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BLOOD METHANOL CONCENTRATIONS DURING EXPERIMENTALLY INDUCED ETHANOL INTOXICATION IN ALCOHOLICS[1]

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ABSTRACT

MAJCHROWICZ, EDWARD AND JACK H. MENDELSON: Blood methanol concentrations during experimentally induced ethanol intoxication in alcoholics. J. Pharmacol. Exp. Ther. 179: 293–300, 1971. Accumulation of methanol in blood was detected in alcoholic subjects during a 10- to 15-day period of chronic alcohol intake. Blood methanol levels increased progressively from 0.2 to 2.7 mg/100 ml from the 1st to 11th day of drinking, when blood ethanol concentrations ranged between 150 and 450 mg/100 ml. Blood ethanol was eliminated at the rate of 27.2 ± 3 mg/100 ml/hr within 14 to 18 hours after cessation of drinking. Blood methanol levels decreased at the rate of 0.29 ± 0.04 mg/100 ml/hr only after blood ethanol levels decreased to 70 to 20 mg/100 ml. Blood methanol disappearance lagged behind the linear disappearance of ethanol by approximately six to eight hours and complete clearance of blood methanol required several days. The pattern of accumulation and clearance of methanol and ethanol was similar for subjects who consumed either beverage alcohol (bourbon) or methanol-free grain alcohol. Methanol probably accumulates in the blood as a result of competitive inhibition of alcohol dehydrogenase by ethanol and the presence of endogenously formed methanol or its metabolites may contribute to the severity of intoxication and/or the alcohol withdrawal syndrome.

Since the early 1900s there has been considerable controversy as to whether trace amounts of endogenous methanol (Western and Ozburn, 1949) or endogenous ethanol were present in mammalian organisms (McManus *et al.*, 1960). However, recent studies by Eriksen and Kulkarni (1963) with sensitive and specific gas chromatographic techniques showed that methanol can be detected in trace amounts in breath samples obtained from man. In 1965, Axelrod and Daly identified an enzyme system which metabolized S-adenosylmethionine to methanol and S-adenosylhomocysteine. This methanol-forming enzyme system was found to be highly localized in the pituitaries of a variety of mammalian species including man (Axelrod and Daly, 1965).

Although the exact enzymatic mechanisms involved in the metabolism of methanol have not been clearly delineated, it has been well established that ethanol competitively inhibits methanol oxidation (Leaf and Zatman, 1952). The competitive inhibition of methanol oxidation by ethanol underlies the rationale for the treatment of methanol poisoning with ethyl alcohol (Röe, 1955). Thus, it is possible that metabolism of endogenously synthesized methanol might be inhibited during chronic ethanol ingestion with the subsequent accumulation of methanol in body tissues.

In addition to ethanol, most alcoholic beverages contain trace quantities of other short-chain aliphatic alcohols including methanol (Caroll, 1970). Although trace amounts of these substances may be innocuous, accumulation of methanol during chronic ingestion of alcoholic

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beverages might be associated with toxic effects. The purpose of this study was to assess blood methanol levels in alcoholics after consumption of both beverage alcohol (bourbon) and grain alcohol which contains very little or no methanol.

METHODS. *Experimental subjects.* Nineteen adult male volunteers were selected from a group of inmates in an alcohol rehabilitation facility. They had been in that institution for at least one week prior to this study and had ingested no alcoholic beverages during that period of time. All subjects provided informed consent for participation in the study and were free to leave the experiment at any time. The subjects ranged in age from 26 to 49 years and reported a history of alcoholism of 12 to 36 years duration.

The subjects were studied in groups of four to six. They were housed on a research ward and given a standarized 2000-calorie diet with daily multivitamin supplements. After seven days of acclimation to the research ward, a 10- to 14-day drinking period was initiated. During the drinking period, subjects were permitted to consume up to 32 ounces per day of beverage alcohol (bourbon containing 50% ethanol) or 50% U.S.P. ethyl alcohol (grain alcohol) on a spontaneous drinking regimen. After cessation of drinking, the subjects remained on the research ward for 7 to 10 days. At the time of discharge they showed no evidence of withdrawal signs or intercurrent illness.

Gas chromatographic analyses. Determinations of ethanol, methanol, acetaldehyde and acetone in fingertip blood were carried out during the entire course of the study including three days of the predrinking period. The blood samples were usually taken between 7:45 and 8:45 A.M. unless inidcated otherwise. For the determination of these compounds we utilised a modification of the gas chromatography methods of Roach and Creaven (1968) and Baker *et al.* (1969). Fingertip blood samples were drawn by gravity into precooled heparin-treated micropipettes. The samples (0.1 ml) were transferred to an ice-cold solution of zinc sulfate (0.1 ml, 5%) diluted with 0.1 ml of water. The tubes were capped and barium hydroxide solution (0.1 ml, 0.3 N) was injected. After centrifuging at 10,000 × g for 10 minutes, a few drops of clear supernatant were rapidly decanted into precooled tubes which were stoppered immediately. The temperature of the samples never exceeded 2°C. Five-microliter aliquots of the supernatant were injected into a replaceable injection port of a 1.8-m column packed with Porapak Q, mesh 80 to 100. Helium was used as a carrier gas. Temperatures of the analysis were: injection port, 210°C; column, 140°C; and detector, 210°C. Since during long-term ethanol consumption there are several peaks of varying magnitude, it is impractical to use internal standards. Therefore, the samples were run alternatively with composite standards containing varying amounts of methanol, ehanol and acetone. Typical retention times seen were 34, 100 and 206 seconds for methanol, ethanol and acetone, respectively. Quantitative results could be obtained for biological fluid samples containing 0.1 mg of methanol per 100 ml. However, much lower concentrations of methanol could be detected qualitatively with this method. Several reagent blanks and water samples were included for each determination. Since regular laboratory distilled water may be frequently contaminated with traces of various volatile substances, all standard solutions and reagents were prepared with water redistilled from alkaline potassium permanganate.

Identification of methanol. The presence of methanol in human breath was first identified by Eriksen and Kulkarni (1963) with three separate column packings of quite different physical properties. Later the identity of methanol formed enzymatically by human and animal pituitaries was established by Axelrod and Daly (1965) with radioactively labeled $^{14}CH_3$-S-adenosylmethionine, which upon metabolic degradation gave rise to labeled methanol.

The specificity of the gas chromatographic method of Baker *et al.* (1969) was confirmed in this study. The recoveries of methanol added to blood samples were 95 ± 10%. The variations of temperatures of columns, detector and injector and variations in the rates of carrier-gas flow had no effects on the retention times of methanol in biological fluids as compared with the standards. Furthermore, identical results were obtained with Chromosorb 101 or Porapak Q-S as column packing. Methanol is eluted before acetaldehyde and ethanol (see also Baker *et al.*, 1969); therefore, concentrations of ethanol as high as 500 mg/100 ml had no effect on the shape and elution time of methanol.

Since acetoin may be formed during the metabolism of ethanol, we have tested several compounds of similar structure which were thought to be possible sources of methanol. Thus acetal, acetyl methyl carbinol (acetoin), bis(2-methoxyethoxy)ethyl ether and 2-(2-methoxyethoxy) ether gave no traces of methanol when analyzed under our experimental conditions.

As shown in this study, the blood levels of methanol conform with the kinetics of competitive inhibition of the enzyme by ethanol. There-

fore, this observation further supports the conclusion that the compound found is methanol.

Finally, the identity of methanol was also confirmed chemically utilizing the specificity of the color reaction between chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) and formaldehyde (Eegriwe, 1937) which has been used in numerous modifications of the original procedure. In our studies we utilized a modification by Hindberg and Wieth (1963). Methanol was isolated from 3-ml aliquots of blood or urine in a microdistillation flask fitted with an acetone-Dry Ice cold finger. One milliliter of distillate was treated with 1 ml of 8.1% (w/w) of ethanol and 5 ml of $KMnO_4$. After 10 minutes of oxidation at room temperature, 2.5 ml of 5% (w/v) $NaHSO_3$ were added followed by 0.5 ml of 85% H_3PO_4. One milliliter of the colorless reaction mixture was treated with 1 ml of 1.5% chromotropic acid and 8 ml of 95% H_2SO_4. After oxidation for five minutes in a boiling water bath and subsequent cooling, the optical density was read in a Beckman DBG spectrophotometer at 570 nm.

All samples from the subjects before the commencement of drinking and three to four days after the cessation of drinking, and from control subjects, had negligible amounts of methanol whereas the subjects had variable amounts of methanol during the experimental drinking period.

No significant correlation was found between blood acetone levels and the degree of intoxication during either the continuous drinking phase or during the alcohol withdrawal phase. The results of the acetaldehyde study were reported elsewhere (Majchrowicz and Mendelson, 1970).

RESULTS. Figure 1 presents mean blood methanol concentrations as a function of blood ethanol levels in subjects who consumed bourbon (11 subjects) or 50% grain alcohol (8 subjects) on a free choice basis for 11 and 14 days, respectively. During the predrinking period, blood methanol levels were always less than 0.1 mg/100 ml. On the first day of drinking, blood methanol concentrations were between 0.1 and 0.2 mg/100 ml when the mean range of blood ethanol concentrations ranged between 55 and 354 mg/100 ml. As drinking continued, mean blood methanol levels ranged between 1.1 and 2.7 mg/100 ml when the mean blood ethanol concentrations were between 157 and 435 mg/100 ml. On a few occasions, blood methanol concentrations of 4 mg/100 ml or more were detected in subjects whose blood ethanol concentrations exceeded 500 mg/100 ml. Although blood ethanol levels fluctuated daily, they were usually high enough to induce and maintain a significant degree of clinical intoxication.

Blood methanol concentrations were slightly higher in the bourbon drinkers than in the grain alcohol drinkers. However, the differences were not statistically significant. These data suggest that blood methanol originated primarily from endogenous sources. In addition, the absolute levels of blood methanol were related to duration of drinking and concentrations of ethanol in the blood.

Blood methanol and blood ethanol concentrations after cessation of drinking are shown in figure 2. Blood ethanol values ranged between 200 and 400 mg/100 ml at the initiation of the withdrawal period. Usually, ethanol was not detected in the blood after 14 to 18 hours following cessation of drinking. However, in a few subjects traces of ethanol approximating 1 mg/100 ml were detected up to 20 hours after ethanol withdrawal.

The concentration of blood methanol remained relatively constant in both groups of subjects until blood ethanol levels decreased to approximately 20 mg/100 ml. At this blood ethanol level blood methanol concentrations began to decline and, in most instances, blood methanol was cleared within two days after cessation of drinking. However, in a few cases disappearance of accumulated methanol did not occur until three or four days after termination of drinking.

Figures 3 and 4 present typical blood methanol and blood ethanol curves as a function of time during drinking and withdrawal for subjects who consumed either bourbon or grain alcohol. Blood methanol levels increased progressively from 0.2 mg/100 ml on the first day of drinking to 2.3 mg/100 ml in the bourbon subjects by the 11th day of drinking. A similar pattern of ascending blood methanol levels is related to the duration of ethanol ingestion. The concomitant pattern of increase in blood methanol levels was highly consistent and was categorized into four phases. Low methanol concentrations were found during the first day of drinking (phase I) but as drinking continued, methanol levels progressively increased (phase II). The highest levels of blood methanol were found at the termination of the drinking period. After alcohol withdrawal, blood methanol levels remained relatively stable for about 15 to 18 hours

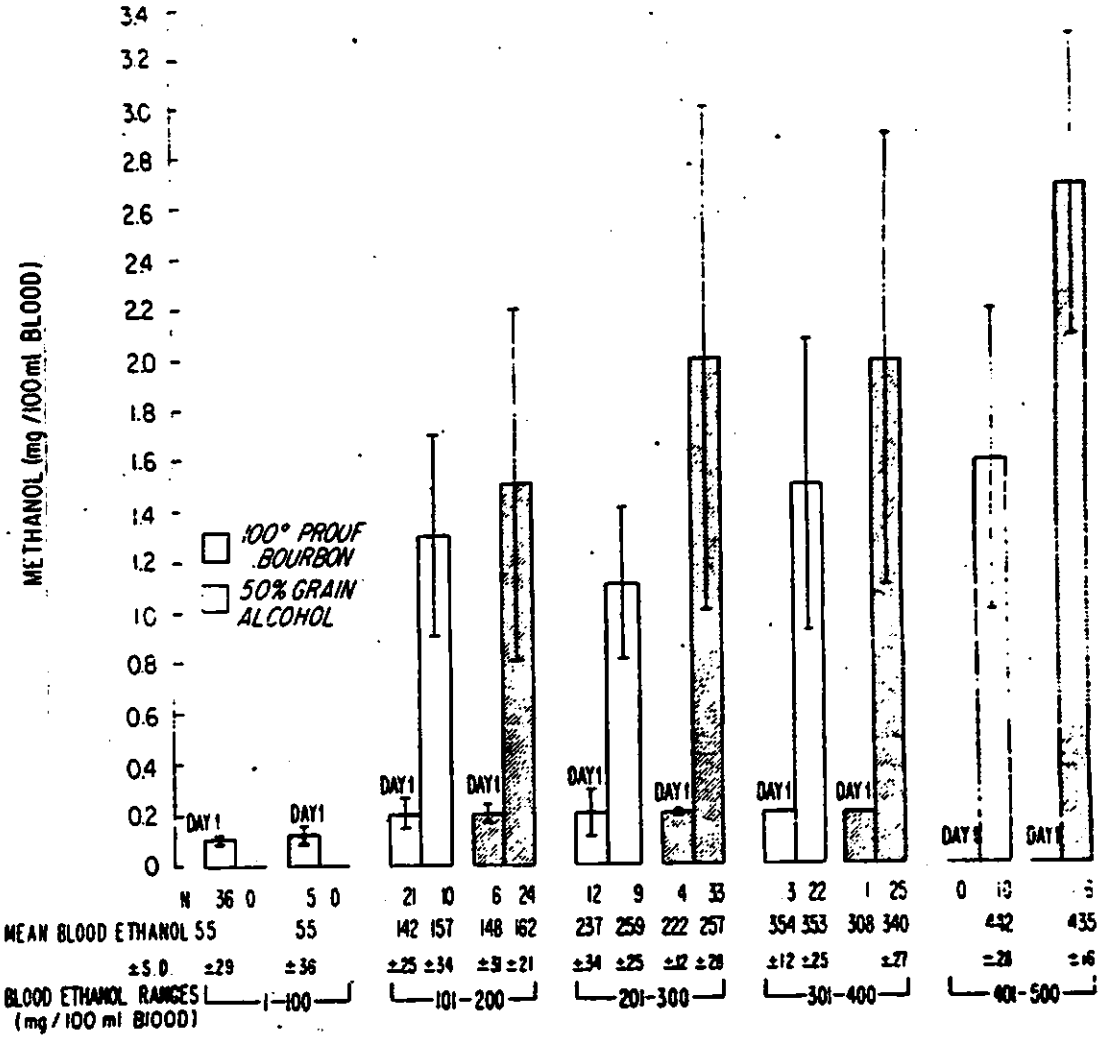

FIG. 1. Blood methanol concentration as a function of blood ethanol levels in bourbon and grain alcohol drinkers. The values are means ± S.D. The left side bar in each group represents the data for the first day of drinking. The right side bar represents the data for the period of continuous drinking. *N* represents the number of subjects whose daily blood ethanol levels were within the respective blood ethanol ranges.

(phase III) but when blood ethanol levels decreased to approximately 70 to 20 mg/100 ml, methanol levels began to decline (phase IV). Thus, blood methanol disappearance lagged behind the linear disappearance of ethanol by approximately six to eight hours and complete clearance of blood methanol accumulation and elimination in grain alcohol drinkers was similar to that of bourbon drinkers.

Rates of methanol and ethanol clearance from blood. These rates were calculated by the method of least squares from linear portions of the disappearance curves. Computation of clearance rates was made only for subjects for which at least three consecutive determinations were carried out. The mean clearance rate for ethanol was 27.2 ± 3 mg/100 ml/hr and is in good agreement with ethanol clearance data for man obtained in previous studies (Westerfeld and Schulman, 1959; Mendelson *et al.*, 1965). The mean clearance rate of methanol was 0.29 ± 0.04 mg/100 ml/hr. These findings are in agreement with those of Leaf and Zatman (1952).

Analysis of beverage alcohol. Gas chromatographic analyses carried out on representative samples of beverage alcohol revealed that meth-

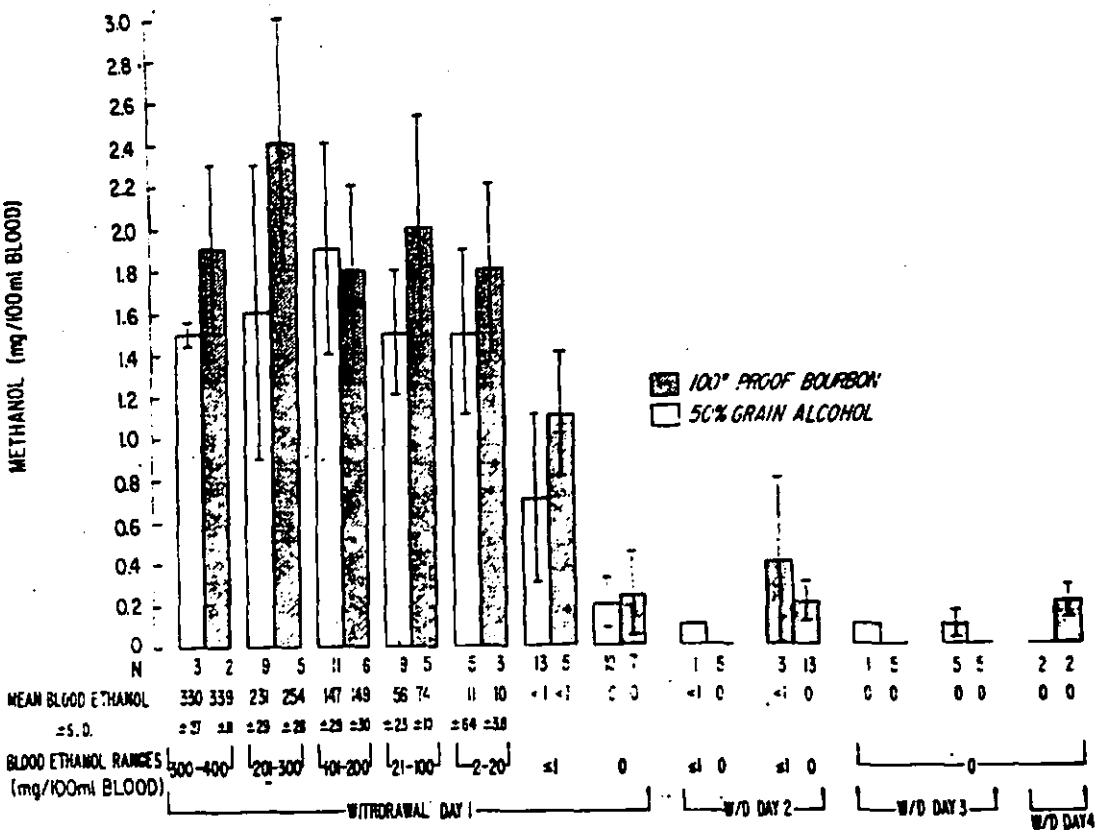

FIG. 2. Blood methanol concentrations as a function of blood ethanol levels in bourbon and grain alcohol drinkers following cessation of drinking. The values are means ± S.D.

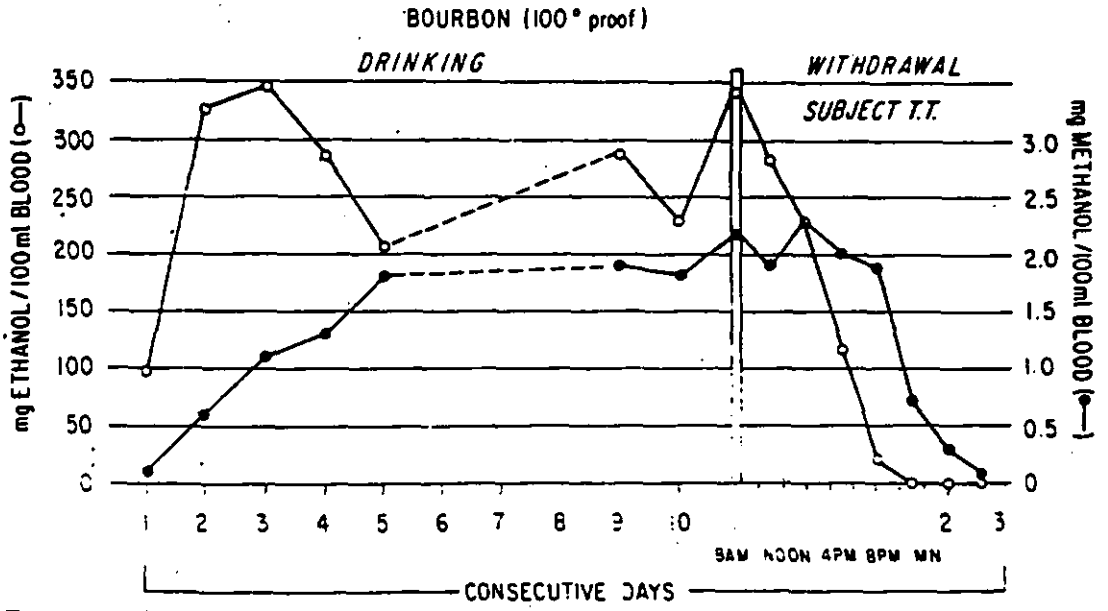

FIG. 3. Blood methanol and blood ethanol concentrations as a function of time in a bourbon drinker.

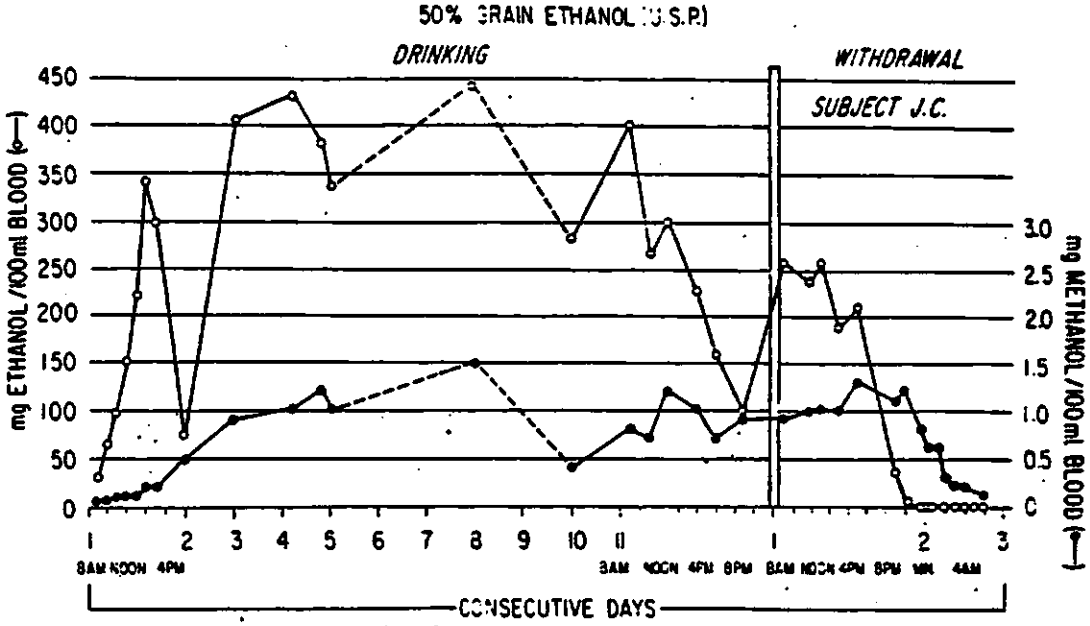

FIG. 4. Blood methanol and blood ethanol concentration as a function of time in a grain alcohol drinker

anol content varied between 40 and 55 mg/l of bourbon. This value is comparable with those reported by the distillers. The concentration of methanol in 50% grain alcohol was approximately 1 mg/l. Average daily consumption of bourbon or grain alcohol was 649 and 781 ml/subject, respectively. Assuming the mean concentration of methanol in the bourbon is 48 mg/l the daily ingestion of methanol was 31 mg/man. After absorption and equilibration, this dose of methanol would result in a concentration of 0.06 mg/100 g of body water in a 70-kg man. Furthermore, if the amount of ingested methanol were unaffected by such variables as the intervals of drinking, the rates of drinking, excretion and metabolic processes, the maximal concentration of methanol after 14 days of drinking would be 0.84 mg/100 g of body water. It is also calculable that the amount of methanol derived from grain alcohol would be undetectable through most days of drinking. It is suggested, therefore, that the presence of small amounts of methanol in the bourbon may explain a somewhat higher blood methanol level in the bourbon drinkers as compared with the grain alcohol drinkers, but not the maximal amount of methanol accumulated with either beverage. Therefore, these data suggest that most of the methanol found in the former group of drinkers and virtually all of the methanol found in the latter group is derived from endogenous sources.

DISCUSSION. Accumulation of methanol in blood was detected in all subjects studied during chronic ethanol intake. Blood methanol levels were only slightly higher in the bourbon drinkers than in the grain alcohol drinkers. This may be explained by the greater content of methanol in the bourbon (40–55 mg/l) as compared to the grain alcohol used (1 mg/l). However, the exogenous source of methanol cannot account for the amount of methanol that accumulated in the blood which suggests that a major fraction, if not all, of the methanol was more than likely derived from endogenous sources. It remains to be established if the methanol-producing enzyme system described by Axelrod and Daly (1965) is capable of producing quantities of methanol in blood similar to those found in this study. Data obtained in this study do not elimate the possibility that methanol may be derived in part from metabolic processes involving intestinal flora.

What are some possible mechanisms which may underlie the accumulation of blood methanol during chronic ethanol ingestion? Although both methanol and ethanol can be oxidized by catalase and alcohol dehydrogenase, there are differences in the major metabolic pathways which affect the disposition of these two alco-

hols. Tephly *et al.* (1964) showed that the peroxidative system involving hepatic catalase had a major role in methanol oxidation in the rat. Kini and Cooper (1961) found that the alcohol dehydrogenase system and not the catalase-peroxide system was responsible for the physiological oxidation of methanol in the monkey. These data have been confirmed in subsequent studies by Goodman and Tephly (1968). In addition, studies of Mani *et al.* (1970) indicate that human alcohol dehydrogenase oxidizes methanol at approximately 1/10 of the ethanol rate. The K_m values of 6.8×10^{-4} M (33 mg/100 ml) and 1.5×10^{-4} M (9.1 mg/100 ml) for methanol and ethanol, respectively, found for human liver alcohol dehydrogenase have good correspondence to the noncompetitive concentrations of ethanol found in this study (approximately 20 mg/100 ml). Since both methanol and ethanol are metabolized by the same enzyme systems and since ethanol competitively inhibits the oxidation of methanol, it is likely that methanol accumulates in the blood because of competitive inhibition of the enzyme which metabolizes both alcohols. This conclusion is supported by the finding that during the early part of the withdrawal phase, blood methanol levels remained relatively constant until blood ethanol levels declined to approximately 70 to 20 mg/100 ml. We also observed that when alcohol consumption was temporarily interrupted or decreased during the phase of continuous drinking (fig. 4), blood methanol levels remained unaffected if blood ethanol concentrations were above 20 to 70 mg/100 ml. These findings suggest that the enzyme which catalyzes the metabolism of both methanol and ethanol is saturated by relatively low concentrations of ethanol. Our data also indicate that levels of blood methanol are related to the concentrations of blood ethanol with time.

At the present time it is not possible to establish any definitive relationship between the degree of intoxication observed during chronic ethanol ingestion and the accumulation of methanol in blood. Similarly, it is difficult to determine the contribution of methanol catabolism to the alcohol withdrawal syndrome after cessation of alcohol ingestion. This question can only be answered by careful correlative studies of clinical phenomena and methanol metabolism in alcoholics after a period of experimentally induced alcohol intake and withdrawal. Such studies are currently underway in our research ward and laboratory.

CONCLUSIONS. Accumulation of methanol in blood of alcoholics was detected during long-term voluntary consumption of both bourbon and grain alcohol. Blood methanol levels are dependent upon time and dose-related concentrations of ethanol, suggesting that it accumulates as a result of competitive inhibition of alcohol dehydrogenase by ethanol. In both groups of drinkers the blood methanol may be derived in large part from endogenous sources.

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