THE SHORT-TERM EFFECTS OF DISULFIRAM (ANTABUSE™) TREATMENT ON NUTRITIONAL STATUS AND BLOOD CHOLESTEROL LEVELS IN ABSTAINING ALCOHOLICS

by

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Introduction

Only two of the approximately twenty alcohol-sensitizing compounds reported have received widespread therapeutic use, disulfiram (tetraethylthiuram disulfide) and calcium carbimide (citrated calcium carbimide.) Of these, only disulfiram has been approved for distribution in the United States (1). Sellers et al. (2) reported that ninety percent of physicians in private practice in this country prescribe drugs for the treatment of alcoholism, with disulfiram the drug of choice for aversion therapy. The goal of alcohol-sensitizing drug treatment is to deter alcohol consumption, thus allowing the patient to participate in accepted behavioral and psychosocial therapy (3).

Disulfiram (Antabuse™, Ayerst Laboratories) was first noted to cause an adverse reaction with alcohol ingestion by Hald and Jacobsen in 1948 (2). Originally, disulfiram was believed to be inert unless alcohol was present, however, several investigations have indicated the possibility of side effects not related to the ingestion of alcohol (1-5).

There is growing concern about potential nutrient-drug interactions among physicians, nutritionists, and pharmacists. Although alterations in serum cholesterol levels, pyridoxine levels, and tryptophan levels have been implicated with the use of disulfiram no studies to date have investigated the nutritional status of the alcoholic in treatment, receiving disulfiram therapy. Such information is vital in insuring the optimal nutritional well being of the alcoholic receiving

disulfiram treatment.

The present placebo-controlled, double-blind, study was undertaken specifically to examine the short-term effects (21 days) of disulfiram (Antabuse) therapy on the nutritional status and serum cholesterol levels (total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; and low-density lipoprotein cholesterol, LDL-C) of abstaining alcoholics. Increased knowledge pertaining to the use of disulfiram (Antabuse) in therapeutic doses (250 mg per day) will aid in determination of concrete benefits and (or) hazards in short-term and long-range therapy.

Review of Literature

Alcohol-Disulfiram Interaction

Disulfiram acts to inhibit aldehyde dehydrogenase (ALDH), the enzyme necessary for the oxidation of acetaldehyde to acetic acid. This inhibition develops approximately twelve hours after administration of the drug and is irreversible (5, 6). Regeneration of ALDH activity depends on de nova enzyme synthesis, thus the alcohol-sensitizing effect of disulfiram can occur up to ten days after cessation of the drug (1,3,5-8). The aversion effect for ethanol is produced when the disulfiram treated individual ingests alcohol and the blood acetaldehyde levels are raised producing the following: dyspnea, tachypnea, tachycardia, flushing, hypotension, headache, nausea, and vomiting (1,3-5,7,9-11).

Disulfiram Related Side-Effects

Several experimental studies have suggested that during short-term administration of therapeutic doses of disulfiram (250-500 mg per day) the alcoholic may experience a variety of side effects not related to alcohol intake. Peachey and Naranjo (3) in an extensive report on the role of drugs in the treatment of alcoholism, discuss several possible effects from the administration of disulfiram: Inhibition of ALDH, dopamine-B-hydroxylase and hepatic mixed-function oxidases (MFO), drug-induced neuropathy, hepatotoxicity, increased serum cholesterol, antithyroid action, neuroendocrine toxicity, and dysmorphogenic effects (hemoglobin, leucocytes, erythrocytes).

Sellers et al. (2) reported that disulfiram treatment could result in hepatotoxicity, hypertension, peripheral neuropathy, and increase in serum cholesterol levels.

Serum cholesterol

Major and Goyer (12) observed an elevation of serum cholesterol levels in alcoholic patients receiving 250 mg or 500 mg disulfiram daily for six weeks. Serum cholesterol increased significantly (193 +/- 16.4 to 227.2 +/- 17.2 mg/dl) after three weeks in those patients receiving 500 mg of disulfiram daily. No statistically significant changes in serum cholesterol were reported after administration of 250 mg of disulfiram daily for three weeks to eight male alcoholics. Determination of the effect of short-term (three weeks) disulfiram therapy on lipid subfractions was not done in these subjects. However, when pyridoxine was administered with disulfiram, serum cholesterol decreased markedly suggesting alterations in pyridoxine metabolism.

Carbon disulfide, the final metabolite of disulfiram, has been shown to produce hypercholesterolemia (9,12). An increase in arteriosclerotic cardiovascular disease from chronic carbon disulfide exposure has been observed in animals and humans (viscose rayon workers) (12). Increased serum cholesterol levels in humans receiving disulfiram are hypothesized to be a result of carbon disulfide induced pyridoxine deficiency (5,12,13,14). Toxic effects can be caused by 30 to 60 mg/m^3 carbon disulfide in the atmosphere which is roughly equivalent to 125 to 250 mg of disulfiram per day (12). Carbon disulfide

reacts with pyridoxine resulting in a pyridoxine deficiency (12-15), suggesting that disulfiram may have a similar effect. But, the mechanism involved in the relationship of increased serum cholesterol to pyridoxine deficiency is unclear.

In an investigation by Perier et al. (16) alcoholics receiving disulfiram and a challenge dose of alcohol daily during a four week detoxification program followed by a weekly dose of disulfiram for one year demonstrated an increase in serum cholesterol (mean-235 mg/dl at 4 weeks to 263 mg/dl at 12 mo., baseline range-135 to 231 mg/dl), increase in LDL-C (mean-168 mg/dl at 4 weeks to 218 mg/dl at 12 mo.), and a decrease in HDL-C (mean-61 mg/dl at 4 weeks to 47 mg/dl at 12 mo.), although this latter lipid subfraction was still significantly high. However, the effects of the alcohol challenge and effects of disulfiram in this study are difficult to seperate.

Rogers and Naseem (17) added 15 mg of disulfiram (per kg of body weight) daily to the diet of twenty Sprague-Dawley rats to elucidate the mechanism of disulfiram-induced hypercholesterolemia. Serum cholesterol increased twenty-five percent after three weeks of disulfiram treatment with a four-fold elevation of hepatic HMG-CoA (3-hydroxy-3-methyl-glutaryl coenzyme A) reductase, the rate limiting enzyme involved in cholesterol biosynthesis. This study clearly showed that the increase in serum cholesterol was not due to variance of dietary intake between test groups and suggested that chronic administration of disulfiram might increase the risk of atherosclerosis and bilary complications.

Triglycerides

"Alcoholic Hypertriglyceridemia" from active alcohol use and subsequent decrease in triglyceride (TG) levels with abstinence, has been extensively reported in the literature (8, 18-20). However, Mach and Janik (13) found a significant elevation of hepatic tissue TG and blood serum TG and cholesterol in male Wistar rats after six weeks of disulfiram administration (150 mg/kg body weight/day), suggesting a need for further study of the effect of disulfiram therapy on triglycerides.

Neurological changes

Mogens et al., (15) reported that disulfiram treatment resulted in subnormal serum tryptophan levels after administration of an oral load of L-tryptophan (100 mg/kg body weight). Serum tryptophan levels six hours after load were 11.9 +/- 1.2 mg/dl in disulfiram treated alcoholics and 14.8 +/- 0.7 mg/dl in non-treated abstaining alcoholics. No differences in fasting serum tryptophan levels were observed, suggesting that disulfiram treated individuals had more rapid removal of tryptophan, an amino acid essential for normal neuromuscular activity, from the blood. Neurological abnormalities have been reported after a few days of disulfiram therapy with some symptoms surfacing after five years of continual usage. However, most symptoms of peripheral neuropathy begin four to six months after initiation of disulfiram therapy (15,21,22). Worner (21) found that most patients who developed peripheral neuropathy (symptomatic of pyridoxine and thiamin deficiency) after one year of 250 mg disulfiram daily,

could resolve the adverse effects with decrease of daily dose to 125 mg. These patients were receiving 300 mg thiamin, 50 mg pyridoxine, and one multi-vitamin daily, which may have been the primary cause of overt symptom resolution rather than decreased dosage of disulfiram.

Cardiomyopathy and generalized myopathy

Major et al. (23) found in their investigation on the effects of prolonged disulfiram treatment in Rhesus monkeys, a marked increase in plasma dopamine-B-hydroxylase (50% increase after six weeks administration of 300 mg/kg body weight), the enzyme necessary for conversion of dopamine to norepinephrine. Disulfiram, as an irreversible inhibitor of aldehyde dehydrogenase, significantly raises blood acetaldehyde, an effective releasor of catecholamine. Thus, this study suggests that disulfiram may directly effect the sympathetic nervous system by increasing sympathetic activity, increasing both diastolic and systolic blood pressure, and prolonging the possible effects of malnutrition and (or) alcoholism on the development of cardiomyopathy (23-25).

Of interest is an investigation by Ekvarn et al. (26) on 30 Sprague-Dawley rats given 120 mg disulfiram/kg body weight daily in water suspension and 30 controls receiving water only. Myocardial degeneration in disulfiram treated rats was characterized by swelling, disintegration and fatty infiltration of the muscle fibers, as was expected. In addition, degenerative changes in biceps, triceps brachii, quadriceps, and gastrocnemius muscles were observed in all animals receiving disulfiram

for either seven or fourteen days.

Effects of Alcohol on Nutritional Status

Coronary heart disease

The relationship between alcohol and increased high-density lipoprotein cholesterol (HDL-C) levels has been the subject of many recent investigations since this cholesterol fraction is believed to be a positive factor in the reduction of risk for atherosclerosis. Alcohol consumption is positively associated with HDL-C and appears to be dose related (18). Data from the five populations participating in the Cooperative Lipoprotein Phenotyping Study (18) shows a positive correlation between high HDL-C levels and decreased incidence of coronary heart disease (CHD). The results of this study, as reviewed by Barborik, Gruchow, and Anderson (27), implicate low HDL-C levels as the major predictive risk factor for CHD. However, they also point out that to accurately analyze this relationship between alcohol consumption and HDL-C levels, epidemiological studies must be conducted on the extent of drinking in the United States.

Data from the Framingham Study indicates that moderate male drinkers (4-9 oz/week) had increased mean serum HDL-C (50.14 mg/dl) compared to abstainers (45.69 mg/dl; 18). Hulley, Mellon, and Dzvonik (28) report a U-shaped association between alcohol consumption and CHD. This suggests that the highest risk of CHD is found in abstainers and chronic abusers with the lowest risk among moderate drinkers (up to approximately 3 "drinks" or 60 ml alcohol per day). However, epidemi-

ological studies on blood lipids and coronary risk factors in 7,735 British and 520 Italian men, while showing a positive correlation between daily alcohol intake and HDL-C levels, found no statistical significance between daily alcohol intake and decrease in positive risk factors; i.e., systolic and diastolic blood pressure, body mass, cigarette smoking, and physical activity (19,29). Even though moderate intake of alcohol has been shown to increase HDL-C levels and fibrinolytic activity, both possible contributing factors to a lower risk of CHD, Segel et al. (24) believe that certain individuals may be unable to restrict their intake to moderate amounts of alcohol, thus counteracting any significant prevention of CHD.

Lipid interactions

High-density lipoprotein cholesterol

The precise mechanisms by which alcohol influences serum high-density lipoprotein cholesterol (HDL-C) are poorly understood. Taskinen et al. (30), Marth et al. (31), and Devenyi et al. (32) all concur that serum HDL-C levels fall to control levels within one to three weeks of abstinence. Of ninety-two patients entering an inpatient treatment facility with a primary diagnosis of alcoholism, 20 percent of males and 57 percent of females evidenced high levels of serum HDL-C. At the end of three weeks of abstinence only one subject was above the upper limit of non-alcoholic control levels (controls-males, 48 +/-14 mg/dl; females, 56 +/- 13 mg/dl; 33). In a more recent study, Haskell et al. (34) found in their investigation of HDL-C subfractions (HDL-C_2 and HDL-C_3) in moderate

drinkers (1.3 +/- 0.6 oz/day), a decrease in HDL-C_3 mass after five weeks abstinence (226.5 +/- 50.3 mg/dl from baseline; 261.5 +/- 49.0 mg/dl), and subsequent elevation in this subfraction (265.0 +/- 49.2 mg/dl) after resumption of moderate alcohol intake. They reported no significant effects from either abstinence or return to alcohol intake on HDL-C_2 mass, the supposed antiatherogenic subfraction. These findings suggest that the proposed protective effect of alcohol on the development of CHD is not mediated by subfraction HDL-C_2 and may be completely unrelated to HDL-C levels.

Triglycerides

Sixty to ninety percent of diagnosed alcoholics have disturbed lipid metabolism affecting both cholesterol and triglyceride (TG) levels (13). Alcohol enhances TG production in the liver and excessive alcohol intake leads to hypertriglyceridemia. Decreased lipid oxidation and increased lipogenesis can be linked to alcohol oxidation and subsequent increase of NADH (reduced form of nicotinamide adenine dinucleotide, NAD). In the liver the NADH/NAD ratio favors the accumulation of hepatic triglycerides by trapping fatty acids through raised concentration of a-glycerophosphate (8). In agreement, were the findings from an investigation by Erkelens and Brunzell (20) undertaken to determine the etiology of hypertriglyceridemia as related to chronic alcohol intake. They evaluated the TG level and removal rate before and after isocaloric replacement of alcohol for the basal diet. Endogenous TG removal rate as related to lipoprotein lipase activated degredation was

estimated by the heparin infusion method. Results indicated TG production was affected, not rate of removal.

Type IV hyperlipoproteinemia (hypertriglyceridemia with elevated very low density lipoproteins, VLDL) is associated with excessive alcohol intake. Marth et al. (31) found that while chronic alcoholics have increased TG levels over controls (135 +/- 52 mg/dl vs. 104 +/- 29 mg/dl, respectively), TC and LDL-C levels for alcoholics were significantly lower than controls at baseline. This suggests that both hepatic TG and intestinal VLDL production may be stimulated by chronic alcohol intake (7,8,20,31).

Alcohol-Nutrient Interactions

The literature is abundant on the nutritional effects of alcohol ingestion. Roe (35) states in Alcohol and the Diet that the effect of alcohol on nutrition is a result of the hypnotic (psycho-active) effect of the drug leading to anorexia and subsequent decrease in nutrient intake, interference with nutrient utilization and absorption, direct toxic effect of ethanol on tissues, and socioeconomic factors.

Eisenstein (7) has cited the following possible results of excessive alcohol intake: glucose abnormalities (alcoholic hypoglycemia and hyperglycemia), alcoholic hyperlipemia, hepatomegaly, hemolytic anemia, acute pancreatitis, alcoholic cirrhosis, low serum albumin (low immunocompetence), amino acid abnormalities (increased aromatic: tryptophan, phenyl-alanine, tyrosine; depressed branched-chain: valine, leucine, isoleucine), peripheral neuropathy, impaired nutrient absorption, and

various other malabsorptive problems. All, or any, of these may lead to a compromised nutritional status.

Abnormal Carbohydrate Metabolism

The effects of alcohol consumption on carbohydrate metabolism have been well delineated during the past several years. While many investigations have implicated chronic alcohol use in decreased insulin response to oral glucose load some investigators suggest that a decrease in insulin production may be secondary to decreased food intake (7).

More commonly seen is alcohol-induced hypoglycemia resulting from inhibition of gluconeogenesis. The inhibition process (or site) in humans has not been defined, but animal studies have positively correlated this inhibition to the reduction of the NAD/NADH ratio related to alcohol metabolism. Thus, severe decrease of nutrient intake leading to acute depletion of hepatic glycogen (i.e., starvation) in concert with chronic alcohol ingestion will impair hepatic glucose output in animals through decreased gluconeogenesis (36).

Arkey, Veverbrants, and Abramson (37) reviewed five cases related to alcohol-induced hypoglycemia. Each of these patients were alcoholic diabetics receiving oral hypoglycemic agents. In all of these patients alcohol seemed to enhance the hypoglycemic effect; mean admitting non-fasting blood glucose 28.0 +/- 10.9 mg/dl. In a subsequent study on healthy males given infusions of 15% ethyl alcohol in saline (2.0 ml/min [236 mg ethyl alcohol/min]) or saline for one hour, followed by 0.1 units of glucagon-free insulin/kg/body weight, they

found similar response among subjects in initial fall from pretest glucose levels, but the rebound phase was significantly depressed in the alcohol-insulin subjects. This study indicates that alcohol interferes with the feed-back mechanisms in insulin-induced hypoglycemia by prolonging dose effect and retarding the normal rise in growth hormone secretion (7,37).

Malnutrition

In a recent publication, Roe (38) summarizes the etiologies of malnutrition in the alcoholic as complex and usually associated with prior alcohol insult to the tissues, in combination with predisposition for certain nutritional deficiencies. Lieber (39), classifies malnutrition in alcoholism as either primary or secondary; primary related to low intake of nutrients due to (in accordance with Roe; 35,38) the anorexic effect of alcohol, majority of limited resources spent on alcohol, and low nutrient value of alcoholic bevererages. The etiology of secondary malnutrition is related to a variety of direct toxic effects of alcohol on the utilization and metabolism of nutrients.

The incidence of malnutrition among the alcoholic population is undetermined. In a study of 62 middle-class alcoholic patients, for whom the mean daily caloric contribution from alcohol and non-alcohol foods was 2,000 kcal, only 12 percent were less than ideal body weight (40). In agreement with this study, Dickson et al. (41) found no severe nutritionnal deficiencies in their investigation of visceral protein status in twenty-five alcoholic inpatients, free of liver disease.

However, several other investigations indicated an increased incidence of protein malnutrition with multiple vitamin deficiencies in lower-income alcoholic populations (40).

Protein. Utilizing anthropometric measurements (height/weight index, triceps skin-fold, arm circumference), dietary reviews, and serum analysis (albumin and transferrin) Simko et al. (42) studied 102 alcoholics with and without liver disease to determine nutritional status. They found that regardless of adequate protein intake, alcoholics (without liver disease) had markedly lower triceps skin-folds (7.4-7.6 mm vs. 12.1 mm in abstainers), body weight (67.3-72.0 kg vs. 80.7 kg in abstainers), and arm muscle circumference (264.0-264.2 mm vs. 297.0 mm in abstainers) than abstainers. These investigators concluded that the poor nutritional status of alcoholics was probably due to the direct toxic effects of alcohol and not decreased intake of dietary calories. However, the prevalent type of malnutrition seen in alcoholics is protein-calorie malnutrition (PCM) often referred to as "Adult Marasmus." Mendenhall et al. (43) found in an investigation of 284 patients with alcoholic hepatitis and 21 alcoholic patients without liver involvement that all patients with liver disease and 62% of patients without evidenced some degree of either marasmus or kwashiorkor. With excessive chronic alcohol intake approximately 20 to 25% of ingested alcohol may be metabolized through a secondary pathway, the hepatic microsomal ethanol oxidizing system (MEOS), delivering less than 7.1 kcal/gm and believed to be an energy wasting process (7,35,44).

Malnutrition is associated with altered RNA (ribonucleic acid) metabolism and a decrease in the synthesis and secretion of serum albumin, a major product of protein synthesis by the liver (45). Serum albumin levels can be severely depressed during trauma, malnutrition, and in conjunction with alcohol related liver disease. To elucidate the effects of chronic alcohol intake on albumin production, livers from fed and fasted rabbits were measured for albumin synthesis 45 to 75 minutes after perfusion with 200 mg% alcohol. From this investigation, Rothschild, Oratz, and Schreiber (45) reported that fasting depressed albumin production and disaggregated the endoplasmic bound polyribosomes. With refeeding, normal albumin synthesis was associated with reggregation of bound proteins. They observed the same results in chronic exposure to and subsequent cessation of alcohol. Reduced albumin synthesis could be reversed in the animal model by delivery of therapeutic amounts of selected amino acids (arginine, ornithine, tryptophan, lysine, phenyl-alanine, alanine, threonine, proline, and glutamine) to the liver. Chronic excessive alcohol use is the primary cause of cirrhosis. However, the loss of hepatic parenchymal cells is responsible for impaired protein synthesis (7). The effects of alcohol and fasting were similar in the previous study which implies that alcohol may act as a pharmacologic fast (45).

Plasma amino acid abnormalities are not uncommon in alcoholics as low protein intake associated with chronic alcoholism depresses branched-chain amino acids (valine, leucine, and

isoleucine) and chronic alcoholism (without cirrhosis) tends to increase the synthesis of these amino acids (39). The evidence from several investigations indicates that alcohol related depression of circulating branched-chain amino acids has a multiple etiology. Shaw and Lieber (46) hypothesized, however, that the primary cause of depressed branched-chain amino acids in subjects without overt liver involvement, was dietary protein deficiency.

Cardiomyopathy and generalized myopathy. Acute alcoholism (chronic excessive alcohol intake) has been associated with heart disease, and may result in alcoholic cardiomyopathy (congestive cardiomyopathy) from the direct toxic effect of alcohol on the heart (24). Rossi (25) argues, however, that the relationship of alcohol abuse and cardiomyopathy is a result of associated dietary deficiencies. The results of his animal investigation indicate that continued exposure to catecholamines (dopamine, epinephrine, and norepinephrine), positively correlated with malnutrition, may play the major role in increased development of cardiomyopathy.

Vitamin and Mineral Deficiencies

Deficiencies of folate, vitamin B-12 (cobalamin), thiamin, and (or) vitamin B-6 (pyridoxine) have all been observed in subjects with chronic alcohol consumption (7,35,38,40,42,44-49). Decreased hepatic concentrations of vitamins are often found in subjects with fatty liver due to decrease in storage space as a result of deposition of fat and fibrous tissue. Alcohol-induced catabolic losses of many vitamins and cellular

degeneration may also reduce hepatic concentrations. Thus, vitamin and mineral deficiencies in alcoholics may result from reduced intake, alterations in metabolism and absorption, decreased storage, and hyperexcretion (35,44,50). Halsted (48) and Mezy (50) found that a significant number of alcoholic subjects without overt liver disease (fatty liver or cirrhosis) also evidenced malabsorption of folic acid, thiamin, and vitamin B-12 without overt clinical signs of deficiency. This malabsorption seemed to correlate positively with recent excessive alcohol intake, and resolved after two to three weeks abstinence combined with an adequate diet.

Folate deficiency. Folate deficiency (the vitamin most commonly deficient in the alcoholic) results in intestinal malabsorption. Eisenstein (7) and Roe (35) list the primary histological changes occurring in the intestinal mucosa from folate deficiency as shortened villi, reduced thickness of intestinal mucosa, and reversible megaloblastic changes in the crypt epithelium of the villi. These changes will adversely affect the absorption of other nutrients.

Jejunal uptake of folic acid (pteroylmonoglutamate [Pte-Glu]) was studied in four chronic alcoholic patients; two patients were maintained on a folate-deficient diet with 256 gm alcohol per day, one patient on a folate-deficient diet with no alcohol dose, and one patient on a folate-adequate diet with addition of 300 gm alcohol per day. Halsted (48) found that compared to baseline values, jejunal uptake of ^{3}H-Pte-Glu, glucose, and sodium were decreased in folate-deficient alcohol

dose patients. Water and sodium uptake were decreased and uptake of ^{3}H-PteGlu and glucose remained unchanged in single challenge patients, indicating that dietary folate deficiency in combination with chronic alcohol intake affects more than one transport/absorption mechanism. Thus a cyclic effect may occur whereby folate malabsorption contributes to folate deficiency which in turn promotes intestinal malabsorption of folate.

Values taken from a study by Mills et al. (47) on twenty-three alcoholic patients, expressed as a percentage of the recommended daily intake for groups of people in the United Kingdom, showed 78% of patients with less than 60% of recommended folate intake. They also reported low levels of serum folate (43% of patients) and red cell folate (13% of patients). In addition to low dietary intake of folate, alcohol exerts a toxic effect on the gut reducing the absorption of several vitamins including folate, thiamin, and vitamin B-12 (38).

Folate, vitamin B-12, and pyridoxine are necessary for adequate cell replication. The primary cause of megaloblastic anemia (commonly identified with chronic excessive alcohol use) is folate deficiency with occasional secondary effect from vitamin B-12 deficiency (50,35). The incidence of folate deficiency in chronic alcoholism has been reported to be as high as 87%, as determined by low serum folate levels (48,50), with an increase of mean corpuscular volume MCV) found to be directly related to folate and (or) vitamin B-12 deficiencies. Mildly elevated (100-110 cuu; normal value 87-103 cuu) MCV has

been found in 25 to 96% of alcoholic patients and is frequently used as a diagnostic marker of alcoholism (49,51).

Vitamin B-12 deficiency. Alcohol exerts a toxic effect on the production of intrinsic factor, a protein synthesized in the gastric mucosa and required for intestinal absorption of vitamin B-12. But pernicious anemia, a "nutritional anemia" caused by vitamin B-12 deficiency, is seen infrequently in alcoholics (35,49,52). Apparently the major contribution of vitamin B-12 deficiency is the enhancement of folate deficiency through the methyl-folate-trap (49,52). This premise is based on the hypothesis that as a result of vitamin B-12 deficiency, folate is trapped and accumulates as 5-methyltetrahydrofolate (5-methyl THF). And, etiologically implicates chronic alcohol intake with pernicious, megaloblastic, and sideroblastic (accumulations of erythroid iron strongly associated with folate deficiency) anemias (49).

Materials and Methods

Patients

Thirty-one patients, admitted to the Chemical Problems Treatment Unit (CPTU) of Colmery-O'Neil Veterans Administration Medical Center for treatment of primary alcoholism, voluntarily participated in this investigation. This study was approved by the Committees on Research Involving Human Subjects at Kansas State University and the Colmery-O'Neil Veterans Administration Medical Center (CO-VMAC).

All consecutive admissions with a primary diagnosis of alcoholism were considered and routine vitamin supplementation was withheld until completion of diagnostic blood work. Subjects were alcohol and abuse drug free for a minimum of three days and without hepatic and (or) pancreatic diseases as determined by standard laboratory tests, medical history, and physical examination.

After initial blood work, subjects received 100 mg thiamin orally once a day for 30 days, 1 mg folic acid orally once a day for 7 days, and 1 multi-vitamin (Stresstabs Tm 600 Advanced Formula High-Potency Stress Formula Vitamins, Lederle Laboratories Division, American Cyanamid Company) once a day for 30 days. Four hundred mcg folic acid and 12 mcg vitamin B-12 (as cyanocobalamin), two variables studied in this investigation, were included in the multi-vitamin preparation.

After obtaining informed consent the subjects were randomly assigned to one of two treatment groups; disulfiram or placebo for 21 days. Nutritional assessment measurements and

laboratory tests were performed at the beginning and end of the 21-day study period.

Study Medication

The study medication and placebo were prepared by the hospital pharmacy. Disulfiram was prepared daily for oral administration by pulverizing and dissolving the treatment dose (Antabuse tablet-250 mg disulfiram) in 4 oz of canned unsweetened orange juice (Juice Bowl Products, Inc.). Subjects in the placebo group received 4 oz unadulterated canned unsweetened orange juice. Study medication (or placebo) was taken under supervision and neither the subject nor the nursing staff were aware of who received disulfiram or placebo.

Nutritional Assessment

Dietary History

A dietary history including 24-hour diet recall, food frequency, socioeconomic variables, known food allergies and (or) intolerances, and nicotine and alcohol use, was taken at the beginning of study participation. Adequacy of recent dietary intake (24-hour recall) was estimated using the revised Daily Food Guide (53).

Anthropometry

Anthropometric measurements including height, weight, mid-arm circumference (MAC), triceps skinfold (TSF), subscapular skinfold (SSF), and thigh skinfold (THSF) were taken at the beginning and end of the 21-day study period (54-57). All measurements were taken by the same investigator; mid-arm

circumference was obtained in cm from the right arm of each subject, with an insertion tape (Ross Laboratories, Columbus, OH) and skinfolds were measured in cm using a Lange Skinfold Caliper (Cambridge Scientific Industries, Cambridge, MD; 57, 58). Mid-arm muscle circumference (MAMC) was calculated from TSF and MAC as follows:

MAMC = MAC - (3.14 X TSF [in centimeters]).

A nomogram for calculation of total body fat in males was used to estimate percent body fat from right side SSF and THSF measurements (59). Weight, expressed as percent of ideal body weight (%IBW) was evaluated against age and sex-specific reference values based on HANES II, 1971-1974 (56). Triceps skinfold, MAC, and MAMC were evaluated against standards reported by Blackburn et al. (56), Bishop et al. (58), Bishop and Ritchey (60), and Frisancho (61).

Data collection

Questionnaires and assessment forms were designed for the specific needs of this investigation following the guidelines of Roe (35), Grant (57), Christakis (62), and Aronson and Fitzgerald (63). Interviews and data collection were conducted using techniques developed for the "reluctant" or stigmatized client (i.e., alcohol and drug dependent; 44,64,65). When possible, obtained data was verified against the nutritional summary taken by the admitting staff and (or) confirmed by relatives.

Laboratory Tests

The following parameters were obtained on fasting blood

samples obtained at the beginning and end of the 21-day study period for each patient: total leukocyte count (WBC), mean corpuscular volume (MCV), hematocrit (Hct), hemoglobin (Hgb), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), calcium, phosphorus, albumin, total protein (TP), glucose, gamma-glutamyl transferase (GGT), folate, and cobalamin (vitamin B-12).

Total leukocyte count, MCV, Hct, and Hgb were measured on the ELT-8 laser-automated cell counter (Ortho Diagnostic, Rariton, NJ). The Abbott-VP (Abbott Industries, Irving, TX) was used for enzymatic assay of serum TG, calcium, phosphorus, albumin, TP, glucose, and GGT. Lipoprotein HDL-C (alpha) and LDL-C (beta) fractions were separated by the micro-affinity column chromatography method developed by Bentzen et al.(66; Isolab Inc., Akron, OH). Total cholesterol (serum cholesterol assays between the CO-VMAC laboratory and the Kansas State University Food and Nutrition laboratory were not significantly different and were highly correlated; $r = 0.985$, $p < 0.0001$), HDL-cholesterol, and LDL-cholesterol were determined enzymatically (modified Sigma Procedure No. 351, Sigma Diagnostics, St. Louis, MO). Percent recovery of lipoprotein fractions was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{HDL-C (mg/dl)} + \text{LDL-C (mg/dl)}}{\text{TC (mg/dl)}} \times 100$$

Serum folate and vitamin B-12 were determined by radioassay using a dual isotope (^{57}Co and ^{125}I) kit produced by

Clinical Assays, Travenol Laboratories, Inc., Cambridge, MA. Purified porcine intrinsic factor and exogenous folate binding protein in bovine milk were utilized to bind free vitamin B-12 and free folate. Samples were counted for 1 minute in a multi-channel gamma counter (Isodata, Inc., Rolling Meadows, IL). Serum collected for folate and vitamin B-12 assay was protected from the light both during freezing and storage.

Analysis

The experiment was carried out as a double-blind completely randomized design with a 2 x 2 factorial combination (2 treatment groups and 2 measurement times). Analysis of Variance (ANOVA) was used to compare initial and final measurements and the difference between these measurements (final - initial). A general linear model (Statistical Analysis System; 67) was utilized for ANOVA least squares means and means separation (least significant difference).

Results

Thirty-one male alcoholics without overt hepatic or pancreatic involvement completed the study. The mean age was 39.5 +/- 10.9 years and average years of alcohol abuse 12.6 +/- 6.9. Fifteen subjects received disulfiram (250 mg/ day) and 16 received placebo (controls). Twelve of the disulfiram group and thirteen of the control group received vitamin/ mineral supplementation.

Nutritional Status

Anthropometric measurements

Average (mean +/- SD) skinfold measurements compared to age/sex specific data from the Health and Nutrition Examination Survey (HANES, 1971-1974; 54) were within acceptable range (85.8-102.5% of standard) at baseline and end of study. Initial and final mean percent of ideal body weight and body fat were within acceptable range with the exception of percent body fat in the control group (65.7-70.7% of standard). Analysis utilizing least squares means (LSM) estimates to minimize standard errors and decrease bias, showed no significant ($p<0.05$) differences (initial-final, I-F) between or within groups (Table I).

Dietary History

Socioeconomic and Abuse History

The disulfiram group had a significantly ($p < 0.01$) higher mean income (12533 +/- 9181 vs. controls 6887 +/- 6811 dollars per year) than the control group. Analysis (LSM) of other variables (employment, years of education, years of al-

TABLE I Anthropometric values of subjects. Initial (I) and final (F) values as means +/- SD.

Variable		Treatment 1 (Antabuse) n=15	Treatment 2 (Placebo) n=16	Differences Between Groups(LSM) (p<0.05)	Standard Values* (male 35-45 years)
Age (years)		43.4 +/-10.0	35.9 +/-11.0	NS	
Body Weight (Kg)	I	71.2 +/- 7.5	69.9 +/- 9.2	NS	
	F	74.3 +/- 7.7	71.4 +/- 8.4	NS	
Ideal Body Weight (%)					(acceptable range)
	I	93.8 +/-13.1	91.8 +/-12.0	NS	80-120
	F	98.0 +/-12.7	93.8 +/-11.1	NS	
Body Fat (%)	I	11.5 +/- 5.7	9.2 +/- 4.0	NS	14-22
	F	12.6 +/- 5.2	9.8 +/- 3.9	NS	
% of Std.+	I	82.1	65.7		
	F	90.0	70.0		
					(50th percentile)
Triceps Skinfold (mm)	I	12.0 +/- 4.8	10.3 +/- 4.7	NS	
	F	12.3 +/- 3.7	11.4 +/- 5.4	NS	12.0
% of Std.	I	100.0	85.8		
	F	102.5	95.0		
Mid-Arm Circumference (cm)	I	30.2 +/- 2.5	29.7 +/- 2.1	NS	32.7
	F	30.7 +/- 2.6	30.3 +/- 1.6	NS	
% of Std.	I	92.3	90.8		
	F	93.9	92.7		
Mid-Arm Muscle Circumference (cm)	I	26.4 +/- 1.6	26.4 +/- 1.6	NS	28.7
	F	27.2 +/- 2.2	26.6 +/- 1.6	NS	
% of Std.	I	91.9	91.9		
	F	94.8	92.7		

* Standard values; Bishop, Bowen, and Ritchey (58), Sloan and de V Weir (59), Frisancho (61), and Pike and Brown (68).
\+ Acceptable % of standard, 80-120%

cohol abuse, years of tobacco use, quantity of tobacco used per day) did not reflect any significant differences between groups (Table II).

Dietary Intake

Estimation of recent dietary intake obtained by 24-hour recall (expressed as percent of recommended daily intake) indicated no significant difference between groups. Mean percent of recommended intake prior to admittance: Disulfiram group, 52.5 +/-34.9% vs. placebo group, 51.4 +/-24.1%.

Laboratory Tests

Hematology

Mean hematocrit values were within normal limits, however, mean hematocrit was significantly lower in the control group ($p<0.01$) on analysis (LSM) of I-F (disulfiram, 47.9 +/-4.5 to 48.6 +/- 3.3 4%; control, 48.2 +/- 2.7 to 46.4 +/-2.6%). Baseline and final hemoglobin and total leukocytes (means +/- SD) values were within normal limits with no significant differences between treatment groups at $p<0.05$ (Table III).

Mean Corpuscular Volumn (MCV), an accepted marker for alcoholism (69), was slightly elevated in both groups at baseline and in the disulfiram group during final testing. On analysis (LSM), there were no significant differences at $p<0.05$ within or between groups (Table III).

Blood (Serum) Studies

As judged by the following laboratory tests: gamma-glutamyl transferase, serum albumin, total protein, glucose, triglycerides, calcium, and phosphorous, the two groups were of

TABLE II Socioeconomic and abuse history factors affecting nutritional status. Values as means +/- SD.

Variable	Treatment No.1 (Antabuse, n=15)	Treatment No.2 (Placebo, n=16)	Differences (LSM) Between Groups ($p < 0.05$)
Unemployed (%)	60.0	43.7	NS
Education (years)	12.9 +/- 2.4	12.3 +/- 0.7	NS
Income ($)	12533 +/- 9181	6887 +/- 6811	$p < 0.02$
Alcohol Abuse (years)	14.4 +/- 7.1	11.0 +/- 6.6	NS
Tobacco smoking (years)	25.1 +/-13.5	20.8 +/-11.8	NS
Cigarettes (packs/day)	1.6 +/- 0.7	1.7 +/- 0.6	NS

similar overall nutritional status (mean values +/- SD) both at baseline and completion of the study. Difference in final glucose levels between groups was significant at $p<0.02$ (disulfiram, 95.2 +/-13.8 mg/dl vs. controls, 87.6 +/- 5.5 mg/ dl). But, there was no significance within groups on analysis of I-F serum levels (Table III).

Serum Cholesterol Levels. Total serum cholesterol levels were assayed using two methods for control of accuracy (Abbott VP, and modified Sigma Procedure No. 351; 66). Values were verified by analyzing significance of correlation (initial, $r = 0.985$, $p< 0.0001$; final, $r = 0.992$, $p< 0.0001$) between assay methods. Results from the modified Sigma procedure are reported in Table III.

Initial difference of means between treatment groups was significant at $p<0.04$ (disulfiram, 203.0 +/- 34.6 mg/dl vs. control, 174.5 +/- 30.1 mg/dl), indicating a slight biasing. Subjects taking 250 mg disulfiram per day for 21 days had a significant ($p< 0.002$) increase in total serum cholesterol (203.0 +/- 34.6 mg/dl to 225.8 +/- 27.1 mg/dl). Control values increased from 174.5 +/-30.1 mg/dl at baseline to 178.0 +/- 29.5 mg/dl at three weeks but the increase was not significant ($p<0.70$). Final serum levels between groups were significant at $p<0.0002$ (Figure I).

High-Density Lipoprotein Cholesterol. Initial and final (mean +/- SD) serum values of high-density lipoprotien cholesterol (HDL-C) were within normal limits for both treatment groups (Table III). The expected decrease with cessation of

TABLE III Laboratory values of subjects. Initial (I) and final (F) values as means +/- SD.

Variable		Treatment 1 (Antabuse) n=15	Treatment 2 (Placebo) n=16	Differences Between Groups(LSM) (p< 0.05)	Normal Values* (male; 35-44 years)
Hematology -					
Hemoglobin	I	15.3 +/- 1.0	15.5 +/- 0.8	NS	14.0-18.0
(gm/dl)	F	15.8 +/- 0.6	15.4 +/- 0.8	NS	
Hematocrit	I	47.9 +/- 4.5	48.2 +/- 2.7	NS	42-52
(%)	F	48.6 +/- 3.3	46.4 +/- 2.6	NS+	
T.Leukocytes	I	8.2 +/- 1.9	7.9 +/- 1.7	NS	5.0-10.0
(cumm X10)	F	9.1 +/- 2.7	9.1 +/- 2.7	NS	
MCV	I	97.7 +/- 5.0	95.6 +/- 7.4	NS	80-94
(cuu)	F	95.7 +/- 2.6	92.4 +/- 5.5	NS	
Blood Studies-					
GGT	I	100.5+/-129.7	38.6 +/-31.5	NS	11-63
(IU/l)	F	43.8+/- 42.8	27.1 +/-13.6	NS	
S. Albumin	I	4.4 +/- 0.3	4.5 +/- 0.4	NS	3.0-5.2
(gm/dl)	F	4.5 +/- 0.4	4.5 +/- 0.4	NS	
T. Protein	I	7.3 +/- 0.8	7.2 +/- 0.5	NS	6.0-8.5
(gm/dl)	F	7.2 +/- 0.4	7.2 +/- 0.3	NS	
Glucose	I	93.9 +/-11.7	91.2 +/- 7.9	NS	65-110
(mg/dl)	F	95.2 +/-13.8	87.6 +/- 5.5	p<0.02++	
TG	I	141.7 +/-57.8	108.3 +/-42.3	NS	20-200
(mg/dl)	F	127.6 +/-34.0	121.3 +/-46.1	NS	
TC	I	203.0 +/-34.6	174.5 +/-30.1	p<0.04	140-280
(mg/dl)	F	225.8 +/-27.1	178.0 +/-29.5	p<0.0002	
HDL-C	I	50.4 +/-13.3	56.8 +/-14.8	NS	29-61
(mg/dl)	F	48.2 +/- 6.6	46.1 +/- 7.6	NS	
LDL-C	I	138.4 +/-21.2	107.8 +/-31.7	p<0.009	
(mg/dl)	F	160.7 +/-19.8	119.8 +/-29.3	p<0.0002	66-178
Calcium	I	9.5 +/- 0.5	9.5 +/- 0.4	NS	8.5-10.5
(mg/dl)	F	9.5 +/- 0.3	9.5 +/- 0.3	NS	
Phosphorus	I	3.5 +/- 1.0	3.3 +/- 0.8	NS	2.5-4.5
(mg/dl)	F	3.7 +/- 0.6	3.8 +/- 0.6	NS	

* Normal Values specific to method used in determination of serum values.

\+ Hct. was significantly lower in the control group (P< 0.01) on analysis (LSM) of I-F. ++ NS on analysis of I-F (LSM).

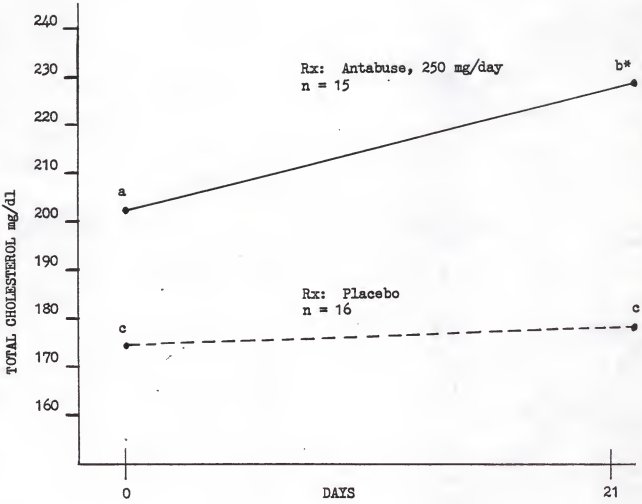

Fig. I Total serum cholesterol of alcoholic patients receiving Antabuse, 250 mg/day (————, n = 15) or placebo (- - - - -, n = 16) for a 21-day treatment period. Means with different superscripts differ significantly, $p<0.05$. * Values significantly ($p<0.0002$) increased above baseline, day 0.

alcohol intake (disulfiram, 50.4 +/- 13.3 to 48.2 +/- 6.6 mg/dl vs. control, 56.8 +/- 14.8 to 46.1 +/- 7.6 mg/dl) showed no significance at $p<0.05$ between or within groups during either trial (Figure II).

Low-Density Lipoprotein Cholesterol. Subjects receiving disulfiram (250 mg day) evidenced a marked increase in serum low-density lipoprotein cholesterol fraction (LDL-C) over the three week test period (increase: disulfiram, 22.3 +/- 19.4 mg/dl vs. control, 12.0 +/- 17.7 mg/dl). Initial LDL-C mean values between groups were significant at $p<0.009$ indicating a slight bias effecting LDL-C as well as total cholesterol. Final (21 days) LDL-C differences (LSM) between treatment groups were significant at $p<0.0002$ (Figure II).

Over the 21 day period there was a significant positive correlation between increase in serum total cholesterol and increase in LDL-C ($r = 0.879$, $p<0.0001$) indicating the possibility of increased LDL-C fraction with disulfiram treatment. The effect of disulfiram on LDL-C final values was significant, but differences in repeated measures were not significant at $p<0.05$.

Vitamin Studies

Serum Folate. Vitamin and mineral supplementation significantly increased ($p<0.002$) the serum folate levels over the three week treatment period in both groups (disulfiram, 5.3 +/- 1.7 to 11.4 +/- 6.9 ng/ml; placebo, 8.6 +/- 5.1 to 12.0 +/- 3.9 ng/ml). Mean values between treatment groups not receiving folic acid therapy were without statistical significance during

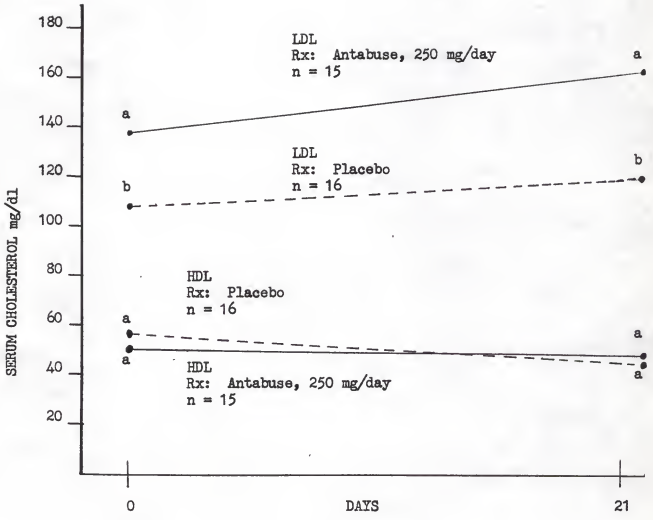

Fig. II Serum low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol of alcoholic patients receiving Antabuse, 250 mg/day (————, n = 15) or placebo (- - - - -, n = 16) for a 21-day treatment period. Means with different superscripts differ significantly, $p<0.05$.

initial and final trials. However, serum folic acid levels decreased during the treatment period with the placebo group falling below normal values (7.9 +/- 1.6 to 6.3 +/- 0.5 ng/ml) of 7-19 ng/ml (Table IV). Interactions between groups (ANOVA 4 X 2 factorial) expressed as means are plotted on Figure III.

Serum Cobalamin. Mean increase of serum cobalamin was markedly lower in the placebo group (n=13) receiving 12 mcg vitamin B-12 (as cyanocobalamin) during the 21-day treatment period (354.6 +/- 134.3 to 373.2 +/- 373.2 pg/ml). Disulfiram treatment group without cobalamin therapy (n=3) evidenced the greatest increase of serum cobalamin (379.3 +/- 110.6 to 446.1 +/- 15.0 pg/ml). Slight biasing possibly due to low sample size (Table IV).

Initial and final differences between groups were not significant at $p<0.05$. Final-initial difference expressed as means within groups were not significant (Figure IV).

TABLE IV Serum Folate (ng/ml) and Cobalamin (pg/ml) concentrations in subjects. Initial (I) and final (F) values as means +/- SD.

Variable		Treatment 1 (Antabuse)	Treatment 2 (Placebo)	Differences Between Groups (LSM, $p<0.05$)	Normal Serum Values*
Without Folic Acid Therapy (ng/ml)		n=3	n=2		
	I	14.2 +/- 13.8	7.9 +/- 1.6	NS	7-19
	F	9.3 +/- 2.8	6.3 +/- 0.5	NS	
		n=12	n=12		
Folic Acid Therapy + (ng/ml)	I	5.3 +/- 1.7	8.6 +/- 5.1	NS	
	F	11.4 +/- 6.9	12.0 +/- 3.9	NS	
	F-I	(difference within groups)		$p<0.002$	
Without Cobalamin Therapy (pg/ml)		n=3	n=3		
	I	379.3+/-110.6	322.6+/-113.4	NS	200-900
	F	446.1+/- 15.0	379.6+/-101.6	NS	
		n=12	n=13		
Cobalamin Therapy+ (pg/ml)	I	379.9+/-144.8	354.6+/-134.3	NS	
	F	442.7+/-121.0	373.2+/- 92.8	NS	
	F-I	(difference within groups)		NS	

* Normal values; Fishbach (51)

\+ One mg folic acid orally once a day for 7 days; 1 multi-vitamin including 400 mcg folic acid and 12 mcg vitamin B-12 (as cyanocobalamin, Stresstabs (R) 600 Advanced Formula, High-Potency Stress Formula Vitamins, Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY) once a day for 30 days.

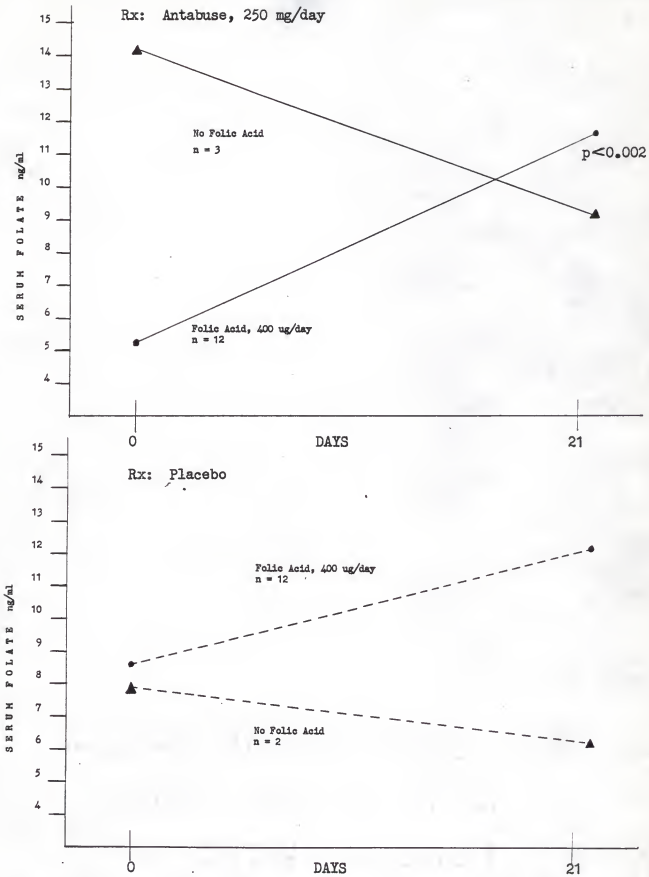

Fig. III Influence of interaction between patients treated with Antabuse, 250 mg/day (———) or placebo (- - - -) and those receiving (●) or not receiving (▲) folic acid supplementation over a 21-day treatment period.

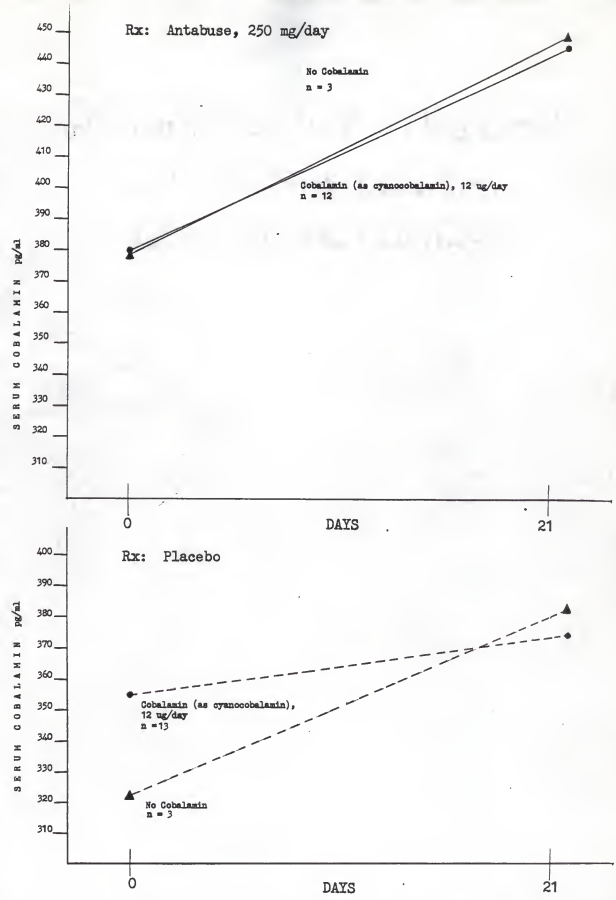

Fig. IV Influence of interaction between patients treated with Antabuse, 250 mg/day (————) or placebo (- - - -) and those receiving (●) or not receiving (▲) cobalamin (as cyanocobalamin) supplementation over a 21-day period.

Discussion and Conclusions

This study has investigated the possible effects of short-term (3 week) disulfiram treatment on the overall nutritional status of abstaining alcoholics in a controlled environment. Special emphasis was placed on the effect of disulfiram treatment on serum cholesterol, folate, and cobalamin levels.

The overall nutritional status of the subjects at baseline demonstated no significant difference in the prevalence of malnutrition when compared to the general population (percent of alcohol abuse unknown). The current investigation supports the findings of Halsted (40), Dickson et al.(41), and Bienia et al. (70) which report no significant differences between alcoholic and nonalcoholic groups in relation to incidence of malnutrition (alcoholic, 36.9%; nonalcoholic, 43.7%; 70).

All study participants were offered the same (2500 calorie) hospital diet. Over the three week treatment period all anthropometric indices increased slightly in both treatment groups as recorded in Table I. Slight increase in all values indicates a refeeding effect (Lieber, 39) with no significant effect from disulfiram treatment or vitamin therapy.

Mogens, Mogens, and Smith (15) report that tryptophan, one of the amino acids recommended for nutritional support in decreased albumin synthesis, has been found to exhibit increased clearance in individuals receiving disulfiram therapy. Serum albumin and total protein were within normal limits at baseline and termination of study participation in all of our subjects, although, Mendenhall et al (43) found that 62% of patients

studied, without liver disease, evidenced some type of protein-calorie malnutrition.

Our current findings corroborate results from animal studies by Rothschild, Oratz, and Schreiber (45) indicating that serum albumin is severly depressed only during liver involvement and malnutrition. Disulfiram administration (250 mg/day) did not appear to contribute to overt changes in serum albumin or total protein during the 3 weeks treatment period.

Eisenstein (7) cited alcoholic hypoglycemia and hyperglycemia as possible results of alcoholism. Williams (36) reported impaired hepatic glucose output in animals with excessive alcohol intake and Arkey, Veverbrants, and Abramson (37) found that hypoglycemia could be induced in healthy adult males given 15% ethyl alcohol infusions. No hypoglycemic or hyperglycemic effect was found in either treatment group in our study. The group receiving disulfiram had a slight increase in glucose levels during the treatment period (93.9 +/-11.7 to 95.2 +/-13.8 mg/ dl) and controls a slight decrease, representing a possible trend. Futher investigation is needed on the effects of disulfiram on glucose levels since disulfiram has been shown to (1,5,14,23) decrease norepinephrine and epinephrine through suppression of dopamine-B-hydroxylase (DBH) and may have an effect on other regulatory hormones.

Prolonged disulfiram therapy in human and animal subjects results in an elevation of serum cholesterol levels (1-3,5,6,9, 11-17,26,32,35). These studies frequently employed disulfiram dose levels of at least 500 mg/day over a period of several

months to years. We were challenged by these findings to examine the possible effects of a therapeutic dose (250 mg/day disulfiram) during initial treatment of alcoholism (21 days).

In a similar study to ours (disulfiram dosage and length of treatment) Major and Goyer (12) reported a significant elevation in total serum cholesterol levels after 3 weeks in patients receiving 500 mg of disulfiram a day ($p<0.02$). An elevation in serum levels was observed after 6 weeks in patients ($n=8$) receiving 250 mg daily, however, no significant changes were observed at 3 weeks. Results of their study suggest that initial disulfiram treatment (3 weeks) of 250 mg/day has no significant effect on serum cholesterol.

In contrast, serum cholesterol levels in our subjects receiving 250 mg disulfiram daily increased significantly ($p<0.002$) over the 3 week treatment period; final serum levels between groups were significant at $p<0.0002$ (Figure I). Difference of means between treatment groups at baseline was significant at $p<0.04$ representing a slight biasing. This variation may have been due to not completely random sampling; difference in mean age between groups (disulfiram, 43.4 +/- 10.0 years vs. controls, 35.9 +/- 11.0 years) and years of alcohol abuse (disulfiram, 14.4 +/- 7.1 years vs. controls, 11.0 +/- 6.6 years).

Dietary intake of cholesterol was not monitored in our study but Rogers and Naseem (17) reported a twenty-five percent increase in serum cholesterol with no variance of dietary intake between groups, after administration of disulfiram (15 mg/

kg of body weight/day) to Sprague-Dawley rats. These investigators observed a four-fold elevation in hepatic 3-hydroxy-3-methyl-glutaryl co-enzyme A (HMG-CoA) reductase in the disulfiram group, suggesting alteration in cholesterol synthesis.

We found very poor correlation between years of alcohol abuse and serum high-density lipoprotein cholesterol (HDL-C) at baseline ($r = 0.232$, $p<0.20$). But, our findings agreed with those of Taskinen et al. (30), Marth et al. (31), and Devenyi et al. (32) that HDL-C levels fall to normal levels within three weeks of abstinence. Initial and final HDL-C levels were within normal range in both treatment groups.

Hegarty et al. (71) and Willet et al. (72) report a significicant depression of HDL-C serum levels in both animal and human studies ($p<0.05$) with cigarette smoking. We found low correlation between initial serum HDL-C levels and years of smoking ($r = 0.382$) and quantity smoked ($r = 0.170$). Changes in HDL-C levels (I-F) were not significant in the disulfiram group and frequency of cigarette smoking did not change between trials, indicating that initial HDL-C levels and subsequent decrease were directly related to alcohol use.

Increased levels of low-density lipoprotein cholesterol (LDL-C) in workers exposed to toxic amounts of carbon disulfide, a final metabolite of disulfiram, have been reported (12). Our findings from investigation into the short-term effects of disulfiram on LDL-C levels support these results.

The group receiving disulfiram had a two-fold increase of LDL-C over the control group ($p<0.0002$) suggesting that the

effect of disulfiram on this fraction is immediate and may contribute to hypercholesterolemia. During disulfiram treatment (21 days), a positive correlation between increases in total cholesterol and LDL-C was significant ($r = 0.879$, $p<0.0001$). Again, similar to values of total cholesterol, we found slight biasing at baseline between groups ($p<0.009$), which may be a result of random variation. These findings prompt further investigation with more repeated measures.

Concurrent with the effect of carbon disulfide on LDL-C is the reported effect on pyridoxine (12-15), resulting in a pyridoxine deficiency. We did not assay serum pyridoxine levels in our subjects. Therefore, we are unable to draw any conclusions on the relationship between pyridoxine and increased LDL-C levels with initial disulfiram treatment.

Initial mean serum folate levels across all treatment groups were within normal range. However, in agreement with Mills et al. (47) who reported low levels of serum folate in 43% of patients studied, 19 subjects had an initial serum folate below normal range.

We did not find any significant effect from disulfiram therapy on serum folate status. Our findings of decreased levels of serum folate without supplementation support the hypothesis of Mills et al. (47) that a cyclic effect may occur with chronic alcohol use whereby folate malabsorption promotes folate deficiency resulting in intestinal malabsorption of folate.

Pernicious anemia is not commonly found in alcoholics

(35,49,52) but, vitamin B-12 deficiency can contribute to folate deficiency through the methyl-folate trap. Only one subject had an initial blood profile symptomatic of overt alcohol related anemia: Serum folate, 5.64 ng/ml; serum cobalamin, 50.6 pg/ml; mean corpuscular volume (MCV), 101.0 cuu. These findings do not agree with Lindenbaum and Roman (49) who reported 62% of 65 alcoholic patients presenting with anemia.

Mean increase of serum cobalamin was not significant within or between any treatment groups (disulfiram, placebo; with cobalamin supplementation, without cobalamin supplementation). The group receiving disulfiram and no supplementation had the greatest increase over the 21 day treatment period. These results reflect possible biasing as a result of low sample size ($n = 3$).

In conclusion, the nutritional status of the alcoholic patients in this study was not compromised by either their prior alcoholic intake or the 21-day treatment with disulfiram. Neither serum folate nor serum cobalamin levels were affected by disulfiram treatment. However, short-term (21-day) treatment with a moderate dose (250 mg/day) of disulfiram did produce a significant increase in total serum cholesterol and an increase in low-density lipoprotein cholesterol fraction. Because of the apparent effect of disulfiram on serum cholesterol, the atherosclerotic risk may be increased in some alcoholics treated with this drug.

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APPENDIX A-

Research Informed Consents

INFORMATION ABOUT

THE SHORT-TERM EFFECTS OF DISULFIRAM (ANTABUSE^{TM}) TREATMENT ON

NUTRITIONAL STATUS AND BLOOD CHOLESTEROL LEVELS IN ABSTAINING ALCOHOLICS

INFORMED CONSENT

Recent studies have suggested that elevation of total serum (blood) cholesterol has been observed in some human subjects receiving disulfiram (Antabuse) treatment to prevent alcohol intake while participating in rehabilitation from alcoholism. At the same time, other effects that the administration of disulfiram may have on nutritional status of the recovering alcoholic are largely unknown.

The purpose of this study is to investigate the short-term effects of disulfiram (Antabuse) treatment on total serum cholesterol, serum HDL-cholesterol, and nutritional status of alcoholic patients during the initial phase of abstinence from alcohol.

Your agreement to participate in this study will involve the following:

1) willingness to be randomized (assigned by chance) into one of two treatment groups. One group will receive 1 disulfiram (Antabuse) tablet (250 mg) and the other group will receive a placebo (inactive substance) each day for 21 days. Subjects in both groups will receive all other benefits associated with the Chemical Problems Treatment Unit (CPTU). All participants will sign the Antabuse (disulfiram) Instructions and Consent Form (VA form 10-63 R (677), August 1980).

2) access to your medical records for information pertinent to the study.

3) drawing of 30 ml of blood at the beginning and end of the study in addition to routine blood tests by the CPTU for biochemical and nutritional evaluation. Nothing is to be taken by mouth except water for 12 hours prior (7 P.M. to 7 A.M.) to drawing blood.

4) physical (anthropometric) measurements, i.e., height, weight, and skinfold measurements that will indicate the percent of body fat and muscle, to be taken within 3 days of entering the CPTU and again after 21 days continuous participation in the study, for evaluation of nutritional status.

Total time required for participation in the study is approximately 3 hours over a 21 day period. You may contact any of the investigators to answer any questions you may have at any point in the study.

Investigators - Roy B. Lacoursiere, M.D. Chemical Problems Treatment Unit
Phone: (913) 272-3111 Colmery-O'Neil Veterans Administration Medical Center
Topeka, Kansas 66604

Robert D. Reeves, Ph.D. Department of Foods and Nutrition
Phone: (913) 532-5508 Kansas State University
Manhattan, Kansas 66506

Emmalyn B. Aiken Graduate Student
Phone: (913) 532-5508 Kansas State University

Discomforts and Risks

If you will be taking disulfiram (Antabuse) there are some things about it that you need to know. Like all medications, disulfiram (Antabuse) may have certain side effects. Disulfiram ((Antabuse) may occasionally cause drowsiness, tiredness, and skin eruptions. Although drowsiness is not common with the dose of disulfiram (Antabuse) used in this study, we nevertheless urge you to be careful driving a car and working around machines or in high places for the first serveral days after beginning the disulfiram (Antabuse) treatment. If the medication causes drowsiness which persists for more than a week or two, tell your counselor or doctor.

If you become a participant and receive disulfiram (Antabuse), it will be necessary for you to AVOID ALL ALCOHOL. The reason is that disulfiram (Antabuse) can cause a SEVERE REACTION WHEN TAKEN WITH ALCOHOL. If you drink alcohol while taking disulfiram (Antabuse), you will get a reaction consisting of flushing, headache, difficulty breathing, nausea, vomiting, dizziness, and fainting. In severe reactions, heart attacks can occur, and this could endanger your life. You must also avoid alcohol in medications and food, and in cosmetics if your skin is very sensitive. If you stop the disulfiram (Antabuse), you must wait up to two weeks before drinking any alcohol.

This study involves only minimal discomfort from drawing blood. The primary discomfort will be the slight pain associated with drawing blood as the sterile needle enters the skin. The staff drawing your blood are experienced and will minimize the discomfort as much as possible. Skinfold measurements will be taken on the arm, shoulder, and thigh. No physical pain is associated with this activity.

Benefits

This study will serve to increase the knowledge of the benefits and/or hazards of administering disulfiram (Antabuse) treatment as a support to reaching optimal physical and behavioral recovery from the illness of alcoholism.

Alternative Action and Confidentiality

You may withdraw at any time from participation in the study without jeopardizing your treatment or other VA benefits. The investigators ask that you meet with them if you wish to leave the study so that any misunderstanding can be clarified. Your identity as a participant will not be revealed in any published or oral presentation of the results of this study.

For Non-Veteran or Non-Eligible Veteran Participants

"In the unlikely event you are injured as a result of participation in this study, the Colmery-O'Neil VAMC is authorized to furnish humanitarian emergency medical care only as provided by Federal Statute. Non-eligible veterans might be entitled to compensation under 38 U.S.G. 351 or to recovery under the Federal Tort Claims Act, depending on the circumstances of the particular situation. Non-Veterans, however, can recover only in situations where negligence occured, which would be covered by the provisions of the Federal Tort Claims Act. For further information, contact the VA District Legal Counsel at (316) 267-6311."

For Veteran Participants

"In the unlikely event you are injured as a result of Participation in this study, the Colmery-O'Neil VAMC will furnish medical care as provided by Federal Statute. Compensations for such injury may also be available for you in some instances, under the provisions of the Federal Tort Claims Act (28-U.S.C. 1346 (B) and 2675). For further information contact the VA District Legal Counsel at (316) 267-6311.

I, _____certify that the above written summary was discussed and explained fully to me by one of the investigators.

Date_____ Subject's Signature_____

Date_____ Investigator's Signature_____

Date_____ Witness_____

PART I-AGREEMENT TO PARTICIPATE IN RESEARCH
BY OR UNDER THE DIRECTION OF THE VETERANS ADMINISTRATION

DATE

I, I, _____ voluntarily consent to participate as a subject
(Type or print subject's name)

in the investigation entitled The Short-Term Effects of Disulfiram (Antabuse™) Treatment
(Title of study)

on Nutritional Status and Blood Cholesterol Levels in Abstaining Alcoholics

2. I have signed one or more information sheets with this title to show that I have read the description including the purpose and nature of the investigation, the procedures to be used, the risks, inconveniences, side effects and benefits to be expected, as well as other courses of action open to me and my right to withdraw from the investigation at any time. Each of these items has been explained to me by the investigator in the presence of a witness. The investigator has answered my questions concerning the investigation and I believe I understand what is intended.

3. I understand that no guarantees or assurances have been given me since the results and risks of an investigation are not always known beforehand. I have been told that this investigation has been carefully planned, that the plan has been reviewed by knowledgeable people, and that every reasonable precaution will be taken to protect my well-being.

4. In the event I sustain physical injury as a result of participation in this investigation, if I am eligible for medical care as a veteran, all necessary and appropriate care will be provided. If I am not eligible for medical care as a veteran, humanitarian emergency care will nevertheless be provided.

5. I realize I have not released this institution from liability for negligence. Compensation may or may not be payable, in the event of physical injury arising from such research, under applicable federal laws.

6. I understand that all information obtained about me during the course of this study will be made available only to doctors who are taking care of me and to qualified investigators and their assistants where their access to this information is appropriate and authorized. They will be bound by the same requirements to maintain my privacy and anonymity as apply to all medical personnel within the Veterans Administration.

7. I further understand that, where required by law, the appropriate federal officer or agency will have free access to information obtained in this study should it become necessary. Generally, I may expect the same respect for my privacy and anonymity from these agencies as is afforded by the Veterans Administration and its employees. The provisions of the Privacy Act apply to all agencies.

8. In the event that research in which I participate involves certain new drugs, information concerning my response to the drug(s) will be supplied to the sponsoring pharmaceutical house(s) that made the drug(s) available. This information will be given to them in such a way that I cannot be identified.

I _____
NAME OF VOLUNTEER

HAVE READ THIS CONSENT FORM. ALL MY QUESTIONS HAVE BEEN ANSWERED, AND I FREELY AND VOLUNTARILY CHOOSE TO PARTICIPATE. I UNDERSTAND THAT MY RIGHTS AND PRIVACY WILL BE MAINTAINED. I AGREE TO PARTICIPATE AS A VOLUNTEER IN THIS PROGRAM.

9. Nevertheless, I wish to limit my participation in the investigation as follows:

VA FACILITY	SUBJECT'S SIGNATURE
Colmery-O'Neil VAMC 2200 Gage Blvd. Topeka, KS 66622	
WITNESS'S NAME AND ADDRESS (Print or type)	WITNESS'S SIGNATURE
INVESTIGATOR'S NAME (Print or type) Roy B.Lacoursiere,M.D.(CPTU,CO-VMAC) Robert D. Reeves,Ph.D.(K.S.U.)	INVESTIGATOR'S SIGNATURE

☐ Signed information sheets attached. ☐ Signed information sheets available at:

SUBJECT'S IDENTIFICATION (I.D. (Hosp or give name - last, first, middle)

SUBJECT'S I.D. NO. | WARD

AGREEMENT TO PARTICIPATE IN RESEARCH BY OR UNDER THE DIRECTION OF THE VETERANS ADMINISTRATION

VA FORM 10-1086A SUPERSEDES VA FORM 10-1086 JUN 1975, WHICH WILL NOT BE

Topeka Veterans Administration Medical Center
Chemical Problem Treatment Unit
Antabuse (Disulfiram) Instructions and Consent Form

As part of your treatment program you may want to start taking Antabuse, chemically called disulfiram. Antabuse is a medicine that works by interfering with the way your body handles alcohol after the alcohol gets into your system. Antabuse slows the breakdown of alcohol at an intermediate stage, and a substance accumulates in your body that causes the reaction. The reaction varies considerably, depending on the amount of Antabuse in your body and how much alcohol is taken. Most of the symptoms are caused by enlargement of the skin blood vessels and a drop in blood pressure. The mildest symptoms are a flushing of the skin with a feeling of heat. This is due to the enlargement of the blood vessels in the skin. Some people experience little more than this. Other symptoms that go with this and with the drop in blood pressure include a feeling of weakness and nausea, headache, sweating, a strong heart beat, and in more severe cases, vomiting and fainting. The reaction can be very severe, especially with large amounts of alcohol and Antabuse. In very rare cases the Antabuse-alcohol reaction has led to death.

On the dosage we usually prescribe, patients who drink alcohol on top of the Antabuse usually experience only a moderate amount of discomfort, but it depends on how much alcohol is taken. This discomfort is enough to keep you from drinking according to your old patterns. You won't be able to drink to feel good. You won't be able to drink to get rid of anxiety or to get up your courage. You won't be able to drink just to be friendly or because there doesn't seem to be any reason not to drink. Antabuse will give you another reason to refuse a drink. If you take Antabuse in the morning that settles for the day the question of whether or not to drink. When friends ask you to take a drink you will be able to tell them you are taking Antabuse and if they still insist that you drink, you will know they are not your friends.

When taken daily Antabuse builds up in the body fluids and will remain in your body for several days after you stop taking it. Some people have an Antabuse reaction as much as a week or two after they stop taking Antabuse, if they drink alcohol. This means that you can't stop taking Antabuse today and start drinking the old way tomorrow. You have to wait several days, and hopefully in that period of time you will control the urge to drink and start back on Antabuse. It is a kind of insurance policy.

There are certain precautions that you have to take when you are on Antabuse. It is very unusual for people to have any serious symptoms from Antabuse itself, but occasionally patients will be drowsy when first on Antabuse, or develop a rash or some other symptom while they are taking this medicine. Report any kind of symptoms you develop after you have started on the medicine. In addition you have to be careful not to drink alcohol accidentally. You will react to alcohol in any form, not only in alcoholic beverages. Years ago, when high doses of Antabuse were used, some people claimed that they saw reactions due to alcohol rubs, but we have seen no reactions to alcohol absorbed through the skin or through the lungs with doses of Antabuse that you will be taking. However, in things that you eat or drink, even in medicine, it is important for you to avoid alcohol. Any time a doctor treats you, tell him that you are on Antabuse and that you cannot take any medicine that contains alcohol. When you have prescriptions filled, also tell the pharmacist that this is the case and that you must not have any medicine containing alcohol. (For example, most cough and cold medicines contain large amounts of alcohol.) It is important for you not to drink from a punch bowl and not to take drinks when you don't know what is in them. Even foods containing alcohol must be

avoided unless you are certain that the alcohol content has been cooked out. The one drug other than alcohol that might cause an Antabuse reaction is paraldehyde, so it must also be avoided. (Paraldehyde is an old sleeping medication that is not used very often anymore.) If at any time when you are on or going to take Antabuse and you have any medical concerns about Antabuse and your taking it, discuss these with your doctor. Also, for your safety, we will give you a card to carry with you that says you are on Antabuse.

If you should have an Antabuse reaction, that is, if you should drink alcohol while you are taking Antabuse, and then have some symptoms, it is likely that you will want to discontinue your activities at the time, and you will probably want to lie down. Most people will feel nauseated enough that they will want to have some kind of container nearby in case they vomit. It is usual for someone with an Antabuse reaction to lie down and finally fall asleep and to feel well when he or she awakens a few hours later. Lying down is the best thing you can do, since this counteracts the effects of the lowered blood pressure. Certainly while you are feeling ill you should not try to do things like driving a car or climbing a ladder or other activities that would endanger you or others because of your feeling of weakness and discomfort. If a reaction makes you extremely ill, or if you become concerned, it would be appropriate for you to call a doctor or go to a hospital emergency room. Do not try to drive during a reaction. If you don't take any alcohol, you won't have a reaction.

It is a good idea to get used to a regular pattern in taking your Antabuse. Perhaps you can combine it with some other activity that you rarely forget. If you are married or have a family member or other helpful person with you regularly, it is a good idea to take your medication at a time when they see you take it so that if you forget he or she can remind you about taking it each day. Also, by informing your spouse or other close person(s) that you are on Antabuse, this will help them to not give you anything to drink or eat containing alcohol. If you forget to take it at the regular time you should take it when you do remember. Lots of people ask how long they will have to stay on this medicine. We have discovered that most people don't like the idea of being on medicine indefinitely, and decide in their own minds that they will give this a brief trial. We recommend that you take the medication for one year, then decide at that time if you should continue. In any case, we recommend that you talk with your doctor or counsellor before you consider stopping your Antabuse.

Years ago people were given a "challenge." They were given alcohol after they were started on Antabuse so they would experience an "Antabuse reaction." We don't see any need for this. You have been told what will happen if you drink alcohol when on Antabuse, and we think that is sufficient.

(If further questions should come up in your mind about Antabuse, please be sure to ask about them.)

I,_____, hereby ask for and consent to Antabuse

(disulfiram) treatment as explained in this material and by Dr. _____.

Witness_____ Patient's Signature_____.

Date_____

VA Form 10-63 (677)
August 1983

APPENDIX B-

Assessment Forms

By_____ NUTRITIONAL ASSESSMENT FORM - DISULFIRAM-NUTRITION

Date_____

Subject Code No._____Date Admitted_____Age_____Sex_____

Education_____Occupation_____Income_____

Ethnic Background_____ Marital Status_____

Number of persons living in household_____

Medical History:

Date of last examination before admittance and reason_____

Food Intolerance &/or Allergies_____

Hyperlipidemia_____

Cardiovascular Disease_____

GI Disorders_____

History of Alcohol Intake_____

_____

History of Abuse Drug Intake_____

History of Prescription Drug Use_____

_____

Renal, Pancreatic &/or Hepatic Disease_____

Other diseases_____

Hospitalization (s) and Diagnosis_____

Current diagnosis (s)_____

Current Symptoms and Clinical Signs (that may affect nutritional status):

Symptoms	Clinical Signs
Anorexia_____	Skin (dermatitis)_____
Vomiting_____	Eyes_____
Diarrhea_____	Hair_____
Stomach Pain_____	Teeth-Gums_____
Excessive Fatigue_____	Tongue_____
Peripheral Numbness, Tingling, or Pain_____	Mouth-Lips_____
Loss of Taste Acuity_____	Nails_____
	Neurological (confusion)_____
Other_____	Other_____

Current Medication (s)_____

Vitamin/Mineral Supplements_____

Tobacco Smoking; Length of time_____Quantity per day_____

Dietary Information:

Current Diet Order_____

_____

Trouble Chewing or Swallowing_____

Review of Diet History (Food Frequency, 24-hour recall, etc) expressed as total percent of recommended intake for adult:

Foods Eaten	% of Recommended/Day	Comment
Meat, Fish or Poultry	_____	_____
Milk or Milk Products	_____	_____
Fruits &/or Vegetables	_____	_____
Breads &/or Cereals	_____	_____
Alcohol Containing Beverages	_____	_____
Total % of Recommended Intake	_____	_____

Daily intake of Coffee, Tea, Cola _____

Recent changes in food intake and meal planning_____

_____

Cultural/religous dietary practices_____

Vitamin/mineral supplements (taken before admittance)_____

_____

Physical Activity (level)_____How often and what activities_____

Food preparation and storage facilities_____

Who purchases and prepares food?_____Participation in Food Assistance Programs?_____When?_____

NUTRITIONAL IMPLICATIONS OF PATIENT HISTORY (medical, socioeconomic, dietary):

Anthropometrics:

Height_____ Body Weight: Admitting_____ Initial_____ (study)

Termination_____ Ideal Body Weight(IBW)_____ % IBW_____/_____

Triceps Skinfold_____/_____ % Std._____/_____

Mid Arm Circumference_____/_____ % Std._____/_____

Mid Arm Muscle Circumference_____/_____ % Std._____/_____

Subscapular Skinfold_____/_____ % Std._____/_____

Thigh Skinfold_____/_____ % Std._____/_____

Body Fat_____/_____ % Std._____/_____

Laboratory Values:

Test	Initial	Termination	Normal
Hemoglobin			14.0-18.0 gm/dl
Hematocrit			42-52%
Total Leukocytes (TLC)			5.0-10.0 $X10^3$
Mean Corpuscular Volume (MCV)			80-94 um^3
GGT			11-63 IU/L
S. Albumin			3.0-5.2 gm/dl
Total Protein			6.0-8.5 gm/dl
Fasting Glucose (blood)			65-110 mg/dl
Triglycerides			20-200 mg/dl
Total cholesterol			140-280 mg/dl
HDL-cholesterol			29-61 mg/dl
LDL-cholesterol			66-178 mg/dl
Calcium			8.5-10.5 mg/dl
Phosphorus			2.5-4.5 mg/dl
Serum Folate			7-19 ng/ml
Cobalamin (B-12)			200-900 pg/ml

NUTRITIONAL IMPLICATIONS OF PHYSICAL EXAM (anthropometrics, clinical findings):

NUTRITIONAL IMPLICATIONS OF LABORATORY VALUES:

NUTRITIONAL IMPLICATIONS OF DRUG-NUTRIENT INTERACTIONS:

DISULFIRAM-NUTRITION STUDY

DIET HISTORY FORM

CLIENT NO._____

24-HOUR RECALL

Date	Time	Food/Amount	Where	With Whom	Associated Activity

Trouble chewing or swallowing_____

Meal times (usually) AM_____PM_____

Snacks_____What times?_____

Recent changes in food intake or meal patterns_____

_____

Vitamins/mineral supplements_____How Often?_____

Why?_____

"Health foods", dietetic foods or convenience foods_____

How often and why?_____

Cultural/religious dietary practices_____

Food preparation and Storage facilities_____

Who purchases and prepares food?_____

Storage time of fresh fruits and vegetables_____

How prepared?_____

What type of cooking utensils used?_____

Fried foods_____Frequency_____

Participation in Food Assistance Program(s)_____

_____

Meals eaten away from home_____Where?_____How often?_____

Wt._____Wt.,10 years_____Wt., 20 years_____Desired Wt.___

Maximum Wt.(age)_____Minimum Wt. (age)_____

Physical activity_____

How often and what activity?_____

Hobbies/recreational activities_____

_____

What periods of your life have you been overweight?_____

Overweight relatives_____

DHx -3- (D/NS)

Do you include the following foods in the diet every day?

2 servings meat, fish, or poultry_____

2 servings milk or milk products_____

4 servings fruits and vegetables_____(vitamin C)_____

4 servings breads and/or cereals_____

2 servings butter, margarine or oil_____

Additional comments:

FOOD FREQUENCY SCHEDULE - DISULFIRAM-NUTRITION STUDY

Client No._____

FOOD	DON'T EAT	DO EAT TIMES/DAY	DO EAT TIMES/WEEK
I. MEAT GROUP			
Chicken			
Beef, hamburger, veal			
Liver, kidney, tongue, Etc.			
Lamb			
Coldcuts, hot dogs			
Pork, ham, sausage			
Bacon			
Fish			
Kidney beans, pinto beans, lentils (all legumes)			
Soybeans			
Eggs			
Nuts or seeds			
Peanut butter			
Tofu			
II. MILK GROUP			
Milk (fluid, dry, evaporated)			
Cottage Cheese			
Cheese, all kinds other than cottage			
Condensed milk (sweetened)			
Ice cream			
Yogurt			
Pudding and custard			
Milk shake			
Sherbet			
Ice milk			
III. BREAD AND CEREAL GROUP			
Whole grain bread			
White bread			
Rolls, biscuits, muffins			
Bagel			

FSS,2 FOOD	DON'T EAT	DO EAT TIMES/DAY	TIMES/WEEK
Crackers, pretzels			
Pancakes, Waffles			
Cereals (cooked or dry)			
White rice			
Brown rice			
Noodles, macaroni, grits			
Tortillas (flour)			
Tortillas (corn)			
Corn			
Sweet potato or yam			
Green (sweet) peas			
Lima beans			
Hominy			
Cakes, pies, cookies			
Sweet rolls, doughnuts			
IV. FRUITS AND VEGETABLES			
Tomato or tomato juice			
Stewed tomato or tomato sauce			
Orange or orange juice			
Tangarine			
Grapefruit or grapefruit juice			
Mango, avacado			
Lemonade			
Turnip			
Peppers (green, red, chili)			
Strawberries			
Dark green or red lettuce			
Asparagus			
Swiss Chard			
Bok Choy			
Cabbage			
Broccoli			
Brussels sprouts			
Onions			
Scallions			

FSS,3 FOOD	DON'T EAT	DO EAT TIMES/DAY	TIMES/WEEK
Spinach			
Greens (beet, collard, kale, turnip mustard)			
Carrots			
Artichoke			
Zucchini			
Summer squash			
Green and wax beans			
Beets			
Cucumbers or celery			
Peaches			
Apricots			
Apples			
Bananas			
Pineapple			
Cherries			
Plums			
Dates			
Raisins			
V. OTHERS (MISCELLANEOUS) Candy			
Sugar or honey			
Carbonated beverages			
Coffee or tea			
Cocoa			
Wine, beer, cocktails			
Fruit drinks			
Irish Potatoes (HOW PREPARED?)			

APPENDIX C-

Laboratory Procedure

September 1984

CHOLESTEROL DETERMINATION (TOTAL, HDL, LDL) WITH MICRO-SCALE

AFFINITY CHROMATOGRAPHY COLUMNS

Column separation of alpha and beta fractions per Isolab instructions using Isolab columns and elution fluids.

Determination of cholesterol can be done with other enzyme methods as long as samples are diluted correctly and the ratio of serum to enzyme is maintained (appropriate to the reagent directions.)

Sigma quantitative, enzymatic determination of total and HDL cholesterol in serum at 500nm (procedure No. 351)
Sigma procedure modified as follows:

1. Macro-method (greater than 1 ml reaction volume) for total cholesterol used for all determinations so that Brinkmann probe colorimeter could be used.

2. Total cholesterol serum samples and standards (50 and 200mg) diluted 1:6 to match dilution factor of alpha and beta eluates.
   i.e. 0.2 ml serum or standard
   1.0 ml saline
   1.2 ml total volume = eluate volume and concentration

3. Once all serum, eluates and standards are at similar dilution, 0.12 ml is added to 1.0 ml of reagent so that correct ratio of serum to reagent is maintained (i.e. original Sigma method: 0.02 ml serum to 1.0 ml reagent = 1:50, therefore, diluted serum and standards use 0.12 ml (6x) to 1.0 ml reagent = 1:50).

4. More consistent results obtained if reagent is reconstituted (50 ml deionized water/bottle) several hours (or overnight) before use.

PROCEDURE: Separation of alpha and beta fractions (HDL and LDL cholesterol)

Isolab LDL-Direct Cholesterol Audit System
#QS-8160 (60 test)
Isolab Inc.
Innovative Biochemical Methodology
Drawer 4350
Akron, OH 44321 800-321-9632

Bentzen, C.L., Acuff, K.J., Marechal, B., Rosenthal, M.A. and Volk, M.E. (1982) Direct determination of lipoprotein cholesterol distribution with micro-scale affinity chromatography columns. Clin Chem 28(7): 1451-1456.

1. Remove first the column's top cap, then the bottom closure. This order of opening is important - otherwise air will enter the column tip, interfering with free liquid flow.

2. Use the wide end of a Pasteur pipette to push the upper disc down until it contacts the top of the resin bed. Do not compress bed.

3. Allow the column to drain until the liquid level reaches the top disc, where flow will automatically stop.

4. Check to determine whether air may have entered the column during shipment. A few small air bubbles will not affect its performance. However, large volumes of air should be removed by tilting the top disc until the bubble escapes, then returning the disc to its original position.

5. Equilibrate the column bed by adding 1.0 ml of Alpha Fraction Elution Agent (Reagent #1) to the column. Allow column to drain. Discard eluate.

6. With the column positioned over a test tube (12 x 75mm, 5 ml), add 0.2 ml patient serum to the column, near or on the upper disc. Collect the eluate.

7. Add 1.0 ml of Alpha Fraction Elution Agent (Reagent #1) and collect the entire volume in the same test tube, for a total fraction volume of 1.2 ml. Mix well.

8. Place the column over a clean 12 x 75 mm tube.

9. Add 1.2 ml of Beta Fraction Agent (Reagent #2) and collect the entire volume. Mix well
Fill column with saline or eluted Alpha Elution Reagent, recap and store for possible regeneration.

PROCEDURE: Determination of Cholesterol (total, HDL, LDL)

Cholesterol, Total and HDL Quantitative, Enzymatic Determination in Serum or Plasma at 500 nm (Procedure No. 351). Cholesterol Reagent, Catalog No. 351-50, 50 ml size
Sigma Diagnostics
P.O. Box 14508
St. Louis, MO 63178
800-325-3010 (order)
800-325-8070 (service and technical information)

Cholesterol Standards:
Dow Diagnostics
The Dow Chemical Company
Indianapolis, IN 46268
200 mg/dl - 212688 lot # 3M4L
expiration Dec. 1985
50 mg/dl - 213967 lot # 3M4M
expiration Dec. 1985
Cholesterol in solution of ethylene glycol monomethyl ether and stabilizer

Procedure

Incubator at 37° C

Reagent should be reconstituted (50ml deionized distilled water/bottle) several hours or overnight before assay.

Disposable glass tubes 12x77 mm (5 ml culture tubes)

A. Dilution of standards and serum for total cholesterol (1:6 to match dilution of alpha and beta fractions during separation).

1. Pipette 0.2 ml of each STANDARD (50 and 200 mg/dl) or SERUM SAMPLE for total cholesterol into tubes.

2. Add 1.0 ml SALINE to each of the above tubes and vortex.

B. Cholesterol Determination

1. Pipette
0.12 ml saline for blank (or distilled water)
0.12 ml diluted serum for total cholesterol
0.12 ml diluted standards
0.12 ml alpha fraction
0.12 ml beta fraction
(run duplicates of each, except blank)

2. To each tube add 1.0 ml cholesterol REAGENT

   Cover with parafilm and invert several times to mix (gently)

3. Incubate all tubes at 37°C for 12-15 minutes.

4. Following incubation, add 1.0 ml SALINE to all tubes and vortex gently. (can add 2.0 ml saline to increase volume to 3 ml to read in Spec. 20).

5. Read BLANK - 100% transmittance, 0% absorbance as reference at 545 nm (direction indicate 500 ± 15 nm but Brinkmann probe has filter at 545nm). Read and record absorbance of STANDARD and SAMPLES.

   COMPLETE ALL READINGS WITHIN 30 MINUTES OF INCUBATION

6. Calculate cholesterol as follows:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Standard}}} \times \text{Concentration of standard (50 or 200)}$$

7. Calculate percent recovery of alpha and beta fractions:

$$\text{\% Recovery} = \frac{\text{HDL (mg/dl)} + \text{LDL (mg/dl)}}{\text{Total Cholesterol (mg/dl)}} \times 100$$

(recovery generally 94-97%)

THE SHORT-TERM EFFECTS OF DISULFIRAM (ANTABUSE™) TREATMENT ON NUTRITIONAL STATUS AND BLOOD CHOLESTEROL LEVELS IN ABSTAINING ALCOHOLICS

by

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B.S., Southwest Missouri State University, 1983

AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1985

Abstract

Disulfiram (tetraethylthiuram disulfide) is an alcohol-sensitizing compound used to encourage the alcoholic to maintain abstinence while receiving primary therapy for alcoholism. Several investigations have suggested that administration of disulfiram in therapeutic doses (500 mg per day) may be associated with increased serum cholesterol levels and subsequent increased risk for atherosclerosis and bilary complications. However, little is known about the effects of disulfiram therapy on the nutritional status of the alcoholic. The purpose of this 21 day, double-blind study was to investigate the short-term effects of disulfiram on the nutritional status and serum cholesterol of abstaining alcoholics.

No significant changes in overall nutritional status were observed in 15 male alcoholic subjects receiving 250 mg disulfiram (Antabuse™) daily for 21 days. Disulfiram treated subjects had increased mean fasting blood glucose levels (93.9 to 95.2 mg/dl) while the placebo group (n=16) had a mean decrease of 3.6 mg/dl, indicating the need for further investigation. Serum folate increased significantly in all groups receiving folate supplementation ($p<0.002$), but showed no effect from disulfiram administration.

Administration of 250 mg of disulfiram per day increased mean total serum cholesterol from 203.0 +/-34.6 mg/dl to 225.8 +/- 27.1 mg/dl ($p < 0.0002$ between treatment groups) and mean low-density lipoprotein cholesterol 138.4 +/- 21.2 mg/dl to

160.7 +/-19.8 mg/dl ($p < 0.0002$ between groups) after three weeks. The increase in low-density lipoprotein cholesterol was numerically equal to the increase in total serum cholesterol, implicating that fraction in the elevation of total cholesterol. Results from this study indicate an immediate effect (first 21 days) on total serum cholesterol and low-density lipoprotein cholesterol from disulfiram therapy with possible increased risk of hypercholesterolemia and atherosclerosis.