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Note

Measurement of breath carbon disulfide during disulfiram therapy by gas chromatography with flame photometric detection

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The measurement of carbon disulfide (CS_2) in the breath is useful for monitoring compliance with disulfiram therapy [1]. Disulfiram (tetraethyl thiuram disulfide, Antabuse®) is often prescribed to alcoholics to help them maintain sobriety, since anyone who drinks ethanol after pre-treatment with disulfiram will experience the disulfiram—ethanol reaction, a subjectively unpleasant syndrome of flushing, malaise, nausea, dyspnea and palpitations [2]. In theory, fear of this reaction should persuade alcoholics to maintain long-term sobriety. In reality, many cease taking their disulfiram and resume drinking after the effects have worn off [3]. It is therefore clinically desirable to know if alcoholic patients are taking their disulfiram tablets as prescribed, and this can be confirmed by the presence of CS_2 (a metabolite of the drug) in the breath [4].

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Earlier breath assays have required "wet chemistry" techniques to form a derivative of CS_2 for subsequent assay. Rogers et al. [4] described a method of bubbling breath through a reagent (McKee's solution) which reacted with CS_2 to form a yellow-colored derivative. The intensity of the color change varied with the amount of CS_2 in the breath sample and was assayed spectrophotometrically. Although this method provided useful information about the absorption and metabolism of disulfiram, its clinical use was limited by relative insensitivity and the requirement for the patient to blow into the apparatus for several minutes.

Wells and Koves [5] described an advance in analytical methodology using a gas chromatographic assay. However, their method also required a wet chemistry step wherein the patient's breath was bubbled through a reagent to form a CS_2 derivative, which was then assayed by gas chromatography with flame ionization detection (FID).

The introduction of flame photometric detection (FPD) as an adjunct to gas chromatography has now made it possible to assay sulfur-containing compounds such as CS_2 with a high degree of sensitivity and without forming a chemical derivative beforehand [6].

We describe here a method for measuring the concentration of CS_2 in breath by gas chromatography with FPD, and the results obtained with the method in a patient treated with disulfiram.

EXPERIMENTAL

Breath collection tubes

Breath was collected in Pyrex® gas-collecting tubes (average volume 132 ml) sealed at each end with Teflon® taps. A side-port sealed with a Neoprene® rubber septum permitted aspiration of breath samples for assay. The structure of the tube, as well as the techniques for heating the tube (to prevent condensation) and collecting the breath specimen, have been described [7]. Prior to use, a 2.0-ml sample was aspirated and assayed (as described below) to exclude contamination, after the tube had been flushed first with compressed air and then with pure nitrogen.

CS_2 standards

Fresh standards were prepared each day. Carbon disulfide (Fisher Scientific, Itasca, IL, U.S.A.; reagent A.C.S. Lot No. 850527) was diluted in absolute ethanol (Pharmco, Publicker Industries, Linfield, PA, U.S.A.) to $1.66 \cdot 10^{-2}$ *M* and serial ethanolic dilutions (× 2) were prepared down to $5.2 \cdot 10^{-4}$ *M*. A 30-μl aliquot of each standard solution was pipetted into a breath tube via the side-port, which was then immediately sealed with a rubber septum. The breath tube was then heated (as described above) to volatilize the CS_2—ethanol solution, so that the final concentration of CS_2 in the vapor phase ranged from a high of $3.8 \cdot 10^{-6}$ mol/l down to a low of $1.16 \cdot 10^{-7}$ mol/l.

Gas chromatographic analysis

A gas-sampling syringe (Pressure-Lok, Series A-2, Alltech Assoc., Deerfield, IL, U.S.A.) was heated to prevent any condensation of the specimen. The

needle was then inserted through the septum of the side-port to aspirate a 2.0-ml sample of vapor, which was injected into the gas chromatograph. A Shimadzu Model GC-9A was used (Shimadzu Scientific, Columbia, MD, U.S.A.) equipped with both a flame ionization detector and a flame photometric detector. The injected sample was split at a T-junction in the oven into two approximately equal portions, each leading to a stainless-steel column (1.5 m × 3.2 mm I.D.) packed with Porapak Q (80—100 mesh). The sample was split in order to facilitate simultaneous assay by FPD and FID. The flow-rate of the carrier gas (nitrogen) was 60 ml/min. The column temperature was maintained isothermally at 165°C, and the injection port and flame photometric detector temperatures were both 225°C. The signal from the FPD channel was attenuated × 16.

Construction of standard curve

A two-point calibration curve was constructed from the highest and lowest CS_2 standards, using the exponential calibration program of the Chromatopac CR2AX microprocessor unit of the Shimadzu GC9A gas chromatograph. The linearity of this two-point curve was evaluated by injecting a series of intermediate dilutions of the standards and observing the concentration of CS_2, which was calculated automatically by the microprocessor unit. These calculated values were then plotted as a function of the actual concentration of CS_2 in the sample.

Clinical study

A male alcoholic subject (aged 44) was studied on the Detoxification Ward of the Veterans Administration Medical Center (North Chicago, IL, U.S.A.). He was medically stable and had been prescribed treatment with disulfiram as part of his program of rehabilitation. On the first day of therapy (after fasting overnight), he was given 250 mg of disulfiram (Antabuse, Ayerst Labs., New York, NY, U.S.A.) with 100 ml of water. Breath samples were collected at time zero, then at hourly intervals for the next 7 h, and also at 24 h. The samples were assayed for CS_2 content on the same day that they were collected, by the method described above.

Reproducibility

The reproducibility of the assay was determined by loading six breath collection tubes with a known quantity of CS_2 standard solution, so that the final concentration in the gaseous phase (after heating the tube) was $9.75 \cdot 10^{-7}$ mol/l. A 2.0-ml sample from each tube was assayed as described above, and the mean value of the assays (± S.D.) and the coefficient of variation were calculated.

RESULTS

There was a linear correlation between the concentration of CS_2 in the sample and the detector response ($r^2 = 0.99$). The assay readily detected levels of CS_2 as low as $1.0 \cdot 10^{-7}$ mol/l.

The results of the clinical study are shown in Fig. 1. No CS_2 was detected in

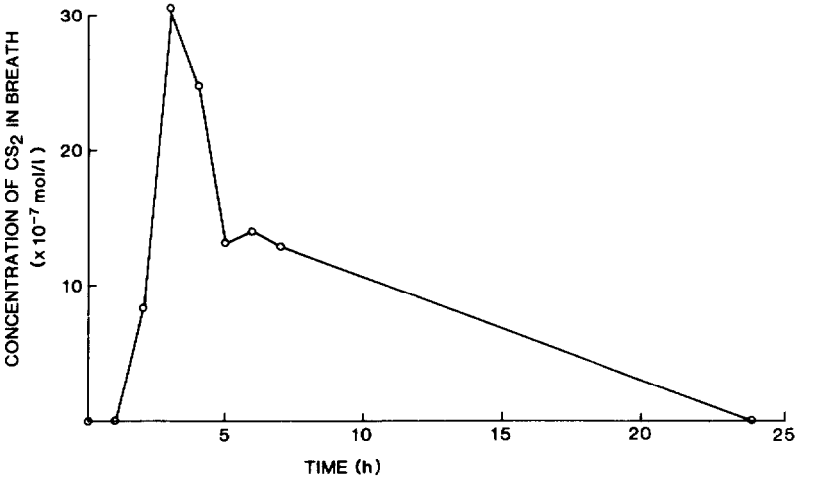

Fig. 1. Results of human study, showing concentration of CS_2 in breath over time following a single oral dose of 250 mg of disulfiram.

the breath until 2 h after the disulfiram tablet had been taken. The breath concentration of CS_2 peaked at 3 h (at $30.45 \cdot 10^{-7}$ mol/l) and was undetectable at 24 h. No signal was observed on the FID channel during this study.

The reproducibility study of six samples with a CS_2 concentration of $9.75 \cdot 10^{-7}$ mol/l yielded a mean value of $9.14 \cdot 10^{-7}$ mol/l, with a standard deviation of 0.95 and a coefficient of variation of 10.40%.

DISCUSSION

The assay yielded a linear response to CS_2 over the concentration range studied. In addition, it quantitated concentrations of CS_2 as low as $1.0 \cdot 10^{-7}$ mol/l. This degree of sensitivity is considerably greater than that reported with other breath assays for CS_2.

The clinical study demonstrated that this method detected CS_2 in the breath of a patient after he had taken 250 mg of disulfiram. The time course of the CS_2 level in the breath was similar to that described by Rogers et al. [4] using a different method.

This technique appears to provide an improvement in measuring CS_2 in the breath. It is convenient for patients because it requires only a "single puff" of breath into a tube. The assay is rapid, sensitive and reproducible, and does not require the formation of a chemical derivative of CS_2. This assay has potential applications in monitoring compliance with disulfiram therapy, and possibly in detecting environmental exposure to CS_2.

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