J. Pharm. Pharmac., 1969, **21**, 283–286 *Received October* 2, 1968

The determination of bromisoval and carbromal in biological material

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The bromine determination (van der Meulen) has been adapted to the assay of bromisoval and carbromal in biological material. Interfering bromide is eliminated by pretreatment with silver nitrate. The ashing procedure was simplified by previous precipitation of proteins by acetone. Interference by bromine-containing metabolites of the drugs is absent or minimal. The method is applicable in the range of 0·1 to 1 μmol. Recovery in blood was 95%. The standard deviation of the determination is approximately 5%.

The distribution and biotransformation of the bromocarbamides (bromoureides) were examined early in this century (Takeda, 1911; Kwan, 1912; Impens, 1912; Gensler, 1916). These drugs were largely superseded by the barbiturates, and their place has been only in household medicine in some countries. Some 15 years ago manifestations of bromocarbamide toxicity revived interest, mainly in forensic science laboratories (Schmidt, 1954; Vidic, 1959; Curry, 1963). The methods then developed were qualitative or semiquantitative and not useful for serial work. A method for the determination of bromisoval or carbromal adapted to process larger numbers of samples of biological material in an investigation of bromocarbamide metabolism is now described. After some trial (Rauws, 1968), a procedure was evolved, by which the bromine compounds, after hydrolysis, were determined as bromide. A titration procedure was arrived at which later was recognized as being essentially the same as that developed by Hunter (1953).

Both bromocarbamides are almost wholly degraded in the body with the formation of bromide. It is therefore necessary to separate them from bromide before the determination. Complete extraction of carbromal from blood or plasma with chloroform or ether after denaturation with trichloroacetic acid or wolframate proved to be difficult because of the adsorption of the drug to the precipitated proteins. A complicating factor was its unexpected property of partly volatilizing during evaporation of the extracts. To prevent this, the proteins were precipitated with a water miscible solvent (ethanol, acetone) in which the bromocarbamides are soluble. From the resulting extract, bromide was precipitated with silver nitrate according to Wollheim (1958) and the remaining bromine compounds were determined as bromide after alkaline hydrolysis.

This method has been used for determinations in plasma, blood, brain and liver. For determinations in fat tissue a modified extraction procedure was necessary.

EXPERIMENTAL

Reagents. (Unless otherwise stated the reagents were of analytical grade.) Acetone; 200 mM aqueous solution of silver nitrate; 220 mM aqueous solution of potassium chloride (Merck "bromfrei"); 2 M aqueous solution of sodium hydroxide; 40% w/v aqueous solution of sodium dihydrogen-phosphate monohydrate; 1·0 M sodium

hypochlorite in aqueous 0·1 M sodium hydroxide (BDH, "Low in bromine"); 50% w/v aqueous solution of sodium formate; 6·0 N aqueous solution of sulphuric acid; 10% w/v aqueous solution of potassium iodide, freshly prepared; 0·006 N aqueous solution of sodium thiosulphate, freshly diluted from 0·1 N stock solution; sodium starch glycollate indicator solution, prepared according to Vogel (1951); potassium bromide standard, 1 mM aqueous solution; potassium bromate standard, 1 mM aqueous solution; bromisoval (Ph.Ned.Ed.VI) 10 mM fresh solutions in ethanol or propylene glycol; carbromal (Ph.Ned.Ed.VI) 10 mM fresh solutions in ethanol or propylene glycol.

Separation procedures

Plasma or blood. Plasma or blood (1·0 ml) is added dropwise to acetone (4·0 or 4·5 ml respectively) in a centrifuge tube. After mixing on a vortex mixer, the contents are centrifuged for 10 min in a tabletop centrifuge at 3000 rev/min. The supernantant (4·0 ml) is pipetted into a centrifuge tube and silver nitrate (1·0 ml) added. The contents of the tube are thoroughly mixed and, after 30 s, potassium chloride (1·0 ml) is added. Again the contents are mixed thoroughly and the tube is closed with "Parafilm" to prevent evaporation and centrifuged at 3000 rev/min for 30 min. The silver halide supernatant (5·0 ml) is used for the next steps in the analytical procedure. The precipitation of the silver halides is carried out in semi darkness.

Soft tissues (brain, liver). 1·0 g or less tissue is homogenized in acetone (3–4 ml). The volume of the homogenate is adjusted to 5·0 ml. After centrifugation at 3000 rev/min for 10 min the supernatant (4·0 ml) is treated as for the corresponding blood supernantant.

Fat tissue. Fat tissue (approximately 1 g) is cut in small pieces. It is then extracted three times with chloroform (50 ml). The extract is evaporated from pre-weighed flasks, and the extracted fat is weighed*. The fat is dissolved in light petroleum (b.p. 80–100°) (5 ml) and transferred into a stoppered centrifuge tube. Ethanol (4·0 ml) is added and the contents are mixed. To the homogeneous solution water (1·0 ml) is added. The two phases separate after shaking. An aliquot of the ethanol-water phase (4·0 ml) is processed further.

Analytical procedure

The silver halide supernatant (4·0 or 5·0 ml) is pipetted into a nickel crucible (capacity 25 ml) and 2 drops of sodium hydroxide are added. The mixture is then dried quickly at its boiling point where, for carbromal, hydrolysis competes succesfully with volatilization. The crucible with its dried contents is heated in an electrical oven to dull red for 45 s. The contents are dissolved in 1 ml water and are transferred quantitatively to a 100 ml Erlenmeyer flask. The total volume of the washings (e.g. 3 × 1·5 ml of water) should not exceed 5 ml. Sodium dihydrogenphosphate (5 ml) and sodium hypochlorite (3 ml) are added and the mixture is heated for 20 min in a boiling water bath. After 15 min, sodium formate (1 ml) is added and the mixture stirred. Excess hypochlorite is destroyed and carbon dioxide is evolved. The flask is taken from the bath and cooled to approximately 15°, sulphuric acid (5 ml) and potassium iodide (1 ml) are added. The iodine formed in the reaction is titrated with 0·006 N thiosulphate with a few drops of starch glycollate as an indicator. 1·0 ml thiosulphate is equivalent to 1 μmol of bromine.

* The fat content of the fresh wet tissue of the rats used was 34 ± 5%.

The blank value depends on the varying quality of the hypochlorite reagent. Blood blanks varied between 0·050 and 0·090 ml.

RESULTS

The average recovery from blood, plasma and brain tissue was approximately 95% (Table 1). With fat tissue recovery was less satisfactory. Using olive oil as a model approximately 85% of the added bromocarbamides was recovered (Table 2).

Table 1. *Accuracy and recovery of bromocarbamide determination in blood*

	Titrated (ml)	Concentration determined (μmol/ml)	Bromocarbamide added (μmol/ml)	Recovery (%)
Blank	0·090*	—	—	—
Bromisoval	0·305	0·355		95
	0·300	0·345		92
	0·315	0·370	0·375	99
	0·315	0·370		99
	0·285	0·320		85
mean ± s.d.		0·350 ± 0·020		94 ± 6
Carbromal	0·310	0·365		92
	0·310	0·365		92
	0·315	0·370	0·395	94
	0·325	0·390		99
	0·325	0·390		99
mean ± s.d.		0·375 ± 0·015		95 ± 4

* Volumes and concentrations are rounded off to 0·005 units.

Table 2. *Accuracy and recovery of bromocarbamide determination in olive oil*

	Titrated (ml)	Concentration determined (μmol/ml)	Bromocarbamide added (μmol/ml)	Recovery (%)
Blank	0·045*	—	—	—
Bromisoval	0·380	0·420		84
	0·370	0·405	0·500	81
	0·375	0·410		82
mean		0·410 ± 0·010		82
Carbromal	0·390	0·430		86
	0·385	0·425	0·500	85
	0·380	0·420		84
mean		0·425 ± 0·005		85

* Volumes and concentrations are rounded off to 0·005 units.

The standard deviation for the determination of bromide at 0·5 μ-equiv/ml was 0·010 μ-equiv/ml and at 0·1 μ-equiv/ml it was 0·005 μ-equiv/ml. It thus varied between 2 and 5% of the values to be determined. The determination of covalently bound bromine was, as had been expected, less accurate. In the concentration range of 0·1 to 0·5 μmol/ml the standard deviation varied from 0·010 to 0·020 μmol/ml i.e. maximally 10% of the value to be determined (Table 1). This was also the concentration range found in animal experiments. As the molecular weight of bromisoval is 223 and that of carbromal 237, it corresponds to a range of approximately 2 to 10 mg/100 ml.

DISCUSSION

The method does not discriminate between covalently bound iodine and bromine. Iodine compounds in as far as they are not precipitated with the proteins are determined together with the bromine compounds. Interference however, is not to be

expected, as physiological and pharmacological concentrations of iodine compounds are negligible except perhaps shortly after an intravenous injection of radio-opaque media. Physiological bromine compounds are rare in terrestrial animals. A qualitative analysis will detect external bromine-containing compounds which unexpectedly might be present. In general however an external interference is not to be expected.

What could be called internal interference might be caused by bromine-containing metabolites. Those found are: 2-bromo-2-ethyl-butyric acid (Jindra, Slámová & others, 1964), hydroxycarbromal or 2-bromo-2-ethyl-3-hydroxy-butyryl-carbamide (Butler, 1964) from carbromal, and 2-bromoisovaleric acid (Rauws, 1968) from bromisoval. In acid extracts of blood taken from rats and mini-pigs after administration of bromisoval or carbromal, no hydroxycarbromal or bromoethylbutyric acid have been detected. However, a trace of bromoisovaleric acid has been found. This does not interfere with the quantitative determination since it is precipitated together with the bromide by silver nitrate. Likewise bromoethylbutyric acid will not interfere. But hydroxycarbromal will be determined together with carbromal when present in blood. In mice, appreciable blood levels of this compound have to be reckoned with (Butler, 1964). If a separation of hydroxycarbromal from carbromal is needed, chromatographic separation or extraction will be necessary. The same applies to separation of bromisoval and carbromal after their simultaneous administration. After a tenfold dilution of the acetone supernatant with water, most of the carbromal may be recovered by extracting three times with two volumes of light petroleum. The bromisoval may be extracted next with chloroform (three times the same volume). From the chromatographic properties of hydroxycarbromal compared with those of bromisoval it may be expected that the former could be separated from carbromal by a similar procedure.

The methods described have been applied successfully to a comparative investigation of the distribution and biotransformation of bromisoval and carbromal in which series of 30 to 50 samples at a time were processed. For clinical toxicological purposes the method is time-consuming and gas chromatography would be the method of choice.

This work is part of a doctor's thesis at the University of Leiden. The full text is published in Dutch.

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