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Note

The measurement of methylamines in biological material using a gas chromatographic head space gas technique

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The three methylamines, methylamine, dimethylamine and trimethylamine, are produced by bacterial action in the gut and are excreted in the urine and faeces after some modification. The complete details of this process have yet to be elucidated. To facilitate the further study of these amines a simple method for their detection is desirable.

Various methods have been described for the measurement of methylamines by gas chromatography (GC) and many different column packings have been tried, e.g. Carbopak B [1], Chromosorb 103 [2] and Pennwalt 223 [3]. Some have involved derivatisation of the amine and others the use of a precolumn. All these methods are unsatisfactory in some way for the detection of the three amines in biological material.

Trimethylamine has also been assayed in the urine of subjects with trimethylaminuria. Marks et al. [4] used a column of 10% free fatty acid phase and 5% potassium hydroxide on 80—100 mesh Chromosorb W and injected urine directly onto the column. However, there is no mention of the analysis of methylamine and dimethylamine.

Dunn et al. [5] have described a system for the detection of amines in the ultrafiltrate of biological fluids using a packing of 10% amine 220 and 10% potassium hydroxide on 80—100 mesh Chromosorb W. This method does not separate methylamine and dimethylamine.

Kuwata et al. [6] have shown that a simple column packed with Chromosorb 102 treated with 5% trimethylchlorosilane and coated with potassium hydroxide can separate aqueous mixtures of all three amines at low concentration.

Head space analysis combined with GC can be used to analyse volatile substances which have become extracted from the general medium. Using a

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head space analysis technique a method was developed for the detection of methylamine, dimethylamine and trimethylamine in biological material, using isopropylamine as an internal standard.

EXPERIMENTAL

Standards

Standard solutions of methylamine, dimethylamine, trimethylamine and isopropylamine were made from their hydrochloride salts (BDH, Poole, U.K.). Aliquots of a stock solution of 1 mg/ml were neutralized and diluted to the required concentrations.

Samples

Urine, faeces and rat gut contents have been used as biological samples. These were collected into 1 *M* hydrochloric acid to trap the amines as hydrochloride salts. The amines were extracted from faeces and gut contents by mixing them 4—6 times with 5-ml aliquots of 1 *M* hydrochloric acid for 30 sec followed by centrifugation at 1600 *g* for 5 min. The supernatants from these washings were pooled. Acidified urine was used without further modification. All samples were stored at −20°C until analysed.

Head space analysis

Aliquots of 1 ml (for dimethylamine and trimethylamine) or 5 ml (for methylamine) of the sample or standard were placed in 2 cm × 4 cm screw-top bottles with rubber seals and pierced metal caps. Samples previously acidified with hydrochloric acid were neutralized with potassium hydroxide (10 *M*). Isopropylamine hydrochloride was added as an internal standard; 20 μl of 10 *N* ammonia and 2 g of potassium carbonate per ml of sample were added. The containers were heated in a water bath at 50°C for 40 min.

The vapour above the liquid was sampled with a gas-tight syringe and injected directly onto the column.

Chromatography

A Carlo Erba 4200 Fractovap gas chromatograph fitted with a Carlo Erba NPD 40 nitrogen detector was used for the chromatography. The column was packed with Chromosorb 102 coated with 5% trimethylchlorosilane and 5% potassium hydroxide (Chromatography Supplies, Wirral, U.K.). The working conditions were: nitrogen carrier gas flow-rate, 50 ml/min; injector temperature, 160°C; column temperature, 160°C (isothermal), detector temperature, 200°C; detector hydrogen and air pressures, 0.8 kg/cm^2; sample size, 0.5 ml.

RESULTS AND DISCUSSION

Fig. 1a and b shows the chromatogram of the vapour collected from the head space of a 1-ml and a 5-ml incubation of a 1 μg/ml standard. The retention times of each peak (mean ± standard deviation (S.D.) for seven repeated injections) are: ammonia, 0.64 ± 0.02 min; methylamine, 1.16 ± 0.02

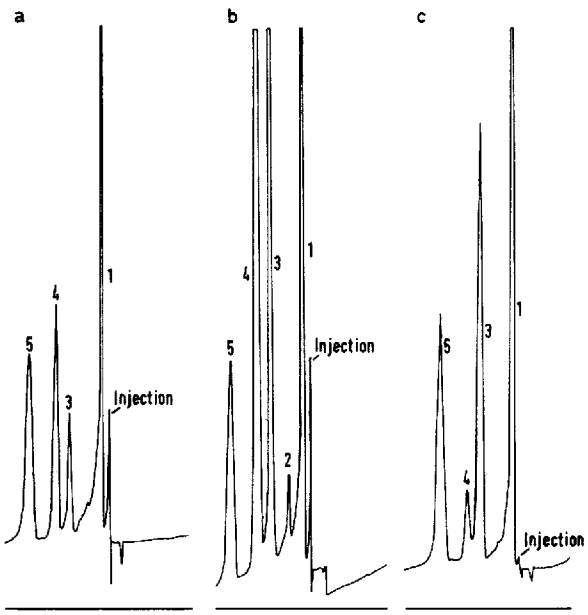

Fig. 1. Chromatogram of the head space above (a) a 1-ml incubation and (b) a 5-ml incubation of a standard solution containing methylamine, dimethylamine and trimethylamine each at a concentration of 1 μg/ml and (c) a 1-ml incubation of human urine. Peaks: 1 = ammonia; 2 = methylamine; 3 = dimethylamine; 4 = trimethylamine; 5 = isopropylamine.

min; dimethylamine, 1.73 ± 0.03 min; trimethylamine, 2.23 ± 0.03 min; isopropylamine, 3.16 ± 0.02 min.

A straight-line calibration graph is obtained for standards in the concentration range 0.25—200 μg/ml with correlation coefficients between 0.99 and 1.00. Fig. 2 shows a calibration graph in the range 0.5—30 μg/ml for dimethylamine. Each point shows the mean ± S.D. for five incubations and injections of that concentration. Similar calibration graphs were obtained for methylamine and trimethylamine.

A larger sample is required for methylamine since it is more soluble in water and less readily vaporised.

Recoveries of 100—102% were obtained on adding the pure amines to urine.

The detection limit for methylamine is 0.25 μg/ml (limited by its solubility), while that for dimethylamine and trimethylamine is considerably lower (ca. 0.01 μg/ml). This is an improvement on the method described by Dunn et al. [5] and is probably due to the use of a nitrogen detector.

Potassium carbonate and ammonia are necessary to drive the very soluble amines out of solution. Potassium carbonate is a non-reactive, very soluble alkaline salt and is thus suited to this purpose. The amount of ammonia added is critical since higher concentrations interfere with the methylamine peak and also cause the resolution and response of other peaks to deteriorate.

Direct injection of aqueous solution of standards and samples as used by Kuwata et al. [6] was also tried. The resulting water peak could not be separated from the methylamine peak. The water also caused a negative peak

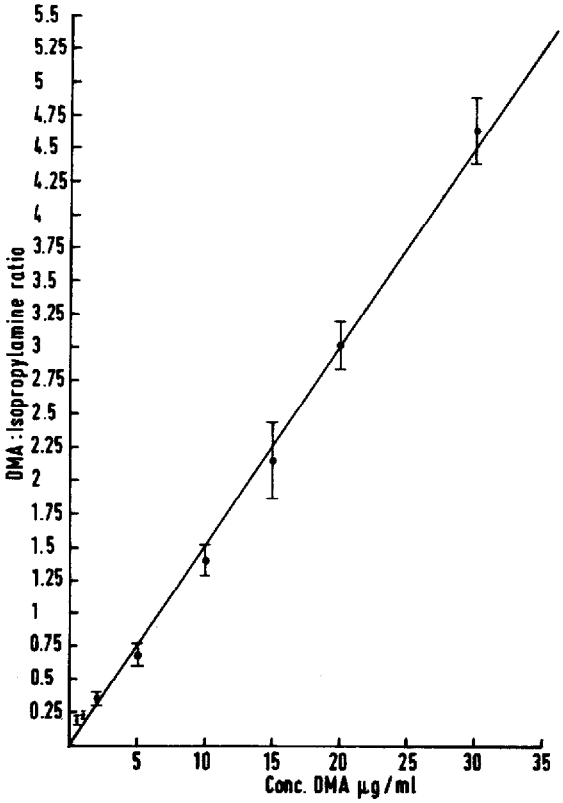

Fig. 2. A calibration graph for dimethylamine. Each point shows the mean ± S.D. for five separate incubations and injections.

at high sensitivities which interfered with the dimethylamine peak. Head space analysis overcame these problems.

Fig. 1c shows a chromatogram of the vapour sampled from the head space over a 1-ml sample of human urine. Chromatograms of similar quality were obtained for extracted rat faeces and rat gut contents. The repeated analysis (seven separate incubations of a single human urine sample) gave a mean ± S.D. value of 18.3 ± 1.8 µg/ml for dimethylamine, 1.7 ± 0.3 µg/ml for trimethylamine and 1.8 ± 0.2 µg/ml for methylamine.

The amounts of amines found in rat urine, rat faeces and various parts of the rat gut are shown in Table I. These rats had been fed Rat Maintenance Diet No. 1 supplied by Special Diet Services (Witham, U.K.) which contains 2.4% fat, 13.6% protein, 18.6% dietary fibre, 38% starch, 10.4% sugars and an adequate supply of minerals and vitamins.

A simple method has been developed for the detection of aliphatic amines in biological materials which is both more sensitive and gives greater resolution than the ultrafiltration technique of Dunn et al. [5]. Use of head space samples avoids the possibility of interference from other water soluble, biological substances in the chromatography.

TABLE I

AMOUNTS (μg) OF METHYLAMINES FOUND IN RAT URINE, RAT FAECES AND VARIOUS PARTS OF THE RAT GUT

Values are mean ± S.D. for eight rats.

	Trimethylamine	Dimethylamine	Methylamine
Rat urine 24 h	57 ± 27	410 ± 200	84 ± 35
Rat faeces 24 h	11.7 ± 9.8	9.8 ± 4.8	14.1 ± 14.4
Rat upper small bowel	0.9 ± 0.6	3.7 ± 0.4	trace
Rat lower small bowel	2.5 ± 1.5	8.7 ± 4.4	trace
Rat caecum	11.8 ± 6.1	4.4 ± 0.4	35.4 ± 11.6
Rat colon	8.7 ± 5.4	6.0 ± 1.7	24 ± 11.1

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REFERENCES

1 A. Di Corcia, R. Samperi and C. Severini, J. Chromatogr., 170 (1979) 325.
2 C.E. Andre and A.R. Mosier, Anal. Chem., 45 (1973) 1971.
3 M. Dalene, L. Mathiasson and J.Å. Jönsson, J. Chromatogr., 207 (1981) 37.
4 R. Marks, M.W. Greaves, C. Prottey and P.J. Hartop, Brit. J. Dermatol., 96 (1977) 399.
5 S.R. Dunn, M.L. Simenhoff and L.G. Wessen, Jr., Anal. Chem., 48 (1976) 41.
6 K. Kuwata, Y. Yamazaki and M. Uebori, Anal. Chem., 52 (1980) 1980.