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HENRY S. CABIN

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The Effects of Ethanol on  
Ketone Body Metabolism of Fasted Rats

by  
Henry S. Cabin  
B.A. University of Pennsylvania, 1971

Presented in partial fulfillment of the requirements  
for the degree of Doctor of Medicine,  
Yale University School of Medicine  
-March, 1975-





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To Jim Gilliam- for his invaluable help with the free fatty acid assay.

To my wonderful parents

To Florence



## INTRODUCTION

Alcoholic ketoacidosis is a clinical entity with a significant morbidity (1-5). It is apparently rare, considering the small number of reported cases and the large population of alcoholics, but its incidence is probably underestimated. The nitroprusside test (Acetest tablets), which is the common method of testing serum and urine for ketones, measures acetoacetate but not B-hydroxybutyrate. Alcoholic ketoacidosis is characterized by a relatively high ratio of B-hydroxybutyrate to acetoacetate (3). Thus alcoholics who present with severe acidosis and a large anion gap may have markedly elevated B-hydroxybutyrate and total ketones, but only insignificant or moderate amounts of ketones in their urine and serum when tested with Acetest tablets. Consequently, the acidosis may then be attributed to lactate or an exogenous anion (3).

The fact remains, however, that a minority of non-diabetic alcoholics develop ketoacidosis, while most do not. The answer to this can only be found when the pathogenesis of alcohol-induced ketosis is understood. Controlled animal studies are consequently needed in which dietary protocols and patterns of alcoholic ingestion parallel the usual clinical picture of alcoholic ketoacidosis (i.e. fasting with repeated ingestion of large doses of ethanol).

The current study was undertaken as an attempt to establish an animal model of alcoholic ketoacidosis, and then study free fatty



acids, insulin, and glucose levels associated with the alcohol-induced hyperketonemia in order to begin to understand the pathogenesis of this disorder.



## LITERATURE REVIEW

### I. Case Reports of Alcoholic Ketoacidosis

The first documented cases of non-diabetic acidosis attributable to increased ketones in individuals, with a history of chronic ethanol ingestion, were reported in 1940 by Dillon (1). Since that time, a total of fifteen cases have been presented in the literature (2-5). The latest report by Cooperman et al (5) suggests that alcoholic ketoacidosis may be considerably more common than is generally appreciated, and that as many as one out of five cases of ketone body acidosis in the Beth-Israel hospital population are diagnosed as non-diabetic alcoholic ketoacidosis. Dillon in 1940 observed (1) "If the combination of liver damage and food deprivation, occurring most frequently in association with acute alcoholism, can cause such severe ketone acidosis as we have described, the question naturally arises as to why this state is not more frequently observed. To this we have no answer, except that we have diagnosed such cases more frequently since being aware of their existence."

Certain historical features, physical findings and laboratory data are present often enough in the reported cases (1-5) to be considered helpful in making the diagnosis of alcoholic ketoacidosis. Other data, although present in all cases in a particular report, do not prove to be consistent features of alcoholic ketoacidosis throughout the rest of the literature.

Sixteen of twenty-two reported cases in the literature were





females and more than half of these were younger than forty. Cooperman reported one case of a pregnant thirty-one year old with documented episodes of alcoholic ketoacidosis at twenty-eight and thirty-two weeks of gestation (5). Of the six males, four were over the age of fifty. The average age of all patients was forty-two with a range of twenty-six to sixty-nine. Five patients had more than one admission for non-diabetic ketoacidosis (1-3,5), and one had seventeen episodes of alcoholic ketoacidosis over a period of seven years (2).

All patients gave a history of chronic ethanol ingestion. Most described an increase in alcohol consumption for a period of days or weeks which was terminated by anorexia, nausea and vomiting with decreased food intake for a period of twenty-four to seventy-two hours prior to admission. Three patients reported an ethanol "binge" followed by decreased food intake without nausea or vomiting (3,4). Although most patients had not consumed any alcohol for at least twenty-four hours prior to admission, a few had continued drinking up until the time of admission (3,4,5). None of the patients reported a definite personal or family history of diabetes. One individual had a history of glucosuria only when pregnant (2). The most common reasons for seeking medical attention were abdominal pain, usually epigastric, nausea and vomiting with decreased food intake, and weakness. Several patients complained of shortness of breath and one individual came to the emergency room because of a transient episode of blurred vision (3). Three patients presented in deep coma which proved to be secondary to hypoglycemia in two of the three patients (1). Despite the severe pH abnormalities,



all other patients were conscious on admission. Many, however, exhibited variable degrees of confusion and disorientation. Review of physical exams revealed no uniformly present findings. Those findings that were present in many, but not all, individuals included: poor skin turgor and other evidence of dehydration, rapid respiration, often of the Kussmaul type, odor of acetone on breath, tender epigastrium, and an enlarged liver. All subjects for whom pH,  $p\text{CO}_2$  and bicarbonate were measured demonstrated a partially compensated metabolic acidosis with pH's ranging from 6.96 to 7.29,  $p\text{CO}_2$ 's from less than 10 to 32 and bicarbonates from 5 to 19. Urine and serum tested with Acetest tablets for ketones, frequently did not reflect the degree of ketosis as determined by specific assays of B-hydroxybutyrate and acetoacetate (3,5). In some instances ketones were not detected in undiluted serum or urine, or were only weakly positive. As was mentioned earlier, this can be explained by the increased ratio of B-hydroxybutyrate to acetoacetate in alcoholic ketoacidosis. Cooperman (5) found that the B-hydroxybutyrate to acetoacetate ratio was inversely proportional to the pH; and the mean B-hydroxybutyrate to acetoacetate ratio was 5.2, with a mean B-hydroxybutyrate of 8.7 meq./l., and a mean acetoacetate of 2.1 meq./l. In Levy's series (3), the mean B-hydroxybutyrate to acetoacetate ratio was 7.2, with a mean B-hydroxybutyrate of 10.8 meq./l. and a mean acetoacetate of 2.5 meq./l. Admission glucoses generally showed mild hyperglycemia, but they ranged from 20 to 330. The majority, however, were between 120 and 200. Only three patients had admission glucoses less than 30 and two of these were in Dillon's original series (1). Initial serum insulin concentrations



have been less than 12 uunits/ml. [6.4 uunits/ml. (simultaneous glucose of 145 mg./100ml.), 12 (190), 6 (25), 6 (202), 7 (194), and less than 5 uunits/ml. in two cases with a mean glucose of 143 mg./100ml.] These are appropriate for the normal fasting state but in all, except the individual with a glucose of 25 mg%, they are inappropriately low for the correspondingly elevated glucoses. Cortisol was measured in six patients (3,5) and was markedly elevated in all (71 to 115 ug./100ml.). Growth hormone, in the same patients, was also generally increased, but, was more variable (2.8 to 29 ng./ml.). Serum free fatty acid concentration was measured in six cases (3,5), and was extremely elevated in all (1,808 to 3,789 ueq./l.). Plasma lactate was very high in only one case (3) and normal in several others. Patients generally had evidence of some liver disease with at least one abnormal liver function test, but none had evidence of severe disease. Although some patients had evidence of pancreatitis with elevated amylases, 50% had normal values.

In 1940, Dillon (1) recognized that non-diabetic ketoacidosis in alcoholics was as effectively treated without insulin as with it, but that glucose was essential. This has been borne out in subsequent reports. The acidosis is usually corrected in twelve to twenty-four hours by intravenous glucose and water. Sodium bicarbonate is usually administered in relatively small amounts, considering the severity of the acidosis; and, there are instances with favorable outcomes where it has not been used at all. In Cooperman's series (5), the mean bicarbonate administered was 133 meq. with a range of 44 to 272 meq. All patients survived, and were discharged from the hospital except for two patients, in Levy's series (3), who died several days after the acidosis had been



corrected of causes not directly related to the ketoacidosis.

Several patients were tested for glucose tolerance a few days to weeks after their acidosis had been corrected. Cooperman (5) reported that serum glucose and/or two hour post cibum glucose values were normal in all patients during convalescence. Levy (3) found that patients that were hyperglycemic on admission had normal or equivocal glucose tolerance tests or two hour post-prandial glucoses several days to months after the episode of acidosis. Jenkins (2) found that all three of his patients had abnormal glucose tolerance tests when tested within a week of admission, but that the abnormality decreased with increasing time interval from the hospital admission. Dillon (1) reported normal glucose tolerance tests in some of his patients while others had evidence of "chemical diabetes". Thus, an abnormal glucose tolerance test, when performed after the acidosis is corrected and the patient is clinically stable, is present in some cases, but does not seem to be an absolute prerequisite for the development of ketoacidosis.

Two patients with multiple episodes of alcoholic ketoacidosis (1,2) were challenged with ethanol, after a period of abstinence, in order to see if ketoacidosis could be induced in these susceptible individuals in a controlled setting. Dillon (1) administered small doses of whiskey for four days along with a high fat diet and he induced 3+ ketonuria on day two and three. Jenkins (2), however, found that starvation ketosis was not enhanced by the administration of two doses of 100 ml. of alcohol during a sixty hour fast. The ethanol also had no significant effect on free fatty acids or glucose.

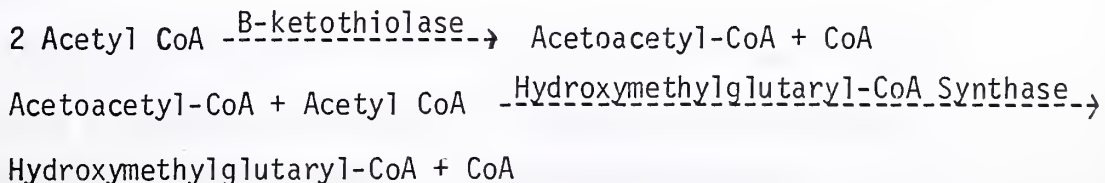




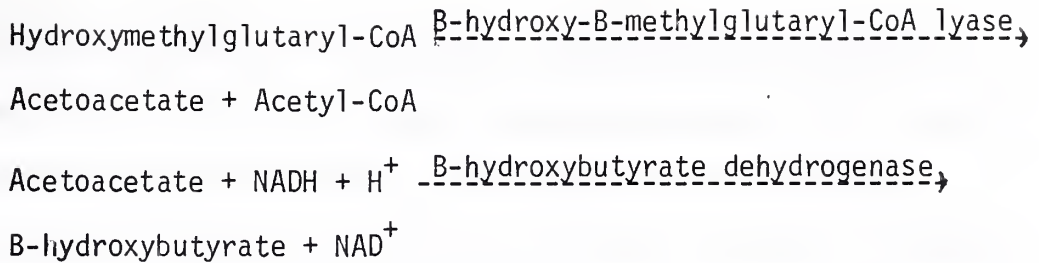
In summary, alcoholic ketoacidosis should be suspected in an individual with a partially compensated metabolic acidosis, who has a low, normal, or mildly elevated blood glucose, no glucose in the urine, and positive ketones by Acetest tablets in urine and undiluted serum (although initially this test may be negative). The patient will usually be conscious despite the severity of the acidosis, he will give a history of chronic ethanol ingestion with a recent increase in intake, and he will often describe nausea, vomiting and anorexia for one to two days or longer prior to admission. He will frequently complain of abdominal pain and may describe shortness of breath of recent onset. Further laboratory studies will reveal normal to only moderately elevated lactate with markedly elevated B-hydroxybutyrate and acetoacetate and an increased B-hydroxybutyrate to acetoacetate ratio. The acidosis is rapidly corrected by intravenous glucose and water. Relatively small amounts of bicarbonate may also be administered, but insulin is not required. The patient may have an abnormal glucose tolerance test several days after the acidosis has been corrected, although many will have an entirely normal response to a glucose load without evidence of a metabolic derangement. Some individuals will go on to have recurrent episodes of non-diabetic ketoacidosis with a history and course similar to the initial episode.

## II. Pathogenesis and Regulation of Ketone Body Formation

The following series of reactions comprises the main pathway for ketogenesis (7-10):







High levels of B-hydroxy-B-methylglutaryl-CoA-synthase, which is the rate limiting enzyme of this sequence (7), is unique to the liver (11). This provides a biochemical basis for the liver's role as the primary ketogenic organ. Further evidence for the liver's central role in ketogenesis is that functional hepatectomy will result in a rapid decrease in circulating ketones to undetectable levels and will abolish interconversion of acetoacetate and B-hydroxybutyrate (12).

The overall pathway for the generation of ketone bodies can be broken down into steps. Each one represents a potential regulatory point in ketogenesis:

1. Fatty acid release from adipose tissue and uptake by the liver.
2. Transfer of free fatty acids into the mitochondria and oxidation to acetyl CoA.
3. The entry of acetyl CoA into the hydroxymethylglutaryl-CoA pathway producing B-hydroxybutyrate and acetoacetate.
4. Peripheral utilization of ketone bodies.

In order to determine regulatory sites in ketogenesis each step must be examined.

It is well known that free fatty acids, mobilized from the periphery, are a necessary substrate for ketone body production and that depletion of fat stores results in decreased ketogenesis (7, 13). Ontko (16) was able to increase serum ketones in diabetic and fasting



animals by infusing corn oil and heparin which increased serum free fatty acid levels. It has been demonstrated by Bieberdorf (14), however, that increasing plasma free fatty acid concentration by infusing chylomicrons and heparin into animals not already in a state of enhanced ketogenesis does not lead to increased ketone body formation. In the same study, insulin infusion decreased ketogenesis in the presence of a sustained high plasma free fatty acid concentration. McGarry (21) found that rats starved for six hours showed no significant increase in plasma ketone concentration even though their plasma free fatty acids had doubled in this time. Thus, although an adequate supply of free fatty acids is necessary for ketogenesis, there must also be a change in the metabolic set of the liver before there will be an increase in ketone body synthesis (15).

Once the fatty acid is taken up by the liver it can either enter the mitochondria via acyl-carnitine transferase and undergo  $\beta$ -oxidation, or it can remain in the cytoplasm and be esterified. McGarry and Foster (17) demonstrated that isolated livers from fed and fasted rats removed similar quantities of oleic acid from the perfusate, but that the fate of the oleate was different. It was converted primarily to ketones in the fasted rat liver, and triglycerides in the fed rat liver. It had been the thesis of many investigators that the rate of free fatty acid incorporation into triglycerides in the liver determined the rate of free fatty acid oxidation by controlling the availability of substrate, and thereby regulated the rate of ketogenesis (18). According to this thesis, normal rats had a lower rate of ketogenesis than starved rats because of the greater capacity of the former to



esterify incoming fatty acids (17). The observation that the ketosis of fasting is associated with decreased triglyceride synthesis in the liver and that its reversal is accompanied by increased free fatty acid esterification (17) is consistent with this view. It was felt that the rate of triglyceride synthesis was in turn regulated by the concentration of free sn-glycero-3-phosphate (19,20). McGarry (17,20) has questioned this view, however, on the basis of the following experimental observations:

1. Despite previous reports to the contrary, whole liver concentrations of sn-glycero-3-phosphate were elevated in fasted animals (17). This is in accordance with the observations of Veech (22).
2. Perfusion of livers from normal and starved rats with oleic acid led to a five fold greater rate of esterification in the former, with similar levels of sn-glycero-3-phosphate in both sets of rats (17).
3. No correlation has been found between the reversal of starvation ketosis by various compounds and their effects on the levels of sn-glycero-3-phosphate in liver (17,23).
4. The development of diabetic ketoacidosis is associated with increased hepatic triglycerides. This is a consequence of increased hepatic triglyceride synthesis from incoming free fatty acids (24). This finding is incompatible with the thesis that a decrease in triglyceride synthesis from incoming free fatty acids is necessary for an increase in  $\beta$ -oxidation and ketogenesis to take place. In addition, the increased ketosis occurs prior to a significant change in liver triglyceride content (24). This is evidence against the theory that the initial event is an increase in the esterification pathway with an increase in ketosis occurring only when there is spillover of free fatty acid into the mitochondria secondary to a saturation of the triglyceride synthesis pathway.
5. Additional evidence against the theory that a defect in the triglyceride synthesizing machinery in the liver





is necessary for increased ketogenesis to occur came with McGarry, Meier and Foster's work with decanoyl-carnitine (21). This is an inhibitor of long chain acyl-carnitine transferase, which is the enzyme involved in transporting long chain free fatty acids into the mitochondria for oxidation. When (+)decanoyl-carnitine was used to block fatty acid oxidation in livers of fasted rats, all of the oleic acid taken up from the perfusate was esterified. This occurred in the absence of any added precursor of sn-glycero-3-phosphate. Thus, there was no apparent primary defect in the esterification pathway in livers from fasted rats.

In light of these observations, McGarry and his group have suggested that the rate of triglyceride synthesis is dependent upon the rate of free fatty acid uptake into the mitochondria for oxidation and not vice versa (7,17,21,24). Thus, in the starved state, ketogenesis is enhanced because of the activation of the oxidative sequence and the free fatty acids are channeled away from triglyceride synthesis. They suggest that in diabetic ketoacidosis this same sequence occurs initially, but because of the increased free fatty acid load presented to the liver, the oxidative pathway is saturated. This results in an increase in esterification as well as oxidation.

If the enhancement of ketone body production induced by starvation and diabetes is due primarily to activation of the oxidative sequence, it would be unlikely that the  $\beta$ -oxidation pathway itself is involved in the regulation (7,17,21,24). McGarry and Foster (26) found that octanoic acid, which bypasses the acyl-carnitine transferase reaction, is oxidized at similar rates in livers from diabetic, starved and normal rats even though the fate of the acetyl CoA generated was different.

McGarry's group suggests that the site of control is probably the transfer of long chain fatty acyl groups into the mitochondria which



is catalyzed by acyl-carnitine transferase (7,17,21,24,25). Williamson (25) demonstrated that decanoyl-carnitine completely blocked the rise in acetyl CoA produced by perfusion of livers with oleic acid. McGarry and Foster (15) showed that decanoyl-carnitine could produce a rapid reversal of severe ketosis in alloxan diabetic rats in situations where large doses of insulin had little effect. They have also demonstrated that decanoyl-carnitine reverses starvation ketosis in vitro (21). Thus, by use of this specific blocker, Williamson and McGarry have shown that the acyl-carnitine transferase reaction is an obligatory step in producing starvation and diabetic ketosis. Norum (27), in 1965, provided suggestive evidence of a role for acyl-carnitine transferase in ketogenesis by developing a specific assay for carnitine palmityl-transferase and measuring levels of this enzyme in the liver. He found significantly increased activity in livers from fat-fed, diabetic and fasted rats when compared with the activity in normal rat livers. The increase was not blocked by inhibitors of protein synthesis suggesting that the increase in activity was not due to de novo synthesis, but was caused by activation of preformed enzyme. This data, along with the aforementioned work with decanoyl-carnitine, as well as the experiments with octanoic acid, provides strong evidence of a role for acyl-carnitine transferase in the regulation of the amount of long chain free fatty acids that undergoes B-oxidation. Presumably, the enzyme is inhibited in the normal fed state resulting in the shunting of free fatty acids into the esterification pathway; and it is activated in the starved and diabetic state resulting in an increased oxidation of free fatty acids with a secondarily increased generation of acetyl CoA and ketone bodies.



The regulation of ketogenesis is not only a function of the rate at which the liver generates acetyl CoA, but also is determined by the rate of disposal of acetyl CoA in nonketogenic pathways. The three routes acetyl CoA can take within the liver are:

1. Combination with oxaloacetate and subsequent oxidation to  $\text{CO}_2$  within the tricarboxylic acid cycle
2. Fatty acid synthesis
3. Ketone body formation

In order for ketone body synthesis to take place, the acetyl CoA to CoA ratio must be high since the initial reaction in the synthesis of acetoacetate and B-hydroxybutyrate ( $2 \text{ Acetyl CoA} \rightleftharpoons \text{Acetoacetyl CoA} + \text{CoA}$ ) is strongly displaced to the left (9). The rates of the reactions in the tricarboxylic acid cycle and in fatty acid synthesis will determine levels of available acetyl CoA and thus, affect the rate of ketone body formation. McGarry and Foster (26) studied this aspect of ketogenesis by using octanoic acid which does not serve directly as a substrate for triglyceride formation (28) and is not dependent on acyl-carnitine transferase for its oxidation (25). They perfused livers from normal, starved and diabetic rats with octanoate  $1-^{14}\text{C}$  and found:

1. Approximately equal amounts of labelled octanoate was taken up in all three groups of livers; however, considerably more radioactivity appeared in ketones and  $\text{CO}_2$  in the diabetic and starved groups than in the normal group.
2. The incorporation of isotope into phospholipids and triglycerides was markedly decreased in the livers from fasted and diabetic rats compared to the normal group. This diminished flow of acetyl CoA into fatty acid synthesis was of a magnitude sufficient to account for most of the increased flux of acetyl CoA into ketone bodies in the starved group.



3. The increased flux of acetyl CoA into ketone bodies could not be accounted for by decreased flow through the tricarboxylic acid cycle.
4. The total recovery of radioactivity in ketones, CO<sub>2</sub> and lipids was essentially identical in all three groups, suggesting that the rate of oxidation itself does not vary with a constant flow of free fatty acid substrate.

The important conclusion from this study is that decreased lipogenesis from acetyl CoA can account for increased ketogenesis in certain instances, and that increased rates of ketone body synthesis do not always require a concomittant depression in tricarboxylic acid cycle activity.\*

Although lipogenesis was inhibited to an equal extent in livers from fasted and diabetic rats, total ketone levels were higher in the latter group. Thus, when acetyl CoA levels are markedly elevated, as in the diabetic group, the contribution of decreased lipogenesis to ketone body production is not nearly as significant as in the starved rat, where lower levels of acetyl CoA are present (26).

It is apparent that the regulation of ketone body production is a function of more than one step in the ketogenic pathway. It depends upon free fatty acid delivery to the liver, ability of the liver to oxidize the incoming free fatty acid to acetyl CoA, and its ability to dispose of the acetyl CoA through nonketogenic pathways.

An additional factor that may be significant in the development

\*The secondary importance of decreased tricarboxylic acid cycle activity in ketogenesis was emphasized by Engel and Amatruda in 1963 (8). Spencer (29) reported that citrate levels in diabetic and starved rats were not depressed when compared to levels in normal rats. Cahill (30), however, in his discussion of human starvation, emphasized the importance of decreased tricarboxylic acid cycle activity in the increased ketogenesis of starvation.





of ketosis, is the rate of utilization of ketone bodies by peripheral tissues. Garber (31) demonstrated that ketone output by the liver in humans, after three days of starvation, was the same as that noted after a five to six week fast, but the degree of hyperketonemia was three times greater after the longer fast. This suggests that a decrease in peripheral utilization of ketones in a prolonged fast plays an important role in the increased ketosis of that state. McGarry, Guest, and Foster (12) studied ketone turnover rate in starved and alloxan diabetic rats. They found a 1.6 times greater turnover rate in the diabetic rats compared to the starved rats, but the plasma total ketone concentration was 3.4 times greater. Thus, the increased rate of ketogenesis does not totally explain the difference in the degree of ketosis. They postulated that in the diabetic state, the peripheral utilization mechanisms are saturated, so that small differences in production result in large differences in plasma ketone concentration.

The effects of insulin on ketogenesis are extensively studied and discussed in the literature. A widely accepted theory is that with fasting, insulin falls secondary to decreasing glucose and this fall in insulin is a key factor in initiating ketone production (8,13,32). Engel and Amatruda, in their review of the hormonal aspects of ketosis (8), stated that during a prolonged fast in the rat, ketosis is maximal when the blood sugar is lowest, and declines as the blood sugar rises towards normal. The high point in ketosis corresponds with the expected minimum of insulin secretion. As gluconeogenesis increases, the glucose and insulin levels rise and the ketone level stabilizes, thereby protecting the animal from developing severe ketosis. In the adrenalecto-



mized rat, gluconeogenesis is severely decreased and these rats develop progressive hypoglycemia and ketonemia. A minute dose of insulin administered to the fasting, severely hypoglycemic and ketotic, adrenalectomized rat immediately lowers ketone levels without any effect on blood sugar.

Wildinoff (32) studied normal diurnal variations in ketone levels and found that they transiently drop, postprandially, coinciding with the rise in glucose and insulin levels. Ketones then begin to increase five hours postprandially, preceded by a decrease in glucose and insulin and an increase in free fatty acids. A constant glucose infusion wiped out this rise in ketones and free fatty acids.

Potential sites for insulin exerting a regulatory role in ketogenesis are:

1. Free fatty acid release from adipose tissue
2. Relative rates of triglyceride synthesis and fatty acid oxidation in the liver
3. Determination of the rates of reactions in the tricarboxylic acid cycle and in the pathway for free fatty acid synthesis, thereby controlling the availability of acetyl CoA for ketogenesis
4. Peripheral utilization of ketones

Bieberdorf (14) reported that insulin deprivation increased, and insulin ingestion decreased, the release of free fatty acids from body tissues. In the same study, he also found that insulin increased ketogenesis in the presence of sustained high free fatty acid concentration. Thus, insulin can apparently decrease ketogenesis by decreasing free fatty acid supply to the liver, and, independent of that, by some change in the metabolic set of the liver. It has not been determined



where, in the liver, insulin acts to decrease ketogenesis (15). McGarry and Foster (15) found that (+)decanoyl-carnitine caused a rapid reversal of ketosis under conditions where large doses of insulin had little effect. A combination of the two agents, however, was more effective in lowering plasma ketone levels than (+)decanoyl-carnitine alone. This raises the possibility that insulin also effects the acyl-carnitine transferase reaction (15). Indirect support for this hypothesis is that increased levels of the enzyme are found in livers from diabetic and fasted rats- conditions associated with relatively low or absent insulin. Additional evidence, for insulin exerting its effect on ketogenesis at the acyl-carnitine transferase step, is provided by McGarry and Foster (26). They found that levels of insulin sufficient to reverse starvation ketosis in a rat, did not impair subsequent ketogenesis induced by infusion with octanoate, a fatty acid that bypasses the acyl-carnitine transferase step.

It is well known that insulin inhibits gluconeogenesis, and some investigators have suggested that the rate of gluconeogenesis is directly related to the rate of ketogenesis (33). Thus, insulin would effect ketogenesis by influencing the rate of gluconeogenesis. Insulin also affects hepatic ketone production by affecting the balance between  $\text{CO}_2$  and ketoacid production from acetyl CoA (13).

It has been postulated that insulin increases the rate of peripheral utilization of ketone bodies, and, thereby, lowers plasma ketone concentration (13). Balasse and Havel (34) showed that administration of insulin and glucose to normal dogs increased peripheral uptake of infused ketones.

Finally, ketone production may be regulated by ketone bodies



themselves (35-37). Mebane and Madison (35,36) have found that pancreatic and venous insulin concentrations rise after peripheral venous or pancreatic arterial infusion of ketones. They suggest that this feedback on islet cells may be important in preventing progressive ketoacidosis in starvation. Other authors have refuted the idea of an effect of ketones on insulin release, and instead, postulate a direct inhibitory effect of ketones on lipolysis, hepatic glucose production, and on peripheral glucose utilization (38,39).

It is obvious from this discussion of ketogenesis, that its initiation and regulation are complex, and not fully understood. Multiple factors are probably involved. The effect of ethanol on ketone body production will be discussed in a later section.

### III. The Metabolism of Ethanol

Two to ten percent of ingested ethanol can be excreted through the kidneys and lungs. The remainder must be oxidized, and this occurs primarily in the liver (40). It is this organ specificity that helps to explain the significant degree of metabolic alteration that occurs with ethanol intake (40).

The main metabolic pathway for ethanol metabolism involves alcohol dehydrogenase, an enzyme in the cytoplasm which catalyzes the conversion of ethanol to acetaldehyde with the reduction of  $\text{NAD}^+$  to NADH. The acetaldehyde is oxidized to acetate so that, from each molecule of ethanol, two molecules of NADH are formed (40-42). The acetate is released into the circulation and mainly disposed of in extrahepatic





tissues (40,43). The human myocardium increases its uptake of acetate five fold during ethanol infusion with a concomitant decrease in fatty acid utilization (44). Skeletal muscle also uses a large amount of the acetate produced by ethanol metabolism (43). The reoxidation of the NADH, generated from ethanol, is the rate-limiting step for ethanol metabolism (42). This is effected primarily by the respiratory chain in the mitochondria. The mitochondrial membrane is impermeable to NADH and the malate/aspartate "shuttle" is the main means of transporting the NADH into the mitochondria.

Approximately twenty to twenty-five percent of ethanol metabolism takes place in a microsomal ethanol oxidizing system (40,45). The importance of this pathway varies with the concentration of ethanol. There is increased activity at a high ethanol concentration because its  $K_m$  is approximately four times that of alcohol dehydrogenase (40). The microsomal pathway utilizes reduced cofactors ( $\text{CH}_3\text{CH}_2\text{OH} + \text{NADPH} + \text{H}^+ + \text{O}_2$  microsomal ethanol oxidizing system  $\text{CH}_3\text{CO} + \text{NADP}^+ + 2 \text{H}_2\text{O}$ ) and thus, may be another route for disposal of the hydrogen generated from the oxidation of ethanol by alcohol dehydrogenase (40,41). Chronic ethanol ingestion results in increased microsomal ethanol oxidizing system activity associated with a proliferation of the smooth endoplasmic reticulum and thus may, in part, explain the adaptation of alcoholics to ethanol (40,41). In addition, other drugs that stimulate microsomes (i.e. barbiturates) enhance the rate of ethanol clearance (40).

In summary, the cytoplasmic alcoholic dehydrogenase is the main site of oxidation of ethanol, but, particularly with high doses of ethanol and chronic ingestion, the microsomal ethanol oxidizing system



plays a significant role in ethanol metabolism.

#### IV. The Effects of Ethanol on Glucose Metabolism

In the past several years, there have been numerous studies investigating the interactions of ethanol with carbohydrate metabolism. In the postabsorptive state, when liver glycogen stores are adequate, ethanol has been shown to cause an increase in serum glucose within one hour of ingestion (43). This effect can be prevented by ganglionic blockade, and it presumably results from adrenergic stimulation leading to increased glycogenolysis (43).

There have been many case reports of hypoglycemia following ethanol ingestion (46,47), and these episodes generally occur in chronic alcoholics with poor dietary intake. The syndrome has been reproduced and studied in patients who have been admitted to the hospital for alcohol hypoglycemia (46,47) and in normal humans and animals (48-56).

A decline in serum glucose can be produced by a decrease in hepatic glucose output, or by an increase in peripheral utilization. It is generally accepted that ethanol inhibits gluconeogenesis. Searle (50) demonstrated a sixty percent decrease in hepatic glucose production following ethanol infusion into fasted humans; and Field and Williams (48) found a fifty percent decrease in glucose and urea production in ethanol perfused livers from rats. In both of these studies, the decreased glucose production was presumably secondary to decreased gluconeogenesis (48,50). A decrease in gluconeogenesis will only result in diminished hepatic glucose output and hypoglycemia if the liver is depleted of glycogen. In the presence of adequate glycogen stores, the liver will



maintain its glucose output, via glycogenolysis (43,47-49,51,58). It has been repeatedly demonstrated in humans that ethanol will inhibit gluconeogenesis in the fed state (50,57,58). Hypoglycemia, however, cannot be induced by ethanol unless the individual has been fasted for a long enough period of time (usually at least twenty-four to forty-eight hours) to deplete his liver of glycogen (41,48,50,54). Decreased or absent hepatic glycogen has been found in liver biopsies from individuals presenting with the clinical picture of alcohol hypoglycemia (43). Additional evidence for absent glycogen stores in alcohol hypoglycemia is that the hypoglycemia does not respond to glucagon infusion (47). Ethanol's inhibition of gluconeogenesis is generally felt to be secondary to the increased ratio of NADH to NAD resulting from the metabolism of ethanol (48-57). Leevy (59) showed that the maximum increase in hepatic NADH occurred approximately four hours after ethanol ingestion, with a negligible increase when low doses of ethanol were administered (less than 3g./kg. in rats), and a large increase when high doses were given (greater than 6g./kg. in rats or 1.5g./kg. in humans). The NADH to NAD ratio began to return to pretreatment levels at twelve hours, but this was prolonged when there was evidence of mitochondrial damage or fragmentation of the endoplasmic reticulum which occurred after chronic alcohol ingestion (59). The effects of an increased NADH to NAD ratio on NAD dependent steps in gluconeogenesis include (42,43,55):

1. an increase in the lactate to pyruvate ratio resulting in a decreased availability of pyruvate for regeneration of glucose. Krebs (60) showed that a moderate intake of ethanol delayed post-exercise clearance of lactate, which he ascribed to decreased lactate conversion to glucose by the liver.



2. a decreased conversion of glutamate to  $\alpha$ -ketoglutarate by glutamic dehydrogenase
3. a decreased conversion of  $\alpha$ -ketoglutarate to succinate and of malate to oxaloacetate, with the net effect of decreased oxaloacetate for glucose production via phosphoenolpyruvate. Madison, Lochner and Wulff (55) found that infusion of large amounts of  $\alpha$ -ketoglutarate and glutamate failed to increase hepatic gluconeogenesis that was already suppressed by ethanol.
4. a decreased conversion of L-  $\alpha$ -glycerophosphate to dihydroxyacetone-phosphate which decreases glucose production from glycerol

If the increased NADH to NAD ratio is in fact the cause of the ethanol induced inhibition of gluconeogenesis, then an experimentally induced decrease in this ratio would be expected to stimulate gluconeogenesis. When Madison, Lochner and Wulff (55) infused methylene blue, a dye capable of oxidizing NADH to NAD, they reversed the ethanol-induced suppression of gluconeogenesis and restored hepatic glucose output to normal. They were also able to prevent a reduction in gluconeogenesis by administering methylene blue simultaneously with ethanol (55).

Kreisberg (57) and Kalkhoff (54) have reported that an additional site, at which ethanol may inhibit gluconeogenesis, is at the level of alanine release from muscle. Alanine is the primary amino acid precursor in gluconeogenesis. It accounts for approximately fifty percent of amino acid uptake by the splanchnic bed, and the hepatic capacity, for glucose production from alanine, is greater than that for all other amino acids (61). Approximately thirty percent of the total amino acids released from muscle is alanine, but only seven to ten percent of protein amino acids in skeletal and cardiac muscle can be





ascribed to alanine. Thus, the majority of alanine released must come from de-novo synthesis, via transamination of pyruvate (61). Kreisberg (51) found a twenty-five percent decrease in plasma alanine concentration following ingestion of ethanol by humans. He postulated that the decrease in plasma alanine concentration was due to decreased alanine synthesis from pyruvate. This effect could be mediated by acetate and/or lactate arising directly or indirectly from the metabolism of ethanol. Both of these substances have been demonstrated to interfere with peripheral glucose utilization (57), which would decrease the availability of pyruvate for alanine synthesis. Thus, there is evidence that ethanol-induced hypoglycemia results from decreased substrate availability for glucose production, as well as from inhibition of intrahepatic pathways of gluconeogenesis.

An additional factor that could affect serum glucose concentration is the rate of peripheral utilization. Lochner (51) measured hepatic glucose output and peripheral uptake of glucose after an infusion of ethanol into fasted dogs with chronic end-to-side portacaval shunts. He found a prompt sixty-five percent fall in mean hepatic glucose output and a twenty-five percent inhibition of peripheral glucose utilization. Not all of his dogs became hypoglycemic, because, in order for the serum glucose concentration to drop, the magnitude of the decrease in hepatic glucose output had to exceed the magnitude of inhibition of peripheral glucose utilization (51). Other investigators have also reported that ethanol decreases peripheral glucose utilization (43,57, 58). Phillips and Safrit (6) fed humans alcohol, and found a decreased glucose tolerance with an increased insulin response to glucose. This



suggests that ethanol resulted in a decreased ability to dispose of serum glucose despite adequate amounts of insulin (6). Searle, however, recently published a study (50) in which he found an increase in the rate of peripheral glucose utilization in individuals of normal weight who were fasted for three days and then received ethanol. In his obese subjects, who were treated in a similar fashion, he found a decrease in peripheral glucose utilization. The normal weight individuals showed a significantly greater rate of decrease in plasma glucose concentration following ethanol administration compared to the obese subjects. He explains this by the differences observed in the rate of peripheral glucose utilization.

It can be concluded from this review that ethanol produces hypoglycemia in man and in certain animals after an appropriate fast. The mechanism for this seems to be an inhibition of intrahepatic gluconeogenic pathways via an alteration in the redox state of the hepatocytes, secondary to the oxidation of ethanol. In addition, there is an apparent decline in peripheral alanine production after ethanol ingestion, resulting in a decrease in gluconeogenic substrate supply to the liver. Because of conflicting reports, no conclusion can be drawn about the role of peripheral glucose uptake in the production of ethanol hypoglycemia.

#### V. The Effects of Ethanol on Insulin Release

Ethanol has been reported to both increase (6,67-72) and decrease (62-66) insulin release via direct and indirect mechanisms. Ethanol-induced hypoglycemia is associated with a fall in serum insulin



levels (64-66). The decrease in insulin is not a direct effect of ethanol since there is no decline in insulin concentration when glucose is infused along with ethanol (64). There is also no decline in insulin concentration when normal, fed individuals are given ethanol (66). The fall in insulin, therefore, is probably mediated by the decline in plasma glucose induced by ethanol in fasted individuals.

Malaisse (63) reported that ethanol had a direct inhibitory effect on insulin release from  $\beta$  cells in vitro, and he postulated that this was secondary to an inhibition of a microtubular, microfilamentous system in the  $\beta$  cell involved in the insulin secretory process. Colwell (65) reported that infusion of ethanol alone into the pancreatic artery did not effect insulin release, but that ethanol blocked the immediate release of insulin that occurred when cyclic AMP was infused.

Kuo (67), however, reported that pancreatic perfusion with a low concentration of ethanol (.05% V/V) stimulated islet adenylate cyclase. Metz (68), Siegal (71), Friedenbergl (70), and Kuhl (72) all reported that, although ethanol alone had no effect on insulin release, prior ethanol infusion potentiated the glucose induced insulin response. Metz (68) reported a more rapid peripheral clearance of glucose with the potentiation of insulin release by ethanol, thereby improving glucose tolerance. Phillips (69), however, found a decreased glucose tolerance associated with increased levels of insulin in subjects fed ethanol. He suggested that ethanol led to decreased peripheral glucose utilization resulting in higher glucose levels after ethanol infusion. The increased insulin was, therefore, a result of the increased glucose, and not a primary effect of ethanol.



## VI. The Effect of Ethanol on Cortisol Levels

Single large doses of ethanol have resulted in elevated levels of corticosteroids (43,73-75). Jenkins (74) reported that ethanol had no effect on cortisol levels in patients with pituitary lesions, who had a normal response to exogenous ACTH, suggesting that ethanol's effect on cortisol levels is mediated through the pituitary (74). Myerson (75) reported that, in contrast to the increase in plasma cortisol levels seen following ethanol administration to normal humans, chronic alcoholics did not always manifest an acute elevation of cortisol after ethanol intake. Margraf (76), however, reported that plasma cortisol levels were significantly elevated in alcoholics when they were inebriated. He also found that alcoholics showed a diminished serum cortisol response to ACTH (76).

## VII. The Effects of Ethanol on the Mobilization of Free Fatty Acids from Peripheral Fat Deposits

Ethanol has been reported to increase serum free fatty acid levels (56,73,77-79), decrease free fatty acid levels (80-81), and cause no significant change in free fatty acids (82-83). An analysis of these reports shows that the net effect on free fatty acid levels depends upon the balance between two indirect effects of ethanol on fatty acid release from adipose tissue (40-41,43,45). With low or moderate doses of ethanol, there is a decrease in free fatty acid mobilization. This is a result





of the inhibitory effect of acetate, generated from ethanol metabolism, on lipolysis (40-41,43,45). Crouse (81) reported that sodium acetate, given orally to normal fasting humans, caused a significant fall in plasma free fatty acids. The plasma acetate levels were less than, or equal to, those reached after ethanol (81). With high doses of ethanol, there is an adrenergic response to intoxication, causing increased lipolysis and free fatty acid mobilization (40-41, 43,45,73,78,79). Mallov (78) reported that urinary excretion of epinephrine and norepinephrine were increased in ethanol intoxicated dogs. Brodie (73) found that he could prevent the rise in free fatty acids, following ethanol intoxication, by administering ganglionic blocking agents. Adrenal demedullation, however, did not prevent the increase in free fatty acids (73). Although the rise in free fatty acids is apparently mediated by catecholamines, it does not occur without an intact pituitary-adrenal axis (84). Maickel (84) found that the normal response in adrenalectomized rats could be restored by pretreatment with cortisone.

In summary, with low to moderate doses of alcohol, the predominant effect is one of inhibition of free fatty acid mobilization from adipose tissue. This is mediated by the increase in serum acetate produced by the metabolism of ethanol. With intoxicating doses, the catecholamine effect predominates and adipose tissue lipase is stimulated, resulting in increased serum free fatty acid levels.

#### VIII. The Effect of Ethanol on Ketogenesis

Studies of the relationship between ethanol and ketone metabolism



have yielded conflicting results.

Ethanol has been referred to as an antiketogenic agent (17). In the preinsulin era, diminutions in glycosuria and ketonuria were found in ethanol treated diabetics (47). Arky and Freinkel (49) withheld food and insulin for twelve hours from juvenile diabetics and infused 118 grams of ethanol over eight hours. They found that the ethanol arrested or dampened progressive ketonemia as well as the rise in glucose. In some recent studies, ethanol has been shown to decrease hepatic ketone production in vitro (17,85-87). Blomstrand (87), Ontko (86), and McGarry (17) have shown, with labelled long chain fatty acids, that ethanol inhibits ketogenesis and  $\text{CO}_2$  formation with enhanced recovery of label in triglycerides and phospholipids.

There are several reports of ethanol decreasing free fatty acid oxidation and increasing triglyceride synthesis (40-41,45,73,82,82,88); both would be expected to decrease ketogenesis. Lieber (40,41), Fellenius (85), Ontko (86), and Blomstrand (87) suggest that the mechanism for the inhibition of  $\beta$ -oxidation is an increase in the intramitochondrial NADH to NAD ratio secondary to the production of reducing equivalents in the cytoplasm from the oxidation of ethanol. Since NAD is a necessary cofactor for  $\beta$ -oxidation, a diminution in the NAD to NADH ratio would be expected to inhibit  $\beta$ -oxidation. The competitive nature of the interaction between ethanol and free fatty acid oxidation is supported by the finding that ethanol oxidation is inhibited by oleate (85). The increased esterification of free fatty acids can be explained by increased availability of substrate secondary to the inhibition of  $\beta$ -oxidation or by an accumulation of  $\alpha$ -glycerophosphate, which has been shown to occur following ethanol administration (89).



In contrast to the preceding reports, ethanol administration in several in vivo and in vitro studies has been shown to increase hepatic ketone production and serum ketone levels (45,89-96). Most investigators have reported that with acute single-dose ethanol administration, B-hydroxybutyrate increased markedly, while acetoacetate increased only slightly or not at all (89,90,94-95). This would be expected from the shift in the redox state of the cell induced by ethanol metabolism.\* The explanation given for the increased ketogenesis following acute ethanol administration in vivo and in vitro is also based upon the shift in the NADH to NAD ratio induced by ethanol (89). Williamson (89) reported that, despite a thirty-seven percent inhibition of  $\beta$ -oxidation following addition of ethanol to a liver perfusate containing oleate, ketogenesis was stimulated. He suggests that this is due to an inhibition of the citric acid cycle secondary to the decreased NAD to NADH ratio. This results in an increased availability of acetyl CoA for ketone production (89). The inhibition of the tricarboxylic acid cycle by ethanol has also been reported by other investigators (41,43,85-86,90,94).

Ethanol has been shown to increase ketone production when administered chronically to humans and animals maintained on a high fat intake. The peak of ketogenesis occurred in the fasting state when alcohol had disappeared from the blood, and when its metabolic effect



An increase in NADH to NAD ratio shifts this reaction to the right, resulting in increased conversion of acetoacetate to B-hydroxybutyrate.



on the redox potential of the cell had disappeared, indicated by a normal B-hydroxybutyrate to acetoacetate ratio (93). Lefevre, Adler, and Lieber (93) administered ethanol in non-intoxicating doses, along with an adequate diet in which thirty-six percent of the calories were lipid. They found that after one week of alcohol, marked ketonuria and ketonemia developed, which was three times greater than that induced by a high fat diet alone, and thirty times greater than in the control group. This hyperketonemia was present in the postprandial period, but was most marked after an overnight fast, when ethanol was no longer detectable in the blood. Alcohol did not induce hyperketonemia, when given with a low fat diet (five percent of the total caloric intake). Similar findings were reported in their studies with rats who developed ketonemia after receiving subintoxicating doses of alcohol (3g./kg.) for three days. They also found that neither in vivo pretreatment with ethanol, nor in vitro addition of alcohol had a significant effect on uptake of acetoacetate by rat diaphragm. Thus, they provided evidence for a delayed effect of ethanol on ketone metabolism. This effect was not secondary to the immediate alteration of the cells' redox potential, and was most marked when ethanol was no longer present (93). Zoth (91) suggested that a mechanism for this delayed effect was mitochondrial damage from chronic ethanol ingestion, resulting in an alteration of fatty acid metabolism. He studied the oxidation of fatty acids in mitochondria, isolated from livers of rats fed a diet consisting of fat (thirty-five percent of the total calories), and carbohydrate or isocaloric amounts of ethanol (thirty-six percent of the calories) for twenty-eight days. Zoth found that oxidation of fatty acids to CO<sub>2</sub>





was decreased twenty to thirty-eight percent in mitochondria from ethanol fed rats, but that mitochondrial  $\beta$ -oxidation was increased by thirty percent. The net result of this could be heightened ketogenesis from the higher levels of acetyl CoA generated from increased  $\beta$ -oxidation of free fatty acids (91).

The current study was undertaken to investigate further the effects of chronic ethanol ingestion on ketogenesis.



## MATERIALS AND METHODS

## I. Animals

Male, Sprague-Dawley rats weighing 200-300 grams were used in this study. The animals were kept in a controlled environment with a constant temperature of 75-80 degrees F. and with lighting from 7:00 a.m. until 7:00 p.m. For at least 3 days prior to beginning the experiment the animals were allowed water and food ad libitum. Their diet consisted of Purina Laboratory Chow which contained not less than 23% protein and 4.5% fat.

## II. Experiments

The rats were divided into two groups. One group was to receive ethanol by nasogastric tube, in a dose of 6 gms./kg. diluted 1:1 (V/V) with tap water. Rats became grossly intoxicated at this dose. They were unable to maintain themselves in an upright position and were only weakly responsive to noxious stimuli. A similar volume of tap water was administered by nasogastric tube to a control group of rats.

At 9:00 a.m., on day one of the experiment (0 hours), all rats were placed in individual cages and food was removed. Rats from both groups were allowed water ad libitum but received no food throughout the experiment. Tube feedings were administered at 3 hours, 27 hours, 51 hours, and 75 hours into the fast. Five normal, untreated rats were sacrificed at 0 hours and five or six rats from the ethanol-fed group and a similar number from the water-fed group were sacrificed 27-31 hours



after each tube feeding, except for a final group of ten rats that were sacrificed six to eight hours after the last tube feeding. Only this final group contained rats that were intoxicated at the time of sacrifice. It can be assumed that the blood ethanol level was essentially zero in all other rats when they were sacrificed (93). Thus, rats that were sacrificed at 30-34 hours received one dose of ethanol or water at 3 hours into the fast; rats sacrificed at 54-58 hours received a dose at 3 hours and at 27 hours; rats sacrificed at 78-81 hours were tube fed at 3 hours, 27 hours, and 51 hours; and rats sacrificed at 81-83 hours were tube fed at 3 hours, 27 hours, 51 hours and 75 hours. The table below is an outline of the experiment:

	Rat#	Fed Group	Ethanol Group				Water Group			
		1-5	6-11	12-17	18-23	24-28	29-34	35-40	41-46	47-51
<u>Day 1</u>										
	9a.m.-0hrs.	*								
	noon-3hrs.		+	+	+	+	+	+	+	+
<u>Day 2</u>										
	noon-27hrs.			+	+	+		+	+	+
	3p.m.-30hrs.		*				*			
<u>Day 3</u>										
	noon-51hrs.				+	+			+	+
	3p.m.-54hrs.			*				*		
<u>Day 4</u>										
	noon-75hrs.					+				+
	3p.m.-78hrs.				*			*		
	6p.m.-81hrs.					*				*
		+	-	<u>tube fed</u>						
		*	-	<u>sacrificed</u>						



At the time of sacrifice, the rat was placed in a jar containing ether, and was removed when he was lightly anesthetized. The abdominal cavity was then entered anteriorly, and a 22 gauge needle, attached to a heparinized syringe, was placed into the aorta at its bifurcation. Blood was removed until the animal was exsanguine. Samples were put into separate tubes for insulin, glucose, free fatty acids and ketone analysis. The insulin and free fatty acids specimens were centrifuged at 4 degrees centigrade, and the serum was removed and stored in the frozen state. Ketone samples were deproteinized by adding an equal volume of 6% perchloric acid solution to the blood sample. This mixture was vortexed and then centrifuged at 4 degrees centigrade. The supernatant was removed and stored frozen. All samples were assayed within one month of collection. Specimens for glucose measurement were collected in pediatric capillary tubes and were kept on ice for not longer than two hours before being assayed.

### III. Assays

All glucose measurements were performed by the Yale-New Haven Hospital Clinical Chemistry Laboratory, using an automated glucose oxidase method. Acetoacetate and B-hydroxybutyrate were determined by the methods of Mellanby and Williamson (97-98). Insulin was measured by radioimmunoassay employing talc to separate bound from free insulin (100). Free fatty acids were determined by the colorimetric method of Novak (99) with the following modifications:

1. The chloroform-heptane solution was prepared in a ratio of 4 to 1 (V/V) instead of 5 to 1, in order to improve the phase separation.





2. All samples and reagent volumes were doubled through the second centrifugation to facilitate removal of supernatants.
3.  $K_2SO_4$  solution was prepared by adding 12 grams of  $K_2SO_4$  to 100cc. of distilled water at room temperature. The solution was stirred overnight and filtered in the morning.
4.  $Na_2SO_4$  solution was prepared by adding 15 grams of  $Na_2SO_4$  to 100cc. of distilled water at room temperature.

#### IV. Statistical Methods

The Student T-test was utilized for determinations of the significance of differences between means

All values are reported as  $\pm$  standard error of the mean.



## RESULTS

The B-hydroxybutyrate, acetoacetate, and total ketone levels obtained in the water-fed and ethanol-fed rats are reported in Table 1 and are depicted in Figures 1 and 2. All rats, except for the final group, received their last tube feeding 27-31 hours prior to sacrifice. The final group of rats, sacrificed at 81 hours, received their last intragastric dose of ethanol or water 6-8 hours prior to sacrifice.

In the control group, B-hydroxybutyrate and acetoacetoacetate increased markedly from 0 to 30 hours of fasting. B-hydroxybutyrate increased ten fold, from  $0.095 \pm 0.020$   $\mu\text{mol./ml.}$  at 0 hours to  $1.091 \pm 0.091$   $\mu\text{mol./ml.}$  at 30 hours. Acetoacetate increased eight fold, from  $0.021 \pm 0.005$   $\mu\text{mol./ml.}$  to  $0.164 \pm 0.022$   $\mu\text{mol./ml.}$  Between 30 hours and 54 hours of fasting total ketones tended to level off with no significant change in B-hydroxybutyrate or total ketones. Acetoacetate levels, however, continued to rise, with an increase of approximately fifty percent ( $p < .02$ ). From 54 to 78 hours, mean total ketones in the control group fell forty-two percent ( $p < .05$ ) and the total decline from 54 to 81 hours was fifty-nine percent ( $p < .001$ ). Thus in the control group, total ketones increased markedly with 30 hours of fasting, peaked at 54 hours of fasting and then declined throughout the remainder of the study.

B-hydroxybutyrate and acetoacetate levels in the ethanol fed rats were not significantly different from the controls at 30 hours, 54 hours and 78 hours, after receiving one, two or three prior tube feedings, respectively. A final tube feeding of ethanol at 75 hours, however,



TABLE 1

Duration of Fast (hours)	Total Ketones (umoles/ml)	B-hydroxybutyrate (umoles/ml)	Acetoacetate (umoles/ml)	Ratio of B-hydroxybutyrate to Acetoacetate
0	0.116 $\pm$ 0.023	0.095 $\pm$ 0.020	0.021 $\pm$ 0.005	4.50
30(control)	1.254 $\pm$ 0.107	1.091 $\pm$ 0.091	0.164 $\pm$ 0.022	6.60
30(ethanol)	1.119 $\pm$ 0.130	0.972 $\pm$ 0.124	0.147 $\pm$ 0.024	6.60
t-test (p)	N.S.	N.S.	N.S.	
54(control)	1.574 $\pm$ 0.255	1.324 $\pm$ 0.252	0.250 $\pm$ 0.008	5.30
54(ethanol)	1.244 $\pm$ 0.178	1.025 $\pm$ 0.163	0.218 $\pm$ 0.020	4.88
t-test (p)	N.S.	N.S.	N.S.	
78(control)	0.910 $\pm$ 0.131	0.758 $\pm$ 0.119	0.152 $\pm$ 0.032	4.99
78(ethanol)	0.856 $\pm$ 0.077	0.712 $\pm$ 0.070	0.144 $\pm$ 0.010	4.94
t-test (p)	N.S.	N.S.	N.S.	
81(control)	0.648 $\pm$ 0.066	0.562 $\pm$ 0.069	0.086 $\pm$ 0.011	6.53
81(ethanol)	1.541 $\pm$ 0.092	1.383 $\pm$ 0.056	0.158 $\pm$ 0.042	8.75
t-test (p)	p < .001	p < .001	.05 < p < .10	

All values are  $\pm$  S.E.M.



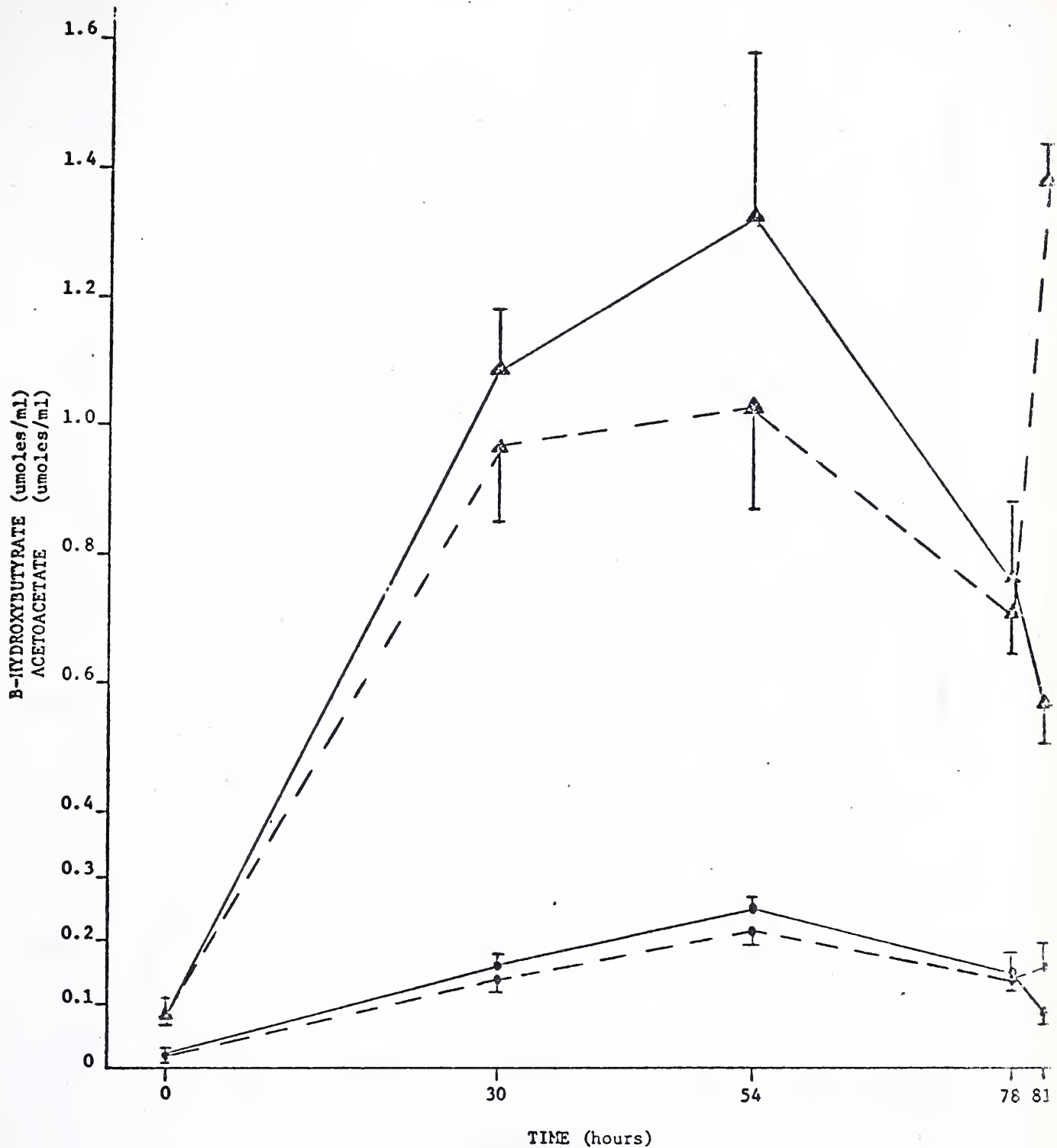


Figure 1- Mean serum B-hydroxybutyrate ( $\blacktriangle$ ) and acetoacetate ( $\blacklozenge$ ) levels ( $\pm$ S.E.M.) in water-fed rats (solid line) and ethanol-fed rats (broken line). At 81 hours, ethanol produced a marked rise in B-hydroxybutyrate with no significant change in acetoacetate. At all other times there was no significant difference between the two groups.





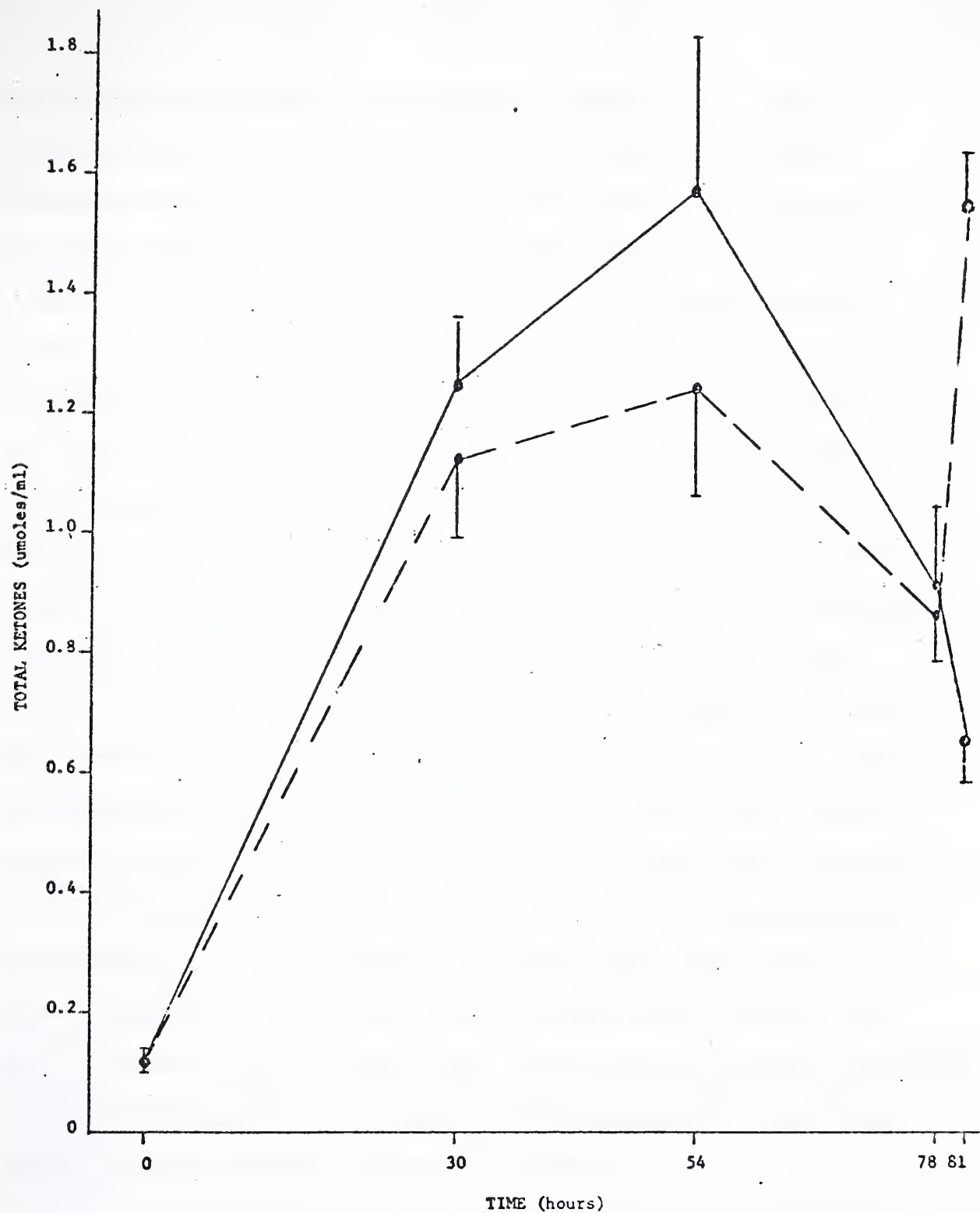


Figure 2- Mean total ketone body levels ( $\pm$ S.E.M.) in water-fed rats (solid line) and ethanol-fed rats (broken line). At 81 hours, ethanol produced a marked rise in total ketones. At all other times there was no significant difference between the two groups.



resulted in a marked rise in serum ketones 6 hours later. There was no significant change in total ketone levels in the control group during the same time period. The B-hydroxybutyrate in the ethanol rats increased from a mean level of  $0.712 \pm 0.070$   $\mu\text{mol/ml}$ . at 78 hours, to  $1.383 \pm 0.056$   $\mu\text{mol/ml}$ . at 81 hours ( $p < .001$ ). The B-hydroxybutyrate level in the control group decreased from a mean of  $0.758 \pm 0.119$   $\mu\text{mol/ml}$ . at 78 hours to  $0.562 \pm 0.069$   $\mu\text{mol/ml}$ . at 81 hours, but this decrease was not statistically significant. There was a two to three fold difference in B-hydroxybutyrate levels between the ethanol and water groups at 81 hours ( $p < .001$ ). The mean acetoacetate level did not change significantly from 78 to 81 hours in the ethanol-fed group ( $0.144 \pm 0.010$   $\mu\text{mol/ml}$ . to  $0.158 \pm 0.042$   $\mu\text{mol/ml}$ .) and decreased in the water-fed rats from  $0.152 \pm 0.032$   $\mu\text{mol/ml}$ . to  $0.086 \pm 0.011$   $\mu\text{mol/ml}$ . ( $.05 < p < .10$ ). There was a two fold difference in acetoacetate between the two groups ( $.05 < p < .10$ ). Total ketones at 81 hours were  $0.648 \pm 0.066$   $\mu\text{mol/ml}$ . in the water-fed group and  $1.541 \pm 0.092$   $\mu\text{mol/ml}$ . in the ethanol group, which represented a two to three fold difference ( $p < .001$ ). The ratio of B-hydroxybutyrate to acetoacetate at 81 hours was 8.8:1 in the ethanol group which was elevated compared to a ratio of 4.9:1 in the ethanol rats at 78 hours and 6.5:1 at 81 hours in the control group. There was no significant difference in the B-hydroxybutyrate to acetoacetate ratio between the ethanol and control groups at 30 hours, 54 hours, or 78 hours.

Free fatty acid levels in the ethanol and water rats at the various time periods studied, are reported in Figure 3 and Table 2. In the control group after 30 hours of fasting, free fatty acids rose three



TABLE 2

Duration of Fast (hours)	Total Ketones (umoles/ml)	Free Fatty Acids (umoles/ml)	Glucose (mg/100ml)	Insulin (uunits/ml)
0	0.116 $\pm$ 0.023	0.283 $\pm$ 0.045	178 $\pm$ 2.99	78.8 $\pm$ 5.66
30(control)	1.254 $\pm$ 0.107	1.054 $\pm$ 0.046	109 $\pm$ 6.02	25.2 $\pm$ 5.26
30(ethanol)	1.119 $\pm$ 0.130	0.974 $\pm$ 0.029	116 $\pm$ 6.66	30.0 $\pm$ 5.81
t-test (p)	N.S.	N.S.	N.S.	N.S.
54(control)	1.574 $\pm$ 0.255	1.121 $\pm$ 0.043	141 $\pm$ 5.58	22.7 $\pm$ 3.79
54(ethanol)	1.244 $\pm$ 0.178	1.229 $\pm$ 0.035	154 $\pm$ 5.64	36.6 $\pm$ 6.13
t-test (p)	N.S.	.05 < p < .10	N.S.	N.S.
78(control)	0.910 $\pm$ 0.131	0.932 $\pm$ 0.111	171 $\pm$ 10.7	32.7 $\pm$ 3.72
78(ethanol)	0.856 $\pm$ 0.077	0.973 $\pm$ 0.098	164 $\pm$ 1.87	29.9 $\pm$ 4.65
t-test (p)	N.S.	N.S.	N.S.	N.S.
81(control)	0.648 $\pm$ 0.066	0.777 $\pm$ 0.134	163 $\pm$ 6.06	32.3 $\pm$ 6.85
81(ethanol)	1.541 $\pm$ 0.092	0.896 $\pm$ 0.054	168 $\pm$ 16.5	37.0 $\pm$ 3.84
t-test (p)	p < .001	N.S.	N.S.	N.S.

All values are  $\pm$  S.E.M.



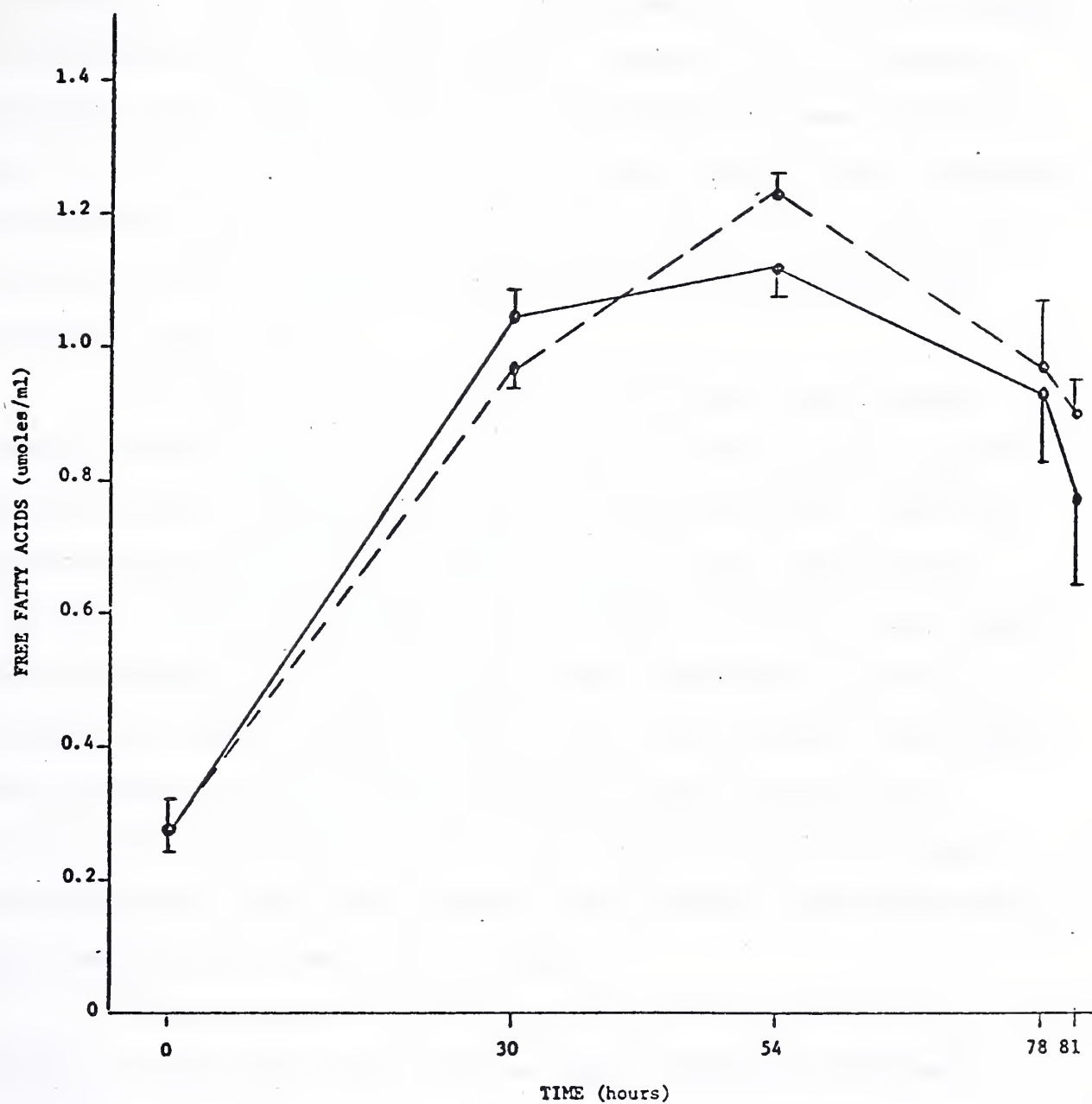


Figure 3- Mean serum free fatty acid levels ( $\pm$ S.E.M.) in water-fed rats (solid line) and ethanol-fed rats (broken line). All rats were fasted for the entire study. There was no significant difference in FFA levels between the two groups at any time.





to four fold from  $0.283 \pm 0.045$   $\mu\text{mol./ml.}$  at 0 hours to  $1.054 \pm 0.046$   $\mu\text{mol./ml.}$  at 30 hours. From 30 to 54 hours of fasting there was no significant change in free fatty acids in the control group. Beyond 54 hours of starvation, free fatty acids tended to decline with the difference between the 54 hour and 81 hour level significant at the six percent confidence level ( $.05 < p < .10$ ). The mean free fatty acid level in the control group at 81 hours was  $0.777 \pm 0.134$   $\mu\text{mol./ml.}$  which was still significantly elevated compared to the level at 0 hours ( $p < .01$ ).

The mean free fatty acid level in the ethanol group also rose markedly between 0 and 30 hours and the level at 30 hours was not significantly different from the control group. From 30 to 54 hours, free fatty acids increased from  $0.974 \pm 0.029$   $\mu\text{mol./ml.}$  to  $1.229 \pm 0.035$   $\mu\text{mol./ml.}$  ( $p < .001$ ). The difference between mean free fatty acid levels in the ethanol and control group at 54 hours was small and of questionable significance ( $.05 < p < .10$ ). Beyond 54 hours, the free fatty acids declined in the ethanol rats and there was no significant difference between the ethanol and control rats at 78 hours or 81 hours. Thus, despite the marked increase in ketone levels that occurred between 78 and 81 hours in the ethanol group, the free fatty acid level did not increase.

Glucose levels are reported in Table 2 and are depicted in Figure 4. At no point in the study was there a significant difference in glucose levels between the ethanol-fed group and the control group. In both sets of rats, there was a thirty-five to forty percent decline in serum glucose concentration during the first thirty hours of the fast. Glucose in the water-fed rats fell from a pre-fasting level of  $178 \pm 2.99$   $\text{mg./100ml.}$  to  $109 \pm 6.02$   $\text{mg./100ml.}$  at 30 hours ( $p < .001$ ) and the level



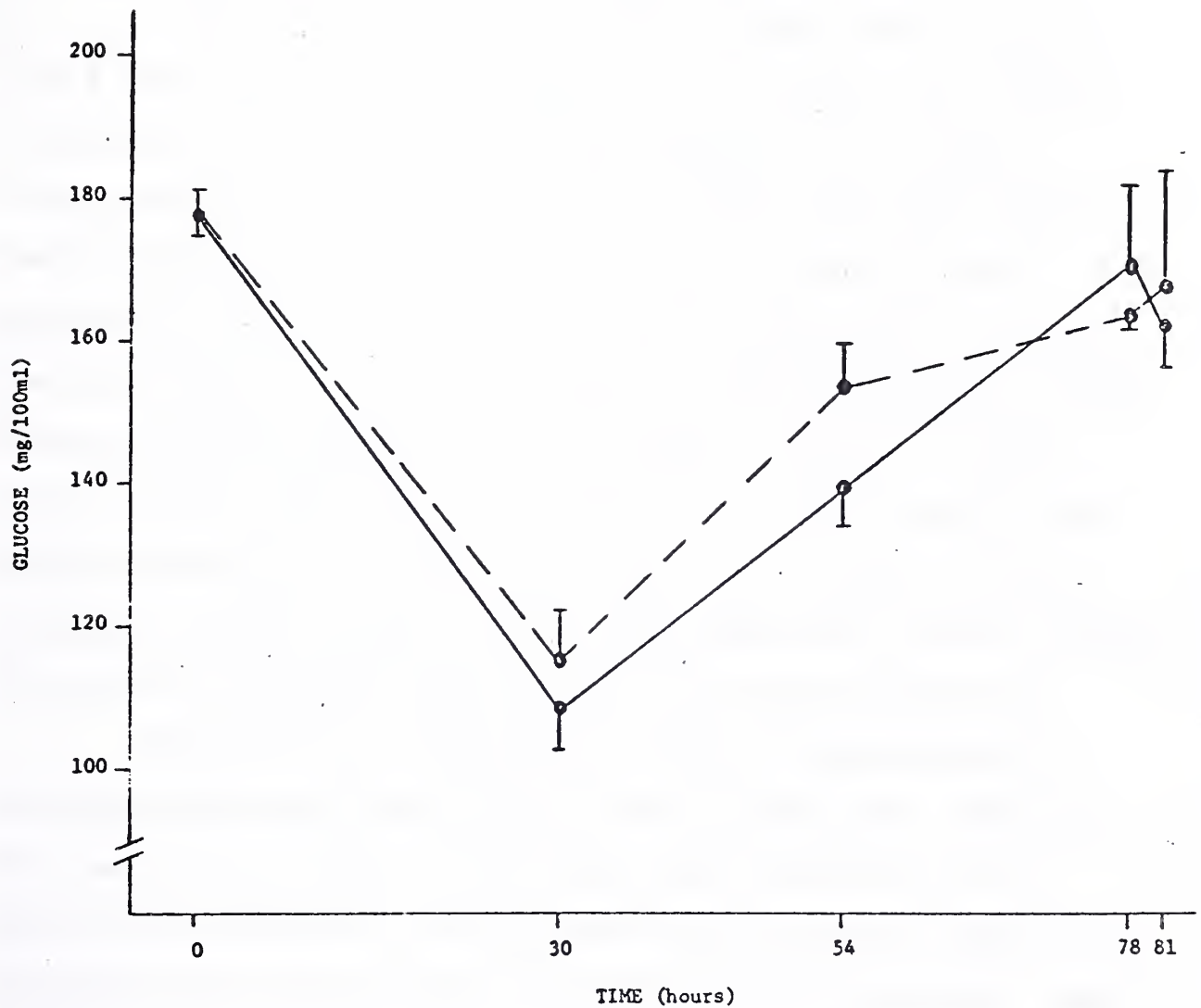


Figure 4- Mean serum glucose levels ( $\pm$ S.E.M.) in water-fed rats (solid line) and ethanol-fed rats (broken line). All rats were fasted for the entire study. There was no significant difference between the two groups at any time.



at 30 hours in the ethanol-fed rats was  $116 \pm 6.66$  mg./100ml. ( $p < .001$ ). Beyond 30 hours of starvation, glucose increased in both the ethanol and water rats and approached pre-fasting levels by 78 hours. There was no significant change in either group between 78 and 81 hours.

Insulin (Figure 5, Table 2) initially showed a marked decline from a 0 hour level of  $78.8 \pm 5.7$  uunits/ml. to a 30 hour level of  $30.0 \pm 5.8$  uunits/ml. in the ethanol rats ( $p < .001$ ) and  $25.2 \pm 5.3$  uunits/ml. in the control rats ( $p < .001$ ). There were no further significant changes noted in the mean insulin level in either group. In addition, there were no significant differences in mean insulin levels between the ethanol and control rats at 30, 54, 78, or 81 hours. The initial decline in insulin occurred during the same time period as the fall in glucose. The final rise in glucose, however, was not accompanied by a rise in insulin. This apparent abnormality in insulin responsiveness to an increase in glucose is present in both the water group and ethanol group, and, therefore, cannot be related to the relative hyperketonemia in the 81 hour ethanol rats.

Repeated doses of ethanol had no effect on the percentage of the rat's initial weight that was lost after an 81 hour fast. Water-fed rats that were sacrificed at 81 hours were noted to have lost 24.5% of their initial body weight, and the ethanol-fed rats that were fasted for the same period of time had lost 24.4% of their initial body weight. Table 3 shows the mean percentage of initial body weight lost in both groups at all time periods that were studied.



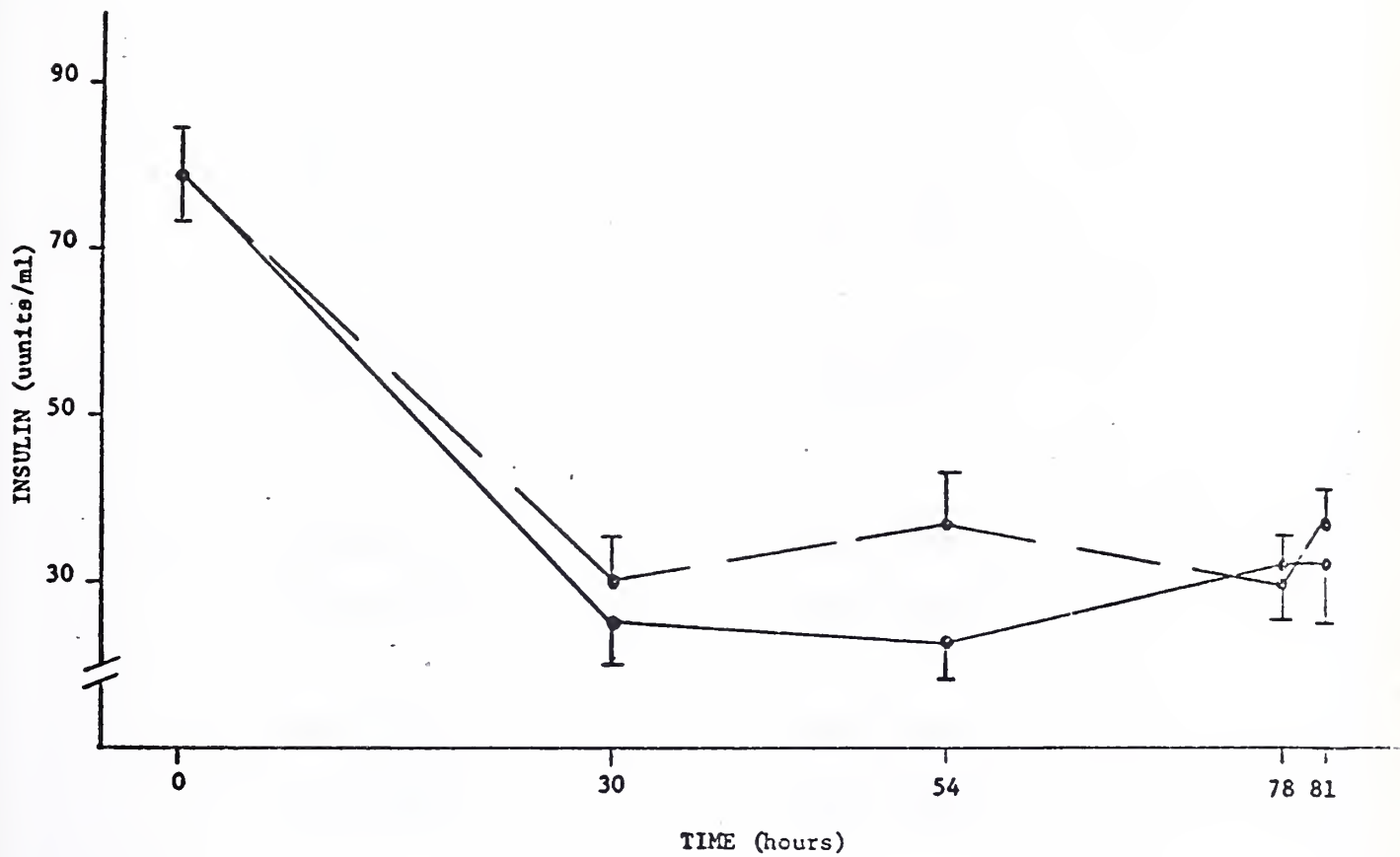


Figure 5- Mean serum insulin levels ( $\pm$ S.E.M.) in water-fed rats (solid line) and ethanol-fed rats (broken line). All rats were fasted throughout the study. There was no significant difference between the two groups at any time.





TABLE 3

Duration of Fast (hours)	Percentage of Initial Body Weight Lost
0	0
30 (control)	14.3 $\pm$ 1.20
30 (ethanol)	14.3 $\pm$ 0.87
54 (control)	21.1 $\pm$ 0.48
54 (ethanol)	17.9 $\pm$ 0.76
78 (control)	22.5 $\pm$ 0.61
78 (ethanol)	24.8 $\pm$ 0.49
81 (control)	24.5 $\pm$ 1.24
81 (ethanol)	24.4 $\pm$ 0.74

All values are  $\pm$  S.E.M.



## DISCUSSION

In the present study, fasted rats received intoxicating doses of ethanol in order to investigate two different aspects of the interaction of alcohol with ketone metabolism. The first problem investigated, was the effect of daily doses of ethanol on the ketone level of rats when it was measured one day after the preceding dose of alcohol. It was felt that this experimental design would reproduce aspects of the usual clinical picture of patients with alcoholic ketoacidosis (i.e. chronic ingestion of large doses of ethanol associated with anorexia and decreased food intake for 24-72 hours prior to admission). In both the ethanol and control rats, the ketone levels rose to a peak at 55 hours and then began to decline. These findings are consistent with previous observations of ketone levels in rats during a prolonged fast (14,102). No effect of ethanol on serum ketone levels was noted when blood samples were taken 27 hours after the last dose of alcohol. Thus, an effect of chronic ethanol ingestion on ketone metabolism, that would persist beyond the period when ethanol was still being metabolized, was not demonstrated in fasted rats.

The second aspect of ethanol's interaction with ketone metabolism, investigated in this study, was the acute effect of a large dose of alcohol on a rat experiencing a prolonged fast and primed with daily intoxicating doses of ethanol. After 75 hours of fasting and daily doses of ethanol, a final intoxicating dose was administered. Blood was drawn 6 hours later and a marked rise in total ketones was demonstrated.



There was an increased B-hydroxybutyrate to acetoacetate ratio with a sharp rise in B-hydroxybutyrate and no change in acetoacetate. The total ketone level was significantly greater than the level found in rats starved 81 hours and tube fed water, but was similar to the peak starvation levels, which occurred at 54 hours of fasting. Despite the rise in ketones at 81 hours, the level reached was still several times less than the ketone concentrations found in alloxan diabetic rats (14,24). Thus, the ketosis induced by ethanol was significant, relative to the ketosis induced by 81 hours of fasting, but was no greater than the maximum ketone levels produced by starvation and certainly did not represent more than a minimal ketoacidosis (13).

The only prior report in the literature, that investigated the in vivo effect of chronic ethanol ingestion on ketone levels in animals, was by Lefevre in 1970 (93). His rats were fed a calorically adequate diet and received subintoxicating doses of ethanol. He reported an increase in serum ketone levels shortly after ingestion of alcohol and the diet; and, in contrast to the present study, the hyperketonemia was more marked after an overnight fast when ethanol levels were zero. In a similar study carried out by Lefevre with human subjects (93), the delayed effect of alcohol on ketone metabolism was well documented. Ethanol led to an increased ketosis after chronic ingestion of subintoxicating doses, and the increase in B-hydroxybutyrate and acetoacetate was more marked fifteen hours after the last dose of alcohol was ingested. In rats, however, his data is less conclusive because only acetoacetate was measured. It has been well established that, following ethanol ingestion, there is an increase in the intracellular NADH to NAD ratio (48-57,59) which



causes an increase in the B-hydroxybutyrate to acetoacetate ratio (3,94). Therefore, as the redox potential of the cell returns to normal, during the fifteen hour period after ethanol ingestion, a rise in acetoacetate is consistent with no change or even a decrease in total ketone levels since it may be associated with a decreasing B-hydroxybutyrate level. A comparable situation occurs during the correction of alcoholic ketoacidosis (3,5). Thus, although Lefevre (93) has documented a post-ethanol rise in acetoacetate in rats, which persisted into the fasted state, he has not established that the magnitude of the ketosis is greater when the ethanol level is zero. In the current study, a delayed effect of ethanol on ketone metabolism was not found. Ketone levels, measured 27 hours after alcohol was administered, were no higher than in fasted controls. Although this contrasts with Lefevre's results, the two studies are not comparable. Rats in Lefevre's experiment received a high fat diet and were only fasted overnight. The rats in the current study received no food with their ethanol feedings and were fasted for 30 to 81 hours. It is possible that in the current study, the 27 hour period separating the administration of ethanol, and the collection of blood samples was too prolonged to detect a delayed but evanescent effect of ethanol. This can only be evaluated with further studies of fasted rats, that vary the length of time between ethanol feeding and sacrifice of the rat.

In addition to the delayed effect of ethanol on ketone metabolism, reported by Lefevre (93), several investigators have demonstrated an acute effect of ethanol on hepatic ketogenesis with increased hepatic output of ketone bodies and increased serum levels of ketones (89-95). Most of these studies have explained the increased ketogenesis on the





basis of the increased NADH to NAD ratio induced by the metabolism of ethanol (described in the Literature Review earlier in this paper). In the current study, the effect of ethanol on two additional factors, that could influence ketone metabolism were studied- free fatty acids, and insulin.

Free fatty acids are a necessary substrate for ketogenesis (7,13). An increase in free fatty acids, in a normal, well fed animal, will not increase ketone body formation (14), but a similar elevation in a fasting or diabetic animal will result in an increase in the serum ketone level of that animal (16). In the present study, serum free fatty acid levels paralleled the ketone levels at 30, 54, and 78 hours of fasting in both the ethanol-fed and control rats, with no significant difference between the two groups. These results are consistent with findings reported in other investigations of prolonged starvation in rats (14,102). In contrast, at 81 hours, which was shortly after the last dose of ethanol, ketones rose, but free fatty acids continued to fall. This suggests that the increase in ketones resulted from an effect of ethanol on hepatic ketogenesis or on peripheral ketone utilization, and was not a result of increased mobilization of free fatty acids. The declining free fatty acid level, and the failure of an intoxicating dose of ethanol to increase free fatty acid levels, may have been due to depletion of peripheral fat stores, which is known to occur in rats after a three to four day fast (13). The declining serum ketone concentration, prior to the final dose of ethanol, may also have been a consequence of the decline in free fatty acid substrate for ketogenesis. The interplay between the serum free fatty acid level and the metabolic set of the liver, and the importance of both of these factors



in determining the level of ketogenesis, is highlighted by the results of the present study. The ethanol-induced rise in ketogenesis without an increase in serum free fatty acids, suggests that ethanol changed the metabolic set of the liver so that a greater proportion of incoming fatty acids were taken up by the mitochondria and oxidized; or that a greater proportion of the acetyl CoA, generated by the  $\beta$ -oxidation of fatty acids, was diverted into ketogenic pathways.

Insulin and glucose were also studied to determine if the acute ethanol-induced rise in total ketones, at 81 hours of fasting, was associated with a change in the serum concentration of these substances. The key role of insulin in controlling the production and utilization of ketoacids is well established (8,13,22). Insulin can affect serum ketone levels by controlling adipose tissue lipolysis (14), by affecting the flow of acetyl CoA into ketogenic pathways (13), and by controlling the rate of peripheral utilization of ketones (13,34). In addition, insulin may effect the acyl-carnitine transferase step (15,26), and other, as of yet, undefined sites of regulation of hepatic ketone production. Because of insulin's significant role in the regulation of ketogenesis associated with starvation and diabetes, it is important to determine the effect of ethanol ingestion on insulin levels, particularly when the ethanol administration results in hyperketonemia. Ethanol has been reported to increase (6,67-72) and decrease (62-66) serum insulin levels and islet cell release of insulin. The hypoglycemic effect of ethanol in the fasting state has been well documented and extensively studied (46-56), and has been associated with a fall in insulin concentration. In the present study, no significant effect of ethanol on insulin or glucose levels was demonstrated. The



final dose of ethanol, administered after 75 hours of fasting, was associated with a marked rise in total ketones, but there was no significant change in insulin or glucose levels. Failure to demonstrate an ethanol-induced decline in glucose and a secondary fall in insulin would seem to be inconsistent with previous studies of the effects of ethanol on carbohydrate metabolism (46-56). However, the in vivo studies, in which fasted subjects became hypoglycemic after ethanol intake, were done with humans or with animals other than rats; and there is evidence that the metabolic response of a rat to starvation is different from that of larger animals and humans. In humans, there is an initial fall in glucose followed by a maintenance of the glucose concentration at this lower level during a prolonged fast (103). Owens (102) found that in rats, the glucose fell during the initial forty-eight hours of a fast, but with continued starvation, it rose and approached values for fed animals. This observation is confirmed in the present study. Cahill (13) stated that gluconeogenesis, instead of becoming attenuated as in man, progressively increases during starvation in the rat since the non-obese rat has more calories in muscle nitrogen than in his limited supply of adipose tissue. Thus, prolonged starvation of a rat is characterized by hypergluconeogenesis relative to the response of normal man to fasting. There is evidence that heightened gluconeogenesis in fasted humans and dogs, is characterized by a resistance to the hypoglycemic action of ethanol. Freinkel (47) reported that administration of 75mg. of cortisone every eight hours to normal humans, as a means of activating gluconeogenesis, prevented hypoglycemia when ethanol was ingested after a three day fast. A prolonged fast in a dog would also be expected to result



in an increase in gluconeogenesis, as fat stores were exhausted. It has been found that acute doses of ethanol, that elicited hypoglycemia in dogs fasted for three to six days, did not produce hypoglycemia in dogs fasted for 14 days (47). Therefore, the failure of ethanol, administered after a 75 hour fast, to induce hypoglycemia and hypo-insulinemia in the current study, may have been a result of the heightened gluconeogenesis which is present in a rat after a prolonged fast.

Insulin levels remained low after their initial decline despite the rise in glucose that occurred in both groups after thirty hours of fasting. The failure of the animals to increase their serum insulin levels in response to the increase in glucose, may have been an effect of prolonged starvation. It was definitely not related to ethanol ingestion, since it was present to an equal extent in both the ethanol-fed and control rats. Cahill (103) reported that if normal humans undergo an eight day fast, they will have an abnormal glucose tolerance after the fast. Fasted subjects, however, still exhibited a brisk insulin response, but of a lesser magnitude than when tested prior to the fast.

The present study has demonstrated that, although ethanol induces an acute rise in total ketones when administered after a prolonged fast, it does not produce a detectable increase in serum free fatty acids or a decrease in insulin to explain its hyperketotic effect. Ethanol did produce an increase in the B-hydroxybutyrate to acetoacetate ratio, implying a change in the redox potential of the hepatocyte. Prior studies have postulated that this increase in the NADH to NAD ratio inhibits the tricarboxylic acid cycle, and channels the acetyl CoA generated from





$\beta$ -oxidation into ketogenic pathways (89-90). Another possible mechanism, for the increase in ketone levels, is that the acute administration of ethanol decreased peripheral uptake of ketones resulting in hyperketonemia without a change in hepatic ketogenesis. Lefevre (93), however, reported that neither in vivo pretreatment with alcohol nor in vitro addition of alcohol had any significant effect upon consumption of acetoacetate by rat diaphragm. A third possibility is that ethanol had an acute effect on mitochondrial pathways of fatty acid uptake and oxidation, possibly via an effect on acyl-carnitine transferase. Evidence for the importance of this enzyme in the production of starvation ketosis and diabetic ketosis has been provided (7,17,21,24,25), and similar studies are needed to evaluate its role in ethanol-induced ketosis.

We have not demonstrated an effect of ethanol on the ketogenesis of a fasted rat, that persists beyond the period of metabolism of the ethanol. Further investigation into the delayed effect of ethanol is needed since this is particularly relevant to an understanding of the pathogenesis of alcoholic ketoacidosis. The effects of ethanol on the ketogenesis of a fasted rat could be studied after a period of days or weeks of ingestion of low doses of ethanol (3g./kg.) along with an adequate diet. Such an experimental design would hopefully modify the hepatocyte so that a ketogenic state could be induced that would not require the immediate presence of ethanol to sustain. It would then be possible to evaluate specific sites in the ketogenic pathway (i.e. use decanoyl carnitine to evaluate the importance of the acyl-carnitine transferase step) in order to determine their role in the production of ethanol-induced ketosis.



## SUMMARY

1. Daily intoxicating doses of ethanol were administered to fasted rats to evaluate the pathogenesis of alcoholic ketoacidosis

2. After three daily tube feedings of ethanol, a final dose of ethanol on the fourth day of fasting resulted, within six hours, in a two fold increase in B-hydroxybutyrate and total ketones. There was an increase in the B-hydroxybutyrate to acetoacetate ratio with no significant change in acetoacetate levels. Total ketones were two and one half times the level in control rats that were fasted for 81 hours. In animals studied at earlier points in the fast and at intervals of 24 hours or more after ethanol intake, there was no ethanol-induced augmentation in ketogenesis.

3. The increased ketone level, induced by ethanol, was not associated with a change in free fatty acids, insulin or glucose, and levels of these three substances were not significantly different in control rats that were fasted for the same period of time but did not receive ethanol.

4. These data indicate that ethanol enhances starvation-ketosis independent of changes in lipolysis, insulin secretion or blood glucose, suggesting a direct effect on hepatic ketogenesis.

5. This effect may be short-lived as it was observed only within six to eight hours of alcohol treatment.



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