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Note

Gas chromatographic determination of volatile sulfur compounds in the expired alveolar air in hepatopathic subjects

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Characteristic halitosis of patients in hepatic coma has been described as being of diagnostic value by many clinicians, since early times. In previous literature, methyl mercaptan was isolated from the urine of patients in hepatic coma and exhibiting *fetor hepaticus* following acute massive necrosis of the liver. It was speculated that the breath odor was caused by a mixture of mercaptans and dimethyl sulfide [1, 2]. Subsequently, the concentration of methyl mercaptan was reported to be significantly elevated in the breath of patients with hepatic cirrhosis. A correlation between the breath odor and the dimethyl sulfide concentration in the breath of cirrhotics was also demonstrated after oral administration of methionine [3].

In recent gas chromatographic analysis, the flame photometric detector has been extensively used for the selective detection of sulfur and phosphorous compounds. By the application of this apparatus, trace amounts of volatile sulfur compounds have been detected in the field of environmental hygiene and toxicology, especially in analyses of atmospheric air pollutants and pesticides.

This paper presents our gas chromatographic procedure for the analysis of human expired alveolar air and the application of this technique in the determination of the overnight fasting level of volatile sulfur compounds, mainly methyl mercaptan and dimethyl sulfide, in the expired adveolar air in patients with diseases of the liver.

EXPERIMENTAL

Apparatus

A gas chromatograph (Model GC-5AP$_S$TFF$_p$, Shimadzu, Kyoto, Japan)

equipped with a flame photometric detector (FPD) and with a flame ionization detector (FID) monitor, was used for analysis. The cryogenic vapour preconcentration device and subsequent heat-desorption transfer system (flash sampler) are schematically illustrated (Fig. 1). The sample tube, column, connecting glass tubing and gas-tight syringe were all treated with 0.05 *N* phosphoric acid. The contact of metal surfaces by the sample was avoided, except for the needles of the connector and the injection syringe.

The glass column (3 m × 3 mm I.D.) was packed with 10% polyphenyl ether (5 rings) OS-124 on Shimalite TPA 60—80 mesh. Column temperature was initially isothermal at 40° for 5 min, then increased to 90° at the rate of 10°/min with a hold at 90°. For routine examination, the procedure takes about 30 min from the gas sampling to the end of cooling process. The FPD with a 394 mμ filter was operated at 750 V. Detector temperature was 140°. The gas flows were nitrogen as a carrier 50 ml/min, hydrogen 50 ml/min, and air 50 ml/min.

Reagents

Nitrogen-balanced standard hydrogen sulfide (H_2S) gas (13.8 ppm) was prepared by Nihon Sanso (Tokyo, Japan). Standard benzene solutions of methyl mercaptan (MM) (1μg/μl) and dimethyl sulfide (DMS) (0.1 μg/μl) were obtained from Wako (Osaka, Japan). Special grade ethyl mercaptan (EM) and dimethyl disulfide (DMDS) were also obtained from Wako. Each of these solutions was further diluted and adjusted to a suitable concentration for daily calibration.

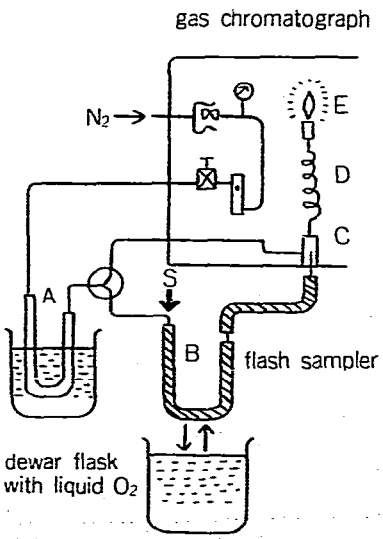

Fig. 1. Schematic diagram of cold-trap preconcentration and analytical system. A = Freeze-out trap for carrier gas (N_2) packed with molecular sieve 5A, 30—60 mesh, stainless steel; B = Sample tube packed with 1, 2, 3, tris (cyano ethoxy) propane (TCEP) 25% on Shimalite, 60—80 mesh, 31 cm × 4 mm I.D., glass, C = Injection port; D = Column; E = FPD with FID monitor; S = Sample injection by gas-tight syringe.

Separation of volatile sulfur compounds

The retention times in minutes are as follows; H_2S 1.67, MM 3.18, EM 4.18, DMS 4.68, and DMDS 15.17, respectively. Fig. 2 shows the typical chromatograms obtained from a 75-year-old male patient with hepatic cirrhosis and secondary diabetes.

Calibration of volatile sulfur compounds

Between 0.2 and 50 ng of these compounds were injected into U-shaped sample tubes using Hamiltone gas-tight syringes of 10 ml capacity (Hamiltone Whittier, Calif., U.S.A.) or SGE microsyringes of 5 μl capacity (Scientific Glass, North Melbourne, Australia). The line produced by plotting the logarithm of dose vs. the logarithm of peak height was used for calibration.

Procedure

Routinely, 100 ml of expired air was collected in the pharyngeal region with a gas-tight syringe of 100 ml capacity (TOP Surgical Manufacturing Co., Tokyo, Japan) towards the end of a prolonged uninterrupted expiration subsequent to 20-sec breathholding. In such samples, the composition of the alveolar air is in equilibrium with the air dissolved in alveolar capillary blood, and the alveolar carbon dioxide tension is estimated as equivalent to the carbon dioxide tension in oxygenated mixed venous blood. The specimen was immediately injected into the sample tube (↓S: Fig. 1).

Reproducibility of the quantitation of volatile compounds in the expired alveolar air

Table I shows duplicate analyses in five subjects. In order to obtain reliable

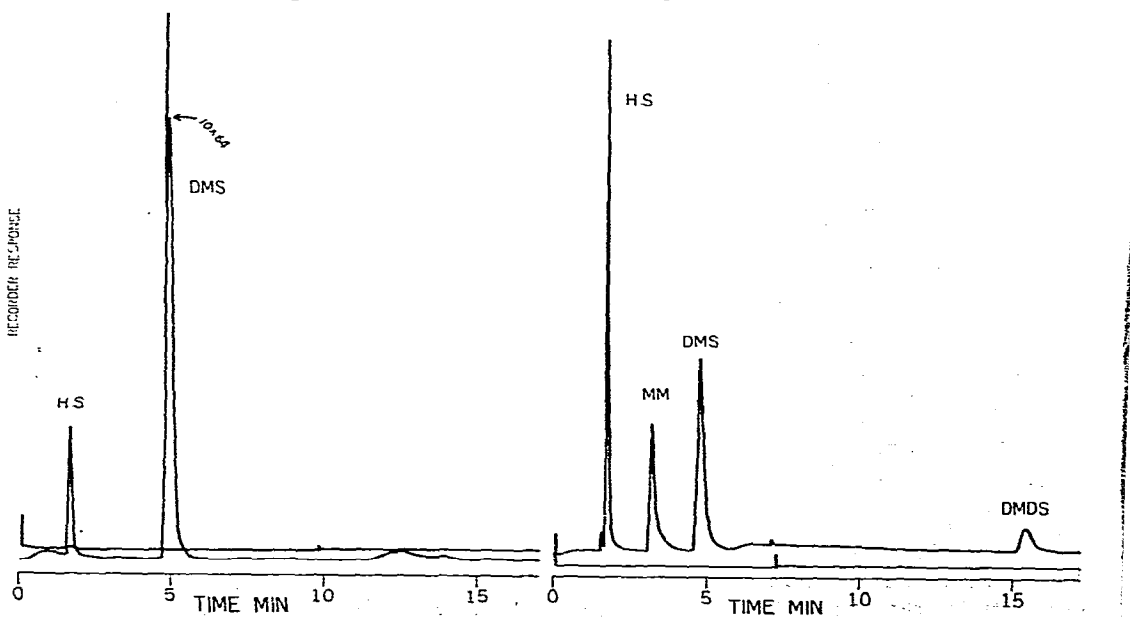

Fig. 2. Gas chromatogram obtained from a 75-year-old male with hepatic cirrhosis and secondary diabetes. Left: expired alveolar air; right: urinary headspace gas; sample size: 20 ml; detector sensitivity and range: FPD = 10 × 32, FID = 10^3 × 256.

and reproducible data in such an experiment, we must emphasize the importance of the sampling procedure for the expired alveolar air as described above. In other experiments on the gas chromatographic quantitation of acetone or ethanol, duplication has been clearly demonstrated [4, 5].

Subjects

Concentrations of MM and DMS in the expired alveolar air were determined after 12-h fasting in 97 subjects; 53 normal controls and 44 patients with diseases of the liver (13 acute hepatitis; 11 chronic hepatitis; and 20 cirrhosis of liver).

RESULTS AND DISCUSSION

Since DMS concentration was sufficiently detectable in 100 ml samples of expired alveolar air, for the requirements of the analyses, we repeated our estimation every 30 min, routinely determining both DMS and MM. By means of the t-test, the concentration of DMS was shown to be significantly elevated in cirrhotics (Table II).

Previously, sulfur compounds were collected from 60—80 l of tidal air as mercuric salts and analyzed by gas chromatography and flame ionization detection [3]. Their fasting average values were: in 7 normal subjects, MM 0.8 and EM 5.9; in 6 compensated cirrhotics, MM 3.0 and EM 4.7; and in 12 severely decompensated cirrhotics, MM 4.4 and EM 11.5 ng/l. But, since several kinds of volatile sulfur compounds have been detected from the oral cavity [6—8], the contamination by mouth air of the expired alveolar had to be carefully excluded.

Because of improvements in expired alveolar air sampling, use of cold-trap preconcentration procedure, utilization of all-glass and PTFE tubing, and the selectivity of FPD with FID monitor, the time required for sampling, pretreat-

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF VOLATILE SULFUR COMPOUNDS IN EXPIRED ALVEOLAR AIR (ng/dl)

N = normal subject; LC = patient with liver cirrhosis

Patient	MM	DMS
M.H. (N)	—	0.45
	—	0.46
H.K. (N)	2.25	0.58
	2.20	0.64
A.K. (LC)	—	3.9
	—	3.9
S.I. (LC)	3.18	15.6
	4.80	14.7
T.M. (LC)	1.10	0.73
	1.15	0.73

TABLE II

FASTING LEVELS OF MM AND DMS IN THE EXPIRED ALVEOLAR AIR (MEAN ± SE, ng/dl)

Experimental group	MM	DMS
53 Normal (control)	0.71 ± 0.21	1.54 ± 0.09
13 Acute hepatitis	0.48 ± 0.27	1.48 ± 0.50
11 Chronic hepatitis	0.23 ± 0.23	2.30 ± 0.86
20 Liver cirrhosis	0.94 ± 0.33	4.05 ± 1.06*

*vs. normal control, $t = 2.3611$, $P < 0.05$.

ment and gas chromatographic analysis has been markedly shortened, and the concentrations of MM obtained by us were about ten times higher than previous workers had obtained [3].

Volatile sulfur compounds constitute the *fetor hepaticus* and they seem to play an important role in the induction of hepatic encephalopathy, as do ammonia, short chain fatty acids, etc. [9]. Methionine toxicity in liver cirrhosis has been studied in relation to mercaptans and dimethyl sulfide [3, 10—12]. The physiological significance of these compounds in normal and pathological states must be further studied.

As the quantitative analyses of trace amounts of volatile sulfur compounds in blood are still complicated and uncommon [13, 14], it is important to take full advantage of the expired alveolar air analysis in the field of clinical biochemistry.

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