Technical methods

Simple colorimetric method for the determination of bromide in urine

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Clinical studies on an oral diuretic by Wooster, Dunlop, and Joske (1967) required the determination of urinary and plasma bromide concentrations and bromide clearances.

A modification of the gold chloride method of Barbour, Pilkington, and Sargent (1936) for plasma bromide was already in routine use at Royal Perth Hospital and, for convenience, further modification of the technique for the determination of urinary bromide was undertaken. This paper describes the adapted method for urinary bromide estimation.

REAGENTS

- 1 NaCl 0.5% (w/v)
- 2 Trichloroacetic acid (T.C.A.) 30% (w/v)
- 3 Gold chloride 0.5% (w/v)
- 4 Standard: 100 mg. Br/100 ml. Dissolve 128.8 mg. NaBr (dried) in 100 ml. H₂O. Discard if a straw colour develops.
- 5 Activated charcoal powder (B.D.H.)

PLASMA BROMIDE DETERMINATION The major modifications to the method were: (1) the use of 0.5% NaCl, rather than H₂O, as a diluent; (2) quantitation by means of a Hilger Spekker absorptiometer using filters Kodak no. 2 (470 mμ); (3) the increase in T.C.A. concentration to provide the correct pH for colour development and to produce a clear, protein-free filtrate.

Plasma (1 ml.) was added to a mixture of 6 ml. NaCl and 1 ml. T.C.A., then filtered. Gold chloride, 0.5 ml., was added to 4 ml. filtrate. A standard was prepared in the same way and the developed colour read against a reagent blank in the Spekker absorptiometer.

URINARY BROMIDE DETERMINATION Approximately 0.8 g. (AutoAnalyzer cup) of activated charcoal was added to 8 ml. urine. The urine and charcoal were mixed, allowed to stand for a few minutes, then filtered.

Standard solutions were prepared by adding 1 ml. NaBr standard to 7 ml. normal urine and 2 ml. standard to 6 ml. urine. Charcoal, 0.8 g., was added to both the

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standard solutions and to 8 ml. normal urine (reagent blank). After mixing and filtering, 2 ml. filtrate of each of the two standard solutions and the reagent blank were pipetted into tubes each containing 2 ml. NaCl and 0.5 ml. trichloroacetic acid.

An appropriate aliquot (usually 1 or 2 ml.) of the test urine filtrate was measured into a tube containing 2 ml. NaCl and 0.5 ml. T.C.A. and the volume made up to 4.5 ml. with H₂O if necessary.

Gold chloride, 0.5 ml., was added to each tube immediately before reading on the Spekker absorptiometer. The bromide concentration was read from a plot of the standards and the dilution of the test urine included in the calculation.

RESULTS AND COMMENTS

The reproducibility of the methods for both plasma and urine was tested. On 19 pairs of duplicates, the bromide concentrations ranging from 7 to 163 mg./100 ml. urine and 1 to 196 mg./100 ml. plasma, the standard deviations

(calculated as $\sqrt{\frac{\sum d^2}{2n}}$ where d = difference between pairs and n = number of pairs) were 1.4 for plasma bromide and 1.3 for urinary bromide methods.

The gold chloride method for estimating bromide has been criticized by Gray and Moore (1942) who found nine, of 76 normal subjects, with plasma bromide levels ranging from 16 to 53 mg./100 ml. Of 25 normal subjects tested during our study on diuretics only one had a level greater than 4 mg./100 ml. and this volunteer was subsequently found to be taking carbromal, a brominated mono-ureide.

The urinary bromide estimations were first attempted unsuccessfully by the plasma bromide method. Pigments present caused interference and the colour of the gold bromide formed was rapidly discharged. The adaptations required to produce a precise estimation of urinary bromide concentration were (1) treatment with activated charcoal and (2) the addition of chloride to ensure uniform colour production.

The effect of pH on colour development, noted by Wolf and Eadie (1950), could cause serious errors in urinary bromide measurements because of variations in urinary pH. The T.C.A. was therefore added to bring the final solutions to pH 1.

It was found, by treating the urine with activated charcoal, that the recovery was 90% at the 100 mg. Br/100 ml. level and the colour formed was stable for 2 min. with a 5% decrease in bromide occurring after 10 minutes. (The colour is stable for several hours for the plasma bromide determination.)

Hunter, Smith, and Taylor (1954) tested many interfering substances, including ascorbic acid, in the formation of gold bromide. Ascorbic acid, which is oxidized to dehydroascorbic acid by the charcoal, was added to

FIG. 1. The effect of chloride on the gold bromide colour development.

The optical densities are recorded on increasing chloride concentration and maintaining the bromide level at: A, 200 mg./100 ml.; B, 100 mg./100 ml.; C, 0 mg./100 ml.

normal urine and standard lines prepared from both normal and the ascorbic-acid-supplemented urine were compared. The lines obtained were identical.

The chloride concentration has a marked effect on the gold bromide colour development. This effect was noted by Malamud, Mullins, and Brown (1933), who added sodium chloride to serum until the serum chloride concentration equalled that of the cerebrospinal fluid before measuring the bromide concentrations. Wolf and Eadie (1950) diluted urine, with no prior treatment, and plasma to a constant halide concentration, a technique requiring tedious preliminary total halide measurements and dilutions. Figure 1 shows that the colour development falls to a constant rate at a chloride concentration of 44 mEq./l. In the method described the inclusion of 2 ml. 0.5% NaCl produced the required chloride concentration and the large variations encountered in urinary halide levels caused no change in colour development. The NaCl was also found to prevent turbidity in the plasma bromide estimation.

Because of the 90% recovery for urinary bromide the standards were prepared from normal pooled urine. Urines of varying pH and pigment content were used to prepare standard lines which were identical. Similarly standards were prepared from the test urines, after treatment with Dowex 2-x8 (200–400 mesh) to remove the bromide. These standards replicated those obtained from the series of normal urines. Calibration is linear up to 300 mg. Br/100 ml.

An automated plasma bromide method based on the development of gold bromide colour has been advanced

Screening for metachromatic leucodystrophy¹

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Lake (1965) proposed a diagnostic test for metachromatic leucodystrophy based on the detection of sulphatides within renal epithelial cells in urinary deposits. These sulphatides react with certain dyes to give a brown metachromasia.

The urinary deposit often contains much debris, consisting mainly of bacteria and phosphates desired and this obscures the few cells that may be present. The method described below overcomes these difficulties.

REAGENTS AND MATERIALS

1 1% aqueous solution of cresyl fast violet (Merck) the pH adjusted to 3.5–3.6 with acetic acid.

2 Hemming² filter containing Whatman No. 41 filter paper.

3 Glycerin albumin.

PROCEDURE

The urine, which must be fresh and should not be an early morning specimen, is centrifuged at about 1,500 r.p.m. for five minutes. Most of the supernatant is decanted and the sediment resuspended in the remaining

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²Beaumaris Instrument Co. Ltd., Rosemary Lane, Beaumaris, Anglesey. It is described in the *Oxoid Manual* (2nd ed., 1963).

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Simple colorimetric method for the determination of bromide in urine—concluded

by Technicon Corporation but cannot be directly applied to urine because of the variation in chloride concentration and interference by urinary pigments.

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