ABSTRACT

Stuart McGuire Thomson Jr. AN ANALYSIS OF URINARY METABOLITES OF TRYPTOPHAN AS RELATED TO ALCOHOLISM. Department of Biology, August, 1986.

Alcoholism is a disease that has been a problem throughout recorded history. It was recognized even during early times that there is an hereditary component to alcoholism. An hereditary component means that there is some physical trait that can be genetically transmitted from generation to generation that predisposes one to alcoholism or makes one more vulnerable to becoming alcoholic. At least one manifestation of this trait seems to be an impaired serotonin metabolic pathway of tryptophan metabolism. Investigators, using many different types of tests, have indicated that people with the most severe form of alcoholism have an impaired serotonin metabolic pathway of tryptophan metabolism. However, the tests that were used were too complicated, cumbersome, or painful to be good screening tests. The purpose of this study was to develop a non-invasive and easy to use test, that can be used for screening. It was felt that the ratio between tryptophan metabolites in different metabolic pathways would remain approximately the same even though the amounts of the metabolites present in the urine would be expected to vary with the concentration of the urine and other

factors. A test was developed, based on this assumption, that uses an HPLC with an electrochemical detector to quantify tryptophan metabolites. The test uses one urine collection and it requires a minimal of sample preparation. A ratio is determined between 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, and a tryptophan metabolite in another metabolic pathway such as indoleacetic acid (IAA). A person who has an impaired serotonin metabolic pathway would be expected to have a lower ratio than a person whose serotonin metabolic pathway was not impaired. In order to determine whether or not this test could discriminate between an alcoholic group and a non-alcoholic group, 5-HIAA/IAA and 5-HIAA/AA ratios were compared between these two groups. Statistical analysis indicated that these ratios can differentiate between alcoholic and non-alcoholic populations.

AN ANALYSIS OF
URINARY METABOLITES OF TRYPTOPHAN
AS RELATED TO ALCOHOLISM

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CHAPTER I

INTRODUCTION

Alcohol consumption has been an enigma from earliest history. Some individuals can consume alcohol in a social environment without any apparent adverse effect. Others become alcoholic under a similar environment. The Department of Health, Education, and Welfare compiled a comprehensive report of the various effects of alcohol abuse on the American public (DHEW, 1977). Some of the factors mentioned are listed below.

An estimated 10 million people in the United States have alcoholic problems. This is expanded to include 36 million Americans caught up in the web of alcohol abuse involving unhappy marriages, divorce, broken homes, desertion, impoverished families, and deprived and displaced children. Additionally alcohol is related to many traffic fatalities. Approximately one-third of adult pedestrian fatalities and about 45 percent of fatally injured drivers have a blood alcohol content of 0.10 percent or higher. The report also indicated a positive correlation between the consumption of alcohol and crime. Sixty-four percent of all murders, 41 percent of all assaults, and 34 percent of all forcible rapes were

associated with alcohol consumption. Approximately 50 percent of all arrests and one-third of all suicides were alcohol related.

DHEW (1977) estimated that alcohol misuse and alcoholism cost the American society $25 billion annually in lost production, health and medical costs, property damage, welfare, and criminal justice system costs.

These adverse effects of excessive alcohol consumption have not gone unnoticed. One of the earlier "solutions" was to remove the alcoholic from the public. This meant confinement and was never more than a temporary solution. A more serious approach was an attempt to determine the possible influence of heredity on alcohol consumption. This was to review the family history of alcoholics. This approach implies that excessive consumption of alcohol goes beyond "societal pressures". While this may help one explain alcoholism, it does not correct the problem. Another approach to this problem is to reduce the consumption of alcohol by using drug therapy. One drug, bis(diethylthiocarbamoyl) disulfide which is commonly called disulfiram, is being successfully used on alcoholics to reduce their consumption of alcohol. This drug blocks the oxidation of alcohol at the acetaldehyde stage. The accumulation of acetaldehyde in the blood produces a highly

unpleasant reaction (Ayerst, 1985). This has been a widely accepted method used in many rehabilitation centers.

While the use of disulfiram has been very beneficial to those working with alcoholics, other approaches are being investigated. Recently the biochemical study of the relationship between certain neurotransmitters and alcoholism has been indicated. One such approach is the study of the relationship between an impairment of the serotonin metabolic pathway of tryptophan metabolism and chronic alcoholism (Olson et al., 1960; Akhter et al., 1978; Ballenger et al., 1979; Banki, 1981). Some investigators hypothesize that there is a preexisting defect in tryptophan metabolism that is present in some people that causes them to have lower than normal levels of serotonin. Lowered serotonin predisposes them to chronic alcoholism as well as a spectrum of other depressive illnesses (Olson et al., 1960; Ballenger et al., 1979; Oreland, 1980). Recently von Knorring et al. (1985) suggested that there are two types of alcoholism: (1) Type-I which has a late onset and few social complications and, (2) Type-II alcoholism which has an early onset with severe social complications such as loss of a job due to alcoholism, arrests while intoxicated, arrests for drunk driving, fights while intoxicated, and drug abuse problems. Type-I alcoholism is not known to have a biological predisposing factor or marker. Type-II alcoholics have low

blood platelet monoamine oxidase (MAO) activity for a biological marker. It should be noted that low blood platelet MAO activity is linked to low levels of serotonin in the central nervous system (Oreland, 1980).

Most investigators measure serotonin levels indirectly by measuring 5-hydroxyindoleacetic acid (5-HIAA) either in spinal fluid or in 24 hour urine collections. They either use the method of Udenfriend et al (1955) or a similar one. These methods are time consuming, costly, and cumbersome to use. Thus, these tests are precluded from being used in general clinical practice as screening tools. Some investigators have tested the platelet MAO activity in blood as an indirect method of determining serotonin levels. This relationship is not as clearly defined as is the determination of 5-HIAA levels as an indicator of serotonin activity.

The present investigation is an attempt to develop an analytical test that uses one random urine collection to screen for an impaired serotonin pathway of tryptophan metabolism. This test will be used to determine impairment of the serotonin pathway in control and alcoholic subjects. The test will be considered to be validated if it indicates statistically significant differences in serotonin pathway function between a control group and an alcoholic group.

CHAPTER II

REVIEW OF LITERATURE

Alcoholism has been a problem since early civilization began. Since these early times it was noted that a predisposition toward becoming alcoholic seemed to be inherited. Aristotle made the observation that drunken women "bring forth children like themselves" (Goodwin, 1979). During the "gin epidemic" in eighteenth century England the notion arose that hereditary influences, as well as familial influences, were involved in alcoholism. Modern studies have indicated that there is an inherited predisposition towards becoming an alcoholic (Goodwin, 1979; Cloninger et al., 1981; Bohman et al.; 1981; Goodwin et al., 1973; Goodwin et al., 1974). Sons of alcoholics have about a 25% risk of becoming alcoholic whereas sons of non-alcoholics have only a 5% risk of becoming alcoholic. Daughters of alcoholics have about a 5% risk of becoming alcoholic whereas daughters of non-alcoholics have only a 1% risk of becoming alcoholic. An explanation for the sex differences could be that most societies have different role expectations for the two sexes and different societal prohibitions for the two sexes. There has been a consistent tendency for rates of depression to be greater

among females than males (Baldessarini, 1983). He cited a study of the Amish people in Pennsylvania which indicated that the rate of depression was the same in both sexes of the Amish, whose culture strongly discourages alcoholism and antisocial behavior. Alcoholism and antisocial behavior may be alternative behavior patterns for males in most cultures.

A review article by Goodwin (1979) cited twin and adoption studies by many different investigators. The following references on twin and adoption studies are from this source.

Twin studies have indicated genetic control of drinking behavior. A study by Kaij in 1960 found that identical twins were more concordant for alcoholism than were fraternal twins. The more severe the alcoholism, the greater the discrepancy was between concordance rates in identical vs fraternal twins. A study by Partanen, Bruun, and Markanen in 1966 indicated that identical twins were more concordant for quantity and frequency of drinking than fraternal twins. In 1968, Jonsson and Nilsson reported findings based on questionnaire data from 7,500 twin pairs in Sweden that identical twins were more concordant with regard to the quantity of alcohol consumed than were fraternal twins.

A series of Danish adoption studies that began in 1970 studied individuals separated from their biological parents soon after birth and raised by non-relative foster parents. They found that sons of alcoholics were about four times more likely to be alcoholic than were sons of non-alcoholics, whether they were raised by non-alcoholic foster parents or by their own biological parents. These findings indicate a genetically inherited predisposition towards becoming alcoholic. An inherited predisposition towards becoming alcoholic indicates that there is a physical trait that can be inherited that predisposes one or makes one more vulnerable to becoming alcoholic.

At least one expression of this physical trait seems to be an impaired serotonin metabolic pathway that causes one to have lower than normal levels of serotonin in the central nervous system. It was suggested that a segment of the population has a defect in the metabolism of tryptophan that causes a lowered concentration of serotonin and may indicate a predisposition toward chronic alcoholism (Olson et al., 1960; Ballenger et al., 1979; Oreland, 1980).

Studies indicate that there is a metabolic defect in tryptophan metabolism in the pathway between tryptophan and 5-hydroxyindoleaceticacid (5-HIAA) but not in the other pathways of tryptophan metabolism of chronic alcoholics (Olson et al., 1960; Akhter et al., 1978; Ballenger et al.,

1979; Banki, 1981). Chronic alcoholics were reported to have excreted about one-half the normal amount of 5-HIAA but they excreted the normal amount of N-methylnicotinamide (Olson et al., 1960). This indicated an impairment in the serotonin pathway but not in the kynurenine pathway (Appendices A, B, D).

A later study (Murphy et al.,1962) did not confirm the findings of Olson; however, this may have been related to the less rigorous choice of experimental subjects selected by Murphy.

It has been shown that the tryptamine : 5-HIAA ratio is higher in the alcoholic group than in the normal group (Akhter et al., 1978). This study indicated an impairment of the serotonin pathway but not of the tryptamine pathway (Appendices A, C). No difference was shown in levels of blood serum indole-3-acetic acid (IAA) between the alcoholic and control groups (Friedman et al., 1984). This indicated no impairment of the tryptamine pathway. Decreased 5-HIAA levels were shown in alcohol dependent patients but no decrease was shown in their tryptophan levels (Banki, 1981). This study also indicated a defect in the serotonin pathway of chronic alcoholics. Cerebrospinal fluid (CSF) drawn from alcoholic patients contained less 5-HIAA than CSF drawn from a non-alcoholic group (Ballenger et al., 1979). This also indicated a

defect in the serotonin metabolic pathway.

Ballenger et al. (1979) postulated that alcoholism may be due to an effort to raise the brain serotonin levels but that these levels are gradually depleted as a consequence of repeated drinking. This alcohol related depletion of serotonin makes the desire for alcohol even greater. The alcoholic consumes even more alcohol to pharmacologically modify the central indoleamine defect.

Alcoholic subjects that had not consumed alcohol for periods of three or six weeks had 5-HIAA levels that were lower than those of non-alcoholic controls (Borg et al., 1985). During periods of intoxication, 5-HIAA correlated positively to blood ethanol concentration. These results supported the assumption of subnormal serotonergic activity in abstinent alcoholics and an activation of serotonergic activity during abuse.

Some investigators have studied serotonin levels indirectly by measuring blood platelet MAO levels. Blood platelet MAO levels are directly related to serotonin levels in the central nervous system (Oreland, 1980). The study by Major and Murphy (1978) found that their alcoholic group had lower platelet MAO levels than their non-alcoholic group.

A positive correlation between blood platelet MAO activity and both the concentration of serotonin and the concentration of 5-HIAA was found (Oreland, 1980). An association between low thrombocyte MAO activity, alcoholism, and suicidal behavior was also found . Low MAO activity was shown in both the brain and the thrombocytes in patients with suicidal behavior. It was speculated that low MAO activity is already present prior to alcohol abuse and therefore should indicate a constitutional disposition for abuse. It was also speculated that low serotonin turnover can mean a disposition for suicidal behavior, alcoholism, cycloid psychosis, bipolar affective illness, and possibly for phobic states. Defined behaviors such as ethanol abuse and suicidal behavior seem to correlate better to biochemical findings than the conventional psychiatric catagories.

The activity of human platelet and brain MAO is lower in alcoholics than in controls (Oreland, 1983). These results and others indicate a common control of brain and platelet MAO, which would support an hypothesis that there is a common ontogenetic origin of platelets and brain tissue. Platelet MAO activity is also correlated with cerebrospinal fluid 5-HIAA and HVA, which is consistent with the hypothesis that low platelet MAO activity reflects a "weak" brain monoaminergic system.

von Knorring et al. (1985) divided the alcoholic population into two subpopulations that he called "Type I" alcoholism and "Type II" alcoholism. Type I alcoholism is characterized by having a late onset and having few social complications. Type II alcoholism is characterized by having an early onset and having more social complications such as loss of a job due to alcoholism, arrests while intoxicated, arrests for drunk driving, fights while drinking, and drug abuse problems. It was also noted that the Type II alcoholics had more alcoholism and depression among their first degree relatives than did the Type I alcoholics. The two types of alcoholism were separable by means of a biological marker for Type II alcoholism, low blood platelet MAO levels. Blood platelet MAO levels were normal in Type I alcoholics and are decidedly low in Type II alcoholics. This study determined that low blood platelet MAO activity is a "biological marker" for "Type II" alcoholism but not for "Type I" alcoholism.

Studies have shown that drinking behavior can be altered by manipulating serotonin levels. Animal studies have indicated that lowered levels of serotonin cause increased ethanol intake. It has been demonstrated that the selective destruction of serotonergic neurons in rat brains caused the animals to increase their ethanol intake in a free choice situation (Myers and Melchior, 1975).

Other studies have indicated that voluntary consumption of ethanol is decreased by the administration of serotonin or a serotonin agonist. A study indicated that intraventricular injections of serotonin would lower voluntary ethanol consumption by rats in a free choice situation without altering their water intake (Hill, 1974). It was demonstrated that treatment with 5-hydroxytryptophan, a serotonin precursor, reduced ethanol consumption of rats in a free choice situation (Myers et al., 1972). A serotonin agonist, zimeldine, which is a specific serotonin reuptake inhibitor, decreased voluntary consumption of ethanol in both rats (Gill et al., 1985; Rockman et al., 1979) and humans (Naranjo, 1984a,b)

Daoust et al. (1984) compared the effects of several different antidepressant drugs with different specificities of action on voluntary ethanol consumption. This study found that the antidepressant drugs that were serotonin reuptake blockers or were norepinephrine reuptake blockers inhibited the voluntary consumption of ethanol but that the drugs that were dopamine reuptake blockers did not reduce the voluntary consumption of ethanol. The drugs that reduced the intake of alcohol were clomipramine and doxepine, which are serotonin reuptake inhibitors, and desipramine and metapramine, which are norepinephrine reuptake inhibitors. Nomifensine and maprotiline, which are dopamine reuptake inhibitors, did not decrease ethanol

consumption. This study indicates that norepinephrine as well as serotonin may be involved in the control of drinking behavior. It is noted that nomifensine and maprotiline also inhibit the reuptake of norepinephrine to a degree.

Studies using para-chlorophenylalanine as a serotonin depleter yielded contradictory results (Stein et al., 1977). Various studies indicated a decrease in voluntary ethanol consumption, no change in voluntary ethanol consumption, a decrease in voluntary ethanol consumption followed by an increase in voluntary ethanol consumption. There may be several factors that account for these contradictions. One factor may be taste aversion since it has been shown that para-chlorophenylalanine is capable of producing taste aversion comparable to lithium chloride when associated with novel solutions. Another factor may be that at the dosages usually given, para-chlorophenylalanine inhibits tyrosine hydroxylase as well as tryptophan hydroxylase thereby reducing the amount of catacholamines present as well as the amount of serotonin present (Stein et al., 1977). It is evident that the results from any study that used para-chlorophenylalanine as a serotonin depleter should be carefully evaluated and not accepted on face value since it is highly likely that the results of these studies are due

to factors other than lowered levels of serotonin.

It seems that from the sources cited that there is an inherited predisposition to becoming alcoholic. The genetic nature of this predisposition towards becoming alcoholic has been demonstrated by the use of twin studies and adoption studies. An inherited predisposition towards becoming alcoholic indicates that there is a physical trait that can be inherited that predisposes one or makes one more vulnerable to becoming alcoholic. At least one expression of this physical trait seems to be an impaired serotonin metabolic pathway that causes one to have lower than normal levels of serotonin in the central nervous system. The relationship between low levels of serotonin and alcoholism was demonstrated both by studies measuring 5-HIAA as an indicator of serotonin levels and by studies measuring blood platelet MAO activity as an indicator of serotonin levels. Once the relationship between low serotonin levels was demonstrated experiments were performed to test the causality of the relationship. A study that experimentally lowered serotonin levels caused an increase in voluntary alcohol consumption. Studies that experimentally raised serotonin levels caused a decrease in voluntary alcohol consumption.

Investigators have used various methods or tests to indicate the functioning of the serotonin metabolic

pathway. Some investigators measured 5-HIAA in the urine. The method of Udenfriend et al. (1955) has been used to quantify 5-HIAA in 24 hour urine collections (Olson et al., 1960; Murphy et al., 1962) and to determine the amount of 5-HIAA in random urine collections (Akhter et al., 1978). Akhter et al. (1978) related the amount of 5-HIAA in random urine samples to creatinine. The ratio of tryptamine per mg creatinine to 5-HIAA per mg creatinine was also calculated. All of these methods required a long rather complicated chemical extraction and most required a 24 hour urine collection.

Other investigators measured 5-HIAA in cerebrospinal fluid. A high performance liquid chromatography system (HPLC) with a fluorometric detector was used to measure 5-HIAA in cerebrospinal fluid (Ballenger, 1979). A slightly modified method of Kemerer et al. (1979) has also been used to quantify 5-HIAA in cerebrospinal fluid (Banki, 1981). Mass fragmentographic methods have been used to measure 5-HIAA in cerebrospinal fluid (Borg et al., 1985). All of these methods have the disadvantage of requiring a spinal tap, which is a painful procedure, in order to obtain cerebrospinal fluid to analyze.

Still other investigators have indirectly measured serotonin levels in the central nervous system by measuring blood platelet MAO activity which required a radioactive

binding assay (Major and Murphy, 1978; Oreland, 1980; von Knorring et al., 1985).

The tests used by the investigators cited are not suitable for use as clinical screening tests. The investigators that measured 5-HIAA in the urine used a complicated chemical extraction procedure to measure the 5-HIAA. Olson et al. (1960) and Murphy et al. (1962) used a 24 hour urine collection. A 24 hour urine collection usually requires hospitalization to insure accuracy.

Tests that measured tryptophan metabolites in cerebrospinal fluid all caused pain to the experimental subjects during the collection of the cerebrospinal fluid since they required a spinal tap.

Tests that measured blood platelet MAO activity caused some discomfort during the collection of blood. The assay for blood platelet MAO activity was somewhat complicated even though it was much simpler than the chemical extraction method of Udenfriend et al. (1955).

It is noted that modern HPLC methods using fluorometric or amperometric detectors offer the possibility of determining levels of tryptophan metabolites in the urine with a minimal of sample preparation.

It is apparent that a test that is easy to use and that is non-invasive is badly needed. A simple

non-invasive test that could be used for screening purposes could aid in the early treatment or prevention of alcoholism. The purpose of this study is to develop such a screening test.

CHAPTER III

TEST DEVELOPMENT AND VERIFICATION

CONCEPTUAL BASIS

The serotonin metabolic pathway of tryptophan metabolism is impaired in most chronic alcoholics, e.g. Type II alcoholics (Olson et al., 1960; Akhter et al., 1978; Balenger et al., 1979; Banki, 1980; von Knorring et al., 1985). These investigators demonstrated that the other metabolic pathways of tryptophan metabolism are not impaired. Each person has some finite amount of tryptophan present, depending upon dietary intake and metabolic degradation. It is reasonable to assume that differing percentages of the available tryptophan will be metabolized in each metabolic pathway of tryptophan. If this assumption is correct, it means that the ratio between a tryptophan metabolite in one pathway and a tryptophan metabolite in another pathway will remain approximately the same regardless of the amount of metabolite excreted.

Based on this assumption, a test was developed to differentiate alcoholics from controls using the 5-HIAA/IAA ratio to indicate altered serotonin (5-Hydroxytryptamine) metabolism. The major metabolite of serotonin, 5-HIAA, was selected since it is present in the urine and can be

detected with an electrochemical detector. From the tryptamine pathway, IAA was selected to be the other metabolite. The advantages of IAA are that it comes from the shortest of the tryptophan metabolic pathways, it is also found in urine, and it can also be detected with an electrochemical detector. A ratio between 5-HIAA and IAA or a ratio between 5-HIAA and anthranillic acid (AA) could be used to differentiate between an alcoholic population and a non-alcoholic population since a low ratio should indicate an impaired serotonin metabolic pathway. A high ratio would indicate greater use of the serotonergic pathway.

TEST DEVELOPMENT

Once the theoretical part of the test was developed, a practical method of measuring 5-HIAA, IAA, and AA was needed. It seemed logical that a method that used a HPLC with an electrochemical detector would be ideal. This is because the metabolites that needed to be measured occur in minute amounts in urine and amperometric detection is sensitive enough to measure these very small amounts. This type of procedure also requires very little in the way of sample preparation.

INSTRUMENTATION

A High Performance Liquid Chromatography (HPLC) system was set up as follows. An Altex model 500 automatic injector was connected to a Gilson model 302 pump. These in turn were connected to a Gilson model 802B manometric module. These were connected to a Brownlee Labs MPLC guard column (RP-18) (ODS 5 um, I.D. 4.6 mm, length 3 cm) which was connected to an Altex, ultrasphere column (ODS 5 um, I.D. 4.6 mm, length 25 cm). These were connected to an ASI/Taccussel ED-110 electrochemical detector which was connected to a Perkin Elmer model 56 chart recorder. The pumping speed was set at 1 ml/min. The sensitivity of the electrochemical detector was set at 10 nA full range and the oxidizing potential was set at +1.000 V. The chart recorder speed was set at 5 mm/min.

BUFFER DEVELOPMENT

It became necessary to develop a buffer that would allow 5-HIAA, IAA and AA to be quantified using a single sample injection. The buffer was developed by adjusting the pH and the percentage of organic solvent present until a formulation was found that allowed for the quantification of the 5-HIAA and AA peaks, with out interference by other

peaks, and that allowed the IAA peak to come off the column at a time that was not unacceptably long.

BUFFER PREPARATION

The buffer was prepared by mixing together the following ingredients in the amounts indicated.

350 ml 0.1 M Sodium Phosphate, monobasic
5 ml 0.1 M Acetic Acid
11 mg Na2EDTA
18.6 ml Methanol

The pH of the above solution was adjusted to 4.35 by the addition of a few drops of 1 N potassium hydroxide, thereby giving a buffer that was 5 percent methanol with a pH of 4.35. The buffer was filtered through a Millipore GS 0.22 uM filter and degassed under vacuum before it was used.

VERIFICATION OF METABOLITES MEASURED

In order to insure that the metabolites of interest were indeed the ones being measured, the following

procedures were performed. A standard was prepared that contained a known amount of a metabolite of interest in a concentration that was similar to that of the naturally occurring metabolite in urine. It was injected into the HPLC system. The graph from the chart recorder was read and the time between injection and the occurrence of the peak was noted. Then a urine sample was prepared and injected. The investigator noted if a peak occurred at the same time after injection as the standard. If a peak did occur at the same time after injection as the standard, it was suspected that the peak might be that of the metabolite of interest. In order to test to see if this really was the peak, a urine sample was spiked with a known amount of the metabolite and was injected into the system. If the peak "grew" after the injection of the spiked urine without showing signs of overlapping, the peak was considered to be that of the metabolite of interest.

TEST VERIFICATION

After the test had been developed, it needed to be validated. The test would be considered to be a valid test if it could distinguish between an alcoholic group and a non-alcoholic group. The following segments of this chapter will give the materials, methods, and results of

the test validation trial.

EXPERIMENTAL SUBJECTS

The experimental subjects consisted of males undergoing treatment for alcoholism at Walter B. Jones Alcoholic Rehabilitation Center. Their ages ranged from 20 years to 60 years with the average age being 37.4 years. They were all detoxified and had a minimum period of at least two and one-half weeks without consuming alcohol. Most had not consumed alcohol for a period of three to over four weeks prior to testing. They were taking multiple vitamin supplements and disulfiram (Antabuse) at a dosage of 250 mg per day. Disulfiram was reported not to affect 5-HIAA excretion in man (Ballenger, 1979). Patients taking anti-depressant medication were excluded from the experimental group. The experimental subjects were asked to answer a set of questions consisting of the Michigan Alcoholism Screening Test. They were also asked to sign a form indicating that they were voluntarily consenting to participate in this experiment.

CONTROL SUBJECTS

The control group consisted mostly of males who were employed by Tideland Mental Health Center in Washington, North Carolina or by East Carolina University in Greenville, North Carolina. There was one East Carolina University Student included in the group in order to more closely match the ages in the experimental group. Members of the control group had ages that ranged from 19 years to 61 years with the average age being 37.6 years. Most of the control subjects were moderate drinkers even though a few in the control group were non-drinkers. People taking anti-depressant medication were excluded from the control group. The control subjects were asked to answer a set of questions consisting of the Michigan Alcoholism Screening Test. Anyone scoring more than 10 on the Michigan Alcoholism Screening Test was excluded from the control group. The subjects were asked to sign a form indicating that they voluntarily consented to participate in this experiment.

MICHIGAN ALCOHOLISM SCREENING TEST ADMINISTRATION

A set of questions containing the Michigan Alcoholism Screening Test (MAST) was administered to both the

experimental and control subjects between 9:00 a.m. and 11:00 a.m. on the days samples were collected. The test was typewritten and mimeographed. However, the questions were verbally read to anyone who had difficulty reading the questions. The test was administered just before the subjects were asked to donate a urine sample. Each subject was given a test marked with a number that corresponded to the number on the urine collection cup given that subject.

SAMPLE COLLECTION

Two 12 ml plastic capped test tubes were numbered for each urine collection cup with a number corresponding to that cup. The tubes were prepared prior to urine collection by adding 6 ml of a preservative consisting of 1% sodium metabisulfite dissolved in 1 N hydrochloric acid. Urine samples were collected between 9:00 a.m. and 11:00 a.m. on weekday mornings. Immediately upon receiving a urine sample, 6 ml of urine was added to each tube by filling the prepared tubes to the 12 ml mark with urine. Excess urine was then discarded. This procedure resulted in the collection of two tubes each containing 12 ml of a mixture of 50% urine and 50% preservative for each test subject.

SAMPLE PREPARATION

One ml of urine and preservative mixture was removed from one of each subject's two 12 ml sample tubes. The remaining samples were then frozen at -80 deg. C in order to preserve them for future use. The 1 ml of urine and preservative mixture was placed in a microcentrifuge tube and centrifuged for 6 minutes in a Fisher microcentrifuge in order to remove any particulate matter present. A 12.5% urine solution was produced by placing 200 ul of the centrifuged urine and preservative mixture in a serum bottle and adding 600 ul of distilled water. The serum bottles were then capped with teflon lined aluminum serum caps. The capped serum bottles were then ready to be loaded into the magazine of the automatic injector.

STANDARD PREPARATION

Stock solutions containing 1 mg/ml were prepared for each of the following substances: 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 3-hydroxyanthranilic acid (3-HAA), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), anthranilic acid (AA), indoleacetic acid (IAA), and xanthurenic acid. Then 2 ml of 0.1 M phosphoric acid was placed in a test tube and

10 ul of the desired stock solution was added. After thorough mixing by a vortex machine, 100 ul of the new solution was placed in a test tube with 900 ul of 0.1 M phosphoric acid and mixed thoroughly. One ml of this solution was placed in a serum bottle and capped with a teflon lined aluminum serum cap. The capped serum bottles containing the standards were then ready to be loaded into the magazine of the automatic injector.

SAMPLE RUNS

The Altex Model 500 automatic sample injector was set for 75 minute runs of 1 run per sample. The magazine of the injector was loaded by alternating samples with standards. Each serum bottle containing a urine sample was followed by a serum bottle containing a standard. This had the effect of making each urine sample run 150 minutes long. This was necessary since the maximum time per sample allowed by the automatic injector was 99 minutes and since the indoleacetic acid (IAA) was eluted at a later time. This alternating arrangement also allowed for the placing of an external standard in the middle of a sample run. The automatic injector had a 20 ul sample loop. Therefore 20 ul of sample was injected during each individual injection.

DATA COLLECTION

The data was collected as tracings made by a Perkin-Elmer Model 56 chart recorder on graph paper. The peak heights of the substances of interest were measured and recorded.

EVALUATION OF DATA

Ratios between 5-HIAA and IAA and ratios between 5-HIAA and AA were determined by dividing the peak height of 5-HIAA by the peak height of IAA and AA for each urine sample (Appendices I & J). A two way analysis of variance statistical program was run that compared the variance within groups to the variance between groups in order to determine if there were statistically significant differences between 5-HIAA/IAA ratios and 5-HIAA/AA ratios for the alcoholic and control groups.

CHAPTER IV

RESULTS

Previous chapters indicated that a test was developed to screen for an impaired serotonin metabolic pathway. They indicated the theoretical basis for the test and gave the methods used to gather data. The data obtained was used to differentiate between an alcoholic population and a non-alcoholic population. This chapter will give the data that was collected and will correlate the data obtained with the ability of this test to differentiate between alcoholic and non-alcoholic populations.

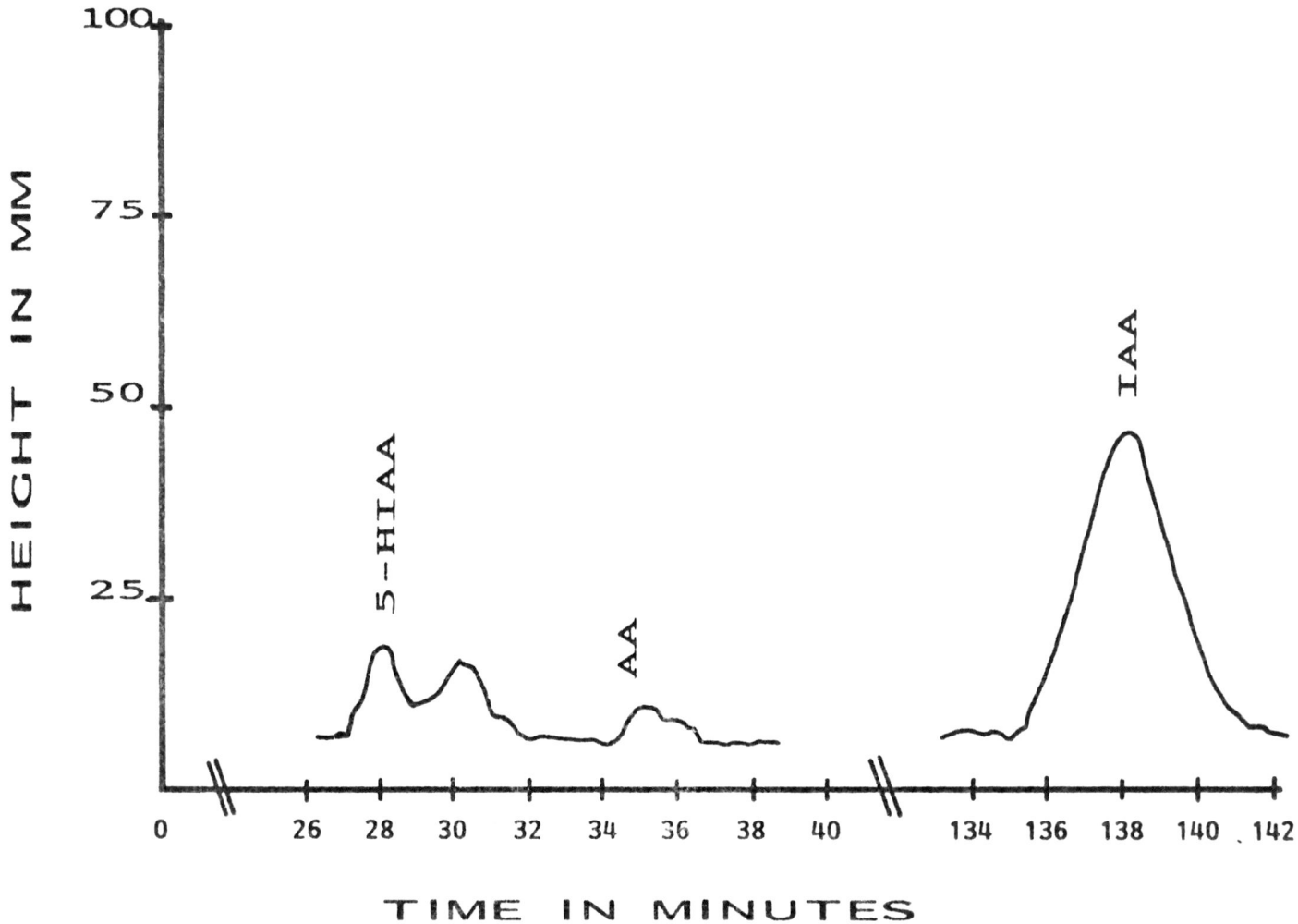
The data given will only be used to evaluate the test as many investigators, such as those cited in the "Review of Literature", have already shown that most alcoholics have an impaired serotonin metabolic pathway of tryptophan metabolism.

The data obtained (Figure 1) were from tracings made by a Perkin Elmer 56 chart recorder. The data collected from the alcoholic group is listed in Appendix F, that for the control group is listed in Appendix G.

The MAST scores were collected and compiled equally for both the experimental and control subjects (Appendices

FIGURE 1

A representation of the peaks of interest produced by the chart recorder. Due to space limitations the time scale was compressed. Peaks of no interest were omitted to avoid confusion. A time scale is given along the X axis so the reader may know at what time after injection the metabolite of interest came off of the column. The peak heights indicate the relative amounts of metabolites that are present.


HEIGHT IN MM
100
75
50
25
5-HIAA
AA
IAA
0
26
28
30
32
34
36
38
40
134
136
138
140
142
TIME IN MINUTES

F & G). The scores on the MAST ranged from 8 to 115 for the members of the alcoholic group with 55.5 being the average score. The MAST scores for the control group ranged from 0 to 5 with an average of 0.9. There were two subjects excluded from the control group for having scores greater than 10.

As shown in Appendix A tryptophan may be converted to a number of indole derivatives. The principle end products of these conversions which appear in the urine are 5-HIAA for the serotonin pathway and IAA for the tryptamine pathway. A metabolite derived from the kynurenine pathway that can be measured is AA. These three metabolites can be measured in the urine using amperometric methods. They were therefore chosen to be the metabolites measured in this study.

One of the major advantages of this test is that it frees the user of the test from the necessity of calculating exact amounts of metabolites excreted in the urine. Peak heights, which can be easily measured, are used as indicators of the relative amount of metabolite present. The ratios that are used in the test are obtained by dividing the peak height of 5-HIAA by the peak height of IAA and of AA. The ratios give in indication of the amount of one metabolite relative to the amount of another

metabolite.

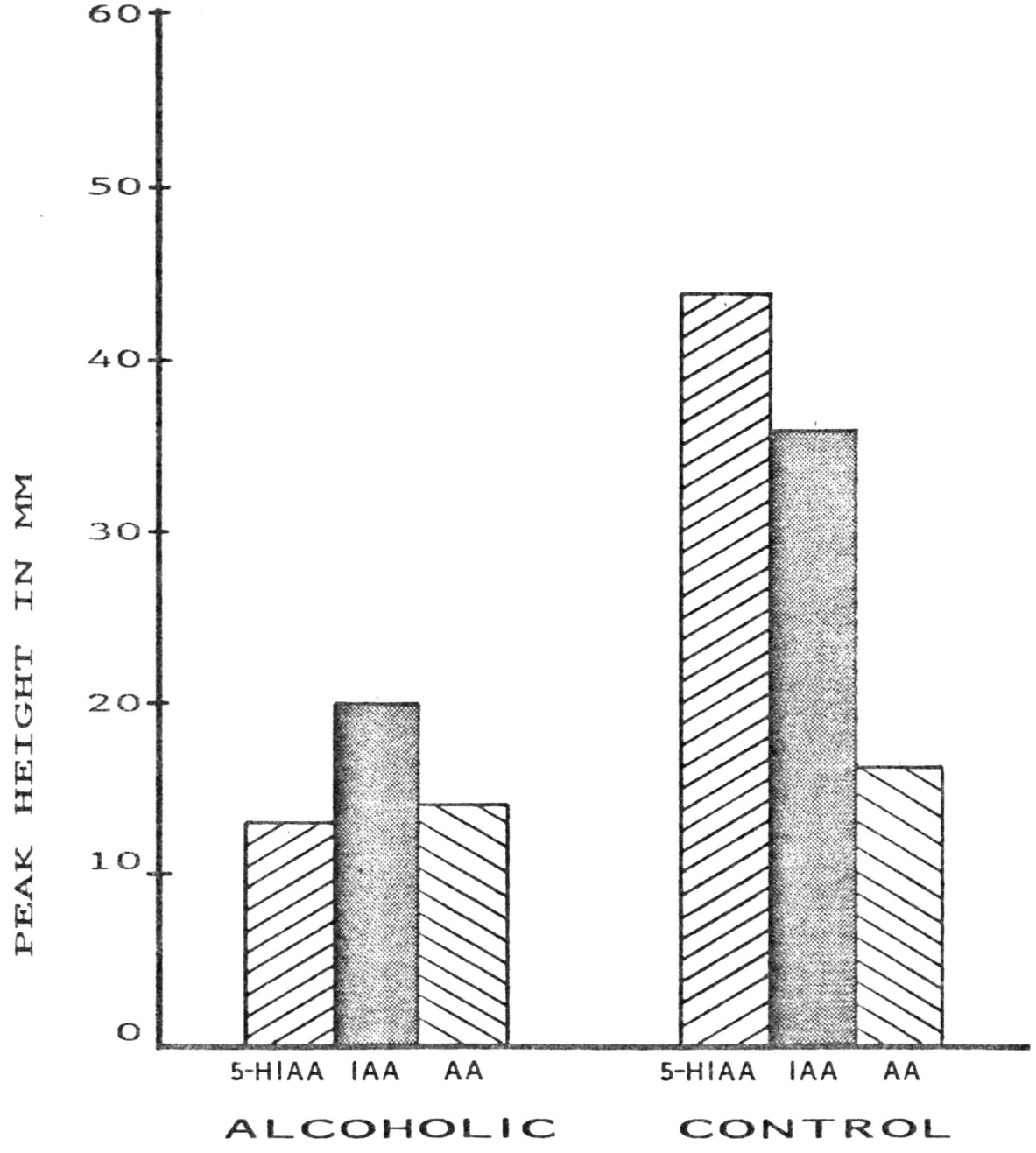
The heights of the 5-HIAA peaks ranged from 3 mm to 35 mm for the alcoholic group with an average of 12.8 mm (Figure 2). The heights of the 5-HIAA peaks of the control group ranged from 17 mm to 135 mm. Two of the peaks were so high that they ran off the scale and could not be measured. The average height of the peaks that could be measured was 43.5. The significant differences in the levels of 5-HIAA between these two groups is in agreement with other investigators.

The heights of the IAA peaks ranged from 4 mm to 40 mm for the alcoholic group with an average height of 20.2 mm (Figure 2). The heights of the IAA peaks of the control group ranged from 8 mm to 104 mm with an average height of 35.6 mm. No significant difference was found in the peak heights of IAA between the two groups.

In the alcoholic group, the heights of the AA peaks ranged from 4 mm to 34 mm with an average height of 14.3 mm (Figure 2). The height of the AA peak was too low to be measured for one subject. The heights of the AA peaks ranged from 2 mm to 48 mm for the control group with an average height of 16.1 mm. As expected, no significant difference was detected in the amount of AA present in the urine between these two groups.

FIGURE 2

A comparison of the average peak heights of the metabolites measured. It should be noted that no corrections have been made for differences in the concentration of the urines. The average peak height of 5-HIAA is significantly lower for the alcoholic group than for the control group. There is no significant difference between the levels of AA in the alcoholic and control group. The difference in IAA levels between the two groups approached a level of significance. However, there was no significant difference at the 5% level.


PEAK HEIGHT IN MM
60
50
40
30
20
10
0
5-HIAA
IAA
AA
5-HIAA
IAA
AA
ALCOHOLIC
CONTROL

The ratios of 5-HIAA to IAA ranged from 0.2308 to 1.3462 with an average of 0.7024 for the alcoholic group (Appendix H). The ratios of 5-HIAA to IAA for the control group ranged from 0.5227 to 3.2667 with an average of 1.5162 (appendix I). A better comparison of the 5-HIAA/IAA ratios can be seen in Figure 3. An analysis of variance test that was run using this data indicated that there is a statistically significant difference between the 5-HIAA/IAA ratios in the alcoholic group and those in the control group (Appendix J). The F ratio equalled 14.1607 and the probability of chance was 0.001.

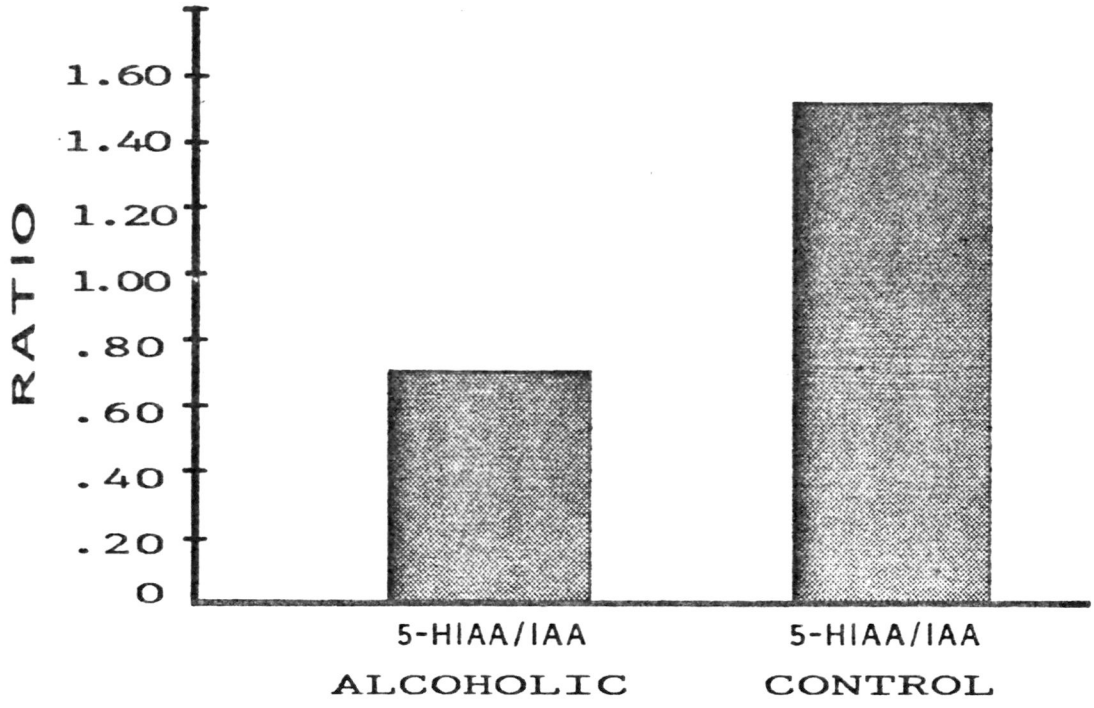
The ratios of 5-HIAA to AA ranged from 0.3000 to 4.0000 with an average of 1.2746 for the alcoholic group (Appendix H). The ratios of 5-HIAA to AA for the control group ranged from 0.6667 to 15.0000 with an average of 5.1178 (Appendix I). The data as plotted in Figure 4 shows the contrast between the two groups. An analysis of variance test was run using these data. It indicated that there was a statistically significant difference between the 5-HIAA/AA ratios in the alcoholic group and the 5-HIAA/AA ratios in the control group (Appendix K). The F ratio was 8.2765 and the probability of chance was 0.008.

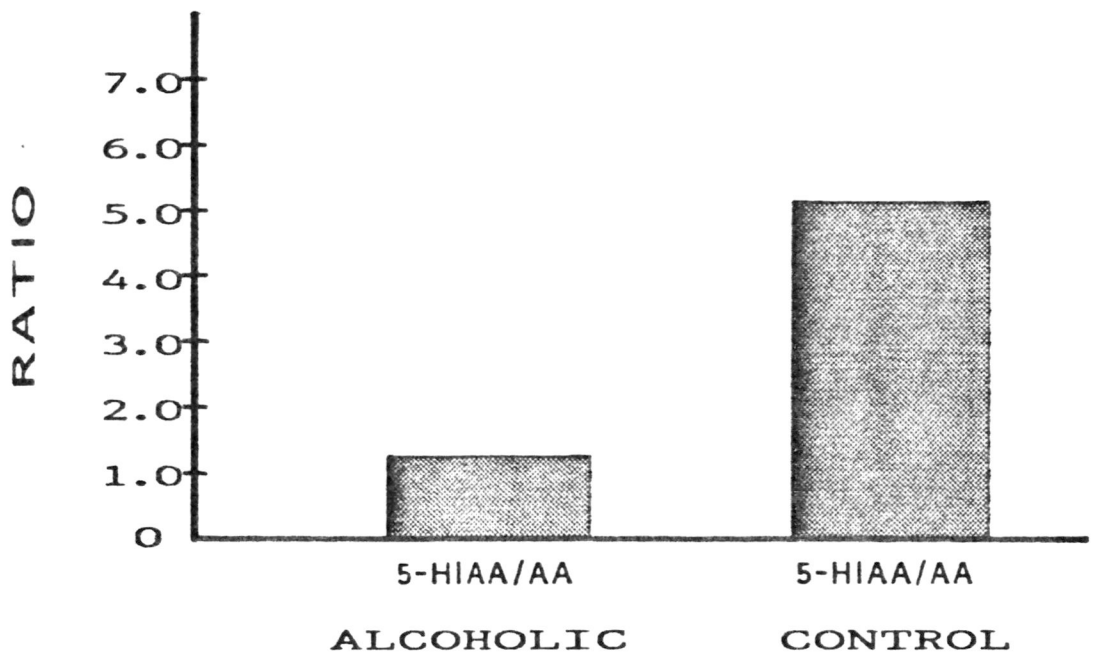
FIGURE 3

A comparison of the average ratios of 5-HIAA to IAA between the alcoholic group and the control group. Note that the average of the ratios for the alcoholic group is less than one-half that of the control group.

FIGURE 4

A comparison of the average ratios of 5-HIAA to AA between the alcoholic group and the control group. Note the large difference in the average of the ratios.


RATIO
1.60
1.40
1.20
1.00
.80
.60
.40
.20
0
5-HIAA/IAA
ALCOHOLIC
5-HIAA/IAA
CONTROL


RATIO
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
5-HIAA/AA
ALCOHOLIC
5-HIAA/AA
CONTROL

CHAPTER V

DISCUSSION

In this study a test was devised that used urinary metabolites of tryptophan in order to detect an impaired serotonin metabolic pathway of tryptophan metabolism. People who have an impairment of this pathway are thought to be more vulnerable to becoming alcoholic or to be predisposed to becoming alcoholic. Studies have shown that almost everyone who has the most severe form of alcoholism has this trait. The major metabolite of tryptophan, 5-HIAA, was chosen to be the metabolite to indicate the serotonin pathway function. This metabolite was selected to compare, by ratio, to two other tryptophan metabolites. The assumption being that 5-HIAA would be significantly lower in alcoholics, whereas, IAA and AA (also metabolites of tryptophan metabolism) would remain rather constant. A low ratio between 5-HIAA and either one of these other two metabolites would indicate an impairment of the serotonin pathway of tryptophan metabolism. The ratio of 5-HIAA/IAA was made for each subject in the alcoholic group (Appendix J) and for each subject in the control group (Appendix K). A break point was selected that would place approximately 95% of the known alcoholics in the alcoholic group. This point was determined to be 1.2. This point gave the best

separation between the two groups. Figure 5 graphically demonstrates the results when a break point of 1.2 is used. There was only one member of the alcoholic group that had a ratio of 5-HIAA/IAA above 1.2. Four members of the non-alcoholic group had scores below 1.2. The results indicated a greater amount of variability in the control group than in the experimental group. This greater variability in the control group was about as expected. Low serotonin may predispose one to becoming alcoholic - it does not force one to become alcoholic. People who have lower than normal levels of serotonin are thought to be predisposed to bouts of depression and to be at a greater risk for suicide than those who have higher levels of serotonin.

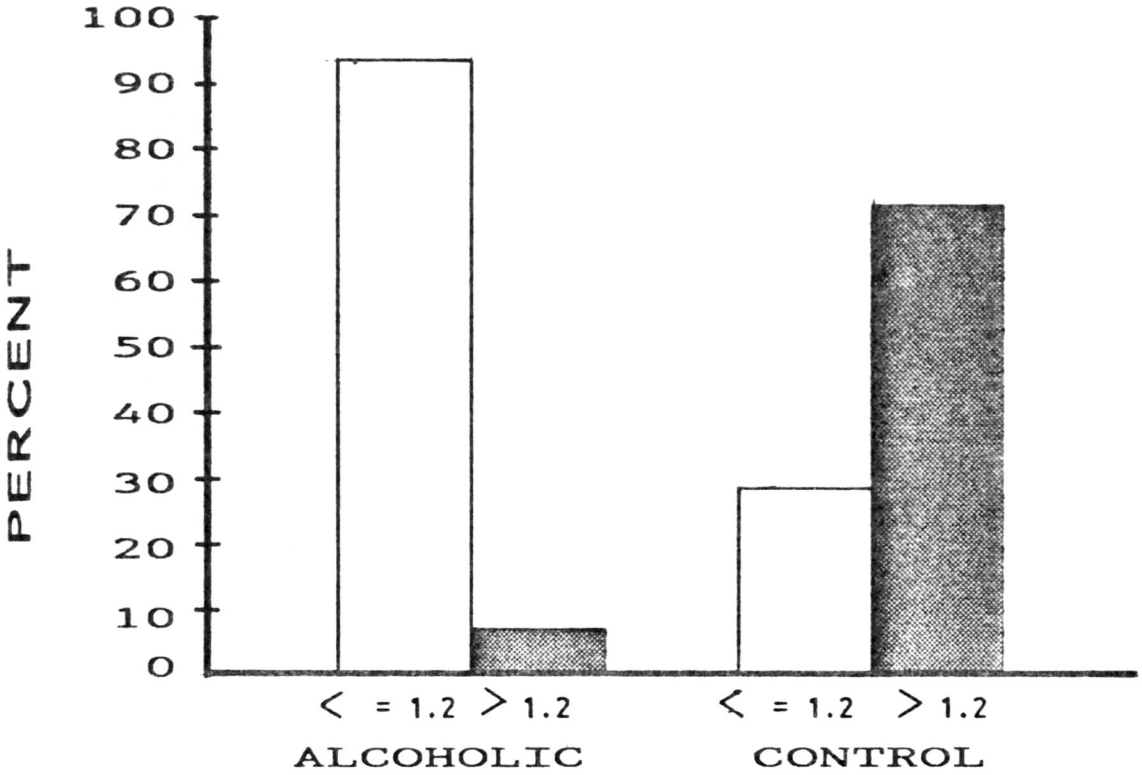
An additional check was made to determine whether or not the 5-HIAA/AA ratio could discriminate between an alcoholic and a non-alcoholic group. An analysis of variance indicated that it could discriminate between the two groups. A break point of 2.4 was determined to be the most optimal break point by using the methods previously described for determining the 5-HIAA/IAA break point. Only one member of the alcoholic group had a ratio higher than 2.4; whereas, five members of the control group were at 2.4 or below. This again indicated the obvious, that there is greater variability within the control group. The control

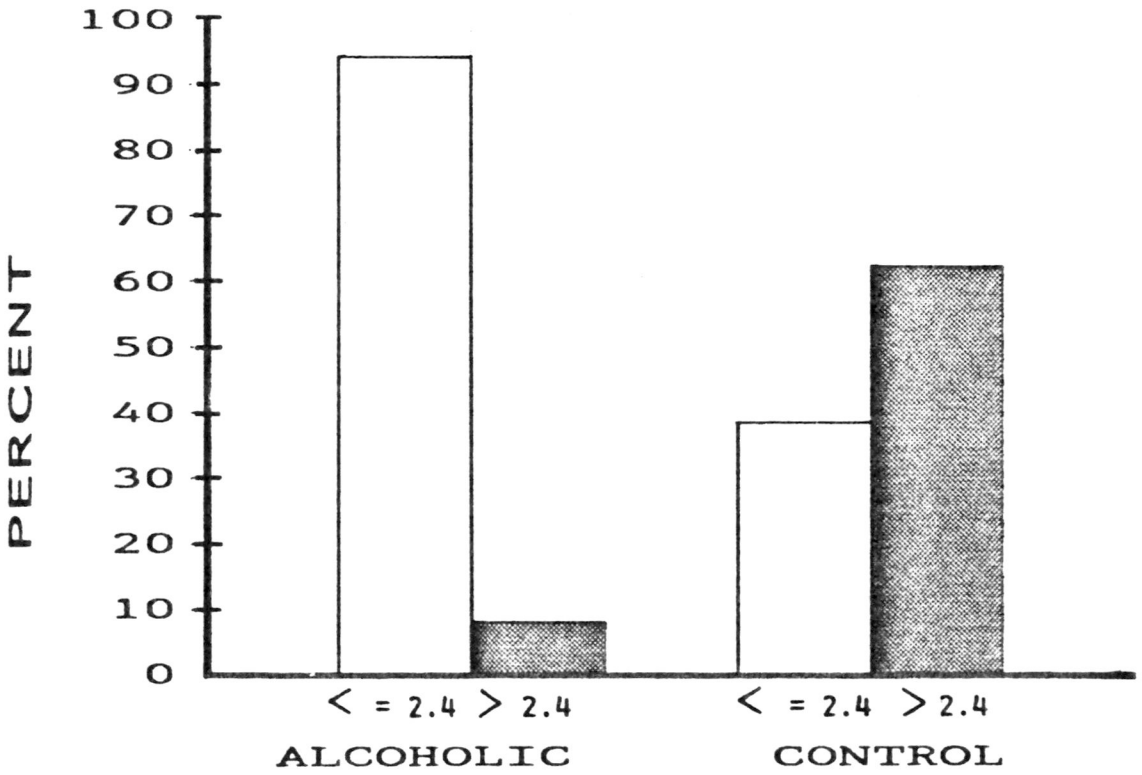
FIGURE 5

A comparison showing the difference in the percentages of people with 5-HIAA/IAA ratios above and below 1.2 in both the alcoholic and control groups. Note that about 94% of the people in the alcoholic group had a 5-HIAA/IAA ratio less than or equal to 1.2.

FIGURE 6

A compaarison showing the difference in the percentages of people with 5-HIAA/AA ratios above and below 2.4 in both the alcoholic and control groups. Note that about 94% of the people in the alcoholic group had a 5-HIAA/AA ratio less than or equal to 2.4.


100
90
80
70
60
50
40
30
20
10
0
PERCENT
< = 1.2
> 1.2
< = 1.2
> 1.2
ALCOHOLIC
CONTROL


100
90
80
70
60
50
40
30
20
10
0
PERCENT
< = 2.4
> 2.4
< = 2.4
> 2.4
ALCOHOLIC
CONTROL

group presumably includes individuals that may be predisposed to becoming alcoholic and, at the other extreme, those that have little or no desire for alcohol. Figure 6 gives a graphic representation of the results when a breakpoint of 2.4 is used.

The standard error of every metabolite measured is smaller for those in the alcoholic group than for those in the control group (Figure 2). This finding was expected. Therefore, the control group may be expected to contain both people who are predisposed to becoming alcoholic as well as those who are not predisposed to becoming alcoholic.

The data for 5-HIAA, IAA, and AA were as expected and were consistent with findings reported in the literature. The literature indicated that the amount of 5-HIAA excreted by the members of the alcoholic group should be about one-half that of the control group (Olson et al., 1960). It was about one-half of that of the control group in the current study. The literature indicated no significant differences in the other pathways of tryptophan metabolism (Olson et al., 1960; Akhter et al., 1978; Ballenger et al., 1979; Banki, 1980; Friedman et al., 1984). There was no significant difference, at the 5% level of significance, in the amount of IAA or AA excreted by the alcoholic or the control groups. However, the difference in IAA levels did

approach significance.

The results of the 5-HIAA/IAA test were compared to those of the 5-HIAA/AA test in order to see if the two tests were identifying the same individuals as having an impaired serotonin metabolic pathway (Figure 7). There was a high degree of concurrence between the two tests for the alcoholic group. Fourteen out of sixteen or 87.5% of the alcoholic subjects were identified by both tests as having an impaired serotonin metabolic pathway. Two of the subjects were identified as having an impaired serotonin metabolic pathway by one test but not by the other. Every member of the alcoholic group was identified as having an impaired pathway by at least one test. No one in the alcoholic group was identified as not having an impaired pathway by both tests.

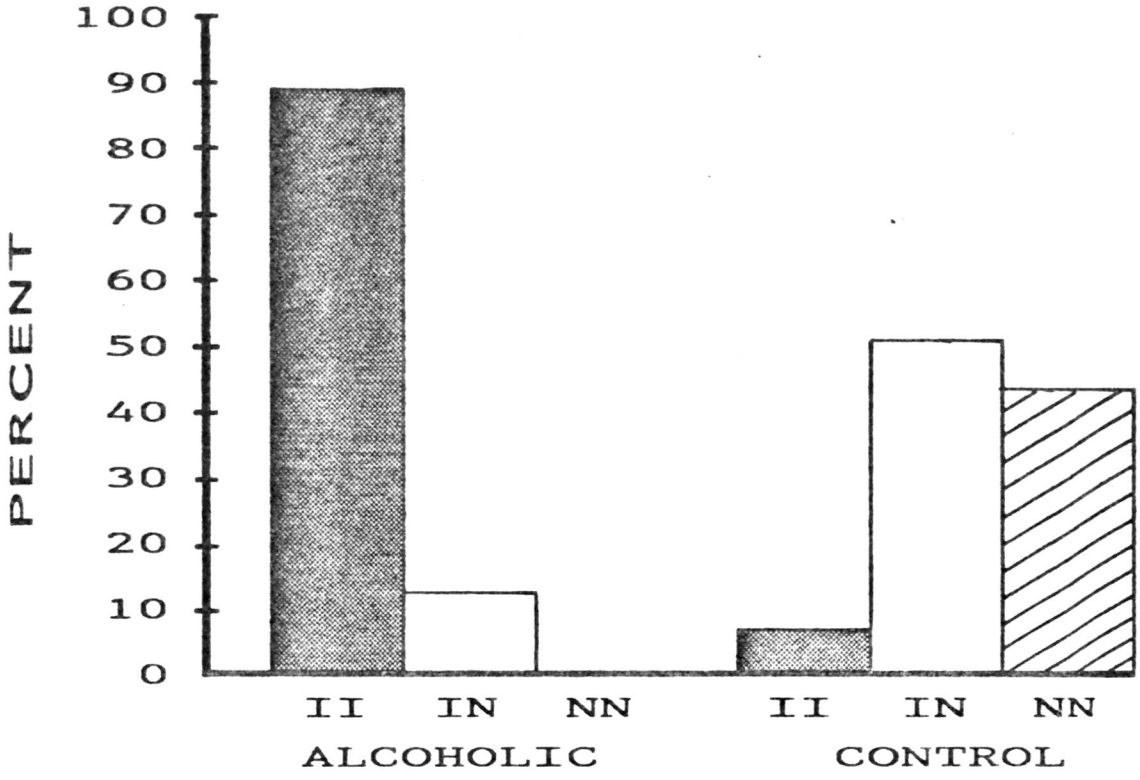
There was a large amount of variability identified in the control group. There was only 50% concurrence between the tests on the control group. One individual in the control group was identified by both groups as having an impaired serotonin metabolic pathway. There was disagreement between the two tests on seven out of the fourteen control group members (50%). Six of the control group members were identified by both tests as not having impaired serotonin metabolic pathways.

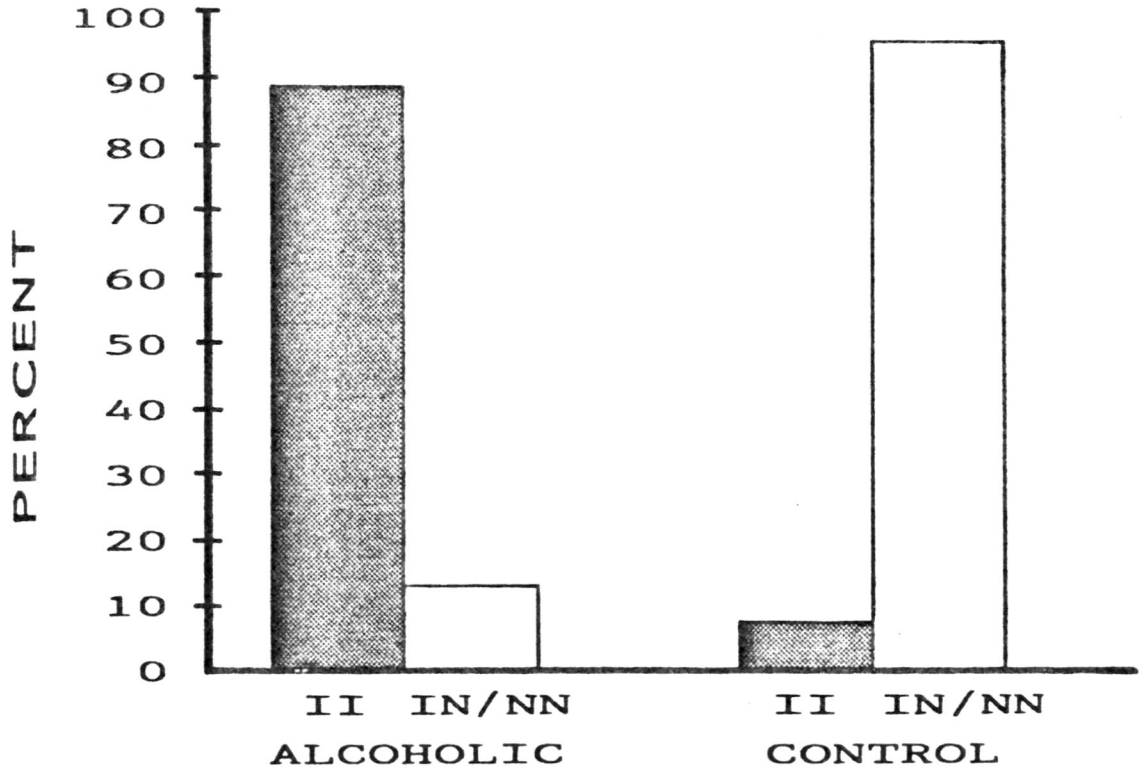
FIGURE 7

A comparison showing the percentage of subjects in both the alcoholic and control groups in which there is agreement or disagreement as to classification. II indicates that both tests indicated an impaired serotonin metabolic pathway. IN indicates that one test indicated an impaired pathway and the other did not. NN indicates that both tests indicated no impairment of the serotonin metabolic pathway. Note the high percentage of people in the alcoholic group that both tests identified as having an impaired serotonin metabolic pathway.

FIGURE 8

A comparison showing the differences between the alcoholic and control groups when the findings of the 5-HIAA/IAA test and the 5-HIAA/AA test are combined in a manner that indicates an impaired serotonin metabolic pathway if both tests agree that there is an impairment and that indicates no impairment if at least one test indicates no impairment.


100
90
80
70
60
50
40
30
20
10
0
PERCENT
II
IN
NN
II
IN
NN
ALCOHOLIC
CONTROL


100
90
80
70
60
50
40
30
20
10
0
PERCENT
II
IN/NN
II
IN/NN
ALCOHOLIC
CONTROL

Superior results in differentiating between the alcoholic group and the control group were obtained when the results of the 5-HIAA/IAA test were combined with those of the 5-HIAA/AA test (Figure 8). Combining the tests in this manner seemed to eliminate most of the readings that were felt to be falsely positive for an impaired serotonin metabolism. A subject was considered to have an impaired serotonin pathway only if both the 5-HIAA/IAA and the 5-HIAA/AA test indicated an impaired pathway and considered to have no impairment of the pathway if at least one of the tests indicated no impairment. Results showed that 87.5% of the alcoholic group was identified as having an impaired serotonin pathway and 12.5% of the alcoholic group was identified as not having an impaired pathway. In the control group, 93% were identified as not having an impaired pathway and only 7% were identified as having an impaired pathway.

This test can be used as a routine medical screening test much as testing the urine for glucose is now used. It is thought that this test could identify people who are at high risk of becoming alcoholic before they become alcoholic. This would allow for early medical intervention. The test also suggests a new method for treating people with alcohol problems. It suggests that treating these people with antidepressants that are serotonin reuptake inhibitors or with l-tryptophan might

help them remain off of alcohol or to lessen their drinking. It is thought that this use of the test as a routine medical screening test could save many lives, prevent many injuries, and prevent many careers from being ruined.

Another use of the test that has the potential for saving lives, preventing injuries, and reducing property damage, is for the states to use the test as a basis for granting drivers licences. Everyone wanting a drivers licence would be required to be tested. The people that the test identified as having an impaired serotonin pathway would be required to submit medical evidence each year indicating that they are not alcoholic in order to continue having a driver's licence. This is the same procedure that people with epilepsy currently use to obtain a driver's licence. This use would be expected to remove many alcoholics from the roads that are now driving and would be expected to greatly reduce the number of traffic accidents.

Insurance companies could use the results from this test to identify people who are at high risk of injury or disease due to alcoholism. Identifying these high risk people would allow the insurance companies to take measures to reduce losses due to excessive claims.

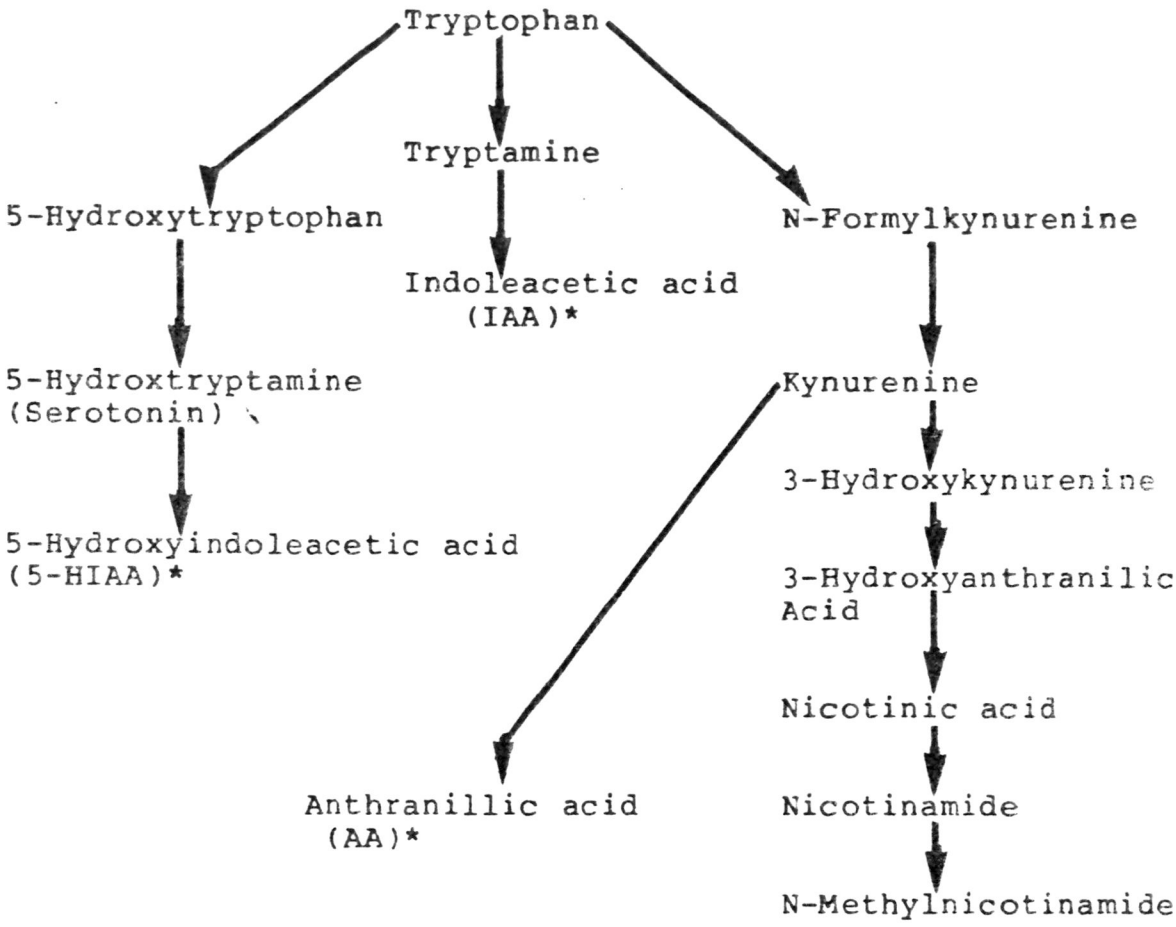
Employers could use the test to reduce absenteeism and to increase productivity. Employees could be required to

be tested as a requirement for employment. Those who were identified as having an impaired serotonin pathway could receive special help to insure that they did not have excessive absences due to "sickness" and to insure that they did not come to work too hungover to properly perform their jobs.

In conclusion, it is obvious that a test which can identify people who are biologically predisposed toward alcoholism by analyzing one random urine sample can be of great benefit to the medical profession. All that is needed is for the test to be validated through further investigations and reduced to clinical practice. The purpose of this investigation was to indicate that it is possible for such a test to be developed.

APPENDIX A

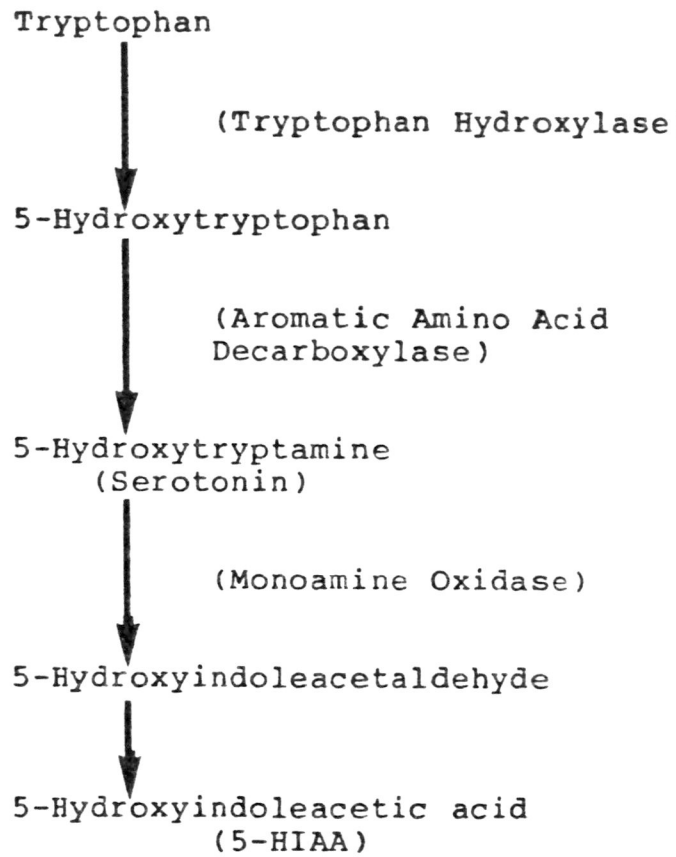
METABOLISM OF TRYPTOPHAN



* Indicates the metabolites that were measured.

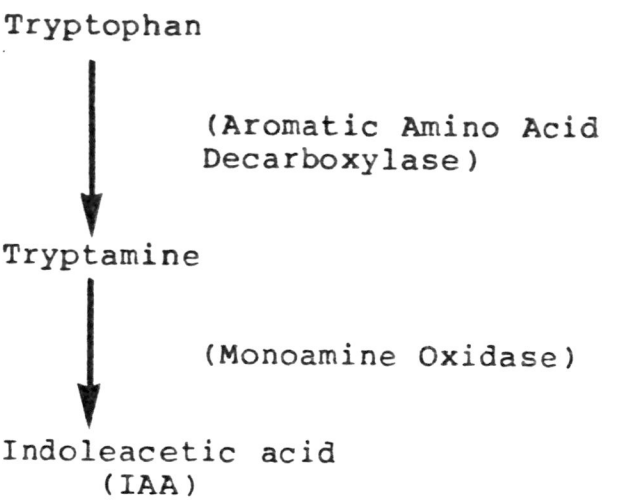
APPENDIX B

SEROTONIN METABOLIC PATHWAY



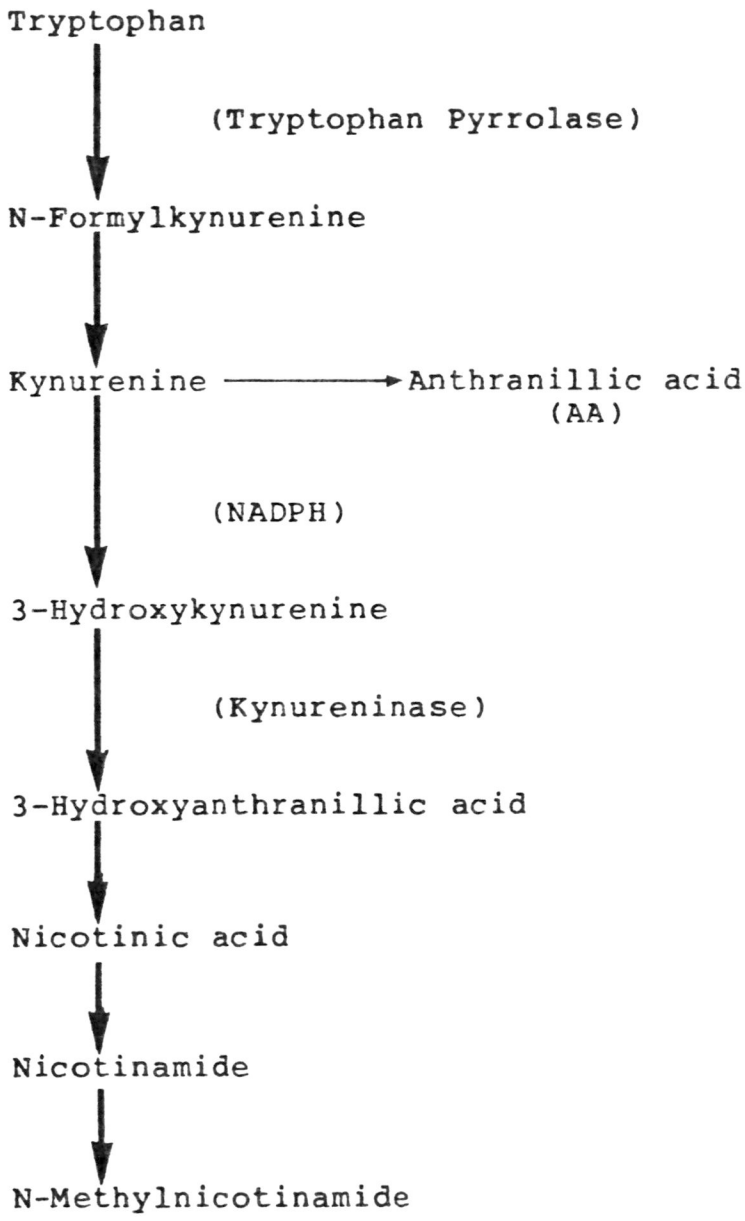
APPENDIX C

TRYPTAMINE METABOLIC PATHWAY



APPENDIX D

KYNURENINE METABOLIC PATHWAY



APPENDIX E

MICHIGAN ALCOHOLISM SCREENING TEST

Melvin L. Selzer, M.D.

Professor of Psychiatry
University of Michigan

Score Points	Question	Response
0	0. Do you enjoy a drink now and then?	Y/N
2	*1. Do you feel you are a normal drinker? (By normal we mean you drink less than or as much as most other people).	Y/N
2	2. Have you ever awakened the morning after some drinking the night before and found that you could not remember a part of the evening?	Y/N
1	3. Does your wife, husband, a parent, or other near relative ever worry or complain about your drinking?	Y/N
2	*4. Can you stop drinking without a struggle after one or two drinks?	Y/N
1	5. Do you ever feel guilty about your drinking?	Y/N
2	*6. Do friends or relatives think you are a normal drinker?	Y/N
2	*7. Are you able to stop drinking when you want to?	Y/N
5	8. Have you ever attended a meeting of Alcoholics Anonymous (AA)?	Y/N
1	9. Have you gotten into physical fights when drinking?	Y/N

2	10. Has drinking ever created problems between you and your wife, husband, a parent or other near relative?	Y/N
2	11. Has your wife, husband (or other family member) ever gone to anyone for help about YOUR drinking?	Y/N
2	12. Have you ever lost friends because of your drinking?	Y/N
2	13. Have you ever gotten into trouble at work because of your drinking?	Y/N
2	14. Have you ever lost a job because of drinking?	Y/N
2	15. Have you ever neglected your obligations to your family, or your work for two or more days in a row because you were drinking?	Y/N
1	16. Do you drink before noon fairly often?	Y/N
2	17. Have you ever been told you have liver trouble? Cirrhosis?	Y/N
2	**18. After heavy drinking have you ever had delerium tremens (DT's) or severe shaking or heard voices or seen things that really weren't there?	Y/N
5	19. Have you ever gone to anyone for help about your drinking?	Y/N
5	20. Have you ever been in a hospital because of drinking?	Y/N
2	21. Have you ever been a patient in a psychiatric hospital or on a psychiatric ward of a general hospital where drinking was a part of the problem that resulted in hospitalization?	Y/N

2 22. Have you ever been seen at a psychiatric or mental health clinic or gone to any doctor, social worker or clergyman for help with any emotional problem where drinking was a part of the problem? Y/N

2 ***23. Have you ever been arrested for drunk driving, driving while intoxicated, or driving under the influence of alcoholic beverages? Y/N

(If yes, how many times? -------)

2 ***24. Have you ever been arrested, or taken into custody,even for a few hours, because of your drunken behavior? Y/N

(If yes, how many times? -------)

LEGEND

* Alcoholic (scoreable) response is a "no" response.
** 5 points for Delerium Tremens.
*** 2 points for each arrest.

SCORING

In general, five points or more would place the subject in an alcoholic catagory. Four points would be suggestive of alcoholism. Three points or less would indicate the subject was not alcoholic.

NOTE

Programs using the above scoring system find it very sensitive at the five point level and there is a tendency to find more people alcoholic than anticipated. However, it is a SCREENING test only, and should be ultra-sensitive.

APPENDIX F

DATA - ALCOHOLIC GROUP

Subject Number	Subject Age	MAST Score	5-HIAA Height	IAA Height	AA Height
1	44	63	8	29	8
2	34	49	10	36	8
3	31	36	14	21	12
4	60	38	14	25	14
5	34	94	3	13	10
6	59	55	35	26	34
7	20	8	11	12	32
8	31	65	16	20	4
9	39	53	12	40	5
10	32	74	10	12	9
11	28	53	19	18	13
12 *	51	115	4	4	N.M.
13	29	40	11	14	8
14	23	51	7	17	9
15	37	71	14	18	17
16	46	23	18	18	31

N.M. = Not measurable
* Subject excluded from 5-HIAA/AA study since AA was not measurable.

APPENDIX G

DATA - CONTROL GROUP

Subject Number	Subject Age	MAST Score	5-HIAA Height	IAA Height	AA Height
18	37	0	23	44	6
19	25	0	40	32	28
20 *	35	0	O.S.	40	6
21	28	0	28	12	2
22 **	45	22	56	18	10
23 ***	57	2	21	33	15
24	52	0	19	29	7
25	43	5	24	13	28
26	27	0	32	54	48
27 **	35	30	17	7	6
28	43	0	26	16	4
29	44	0	18	8	17
30	41	0	28	23	12
31	61	2	41	20	16
32	33	0	98	30	19
33	38	0	71	104	28
34	19	4	26	17	2
35	36	2	135	96	9
36 *	44	0	O.S.	10	15

O.S. = Off Scale. * = Excluded from study since 5-HIAA was off scale. ** = Excluded from study due to MAST score >10. *** = Excluded from study due to taking antidepressant.

APPENDIX H

CALCULATED DATA - ALCOHOLIC GROUP

Subject Number	5-HIAA/IAA Ratio	5-HIAA/AA Ratio
1	.2759	1.0000
2	.2778	1.2500
3	.6667	1.1667
4	.5600	1.0000
5	.2308	.3000
6	1.3462	1.0294
7	.9167	.3438
8	.8000	4.0000
9	.3000	2.4000
10	.8333	1.1111
11	1.0556	1.4615
12 *	1.0000	N.C.
13	.7857	1.3750
14	.4118	.7778
15	.7778	.8235
16	1.0000	.5806

N.C. = Not calculable
* = Subject excluded from 5-HIAA/AA study since 5-HIAA/AA ratio was not calculable.

APPENDIX I

CALCULATED DATA - CONTROL GROUP

Subject Number	5-HIAA/IAA Ratio	5-HIAA/AA Ratio
18	.5227	3.8333
19	1.2500	1.4286
20 *	N.C.	N.C.
21	2.3333	14.0000
22 **	3.1111	5.6000
23 ***	.6364	1.4000
24	.6552	2.7143
25	1.8462	.8571
26	.5926	.6667
27 **	2.4286	2.8333
28	1.6250	6.5000
29	2.2500	1.0588
30	1.2174	2.3333
31	2.0500	2.5625
32	3.2667	5.1579
33	.6827	2.5357
34	1.5294	13.0000
35	1.4063	15.0000
36 *	N.C.	N.C.

N.C. = Not calculable. * = Excluded from study since ratios were not calculable. ** = Excluded from study due to MAST score >10. *** = Excluded from study due to taking antidepressant medication.

APPENDIX J

ANALYSIS OF VARIANCE

SUMMARY TABLE (5-HIAA/IAA)

SOURCE	SS	DF	MS
TOTAL	14.7244	1	
BETWEEN	4.94556	1	4.94556
WITHIN	9.77885	28	.349245

F-RATIO = 14.1607

DEGREES OF FREEDOM = 1 & 28

PROBABILITY OF CHANCE = 0.001

GROUP STATISTICS

GROUP	N	MEAN	S.D.
ALCOHOLIC	16	.702394	.332073
CONTROL	14	1.51624	.790558

APPENDIX K

ANALYSIS OF VARIANCE

SUMMARY TABLE (5-HIAA/AA)

SOURCE	SS	DF	MS
TOTAL	455.851	1	
BETWEEN	106.950	1	106.950
WITHIN	348.901	27	12.9223

F-RATIO = 8.27646

DEGREES OF FREEDOM = 1 & 27

PROBABILITY OF CHANCE = 0.008

GROUP STATISTICS

GROUP	N	MEAN	S.D.
ALCOHOLIC	15	1.27463	.921158
CONTROL	14	5.11773	5.09163

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