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Note

Bromide measurement in serum and urine by an improved gas chromatographic method

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Bromide ion is an end product [1] of halothane metabolism following halothane anaesthesia in man. During an investigation of halothane metabolism we required a rapid and convenient method, suitable for routine use, to measure bromide ion in serum and urine in the 10—100 μg Br^-/ml range. Various techniques have been reviewed [2]. Most involve lengthy procedures and are either not sensitive enough [3, 4] for our requirements, or use specialised equipment [5, 6]. Gas chromatographic (GC) methods appeared to offer the best approach. Various reagents have been tested [7—9] but again we required a simpler and more sensitive procedure. A further potentially useful GC method for serum bromide has been reported [2, 10]. This method utilised the principle of oxidation of bromide to bromine followed by coupling the bromine to an unsaturated compound and assaying the bromo derivative by GC. In this paper we present an improved GC method based on the above work [2, 10] but having a tenfold higher sensitivity. Our method has been applied to the routine measurement of bromide at the 10—100 μg/ml level in both serum and urine from subjects undergoing halothane anaesthesia, and a report of the detailed clinical findings is in preparation.

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EXPERIMENTAL

Reagents

Sodium bromide, trichloroacetic acid (TCA), activated charcoal (powder, acid washed, for decolourising purposes), *n*-hexane, bromine and sulphuric acid were purchased from BDH, Poole, Great Britain; sodium hypochlorite (15% available chlorine), petroleum spirit (b.p. below 40°) and 2,4-dimethylphenol were from Koch-Light, Colnbrook, Great Britain; 2-cyclohexen-1-ol was from Aldrich, Milwaukee, Wisc., U.S.A. Standard sodium bromide solution was 1.29 mg/ml (1.0 mg/ml with respect to bromide ion) in water for use in standard curves and reproducibility tests. Sulphuric acid (2 *N*), TCA (30% in water) and 2,4-dimethylphenol (DMP; 200 mg in 20 ml petroleum spirit) were freshly prepared for each batch of analyses. Purity of the solvents and of DMP was checked using the GC procedure below and purification, where necessary, was achieved by alumina chromatography, or redistillation, or both.

Internal standard, 2,3-dibromocyclohexanol (DBCH)

The standard was prepared [11] by addition of a slight excess of bromine in CCl_4 to a solution of 2-cyclohexen-1-ol in CCl_4. After removal of excess bromine and concentration of the sample, the product (m.p. 56°) was recrystallised from CCl_4 at - 10° and characterised by gas chromatography—mass spectrometry (GC–MS). [M^+ group *m/e* 260, 258, 256 (1:2:1); M—Br at 179, 177; M—(Br+OH) at 160, 162; M—(Br_2+H) at 97, corresponding to $C_6H_9(OH)Br_2$. (GC on a 1.5m × 4 mm glass column packed with 3% OV-1 at 150°, with He as carrier gas, 40 ml/min, a retention time of 4.0 min and MS electron impact 25 eV, 300 μA.)]

Between 30 and 60 mg of DBCH was dissolved in 1 ml of ethanol. The concentration of DBCH solution was adjusted so that 10 μl of stock added to the sample before extraction gave approximately the same peak area in the GC analysis as that of the bromo product formed from 40 μg/ml bromide (Fig. 1).

Samples

Serum (10 ml) and urine (20-ml portions from pooled 24-h samples) were collected from patients undergoing orthopaedic surgery under halothane anaesthesia. Pre-operative control sera and further control sera and urines from healthy laboratory staff were also collected. Samples were stored at - 20°.

Extraction procedure

Samples were thawed and each mixed thoroughly before portions were taken for assay.

Serum. To 1.0 ml of serum in a glass centrifuge tube add 2.0 ml 30% TCA solution and mix on a vortex mixer. Add 50 mg of charcoal, mix thoroughly and leave for 5 min. Centrifuge down the denatured protein and charcoal (bench centrifuge, e.g. MSE Minor, speed 7; 200 *g* for 2—3 min). Filter the supernatant through a glass wool plug packed into a pasteur pipette and transfer 1.0 ml of filtrate to a capped vial (2.0 ml size). Add 10 μl of internal standard DBCH and 30 μl of sodium hypochlorite, mix well, then add 0.3 ml of DMP solution. Close the vial securely and extract by shaking vigorously for 1

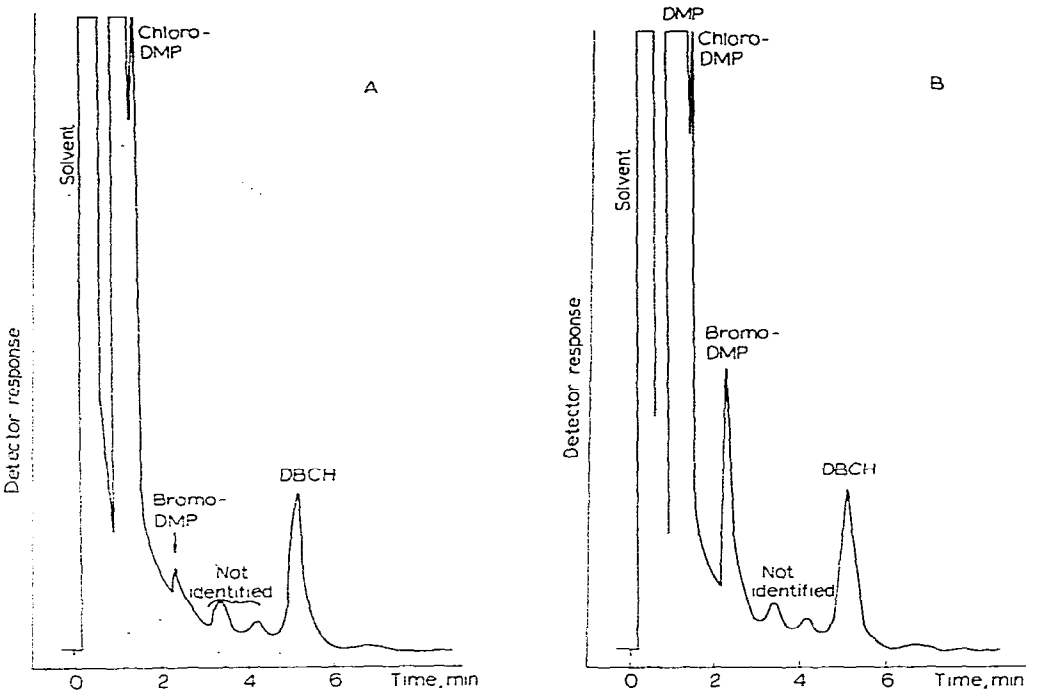

Fig. 1. GC traces from the analysis of: (A) pre-operative serum control; (B) 6 days post-halothane anaesthesia showing a level of bromo-DMP equivalent to 29 μg Br^-/ml serum. Phase OV-17 at 145°, bromo-DMP is measured derivative, DBCH is internal standard.

min. After separation of the solvent layers (by brief centrifugation if necessary), transfer the upper petroleum phase, containing the bromo-DMP product, to a small vial (800-μl Pye-Unicam Autojector sample holders are suitable). The solvent is evaporated off under a stream of dry nitrogen and the samples are stored, if this is necessary, without solvent at −10°. Hexane (20 μl) is added and the samples warmed to room temperature for 10 min before the GC analysis.

Urine. Add 0.15 ml of 2 *N* H_2SO_4 to 1.5 ml of urine in a glass centrifuge tube. Mix well, and add 75 mg of charcoal. Stand 6 min with occasional shaking. Centrifugation and subsequent steps are the same as for serum above except that only 20 μl of sodium hypochlorite is used.

Controls, standard curves and other tests. Pre-operative sera or pooled blank serum and urine were used for controls and standard curves. Standard curves, multiple replicate tests and yield tests were performed by adding the appropriate volume of standard NaBr (1 μg/μl Br^- equivalent) at the beginning of the procedure or to the 1.0-ml aliquot taken after filtration.

Gas chromatography and assay procedure

The bromo-DMP product was assayed, using a Pye 104 chromatograph fitted with a flame ionization detector (FID) on a 2.1 m × 2 mm I.D. glass column packed with 3% OV-17 on Diatomite CQ, 100—120 mesh. Conditions were: column temperature, 145°; injector, 200°; carrier gas, nitrogen at a flow-rate of 15 ml/min; sample volume, 2 μl. Retention times: bromo-DMP, 2.1 min;

DBCH, 5.0 min. The GC column was purged for 15 min at 230° after 20 to 25 samples had been run.

Peak areas were measured by electronic integration with baseline correction, and the bromo-DMP values adjusted to constant internal standard recovery. A linear regression programme was used for the standard curves and the sample values read directly from this using a programmable calculator.

RESULTS AND DISCUSSION

GC traces from the analysis of pre-operative serum control (Fig. 1A) and six days post-halothane anaesthesia (Fig. 1B) are shown for patient PL. The bromo-DMP level in Fig. 1B is equivalent to 29 μg Br^-/ml. Small amounts (usually <5μg/ml) of bromide were found in all control sera. Identification of the peaks marked was made from standards and confirmed by GC—MS (conditions as given for DBCH). The phase OV-17 was preferred to Poly A-103 [10] because bromo-DMP and the internal standard both elute in clear areas; also a rapid analysis time of 6 min is possible. Any effect on the bromo-DMP measurement caused by the proximity of the large DMP plus chloro-DMP peak is corrected by an angular baseline correction applied by the integrator. The chloro-DMP arises by reaction of DMP with chlorine released from the sodium hypochlorite.

Table I summarizes the tests on recovery, precision and the results from applying the method to serum and urine analysis. Linearity of the standard curves was obtained over the range 15—150 μg/ml, but values became inaccurate under 10 μg/ml (see below). Few samples had Br^- values exceeding 100 μg/ml, so the routine standard curves to 100 μg/ml were extrapolated for values above 100 μg/ml. Recovery and reproducibility were considered to be adequate for our investigations because of the fairly large changes of bromide concentration observed in the experimental samples.

Some results showing these changes in serum bromide concentration are given in Table II. The overall recovery (73%) of bromide from serum was

TABLE I

SUMMARY OF EXPERIMENTAL FINDINGS

Parameter	Serum	Urine
Range of samples tested (μg Br^-/ml)	<10—123	<10—65
Mean of sample values (μg Br^-/ml)	32	25
Number of samples assayed	56	50
Variation limits (%) in replicate analyses (10 samples each at 20 μg Br^-/ml and 40 μg Br^-/ml)	± 3.9	± 5.8
Overall recovery of bromide (%)	73	57
Working range (μg/ml)	10—150	

TABLE II

SOME OBSERVED CHANGES IN SERUM BROMIDE LEVELS (μg/ml) AFTER HALOTHANE ANAESTHESIA

Patient	Pre-operative (control)	Post-operative		
		Day 2	Day 6	Day 9
MC	<0.5	21	123	97
RK	<0.5	12	42.2	8.7
AP	<0.5	28	27	9
PL	<0.5	4	29	37
SC	<0.5	5	15	<0.5

similar to that found previously [10], indicating that in the 10—1,000 μg/ml range, a constant proportion of the bromide is not recovered. Recovery from urine was lower (57%) but the proportion remained constant. The effect possibly contributes to the lower accuracy for urine measurements, for below about 15 μg/ml the quantitative recovery of bromide falls away markedly. This may be due to a generally poor recovery, or high adsorption of bromide to protein or charcoal at these low levels. Calculated results therefore become inaccurate below this level, and 10 μg/ml is essentially the lower limit of accuracy (but not necessarily detection) of the method.

The internal standard is in effect an extraction standard. Initial experiments showed that variation between replicate analyses was attributable to the derivative formation and extraction stage. A standard was found which was soluble in both water and petroleum spirit. Trials showed that the efficiency of formation and recovery of bromo-DMP was paralleled by the amount of DBCH extracted into the petroleum phase. Thus DBCH is a reliable internal standard for use in quantitative measurements. When the "trial" serum procedure (modified from ref. 10) was applied to urine no bromo-DMP was formed. This was presumably due to interference by unsaturated compounds in urine; this interference noted previously [12], provided the basis for development of the coupling method for serum.

The problem of how to remove these unsaturated compounds was solved simply by the addition of activated charcoal. An enhancement of the yield was found also on the addition of charcoal to the serum samples; therefore, the charcoal step was included in both procedures. One drawback to the use of charcoal is that the internal standard cannot be added until after the removal of charcoal.

GC methods [13] for analysis of μg/ml levels of inorganic anions are often unsatisfactory for biological samples. The available GC methods for bromide [2, 10] have lower detection limits of about 100 μg Br^-/ml. By using charcoal adsorbent and an effective internal standard, we have increased the sensitivity to a lower limit of 10 μg Br^-/ml. This gives a method which is rapid enough to be suitable for routine measurements of normal post-operative bromide levels after normal anaesthetic doses of halothane. These bromide levels (10—

100 μg/ml) previously could only be measured by activation analysis or X-ray fluorescence.

The system described, therefore, provides a method for the measurement of bromide in serum and urine. We anticipate that the method could be applied also to bromide determinations in other fluids, both biological and chemical.

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REFERENCES

1 K. Rehder, J. Forbes, H. Alter, O. Hessler and A. Steir, Anesthesiology, 28 (1967) 711.
2 A.W. Archer, Analyst (London), 97 (1972) 428.
3 R.E. Johnstone, E.M. Kennell, M.G. Behar, W. Brummund, R.C. Ebersole and L.M. Shaw, Anesthesiology, 42 (1975) 598.
4 G. Kainz, G. Boehm and G. Sontag, Mikrochim. Acta, 2 (1976) 135.
5 R. Cornelis, A. Speecke and J. Hoste, Anal. Chim. Acta, 78 (1975) 317.
6 R.W. Flint, C.D. Lawson and S. Standil, J. Lab. Clin. Med., 85 (1975) 155.
7 R. Belcher, J.A. Rodriguez-Vazquez, W.I. Stephen and P.C. Uden, Chromatographia, 9 (1976) 201.
8 S.G. Heuser and K.A. Scudamore, Pestic. Sci., 1 (1970) 244.
9 D.R. Matthews, W.D. Shults and J.A. Dean, Anal. Lett., 6 (1973) 513.
10 J. Wells and G. Cimbura, J. Forensic Sci., 18 (1973) 437.
11 E.H. Farmer and S.E. Michael, J. Chem. Soc., (1942) 513.
12 H.V. Street, Clin. Chim. Acta, 5 (1960) 938.
13 W.C. Butts, in K. Blau and G. King (Editors), Handbook of Derivatives for Chromatography, Heyden, London, 1977, Ch. 11, p. 411.