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A Quantitative Analysis of Ethanol and Acetaldehyde Expired by Inbred Mouse Strains

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FIORIGLIO, C., J. WOOD, R. A. HARTLINE AND C. W. SCHNEIDER. *A quantitative analysis of ethanol and acetaldehyde expired by inbred mouse strains*. PHARMAC. BIOCHEM. BEHAV. 12(3) 467–469, 1980.—Expired ethanol and acetaldehyde were measured after an oral injection of ethanol in C57BL/6J and DBA/2J mouse strains by a combination of several techniques in a sequence involving a method for trapping expired radioactive compounds, separation of compounds by gas chromatography, isolation of radioactive ethanol and acetaldehyde, and their quantitative analysis by liquid scintillation spectrophotometry. With the specific activities used in evaluation of the technique (0.1 Ci/mole, acetaldehyde; 1.1 Ci/mol, ethanol) the lower limit of sensitivity using 500 μl from a 10 ml trap is 955 pmoles for acetaldehyde and 101 pmoles for ethanol. However, in the animal experiments, injected ethanol has a specific activity of 1.1 Ci/mol which would make the specific activity of expired metabolically formed acetaldehyde the same. This results in a lower limit of sensitivity for acetaldehyde of 80 pmoles. The two strains were monitored for 80 min following an oral injection of 3.8 g/Kg of (2-^{14}C) ethanol. Comparing the two strains on the expiration of each compound the curves were identical.

Mice strains Expired ethanol and acetaldehyde Analysis

DURING experiments measuring CO_2 evolution to determine whether differences in tolerance between mouse strains C57 and DBA to intragastrically infused ethanol were due to metabolism, it became necessary to measure expired ethanol and acetaldehyde. The capacity of the one strain to rid itself, by expiration, of either or both of these compounds more rapidly than the other strain could account for, or contribute to, differences in tolerance.

To trap and analyze expired ethanol and acetaldehyde, we initially considered the method Forsander [1] used for examining expiration of these compounds by rats. The procedure consists of collecting expired compounds in a cold water trap followed by quantitative evaluation by head space analysis [2]. Since lower limits of analysis were not reported, we took the lowest quantity analyzed (0.02 μmoles of acetaldehyde) to be a possible index of the smallest quantity that could be determined by this method. Whether this value was determined by head space analysis of the total trap volume or calculated from analysis of an aliquot from the trap is not known since the volume analyzed was not reported [1,2]. If it is essential to analyze smaller quantities than Forsander measured [1], analysis of the total trap volume could require increasing the volume 100 to 200 times which would not be practical.

Because we were examining mice rather than rats we reasonably assumed that the amounts of ethanol and acetaldehyde expired would be less and that the method of Forsander [1] may not provide, or be easily modified to provide, the sensitivity needed. To be certain of achieving a lower limit of analysis than the quantities measured by Forsander, we chose to administer radioactive ethanol to the animals, separate the expired radioactive compounds by preparative gas chromatography, determine the radioactivity of each compound by liquid scintillation spectrophotometry, and calculate the quantity of each based on the specific activity of the ethanol administered. With a conservative estimate that we could accurately detect at least 200 cpm above background, we calculated, using the specific activity of the ethanol to be administered (1.1 Ci/mole), that the lower limit of sensitivity would be approximately 80 pmoles.

In this paper we describe the methods of trapping, separating, and analyzing expired radioactive ethanol and acetaldehyde, their reliability and sensitivity, and report on the quantities of ethanol and acetaldehyde expired by the C57 and DBA mouse strains.

METHOD

Chemicals

All chemicals were obtained from commercial sources; the components of the liquid scintillation fluor, Packard Instrument Co.; ethanol, Publicker Industries; paraldehyde, Eastman Kodak Co.; (2-^{14}C) ethanol, ICN; (2-^{14}C) acetaldehyde, Research Products International Corp. The liquid scintillation fluor consisted of 5.5 g of 2,5-diphenyloxazale,

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0.1 g of 1,4-bis-[2(5-phenoxazale)]-Benzene, and 1 liter of Toluene. Acetaldehyde is frequently oxidized to acetic acid during storage. Because we did not want acetic acid present during the separation of components by preparative gas chromatography, acetaldehyde used in the collecting traps was generated when needed by distilling paraldehyde containing 1 drop of mineral acid at 95–100°C and collecting the acetaldehyde in an ice-salt bath.

Trap for Collecting Expired Ethanol and Acetaldehyde

A 15 cm bacterial culture tube (1 cm i.d.) containing 10 ml of a 7:2 ethanol-acetaldehyde mixture immersed in a chloroform-liquid nitrogen slurry (−73°C) served as a trap for collecting expired ethanol and acetaldehyde from a metabolic chamber.

The metabolic chamber consisted of a 1/4 in. thick Plexiglas cylinder 11 cm long with an inside diameter of 7.5 cm. One end had a funnel shape exit port, 8 cm wide and 9 cm long that tapered to 0.5 cm i.d., cemented permanently to the cylinder. The entrance port contained an identical removable funnel made airtight by a large brass fitting that could be screwed onto threads cut into the end of the Plexiglas cylinder. Air flowed into the system from a compressed air tank at a steady rate (50 cc/min) measured by a Fisher-Porter Tri-Flat Variable Area Flowmeter set at 3. Air flowing through the chamber was directed into the trapping tube by connecting a glass tube, directed to the bottom of the trap, to the exit port of the chamber with Tygon tubing. Samples of 500 μl were taken from the trap for separation of the alcohol and acetaldehyde by preparative chromatography. There are two advantages in using a cold ethanol-acetaldehyde trapping solution. One, it minimizes the amount of water present and introduces no additional kinds of molecules in the subsequent gas chromatography. Both of these could interfere with separation. Two, it does not freeze at the temperature of the chloroform-liquid nitrogen bath. The advantage of the low temperature is that it eliminates loss due to evaporation during the trapping period.

Separation, Recovery, and Quantitative Analysis of Ethanol and Acetaldehyde

Separation of ethanol and acetaldehyde with a Porapak Q and Porapak P (1:1) packing was not possible with the large samples taken from the trap [5, 8, 10]. Therefore, to separate the ethanol and acetaldehyde in the 500 μl samples of trapping solution we used a Hewlett Packard Preparatory Gas Chromatograph Model 700 equipped with a thermal detector and an aluminum column (20 feet long, i.d. 1/4 in.) packed with carbowax 20 m on a chromasorb W support. Conditions were: injection port temperature, 115°C; column temperature, 100°C; detector temperature, 115°C; bridge current, 150 mA; carrier gas, helium at a flow rate of 40 ml/min. Separated components were recovered by attaching a Teflon tube to the exit port of the chromatograph and inserted into the bottom of a 5 ml Varian chromatographic collection vial containing 5 ml of scintillation fluor and immersed in a chloroform-liquid nitrogen slurry. The Teflon tube leading into the collection vial was removed from the chromatograph outlet, connected to a syringe, and the contents of the vial were rapidly transferred into a liquid scintillation vial and the radioactive content determined on a Packard liquid Scintillation Spectrophotometer Model 3225.

Subjects

Eight male mice, 70–80 days old, from each strain (C57BL/6J and DBA/2J) served as subjects. Each animal was injected orally with 3.8 g/kg of (2-^{14}C) ethanol (specific activity 1.1 Ci/mole) in distilled water and placed immediately into the metabolic chamber where they reached anesthesia within 3 min. Samples of expired air were taken at 5 and 10 min and at 10 min intervals thereafter for 80 min.

RESULTS AND DISCUSSION

Requirements for trapping expired compounds are that the metabolic chamber be air tight and that during collection minimal amounts of the volatile components are lost from the trap due to the flow of air through the trapping solution. The metabolic chamber was checked for leaks by examining for bubble formation when a soap solution is applied to the connections between the chamber and trap while air is flowing through the chamber. To examine for loss of ethanol or acetaldehyde from the trap during the collection time, radioactive ethanol or acetaldehyde was added to the trapping solution and an aliquot immediately counted to determine its radioactive content. Air from the metabolic chamber was bubbled through the solution at 50 cc/min for 10 min and another aliquot of the solution counted. After five trails each with radioactive ethanol and acetaldehyde, no loss of either of these compounds from the trapping solution was observed (data not shown).

Retention times of the preparative gas chromatography were: Air 4.5 min; acetaldehyde, 7.5 min; ethanol 12.5 min. These long retention times were necessary for complete separation of the components in the relatively large 500 μl samples injected. Attempts at achieving good separation of larger volumes (750 μl) were unsuccessful.

To determine the efficiency and linearity of the quantitative analysis following separation and recovery, three trials of nine different quantities, in terms of radioactive molecules, of acetaldehyde with a specific activity of 0.1 Ci/mole (from 955 pmoles of 24 nmoles, 212 to 5300 cpm) or ethanol with a specific activity of 1.1 Ci/mole (from 101 pmoles to 2.5 nmoles, 248 to 6200 cpm) in 500 μl of the trapping solution were injected into the chromatograph. The compounds were recovered at the exit port and their radioactive content determined. The three trials at each quantity were averaged and showed a recovery of radioactivity over the ranges examined of 75.3 to 78.7% and 93.1 to 97.7% for acetaldehyde and ethanol, respectively. The relationship between the quantities injected and the percentage recovered was 77% for acetaldehyde and 95% for ethanol. Results were the same with mixtures of ethanol and acetaldehyde. A least squares linear analysis of the quantities injected versus the amounts recovered based on the determined average percent recovery showed linear correlation coefficients of 0.9995 for acetaldehyde and 0.9998 for ethanol.

The lower limit of sensitivity using 500 μl from a 10 ml trap is 955 pmoles (199 nmoles in the trap) for acetaldehyde and 101 pmoles (2 nmoles in the trap) for ethanol with the specific activities used in these studies; 0.1 Ci/mole, acetaldehyde; 1.1 Ci/mole, ethanol. These specific activities were used either because of necessity for future metabolic studies or were the highest available. Since the quantitative analysis depends solely on the number of radioactive molecules, higher specific activities provide lower limits of sensitivity.

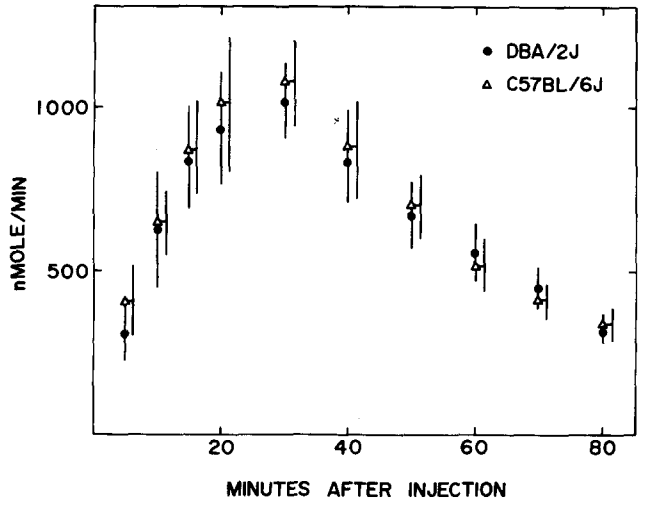

FIG. 1. Expired ethanol for two mouse strains at various intervals following oral injection. Vertical bars indicate SD.

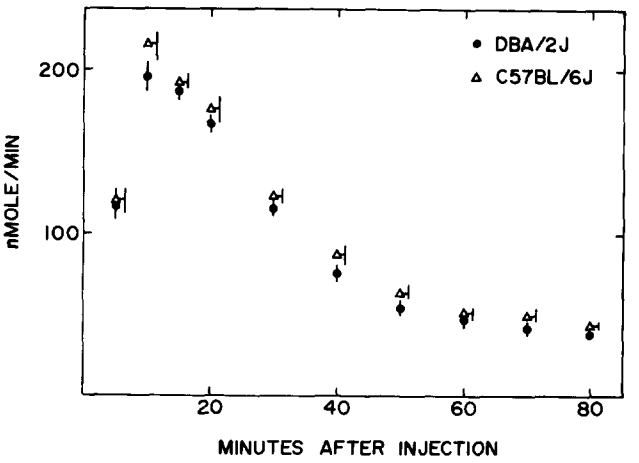

FIG. 2. Expired acetaldehyde for two mouse strains at various intervals following oral injection of ethanol. Vertical bars indicate SD.

Therefore, in these studies where the radioactive ethanol has a specific activity of 1.1 Ci/mole, acetaldehyde metabolically formed would be from the ethanol since it is not generated from any other molecule and would have a specific activity of 1.1 Ci/mole. Comparing the metabolically generated acetaldehyde (specific activity of 1.1 Ci/mole) with the acetaldehyde used in ascertaining the validity of the analysis technique (0.1 Ci/mole, the highest available commercially) 212 cpm represents 86 pmoles of metabolically generated acetaldehyde rather than 955 pmoles. This low limit of sensitivity (80 pmoles) represents a greater analysis sensitivity than the 0.02 μmole value Forsander reported for expired acetaldehyde by rats [1]. Compared to the Forsander method [1], the method reported here involves an additional step (separation by preparative gas chromatography) prior to quantitative analysis of the expired products. However, the sensitivity of analysis more than offsets the relatively minimal effort and time expended to do the separation.

Figures 1 and 2 show the levels of ethanol and acetaldehyde expired during the 80 min following the oral injection. Differences between the strains are small, and the pattern of expiration is identical. Both strains reached their maximum level of ethanol expiration 30 min after injection and the maximum level of acetaldehyde expiration 10 min after injection. It is well-known that these two strains differ in their tolerance to ethanol and other alcohols [3, 4, 6, 7, 9] and much of the data suggest that the difference is due to some characteristic in the response of the nervous system rather than the rate of metabolism [4, 6, 7]. The results we have obtained indicate further that the difference in tolerance between the strains cannot be linked to differences in expiration of ethanol or its first metabolic product.

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