D-GLUCARIC ACID EXCRETION: ITS POSITIVE ASSOCIATION WITH GENDER, TOBACCO CAFFEINE, MARIJUANA, AND VEGETARIANISM IN HUMANS

by

Elizabeth Ellen Kyle

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Human Nutrition and Foods

APPROVED:

Phyllis B. Bowen, Chairperson

Julein M. Axelson

Richard E. Ebel

July, 1982
Blacksburg, Virginia

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the following people for their help in the completion of my thesis work:

To Dr. Phyllis E. Bowen, my advisor, for all the outstanding guidance and support she has given me;

To Dr. J. Axelson and Dr. R. Ebel for serving on my Graduate Committee;

To Leslie Reynolds for her assistance with the creatinine analysis;

To Dr. R. Schulman for his help with all the statistical procedures performed;

To Hardy Mason for typing this thesis and all the love and patience he has shown me during this time;

And to my parents for their encouragement and support of my work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION AND OBJECTIVES

Humans are continuously exposed to foreign compounds, broadly defined as xenobiotics, from dietary and environmental sources which may pose adverse health effects. The intensity and duration of the effects of xenobiotics, especially many drugs, depends on the rate of enzymatic degradation by the host. The hepatic mixed-function oxidase system, referred to as the MFO system, is primarily responsible for drug deactivation (1). This drug metabolizing system can metabolically inactivate the parent compound by reducing its initial activity and convert lipophilic substances into polar derivatives which are soluble in water and can be eliminated by the kidney(2).

There are more than 200 drugs, carcinogens, and other chemicals which have been shown to affect drug-metabolizing enzymes in animals and man. Many <u>in vivo</u> studies have shown that induction of the MFO system, resulting in an increase in enzyme activity, helps protect against challenges by harmful chemical carcinogens including benzopyrene (3,4) and aflatoxin (4,5), as well as some environmental toxins such as pesticide residues (3). MFO induction also alters drug metabolism, so that monitoring these changes could be pharmacologically important in drug therapy.

Activity of drug metabolizing enzymes varies in response to changes in the quality and quantity of dietary protein (6,7), carbohydrates (8,9,10), lipid (11-13), and fiber (14). Small amounts of xenobiotics such as the food additives, BHA and BHT (4,15), natural plant indoles found in the cruciferous (or cabbage) family (4,5,15), and the polycyclic hydrocarbons in charcoal-broiled beef (16-18) have all been reported to induce the MFO system. Therefore, diet can affect the body's ability to metabolize ingested drugs and other foreign compounds. Other factors which affect the MFO system include age, sex, genetic makeup, and hormonal status (19-21).

It is known that a large variety of xenobiotics are capable of stimulating carbohydrate metabolism via the D-glucuronic acid pathway (22,23). This results in an enhanced excretion of the urinary metabolites L-ascorbic acid in rats and D-glucaric acid (D-GA) in man and the guinea pig (23-32). It has been suggested therefore that measuring D-GA excreted in urine may provide an index of hepatic MFO induction. To date, numerous studies have found a strong, positive correlation between MFO induction and the stimulation of urinary D-GA excretion in both man and animals (24,30-37).

The influence of enzyme induction on human drug metabolism has been widely studied, yet little is known about the extent to which the substances consumed on a daily basis in our diet cause changes in D-GA excretion. Chronic treatment with compounds, such as alcohol (38-42) and marijuana (43-45), can alter ones tolerance to the chemical, accelerating their metabolism and decreasing their effects. Cigarette smoke (46-51), caffeine consumption (52-56), and vegetarian dietary habits (57-59) can also alter a patient's metabolic response to a drug.

Therefore, enzyme induction - resulting from ingestion of certain dietary constituents - may seriously affect the performance of therapeutic agents. Most of the work on enzyme induction has been carried out in laboratory animals, due to the need for tissue biopsies or timed blood samples. However, the results of animal studies may not be valid for man since the inducing effect of a compound differs from species to species (60). By analyzing for urinary D-GA as an indirect monitor of hepatic MFO induction, this survey study was designed to assess the effect of various dietary components and common habits involving substance use on the excretion of D-GA in humans.

The main objectives of this study were:

1. to scan a large sample of the population to determine whether correlations exist between the amount of D-GA excreted and the exposure to the substances tobacco, marijuana, caffeine, and alcohol, and to vegetarian diets, and to ascertain the relative importance of each;

2. to determine whether there is a difference in urinary D-GA excretion between males and females after body size adjustments; and

3. to determine the normal excretion of D-GA for a free-living population sample with a wide variety of dietary habits.

If D-GA excretion is responsive to these dietary factors, then it may be considered a sensitive indication of induction, and further research to characterize the connection between D-GA and the MFO system would be warranted.

Chapter II

REVIEW OF LITERATURE

2.1 THE METABOLISM OF XENOBIOTICS

Man is continually exposed to a large variety of harmful exogenous chemicals through his environment and diet (61). The body's principal defense against all these xenobiotics is metabolism to less toxic forms which can be eliminated from the body. It is well established that the hepatic endoplasmic reticulum contains a group of nonspecific enzymes, termed microsomal drug metabolizing enzymes, which are responsible for the biotransformation of a large number of chemicals including drugs, pesticides, carcinogens, herbicides, food additives, and dietary constituents (1,2,22). Metabolism occurs in two phases. Phase I metabolism catalyzed by enzymes of the cytochrome P-450 mixed function oxidase (MFO) complex transforms a lipophilic molecule into a more polar form. This enzyme system is predominantly found in the smooth endoplasmic reticulum of the liver, but it has also been measured in kidney, lung, brain, intestine, and skin (1,2). Phase II metabolism adds an endogenous acceptor onto the newly exposed functional groups of the Phase I metabolites, making them more water soluble and hence more readily excreted from the body. The Phase II reactions use such endogenous acceptors as sulfate,

glutathione, glycine, and other amino acids. The most important reaction involves the carbohydrate D-glucuronic acid (3). The glucuronic acid pathway is a system of enzymes which facilitate the conversion of a hexose to UDP-glucose, then to UDP-glucuronic acid and D-glucuronic acid (see Figure 1). The final end products, besides xenobiotic glucuronides, include L-ascorbic acid in rats, D-glucaric acid in man and guinea pigs, and L-xylulose in patients with pentosuria (4,24). Both Phase I and II reactions will be referred to as the MFO system, although MFO more accurately refers only to cytochrome P-450 dependent reactions.

Many of the xenobiotics found in our environment are known to stimulate the activity of hepatic microsomal MFO enzymes, a process known as induction. Enzyme induction increases the metabolism of the inducing agent, often allowing for the more rapid detoxification of that compound or drug (3). This effect has pharmacological importance when it causes drugs to become less effective during repeated administration. It has been shown also that induction of MFO enzymes by phenobarbital, and polycylic aromatic hydrocarbons provides protection from the carcinogenic effects of such harmful chemicals as benzo(a)pyrene, aflatoxin, and aminoazodyes, to name just a

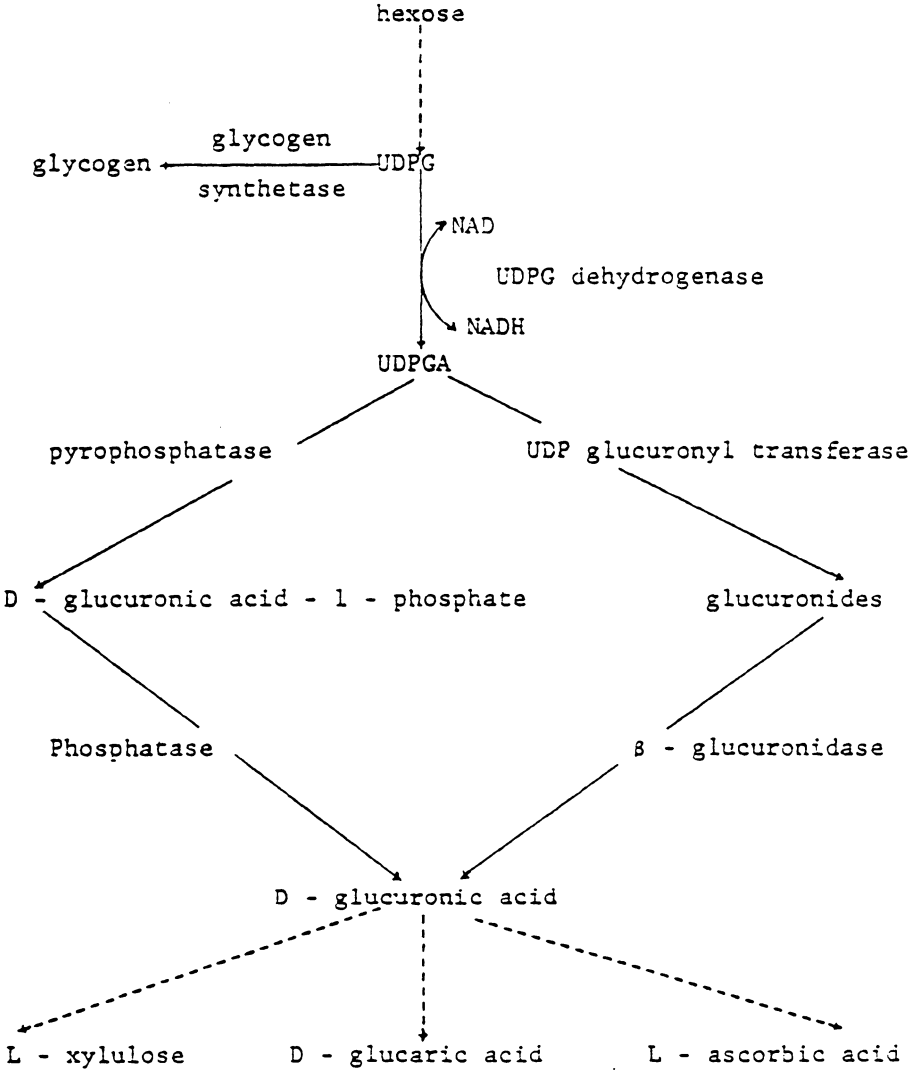


Figure 1: The Glucuronic Acid Pathway (24)

few (3,4,5). These chemicals are thought to act by stimulating the biotransformation of carcinogens to more benign metabolites which are quickly excreted.

The microsomal MFO system has the ability both to detoxify a chemical carcinogen and to activate the chemical or drug to a more toxic form. This activated form would be more hazardous to the host (60). However, data clearly showing such an increase in the carcinogenic effect *in vivo* have not yet been published (4,62). For the purpose of this study, it will be assumed that induction reflects an adaptive response of the organism to xenobiotic exposure, resulting in a protective effect.

2.2 RELATIONSHIP BETWEEN D-GA EXCRETION AND MFO SYSTEM

There have been a variety of specific tests to measure hepatic enzyme induction. The MFO system is bound to the membrane of the smooth endoplasmic reticulum and contains the hemoprotein cytochrome P-450. Direct tissue enzyme assays measuring cytochrome P-450 activity or content in man are limited by the requirement for liver biopsy samples. Since the enzyme inducing action of chemical agents differ from species to species(8,60), results from animal tests may

not be valid when applied to man. Induction, therefore, must be studied in human subjects for accurate assessment of liver enzyme induction.

Several indirect methods are considered satisfactory indices of the activity of drug metabolizing enzymes. These include measuring the concentration of plasma gamma-glutamyl transpeptidase activity (63,64), serum drug disappearance rates (38,65,66), and urinary excretion of hepatic metabolites such as 6-beta-hydroxycortisol and D-glucaric acid (30-37,64,67). Drug disappearance tests, the most common one using antipyrine clearance kinetics, are probably the most frequently used method. However, this type of test is not practical when surveying large segments of a population due to the need for repeated, timed blood or saliva samples.

There is evidence that certain drugs, capable of MFO induction, have a stimulating effect on carbohydrate metabolism via the glucuronic acid pathway (22,23), resulting in an enhanced excretion of urinary metabolites. L-ascorbic acid is one of the end metabolites of the glucuronic acid pathway in rats, and has been used as an index of enzyme induction (28,29,68). Man and guinea pigs, unable to synthesize L-ascorbic acid, excrete D-GA (24,69).

There is growing support that the measurement of D-GA excreted in urine is one of the more reliable indirect tests for assessing the specific induction of xenobiotic metabolizing enzymes. D-GA excretion in man has been shown to increase in a dose responsive fashion to a large array of drugs and chemical challenges which are known potent inducers of the MFO system (31,37). Sotanieni et al. (32) found that the amount of D-GA excreted by 41 hospitalized patients was dependent on the type of drug administered. Such drugs as barbiturates, antihistamines, and cortisone had an inducing effect, and when given to patients increased their urinary D-GA above that of other patients.

A significant correlation exists between hepatic cytochrome P-450 content and urinary excretion of D-GA in guinea pigs and man. Hunter et al. (24) showed a positive correlation between D-GA excreted in 24 hours and hepatic cytochrome P-450 content in 20 guinea pigs treated with phenobarbital. Lecamwasam et al. (35) were able to obtain liver biopsy samples on 10 patients with Hodgkins disease before and after preoperative treatment with phenobarbital. When results were compared between treated and untreated patients, there was a significant increase in cytochrome P-450, microsomal protein content, and D-GA excretion as a result of administering phenobarbital for seven days.

There also exists a positive correlation between increased serum antipyrine clearance and D-GA excretion (37,65,66). Researchers have presented convincing evidence that D-glucaric acid, a normal constituent of human urine (69), is a useful indication of hepatic MFO enzyme activity, possibly reflecting the exposure to certain xenobiotics.

Although there seems to exist some correlation between hepatic xenobiotic-enzymes and the excretion of D-glucuronic acid metabolites, the relationship is an indirect one since the MFO enzymes represent Phase I reactions and the d-glucuronic acid pathway enzymes comprise part of Phase II conjugating reactions. Even though D-GA excretion is very responsive to varying doses of drugs such as phenobarbital, antihistamines, cortisone, and other barbiturates the mechanism by which these drugs enhance D-GA excretion in humans is still unknown.

Several hypotheses have been proposed to explain the correlation between cytochrome P-450 induction and D-GA excretion. Three enzymes, uridine diphosphoglucose dehydrogenase, glucuronolactone dehydrogenase, and D-glucurone-lactone hydrolase, all involved in D-GA synthesis (25,31), are induced by phenobarbital. Notten and Henderson (28) suggest that stimulation of D-glucuronic acid

excretion is based on an increased availability of UDP glucose in the liver, which in turn is the result of an inhibition of glycogen synthesis. Yet another theory maintains that inducing agents may be related by a feedback mechanism to the activity of the MFO system via NADPH production in the pentose-phosphate cycle, since NADPH is a cofactor for cytochrome P-450 reductase (62).

2.3 EFFECTS OF THE VARIABLES EXAMINED ON VARIOUS PARAMETERS USED TO ASSESS MFO ACTIVITY

Even though the microsomal cytochrome P-450 system is actually involved only in Phase I reactions, its activity is often used as an indication of the entire xenobiotic metabolism. Compounds which induce the cytochrome P-450 system include nondietary substances such as organochlorine pesticides (70), a wide range of anticonvulsant drugs (31,67), and polycyclic aromatic hydrocarbons found in tobacco smoke (46,48,54). Common dietary constituents in foods, such as caffeine (52-56), indoles (4,5,15), and the food additives BHA and BHT (4,15) have all been implicated in enhancing drug metabolizing enzymes. The activity of MFO enzymes has also varied in response to changes in the quality and quantity of dietary protein (6,7), carbohydrate (8-10), lipid (11-13), and fiber (14). Therefore, nutritional status can affect changes in the body's ability to metabolize ingested drugs and other foreign compounds.

2.3.1 Tobacco

Cigarette smoke is recognized as a causative factor in diseases such as lung cancer, emphysema, and bronchitis. Chronic cigarette smokers are exposed to high levels of the habit-forming drug nicotine, as well as a wide variety of polycyclic hydrocarbons, including the carcinogens 3,4-benzo(a)pyrene and benz(a)anthracene (3,17). In view of the prevalence of the tobacco habit in our society, it seems pertinent to investigate what effects exposure to tobacco smoke has on the activity of drug-metabolizing enzymes.

One established group of hepatic MFO inducers includes the polycyclic hydrocarbons, 3-methylcholanthrene and benzo(a)pyrene. These compounds increase certain drug metabolizing enzymes in both laboratory animals and man. Studies in pregnant rats show that benzo(a)pyrene hydroxylase, responsible for the hydroxylation of benzo(a)pyrene, was significantly increased in maternal liver, lung , and intestine, as well as in the placenta of rats exposed to cigarette smoke (17,47,51).

Induction of these enzymes results in enhanced metabolism of numerous drugs *in vivo*. Since the metabolism of phenacetin occurs by the polycyclic hydrocarbon inducible enzymes, Pantuck *et al.* (51) examined the metabolism of

phenacetin in smokers and nonsmokers. They found a 20% lower mean plasma concentration of phenacetin after five hours of oral administration of the drug in 9 smokers as compared to 9 nonsmokers.

Other drugs, metabolized by the hepatic MFO system, have also been examined in smokers. Theophylline is metabolized via the N-demethylation pathway (48). Grygiel and Birkett (48) reported that the mean plasma theophylline concentration was lower and the plasma theophylline clearance was 78% higher in the group smoking more than 20 cigarettes per day. The mean plasma theophylline concentration in smokers was 4.8 mg/l, significantly less than 8.1 mg/l in the nonsmokers during the first 8 hours after an oral morning dose of 125 mg.

In man, 30-40% of administered antipyrine is hydroxylated to 4-hydroxy-antipyrine and conjugated with glucuronic acid (56,71). Vestal et al. (56) reported that antipyrine clearances were faster in moderate and heavy cigarette smokers than in the nonsmokers. All these investigations assume that faster clearance of the drug reflects greater induction of the metabolizing enzymes.

People who smoke one package of cigarettes per day absorb approximately one mg nicotine/kilogram body weight/day (49).

However, the effect of nicotine and its metabolites is less well studied than that of polycyclic hydrocarbons. Beckett and Triggs (50) concluded that the metabolism of nicotine was greater in smokers than nonsmokers on the basis that the amount of unchanged nicotine excreted in urine was less in the smokers. Ruddon and Cohen (49) have noted a 30-50% increase in protein synthesis by liver microsomes, corresponding to faster metabolism of nicotine and other drugs in rats after five weeks of chronically administering nicotine in their drinking water. Mitra et al. (47), using tobacco leaf extracts orally administered to rats, also observed an increased activity of hepatic enzymes, including B-glucuronidase, microsomal N-demethylase and benzpyrene hydroxylase. This induction was evident in as little as seven days, and persisted even after 62 days of treatment. They hypothesized that "modification of drug-metabolizing and lysosomal enzyme activity, as observed from the results of the present study, may in part be responsible for the toxic manifestations and carcinogenesis associated with the use of tobacco" (47). Nicotine seems to be a weaker inducer than polycyclic aromatic hydrocarbons. The combination of these two substances found in cigarette smoke may result in a significant increase in microsomal drug metabolizing enzymes in man.

Contrary to most of the literature dealing with the inductive effect of tobacco smoke, Fiedler *et al.* (72), performed the only study examining D-glucaric acid excretion as an index of hepatic microsomal enzyme induction and found no significant difference between 28 smokers and nonsmokers. In view of this discrepancy, the association between tobacco use and D-GA excretion will be re-examined.

2.3.2 Marijuana

Marijuana usage has become prevalent in our society, and the effects of long-term smoking on the disposition of both therapeutic and social drugs is under investigation. In most studies with delta^9-tetrahydrocannabinol (THC), the active compound present in marijuana extracts, it appears that the cannabinoids in the residue, including THC, cannabinol (CBN), and cannabidol (CBD) are metabolized by liver microsomal monooxygenase systems (73-76). However, the literature appears to be almost equally split in deciding whether marijuana induces or inhibits MFO activity.

Kupfer *et al.* (75) reported that the intraperitoneal or oral administration of delta^1-THC for 3 to 9 days did not affect the activity of *in vitro* microsomal N-demethylation of aminopyrine or the oxidative metabolism of ^{14}C-delta^1-THC. These were the only researchers to find no effect of THC on hepatic microsomal enzymes.

Some drug tolerance data indirectly supports the hypothesis that THC stimulates hepatic drug metabolizing enzymes. Drug tolerance is defined as a state of diminishing responsiveness to the pharmacological effect of a drug as a consequence of prior exposure to that drug (44). Drug tolerance usually results from induction of enzymes responsible for the metabolism of that drug, either by increasing the amount or activity of the enzymes. McMillan et al. (77) demonstrated in pigeons that a dose of 1.8mg/kg of delta^9-THC completely disrupted a behavioral task involving a food reward. With successive daily doses the birds' performance returnedby day 6, to what it was before the onset of delta^9-THC administration.

Tolerance indicative of induction is more difficult to measure in man. In 1971, Lemberger et al. (45) reported that five chronic marijuana smokers metabolized ^{14}C-delta^9-THC faster than nonusers. The smokers plasma half-life was 28 hours compare to 57.5 hours in nonsmokers. These findings suggest that hepatic microsomal enzymes are induced after chronic usage.

Theophylline clearance is often used as an indirect measure of hepatic metabolism rates since only 10% of a dose is excreted by the kidneys unchanged. Jusko et al. (43)

found that chronic use of marijuana was associated with an increased theophylline clearance. However, all the marijuana users were also tobacco smokers. Both cigarette and marijuana smoke contain small amounts of polycyclic hydrocarbons (43,78). Since cigarette smoke also hastens theophylline metabolism (43,46,48), it remains to be determined whether induction occurs as a result of the common constituents in marijuana and tobacco smoke, or some other chemical solely in the marijuana.

Recently, it has been hypothesized that marijuana and its metabolites can cause variable effects in animal systems due to both induction and inhibition of drug metabolizing enzymes, depending on the amounts of THC, CBD and CBN present. Siemens *et al.* (79) examined the effects of two cannabis extracts of different cannaboid compositions on pentobarbital metabolism. Extract I, containing high CBN and CBD residues relative to THC prolonged the pentobarbital sleeping time up to 50% in the rat. Extract II, with lower proportions of CBN and CBD to THC did not have any significant effect on pentobarbital metabolism or sleeping time. Each component added alone to an *in vitro* microsomal preparation inhibited pentobarbital metabolism. These results are similar to other studies in which cannaboids were foun both to inhibit antipyrine demethylation (74) and to prolong serum antipyrine half lives (76,80,81).

No studies were found examining the association between D-GA and marijuana usage.

2.3.3 Caffeine

Caffeine is one of the most widely ingested natural alkaloids, identified as 1,3,7-trimethylxanthene. In a 1972 GRAS survey, 82% of the individuals surveyed were consuming a mean intake of 186mg/day (82). With such high intakes of the drug, surprisingly little research has been done to investigate caffeine's effects on hepatic drug metabolizing enzymes.

It has been observed that caffeine is eliminated very slowly in patients with liver failure (83), indicating the liver is a primary organ in caffeine metabolism. The human newborn is deficient in the ability to oxidatively metabolize a variety of drugs. Researchers in early studies suggested that one of the multiple forms of cytochrome P-450 is involved in caffeine metabolism since the half-life of caffeine in newborns is 90 hours, a 20-fold increase over the 4 hour half-life of caffeine in adults (53).

By using the well-established inductive effect on the MFO system of phenobarbital and benzopyrene, Welch et al. (54) demonstrated that rats, treated with as little as 1.0mg/kg

pentobarbital for 3 days, had an elevated plasma clearance of caffeine. Plasma elimination of caffeine was also accelerated after treatment with benzo(a)pyrene and the polychlorinated biphenyl (PCB) aroclor-1254. He suggested that the metabolic and pharmacologic effects of caffeine may be considerably altered in humans exposed to benzpyrene, polycyclic aromatic hydrocarbons, or PCB's, compounds commonly found in today's environment. Other studies support the concept that caffeine stimulates drug metabolism in rats, since treatment with caffeine increased the rate of zoxazolamine (55) and antipyrine (55,56) metabolism. Not surprisingly, cigarette smokers also have a faster caffeine clearance (155±16mg/KG/hr) than nonsmokers (94±10mg/KG/hr) (52).

No studies were found in the literature dealing with caffeine intake and D-GA excretion.

2.3.4 Alcohol

Alcohol is a common dietary constituent in a large segment of our population, yet the effects of alcohol on drug metabolism does not appear to be well defined. It seems to be common knowledge that chronic alcoholics adapt to the higher level of alcohol in their diet and when sober have a greater tolerance to alcohol and various drugs,

especially seditives and barbiturates (39,40,42,84). Acute binges on alcohol, however, seem to create a greater sensitivity to the same drugs while in the intoxicated state (85). Further confusion results from investigations reporting different effects of the same drug *in vivo* and *in vitro*, as well as differences observed between rat and human models.

Chronic alcohol consumption alters the hepatic smooth endoplasmic reticulum in a way that results in a modified metabolism of drugs administered while alcohol is present in the body (40,41,86). As early as 1968, Lieber and Rubin (87) found an increase in and proliferation of smooth endoplasmic reticulum in hepatocytes after feeding alcohol in the diet of laboratory rats for three days. This increase in endoplasmic reticulum accompanies an elevated level of cytochrome P-450. Kalant *et al.* (41) found that chronic treatment with either phenobarbital or ethanol resulted in an increase in liver size relative to body weight, an increase in total cytochrome P-450 content and elevated rates of drug metabolism *in vitro*. Antipyrine (88), meprobamate (40), and tolbutamide (42) all have decreased plasma half lives when alcohol is administered. Chronic alcoholics who consumed more then 200g alcohol/day had a 2.25 fold faster clearance of tolbutamide from their

blood than the control group (42), indicating an induction of hepatic MFO enzymes.

Not all studies support this inducing effect of alcohol. There have been a few investigations which report no effect on serum drug disappearance rates in man and animals (89,90). In one such study, eight volunteers who maintained a blood alcohol concentration of approximately 20 mmoles/l for 12 hours had no difference in the half-life or clearance of antipyrine in their saliva 2 days before, during, or 2 days after treatment (90). From what we know this study is too short to see an inducing effect if they were alcohol naive to begin with.

In contrast to the studies dealing with chronic alcohol usage, acute alcohol intoxification seems to increase the body's sensitivity to drugs metabolized by the MFO system. Rubin et al. (85) found that acute alcohol ingestion significantly increased the plasma half-life of pentobarbital in 200 alcoholic rats from 70 minutes to 150 minutes. A similar two-fold increase was observed in four humans consuming alcohol (85). Thus it appears that acute alcohol consumption competes with other compounds for hepatic drug metabolism.

Mezey (38) investigated D-glucaric acid excretion among seven chronic alcoholics. Immediately after a drinking binge, when all the alcoholics were drinking in excess of 250gm alcohol/day for one or more weeks, their urinary excretion of D-GA rose to a mean of 58.7 micromoles/day, compared to the mean of 18.2 micromoles/day in controls. Eight days later, after the discontinuation of alcohol, their mean D-GA level fell back to within the normal range (18.5 micromoles/day). Clearly, this demonstrates that actively drinking alcoholics do have elevated D-GA excretion, indicative of MFO enzyme induction.

Since ethanol seems to substantially modify the disposition of certain drugs, those drugs with narrow margins of safety should require dose adjustments when administered to chronic alcohol abusers. Alcohol, being regularly consumed by a large segment of the population, even in moderate doses, should not be overlooked when assessing dietary effects on drug metabolizing enzymes. Therefore, alcohol consumption will be included as a variable possibly associated with D-GA excretion in this study.

2.3.5 Vegetarianism

It is increasingly evident that induction of drug metabolizing enzymes can be greatly influenced by dietary factors. Recently, populations which have different protein consumption patterns have been examined to see if the source of dietary protein affects the body's ability to metabolize ingested drugs. There have been a few studies which focused on antipyrine clearance in vegetarians and nonvegetarians. Fraser et al. (58) assessed the saliva antipyrine levels in 131 Londoners (73 of whom were nonvegetarians, 49 of whom were Asian vegetarians). He found that the vegetarians had a significantly prolonged antipyrine half-life of 16.2 hours compared to the nonvegetarians (10.7 hr). Fraser also reported the antipyrine half-life in 49 Gambians as being 13.6 hr., which is slightly longer than the Caucasian nonvegetarians but shorter than the Asian vegetarians. The Gambian population consumes a large quantity of cola nuts, very high in caffeine. Caffeine, an MFO inducer, could have offset the apparent suppressing effect of the vegetarian diet and decreased the antipyrine half-life. Mucklow et al. (57) support Fraser's work and found that the antipyrine clearance was faster in 20 Asian meat eaters (0.91mg/KG/min) than the 16 Asian lacto-ovo-vegetarians (0.54mg/KG/min). However, dietary protein differed significantly in amount

between the two groups, 77.8gm/day versus 52gm/day, respectively.

Brodie et al. (59), on the other hand, demonstrated that serum antipyrine clearances, as well as paracetamol and phenacetin metabolism in nine white vegetarians, although slightly lower, did not differ significantly from that in white or Asian nonvegetarians. Both groups had similar protein consumption levels (about 78gm/day).

The discrepancy in the drug metabolism rates in these studies remains unexplained; thus vegetarians will be assessed in this study to see if D-GA excretion differs between meat and nonred-meat consumers.

2.3.6 Gender-related Differences in D-Glucaric Acid

Endogenous hormones, such as sex steroids, undergo conjugation through the glucuronic acid pathway (68,91,92). During human pregnancy, a time of increased endogenous metabolite and hormonal generation, D-GA significantly increases from 14.4 micromoles D-GA/gm creatinine in the first trimester to 23.5 in the third (63). Progesterone appears to induce hepatic enzymes in the adult female rat in a similar manner to phenobarbital, with increased liver weight, microsomal protein content, and urinary ascorbic

acid (93). Similarly, women treated with progesterone or estrogen, the active ingredients in oral contraceptive agents, have also been shown to have elevated D-GA excretion (91-93); yet no researchers have detected any pattern of change in D-GA excretion in response to the normal hormonal fluctuations during a menstrual cycle (91,94). One study, however, only examined 7 women who collected 24 hour urine samples every 7 days (91).

In addition to large variations between women, there have been sex related differences in D-GA observed (68,94,95). Male rats excreted significantly higher levels of D-GA than female rats after exposure to the same inducer (68). Men excreted more D-GA than women in a study performed by March et al. (94). They found the mean D-GA of 48 women to be somewhat lower than for a man of the same height, corrected for body weight. Whether these different levels of induction occur because of different steroid levels or because of some other genetically related sex difference is not known.

2.4 SUMMARY

Although there is not an accepted rationale for the mechanism by which D-GA excretion is enhanced after hepatic cytochrome P-450 induction, there is increasing evidence

that a positive correlation does exist. This study is designed to investigate the effects of tobacco and marijuana smoke, vegetarianism, alcohol and caffeine, all known to alter microsomal enzyme induction and possibly D-GA excretion in man.

Chapter III

METHODOLOGY

3.1 RECRUITMENT OF SUBJECTS

This study was designed to survey a self-selected sample of the Blacksburg community population focused primarily at men. The appeal for volunteers was conducted primarily through advertisements from the university radio station, the campus newspaper, and posters within university buildings, as well as through the local newspapers and stores within Montgomery County. Door-to-door recruitment efforts were aimed at the college fraternities and Virginia Tech organizations. A free ticket to a local theater was given to participants as an added incentive to volunteer.

All willing subjects within the age group of 18 to 65 were accepted to participate in the study without any bias toward recruitment of individuals with attributes that might favor a certain level of D-GA excretion. Recruitment began in February, 1981 and continued throughout August, 1981 with a maximum of 25 subjects per week due to the limited number of urine collecting bottles available. Data were obtained on 181 men and 21 women, ranging in age from 18 to 56. Women were encouraged to participate only during the last three months of recruitment efforts. Due to the exclusion of all participants who were currently taking prescription

drugs, the final analysis dealt with 124 males and 18 females. Since there are some questions dealing with the effect of female sex hormones influencing D-GA excretion (67,91-95), all women began collecting urine on the tenth day after the beginning of their menstrual period to minimize hormonal variability between subjects.

3.2 DATA COLLECTION

3.2.1 Urine

Each participant was issued as many one-liter polyurethane bottles as they thought they needed in which to collect their urine. Each bottle contained 1ml of 6NHCl to act as a preservative. Careful written instructions concerning urine collections were given to and individually explained to each subject. All subjects collected their complete urine excretions for three consecutive days, starting each day's urine collection after the first morning voiding, not to start later than 9:00 AM. The majority of the men collected urine beginning on a Monday, continuing through the following Wednesday. The filled urine bottles were returned daily to a large walk-in refrigerator, and new bottles were then picked up for the next day's collection. At the end of each day, the total daily urine collected was mixed together and volumes recorded for each subject. A 10%

aliquot of the total composited urine was taken in duplicate with 10ml graduated pipets. These aliquots were labeled and frozen immediately. The same aliquot bottles were used for all 3 days of collection, so each subject had 2 pooled samples of sequentially layered urine representing 10% of the total 3 days complete collection. These aliquots were kept frozen at -20°C until analysis.

3.2.2 Dietary Records

Every subject was required to complete a consecutive five day record of his/her total food and beverage intake (Appendix A). The subjects were encouraged to consume their normal daily diet. No restrictions were placed on consumption patterns during the duration of the study. They began recording their food intakes two days prior to commencement of urine collecting. Most men began recording dietary records on a Saturday, and continued through the following Wednesday, so there was a weekend meal sample as well as three days of the weekday meals to account for differences in eating and drinking habits during a typical week. The participants returned the diet sheets daily with their urine in order to avoid a five-day recall situation at the end of the study. The women who participated began recording their dietary intakes on the eighth day after the

beginning of their menstrual period, and continued through the twelth day of their cycle.

3.2.3 Additional Forms

Everyone, prior to beginning the study, signed a consent form which had been approved by the Chairman of the Internal Review Board for Human Research at Virginia Tech. In addition, all the subjects were required to complete a questionnaire (Appendix C) designed to assess the typical habits of the individual. It consists of questions aimed at revealing patterns of exposure to certain variables of interest within the previous month. The following variables are suspected of causing changes in D-GA excretions and were included: exposure to tobacco products, alcohol consumption, marijuana smoking, genetic factors dealing with family cancer incidences, vegetarianism, caffeine intakes, and usage of both prescription and over-the-counter drugs. There were questions also dealing with woodsmoke and chemical exposures but these variables were not included in the analysis. In order for the subjects to be guaranteed anonymity, each generated his/her own nine digit code number which consisted of his/her day of birth, height in inches, and the first three digits of their social security number. Hopefully, this allowed for increased honesty and

reliability when answering such questions as drug and marijuana usage.

3.3 LABORATORY ANALYSIS

3.3.1 D-Glucaric Acid

D-Glucaric acid in urine was determined as described by Simmons (96). Urinary D-GA is converted into D-glucaro-1,4-lactone (D-GL) by boiling the urine for one hour at 100°C, pH 3.6. The inhibitory effect of D-GL on the enzyme beta-glucuronidase was determined by measuring free phenolphthalein liberated by the hydrolysis of phenophthalein glucuronide (Sigma). The more D-GA present in the urine, the less phenolphthalein released. There is some confusion in the literature as to the percent conversion of D-GA to D-GL. After boiling the urine, Simmons (96) claims a 100% conversion while March and others (65,68,94) report only a 23-30% conversion. Because of this discrepancy, the results from this study will be reported as D-GL and not D-GA. Specifically, the modified procedure is as follows: 5 ml aliquots (a+b) from each subject's 3 day pooled urine sample were transferred into 2 tubes, one containing 1ml of 2M formate buffer, pH 3.3 (aliquot A), and the other containing 1 ml of Tris buffer, pH 9.0 (aliquot B). The tubes were capped with marbles, boiled in a water

bath for 60 minutes, and cooled to room temperature. One milliliter of Tris buffer was added to the tube already containing formate, and 1 ml of formate buffer was added to the tube containing the alkaline Tris buffer. The volume in each tube was then brought up to 10 ml using 2M acetate buffer, pH 4.8. Three 1 ml aliquots from each tube were transferred to tubes containing 0.8 ml of phenolphthalein glucuronide, and 0.5 ml beta-glucuronidase solution (500 activity units/ml) was added to two of these. To the third, which served as a blank to correct for urine color, 3 ml of 2 M glycine buffer, pH 12.0, was added before the 0.5 ml beta-glucuronidase solution. All tubes were incubated in a water bath at 37°C. The enzymatic reaction in the first 2 tubes was stopped by adding 3 ml of glycine buffer after exactly 40 minutes of incubation. The absorbance of the solutions was measured at 555 nm on a Bausch & Lomb spectrophotometer.

The enzyme in the absence of inhibiting compounds was assayed under similar conditions except 5 ml of water was substituted for urine. A series of 8 standard solutions prepared from D-succharic acid at concentrations of 1, 10, 25, 50, 100, 250, 500, and 1000 (stock) micromoles/liter were taken through the same procedure except that they were boiled using only the formate buffer. The percentage

inhibition (%I) of beta-glucuronidase at different concentrations of D-glucurate was calculated from the equation:

I% = 100(1-Aa/Aw or Ab/Aw), where
Aa = abs of acid treated sample
Ab = abs of base treated sample
Aw = abs of water sample

The concentration of D-glucarate in the test sample was obtained by taking the difference between the apparent concentrations in the acid (a) and alkali (b) treated samples, both of which are read from the standard curve obtained by plotting percent enzyme inhibition against the various standards prepared. The alkaline tube corrects for compounds other than D-GA which inhibited beta-glucuronidase. Published data indicate that a healthy person of either sex excretes D-GA proportionally to creatinine excretion (94), since the hydroxylating activity of the liver is associated with body cell mass. The results will, therefore, be expressed as micromoles of D-GL per gram creatinine. Also by using the D-GL creatinine as the variable to analyze, and not D-GL/24 hours, one eliminates concerns with possibly incomplete urine collections.

3.3.2 Creatinine

A second, identical aliquot of the pooled three day urine collected was thawed and urinary creatinine was determined in duplicate, by the alkaline picrate method (Jaffe reaction) using the Technicon Auto Analyzer. The urine sample stream, segmented with air, was diluted with 1.8% sodium chloride; this combined stream was mixed before it entered the donor side of the dialyzer. The analytical stream consisted of water segmented with air. After emerging from the dialyzer, it was joined with 0.5 N NaOH. These two components were mixed and then joined with picric acid. The three components were then mixed, moved into the colorimeter, and the absorbance of the stream was measured at 505 nm in a 15 mm flowcell.

3.4 VARIABLES TO ASSESS

3.4.1 Tobacco Exposures

The questionnaire (Appendix C) was used to assess the level of tobacco use for each individual. Each person was classified into one of three categories, depending on his/her response to question 1B.

group 1: nonsmokers (< 1 cigarette/day)
group 2: moderate smokers (≤ 10 cigarettes/day)
group 3: heavy smokers (> 10 cigarettes/day)

An "occasional" response pertaining to pipe, snuff, or chewing tobacco use was categorized as group one since Vestal *et al.* (56) found no difference in antipyrine clearance between nonsmokers and "once in a while" pipe or cigar smokers. An "often" response was coded as group two.

3.4.2 Marijuana

Since marijuana is the most commonly used social drug in this population, it was the only one analyzed in this study. Everyone was rated into one of three categories, depending on his/her response to question nine (Appendix C).

1. Infrequent users consisting of those who smoked marijuana two or fewer times within the last month.

2. Moderate smokers, using marijuana at least once a week.

3. Heavy smokers who admitted to smoking marijuana daily or almost daily.

As a check, those who were in group three should have included the intake of marijuana on at least two of the five daily food records. If not, they were included in group two even if they checked the "daily" box.

3.4.3 Caffeine Intakes

Since the metabolic half-life of caffeine is only approximately three hours (82), the daily records were used to obtain the actual intake of caffeine over each subject's five-day study period. The questionnaire (Appendix C) was scanned prior to doing the calculations in order to gain information as to the type of coffee/tea consumed, i.e. decaffeinated or caffeinated. Values were estimated based on ... (82,97).

• instant coffee	60 mg/cup
• percolated/dripped coffee	125 mg/cup
• black bag tea	30 mg/cup
• decaffeinated beverages	-
• herbal tea	-
• glass of ice tea	30 mg/glass
• soft drinks+	50 mg/12oz
• allergy tablets	30 mg/tablet
• most aspirins	30 mg/tablet
• NoDoz/Vivarin	150 mg/tablet

+ an average of the most popular carbonated beverages

The average daily consumption of caffeine in mg/day were reported as a function of body weight and the data was analyzed as milligrams of caffeine per day per kilogram of body weight.

The caffeine variable was plotted against D-GL/g creatinine in order to classify caffeine consumers into categories. The groups were determined by looking for breaks in the data distribution. These corresponded to:

group 1	< 1.0 mg caffeine/day/KGBW
group 2	1.0 - 3.0 mg caffeine/day/KGBW

group 3 > 3.0 mg caffeine/day/KGBW

3.4.4 Alcohol

The total alcohol consumed, recorded on the daily food and beverage records, was determined as grams alcohol/KGBW/day. The following values were provided by the U.S. Brewers Association:

Beers:	High beer, ale, or malt	4.3% alcohol by wt.
	Premiums	3.6%
	Light beers	3.0%
Wine		11.0 gm/4 oz glass

All other alcohol values were obtained from Bowes and Church (98), who had already converted the alcohol contained in mixed drinks, liqueurs, and all other types of liquor into grams.

The mean daily consumptions, divided by body weight, were plotted against the individuals' D-GL/gm creatinine. Three groups were determined according to natural clusters on a scattergram.

Group 1: low consumers (< 0.1 gm ETOH/KGBW/day)
Group 2: moderate drinkers
(0.1 ≤ gm ETOH/KGBW/day ≤ 0.3)
Group 3: heavy consumers (> 0.3 gm ETOH/KGBW/day)

3.4.5 Vegetarianism

Everyone was coded as either being a vegetarian or not, depending on their response to question six on the questionnaire (Appendix C). A vegetarian, for this study, was defined as one who does not eat red meat.

3.4.6 Genetic Factors

Genetic factors are very important in the metabolism of drugs in man and contribute to the wide variability in D-GA excretion among human subjects (21). In this study, genetic factors were assessed only in terms of cancer incidences among family members. If there was no known incidence of cancer, they were placed into group one. Group two included all those subjects who knew of cancer occurring in their family, immediate or distant.

3.4.7 Drug Usage

The present literature indicates that the effects of common prescription drugs on D-GA excretion have not been adequately researched. Since it is known that numerous drugs are potent inducers of the MFO system, anyone consuming a prescription drug or any of the over-the-counter medications other than aspirins was deleted from the statistical anaylses. This was determined by examining their daily dietary records.

3.5 STATISTICAL ANALYSES

The responsiveness of D-glucaric acid excretion to dietary variables was determined by comparing group mean values of D-GA excretion, expressed as micromoles D-gluco,1-4 lactone/gm creatinine. The female population was analyzed separately from the male population in all analyses.

T-tests between sample means were used to compare the mean D-GA excretions of males versus females, vegetarians versus nonvegetarians, and those whose family members had cancer versus those whose families did not. Analyses of variance were performed on all other variables to determine if significant differences ($p<0.05$) existed between D-GA excretions within the same variable class. This included comparing the group one, two, and three D-GA differences in caffeine, alcohol, and marijuana variables. Simple correlation analyses were performed to determine if a correlation existed between total D-GL excreted and weight as well as between D-GL/gm creatinine and weight.

In order to assess the relative importance of each variable as predictors of total D-GA excretion, a stepwise backward elimination multiple regression analysis was used. Two- and three-way chi-square tests were then performed on

the variables which remained in the regression model as having the greatest effect. This was done to test for possible additive effects between the variables and D-GA excretion.

Chi-square tests were also used to determine incidences of marijuana, tobacco, caffeine, and alcohol usage among the vegetarian and nonvegetarian populations. Since 20% of the cells in the 3x2 Chi-square table of tobacco versus vegetarians had less than 5 observations, the tobacco category was further condensed into just smoking and nonsmoking groups for this particular analysis. Finally T-tests and two-way analyses of covariance were used to test for possible interactions between vegetarianism and all other variables examined.

Chapter IV

RESULTS AND DISCUSSION

4.1 POPULATION CHARACTERISTICS

Of the 181 men and 21 women who completed this study, the final analyses dealt with a nonmedicated population of 124 men and 18 women. The 60 subjects who were not included were deleted either due to incomplete data collections or use of what proved to be a wide variety of prescription drugs while on the study.

At the onset of the test period, the male and female mean weights were 75.0±10 Kg and 58.6±7.9 Kg respectively. The majority of the participants were of college age, since most of the recruitment efforts were targeted within Virginia Tech. The males ranged in age from 18 to 56 years, with a mean of 26.05±7.7 years. The female ages ranged from 19 to 40 years, with a group mean of 23.77±4.0 years.

Since there were no dietary restrictions imposed on the subjects, the subjects living in dormitories consumed the standard cafeteria meals. All other participants were encouraged to eat their normal, free-choice diets prepared at home. There seemed to be good compliance with maintaining an accurate daily food and beverage record throughout the five days.

4.2 D-GA AND GENDER

The mean daily D-glucaric acid excretion for the female population was significantly higher than for males (Table 1).

The overall range of values obtained in this study are in close agreement with Hunter et al. (31) who reported the mean daily excretion of D-GA in 4 healthy males as being 14 micromoles D-GL/gm creatinine/day. If we were to adjust these results in accordance with the 30% conversion rate of D-GA to D-GL (69), then they would also be well within the normal range of 40 to 60 micromoles D-GA/gm creatinine excreted reported by other authors (72,94).

Researchers are not reporting consistent trends when comparing urinary D-GA differences between men and women. Simmons et al. (96) found that men excrete significantly more D-GA per day than women. These values, however, have not been adjusted for body size differences. March et al. (94) and Fiedler et al. (72) both reported no significant difference in D-GA excretions between genders after weight adjustments had been made, but in each case the mean DGA excretion for females was higher than that for the males. This study reports a significant difference between the men and women, the women excreting more D-GA relative to creatinine excretion.

TABLE 1

Mean D-Glucaric Acid Excretion

Category	Group[1]	Men n	Men Mean[2]	Men Range	Women n	Women Mean	Women Range
Gender		124	14.3±5.2	6.0-35.5	18	17.0±3.7*	11.5-23.8
Tobacco (cigarettes/day)	1	98	13.5±4.7	6.0-32.5	16	16.9±3.2	12.0-21.9
	2	18	16.0±5.8*	8.7-35.5	2	17.6±6.5	11.5-23.8
	3	8	20.5±6.4	13.1-32.9		-	
Marijuana (frequency/week)	1	104	14.2±4.3	6.0-32.9	16	17.0±3.7	11.5-23.8
	2	12	12.9±4.6	7.2-22.3	1	20.2	
	3	8	17.6±7.8*	11.1-35.5	1	13.2	
Caffeine (mg caf/KGBW/day)	1	48	12.6±4.5	6.3-26.5	4	15.3±3.6	11.5-20.2
	2	53	14.2±4.3	7.2-32.5	9	16.1±3.6	12.0-23.8
	3	23	18.0±6.9*	6.0-35.5	4	18.8±1.6	16.8-20.7
Ethanol (gm ETOH/KGBW/day)	1	62	14.1±5.3	6.0-32.5	9	15.6±3.0	12.0-23.8
	2	28	14.2±5.8	8.0-35.5	7	16.5±3.5	11.5-20.7
	3	34	14.8±4.7	7.2-32.9	2	19.9±1.1	19.1-21.9
Nonvegetarians	0	111	13.9±5.1*	6.0-35.5	14	16.2±3.1	11.5-20.7
Vegetarians	1	13	17.4±5.5*	7.8-26.5	4	19.8±4.6*	13.2-23.8
No cancer in family	1	53	13.5±5.1	6.3-35.5	6	17.8±4.2	13.0-23.8
Cancer in family	2	71	14.4±5.0	6.0-32.9	12	16.6±3.5	11.5-21.9

1 Cut-off points for groups are given in Chapter 3.
2 Mean values given as micromoles D-GL/gm creatinine ± S.D.
* Significantly different among groups ($p<0.05$).
\+ Significantly different between men and women ($p < 0.05$).

The observed gender difference could be related to hormonal differences or some other environmental or dietary factor varying between the men and women in the examined population. Since age was not significantly different between genders in this study, age probably does not account for the observed difference in D-GA excretion.

One plausible explanation for the large mean differences obtained could be that all the women collected urine on days 10 through 13 of their menstrual cycle, coinciding with peak levels of circulating serum estrogen present during the month (99). A high level of steroid hormones such as estrogen and progesterone has been shown to elevate D-GA excretion in women (91-93), yet no known research has found changes in D-GA excreted during a woman's menstrual cycle. Perhaps further studies should re-examine the effect of the rhythmic variations of endogenous hormone levels on female D-GA excretions.

4.3 D-GA AND WEIGHT

The mean weight of the male and female populations were 165±22.2 and 129.5±17.4 pounds respectively. A correlation analysis was performed between micrograms D-GA/day and body weight, as well as micrograms D-GL/gm creatinine and body weight. Unlike other investigators (94,95), we found no

correlation between excreted D-GA/day and body weight in either sex. This could be due to either a high degree of variation in body fatness among our population, or due to incomplete daily urine collections. The latter is the most likely case. Once D-GA was corrected for weight, by dividing by creatinine clearance, there was no correlation between D-GL/gm creatinine and body weight as would be expected. This indicates that there was no added effect of weight on top of the D-GL/gm creatinine measured. Therefore, body weight is not a confounding variable in this study.

4.4 MULTIPLE REGRESSION ANALYSIS

A stepwise backward elimination procedure was used to relate all the independent variables examined as predictors of total D-GA excretion. The variables were deleted, one at a time, from the model until all the variables remaining had significant effects ($p<0.1$) on the observed variation. Alcohol consumption, cancer, and marijuana usage, in that order, dropped out of the model, contributing a nonsignificant amount of predictability to the total variance. Tobacco use, vegetarianism, and caffeine consumption remained in the final model for men. Combined, these accounted for 36% of the total variability in the male

population. Caffeine intakes and vegetarianism affected D-GA excretion strongest of all the variables examined with F values of 24.26 and 8.13,respectively. The same analysis on the female population showed that none of the variables were significant. Even so, vegetarianism and caffeine consumption had the greatest effect on the total D-GA excreted. It is therefore important to examine the vegetarian diet and caffeine effects on D-GA excretion separately, in a more controlled study, to evaluate their individual effects on the excretion of D-GA in a large sample population.

4.5 EFFECT OF TOBACCO

The results displayed in Table 1 show that exposure to cigarette smoke was positively correlated with urinary D-GA excretion in the male population. Eighty-one percent of the population did not smoke cigarettes. This group (group 1) had the lowest mean value for D-GA excretion, while the moderate smokers, smoking fewer than 10 cigarettes per day, had a nonsignificant but higher mean ($p>0.05$). The 8 heaviest smokers, comprising group 3, smoked from 10 to 30 cigarettes each day, with an average of 19 per day. This group excreted the greatest amount of D-GA, significantly more than the low or moderate smokers.

There were only two women who smoked cigarettes, both in moderate numbers. These women had a lower mean D-GA excretion level than did the sixteen female nonsmokers (see Table 1). These results are not consistent with the trend seen in the male population, probably due to the small sample size.

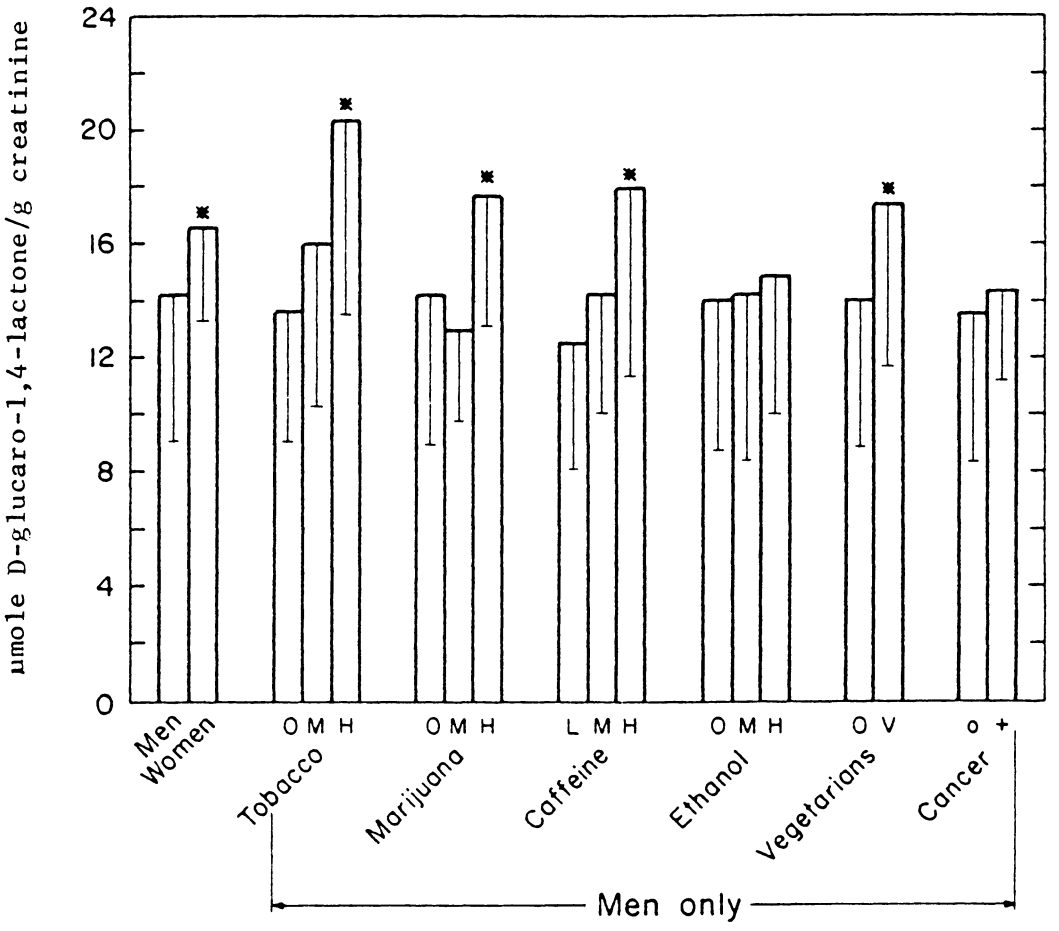
Another study examining D-GA excretion and cigarette smoke found no significant difference between 28 smokers smoking more than 20 cigarettes per day, and 23 controls (72). However, only the first morning urine sample of a single day was used to represent the daily excretion rate of D-GA. Bowen et al. (100) reported that afternoon casual urine samples were more likely to correlate with the daily excretion of D-GA, but any casual sample was a poor representative sample of total D-GA excreted in one day.

The mechanism by which cigarette smoke affects D-GA excretion is unknown. Tobacco smoke contains nicotine and a wide variety of polycyclic hydrocarbons. All of these compounds have been shown experimentally to induce hepatic drug metabolizing enzymes (18,47-51). Researchers consistently find that heavy cigarette smokers have elevated drug clearance rates of antipyrine (56), theophylline (46,48), and phenacetin (51).

The males in this population smoking more than ten cigarettes per day had a significantly elevated D-GA excretion from a three day pooled urine collection. Changes in D-GA are therefore sensitive to exposure of tobacco smoke, supporting the hypothesis that cigarette smoking induces hepatic MFO enzymes and elevates urinary D-GA excretion in man. The four highest D-GA excretors in group three were also large caffeine consumers. This association could have an additive effect on D-GA excretion, responsible for the observed 20.25±6.4 micromoles D-GL/gm creatinine, the highest mean group values of all the variables examined (Figure 2).

4.6 EFFECT OF MARIJUANA

Since there were only two women who admitted frequently smoking marijuana, no analyses were conducted on data from the female population. Among the men, the eight who admitted smoking marijuana daily had significantly higher urinary D-GA levels than either the moderate smokers (those who smoked at least once a week) or those who infrequently or never smoked marijuana (see Table 1). The daily, chronic smokers presumably are inhaling high levels of polycyclic hydrocarbons as well as delta^9-THC, both found in marijuana smoke (43,78). Delta^9-THC is hypothesized to stimulate



* Significantly different among groups ($p < 0.05$)
L&O Low or infrequent exposure
M Moderate exposure
H High exposure
V Vegetarian
o No cancer in family
\+ Cancer in family

Figure 2: D-Glucaric Acid Excretion

drug-metabolizing enzymes since higher tolerance levels are seen when individuals are frequently exposed to the drug (44). Chronic smokers also metabolize delta^9-THC and other drugs faster than nonsmokers (43,45). Often, in these studies, the marijuana user is also a cigarette smoker, possibly contributing to the observed induction. Only three of the daily marijuana smokers in this study were cigarette smokers, none of them smoking more than ten cigarettes per day. Therefore, the elevated D-GA levels seen among the daily smokers of marijuana cannot solely be attributed to the effects of nicotine on the MFO system. These data support the hypothesis that chronic marijuana smoking can elevate D-GA excretion, possibly the result of an activated MFO enzyme system.

4.7 EFFECT OF CAFFEINE

Total caffeine consumption was positively and significantly associated with D-GA excretion levels. Elevated D-GA excretion was observed among the higher caffeine consumers in both men and women (Table 1). Group 3, consisting of the 23 men consuming more than 3 mg caffeine/KGBW had a mean daily caffeine intake of 402.1±181.7 mg caffeine/day, comparable to more than 3 cups

of coffee each day. This group had significantly higher amounts of D-GA in their urine than the moderate and low consumers of caffeine. The same trend was evident in the female population.

These results are in agreement with findings of other researchers who have found that caffeine, when intakes exceed 200 mg, the pharmacologically active dose (82), is a potent inducer and stimulator of the MFO enzymes. Evidence includes the decreased serum halflife of the drugs zoxazolamine and methylpyrene in rats (55) and antipyrine in man (56) following caffeine exposures. A regression analysis revealed that caffeine was one of the strongest variables affecting total D-GA excretion, further supporting the hypothesis that D-GA is a reliable method for monitoring drug-metabolizing activity.

Caffeine is a widely consumed drug, often given in unrestricted amounts to a hospitalized patient. It seems possible that the patient could ingest sufficient caffeine to interfere with or potentiate other medications. Caffeine consumption should, therefore, receive a closer look as to its xenobiotic-drug interactions occurring in a patient receiving other drugs simultaneously.

4.8 EFFECT OF ALCOHOL

There was no association between the amount of alcohol consumed and the excretion of D-glucaric acid in either sex examined (see Table 1). Forty-seven participants abstained completely from alcohol for the five days on the study. They comprised more than half of the 70 people in the lowest group of ethanol consumption (group 1), who drank an average of less than 0.1 gm ETOH/KGBW/day. Group 1 was not significantly different from the highest group (group 3) which consisted of 36 people who drank 21 gm alcohol or more per day. Mezey et al. (38) reported elevated D-GA excretion in the chronic alcoholics, consuming over 250 gm alcohol per day, considerably more than the 21 gm per day minimum for the highest drinkers in this study. There were 2 men who had intakes greater than 1.0 gm/KGBW/day. These men had D-GA excretions of 16.02 and 19.09 micrograms D-GL/gm creatinine, higher than the group 3 mean. However, due to the generally low levels of alcohol being consumed in the population examined, it is not surprising that alcohol had no observable effect on total D-GA excretion.

4.9 EFFECT OF VEGETARIAN DIET

Both the men's and women's mean D-GA excretion were significantly higher in the vegetarian (non-red-meat-eaters)

than in the rest of the population examined (see Table 1). The four women vegetarians were lacto-ovo-vegetarians and did not consume any meat or fish products. Of the 13 men who thought of themselves as vegetarians, seven were lacto-ovo-vegetarians, five consumed chicken and/or fish while on the study, and one consumed several hamburgers, claiming his behavior to be atypical. The observed high D-GA excretion levels seen among this vegetarian population does not agree with other work showing that vegetarians have significantly slower drug clearance rates than red-meat-eaters (57,58).

Since the effect of some inducers of MFO are additive at sub-threshold doses (101,102), T-tests and analyses of covariance were performed to analyze for possible confounding, additive or synergistic interactions existing between vegetarians and the other variables examined. Two of the four female vegetarians were frequent marijuana users, but none smoked cigarettes or drank more than the average amounts of alcohol or caffeine, and still had significantly elevated D-GA in their urine. The male vegetarians were no more likely to be marijuana, tobacco, or caffeine users than the red-meat-eaters. Although the nonvegetarians consumed more than twice as much alcohol, (0.21 versus 0.08 gm ETOH/KGBW/day), the regression analyses

revealed that alcohol had the least effect on D-GA of all the variables examined. Thus, since no other variables seemed to explain the difference between these two groups, this observed difference in D-GA excretion may have been due to other dietary-related inducers in the vegetarian diet.

There have been several types of dietary constituents shown to affect the metabolism of chemicals by the microsomal MFO system. Naturally occurring inducers are present in some plants. Cruciferous vegetables, including Brussels sprouts, cabbage, and cauliflower, contain indoles with inducing activity (4,5,15). These might be consumed in greater quantities in a vegetarian diet, contributing in part to the higher D-GA observed. If so, this effect could be beneficial in retarding malignant tumor formation (3-5). Boyd et al. (5) found that cauliflower fed to rats inhibited aflatoxin-B1 hepatocarcinoma development. Further evidence has shown that dosing rats with indoles reduced chemically induced mammary tumor development (15).

Other dietary constituents might be different in the vegetarian diet, contributing to the elevated D-GA excretion found. Dietary polyunsaturated fatty acids appear to induce MFO activity in rats to a greater extent than saturated fat or fat-free diets (13). It has been found that rats fed

seven percent beef fat metabolized hexobarbital and amino-pyrine more slowly than rats fed the more highly unsaturated fat, corn oil (11,12). The quantity and quality of dietary lipids can therefore affect drug metabolizing enzymes and possibly alter D-GA excretion. Vegetarians consume plant lipids, which generally contain a high amount of unsaturated fats and are cholesterol free. Unfortunately, the polyunsaturated/saturated fat ratios of our subjects are not yet available to test this hypothesis.

Protein variations might also affect D-GA excretions, although the percent protein in most vegetarian diets is surprisingly similar to that in omniverous diets (103). The amount and quality of protein does affect the toxicity and pharmacological activities of chemicals and drugs. Ioannides et al. (62) reported that the feeding of low protein diets to rats led to significant losses in hepatic MFO activity. Children with kwashiorkor exhibit an impaired drug metabolism and depressed D-GA excretion (2,19,104). The activity of drug metabolizing enzymes increases in humans as the protein level increases up to 35% of the total calories in the diet (6). Higher levels of protein (55%) had no effect. The protein levels of this study's vegetarian and nonvegetarian populations await further analysis.

Another speculative cause for the elevated D-GA excreted among the vegetarians could be the high levels of pesticide residues consumed from contaminated market-bought produce. Many pesticides are potent inducers of the MFO system, and detectable levels of pesticide residues have been measured on numerous vegetables examined (105-107). A careful review of the literature, however, revealed no studies comparing total pesticide levels consumed among different populations and dietary patterns. Any or all of these alterations in diet composition may significantly affect drug disposition and D-GA excretion in man. Thus, further research into which of these dietary factors are responsible for the observed difference in D-GA excretion between the two populations is needed.

4.10 EFFECT OF CANCER IN THE FAMILY

There were no differences in D-GA excretion observed between the participants who knew of family members with cancer (group two) from those who did not (group one) (see Table 1). Although genetic factors do affect MFO induction and D-GA excretion (21), the connection between familial tendencies to develop cancer and actual cancer incidences present among family members is probably very weak. Unknown environmental factors, suspected of being responsible for up

to 80% of all human malignancies (61), might strongly affect both the development of cancerous lesions and the state of the liver enzymes. Therefore monitoring D-GA excretion cannot be used as a reliable method for assessing the genetic predisposition to inheriting cancer.

Chapter V

SUMMARY AND CONCLUSIONS

Gender, tobacco, marijuana, caffeine, alcohol, vegetarianism, and cancer incidences among family members were all assessed as to their effect on total D-GA excretion over a three-day period. One hundred twenty-four men and 17 women, all healthy, nonmedicated volunteers, participated in this study. Each participant kept a food and beverage intake record for five consecutive days, and collected daily 24-hour urine outputs for the last three days of the five. D-glucaric acid was determined by an enzymatic assay and recorded as D-glucaro-1,4-lactone/gm creatinine/day.

The females excreted significantly more D-GA than the men, possibly because the females all collected their urine during the peak estrogen level of their menstrual cycle. The mean D-GA excretion in males and females was 14.1±5.4 and 16.8±3.6 micromoles D-GL/gm creatinine, respectively. These values are comparable to the mean D-GA excretion values obtained by other research groups.

There was a significantly elevated amount of total D-GA excreted among the heaviest users of marijuana, caffeine, and tobacco when compared to the moderate and low users of the same drug. This supports much of the recent literature

dealing with dietary inducers of the MFO system. Vegetarians excreted significantly more D-GA than their red-meat-eating counterparts. Analyses of covariance did not reveal any relationship between vegetarianism and the other variables examined, except alcohol. Nonvegetarians drank more than twice the amount of alcohol as the vegetarians. However, the amount of alcohol consumed during this study did not affect D-GA excretion, probably due to the small amounts consumed in this population while on the study. There was also no significant difference in D-GA excretion between those subjects who knew of cancer among their family members and those who did not. A backward elimination, multiple regression analysis showed that caffeine consumption and vegetarianism were the two variables, in both the male and female populations, which contributed most to predictability of total D-GA excretion in this study.

It thus appears that monitoring D-GA excretion in a large population is sensitive enough method to detect differences in dietary habits, possibly reflective of varying levels of liver enzyme induction. This study did not deal with the effects of possible interactions, either positive or negative, existing between any or all of the variables on D-GA excretion. It only served as a survey to identify

which dietary factors can affect D-GA excretion. It is also necessary to consider such well-established associations as the tobacco-alcohol combination and the caffeine-tobacco association when assessing the effects of any one of these variables on the enzymes of the hepatic MFO system. Interactions among these common dietary constituents might substantially alter pharmacokinetic reactions. The The habits of caffeine consumption, cigarette and marijuana smoking, and vegetarianism should, therefore, all be examined singly, in a metabolically controlled study, to determine the individual effect each has on total D-GA excretion among healthy, nonmedicated subjects.

BIBLIOGRAPHY

1. Neal, R.A. Metabolism of toxic substances, Casarett and Doull's toxicology, 2nd Ed., edited by Doull, Klaassen, and Amdur, Macmillan Publishing Co., New York, N.Y., 1980.

2. Remmer, H. The role of the liver in drug metabolism. Am. J. Med. 49:617-629, 1970.

3. Conney, A.H. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19:317-355, 1967.

4. Wattenberg, L.E. Effects of dietary constituents on the metabolism of chemical carcinogens. Canc. Res. 35:3326-3331, 1975.

5. Boyd, J.N., S. Sell, & G.S. Stoewsand. Inhibition of aflatoxin-induced serum alpha-fetoprotein in rats fed cauliflower. Soc. Exp. Biol Med. 161:473-475, 1979.

6. Kato, N., T. Tani, & A. Yoshida. 1980 Effect of dietary level of protein on liver microsomal drug-metabolizing enzymes, urinary ascorbic acid, and lipid metabolism in rats fed PCB-containing diets. J. Nutr. 110:1686-1694, 1980.

7. Campbell, T.C. & J.R. Hayes. The effect of quantity and quality of dietary protein on drug metabolism. Fed. Proc. 35:2470-2474, 1976.

8. Campbell, T.C. & J.R. Hayes. Role of nutrition in the drug-metabolizing enzyme system. Pharmacol. Rev., 1974.

9. Sitar, D.S. & E.R. Gordon. Effect of diet and other drugs on the qualititative and quantitative distribution of cytochromes P-450 in rat liver. Canad. J. Physiol. Pharmacol. 58:331-335, 1980.

10. Campbell, T.C. Nutrition and drug metabolizing enzymes. Clin. Pharm. Ther. 23:699-705, 1977.

11. Wade, A.E. & W.P. Norred. Effect of dietary lipid on drug-metabolizing enzymes. Fed. Proc. 35:2475-2479, 1976.

12. Hietanen, E., H. Vainio, & Q. Hanninen. Dietary fats and properties of endoplasmic reticulum: II dietary lipid induced changes in activities of drug-metabolizing enzymes in liver and duodenum of rats. Lipid 10:467-472, 1975.

13. Wade, A.E., W.P. Norred, & J.S. Evans. Lipid in drug detoxification, Nutrition and drug interactions, J.N. Hathcock & J. Coon editors, Academic Press, New York, N.Y., 1978.

14. Smith-Barbaro, P., D. Hanson, & B. Keddy. Effect of bran and citrus pulp on hepatic small intestinal and colonic HMG CoA reductase, cytochrome P450, and cytochrome b5 levels in rats. J. Nutr. 111:789-797, 1981.

15. Wattenberg, L.W., W.D. Loub, L.K. Lam, & J.L. Speier. Dietary constituents altering the responses to chemical carcinogens. Fed. Proc. 35:1327-1331, 1976.

16. Pantuck, E.J., K.C. Hsiao, R. Kuntzman, & A.H. Conney. Intestinal metabolism of Phenacetin in the rat: effect of charcoal broiled beef and rat chow. Sci. 187:744-745, 1975.

17. Conney, A.H., E.J. Pantuck, K.C. Hsiao, R. Kuntzman, A.P. Alvares, & A. Kappas. Regulation of drug metabolism in man by environmental chemicals and diet. Fed. Proc. 36:1647-52, 1977.

18. Kappas, A., A.P. Alvares, K.E. Anderson, C.B. Pantuck, R. Chang, & A.H. Conney. Effect of charcoal-broiled beef on antipyrine and theophylline metabolism. Clin. Pharmacol. Ther. 22:721-725, 1977.

19. Kato, R. Drug metabolism under pathological and abnormal physiological states in animals and man. Xenobiotica 7:25-92, 1977.

20. Fiedler, K., E. Schroter, & H. Cramer. Glucaric acid excretion: analysis of the enzymatic assay and findings in smokers and non-smokers. Eur. J. Clin. Pharmacol. 18:429-432, 1980.

21. Vesell, E.S. & J.G. Page. Genetic control of drug levels in man: antipyrine. Sci. 161:72-73, 1968.

22. Notten, W.R.F. & P.T. Henderson. The interaction of chemical compounds with the functional state of the liver. Int. Arch. Occup. Envir. Health 38:209-214, 1977.

23. Puzynska, L., F.M. Debets, & J.J. Stirk. Toxicity of hexachlorobenzene with special reference to hepatic P-450 levels and D-GA levles in urine of female rats fed HCB and treated with phenobarbital and diethylmaleate, Chemical porphyria in man. p161-173, 1979.

24. Hunter, J., J.D. Maxwell, D.A. Stewart, & R. Williams. Urinary D-glucaric acid excretion and total liver content of cytochrome P-450 in guinea-pigs: Relationship during enzyme induction and following inhibition of protein synthesis. Biochem. Pharmacol. 22:743-747, 1973.

25. Marselos, M. & O. Hanninen. Enhancement of D-glucuronolactone and acetaldehyde dehydrogenase activities in the rat liver by inducers of drug metabolism. Biochem. Pharmacol. 23:1457-1466, 1974.

26. Notten, W.R. & P.T. Henderson. The influence of n-hexane treatment on the glucuronic acid pathway and activity of some drug-metabolizing enzymes in guinea pig. Biochem. Pharmacol. 24:127-131, 1975.

27. Notten, W.R., P.T. Henderson, & C.M. Kuyper. Stimulation of the glucuronic acid pathway in isolated rat liver cells by phenobarbital. Int. J. Biochem. 6:713-718, 1975.

28. Notten, W.R. & P.T. Henderson. Alterations in the glucuronic acid pathway caused by various drugs. Int. J. Biochem. 6:111-119, 1975.

29. Lake, B.G., R.C. Longland, I.A. Herod, M.A. Collins, & S.D. Gangolli. The effect of 4-hydroxybiphenyl and 2-naphthol on hepatic xenobiotic metabolism and the urinary excretion of metabolites of the D-glucuronic acid pathway in the rat. Biochem. Soc. Trans. 6:1246-1248, 1978.

30. Sotaniemi, E.A., B.O. Pelkonen, & M. Puukka. Measurement of hepatic drug-metabolizing enzyme activity in man. Eur. J. Clin. Pharmacol. 17:267-274, 1980.

31. Hunter, J., M. Carrella, J.D. Maxwell, D.A. Stewart, and R. Williams. Urinary D-glucaric acid excretion as a test for hepatic enzyme induction in man. Lancet, March:572-575, 1971.

32. Sotaniemi, E.A., F. Medzihradsky, & G. Eliasson. Glucaric acid as an indicator of use of enzyme-inducing drugs. Clin. Pharmacol. Ther. 15:417-423, 1974.

33. Kampf, D., I. Roots, A.G. Hildebrandt. Urinary excretion of D-glucaric acid, an indicator of drug metabolizing enzyme activity in patients with impaired renal function. Eur. J. Clin. Pharmacol. 18:255-261, 1980.

34. Aarts, E.M. Evidence for the function of D-GA as an indicator for drug induced enhanced metabolism through the glucuronic acid pathway in man. Biochem. Pharmacol. 14:359-363, 1965.

35. Lecamwasam, D.S., C. Franklin, & P. Turner. Effect of phenobarbitone on hepatic drug-metabolizing enzymes and urinary D-glucaric acid excretion in man. Br. J. Clin. Pharmacol. 2:257-262, 1975.

36. Lecamwasan, S.D. Hepatic enzyme induction and its relationship to urinary D-GA excretion in man. Br. J. Clin. Pharmacol. 2:546-548, 1975.

37. Sotaniemi, E.A., R.O. Pelkonen, & M. Puukka. Measurement of hepatic drug-metabolizing enzyme activity in man: comparison of three different assays. Eur. J. Clin. Pharmacol. 17:267-274, 1980.

38. Mezey, E. Increased urinary excretion of D-GA in alcoholism. Chem. Path. Pharmacol. 15:735-742. 1976.

39. Misra, P.S., A. Lefeure, H. Ishii, & E. Rubin. Increase of ethanol, meprobamate, and pentobarbital metabolism after chronic ethanol administration in man and rats. Am. J. Med. 51:346-350, 1971.

40. Khanna, J.M., H. Kalant, Y. Yec, S. Chung, & A.J. Siemens. Effect of chronic ethanol treatment on metabolism of drugs *in vitro* and *in vivo*. Biochem. Pharmacol. 25:329-335, 1976.

41. Kalant, H., J.M. Khanna, G.Y. Lin, & S. Chung. Ethanol: a direct inducer of drug metabolism. Biochem. Pharmacol. 25:337-342, 1976.

42. Iber, F.L. Drug metabolism in heavy consumers of ethyl alcohol. Clin. Pharmacol. Ther. 22:735-742, 1977.

43. Jusko, W.J., J.J. Schentag, J.H. Clark, M. Gardner, & A.M. Yurchak. Enhanced biotransformation of theophylline in marijuana and tobacco smokers. Clin. Pharmacol. Ther. 24:406-410, 1978.

44. Lemberger, L. & A. Rubin. Cannabis: the role of metabolism in the development of tolerance. Drug Metab. Rev. 8:59-68, 1978.

45. Lemberger, L., N.R. Tamarkin, & J. Axelrod. Delta-9-tetrahydrocannabinal: metabolism and disposition in long-term marijuana smokers. Sci. 173:72-73, 1971.

46. Jenne, J., H. Nagasawa, R. McHugh, & F. MacDonald. Decreased theophylline half-life in cigarette smokers. Life Sci. 17:195-198, 1975.

47. Mitra, G., M.K. Poddar, & J.J. Ghosh. Effect of tobacco-leaf extract administration on liver, lung, intestine, and serum enzymes. Toxicol. Appl. Pharmacol. 52:262-266, 1980.

48. Grygiel, J.J., D.J. Birkett, & D. Phil. Cigarette smoking and theophylline clearance and metabolism. Clin. Pharmacol. Ther. 30:491-496, 1981.

49. Ruddon, R.W. & A.M. Cohen. Alteration of enzyme activity in rat liver following the acute and chronic administration of nicotine. Toxicol. Appl. Pharmacol. 16:613-625, 1970.

50. Beckett, A.H. & E.J. Triggs. Enzyme induction in man caused by smoking. Nature 216:587, 1967.

51. Pantuck, E.J., K.C. Hsiao, A. Maggio, K. Nakamura, R. Kuntzman, & A.H. Conney. Effect of cigarette smoking on phenacetin metabolism. Clin. Pharmacol. Ther. 15:9-17, 1974.

52. Parsons, W.D. & A.H. Neims. Effect of smoking on caffeine clearance. Clin. Pharmacol. Ther. 24:40-45, 1978.

53. Aldridge, A., W.D. Parsons, & A.H. Neims. Stimulation of caffeine metabolism in the rat by 3-methyl cholanthrene. Life Sci. 21:967-974, 1977.

54. Welch, R.M., S.Y. Hsu, & R.L. DeAngelis. Effect of aroclor 1254, phenobarbital, and polycyclic/aromatic hydrocarbons on the plasma clearance of caffeine in the rat. Clin. Pharmacol. Ther. 22:791-798, 1977.

55. Mitoma, C., L. Lombrozo, S. LeValley, & F. Dehn. Nature of the effect of caffeine on the drug metabolizing enzymes. Arch. Biochem. Biophys. 134:434-444, 1969.

56. Vestal, R.E., A.H. Norris, J.D. Tobin, B.H. Cohen, N.W. Shock, & R. Andres. Antipyrine metabolism in man: influence of age, alcohol, caffeine, and smoking. Clin. Pharmacol. Ther. 18:425-432, 1975.

57. Mucklow, J.C., M.T. Caraher, D.B. Henderson, and M.D. Rawlins. The effect of individual dietary constituents on antipyrine clearance in Asian immigrants. Br. J. Clin. Pharmac. 7:416-417, 1979.

58. Fraser, H.S., J.C. Mucklow, C.J. Bulpitt, C. Khan, G. Mould, & C.T. Dollery. Environmental effects on antipyrine half-life in man. Clin. Pharmacol. Ther. 22:799-805, 1978.

59. Brodie, M.J., A.R. Bobis, E.L. Toverud, W. Ellis, S. Murray, S. Webster, & R. Harrison. Drug metabolism in white vegetarians. Br. J. Clin. Pharmac. 9:523-525, 1980.

60. Dutton, G.J., G.J. Wishart, J.E. Leakey, & M.A. Goheer. Conjugation with glucuronic acid and other sugars. In: Drug metabolism - from microbe to man: a symposium, D.V. Parke editor, Guilford, England, pp. 71-90.1974.

61. Arcos, J.C. Cancer: chemical factors in the environment. Amer. Lab., June, p65-73,1978.

62. Ioannides, C. & D.V. Parke. Effect of diet on the metabolism and toxicology of drugs. J. Hum. Nutri. 33:357-366, 1979.

63. Herzberg, M., E. Tenenbaum, B. Fishel, & M.H. Wiener. D-GA and gamma-glutamyltransferase as indices of hepatic enzyme induction in pregnancy. Clin. Chem. 23:596-598, 1977.

64. Davidson, D.C., W.B. McIntosh, & J.A. Ford. Assessment of plasma glutamyl transpeptidase activity and urinary D-GA excretion as indices of enzyme induction. Clin. Sci. Mol Med. 47:279-283, 1974.

65. Perucca, E., A. Hedges, K.A. Makki, & A. Richens. A comparative study of antipyrine and lignocaine disposition in normal subjects and in patients treated with enzyme-inducing drugs. Br. J. Clin. Pharmac. 10:491-497, 1980.

66. Hildebrandt, A.G., I. Roots, G. Heinemeyer, S. Nigam, & H. Helge. Aminopyrine as one of the parameters to measure in vivo drug metabolism activity in man and animals, The induction of drug metabolism symposium, edited by R.W. Estabrook & E. Lindenlaub, F.K. Schalttauer Verlag, New York, N.Y., May, 1978.

67. Latham, A.N. D-glucaric acid as an index of hepatic enzyme induction by anticonvulsant drugs in man. J. Pharm. Pharmac. 26:284-286, 1974.

68. Aarts, E.M. Sex difference in the stimulating effect of drugs on carbohydrate metabolism through the glucuronic acid pathways studied on the rat. Biochem. Pharmac. 17:327-330, 1968.

69. Marsh, C.A. Metabolism of D-glucuronolactone in mammalian systems. Identification of D-glucaric acid as a normal constituent of urine. Biochem. J. 86:77-86, 1963.

70. Hunter, J., J.D. Maxwell, D.A. Stewart, & R. Williams. Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides. Nature 237:399-401, 1972.

71. Kahn, G.C., A.R. Boobis, I. Blair, M.J. Brodie, & D.S. Davies. Amtipyrine as an in vitro probe of mixed function oxidase activity. Br. J. Clin. Pharmac. 8:284, 1979.

72. Fiedler, K., E. Schroter, & H. Cramer. Glucaric acid excretion. Analysis of the enzymatic assay and findings in smokers and non-smokers. Eur. J. Clin. Pharmacol. 18:429-432, 1980.

73. Burstein, S.H. & D. Kupfer. Hydroxylation of trans-D-tetrahydrocannabinol by hepatic microsomal oxygenase. Ann. N.Y. Acad. Sci. 191:61-67, 1971.

74. Fernandes, M., N. Warning, W. Christ, & R. Hill. Interactions of several cannabinoids with the hepatic drug metabolizing system. Biochem. Pharmacol. 22:2981-2987, 1973.

75. Kupfer, D., E. Levin, & S. Burstein. Studies on the effects of D¹tetra-hydrocannabinol and DDT on the hepatic microsomal metabolism of D¹-THC and other compounds in the rat. Chem. Biol. Inter. 6:59-64, 1973.

76. Vesell, E.S. & G.T. Passananti. Inhibition of drug metabolism in man. Drug Metab. Disp. 1:402-410, 1973.

77. McMillian, D.E., W.L. Dewey, R.F. Turk, L.S. Harns, & J.H. McNeil. Blood levels of H-delta⁹-tetrahydrocannabinol and its metabolites in tolerant and non-tolerant pigeons. Biochem. Pharm. 22:383, 1973.

78. Marcotte, J., F.S. Skelton, M. Cote, & H. Witsch. Induction of aryl hydrocarbon hydroxylase in rat lung by marijuana smoke. Toxicol. Appl. Pharmacol. 33:231-245, 1975.

79. Siemens, A.J., H. Kalant, J.M. Khana, J. Marshman, & G. Ho. Effect of cannabis on pentobarbital-induced sleeping time and phenobarbital metabolism in the rat. Biochem. Pharmac. 22:477-488, 1974.

80. Paton, W.D. Pharmacology of marijuana. Ann. Rev. Pharmacol. 15:191-220, 1975.

81. Benowitz, N.L. & R.T. Jones. Effects of delta⁹-hydrocannabinol on drug distribution and metabolism. Clin. Pharmacol. Ther. 22:263-268, 1978.

82. Graham, D.M. Caffeine - its identity, dietary source, intake, and biological effects. Nutr. Rev. 36:97-102, 1978.

83. Statiand, B.E., T. Desmos, M. Danes. Caffeine accumulation associated with alcoholic liver disease. N. Engl. J. Med. 295:110-111, 1976.

84. Rubin, E. & C.S.Lieber. Hepatic microsomal enzymes in man and rat: induction and inhibition by ethanol. Sci. 162:690-691, 1968.

85. Rubin, E., H. Gang, P. Misra, & C.S. Lieber. Inhibition of drug metabolism by acute ethanol intoxification: a hepatic microsomal mechanism. Amer. J. Med. 49:801-806, 1970.

86. Rubin, E., F. Hutterer, & C.S. Lieber. Ethanol increases hepatic smooth endoplasmic reticulum and drug-metabolizing enzymes. Sci. 159:1469-1470, 1968.

87. Lieber, C.S. & E. Rubin. Alcoholic fatty liver in man on a high protein and low fat diet. Am. J. Med. 44:200-205, 1968.

88. Gadehalt, G., J. Aarbakke, E. Dybing, M. Sjoblom, & J. Morland. Hepatic microsomal drug metabolism, glutamyltransferase activity, and *in vivo* antipyrine half-life in rats chronically fed an ethanol diet, a control diet, and a chow diet. J. Pharmacol. Exp. Ther. 213:196-203, 1980.

89. Ioannides, C., B.G. Lake, & D.V. Parke. Enhancement of hepatic microsomal drug metabolism *in vitro* following ethanol administration. Xenobiotica 5:665-676, 1975.

90. Dossing, M. & P.B. Andreasen. Ethanol and antipyrine clearance. Clin. Pharmacol. Ther. 30:101-104, 1981.

91. Wowat, A.P. Developmental effects on liver D-glucuronolactone dehydrogenase levels and on D-glucaric acid excretion in urine; hormonal effects on D-glucaric acid excretion in urine. J. Endocr. 42:585-590, 1968.

92. Hajos, P., I. Berlin, Z. Intody, M. Tornyossy, & A. Kaldor. The effect of oral contraceptives on serum lipids, gamma-glutamyl transpeptidase, and excretion of D-glucaric acid. Int. J. Clin. Pharm. Ther. Tox. 19:117-123, 1981.

93. Fahim, M.S., D.G. Hall, & Z. Fahim. Urinary D-glucaric acid. Am. J. Obst, Gynec. 105:124-126, 1969.

94. March, J., W.J. Turner, J. Shanley, & J. Field. Values for urinary excretion of D-glucaric acid by normal individuals. Clin. Chem. 20/9:1155-1158, 1974.

95. Jung, K., D. Scholz, & G. Schreiber. Improved determination of D-glucaric acid in urine. Clin. Chem. 27:422-426, 1981.

96. Simmons, C.J., M. DAvis, B. Dordoni, & R. Williams. Urinary D-glucaric acid assay by an improved enzymatic procedure. Clin. Chim. Acta. 51:47-51, 1974.

97. Bunker, M.L. & M. McWilliams. Caffeine content of common beverages. Research 74:31-33, 1979.

98. Bowes & Church. *Food values of portions commonly used*. 12th Ed., J.B. Lippincott Co., Philadelphia, 1975.

99. Review of Physiological Chemistry. 17th Ed., H.A. Harper, V.W. Rodwell, and P.A. Mayes editors, Lange Medical Publications, 1979, p. 551.

100. Bowen, P.E., N.H. Nguyen, and F.W. Thye. The effect of diet on daily D-glucaric acid excretion in young men. Am. J. Clin. Nutr. 35:50-55, 1982.

101. McLean, A.E. & H.E.Driver. Combined effects of low doses of DDT and phenobarbital on cytochrome P450 and amidopyrine demethylation. Biochem. Pharmac. 26:1299-1302, 1977.

102. Johansson, S.L. Carcinogenicity of analgesics: long-term treatment of Sprague-Dawley rats with phenacetin, phenozone, caffeine, and paracetamol. Int. J. Cancer 27: 521-529, 1981.

103. Miller, D.S. & P. Mumford. The nutritive value of western vegan and vegetarian diets. Pl. Fds. Hum. Nutr. 2:201-213, 1972.

104. Buchanan, N., C. Eyberg, and M.D. Davis. Antipyrine pharmacokinetics and D-glucaric acid excretion in Kwashiorkor. Am. J. Clin. Nutr. 32:2439-2442, 1979.

105. FAO & WHO. Plant production and protection paper #15: Pesticide residues in food. Rome, 1979.

106. Johnson, R.D. and D.D. Manske. Pesticides in food and feed. Pesticide Monit. J. 11:116-125, 1977.

107. Stein, E.R., C.M. Heald, & A.T. Murray. Residues of Fensulfothian in selected vegetable crops. J. Agric. Food Chem. 28:164-167, 1980.

Appendix A

FOOD AND DRUG INTAKE RECORD

FOOD AND DRUG INTAKE RECORD DAY: Sat Sun Mon Tues Wed (circle one) CODE# ____/____/____

(Record starts when you get up in the morning. Add night-time snacks, drinks, drugs.)

Food Code (leave blank)	Time	Location	Description of Food & Beverages (include preparation)	Amount	Description of Drug Taken	Amount

Did you remember to record everything?

Please answer these questions.*

1. Have you had any exposure to pesticides, solvents or other chemicals today?
   Yes No Don't know
2. Was today's intake different from your usual habits? Yes No Don't know
3. Did you engage in any unusually strenuous exercise today? Yes No Don't know
4. Are you on a special diet?
   Yes No Don't know

*If you answered Yes to any above please explain on back.

Appendix B

POPULATION DATA

The following table gives all the raw data obtained on the 142 non-medicated, healthy subjects who were included in the final analyses. The headings are defined as follows:

DGA:	micromoles D-glucaro-1,4-lactone/day
CREAT:	grams creatinine/day
TOBAC:	average number of cigarettes/day
VEGIE:	0 = non-vegetarians
	1 = vegetarians
MARIH:	marijuana groups:
	1&2 = < 3 times used within the last month
	3 = at least once per week
	4 = used daily
CANCER:	1 = those without family cancer incidences
	2 = those with family cancer incidences
SEX:	0 = male
	1 = female
DAYS:	number of days of complete food records
DGAR:	micromoles DGL/gram creatinine
KGWT:	kilogram body weight
CAFWT:	milligrams caffeine/KGBW/day
ALCOWT:	grams alcohol/KGBW/day

OBS	DGA	CREAT	TOBAC	VEGIE	MARIH	CANCER	SEX	DAYS	DGAR	KGWT	CAFWT	ALCOWT
1	19.5	3.23	0	0	1	2	0	5	6.04	75.0	3.43	0.00
2	12.2	1.92	0	0	1	1	0	5	6.35	63.6	0.00	0.00
3	12.5	1.90	0	0	1	1	0	5	6.58	65.9	0.00	0.00
4	16.5	2.29	0	0	3	2	0	5	7.21	73.6	0.90	0.00
5	11.5	1.59	0	0	1	2	0	5	7.23	88.6	1.69	0.31
6	19.0	2.56	0	0	1	2	0	4	7.42	90.9	0.77	0.00
7	14.0	1.79	0	1	3	1	0	5	7.82	72.7	0.00	0.00
8	16.0	2.03	0	0	3	1	0	5	7.88	79.6	0.07	0.00
9	15.5	1.94	0	0	1	1	0	5	7.99	84.1	0.24	0.19
10	26.0	3.14	0	0	1	1	0	5	8.28	78.2	1.15	0.19
11	17.0	2.05	0	0	1	1	0	5	8.29	68.2	0.85	0.00
12	14.1	1.67	0	0	1	2	0	5	8.44	77.3	1.06	0.09
13	12.3	1.42	5	0	1	2	0	5	8.66	65.9	1.06	0.22
14	18.5	2.13	0	0	1	2	0	5	8.69	61.4	2.28	0.07
15	19.5	2.22	0	0	3	2	0	5	8.78	95.5	0.72	0.29
16	22.0	2.50	0	0	1	2	0	5	8.80	72.7	0.14	0.44
17	15.5	1.73	0	0	1	1	0	5	8.96	75.0	1.33	0.38
18	15.2	1.68	0	0	1	1	0	5	9.05	72.7	1.65	0.00
19	18.0	1.97	0	1	1	1	0	5	9.14	75.9	3.36	0.09
20	15.0	1.64	0	0	1	2	0	5	9.15	78.6	0.38	0.00
21	18.5	1.98	0	0	1	1	0	5	9.34	66.8	0.84	0.02
22	21.5	2.30	0	0	1	2	0	5	9.35	72.7	0.96	0.23
23	12.6	1.32	0	0	1	2	0	4	9.55	100.0	0.15	0.29
24	13.5	1.38	0	0	1	1	0	5	9.78	70.5	1.14	0.26
25	10.4	1.06	0	0	2	2	0	5	9.81	74.1	0.97	0.30
26	29.0	2.92	0	0	1	1	0	5	9.93	79.5	0.62	0.00
27	22.5	2.24	0	0	1	2	0	5	10.04	79.5	1.71	0.00
28	19.0	1.89	0	0	1	2	0	5	10.05	88.6	0.25	0.00
29	23.0	2.23	0	0	1	2	0	5	10.31	88.2	3.02	0.12
30	23.5	2.23	0	0	2	1	0	5	10.54	72.7	1.24	0.12
31	18.5	1.75	2	0	3	2	0	5	10.57	73.6	0.14	0.00
32	12.0	1.12	0	0	2	1	0	5	10.71	68.2	0.00	0.34
33	29.5	2.74	0	0	1	1	0	5	10.77	83.6	0.26	0.00
34	24.5	2.23	0	0	1	1	0	5	10.99	93.2	0.58	0.00
35	11.0	1.00	0	0	2	1	0	5	11.00	75.0	0.21	0.08
36	24.0	2.18	0	0	1	2	0	5	11.01	70.5	1.48	0.23
37	16.2	1.46	0	0	1	2	0	5	11.10	79.5	1.61	0.62
38	16.1	1.45	0	0	4	2	0	5	11.10	67.3	1.64	0.35
39	30.0	2.70	0	0	1	1	0	5	11.11	88.6	0.68	0.35
40	11.5	1.02	0	0	1	2	0	5	11.27	64.5	3.30	0.20
41	22.5	1.98	0	0	1	2	0	5	11.36	75.0	1.76	0.00
42	24.3	2.12	0	0	1	1	0	5	11.46	90.9	2.32	0.09
43	22.5	1.96	2	0	1	2	1	4	11.48	54.5	0.55	0.10

OBS	DGA	CREAT	TOBAC	VEGIE	MARIH	CANCER	SEX	DAYS	DGAR	KGWT	CAFWT	ALCOWT
44	17.5	1.52	0	0	1	1	0	5	11.51	80.9	0.51	0.13
45	26.5	2.28	0	0	1	1	0	5	11.62	72.7	0.25	0.00
46	24.7	2.11	0	0	1	1	0	5	11.71	77.7	2.32	0.00
47	20.0	1.67	0	0	1	2	1	5	11.98	63.6	1.82	0.08
48	26.0	2.16	5	0	4	1	0	5	12.04	73.6	0.62	0.46
49	18.3	1.52	0	1	1	2	0	5	12.04	70.5	0.88	0.08
50	19.2	1.59	0	0	1	1	0	5	12.08	97.7	3.94	0.16
51	25.0	2.07	5	0	1	2	0	5	12.08	65.9	0.15	0.32
52	10.0	0.82	0	0	1	1	0	3	12.20	70.5	0.23	0.58
53	24.0	1.95	0	0	2	2	0	5	12.31	81.8	1.59	0.07
54	28.5	2.30	0	0	1	1	0	5	12.39	68.2	0.88	0.00
55	16.4	1.29	0	0	2	2	0	5	12.71	65.9	0.33	0.33
56	18.7	1.45	0	0	1	1	0	5	12.90	98.6	1.52	0.14
57	20.0	1.55	0	0	2	2	0	5	12.90	65.9	1.61	0.07
58	28.6	2.20	2	0	1	3	0	5	13.00	81.8	1.17	0.20
59	20.5	1.57	0	0	1	1	1	5	13.06	49.1	2.16	0.00
60	14.5	1.11	17	0	2	1	0	5	13.06	70.5	1.42	0.04
61	25.0	1.91	0	0	1	2	1	5	13.09	68.2	1.48	0.00
62	23.8	1.81	0	0	2	1	0	5	13.15	72.7	2.30	0.44
63	15.4	1.17	5	0	1	1	0	5	13.16	75.0	6.09	0.34
64	29.0	2.19	0	1	4	1	1	5	13.24	50.0	2.10	0.27
65	26.0	1.95	0	0	3	1	0	5	13.33	70.5	1.45	0.04
66	14.0	1.02	0	0	1	2	0	5	13.73	67.3	2.97	0.13
67	17.0	1.23	2	0	1	2	0	5	13.82	68.2	0.44	0.09
68	15.6	1.12	0	0	2	2	0	5	13.93	86.4	0.81	0.65
69	24.5	1.75	0	0	2	1	0	5	14.00	75.0	1.44	0.00
70	31.5	2.25	0	0	4	2	0	5	14.00	76.4	1.62	0.53
71	17.8	1.27	0	0	3	1	0	5	14.02	90.9	0.64	0.52
72	11.5	0.82	0	0	1	2	0	5	14.02	68.2	5.87	0.23
73	19.8	1.41	0	0	1	2	0	5	14.04	84.1	0.00	0.22
74	33.0	2.34	0	0	4	3	0	5	14.10	111.4	1.41	0.01
75	31.7	2.24	0	0	1	2	0	5	14.15	77.3	2.14	0.31
76	25.5	1.80	0	0	1	2	0	5	14.17	70.5	1.14	0.04
77	17.6	1.23	2	0	2	1	0	5	14.31	69.1	0.58	0.53
78	30.0	2.09	0	0	1	1	0	5	14.35	79.5	1.89	0.00
79	16.8	1.16	2	0	1	1	0	5	14.48	95.5	1.76	0.08
80	19.0	1.31	2	1	1	2	0	5	14.50	81.8	3.52	0.00
81	21.4	1.47	0	0	1	2	1	5	14.56	65.9	0.85	0.13
82	32.5	2.22	2	0	1	1	0	5	14.64	84.1	1.58	0.00
83	28.0	1.89	0	0	1	2	0	3	14.81	79.5	1.51	0.05
84	28.5	1.91	0	0	3	1	0	5	14.92	68.2	1.26	0.50
85	24.0	1.59	0	0	1	2	1	5	15.09	59.1	0.17	0.00
86	16.1	1.06	0	0	1	2	1	5	15.19	61.4	1.53	0.00

OBS	DGA	CREAT	TOBAC	VEGIE	MARIH	CANCER	SEX	DAYS	DGAR	KGWT	CAFWT	ALCOWT
87	27.5	1.81	0	0	3	2	0	5	15.19	68.2	1.03	0.03
88	14.9	0.98	0	0	1	1	0	4	15.20	63.6	1.76	0.50
89	21.4	1.39	0	0	1	2	0	5	15.40	88.6	0.63	0.18
90	11.0	0.69	17	0	2	2	0	4	15.94	65.9	2.44	0.97
91	19.2	1.20	0	0	1	2	0	5	16.00	68.2	1.32	0.23
92	14.1	0.88	2	0	4	1	0	5	16.02	70.5	2.75	1.32
93	28.5	1.77	0	0	1	1	0	4	16.10	84.1	0.59	0.33
94	22.5	1.39	0	0	3	1	0	5	16.19	78.2	3.84	0.29
95	24.5	1.51	0	1	1	2	0	5	16.23	61.4	0.00	0.00
96	19.0	1.15	10	0	1	2	0	5	16.52	81.8	0.54	0.43
97	14.4	0.86	0	0	1	2	0	4	16.74	86.4	1.76	0.81
98	9.6	0.57	0	0	1	1	1	5	16.8	63.6	4.81	0.08
99	19.6	1.16	0	0	1	2	0	5	16.90	65.9	2.69	0.00
100	24.0	1.42	0	1	1	1	0	5	16.90	72.7	2.85	0.00
101	28.0	1.64	0	0	2	1	0	5	17.07	75.0	1.20	0.00
102	20.2	1.18	0	0	1	2	0	5	17.12	70.5	8.70	0.22
103	20.0	1.15	0	0	2	2	1	5	17.39	61.4	1.89	0.18
104	17.0	0.97	0	0	1	2	0	5	17.53	70.5	4.04	0.50
105	14.9	0.85	25	0	3	2	0	5	17.53	62.7	0.64	0.43
106	40.0	2.28	0	0	1	3	0	5	17.54	65.9	0.55	0.00
107	13.9	0.79	0	0	2	2	0	5	17.59	66.4	1.81	0.00
108	39.5	2.21	0	0	1	3	0	5	17.87	72.7	0.00	0.00
109	11.8	0.66	0	1	1	2	0	5	17.88	77.3	2.92	0.00
110	27.0	1.51	3	0	1	2	0	5	17.88	77.3	1.38	0.13
111	14.1	0.78	0	0	1	2	0	5	18.08	72.7	1.16	0.19
112	11.8	0.64	30	0	1	3	0	5	18.44	86.4	9.00	0.00
113	41.2	2.22	7	0	1	3	0	5	18.56	95.5	3.19	0.68
114	21.2	1.14	0	0	1	3	1	5	18.60	65.9	3.17	0.15
115	27.3	1.46	0	0	1	2	0	5	18.70	75.0	1.79	0.23
116	10.0	0.53	0	0	1	2	0	5	18.87	87.7	2.20	0.00
117	23.6	1.25	0	0	4	1	0	5	18.88	65.9	0.79	0.34
118	22.5	1.18	0	0	2	2	1	5	19.07	55.9	6.04	0.34
119	18.5	0.97	3	1	4	1	0	5	19.07	73.2	1.67	0.00
120	12.6	0.66	0	0	2	1	0	5	19.09	84.1	3.39	1.02
121	21.2	1.10	0	1	1	2	0	5	19.27	70.5	0.40	0.00
122	14.9	0.77	3	0	1	3	0	5	19.35	90.9	7.76	0.10
123	34.0	1.75	0	0	1	2	0	5	19.43	65.0	5.72	0.00
124	19.1	0.98	0	0	1	1	1	5	19.49	47.3	1.73	0.00
125	8.3	0.42	0	0	2	2	1	5	19.7	72.7	1.35	0.13
126	27.5	1.36	0	1	3	1	1	5	20.22	65.0	0.00	0.00
127	17.0	0.83	7	0	1	2	0	5	20.48	65.9	8.31	0.43
128	22.0	1.07	0	0	1	2	0	5	20.56	59.5	2.23	0.00
129	16.7	0.81	0	0	1	1	0	5	20.62	61.4	3.29	0.00

OBS	DGA	CREAT	TOBAC	VEGIE	MARIH	CANCER	SEX	DAYS	DGAR	KGWT	CAFWT	ALCOWT
130	29.5	1.43	0	0	1	1	0	5	20.63	56.4	5.84	0.00
131	12.2	0.59	0	0	2	2	1	5	20.68	56.8	3.94	0.14
132	21.0	0.96	0	1	1	2	1	5	21.8	43.2	1.16	0.34
133	17.3	0.78	0	0	2	2	0	5	22.18	72.7	2.72	0.26
134	11.6	0.52	17	1	3	2	0	5	22.31	65.9	5.61	0.60
135	17.7	0.79	0	1	1	1	0	5	22.41	65.4	0.00	0.00
136	18.4	0.81	0	1	1	2	0	5	22.72	68.2	0.79	0.27
137	18.6	0.78	3	1	1	1	1	5	23.85	55.9	2.29	0.00
138	44.5	1.76	10	0	1	1	0	5	25.28	83.6	6.23	0.00
139	22.5	0.85	0	1	1	2	0	5	26.47	65.9	0.00	0.00
140	35.4	1.09	0	0	1	2	0	5	32.48	79.5	1.56	0.00
141	21.4	0.65	25	0	1	3	0	5	32.92	63.6	13.2	0.91
142	33.7	0.95	7	0	4	1	0	5	35.47	63.6	4.4	0.12

Appendix C

SURVEY QUESTIONNAIRE

General Survey of Environmental Pollutants

In order to match your questionnaire answers with the other information we collect, we must have your assigned number code recorded on this questionnaire. Your number code is _____/_____/_____.

1. We are interested in knowing about your exposure to tobacco products as a possible stimulant for the particular enzyme system being studied. Please circle and fill in the appropriate answers.

   (a) Do you smoke cigarettes? No Yes _____brand name most used
   cigars? No Yes _____brand name most used
   pipe tobacco? No Yes _____brand name most used
   other? No Yes _____describe

   (b) If you smoke, on the average, how many do you smoke each day?

Cigarettes	Cigars	Pipefills
less than 1 per day	less than 1 per day	less than 1 per day
1-5	1	1
5-10	2	2
15-20	3	3
20-30	4	4
30+	5+	5+

   (c) If you smoke, do you regularly inhale the smoke into your lungs of the product you most frequently use?
   (1) Yes - most of the time
   (2) Occasionally - some of the time
   (3) No - I try not to

   (d) If you use other tobacco products, what other forms of tobacco do you use and how frequently do you use them?

	Occasionally	Often	Daily
Snuff	1	2	3
Chewing tobacco	1	2	3
Other _____	1	2	3

   (e) We are often exposed to tobacco smoke because a spouse, roommate or coworker is a smoker. Are you exposed to cigarette smoke on a regular basis even if you do not smoke tobacco products yourself?
   Yes
   No

2. During the past month, on the average, about how many cups/glasses of hot/iced coffee did you drink each day? Circle the appropriate letter.

   a. none or occasionally
   b. 3 or 4 cups a week
   c. 1 or 2 cups a day
   d. 3-5 cups a day
   e. 6-10 cups a day
   f. over 10 cups a day

   Was the coffee consumed usually: a. caffeinated, instant?
   b. caffeinated, brewed?
   c. decaffeinated, brewed or instant (e.g. Sanka)?

3. Please indicate the amount and kind of alcoholic beverages you consumed last month. Circle the number for each product that most closely corresponds to your intake. The meaning of each number is described in the enclosed box below.

0. None the entire month
1. 2-3 times a month
2. once or twice a week
3. 3-4 times a week
4. every day or nearly every day
5. 2 or 3 times a day

(a)	Beer	0	1	2	3	4	5
	Wine	0	1	2	3	4	5
	Mixed drinks	0	1	2	3	4	5
	Brandies (or cordials)	0	1	2	3	4	5

(b) How much of each alcoholic beverage do you consume at one "sitting" or occasion? (Assume that a drink consists of 1/2 cup wine or 12 oz. beer or 1 1/2 oz. whiskey or brandy. Circle the number of drinks or drink-equivalents you consume per drinking occasion.

beer (12 oz)	0	1	2	3	4	5	6+	varies between_____	and_____
wine (1/2 cup)	0	1	2	3	4	5	6+	varies between_____	and_____
mixed drink (1 1/2 oz)	0	1	2	3	4	5	6+	varies between_____	and_____
brandies (1 1/2 oz)	0	1	2	3	4	5	6+	varies between_____	and_____

(c) If you consume alcoholic beverages do you generally consume food with it?
Yes
No

4. During the past month, on the average, about how many cups/glasses of hot/iced tea did you drink? Circle the appropriate letter.

a. none or occasionally
b. 3 or 4 cups a week
c. 1 or 2 cups a day
d. 3-5 cups a day
e. 6-10 cups a day
f. over 10 cups a day

Which type of tea do you usually drink if you are a regular tea drinker?

a. herbal tea (specify brand or type)_____
b. oriental or green teas
c. regular or black teas (such as orange pekoe, Constant Comment, etc.)
Is this tea decaffeinated? ____Yes ____No

5. Do you have a wood-burning stove or fireplace in your home? ____Yes ____No
How many days did you use it last month?
a. none or occasionally
b. 1 or 2 times a week
c. 3 or 4 times per week
d. every day except a few

6. Are you a vegetarian?

(a) no
(b) Yes, I avoid red meat but eat chicken and fish
(c) Yes, I avoid all meats but eat eggs, dairy products and fish
(d) Yes, I avoid all meats and fish but eat eggs and dairy products
(e) Yes, I avoid all animal products including eggs and dairy products
(f) Yes, I consume only grains and fruit
(g) Yes, I follow a macrobiotic diet
(h) Other_____

Remembering that all answers are given anonymously or confidentially, please be as truthful as you can in filling out the following questions. It is important for us to establish some correlation between drug usage and the amount of glucaric acid in your urine.

7. Use of prescription medicine
(a) Over the past month, have you taken any prescription medications for any reason? (e.g. insomnia, colds or flu, nervousness, headaches, pain, seizures) _____Yes _____No

(b) If your answer was yes, name the medication and estimate the amount of medication you have taken by using the form below.

	Name of Drug	Number of days taken last month	Number of Doses or Pills Taken Each Day Used
Example	Ampicillin	10 days	3 tsp.
1.			
2.			
3.			
4.			
5.			

8. Use of non-prescription medication
(a) Over the past month, have you taken any non-presciption medications for any reason? (e.g. cough medicine, aspirin, antacids, vitamin supplements, laxitives, no doz, etc.) _____Yes _____No

(b) If your answer was yes, name the medication and estimate the amount of medication you have taken by using the form below.

	Name of Drug	Number of Days Taken Last Month	Number of Doses or Pills Taken Each Day
Example	Excedrin	20	4
1.			
2.			

3. _____ _____ _____

4. _____ _____ _____

5. _____ _____ _____

6. _____ _____ _____

7. _____ _____ _____

8. _____ _____ _____

9. Use of social drugs

Please check the appropriate box which indicates the relative frequency you have used the following types of drugs in the last month.

	never tried	tried but not used in the last month	used 1-5 times during the last month	used at least once a week	used several times a week	used daily or almost daily
Mari-huana or hash						
Inhalents (glue)						
Hallucino-gens (LSD, mescaline, DMT)						
Cocaine						
Heroine						
Stimu-lants (diet pills, speed, am-phetamines)						
Sedatives or tranqui-lizers (valium, quaaludes, phenobar-bital, nem-butol)						
Other nar-cotics (codeine, THC, opium)						

10. We are interested in knowing if you are exposed to any chemicals during working hours or while pursuing a hobby which might affect your health. Such chemicals may be organic solvents such as xylene, benzene, gasoline, carbon tetrachloride, acetone or agents used in farming such as insecticides and herbicides. Glues, binding agents and paints could also be included. Answer the following questions so that we can assess your level of exposure.

(a) Think through your activities for the last month. Write down the names of substances which you have handled or been exposed to in the last month. If you do not know the name write a description. We are chiefly interested in organic (carbon-based) compounds. Such compounds often have distinctive odors. If you are doubtful about whether your substances qualify, list them and let us decide.

Example acetone

1._____

2._____

3._____

4._____

5._____

6._____

7._____

8._____

9._____

10._____

(b) Place a star beside each substance you have listed in question 10(a) which you have been exposed to in the last two weeks.

(c) We would like to scale the extent of your exposure to the substances you have listed. We have drawn scales below and have identified descriptions of exposures which we suggest might place you in a high, medium, or low exposure category. These descriptions may not fit your circumstances but you likely can judge whether your exposure is fairly high or relatively low. Read the descriptions and rank by checking along the line your exposure to each chemical you have listed in question 10(a) in the order you listed them.

	Exposed daily or almost daily and ventilation is poor or skin is directly exposed (HIGH)	Exposed daily or almost daily; great caution is taken to protect from exposure (MEDIUM)	Exposed weekly or less and precaution is taken to protect from exposure (LOW)
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

11. We would also like to know about your previous history of exposure to organic substances such as pesticides, oil based paints, etc. Answer the following questions by circling the most appropriate answer.

(a) In your memory have you ever been exposed to organic substances for periods of 2 weeks or more?
_____Yes _____No

(b) Have you ever sprayed a cropfield with an herbicide or been downwind of a farm which does?
_____Yes _____No

(c) If you answered yes to either 11(a) or 11(b) how long ago did this exposure take place?
(1) less than 6 months
(2) 6 months to 1 year
(3) 1-5 years
(4) more than 5 years

(d) What was the extent of your exposure at that time? (Refer to question 10(c) for guidance on the definition of high, medium, and low.)
(1) high
(2) medium
(3) low

(e) If appropriate and you can still remember, name the substances to which you have been exposed to at high and medium levels.

_____ _____

_____ _____

12. Has anyone in your **family** had any form of cancer?
_____Yes _____No

Check the relatives who have had cancer in your family. Check only those genetically related to you. Place more than one check when say more than one grand parent has had cancer.

_____mother _____grandparent

_____father _____aunt or uncle

_____brother _____1st cousin

_____sister _____son or daughter

Use this space for any comments or clarifications on the questions you have answered.

The vita has been removed from the scanned document

D-GLUCARIC ACID: ITS POSITIVE ASSOCIATION WITH GENDER, TOBACCO, CAFFEINE, MARIJUANA, AND VEGETARIANISM IN HUMANS

by

Elizabeth Ellen Kyle

(ABSTRACT)

The urinary excretion of D-glucaric acid (DGA) has been used as a nonspecific measure of the induction of hepatic enzymes associated with drug metabolism in man. A survey of 124 nonmedicated men (18-56 years of age), who kept a 5-day food and beverage intake record and collected their total urinary output for the last three days of the five, was conducted to assess the relationship between DGA excretion and various dietary factors. Eighteen nonmedicated, healthy women collected the same data, but started recording on the eighth day from the commencement of menstruation. DGA was determined by an enzymatic assay and recorded as micromoles D-glucaro-1,4-lactone/gm creatinine. There was a positive association between total DGA excreted and the use of marijuana, caffeine, and tobacco products, the heaviest users excreting significantly higher levels of urinary DGA than the moderate or low users of the same substance. Analysis of variance of mean DGA excretion also revealed significant differences between females (17.0±3.7) and males (14.3±5.2); male vegetarians (17.4±5.5) and nonvegetarians

(13.9±5.1); and female vegetarians (19.8±4.6) and nonvegetarians (16.2±3.1). Alcohol consumption and family history of cancer incidences were not significantly related to DGA excretion in either sex. Multiple regression analyses revealed that vegetarianism and caffeine consumption were the two strongest predictors of DGA excretion, while alcohol and marijuana consumption affected DGA the least. These results indicate that dietary and environmental factors can exert a significant effect on DGA excretion, and these associations may identify dietary inducers of hepatic enzymes associated with xenobiotic biotransformations in humans.