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Ethanol-1-C^{14} Metabolism in Alcoholics and Nonalcoholics

Abstract. *Metabolism of ethanol-1-C^{14} was assessed in a group of alcoholic and nonalcoholic male subjects. All subjects were screened for absence of physical derangement. Subjects were also carefully matched by dietary, social, and environmental criteria. No differences in rate of output of $C^{14}O_2$ were detected after ingestion of alcohol which produced concentrations of 50 to 60 milligrams of alcohol per 100 milliliters of blood. These data do not support the hypothesis that alcoholics metabolize ethanol more rapidly than nonalcoholics do.*

The rate of ethanol metabolism is a variable which has been postulated to differentiate alcoholics from nonalcoholics, both in the alcoholic's behavioral tolerance for large quantities of alcohol and as a potentially important factor which may account for differences in susceptibility to alcoholism (*1, 2*). Alcoholics who drink up to 900 ml of beverage alcohol a day for several weeks can function well on a variety of perceptual-motor tasks (*3*). This finding suggests that behavioral tolerance may be related to an induced or innate metabolic efficiency with respect to alcohol. If it could be clearly demonstrated that the rate of ethanol metabolism differentiates alcoholic from nonalcoholic individuals, such data would facilitate the search for a biological mechanism upon which such differences could be based.

An induced increase in the rate of ethanol metabolism after prolonged ingestion of ethanol has been demonstrated in man (*4*). Also, studies with experimental animals have shown that increased rates of ethanol metabolism occur after forced ingestion of alcohol and that this enhanced metabolic rate may be correlated with an increase in activity of alcohol dehydrogenase in the liver (*5*). There have been no studies (with C^{14}-labeling techniques) of alcohol metabolism in alcoholics or nonalcoholics who had taken an acute dose. Although many studies have indicated that there are no significant differences in the rate of ethanol metabolism in alcoholics and nonalcoholics (*6*), two major difficulties related to the method of study prompt a further examination of this problem.

First, most studies have been concerned with the rate of disappearance of ethanol in the blood after acute oral or intravenous administration of ethanol. Because alcohol dehydrogenase acts as a rate-limiting factor in the metabolic degradation of ethanol, it has been assumed that serial measurements of alcohol concentrations in the blood would adequately reflect the overall rate of ethanol metabolism. However, it has been shown that differences in the specific activity of alcohol dehydrogenase (*7*) do not account for the observed interspecies differences in ethanol metabolism. Other factors, such as the rate of regeneration of nicotinamide-adenine dinucleotide from its reduced form in hepatic tissue, appear to be significant in limiting the rate of alcohol metabolism. Therefore, a comprehensive assessment of ethanol metabolism in man should include not only measurement of oxidation of alcohol to acetaldehyde, but also a study of the metabolism of intermediates to carbon dioxide and water.

Second, there has often been inadequate screening and matching of subjects and inadequate detail concerning effects of intercurrent illness and nutritional disorders. For example, many alcoholics have a variety of illnesses (associated with problem drinking) which may affect the rate of alcohol metabolism; these include hepatic, gastrointestinal, and nutritional disorders. Consequently, it is crucial to select subjects who are without evidence of any of these disorders.

In our experiment, alcoholics and nonalcoholic subjects were screened and matched to permit assessment of alcohol metabolism with minimum variables. In addition, the techniques for measurement of ethanol metabolism provide data concerning the total rate in vivo for all intermediates in man. More than 90 percent of the radioactive substance of C^{14}-labeled alcohol can be recovered as $C^{14}O_2$ (*8*). Forsander and Räihä (*9*) found that, after administration of C^{14}-labeled ethanol to intact rats or its addition to an isolated perfused rat-liver system, the label was also present in acetate, acetoacetate, β-hydroxybutyrate, and pyruvate. Goodman and Deykin (*10*) reported that alcohol is also a precursor of the ethyl esters of fatty acids.

Twelve adult male volunteers were selected from a group of inmates in a correctional institution; they had been in the institution for at least 3 weeks before the study and had ingested no alcoholic beverages during that period of time. They were given physical examinations and radiological and laboratory tests to rule out the presence of pulmonary, gastrointestinal (including hepatic), neurological, cardiovascular, or nutritional disease. All subjects received an identical diet and had the same opportunity for physical activity in a similar social milieu for 3 weeks prior to the study.

Six of the subjects were nonalcoholic individuals who had been incarcerated for offenses associated with sexual deviance. The six alcoholic subjects who had been incarcerated for offenses associated with disorderly conduct and alcohol intoxication had a history of at least 5 years of alcoholism and were classified as "gamma alcoholics" according to Jellinek's criteria (*1*). Gamma alcoholism is characterized by (i) recurrent ingestion of large quantities of alcoholic beverages; (ii) loss of control over drinking behavior; (iii) loss of social, family, and occupational resources because of drinking problems; and (iv) occurrence of withdrawal signs and symptoms after cessation of drinking.

The mean age of the alcoholic subjects was 39 years (range, 33 to 45 years); the mean age of the nonalcoholic subjects was 33 (range, 30 to 39 years). None had a history of drug addiction or had received any medication for at least 3 weeks prior to this study. All were of normal intelligence, and none showed evidence of a psychotic disorder.

Subjects did not ingest any food or water for 6 hours before the experiment. At zero time, subjects drank a 43-percent ethanol solution, the dose

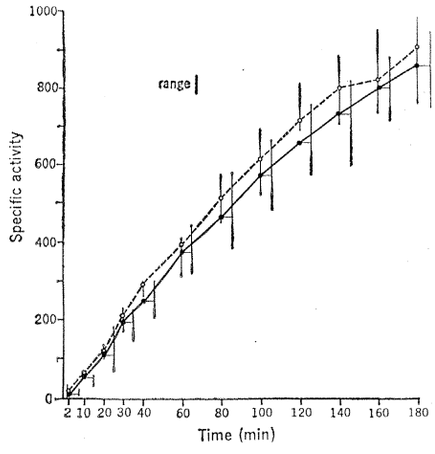

Fig. 1. Output of $C^{14}O_2$ after intake of ethanol-1-C^{14} by alcoholic and nonalcoholic subjects. Specific activity is expressed in picocuries per millimole of CO_2 per liter. Dashed line, six alcoholics; solid line, six nonalcoholics.

being 0.52 g of ethanol per kilogram of body weight. This dose contained 0.024 μc of ethanol-1-C^{14} per gram of nonradioactive ethanol (*11*). Each subject drank his ethanol solution within a 2-minute period of time. Peak concentrations of ethanol in the blood (between 50 and 60 mg per 100 ml) occurred 20 minutes after ingestion for both alcoholic and nonalcoholic subjects. This approximates the optimum ethanol concentration for maximum activity of alcohol dehydrogenase in the human liver (*12*).

After ingestion of ethanol at zero time, expired breath samples were collected at 2, 10, 20, 30, 40, 60, 80, 120, 140, 160, and 180 minutes. Carbon dioxide in breath samples was absorbed in 2*N* sodium hydroxide. Carbon dioxide was regenerated by acidification with concentrated sulfuric acid in a vacuum line apparatus to remove water through differential cooling with liquid nitrogen and dry ice in acetone. Gaseous carbon dioxide was determined manometrically in this apparatus, and a fraction of the CO_2 samples was absorbed in redistilled liquid Primene (*11*).

The counting of $C^{14}O_2$ was carried out in a scintillation-fluid mixture of 0.6 percent 2,5-diphenyloxazole and 0.0075 percent 1,4-bis[2-(5-phenyloxazolyl)]-benzene in toluene in a Packard Tri-Carb scintillation spectrometer with a C^{14}-counting efficiency of 77 percent. No significant quenching occurred as assessed by both internal-standard and channels-ratio methods.

The $C^{14}O_2$ was detected in expired breath samples 2 minutes after ethanol ingestion (Fig. 1). Thereafter, $C^{14}O_2$ increased linearly up to and including 100 minutes. From 100 to 180 minutes, output of $C^{14}O_2$ was curvilinear. This portion of the curve corresponded with concentrations of 20 mg or less of alcohol per 100 ml of blood.

The data indicate that there are no significant differences in the rates of ethanol metabolism in alcoholic and nonalcoholic individuals after administration of a large dose of alcohol. This finding is consistent with those of other investigators who have been unable to find differences in rates of ethanol metabolism in alcoholics as contrasted with those in normal subjects. The enhancement of ethanol metabolism after chronic ingestion of ethanol observed in alcoholics and nonalcoholics (*4*) is not manifested when subjects are abstinent for a period of 3 weeks. It is likely that any enzymatic induction produced by prolonged ethanol ingestion is transitory and does not persist when an individual ceases drinking.

These data do not preclude the existence of a unique pathway for ethanol metabolism in alcoholics. New intermediates in the metabolism of ethanol (*13*) for rat tissue may exist in man; also, even small amounts of such intermediates may have significant pharmacological or pathological effects. However, it is unlikely that any large quantities of new or already identified intermediates accumulate or are retained in alcoholics as contrasted to nonalcoholics. My findings lend support to the conclusions of Westerfeld and Schulman (*8*) that tolerance to alcohol is related to processes of adaptation in the central nervous system rather than to alterations in the rate of metabolism of ethanol.

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13 November 1967

Binuclear Ion Containing Nitrogen as a Bridging Group

Abstract. *A binuclear ion* $[(NH_3)_5RuN_2Ru(NH_3)_5]^{4+}$ *is formed by the direct reaction of* N_2 *with an aqueous solution of* $(NH_3)_5RuOH_2^{2+}$ *at room temperature. The binuclear ion is also formed by the reversible reaction of* $(NH_3)_5RuOH_2^{2+}$ *with* $(NH_3)_5RuN_2^{2+}$. *Solid* $[(NH_3)_5RuN_2Ru(NH_3)_5]$ $(BF_4)_4$ *has been prepared, and its ultraviolet and infrared spectra are reported.*

The discovery by Allen and Senoff (*1*) of the ion $(NH_3)_5RuN_2^{2+}$ is important in showing that a combination of metal ion and N_2 once formed can persist. Of equal significance for the fixation of nitrogen by homogeneous catalysis in solution is Allen and Senoff's (*1*) discovery that coordinated nitrogen is much more readily reduced than free nitrogen is.

The observation (*2*) that $(NH_3)_5RuOH_2^{2+}$ (*3*) reacts spontaneously in aqueous solution with N_2 to form Allen and Senoff's ion demonstrates a remarkable thermodynamic stability for this nitrogen complex and, furthermore, shows that the capacity of a metal ion to combine with N_2 can exceed its capacity to reduce H^+. The fact that the equilibrium constant for the reaction

$$H_2 + 2(NH_3)_5RuOH_2^{3+} = 2H^+ + 2(NH_3)_5RuOH_2^{2+}$$

is greater than 10^3 (*4*) shows that $(NH_3)_5RuOH_2^{2+}$ is a weaker reducing agent than H_2, so that even when H^+ is at unit activity, with H_2 at 1 atm,