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The Determination of Elevated Bromide Levels in Blood by Gas Chromatography

A recent paper by A. W. Archer [*1*] has prompted us to report our results on the gas chromatographic (GC) determination of bromide in blood.

The reported methods for the determination of bromide in blood have been reviewed by Archer and are either nonspecific or inconvenient [*1*]; a GC method which is both convenient and relatively specific would be an obvious asset to the toxicologist. This report describes a method which we developed for the determination of bromide in blood in the 10–100 mg percent range; it has the advantage of employing a standard ¼-in. external diameter column under isothermal conditions.

Experimental

Instrumentation

A Varian 1200 gas chromatograph (Varian Associates, Walnut Creek, Calif.) equipped with a flame ionization detector and a Varian 0-1 mv model 20 recorder was employed. A 6 ft coiled aluminium (¼-in. outside diameter) column packed with 3 percent Poly-A 103 on 80/100 mesh acid washed Gas Chrom Q (Applied Science Labs, State College, Pa.) was used.

The operating conditions were as follows:

Injection port temperature 220°C;
Column temperature 150°C;
Detector temperature 260°C;
Carrier gas (nitrogen) flow rate approximately 50 ml/min.

Confirmatory GC analysis was carried out on a 3 ft aluminium (¼-in. outside diameter) column packed with 3 percent OV 17 on Gas Chrom Q, 80/100 mesh (applied Science Labs) at a column temperature of 130°C.

Materials

Sodium Bromide (B.D.H.) as 10–100 mg percent solutions in water
2,4-dimethylphenol (Eastman Kodak); 1 g dissolved in 100 ml of Benzene
5 percent sodium hypochlorite (B.D.H.)
Trichloroacetic acid solution 20 percent weight/volume (w/v)
Benzene, pesticide grade (Fisher Scientific)

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Procedure

Blood (0.5 ml) was diluted with 0.5 ml of distilled water in a 15 ml centrifuge tube; 3 ml of 20 percent trichloroacetic acid was added and the mixture agitated on a Vortex mixer before being centrifuged. The protein free solution was decanted into a second centrifuge tube to which was added 75 μl of 5 percent sodium hypochlorite followed by 0.5 ml of the 2,4-dimethylphenol solution. The tube was tightly stoppered and the mixture agitated on a Vortex mixer for 2 min before being centrifuged. Two μl of the upper benzene layer was injected into the GC. The peak height of the bromophenol formed was compared directly to that of a standard curve prepared by treating aqueous solutions of sodium bromide in the above manner. If the bromide concentration is greater than 100 mg per 100 ml of blood, the blood should be diluted with a suitable quantity of distilled water.

Results and Discussion

The procedure outlined above produces a gas chromatogram (Fig. 1) containing peak *a* which corresponds to the product formed between the 2,4-dimethylphenol (peak *c*) and

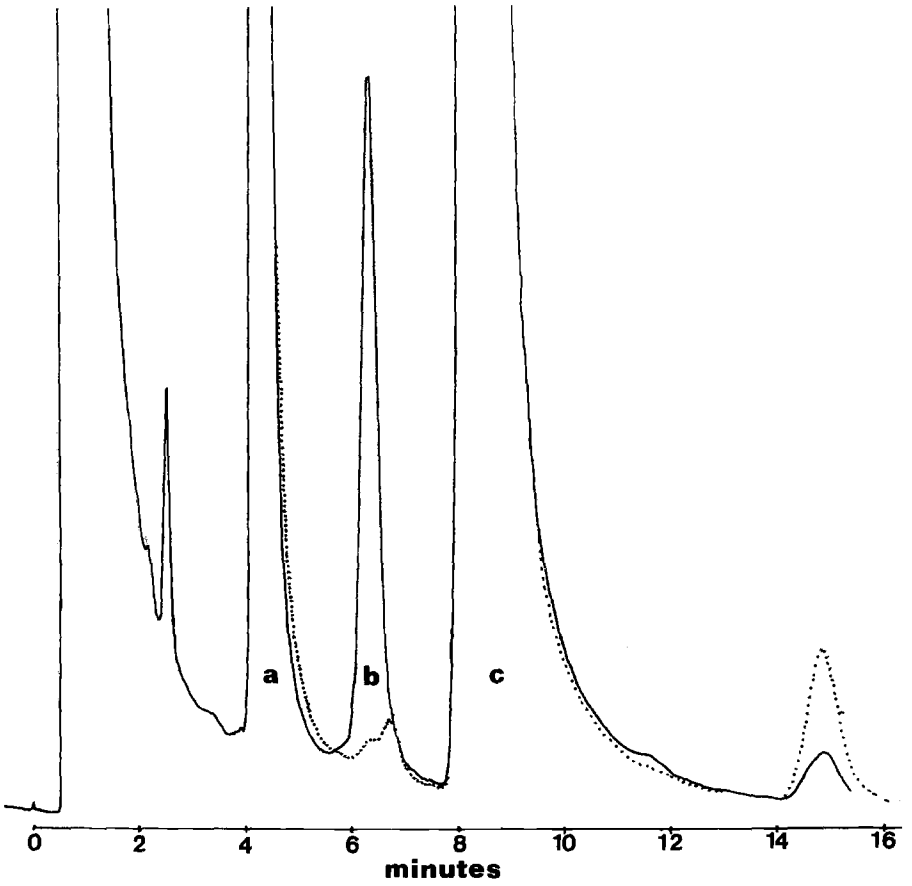

FIG. 1—*GC response (Poly-A103 phase) to a blood sample treated as outlined under "Procedure": The dotted line indicates blank blood. The solid line indicates the same blood to which bromide has been added to give a bromide concentration of 25 mg per 100 ml.*

hypochlorous acid; peak *b* corresponds to the bromo product. The exact nature of the product formed between 2,4-dimethylphenol and bromine is not known but it is probably the 6-bromophenol. A pure sample of the bromo product was obtained by preparative GC and shown to have an infrared spectrum consistent with its formulation as 2,4-dimethyl-6-bromophenol [*2*].

A solution of carbromal (50 mg percent) was processed as above but did not produce any noticeable bromide peak. Confirmation of the presence of the bromophenol was obtained by analysis on the 3 percent OV 17 column (peak order on this column is *c,a,b*). However, the use of this column for the initial screening is not recommended as carbromal does give a peak close to the bromophenol peak under the conditions used.

In five duplicate analyses in the 10–100 mg per 100 ml range, the peak heights of the bromophenol obtained from blood samples to which known concentrations of bromide had been added, averaged 0–5 percent higher than those from the corresponding aqueous solutions which were similarly treated.

The overall efficiency of the oxidation and extraction procedure in the 10–100 mg per 100 ml range was 70 percent, that is, only 70 percent of the theoretical amount of bromophenol that could be produced from the bromide present was obtained. This figure of 70 percent was obtained by comparing the peak heights of the bromophenol obtained from the aqueous standard solutions with the peak heights from a solution of bromophenol (obtained by preparative gas chromatography) in benzene of known concentration.

Figure 2 illustrates a plot obtained from the analysis of blank blood containing known concentrations of bromide ion.

The reproducibility of the method as described under *Procedure* was such that duplicate analyses of 5 blood samples containing known concentrations of bromide in the 10–100 mg per 100 ml range resulted in bromide levels that were within 10 percent of the known bromide concentration.

In the method reported by Archer [*1*], potassium permanganate is used as the oxidant and cyclohexene as the bromide carrier with 1,6-Dibromohexane as an internal standard. The disadvantage of his method, however, is that temperature programming of the GC column (0.2 mm internal diameter) is apparently necessary (70–170°C at a 10°C rise per min).

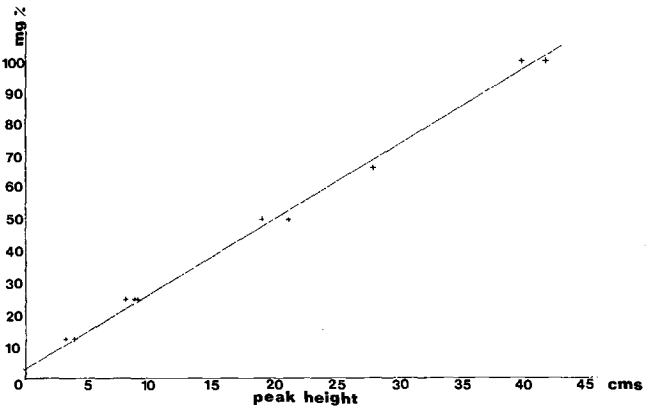

FIG. 2—*Plot of peak height of bromophenol against concentration of bromide ion in blood.*

Summary

A convenient and relatively specific gas chromatographic method is described for the determination of bromide in blood in the 10–100 mg percent range. Oxidation of the bromide ion by hypochlorous acid and subsequent reaction of the bromide with 2,4-dimethylphenol produces a bromophenol which is conveniently detected by a gas chromatograph.

Acknowledgments

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References

[*1*] Archer, A. W., *Analyst*, Vol. 97, 1972, pp. 428–432.
[*2*] Bellamy, L. J., *The Infra-red Spectra of Complex Molecules*, 2nd ed., Methuen & Co., Ltd., London, 1958, pp. 64–82.

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