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Note

Detection of endogenous acetone in normal human breath

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During the nineteenth century, the development of chemical assays made it possible for the first time to detect acetone in the breath of diabetics [1,2]. These pioneer studies were amongst the earliest to demonstrate the value of breath analysis as a tool for the investigation of abnormal metabolism. Hubbard [3], in 1920, developed a more sensitive assay by bubbling breath through a sodium bisulfite solution, thereby concentrating the acetone contained in a large volume of breath into a sample of much smaller volume. Using this method he was able to detect endogenous acetone in the breath of normal volunteers.

It is now possible to detect acetone in the unconcentrated breath of normal subjects using gas chromatography (GC) with flame ionization detection (FID); mean concentrations of 1.1 μg/l (18.9 nmol/l) have been reported [4,5]. However, these methods are limited in practice by the low concentrations of endogenous acetone in normal breath, which are close to the lower detection limits of FID and require the operation of the detector at or near maximal sensitivity. Under these conditions, assays of unconcentrated breath may be difficult to interpret since the signal may be obscured by background noise in the detection system.

If the breath is concentrated beforehand, the sensitivity of the assay may be greatly increased. Three main methods have been described for concentrating the

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volatile compounds in the breath; cryogenic, chemical and adsorptive. The common feature of these methods is that breath is passed through a device in which the organic compounds are trapped while the nitrogen and oxygen in the sample pass through unhindered. This may be accomplished by cold trapping [6,7], chemical interaction [8,9] or adsorptive binding [10,11]. We have recently described a new GC method for the assay of endogenous ethanol, in which the breath sample was concentrated on the GC column by adsorption to the resin packing [12]. During the course of the research, it became apparent that the method could also provide a new technique for the assay of endogenous acetone in the breath. In this report, we describe the assay method, and its evaluation in a group of normal volunteers.

EXPERIMENTAL

Breath collection

The method has been described [12]. In summary, subjects inflated Mylar® bags (of approximately 5 l capacity) with a single breath; the bags had been preloaded with an internal standard (13.2 nmol isopropyl alcohol in 20 μl water). The volume of the breath sample was measured by a respirometer between the mouthpiece and the bag. The first 500 cm^3 of the breath sample were shunted into an auxiliary bag to ensure that the collected sample in the Mylar bag was not contaminated by dead-space air.

Concentration and assay of the breath sample

The Mylar bag was heated in the laboratory to approximately 65 °C to ensure that all the organic compounds in the breath were volatilized. The breath sample was then pumped from the bag to the GC column (Porapak Q, 80–100 mesh) maintained at 35 °C; at this temperature, the nitrogen and oxygen in the breath passed freely through the column, while the organic compounds (including the acetone) were adsorbed onto the packing material. The column was then heated to 120 °C, and the eluted acetone was assayed by FID. The method has been described [12].

Acetone standard curve

Standard solutions of acetone were prepared (Fisher Scientific, Itasca, IL, U.S.A.) in which 20-μl samples contained 13.2 nmol isopropyl alcohol as internal standard and 0, 4.0, 8.0, 12.0, 16.0 and 20.0 nmol acetone, respectively. Triplicate breath collecting bags were inflated with approximately 3.5 l nitrogen after loading with 20-μl samples of the acetone standards. The contents of each bag were assayed as described. The FID output to the microprocessor and printer yielded separate peaks for acetone and the internal standard. The area under curve (AUC) of both peaks was determined automatically, and the AUC ratio ($AUC_{acetone}$/$AUC_{internal\ standard}$) was calculated. The line of best fit (for the relationship between the mean AUC ratio and the quantity of acetone in the sample) was determined by the method of least squares.

Accuracy and precision of assay

Breath collecting bags ($n=6$) were loaded with 20-μl samples containing 20.0 nmol acetone and 13.2 nmol internal standard, and assayed as described. The intra-assay accuracy and precision were determined by calculating the mean, standard deviation and the coefficient of variation (C.V.) of the observed values [7].

Clinical study

Fifteen normal subjects donated breath specimens between 08:00 and 10:00 a.m., and the samples were assayed for acetone on the same day by the method described above. All subjects were laboratory and medical school workers in good health who had fasted from the preceding midnight. All breath samples yielded peaks eluting with the same relative retention times as the acetone peak in the standards, and the concentration of acetone in the breath was determined from the standard curve.

Analysis of data

Data were stored on diskette using a data base management program (Jazz™, Lotus Development, Cambridge, MA, U.S.A.) and a Macintosh™ 512K microcomputer (Apple Computers, Cupertino, CA, U.S.A.); linear regression and descriptive statistics were determined using a statistical analysis program (Statworks™, Cricket Software, Philadelphia, PA, U.S.A.).

RESULTS

Standard curve

There was a linear relationship between the number of nanomoles of acetone in the standard solution (x) and the mean AUC ratio (y): $y=0.022x+0.016$; $r^2=0.99$.

Accuracy and precision of assay

In the determination of intra-assay within-day accuracy and precision, samples containing 20.0 nmol acetone were assayed as containing a mean quantity of 25.0 nmol acetone ($n=6$, S.D. = 2.30 nmol, C.V. = 9.2%).

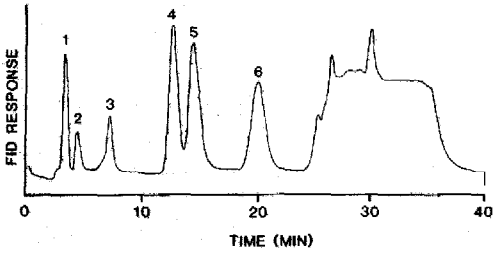

Fig. 1. GC–FID analysis of concentrated breath from a normal subject. The numbered peaks eluted with retention times similar to pure solutions of the following compounds: 1, methanol; 2, acetaldehyde; 3, ethanol; 4, acetone; 5, isopropyl alcohol (internal standard); 6, isoprene. Temperature programming caused the prolonged elevation from 25 to 40 min.

Clinical study

The GC assay of the breath collected from a typical human subject is shown in Fig. 1. The observed concentrations of acetone in fifteen normal subjects ranged from 10.0 to 48.4 nmol/l (mean = 23.2 nmol/l, S.D. = 12.0 nmol/l). Peaks arising from several other compounds (including those with the same relative retention times as pure solutions of acetaldehyde, methanol, ethanol and isoprene) were observed in all subjects.

DISCUSSION

The assay method was found to be highly sensitive and specific for endogenous acetone in normal human breath. The mean concentration in normal subjects (23.2 nmol/l) was similar to the mean value of 1.1 μg/l (18.9 nmol/l) observed by Stewart and Boettner [4]. In practice, the method was highly acceptable to the subjects, with the advantage that samples could be collected at sites remote from the laboratory. In addition, the use of an internal standard in the collection bag minimized potential errors that might have arisen from leakage of the breath sample prior to the assay.

The concentration of acetone in the breath is greatly increased in severe ketoacidosis, when the substance may be readily detected by its odor alone [13]. However, comparatively little attention has been paid to the derangements of acetone metabolism which might accompany the milder degrees of ketoacidosis seen in diabetes mellitus, starvation or alcoholic intoxication. This assay provides a new and potentially useful research tool for the investigation of these conditions.

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