ACETALDEHYDE ADDUCTS OF HEMOGLOBIN AS A

POSSIBLE MARKER OF CHRONIC ALCOHOLISM

by

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ABSTRACT

ACETALDEHYDE **ADDUCTS** OF HEMOGLOBIN AS A
POSSIBLE MARKER OF CHRONIC ALCOHOLISM

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Despite the fact that extensive and prolonged ingestion of alcohol is hazardous to one's health, alcohol abuse and addiction is on the rise and is a serious problem in society. Due to the variation in drinking patterns from culture to culture, it is difficult to define alcoholism since what is normal for one culture may be abnormal for another. Nevertheless, in general, one could define alcoholism as "the intermittent or continual ingestion of alcohol leading to dependence or harm." The abuse of alcohol is increasing and is affecting all age groups of our society; consequently, the number of people requiring treatment for alcoholism and alcohol related pathologies is increasing. Although there are tests for acute alcoholism, there do not appear to be any tests for testing long-term alcoholism. One possible test method to indicate long-term alcoholism employs a fraction of hemoglobin A as an analyte.

Hemoglobin A makes up about 90% of the adult hemoglobin. Chromatography of a red blood cell hemolysate

shows three negatively charged minor hemoglobin components that elute before the main hemoglobin A peak and are hemoglobins A_{1A}, A_{1B}, A_{1C}. Hemoglobin A_{1C} is the most abundant minor hemoglobin and its formation represents a posttranslational glycosylation of Hb A. This process is slow and non-enzymatic and it occurs continuously throughout the 120-day life span of the erythrocyte. The glycosylation of hemoglobin is basically irreversible making the level of Hb A_{1C} directly proportional to the time-averaged concentration of glucose within the erythrocyte. Therefore the cummulative level of Hb A_{1C} is especially important in monitoring the diabetic patient.

The oxidation of ethanol in the liver leads to the formation of acetaldehyde. Acetaldehyde is potentially more toxic than ethanol because of its greater reactivity and lipid solubility. Studies suggest that hemolysates from alcoholic patients have a hemoglobin fraction that has chromatographic properties similar to Hb A_{1C} but they differ from Hb A_{1C} in that they are not bound by boronated agarose. These patients have an elevated concentration of minor hemoglobins but normal amounts of glycosylated hemoglobins. This principle is the basis of this research.

A cation-ion exchange column in tandem with a boronated agarose gel column was used. The cation-ion exchange column binds all hemoglobins except those attached to glucose or acetaldehyde. The effluent is drained into

the boronated agarose gel column. This column binds the glycosylated hemoglobin and only the hemoglobin containing acetaldehyde elutes. These preliminary findings suggest a direct correlation with acetaldehyde-bound Hb and alcohol consumption levels over approximately the past thirty days.

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LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS
ADH	Alcohol Dehydrogenasa	
GGT	Gamma-Glutamyl Transferase	
g	Gram	
mL	Milliliter	$1x10^{-3}$ liter
µL	Microliter	$1x10^{-6}$ liter
nm	Nanometer	$1x10^{-9}$ meter
EtOH	Ethyl Alcohol	
Hb	Hemoglobin	
GHb	Glycosylated Hemoglobin	
AcHb	Acetaldehyde-bound Hemoglobin	
MEOS	Microsomal Ethanol Oxidizing System	
NAD^+	Nicotine Adenine Dinucleotide	
α	Alpha	
β	Beta	
γ	Gamma	
δ	Delta	
ε	Epsilon	
°C	Degrees Centigrade	
min	Minute	
%	Percent	

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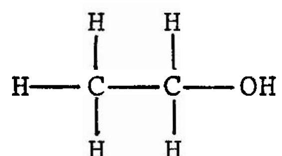
CHAPTER I

INTRODUCTION

A. Abuse of Ethanol

Ethanol, CH_3CH_2OH, is a straight-chained aliphatic alcohol whose chemical structure is as follows:



Of all the aliphatic alcohols, "only ethanol has the appropriate physiological effect, metabolic pathway and low order of toxicity that has resulted in its widespread use as an alcoholic beverage." (1) Ethanol is also widely used as an industrial and laboratory solvent.

Alcoholic beverages have been known to man for 100,000 years and probably were first encountered as a result of the natural fermentation of grapes, grain or honey during their storage. The ancient Egyptians knew about wine since the fifth and forth millenium B.C. The Bible refers to wine in many instances, for example:

> Noah was the first tiller of the soil.
> He planted a vineyard; and he drank of
> the wine, and became drunk,
> and lay uncovered in his tent . . .
>
> Genesis 9:20-1

Despite the fact that extensive and prolonged ingestion of alcohol is injurious to one's health, alcohol abuse and addiction continues and affects all age groups, all societies and all cultures today. The consequences of alcohol abuse represent personal and societal problems. In 1977, alcohol-related problems in the United States cost approximately 45.4 billion dollars. (2)

Lost production, health and medical expenses, motor vehicle accidents, violent crimes, fire losses and social responses are a direct result of alcohol abuse. In 1981, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) reported that alcohol is a factor in ten percent of all deaths in the United States and that approximately one in ten adult drinkers is likely to have a drinking problem or become an alcoholic. (2) These estimates express the magnitude of the problem and the importance of allocating testing, intervention and treatment programs for alcoholism and alcohol related pathologies.

Alcohol abuse results in a wide variety of psychiatric and physical illnesses such as acute alcohol intoxication, psychoses, malnourishment, malabsorption, liver disease, and sexual dysfunction. (3)

The death rate for a given age group is greater among alcoholics as compared to the general population. Of all the age groups, the mortality rate due to alcoholism is greatest in the young and especially females. Diseases of the digestive system such as liver disease, pancreatitis,

and gastritis are the most commonly found diseases in alcohol abuse. The influence of the extent and duration of alcohol abuse resulting in a disease is best seen by the incidence of liver dysfunction. If one's intake of alcohol is less than 160 g per day an increase in the duration from 3.5 to 21 years results in a three-fold increase in the number of cases of liver dysfunction. The consumption of more than 160 g of alcohol per day leads to a rise in the probability of liver dysfunction from 11% to 73% for those whose duration of drinking is 3.5 years to 21 years respectively. (Table 1)

Defining alcoholism can cause confusion, because what one person labels as alcoholic another would not. Alcohol use and abuse covers a wide spectrum with one end involving those who never drink alcohol and the other end involving those who are addicted to alcohol and who may be suffering physical, mental and sociological damage. (3) Therefore, the difficulty in defining alcoholism is in deciding where in this spectrum is normal and beyond what point is abnormal. Social and cultural attitudes influence the concept of normality since what is normal for one culture may be abnormal for another. Generally one could define alcoholism as "The intermittent or continual ingestion of alcohol leading to dependency or harm." (4)

Today, one is faced with the problem of identifying the chronic abuser of alcohol. There is no one reliable

Group	Duration years	BELOW 160 g ethanol/day		ABOVE 160 g ethanol/day	
		Number of cases	% of cases with Liver Dysfunction	Number of cases	% of cases with Liver Dysfunction
1	3.5 ± 1.3	74	6	55	11
2	7.8 ± 1.4	76	16	79	37
3	12.8 ± 1.5	30	17	64	40
4	21.1 ± 5.4	6	17	33	73

Table 1. Influence of Extent and Duration of Alcohol Abuse on Liver Dysfunction (3)

and definitive test method that will identify the alcoholic early. One could look for signs of cirrhosis, or elevated enyzme levels that reflect the degree of tissue destruction, or one could even perform a liver biopsy; unfortunately, the disease must be in an advanced stage in order to be detected by these methods. This research suggests identifying alcoholism by quantifying the levels of acetaldehyde-bound hemoglobin in the blood. This level represents a time-averaged accumulation of acetaldehyde in the blood stream during the 120-day life span of the erythrocyte.

B. Metabolism, Absorption and Excertionof Ethanol

1. Metabolism of Ethanol

Ethanol being polar is miscible with water. It passes easily through the cell membranes by simple passive diffusion due to its small size and polarity.

Ethanol is metabolized primarily in the liver. Alcohol dehydrogenase (ADH), a zinc containing metallo enzyme, is located in the cytosol of the hepatocyte and oxidizes ethanol to acetaldehyde. Catalase and a microsomal ethanol oxidizing system (MEOS) also oxidize ethanol to acetaldehyde. A cytoplasmic aldehyde dehydrogenase oxidizes acetaldehyde to acetate. There are two routes acetate can follow. One is that acetate can be oxidized to carbon dioxide through the citric acid cycle. Secondly, acetyl CoA, the activated form of acetate can be metabolized to form fatty acids, steroids, amino acids, and ketone bodies. (Figure 1)

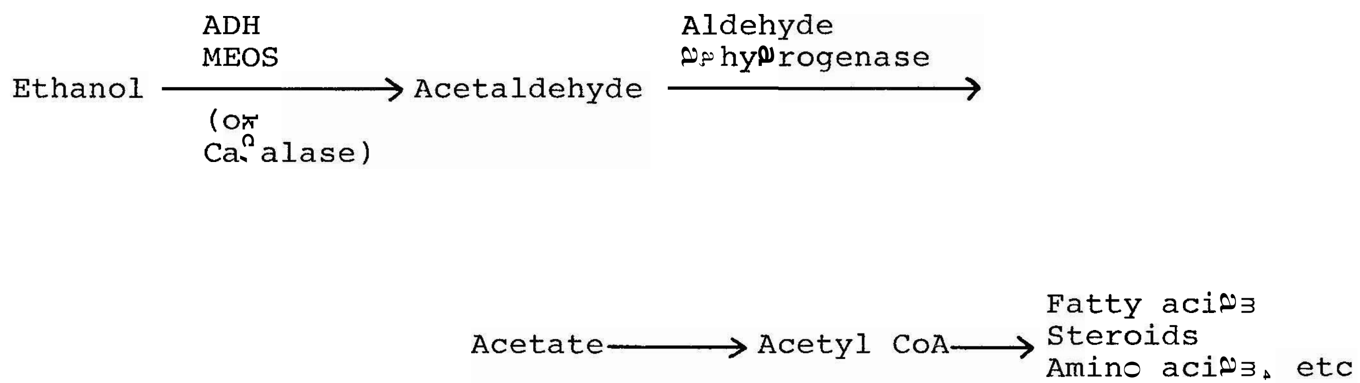


Figure 1. Oxidation of Ethanol

The following reaction is catalyzed by alcohol dehydrogenase:

$$CH_3CH_2OH + NAD^+ \quad NADH + H^+ + CH_3CHO$$

Alcohol dehydrogenase requires nicotine adenine dinucleotide (NAD^+). During the oxidation of ethanol to acetaldehyde, NAD^+ is reduced to NADH. The conversion of NAD^+ to NADH during the metabolism of ethanol results in a significant decrease in the overall NAD^+/**NADH** ratio in the liver. This shift is critical to biochemical reations, thus, accounting for some of the metabolic effects of ethanol. The rate at which ethanol is metabolized depends on the rate at which the **ADH:NADH** complex is reoxidized.

2. <u>Absorption of Ethanol</u>

Ethanol is readily absorbed from the gastrointestinal tract, especially from the duodenum and jejunum and is excreted in a relatively few hours. Once in the gastrointestinal tract, the ethanol diffuses rapidly and uniformly throughout the body water. There is minimal absorption of ethanol from the mouth. Another way ethanol is absorbed into the body is by inhalation of vapor and by passage across the pulmonary epithelium. Ethanol can also be absorbed into the blood by any parenteral route such as intraperitoneal, intrathecal or subarachnoid injection. (Figure 2)

Absorption of ethanol does not occur through intact skin since the keratin layer of the skin is impermeable to ethanol. The urinary bladder is impermeable to ethanol

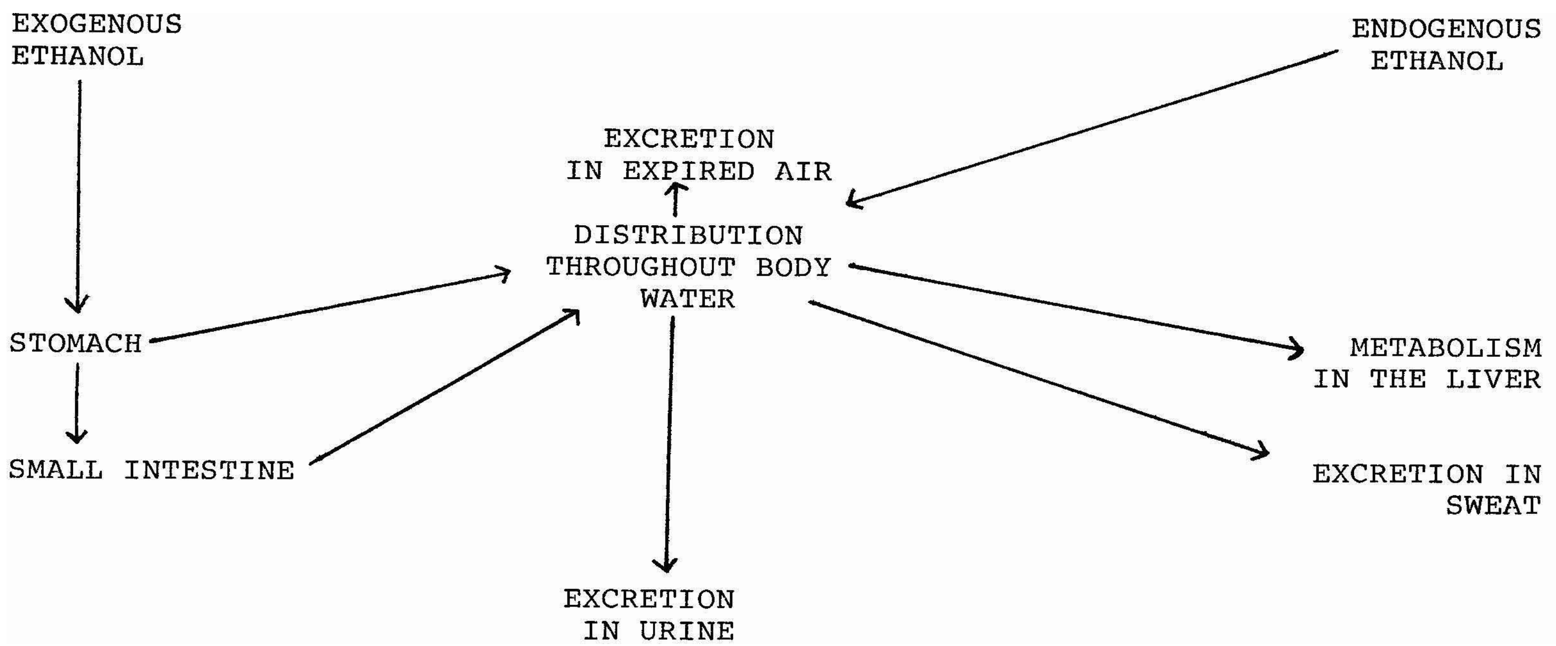


Figure 2. Absorption of Ethanol

and therefore prevents the reabsorption of ethanol contained in urine stored in the bladder.

3. Factors Influencing Ethanol Absorption

As mentioned earlier, ethanol is absorbed through simple passive diffusion; the higher the concentration of ethanol the greater the concentration gradient resulting in a more rapid absorption of ethanol. The laws of diffusion determine the distribution of alcohol in the body. Also any factor which helps maintain the concentration gradient will also enhance absorption. For example, efficient blood flow will quickly remove ethanol from the site of absorption and promote absorption. Concentrations of ethanol above 30 mg/100 mL can cause superficial erosion, hemorrhages, and paralysis of the smooth muscle of the stomach resulting in a decrease in the rate of absorption of ethanol. The rate at which ethanol is ingested will affect the absorption. For example, if an alcoholic beverage is ingested rapidly, the peak blood alcohol level will develop slowly. The absorption of ethanol can be affected by chemicals other than ethanol in an alcoholic beverage. Food in the stomach will delay gastric emptying therefore reducing the rate and efficiency of absorption of ethanol. The amount and type of food also has an effect on the absorption of ethanol. A large amount of food will have a greater effect on ethanol absorption. High protein, high fat, and high carbohydrate-content foods inhibit the absorption of ethanol. Ethanol absorption

is enhanced by rapid emptying of the stomach. Likewise, one who has undergone a gastrectomy will have a rapid absorption of ethanol since the ethanol will reach the highly absorptive small intestine more quickly. Decreasing body temperature and mental or physical exercise will decrease the rate of absorption of ethanol.

4. Distribution of Ethanol in Body Fluids

Blood flow and permeability will affect the rate of equilibrium of ethanol with tissue. Since diffusion is a slow process, an oxgen rich blood supply will speed up this penetration. Therefore, the brain, lungs, kidney and liver will reach equilibrium quickly as compared to skeletal muscle which has a poorer blood supply. The mass of the tissue is also an important factor in that the greater the mass the slower the rate of equilibrium.

Initially, once ingested, alcohol will diffuse into the tissues from the arterial blood. This diffusion will be rapid due to a high solubility of ethanol in blood and tissue. Once this absorption has been completed, the arterial concentration of ethanol will drop and ethanol will diffuse into the capillaries and venous blood. Comparing the level of ethanol in the venous blood to arterial blood, one will observe a higher level in the venous blood because of the lower rates of metabolism and excretion. The level of ethanol will peak in the urine approximately 30-190 minutes after ingestion.

5. Excretion of Ethanol

Usually, up to two percent of alcohol ingested is excreted unchanged through the urine, expired air, and sweat. This value may be higher at elevated temperatures and at high blood alcohol levels.

Excretion of ethanol through the urinary system is a passive process and can have clearances between 0.9 and 12.7 mL/minute. (2)

The movement of alcohol into the expired air is solely a diffusive process. It is not only the alveolar air that is in equilibrium with the lung blood but also the expired air. (5) This is due to the fact that air in the bronchi comes to equilibrium with the alcohol in the blood during respiration. Therefore, one could infer the acute alcohol content of the blood by determining the alcohol content of the expired air. (Figure 3)

6. Hepatic Metabolism of Ethanol

The main site of ethanol metabolism is the liver. Approximately 75% of a dose of ethanol is eliminated by hepatic metabolism. The liver can metabolize up to 2 mmole of ethanol/minute, whereas the total extrahepatic metabolism reaches a maximum of 0.4 mmol/minute.

Alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system and catalase are the three most important emzymes used for the oxidation of ethanol. Alcohol dehydrogenase whose systematic name is alcohol: NAD^+ oxidoreductase, is a dimer containing 2-2.5 atoms of zinc

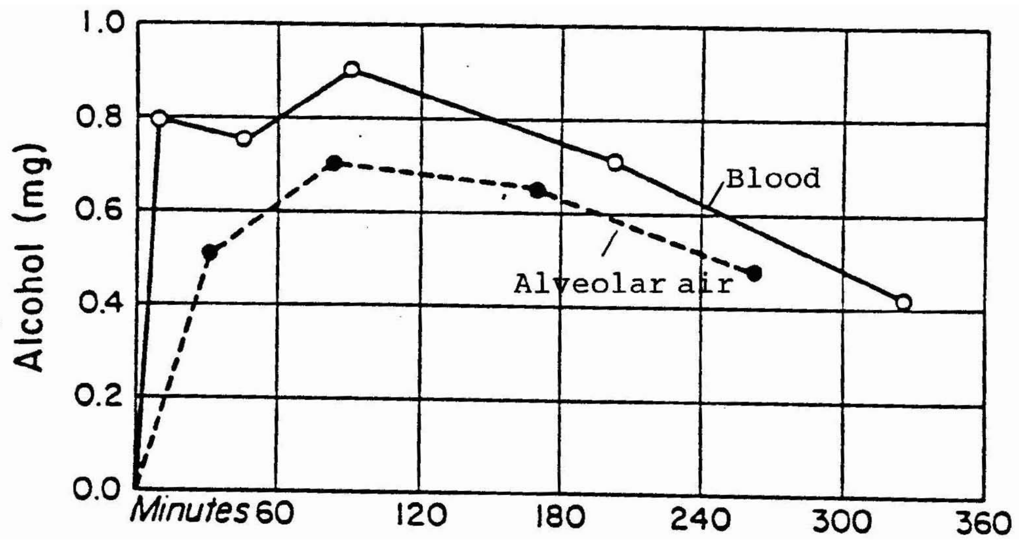


Figure 3. Concentration of Ethyl Alcohol in Blood and Alveolar Air (5)

per molecule. Chromatographic and electrophoretic analyses of this enzyme show that it exists as a mixture of isoenzymes. In addition to the zinc, this enzyme contains two sulfhydryl (-SH) containing polypeptide chains that can be dissociated into two inactive units and recombine to give rise to the isoenzymes. (1) The primary site of action of ADH is the liver although activities have been demonstrated in other organs such as the stomach, lung, and kidney. ADH has the ability to convert a whole range of alcohols to their corresponding aldehydes and vice versa. However, of all the substrates, ethanol is the most readily oxidized substrate.

In addition to ADH, an NADPH dependent microsomal ethanol oxidising system (MEOS) can oxidize ethanol. It has been shown that the activity of MEOS is increased in alcoholic patients as compared to controls. (3) This increased activity persists for possible detection for two to three weeks after withdrawal. There are other microsomal drug metabolizing enzymes that are also stimulated by ethanol. This is the reason sober alcoholics are resistant to such drugs as pentobarbital and meprobamate.

Catalase is primarily located in the peroxisomes. This enzyme along with a hydrogen peroxide generating system such as xanthine oxidase can oxidize ethanol. The activity of catalase is limited by the slow rate of

formation of hydrogen peroxide. (1) Consequently, catalase is not very significant in the metabolism of ethanol.

7. Factors Influencing Elimination and Metabolism of Ethanol

The immature livers of infants possess underdeveloped ADH resulting in a reduced metabolism of ethanol. Low-birth weight infants given ethanol were able to eliminate only 50-60% of the adult value. Fetal livers possess ADH activity of only three to four percent of the adult value, whereas, at birth the ADH activity is 20% of the adult value. The adult value of ADH activity is reached at about five years of age.

Liver damage can also reduce the metabolism of ethanol. Alcoholics with cirrhosis, steatosis, or jaundice have significantly reduced hepatic ADH activities.

Drugs have the ability to alter the rate of metabolism of ethanol by inducing MEOS activity, altering hepatic blood flow, or by increasing the rate of reoxidation of NADH. For example, barbiturates and oral contraceptives increase ethanol metabolism by inducing the mitochondria1 drug metabolizing system, whereas, chloral hydrate inhibits the metabolism of ethanol by inhibiting ADH. (Figure 4) This drug causes peak blood ethanol levels to be higher and to be reached earlier. This is due to the trichloroacetaldehyde which competively inhibits ADH consequently reducing the metabolism of ethanol to acetaldehyde by ADH.

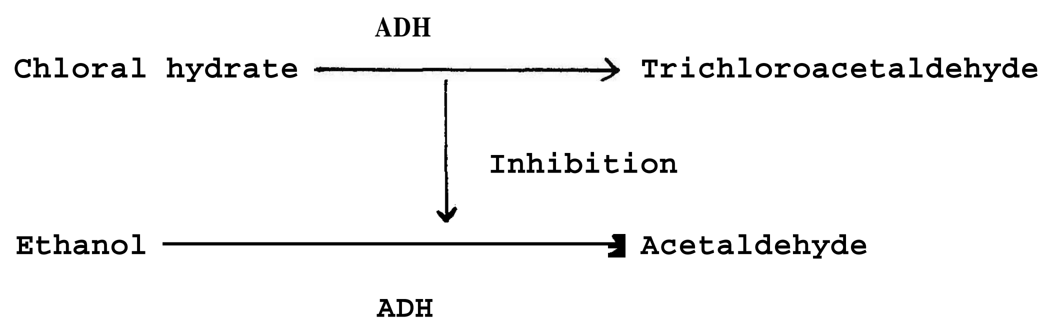


Figure 4. Chloral hydrate Inhibition

Factors such as time of day of testing, phase of the menstrual cycle, age, race, and genetic characteristics affect the interpretation of rates of ethanol metabolism. Repeating ethanol clearance tests on an individual under standardized conditions can give a wide range of results, all making judgement of chronic alcoholism difficult.

C. **Measuring the Rate of Metabolism of Ethanol**

A variety of techniques can be utilized to measure the rate of metabolism of ethanol. One technique deals with the measurement of the rate of $^{14}CO_2$ production from ^{14}C labeled **ethanol.** (6) This measurement deals with the conversion of ethanol to acetaldehyde and acetate and the rate with which acetate is oxidized to CO_2. A second technique involves the measurement of residual ethanol in tissues after a controlled dosage. Finally, the rate of ethanol metabolism can be measured by the rate of disappearance of ethanol from blood.

In general studies seem to agree that alcoholics show less of a response to a given dose of ethanol than does a non-alcoholic resulting in the development of tolerance to alcohol. One proposal deals with an increased rate of alcohol metabolism which results in a decreased cellular response to ethanol. Researchers believe that alcoholism can be an addiction resulting from the role of ethanol or acetaldehyde in the in vivo formation of morphine-type alkaloids such as alkaloid tetrahydropapaveroline (THP). (1)

D. Biochemical Effects of Ethanol

Alcohol can elevate, lower or not affect a biochemical parameter. This is due to a complex interaction of factors such as duration of abuse, extent of abuse, type of alcoholic beverage, solvent effects of ethanol, and the presence of disease secondary to ethanol abuse.

1. Water and Electrolytes

Ingestion of ethanol results in diuresis, which occurs during the first few hours after ingestion while ethanol levels are rising. When the ethanol levels start dropping, a period of water retention follows. Elevated blood levels do not sustain diuresis because the increased blood osmolality following the initial diuresis promotes the release of the anti-diuretic hormone.

Ethanol exerts its diuretic effect by inhibiting the release of ADH by the supraoptical hypophipial system. This increased water excretion is compensated by increased consumption and a shift of water from the intracellular to the extra-cellular compartment.

There has been no significant difference in sodium and potassium levels between alcoholics and nonalcoholics. Generally, any alteration in electrolyte levels in alcoholics is due to altered renal excretion, decreased absorption in the small intestine and diminished dietary intake.

Ethanol also has an effect on magnesium levels. It results in hypomagnesemia which is a reduced

concentration of magnesium. Lactic acidosis, starvation ketoacidosis, protein caloric malnutrition, vomiting and decreased dietary intake are also responsible for this reduced level of magnesium in alcoholics.

Although administration of ethanol reduces blood concentrations of calcium in chronic alcoholics it has no effect on acute ingestion. This reduced calcium level is due to the diminished intestinal absorption.

A zinc deficiency is of great importance in that it could lead to an impairment of the activity of alcohol dehydrogenase which is a zinc metallo-enzyme resulting in impairment of ethanol metabolism. Low levels of zinc have been found in alcoholics with liver disease.

Changes in iron metabolism in alcoholism is due to a direct toxic effect of alcohol on hemoglobin synthesis, negative vitamin balance, liver damage, altered iron absorption, and intake of large amounts of iron contained in some alcoholic beverages. These changes in the metabolism of iron are seen in the anemias associated with alcoholism. There are three common disorders of iron metabolism seen in alcoholism: iron deficiency anemia, megaloblastic anemia with high levels of serum iron, and hemochromatosis.

Following the ingestion of ethanol, there is a shift in the pH of the blood towards acidosis. There are three basic mechanisms explaining this acidosis. One is that ethanol causes a depression of the respiratory center leading to an increased blood pCO_2 resulting in

respiratory acidosis. Another mechanism is that ethanol causes elevated levels of lactate, acetate, free fatty acids and ketone bodies resulting in metabolic acidosis. Finally, the endocrine effects of ethanol can cause an acidosis.

2. Enzymes

The activities of many enzymes are affected by both acute and chronic abuse of ethanol. In a study conducted by Freer and Statland, the activities of the following enzymes were monitored after the ingestion of ethanol: (7) aspartate and alanine aminotransferase, gamma-glutamyl transferase, lactate dehydrogenase, creatine phosphokinase and alkaline phosphatase. (3) The following graph (Figure 5) shows that there was a significant increase in gamma-glutamyl transferase and alanine aminotransferase and a decrease in aspartate aminotransferase activities.

3. Proteins

Most of the studies done on serum proteins have dealt with alcoholics with liver disease. (3) The liver is responsible for the synthesis of most proteins except for the γ-globulins. Therefore, any liver damage will be associated with the inability to synthesize these proteins. Generally, these changes are non-specific. Other than liver damage, malabsorption, malnutrition and infection will cause changes in serum proteins of alcoholics.

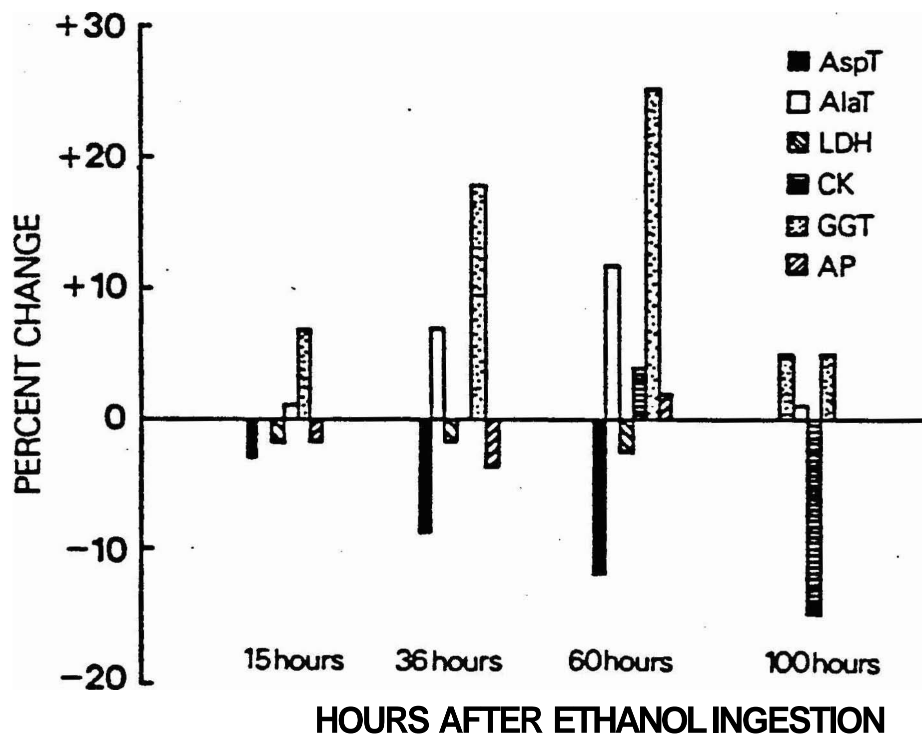


Figure 5. Enzyme Activities (7)

4. Amino Acids

Liver damage will affect the metabolism of amino acids since the liver is the major site of amino acid metabolism. In patients with alcoholic hepatitis, the following amino acids are elevated: phenylalanine, tyrosine, tryptophan, methionine, glutamate and aspartate, whereas, valine, leucine and isoleucine are decreased. (3) Changes in amino acids can be secondary to disorders in carbohydrate metabolism. Therefore the catabolic state of the patient and changes in insulin/glucagon levels can effect the ability of the liver, muscle, and fat to use amino acids as a source of energy. Increased levels of amino acids reflect the degree of hepatic necrosis. Generally, any changes in amino acid metabolism can have an effect on protein synthesis, gluconeogenesis, and the production of neurotransmitters.

5. Carbohydrate Metabolism

Ethanol exerts its effect on the metabolism of carbohydrates to acetate ion, the change in the balance of the NAD^+/NADH redox pair during ethanol metabolism and the effect of ethanol on other metabolic systems.

As mentioned before, ethanol is metabolized to acetaldehyde and then to acetate. Acetyl CoA, which is the active form of acetate, is used by tissue to produce energy by metabolism through the tricarboxylic acid (TCA) cycle. Therefore, acetyl CoA levels will be affected by ethanol metabolism.

The tricarboxylic acid cycle is carried out in the mitochondrion. Its primary functions are the oxidation of pyruvate to carbon dioxide coupled with NADH formation, reoxidation of NADH to NAD^+ by the electron transport chain, and the coupling of NADH oxidation to ATP formation. Ethanol's primary effect is the inhibition of the TCA cycle. For example, the NADH produced during ethanol metabolism can decrease pyruvate levels resulting in decreased levels of oxaloacetate which is the first component of the TCA cycle. Another way the TCA cycle can be inhibited is by the altered redox state affecting the malate/oxaloacetate ratio. These sites of inhibition can be seen in Figure 6.

In the postabsorptive period after ethanol ingestion, there is a hyperglycemic response followed by hypoglycemia. This response is interpreted as an initial glycogenolysis followed by hypoglycemia due to the depletion of glycogen stores.

An increase in serum lipoproteins is seen in alcoholics. This is due to an increased production and release of hepatic lipoproteins or decreased peripheral uptake of lipoproteins due to decreased lipoprotein lipase activity.

An increase in free fatty acids is associated with large doses of ethanol. This is a result of enhanced peripheral fat mobilization, through the stimulation of the sympathetic nervous system. Another cause of this

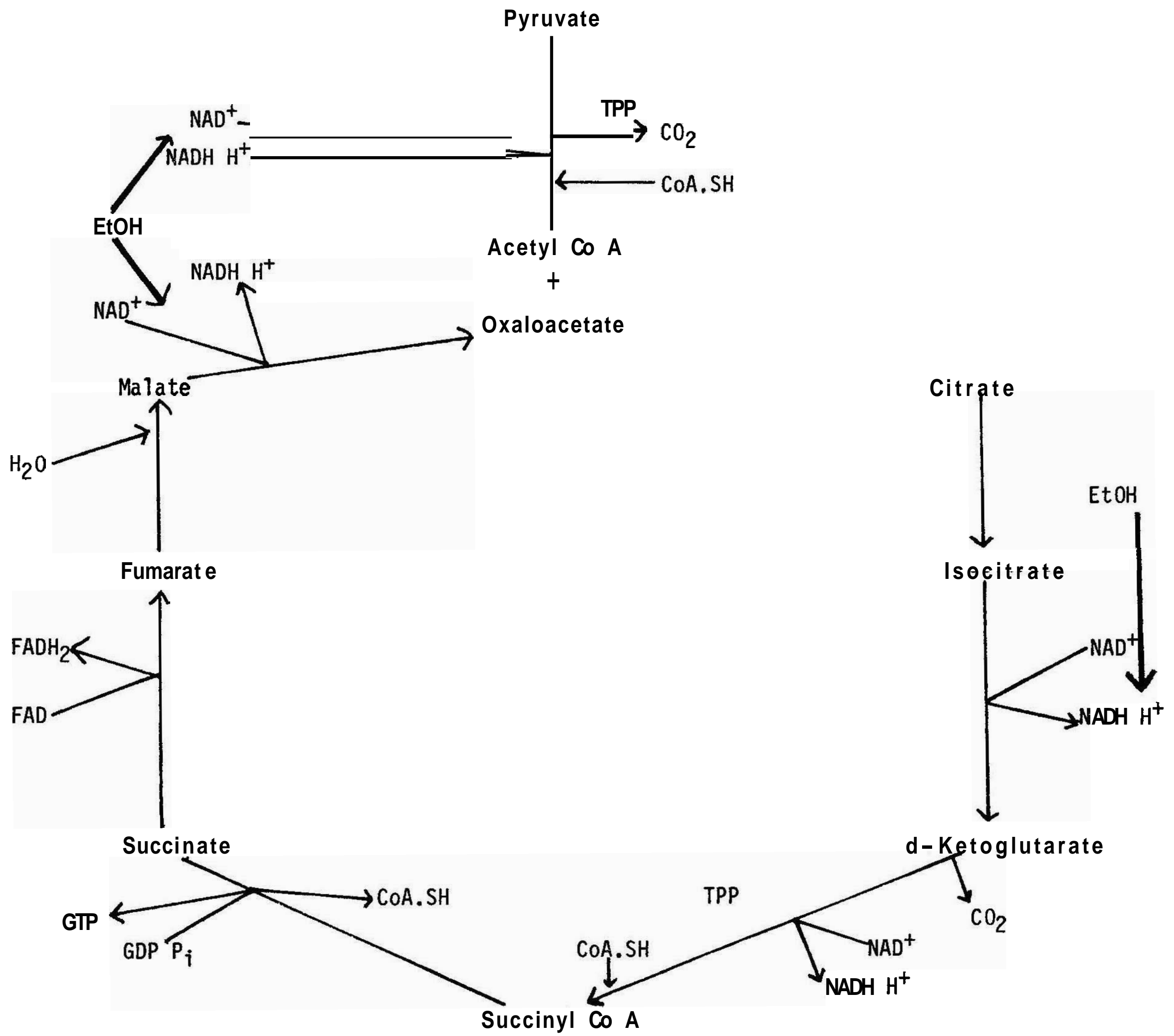


Figure 6. Tricarboxylic acid cycle inhibition by Ethanol

rise in fatty acids is the decreased breakdown of free fatty acids in the liver.

The hyperlipidemia associated with alcoholism leads to an alcoholic fatty liver. There are four major steps leading to an alcoholic fatty liver.

a.) Hepatic mitochondria use ethanol instead of fatty acids for fuel which leads to fatty acid accumulation.

b.) The excess hydrogen generated by ethanol metabolism is used up by increased lipid synthesis. Ethanol metabolism is associated with an increase in NADH and a decrease in NAD^+ levels. This change in cofactor levels inhibits the citric acid cycle resulting in decreased dietary fatty acid oxidation and increased -glycerophosphate levels. This leads to increased triglyceride production. In combination with the acetyl CoA from the metabolism of ethanol, fatty acids are produced. Finally, these transformations lead to the deposit of lipids in the liver.

c.) Fatty acids are mobilized from adipose tissue by ethanol and accumulate in the liver.

d.) Finally, even though there is an increase in lipid transport from the liver, lipids continue to increase in the liver and this can be seen in a biopsy of the liver.

6. Vitamins

Alcoholism leads to a vitamin deficiency through a number of mechanisms such as inadequate diet, reduced absorption of vitamins, reduced storage of vitamins and decreased conversion of vitamins to their metabolicly active forms. (3)

Many metabolic processes are vitamin dependent and a deficiency can result in an impairment of these processes. The metabolic pathways dealing with hepatic oxidation of ethanol and the reoxidation of reduced NAD^+ produced during ethanol metabolism are dependent on a number of vitamins. A deficiency of these vitamins can have an adverse effect on the metabolism of ethanol. Figure 7 shows the important vitamin-dependent pathways of ethanol metabolism.

E. **Testing for Alcohol Abuse**

For a clinical test to be an effective marker of ethanol abuse, it must possess the following characteristics:

1. Chronic ingestion of ethanol should result in a significant and persistent change in the biochemical parameter.

2. This biochemical parameter should be detectable for a period of time after cessation of drinking.

3. The biochemical parameter should not be significantly affected by acute ingestion of ethanol.

4. Dietary factors and other diseases should not affect this biochemical parameter.

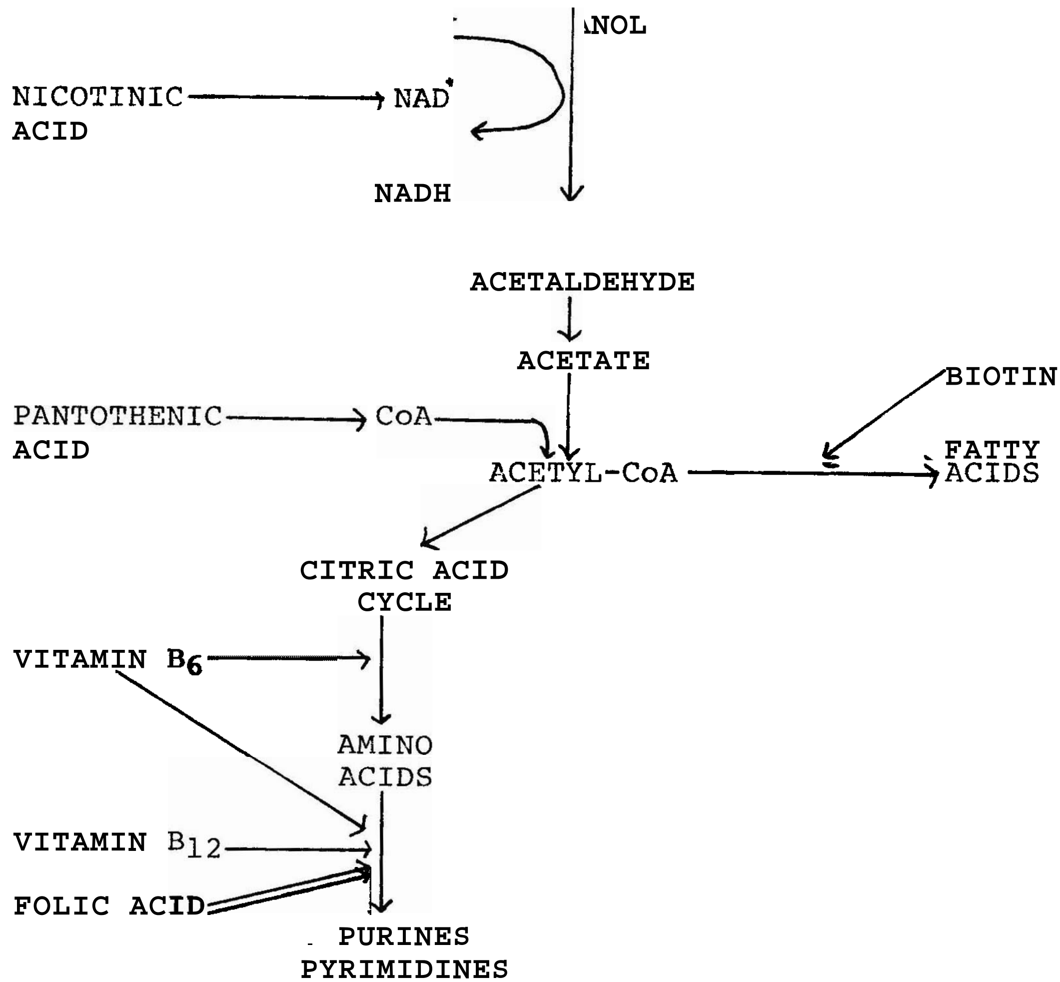


Figure 7. Vitamin Dependent Pathways

5. The clinical test should be reliable, convenient and performed on a readily available biological specimen such as blood or urine.

The measurement of blood ethanol is the most direct method for determining acute alcoholic intoxication.

The main concern is the detection of chronic abuse of alcohol. One method is the measurement of the ratio of the concentrations of the amino acids alpha-amino-n-butyric acid and leucine in plasma. The ratio of these amino acids remains elevated for several weeks after cessation of drinking, it isn't affected by acute ingestion of alcohol and it is not affected by diet. A problem is that the mechanism by which ethanol affects these amino acids is not known. Another method of detecting chronic abuse of alcohol is the measurement of the extent of elevation of enzyme levels. The enzyme level is proportional to the magnitude of tissue damage and can be correlated with the extent and duration of ethanol abuse. The most important enzyme associated with chronic ethanol abuse is gamma-glutamyl transferase (GGT). Others are aspartate aminotransferase and glutamate dehydrogenase. Unfortunately, their activities are affected by drug abuse, and by various other diseases and so do not fulfill the previously mentioned criteria.

Due to the lack of a reliable marker of alcohol abuse, it is difficult to detect chronic alcoholism using laboratory tests. The tests used presently are neither sensitive nor specific. For the time being the tests

used are the mean corpuscular volume (MCV), and the activities of γ-glutamyl transferase and aspartate aminotransferase. A literature review suggests the formation of an acetaldehyde hemoglobin adduct as a reliable marker of alcohol abuse. (8, 9)

F. Storage of Blood Samples

A study was conducted by Winek and Paul to determine the effects of time, temperature, and delayed analysis on the concentration of ethanol in blood samples from living persons. (10) They concluded that alcohol analysis of blood obtained sterilely from living humans can be delayed for as long as 14 days without a significant gain or loss in alcohol concentration. It did not matter if the blood sample was refrigerated or not or whether a preservative is added to the sample. On the other hand, there was a significant change in alcohol content if the blood sample was taken from a deceased individual. They found decreases in alcohol content due to oxidation and increases in alcohol content due to postmortem synthesis of ethanol by microbial fermentation of glucose.

In comparison, a study was conducted by Smalldon and Brown also dealing with the stability of ethanol in stored blood. (11) They found that at no instance was there an increased concentration of ethanol in stored blood. However, they did find three mechanisms of loss of ethanol. One mechanism of ethanol loss in stored blood is the diffusion of ethanol from imperfectly sealed

containers. Secondly, ethanol is lost by its metabolism by the growth of micro-organisms of a preservative. Finally, ethanol levels were lowered in stored blood because of a temperature dependent ethanol oxidation reaction. One solution to producing stable blood ethanol solutions is by storing them in deep freeze. The study also showed that the oxidation of ethanol in stored blood is caused by an oxyhemoglobin intermediate. This oxidation can be prevented by compounds which destroy oxyhemoglobin. This inhibitor of ethanol oxidation should be stable and effective at low concentrations of ethanol. This inhibitor should prevent the growth of micro-organisms. These researchers concluded by suggesting that sodium **azide** is the inhibitor that can effect the least loss of ethanol during its storage in blood samples.

Proper storage conditions of a blood sample not only affect the level of ethanol but also the levels of acetaldehyde and acetaldehyde-bound hemoglobin. For example, if the appropriate inhibitor is not added to stored blood, ethanol levels will decrease due to its oxidation. The oxidation of ethanol results in increased levels of acetaldehyde which will then lead to increased levels of acetaldehyde-bound hemoglobin. Therefore, improper storage of blood samples can affect the actual levels of acetaldehyde and acetaldehyde-bound hemoglobin.

Chapter 2

Review of the Literature

Glycosylated Hemoglobin

Acetaldehyde, the first metabolite of alcohol has been found to form adducts with hemoglobin A resulting in a change in its chromatographic properties. (8) The adducts migrate in the hemoglobin A_{1a-c} region or "fast" hemoglobin or glycosylated hemoglobin fraction. Since acetaldehyde-bound hemogoglobin acts like hemoglobin A_{1c} and since this research involves a modified version of the fast hemoglobin test, it is important for one to understand the chemistry of hemoglobin A_{1c}. (8)

The diversity among proteins is considerably increased by posttranslational modifications. A number of proteins owe many of their functional properties to the covalent attachment of carbohydrates at certain residues in their polypeptide chain. (12) These modifications may provide increased stability or solubility, or even both. The glycosylation of proteins is especially important in the maintenance of the integrity of plasma membranes and in facilitating the secretion of proteins into the extracellular space. (13) However, these types of specific modifications are usually under enzymatic control. On the other hand, certain proteins can undergo nonenzymatic glycosylation. This reaction depends on the presence

of a high concentration of free sugar and nonphysiologic incubation conditions. For example, the "browning" reaction is well known to the dairy industry. (14) The browning reaction occurs when milk is heated for a long period of time. The carbonyl groups on sugars form a Schiff base adduct with the amino groups on proteins such as casein. This type of reaction can also modify insulin and oligopeptides. Unlike the browning reaction, hemoglobin can undergo nonenzymatic glycosylation under physiologic conditions at a specific site on the protein.

Hemoglobin A(HbA) makes up about 90 percent of the hemoglobin found in adults and children above the age of six months. HbA($\alpha_2\beta_2$) is a tetramer composed of two pairs of unlike polypeptide chains. The synthesis of these chains is controlled by separate genes. The same holds true for the formation of hemoglobin A_2($\alpha_2\delta_2$) and HbF($\alpha_2\gamma_2$). On the other hand, the minor hemoglobins found in human red cells are posttranslational modifications of HbA. When a human red blood cell hemolysate is chromatographed on cation exchange resin, three negatively-charged minor hemoglobin components are eluted before the main HbA peak. (15) These three minor hemoglobin components have been designated as hemoglobins A_{1a}, A_{1b}, and A_{1c}. Of the three minor hemoglobin components, HbA_{1c} is the most abundant. Researchers found that HbA_{1c} "is structurally identical to HbA except for an unidentified

group linked to the terminal amino acid of the beta chains by means of a Schiff base. (15) Gabbay, Hasty, Breslow, Ellison, Bun and Gallop demonstrated the presence of glucose on the A_{1c} hemoglobin and that this hemoglobin's formation represents a posttranslational glycosylation of HbA by a slow, nonenzymatic process which occurs continuously throughout the 120-day life span of the human red blood cell. (15) These same researchers showed that both HbA_{1a} and HbA_{1b} are also posttranslational modifications of HbA and may be intermediates in the formation of HbA_{1c}.

The difference between HbA and HbA_{1c} is that HbA_{1c} has a glucose molecule bound to the N-terminus of the beta chains of the normal adult hemoglobin A. (16) (Figure 8)

This reaction is a nonenzymatic condensation of two abundant reactants, glucose and hemoglobin. A schiff base is formed between the aldehyde of the carbohydrate and the aminoterminal valine of the beta chain. The aldemine linkage is further stabilized by an Amadori rearrangement to form a ketoamine bond. (10) This chemical modification is illustrated in figure 9.

The interest in HbA_{1c} geatly increased when it was discovered that there is a two- to three-fold increase in patients with diabetes mellitus which depended on the degree of hyperglycemia. (17) Studies show that the ketoamine linkage characteristic of HbA_{1c} is relatively

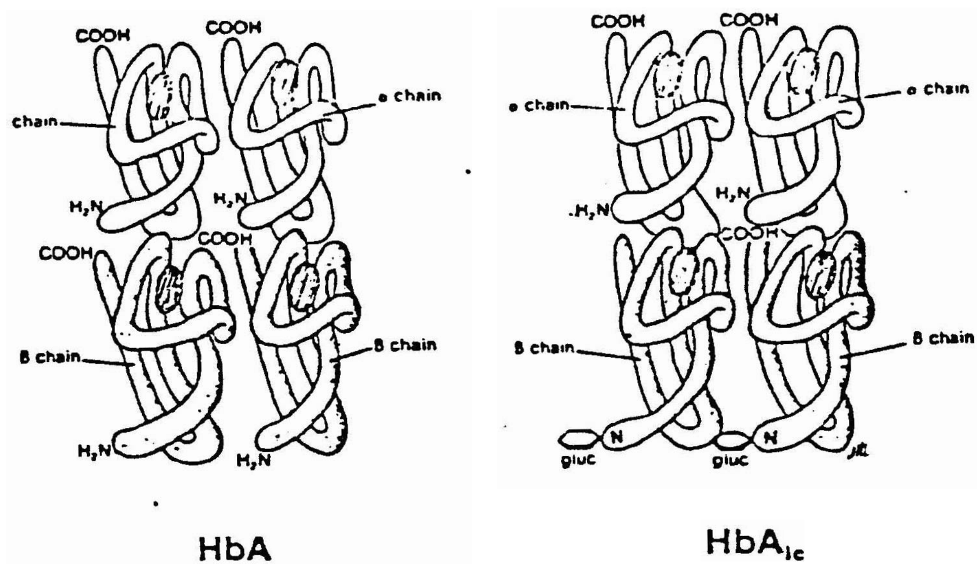


Figure 8. Structure of HbA compared to HbA_{1c} (16)

Aldimine **KETOAMINE**

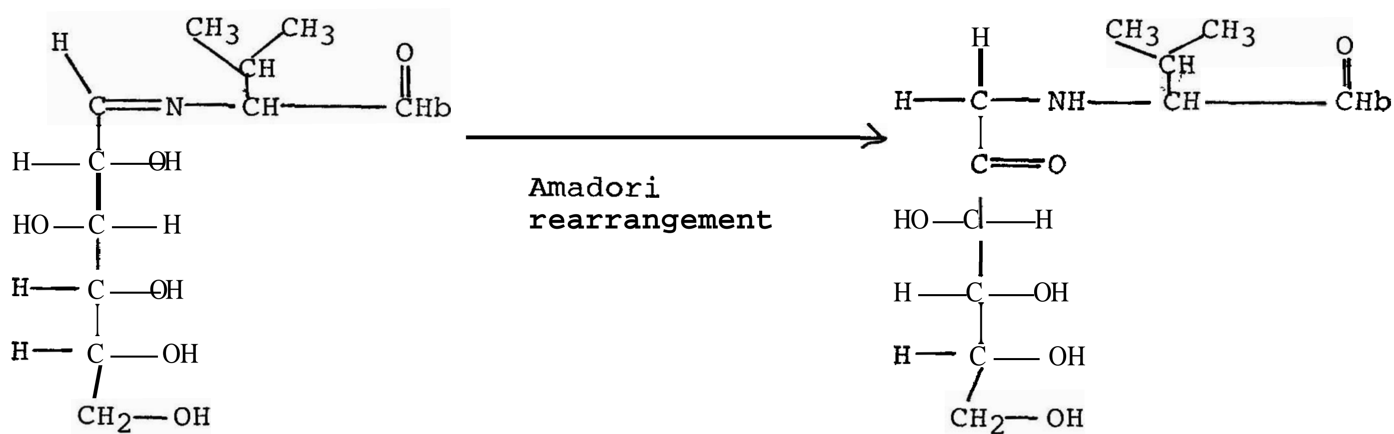


Figure 9. Arnadori rearrangement of Glycosylated Hemoglobin

irreversible so the glycosylated hemoglobins accumulate within the erythrocyte during its 120-day life span. Consequently, the cumulative level of HbA_{1c} is directly proportional to the time-averaged concentration of glucose within the erythrocyte. Therefore, quantification of HbA_{1c} levels represents a potentially valuable index of long-term blood glucose diabetic control. Short term fluctuations in blood glucose will minimally alter the HbA_{1c} level since the reaction progresses so slowly, whereas, determinations of fasting or postprandial blood glucose levels represent only an instant in the course of the diabetic patient. HbA_{1c} levels are not reliable markers of diabetic control in patients with hemoglohinopathies since HbA_{1c} is a modification of HbA. Another case where HbA_{1c} levels cannot be used reliably is for patients having undergone a splenectomy. Here the HbA_{1c} levels will be elevated due to the lengthened red blood cell survival.

High pressure liquid chromatography, gel electrophocusing, radioimmunoassay and colorimetric test methods have enhanced the feasibility of using HbA_{1c} as a clinical test. All of these methods allow for rapid and multiple specimen analysis. Studies done by Abraham and his associates show that no significant amount of HbA_1 is formed when a blood sample is stored for up to one month at 4°C. (12) The graph in Figure 10 demonstrates this finding.

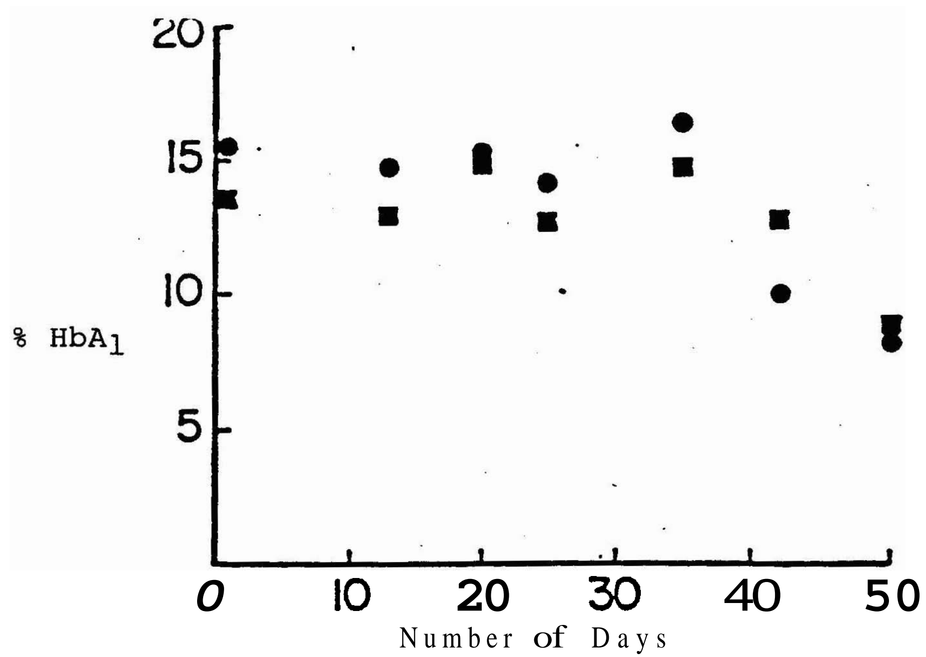


Figure 10. Effect of Storage on % HbA_{1c} (12)

Acetaldehyde

The oxidation of ethanol leads to the formation of acetaldehyde. Acetaldehyde is potentially more toxic than ethanol which is due to its greater reactivity and lipid solubility.

Many studies have been conducted to study the relationship between acetaldehyde and alcohol consumption. (20) One study conducted by Korsten, Matsuzaki, Feinman, and Luber showed that "blood acetaldehyde levels are much higher at elevated than at low concentrations of ethanol and that this difference is greater in alcoholics than in non-alcoholic subjects" (19), whereas, Majchrowicz and Mendelson reported that the concentration of acetaldehyde in the blood is related to the amount contained as congener in alcoholic beverages. (20) Erikson and Schickit found that acetaldehyde levels were higher in alcoholics. (4)

There are three proposals explaining these differences in acetaldehyde levels. (4) One is that these differences are real and not due to an error in measurement. Secondly, the acetaldehyde formation reaction could be more prominent in alcoholics. A third possibility is that the amount of acetaldehyde disappearing from the blood during preparation for analysis differs in alcoholics as compared to controls.

Acetaldehyde-Hemoglobin Adduct

A literature review suggests the formation of an acetaldehyde hemoglobin adduct as a reliable marker

of alcohol abuse. (8, 9) The adducts migrate in the hemoglobin A_{1c} region. Studies done by Stevens found that the minor hemoglobins were elevated in patients of alcohol abuse and yet were not diabetic.(9) This fact leads to the possibility that specific assays can be developed for chronic alcohol consumption based on the way that hemoglobin A_{1c} integrates blood glucose.

The use of acetaldehyde hemoglobin adducts as a marker of alcoholism is a controversial issue. Stevens, Fantl, and Newman support this theory. (11) They found that the amount of hemoglobin adducts formed is a function of the concentration of acetaldehyde to which it is exposed and that these adducts are significantly elevated in alcoholics as compared to non-alcoholics. The sites of reaction on the hemoglobin for acetaldehyde to bind are valine, lysine, and tyrosine. It is felt that the acelaldehyde reacts with the ε-amino group of lysine and the α-amino group of valine through a Schiff's base reaction.

Studies done by Hoberman seem to agree with Stevens' work. (8) Hoberman also found that hemolysates from alcoholics contain a hemoglobin fraction that has chromatographic properties resembling those of the glycosylated hemoglobin (HbA_{1c}) but differing from the glycosylated hemoglobins in that it was not bound by boronated agarose. (8, 21) He also found that this hemoglobin fraction was significantly greater in alcoholics

than in non-alcoholics. This hemoglobin fraction co-chromatographed with the glycosylated hemoglobin on an ion exchange column and gave a negative test for glycosylated hemoglobin. He also suggests that this hemoglobin derivative could serve as a marker of alcoholism since it is formed and accumulated over the 120-day life span of the erythrocyte similar to the formation and accumulation of the glycosylated hemoglobin.

On the other hand, a study done by Homaidan, Kricka, Clark, Jones and Whitehead contradicts Steven's findings. (22) It is felt by them that acetaldehyde hemoglobin adducts are an unreliable marker of alcoholism. They confirmed the findings of Stevens that hemoglobin undergoes a concentration-dependent reaction with acetaldehyde to preduce an increase in the fast hemoglobin fraction. Even though many authors have reported increased acetaldehyde concentrations in the blood of alcoholics after the ingestion of alcohol, Homaidan et.al. suspect this increase in acetaldehyde is due to artifactual acetaldehyde formation from ethanol during protein precipitation and do not feel that enough acetaldehyde accumulates to react with hemoglobin. They also do not expect a stable acetaldehyde-hemoglobin adduct because the reaction of acetaldehyde with the amino groups of protein is a reversible reaction--Schiff base formation. The subsequent Amadori rearrangement that occurs in glycosylated hemoglobin cannot occur with acetaldehyde

bound hemoglobin because there is no α-hydroxyl group in acetaldehyde. These researchers believe that this elevation in minor hemoglobins seen in nondiabetic alcoholic patients is due to adducts formed with products of acetaldehyde, aldol or crotonaldehyde or with impurities present in acetaldehyde. Aldol is formed by an acid-base catalyzed condensation of acetaldehyde which loses water upon warming to form crotonaldehyde. These researchers used cation exchange chromatography which is specific for glycosylated hemoglobin rather than acetaldehyde-bound hemoglobin, whereas, one could use the cation exchange column in conjunction with a boronated agarose column which has the ability to separate glycosylated hemoglobin from acetaldehyde bound hemoglobin.

Even though the use of acetaldehyde hemoglobin adducts as a marker of alcoholism is still under debate, it was attempted in this research to prove that there is a correlation between an acetaldehyde hemoglobin adduct and alcoholism and that this acetaldehyde bound hemoglobin is elevated in chronic abusers of alcohol,

The actual structure of the acetaldehyde hemoglobin adduct has not been illucidated yet, following is the structure assumed in this research:

H_3C CH_3
CH
O
||
Hb-C———C-N=CH
H CH_3

Chapter 3

Methodology

A mixture of solute molecules can be separated by chromatography due to the differences in migration rates through a porous supporting medium. The supporting medium is called an adsorbent. The migration of these solute molecules is effected by the flow of the moving phase which percolates through the stationary phase. In this investigation two physicochemical processes are used to separate the solute molecules: ion exchange and gel affinity filtration.

Ion Exchange Chromatography

In ion exchange chromatography, solutes are separated depending on their differences in sign and magnitude of ionic charge. This type of chromatography is especially used for compounds soluble in aqueous systems, such as hemoglobins, amino acids, proteins and others. (24) The ion exchange resins, whether they be cation or anion exchange, are insoluble polymeric substances containing ionic groups as part of their structure.

In this investigation, a cation-exchange resin packed in columns is used. The kit is called Quik-Sep by Isolab, Inc. (25) The resin has negatively-charged functional groups covalently bound to it. There are also

loosely bound cations which are readily available for exchange with cations of the solute.

A mixture of hemoglobins is separated in the following manner: the positively-charged Hb will exchange with the loosely bound cation and become bound to the negtively-charged resin, a buffer with increased pH is added causing the Hb to become more negatively charged and eluted from the resin by interchanging with the cations. The glycosylated hemoglobins are eluted first and that is why they are called fast hemoglobins. The acetaldehyde-bound hemoglobin will elute with the glycosylated-hemoglobins.

Affinity Chromatography

Affinity chromatography is a technique that isolates proteins by taking advantage of the high affinity of proteins for specific chemical groups. (26) This technique first involves attaching covalently a group, "X", to the column. This group "X" is recognized by the protein. The kit used is called Glyc-Affin GHb by Isolab Incorporated. (27) This kit uses boronated agarose which will bind the glycosylated hemoglobin. Secondly using the appropriate buffer the unbound protein will be eluted. Finally, the bound hemoglobin is elutedby adding a buffer containing a high concentration of boronate which will displace the column-attached glycosylated hemoglobin.

Summary of Method

In this research ion exchange columns and boronated agarose columns are employed. The ion exchange columns are used to separate the glycosylated or fast hemoglobins from the other hemoglobins. The acetaldehyde-bound hemoglobin will elute with the glycosylated hemoglobins. The first effluent from the ion exchange column containing the glycosylated hemoglobin and acetaldehyde-bound hemoglobin is then allowed to chromatograph through the boronated agarose column. There the glycosylated hemoglobins are separated from the acetaldehyde-bound hemoglobin. The boronated agarose will bind the glycosylated hemoglobin and allow the acetaldehyde-bound hemoglobin to elute first. This binding is due to the affinity of boronate groups for cis-diols which is common to glycosylated proteins. It is the acetaldehyde-bound hemoglobin which is of particular interest in this research.

Cyclic voltammetry was used in an attempt to identify the oxidation of ethanol to acetaldehyde, but it failed. Voltammetry "is the measurement of the current which flows at an electrode as a function of the potential applied to the electrode." (23) The current-potentail curve is the equivalent of the spectrum obtained in spectrophotometry. Cyclic voltammetry is used to study mechanisms and rates of oxidation-reduction processes.

Chapter 4

Materials and Apparatus

Materials

Two kits were used, "Quik-Sep" and **"Glyc-Affin Hb"** from Isolab Incorporated.

"Quik-Sep" included the following:

- Columns containing ion exchange resin used to separate fast hemoglobins.
- Sample preparation reagent used to lyse the blood sample.
- Fast Hb Elution Solution used to elute the glycosylated hemoglobins.
- Elution Agent, other hemoglobins used to elute the nonglycosylates hemoglobins.

"Glyc-Affin **GHb"** included the following:

- Columns containing boronated agarose used for separation of hemoglobins.
- Sample preparation reagent used to lyse the blood sample.
- Column preparation solution used to prepare the agarose column prior to sample application.
- First Fraction Elution Agent used to elute the nonglycosylated hemoglobins.
- Second Fraction Elution Agent used to elute the glycosylated hemoglobins.

Apparatus

Columns

The columns used were basically the same except for the packing material which differed. One column used cation exchange resin and the other used boronated agarose. There is also a glass plug above and below the packing material. (Figure 11)

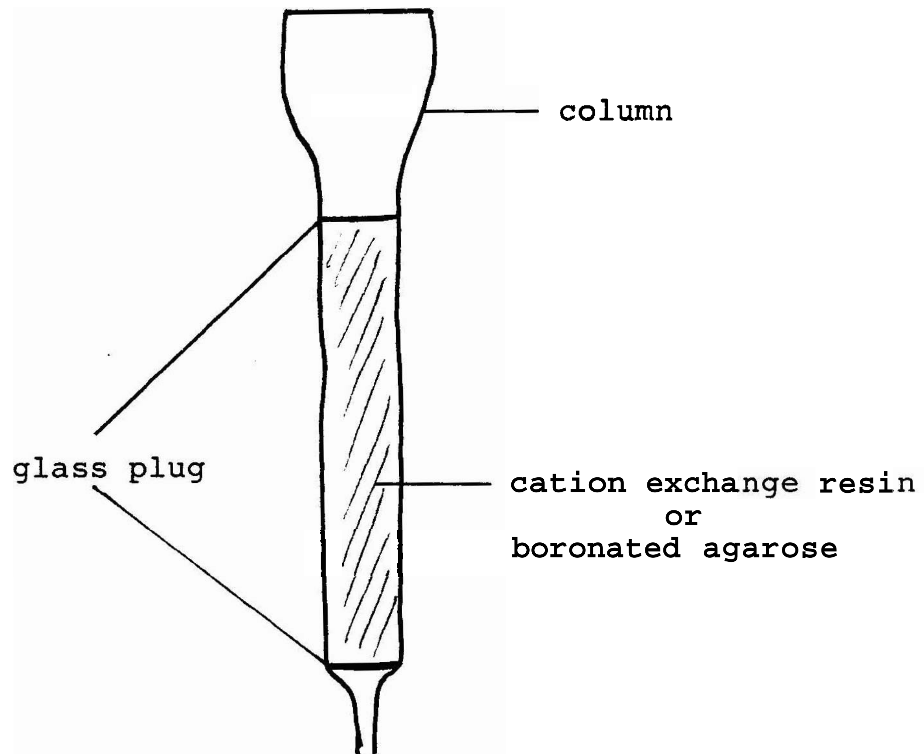


Figure 11. Column and Components

Instrumentation

A double beam, Beckman (Model 26) spectrophotometer was used to measure the absorbance of the effluent at 415 nm.

Chapter 5

Procedure and Results

Procedure

Part A

In the first part of this research a whole blood sample was incubated with ethyl alcohol and alcohol dehydrogenase, ethyl alcohol and acetaldehyde to see if there was an increase in the fast hemoglobin fraction. (Table 2)

1). 50 µL of blood were hemolyzed in 200 µL of the Fast Hb Sample Preparation Reagent.

2). The column is prepared by first taking the top cap off followed by the bottom closure and then allowing the columns to drain.

3). 50 µL of the hemolysate is added and allowed to drain.

4). 200 µL of the Fast Hb Elution Solution is added to initialize the columns and is allowed to drain. The solution eluted thus far is discarded.

5). The fast hemoglobin fraction is eluted by adding 4 mL of the Fast Hb Elution Solution. This effluent is saved for analysis with the spectrophotometer.

6). The other hemoglobin fraction is eluted by adding 4.0 mL of the Elution Agent, Other Hemoglobins. This effluent is diluted with 16 mL of H_2O.

7). The aborbances of the effluent from step

	1	2	3
A			
10% Acetaldehyde (μL)	50	100	250
Whole blood (mL)	0.5	0.5	0.5
B			
10%EtOH (μL)	50	100	250
Whole blood (mL)	0.5	0.5	0.5
C			
ADH (mL)	0.1	0.1	0.1
10%EtOH (μL)	50	100	250
Whole blood (mL)	0.5	0.5	0.5

Table 2. Preparation of Samples.

5 and the diluted effluent from step 6 are measured at 415 nm using a spectrophotometer. (Figure 12)

8). If A = absorbance of the Fast Hb fraction and H= absorbance of the Other Hemoglobins. Then to calculate % Fast Hb = A/(A+5H)100%.

Part B

Whole blood was incubated with acetaldehyde. A sample was taken every day for a week and run through the ion exchange and gel column set up to see if there was an increase in acetaldehyde-bound hemoglobin.

There was a 0.5 mL whole blood to 0.1 mL acetaldehyde ratio.

The procedure is as follows:

1). A Glyc-Affin GHb column is prepared by adding 2.0 mL of Column Preparation Solution. This eluate is discarded.

2). The next five steps are steps one-five from part A except that the glycosylated fraction drains into the preprepared gel-filtration columns. This eluate is also discarded.

3). In order to obtain the nonglycosylated hemoglobin fraction, one adds 2.9 mL of First Fraction Elution Agent to the gel column. This fraction contains all the nonglycosylated hemoglobins. At this point it should only contain acetaldehyde-bound hemoglobin. This fraction is saved for an absorbance reading.

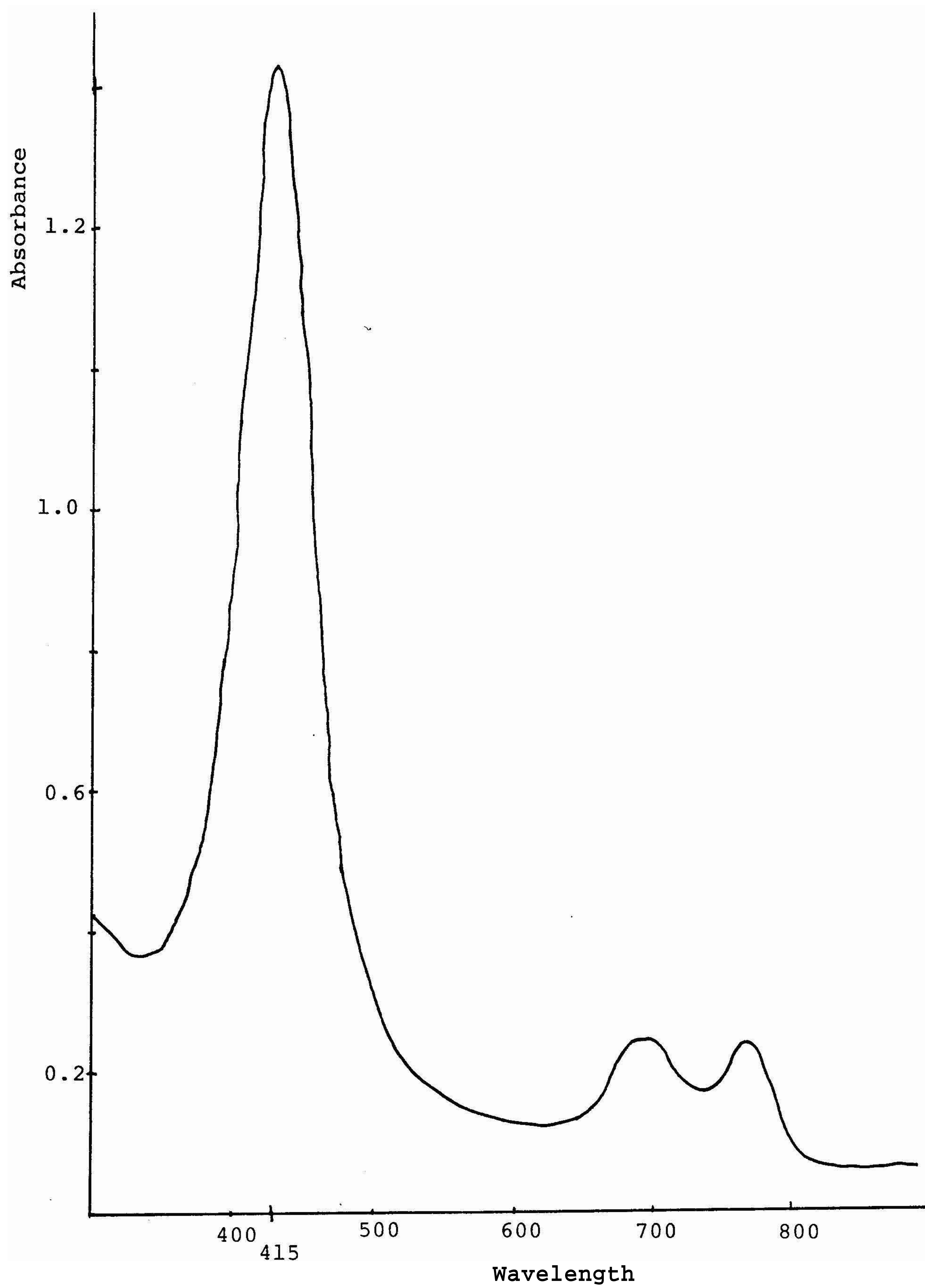


Figure 12. Characteristic Spectrum of Eluate

4). The glycosylated hemoglobin fraction is obtained by adding 2.0 mL of the Second Fraction Elution Agent to the gel column. This fraction is also saved and should contain only glycosylated hemoglobins.

5). The absorbance from the eluates from steps three and four are read using a spectrophotometer set at 415 nm.

Part C

Whole blood was obtained from heavy drinkers and from non-drinkers. The procedure explained in Part B was repeated except that no acetaldehyde was added to these samples.

Xesults

Part A

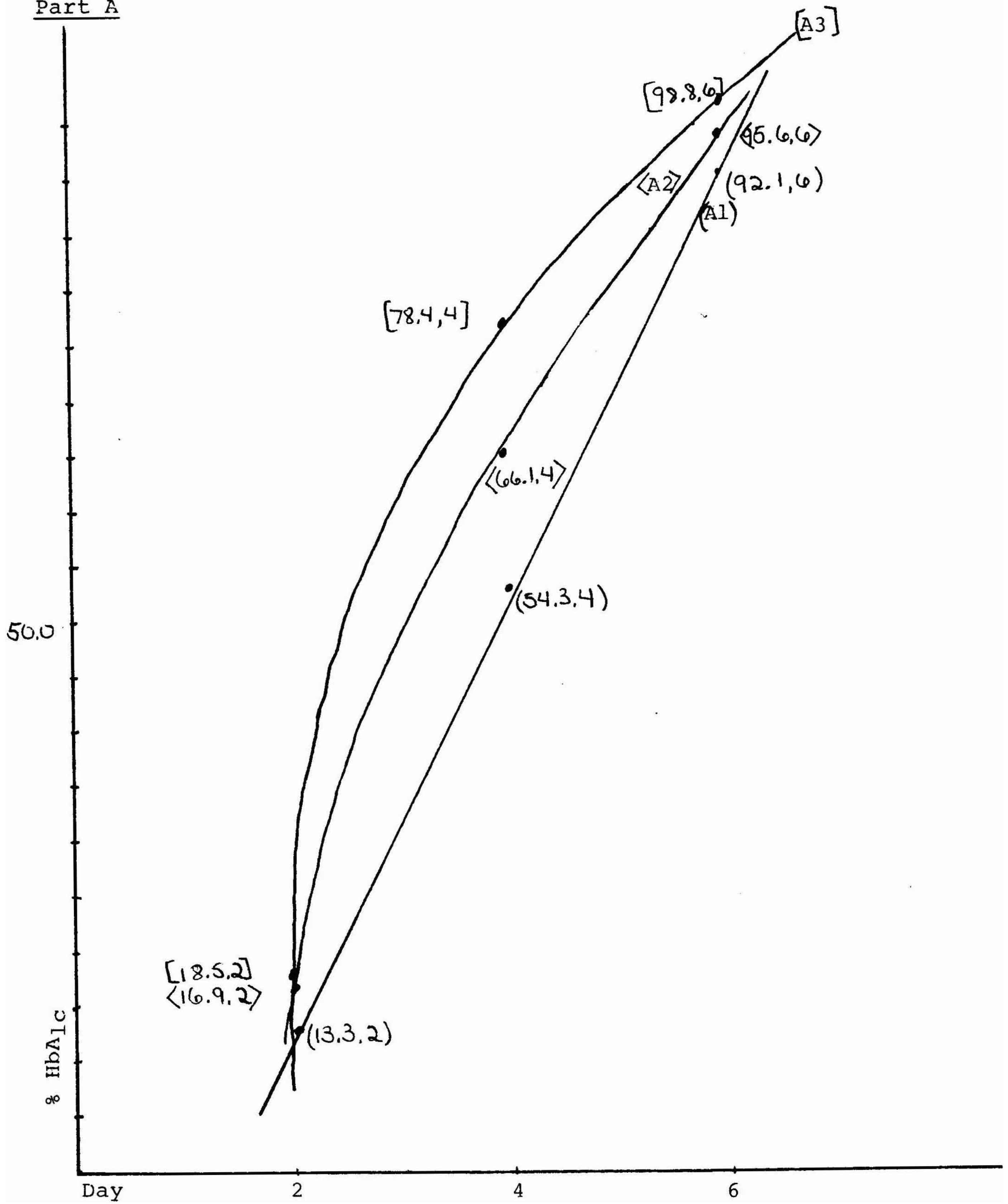


Figure 13. **Effect of Acetaldehyde on Hemoglobin A_{1c}.**

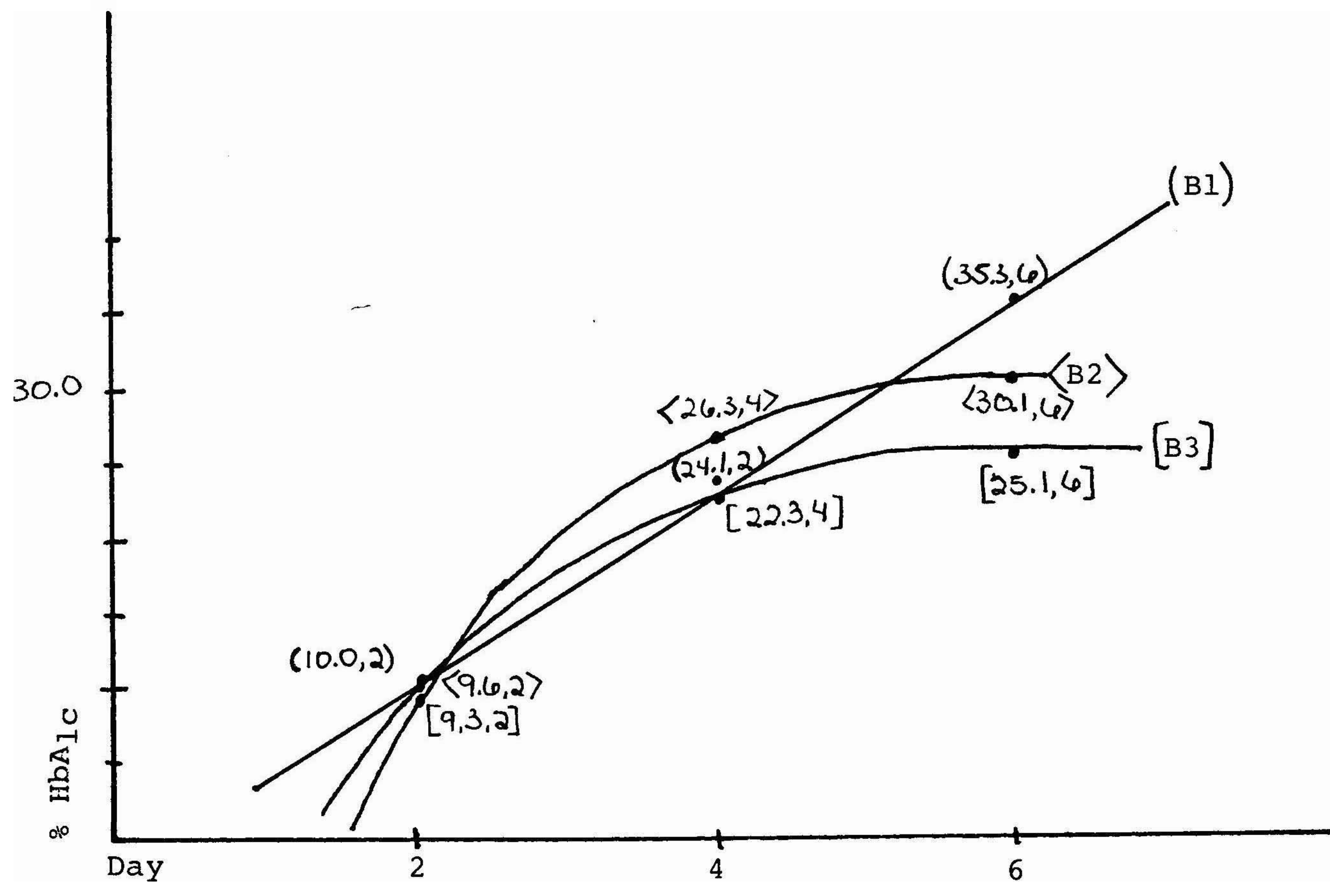


Figure 14. Effect of Ethanol on Henoglobin A_{1c}.

Results

Part A

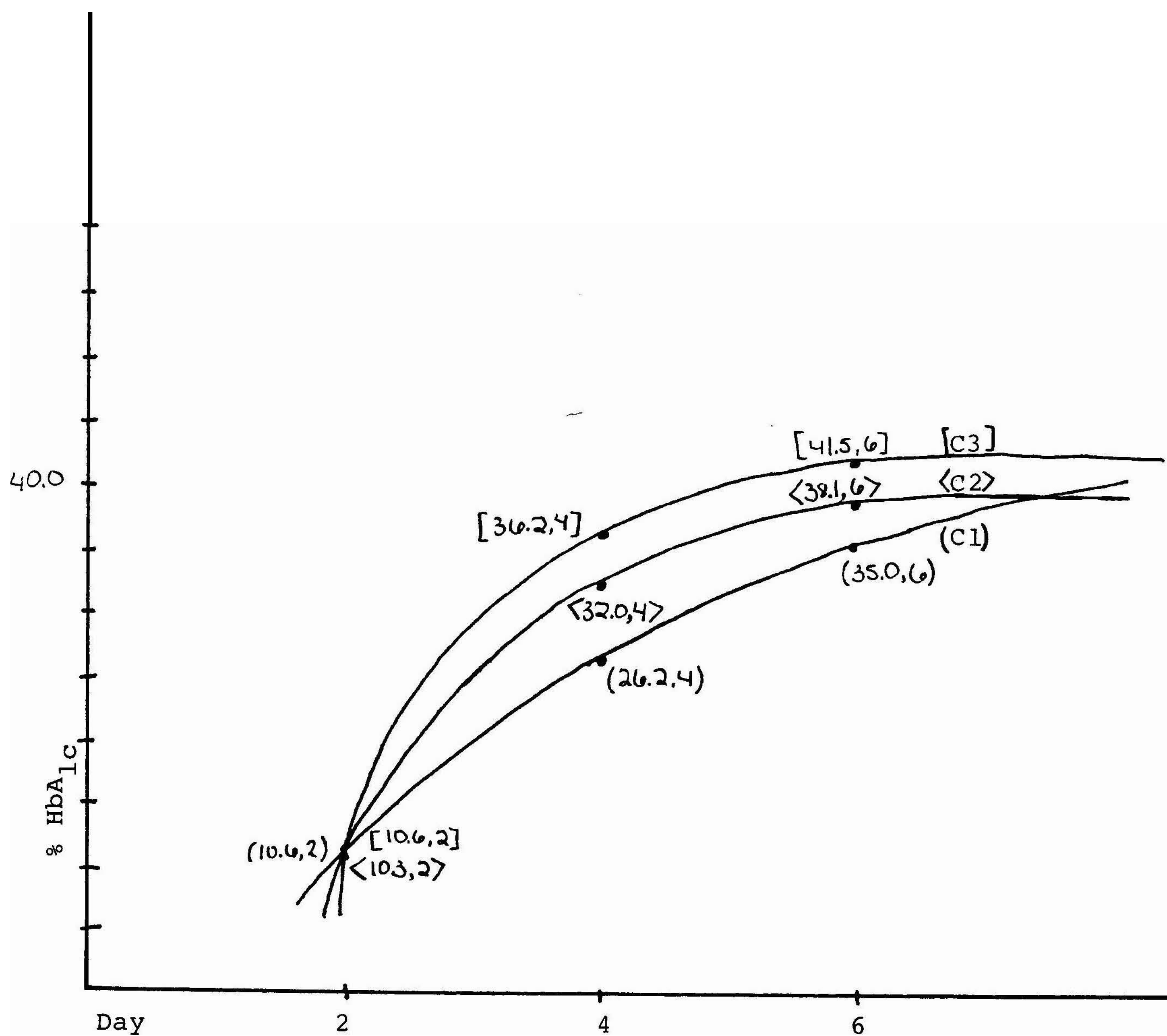
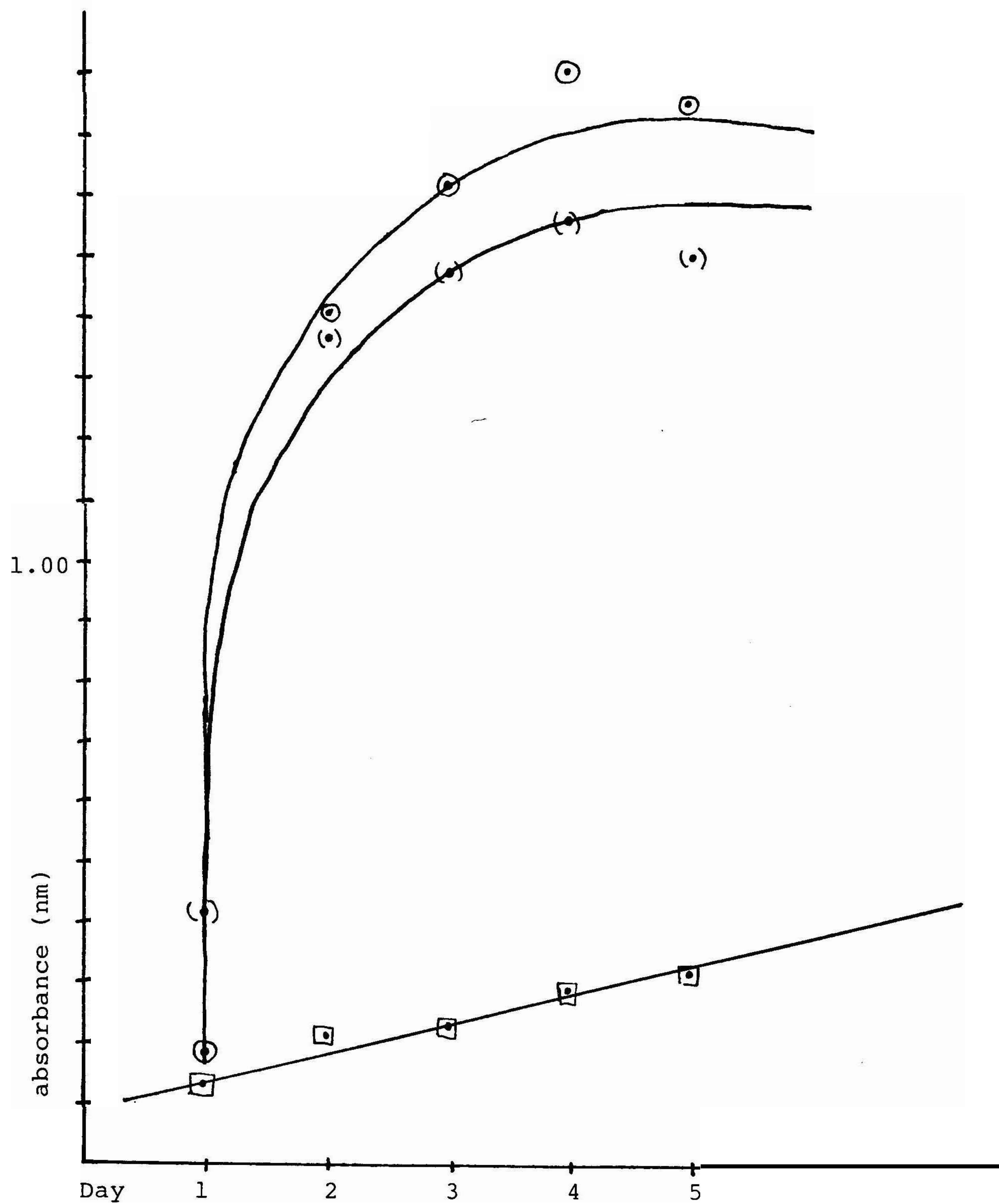


Figure 15. Effect of Ethanol and Alcohol Dehydrogenase on Hemoglobin A_{1c}.

Results

Part B



Fibure 16. Absorbance of Acetaldehyde-bound, Glycosylated, and Total Fast hemoglobins vs. Days.
(⊙) Total Fast Hemoglobin
(•) Nonglycosylated or Acetaldehyde bound hemoglobin
(⊡) Glycosylated Hemoglobin

Results

Part C

Preliminary studies comparing alcoholic to nonalcoholic blood have consistently shown the alcoholic blood to be higher in acetaldehyde-bound hemoglobin. (Table 3)

	average absorbance of alcoholic blood	average absorbance of nonalcoholic blood
total fast hemoglobin	1.284	0.208
nonglycosylated or acetaldehyde hemoglobin adduct	0.470	0.387
glycosylated hemoglobin	0.183	0.119

Table 3. Alcoholic Blood vs. Nonalcoholic Blood

Chapter 6

Conclusion

A clinical test method has been postulated to detect a chronic abuser of alcohol where one tests for the acetaldehyde-bound hemoglobin. It appears that acetaldehyde the first metabolite of alcohol, accumulates in the body and slowly binds to hemoglobin. Therefore, acetaldehyde bound hemoglobin levels should represent the time-averaged blood acetaldehyde level. Acetaldehyde-bound hemoglobin can be compared to glycosylated hemoglobin seen in diabetics in that acetaldehyde and glucose bind to hemoglobin in the same area and acetaldehyde-bound hemoglobin and glycosylated hemoglobin have the same ion chromatographic properties. Therefore, a nondiabetic chronic alcoholic can have a falsely elevated glycosylated hemoglobin level which would be due to the high concentration of acetaldehyde-bound hemoglobin rather than the glycosylated hemoglobin.

The experimental part of this research first involved adding acetaldehyde, ethyl alcohol and ethyl alcohol with alcohol dehydrogenase to three whole blood samples. One was looking for an increase in the fast hemoglobin fraction. As one can see from the graphs (Figures 13, 14, 15), there is a steady increase in the percent of ylysocylated hemoglobin fraction with time.

The next step was to prove that this rise in the fast hemoglobin fraction was actually due to the acetaldehyde-bound hemoglobin or similar species. At this point, the experimenter needed to separate glycosylated hemoglobin from acetaldehyde-bound hemoglobin. This was accomplished using two columns, an ion exchange and a boronated agarose. By using the appropriate buffer, the ion exchange column separated the glycosylated hemoglobins and the acetaldehyde-bound hemoglobins from the "other" hemoglobins. Therefore, only glycosylated and acetaldehyde-bound hemoglobins eluted. The eluate directly drained into the boronated agarose column. This column has the ability to bind the glycosylated hemoglobin and allow the nonglycosylated hemoglobin to elute first. This research assumed that only glycosylated and acelaldehyde-bound hemoglobins eluted to the agarose column. Therefore, from the agarose column, the acetaldehyde-bound hemoglobin or the "nonglycosylated fraction" will elute first, resulting in its separation from the glysocylated hemoglobin. These results can be seen in figure 16. There is a steady rise in the total fast hemoglobin fraction. This rise is due to the increased acetaldehyde-bound hemoglobin because the nonglycosylated fraction rises steadily, whereas, the glycosylated fraction remains basically constant. (Figure 17)

This experiment was performed on whole blood that had acetaldehyde added. A suggestion for further research

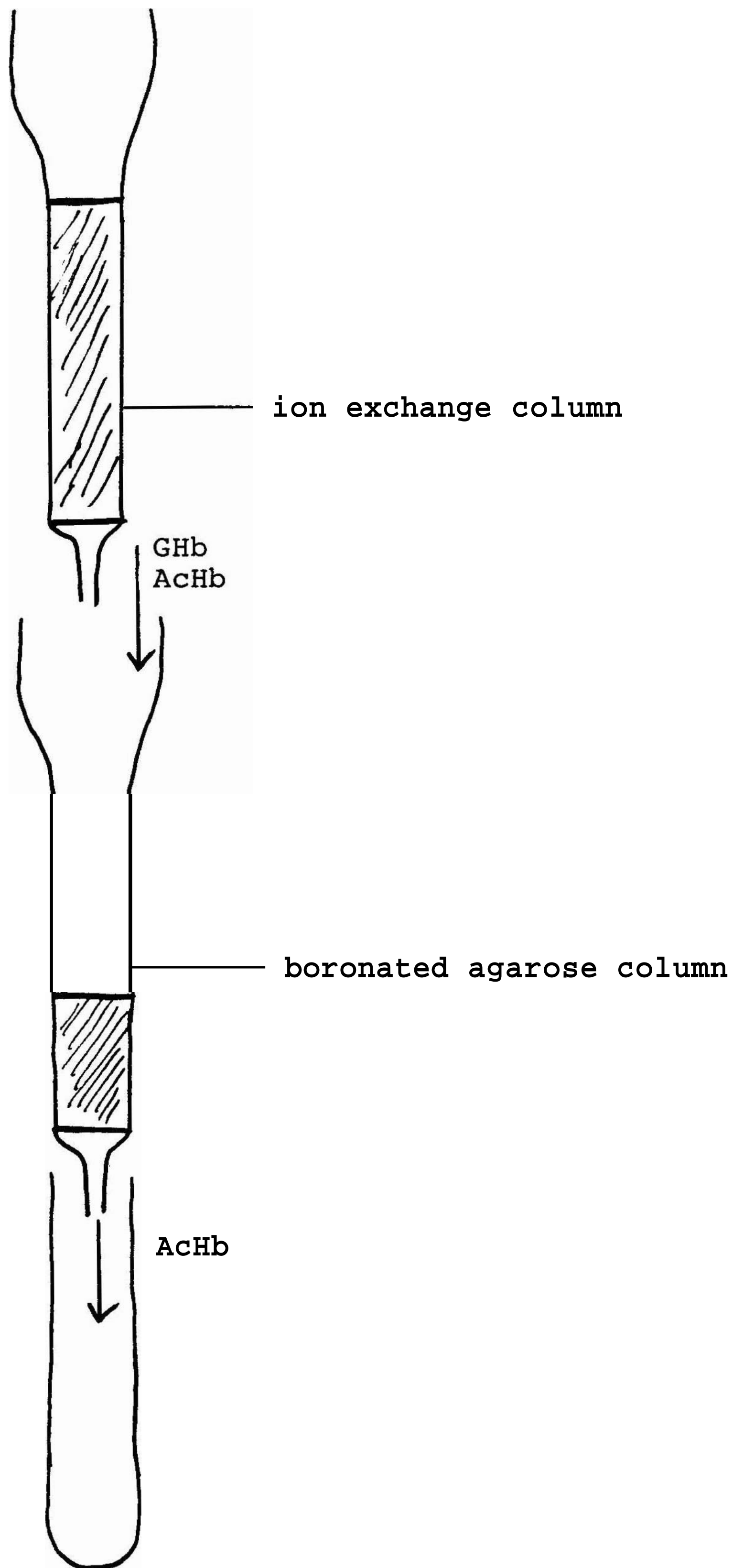


Figure 17. Arrangement of ion exchange and boronated agarose columns.
Glycosylated hemoglobin (GHb)
Acetaldehyde-bound hemoglobin (AcHb)

is to perform this procedure with blood from clinically evaluated chronic alcoholics to see if there is a consistent increase in the acetaldehyde-bound hemoglobin. One could also look for acetaldehyde-albumin adducts using ion exchange chromatography.

A letter requesting whole blood from chronic alcoholics has been sent to Dr. L. M. Pass. (See Appendix A) It is hoped that these samples can be used to further support the results of this thesis.

Detecting elevated levels of acetaldehyde-hemoglobin adducts will aid in screening for chronic alcoholics and also aid in their treatment.

This research devised a method of separating acetaldehyde-bound hemoglobin from the other hemoglobins, especially hemoglobin A_{1c} with which it usually co-chromatographs. As a preliminary step, the bloods of admittedly heavy drinkers and non-drinkers have been compared using this method (Results Part C). A greater hemoglobin content of the eluate from the affinity column is noted for the heavy drinkers than nondrinkers. This is believed to be due to a greater amount of acetaldehyde-bound hemoglobin.

YOUNGSTOWN STATE UNIVERSITY
YOUNGSTOWN, OHIO 44555

APPENDIX A

The College of Arts and Sciences

Dr. L.M. Pass
Youngstown Hospital Association
Youngstown, Ohio

June 22, 1984

Dear Dr. L.M. Pass,

I am a graduate student at Youngstown State University and am working under Dr. D. Mincey on a research project dealing with alcoholism. We would appreciate it if you could provide us with human blood samples from alcoholic patients in order for us to conduct our research. Enclosed is a copy of the corresponding information on our research.

Sincerely,

Eva Diaconis

Eva Diaconis

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