 30 & 40 days of feeding CS diet. Because there were no significant differences in palmitate oxidation over the 40d period. 10d and 40d were chosen to represent short and long term carnitine supplementation. Plasma NEC concentrations were significantly higher in the CS group after 10d of supplementation, but ASAC and AIAC concentrations were not affected. After 40d of supplementation plasma NEC and AIAC concentrations were significantly higher in the CS group and ASAC concentrations were doubled in the CS group but not statistically significant. There were no significant differences between the NS and CS groups in liver concentrations of NEC, ASAC, and AIAC at 10 and 40d. However, 40d was superior to 10d in elevating NEC, ASAC, and AIAC in the liver. Analyses of plasma and liver of NS and CS rats revealed no significant differences in total lipids or triglycerides. It is concluded that in the intact rat, 0.5% L-carnitine supplementation did not significantly alter oxidation of orally administered palmitate to CO_2. Additionally, supplementary carnitine was neither hypolipidemic nor lipotropic in normal rats fed Purina rat chow.

The effect of carnitine supplementation and ethanol administration on palmitate oxidation was determined by feeding rats NS and CS diets for 10d. They were then given an oral dose of [^{14}C(U)]-palmitate followed by an oral dose of ethanol 2h later. Expired $^{14}CO_2$ was then collected for the next 24h then killed. Ethanol administration resulted in decreased palmitate oxidation, at hours 4-24, but dietary carnitine supplementation did not affect palmitate oxidation with or without ethanol administration. Carnitine supplementation significantly increased NEC levels but did not change ASAC and AIAC concentration in the plasma. Liver AIAC concentrations were elevated by ethanol administration but not affected by carnitine supplementation. Liver NEC and ASAC levels, which were elevated 3h after ethanol administration, were not significantly different 24h after ethanol administration. Ethanol administration also resulted in elevated triglyceride concentrations in the liver, but liver total lipids were unchanged. Plasma total lipids and triglyceride concentrations were not significantly affected by ethanol administration and/or carnitine supplementation. It is concluded that ethanol administration reduced the amount of palmitate oxidized to CO_2 over a 24h period, but dietary carnitine supplementation did not prevent the ethanol-induced decrease in palmitate oxidation.

In summary, dietary carnitine supplementation has the following effects on ethanol and palmitate metabolism:

1) It decreases the oxidation of ethanol to CO_2 without affecting the redox state of the liver.
2) It results in higher blood-ethanol and lower liver-ethanol concentrations.

3) It results in a tremendous increase in the urinary excretion of both short and long chain acyl groups following ethanol administration.

4) It does not affect the oxidation of palmitate to CO_2 with or without ethanol administration.

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VITA

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