CCXXXVII. THE DETERMINATION OF BROMINE IN BLOOD.

By EDMUND DENYS YATES.

From the Biochemical Laboratory, Cardiff City Mental Hospital.

(Received October 31st, 1933.)

THE suggested significance of blood-bromine concentration for the diagnosis of the psychoses, particularly of the manic-depressive type [Zondek and Bier, 1932; 1933; Sacristàn and Péraità, 1933], has resulted in much research to obtain a reliable method of blood-bromine estimation [Pincussen and Roman, 1929; Pincussen, 1932; Bier and Roman, 1933; Møller, 1932; Behr *et al.*, 1930; Toxopéus and Van Esvelt, 1930]. The method due to Roman, which has been widely used, has been subjected to some technical criticism [Fleischhacker and Scheiderer, 1933; Hahn, 1933; Holtz and Roggenbau, 1933], and while several accurate methods for the micro-estimation of bromide in the presence of excess chloride have been described [Bobtelsky and Rososkaja-Roseinskaja, 1931; Baitschikov, 1931; Hartner, 1933; Bertram, 1933], no simple method for accurate estimation of bromine in blood seems to be available.

The following method has been elaborated as the result of more than 300 blood estimations, and has been found to give reliable and consistent results.

Protein is first removed by precipitation with tungstic acid. An aliquot portion of the filtrate is treated with potassium hydroxide, evaporated to dryness, and heated at 500° for 20 minutes in a nickel crucible. The extract is acidified and the bromide oxidised to bromine by a chromic-sulphuric acid mixture. This is in such concentration that, while chloride remains unoxidised, bromide oxidation is complete [Bobtelsky and Rososkaja-Roseinskaja, 1931; Evans, 1931]. Bromine is removed by aeration and absorbed in starch-iodide solution. The iodine liberated is titrated with *N*/1000 sodium thiosulphate from a microburette [Pincussen and Roman, 1929].

Apparatus.

The aeration apparatus is similar to that of Hartner [1933] and consists of a 100 cc. conical flask fitted with a two-holed rubber stopper. This carries a tube reaching down to the bottom corner of the flask and drawn out slightly at the end. Maximum aeration of the solution in the flask is thus ensured, when the latter is inclined at an angle of 60°, while small particles of solution are not carried over into the exit tube. The generation of carbon dioxide *in situ* by bicarbonate-acid mixtures, as suggested by Hartner, has been found unnecessary.

The rubber stopper also carries a short glass tube bent at right-angles and connected to a small bubbler. The bubbler used by the author consists of a small test-tube (10 cm. $\times$ 1 cm.), fitted with a rubber bung carrying an exit tube, and an inlet tube drawn out to a V-neck capillary at the bottom. A distance of not more than 2 mm. separates the bottom of this tube and that of the test-tube.

Reagents required.

Solution No. 1. 10 % sodium tungstate.

Solution No. 2. $2/3$ *N* sulphuric acid.

Solution No. 3. Concentrated sulphuric acid (36*N*).

Solution No. 4. 20 g. of chromic oxide and 40 cc. of concentrated sulphuric acid in 120 cc. of distilled water.

Solution No. 5. 10 % potassium iodide solution.

Solution No. 6. 0.5 % starch solution made according to the directions of Nichols [1929].

Procedure.

A. *Treatment of blood.* Measure 5 cc. of oxalated blood into a 125 cc. conical beaker add 35 cc. of distilled water and allow the mixture to lyse for $\frac{1}{2}$ hour.

Add 5 cc. of 10 % sodium tungstate solution, mix, and then slowly and with shaking add 5 cc. of $2/3$ *N* H_2SO_4 . Stand for 10 minutes.

Transfer the bulk of the chocolate-brown mixture to a centrifuge-tube and spin at 3000 r.p.m. for 15 minutes.

Pour off the supernatant liquid through a cotton-wool filter into a measuring cylinder. Place 35 cc. of the protein-free filtrate in a 100 cc. nickel crucible with 1 cc. of 25 % KOH (bromide-free) and evaporate to dryness on a boiling water-bath (1 hour).

Heat the crucible in an electric oven at 500° for 20 minutes.

Dissolve the white residue in 3 cc. of water and pour into a 100 cc. conical flask. Wash out the crucible with two further 2 cc. portions of distilled water, making the total volume in the flask equal to 7 cc. (± 0.1 cc.). Cool to 18° and then proceed as follows.

B. *Estimation of bromine.* Add slowly down the sides of the flask, with shaking and strong cooling under the tap, 2.5 cc. of conc. H_2SO_4 . At least 10 minutes should be taken for this procedure, and the first two drops, causing evolution of carbon dioxide, must be carefully added in order to prevent losses of hydrogen bromide in the gas evolved.

Now add to the cold solution (18°) 4 cc. of the chromic-sulphuric acid solution. Allow the solution to run all over the sides of the flask, quickly wipe the top of the flask, and then connect up to the aeration apparatus.

This latter should contain 1 cc. of KI solution and 4 drops of starch solution.

Aerate for about 2 hours with a steady stream of air (about 30 cc. per minute). Renew the absorption tube, and continue aeration for a further 3 hours.

Titrate the iodine liberated in these tubes with *N*/1000 thiosulphate.

Remarks concerning technique. (The following remarks concern the details given in the paragraphs above.)

1. *Treatment of blood.* Lithium oxalate is usually free from bromide and causes no interference with the estimation.

If the lysed solution is allowed to stand for long periods, some loss of bromine occurs.

Table I. Variation of bromine (γ) found with time of lysis.

Exp.	Time of lysis				
	10 mins.	$\frac{1}{2}$ hour	1 hour	24 hours	6 days
(a)	—	86	—	70	—
(b)	—	150	—	—	94
(c)	284	340	343	—	—

This is in accordance with the findings of Hastings and Van Dyke [1931] and Woodhouse and Pickworth [1932].

Losses occur if sufficient time is not given for the displacement of bromide from protein combination.

Considerable losses are found if the evaporated filtrate is not heated at 500°. All carbonaceous and nitrogenous matter is thus removed. Some representative figures are given below.

Table II. *Variation of bromine (γ) found with and without heating.*

With heating	293	40	265	42	38
Without heating	41	14	94	39	10

The loss of titre found without heating is probably due to a loose combination between the bromine generated during oxidation and an unsaturated substance in blood.

Cyanides are formed by moderate heating and these interfere with the reaction by the formation of bromocyanides [Lang, 1925].

Although, as Bobtelsky [1930] has shown, magnesium and manganese exert a catalytic effect upon the reaction when present in large concentrations, the small amounts present in normal blood cause no catalysis of chloride oxidation with the chromic acid mixture used.

2. *Estimation of bromide.* The starch-iodide solution used for absorption is such that, while no iodine is carried over in the air stream, the blue colour which develops still remains in the presence of 0.4 γ of iodine.

The time taken for the complete removal of bromine from the solution depends on the rate of aeration, but will also be limited by the rate of oxidation, which is slow with small concentrations of bromide. Large amounts may be estimated quite accurately using as short a time as 10 minutes [Hibbard, 1926]. For small amounts of less than 50 γ (1 γ is equivalent to 0.012 cc. of N/1000 thiosulphate), a longer time up to 5 hours is necessary [compare Bobtelsky and Rososkaja-Roseinskaja, 1931]. Some results of the author are given here for comparative purposes.

Table III. *Variation of bromide (γ) found with time of aeration.*

(Figures in cc. N/1000 thiosulphate.)

Titre in 1 hour	14.74	8.52	6.95	3.31	1.17	0.25	0.08
Titre in 5 hours	15.37	9.00	7.10	3.43	1.35	0.38	0.09
Theoretical titre	15.35	9.02	7.15	3.45	1.36	0.38	0.10

Recovery of added bromide.

Some figures for the recovery of potassium bromide added at various stages in the estimation are given below.

Table IV.

Added to whole blood			Added to tungstic acid filtrate			Added after ignition	
Blood value	Added	Recovered	Blood value	Added	Recovered	Added	Recovered
γ	γ	γ	γ	γ	γ	γ	γ
27	20	17	38	50	47	10	9
27	50	50	950	60	57	50	49
186	100	105	25	240	238	70	68
950	100	98	—	—	—	1470	1465
950	60	54	—	—	—	—	—

CRITICAL DISCUSSION.

A. Initial treatment of blood.

The methods for the preliminary treatment of blood for bromide estimation hitherto recommended, have usually involved an ashing process lasting several hours (compare, however, Hastings and Van Dyke [1931]).

Ignition method. The experience of many authors with iodide estimation indicates that some loss occurs with open ashing, even when the temperature is carefully regulated, and the alkali is present in some excess [Orr and Leitch, 1931; Reith, 1930; Allott *et al.*, 1932]. As a result closed combustion has been recommended [Schwaibold and Harder, 1931; Widmann, 1932]. This loss does not appear to take place in bromide estimations [Bier and Roman, 1933], although little systematic work has been published. Pincussen and Roman recommend a temperature of 460–475° in the presence of 2 cc. of 50 % KOH. The blood-extract after ignition has a pale yellow colour which is not removed completely either by charcoal [Bier and Roman, 1933], or barium sulphate [Turner, 1930], and is probably to be attributed to small particles of carbon in a colloidal or semicolloidal state. Prolonged ignition moreover does not remove the colour but causes loss of bromide. Using this method, the author has found that added bromide is not quantitatively recovered from blood when the former is estimated by the chromic acid method described above.

Table V. *Recovery of bromide (γ) from ignited blood-extracts.*

Bromide in blood alone	76	43	198	125
Bromide in blood + 50 γ Br ⁻	110	92	242	170
Recovery of bromide	34	49	44	45

The effect is not due to a reduction in the oxidation potential of the chromic acid mixture by the blood-salts present, nor was any considerable loss observed by using nickel crucibles instead of platinum [compare Behr *et al.*, 1930; Turner, 1930].

Table VI. *Effect of type of ignition vessel (mg. Br⁻ per 100 cc.).*

Platinum	0.54	0.97	2.00	0.98	0.26	5.16	0.79
Nickel	0.52	0.93	1.83	0.98	0.27	4.82	0.77

The losses may possibly be due to the adsorption of bromine ions from the strongly acid oxidation mixture by carbon particles [compare Tezler, 1926].

The relatively low values for blood-bromine usually obtained by the ignition method become more apparent when compared with those obtained by the precipitation technique described below.

Table VII. *Comparison of ignition and precipitation methods for blood-bromide (mg. per 100 cc.).*

Ignition in platinum vessel	0.52	0.14	1.85	0.98	12.10
Precipitation with tungstic acid	0.60	0.18	2.13	0.92	12.72

Precipitation method. The preliminary treatment of blood by the Folin-Wu method (precipitation with tungstic acid) involves certain approximations. It is assumed that the bromine present distributes itself equally between the protein precipitate and the aqueous filtrate. The observation of Pincussen [1929] that 1/3 of the bromine in normal blood is in the state of a non-diffusible organic compound [compare also Guillaumin and Merejkowsky, 1933], suggests that relatively large quantities of bromine may be present preferentially in the protein precipitate, as found with blood-iodine. All the evidence, however, indicates that any organic bromine compound, if present, is readily decomposed either by mild acid or alkali.

Large concentrations of bromide appear to be distributed equally between precipitate and filtrate, and Hastings and Van Dyke [1931], using a modified

Folin-Wu technique, have been able to recover bromide quantitatively (to within 0.02 mg. Br) from serum and cells on this assumption.

The author has investigated the distribution of the much smaller amounts of bromine present in normal blood and finds a similar equality of distribution. The bromine content found by taking a known volume of filtrate is in close agreement with that found after washing the protein precipitate with successive lots of 50 cc. of water.

Table VIII. *Bromide (γ) recovered by washing the precipitate.*

Br found in blood-filtrate (vol. 75/100 cc.)	Br found in first washing of ppt.	Br found in second washing of ppt.	Total Br found	Total Br calc. from that found in blood-filtrate
56	14	4	74	75
47	9	—	56	63
45	9	3	58	60
80	15	—	95	107
183	60	10	253	257

Estimation of bromine in the precipitate by the ignition method gives similar results.

1. Bromine found in blood-filtrate	...	...	...	393 γ
2. Total bromine found by ignition	...	...	...	493 γ
3. Total bromine calc. from (1)	...	...	...	524 γ
4. Bromine found in precipitate (ignition)	...	...	...	118 γ
5. Bromine in ppt. calc. from (1)	...	...	...	131 γ

It is clear that the water-extractable bromine is distributed fairly evenly between the precipitate and filtrate and this is perhaps to be expected in view of the almost complete dissociation of protein salts in dilute solutions [Lewis, 1931], and the large effect of neutral salts in reducing any tendency for the establishment of a Donnan equilibrium [Bolam, 1932].

Treatment of the blood-filtrate with silver nitrate and removal of the silver halides by centrifuging has indicated that, even when the blood-bromide is as high as 78 mg. per 100 cc., only extremely small amounts of bromine remain in the solution (less than 0.1 mg./100 cc.), which may perhaps be accounted for in the method, especially in view of the strong catalytic effect of small concentrations of silver upon the chromic acid oxidation of hydrochloric acid [Bobtelsky, 1930; compare Møller, 1932].

B. *Estimation of bromide.*

A method for the estimation of bromide solutions by a single oxidation must not be sensitive to variations of the concentrations of the other salts present. It must allow for the catalytic effect of metallic ions, if any, while it must have a sufficient H ion reserve to make it insensitive to changes of H ion of the same order as that of the bromide estimated. The only available method of this character appears to be that of Hartner [1933], and this the author has not found to be satisfactory. The chromic acid mixture described above is to be preferred since it allows a greater range of bromide estimation in virtue of its larger concentration of hydrogen ions and of chromic acid, while at the same time it is stable to atmospheric oxidation, does not decompose on keeping and is not catalysed by the small amounts of iron, manganese and magnesium present in blood. Tungstic acid and nickel salts do not interfere. The theoretical

limit of accuracy of the method is 0.5γ Br' in the presence of 100 mg. of Cl', this, in practice, being found to be 2γ Br' [compare Bobtelsky and Rososkaja-Roseinskaja, 1931].

The conditions for the accurate estimation of bromide in the presence of chloride using chromic-sulphuric acid mixture here found are in close agreement with those given by Bobtelsky and Rososkaja-Roseinskaja [*cf.* also, Evans, 1931]. $9.0\text{ }N$ sulphuric acid is recommended, while 3.5% CrO_3 are also present (these authors give 5% CrO_3). The concentration of chromic acid has little effect upon the oxidation potential of the mixture at sulphuric acid concentrations above $4N$.

Results of the estimation of synthetic mixtures of various salts are given below. It has been found that chemically pure potassium chloride is difficult to obtain, the chloride used having a bromide content of 0.014% [*cf.* Bobtelsky, 1930; Hahn, 1933]. Some estimations on chloride samples are given in Table X.

Table IX. *Effect of various ions on bromide estimation.*

Concentration of ions per 100 cc.			"Bromide" found (γ) using H_2SO_4 conc. of				
Br γ	Cl mg.	Other ions	$5.5\text{ }N$	$8.3\text{ }N$	$9.0\text{ }N$	$10.5\text{ }N$	$14.0\text{ }N$
100	—	3.5 g. CrO_3	78	97	99	99	—
60	—	Do.	—	—	58	61	—
50	—	Do.	—	45	48	50	—
10	—	Do.	—	—	9	—	—
100	50	Do.	58	—	105	118	183
50	50	Do.	—	51	58	—	—
0	50	Do.	—	—	7	22	130
100	50	Do. + $N/10\text{ Na}_2\text{SO}_4$	60	—	104	112	120
50	50	Do. + 5 mg. MgSO_4	—	—	52	—	—
100	50	Do. + $N/10$ tungstic acid	—	—	107	—	—
100	50	1.5 g. CrO_3	63	—	90	102	153

Table X. *Estimation of bromide (γ) in potassium chloride.*

Wt. KCl taken mg.	Specimen used		
	B.D.H. (untreated)	A.R.	
10	3	—	—
50	28	15	7
100	53	—	—

Experiments are proceeding to determine the significance of blood-bromine in the psychoses.

SUMMARY.

1. A method for the micro-determination of bromine in 5 cc. of blood is described and critically discussed.

2. The method is accurate to within 2γ for amounts of $5\text{--}1000\gamma$ of bromine.

The author wishes to express his indebtedness to Dr J. H. Quastel, for his advice and criticism and to his medical colleagues for their willing co-operation in the collection of blood samples. His thanks are also due to the Medical Research Council for a grant.

REFERENCES.

- Allott, Dauphinée and Hurtle (1932). *Biochem. J.* **26**, 1665.
Baitschikov (1931). *J. Chem. Ind. Russ.* **8**, 54.
Behr, Palmer and Clarke (1930). *J. Biol. Chem.* **88**, 131.
Bertram (1933). *Biochem. Z.* **261**, 202.
Bier and Roman (1933). *Klin. Woch.* **12**, 391.
Bobtelsky (1930). *Z. anorg. Chem.* **189**, 196.
—— and Rososkaja-Roseinskaja (1931). *Z. anorg. Chem.* **199**, 283.
Bolam (1932). The Donnan equilibrium, p. 64. (Bell.)
Evans (1931). *Analyst*, **56**, 590.
Fleischhacker and Scheiderer (1933). *Monatsh. Psych. Neur.* **84**, 348.
Guillaumin and Merejkowsky (1933). *Compt. Rend. Soc. Biol.* **113**, 1428.
Hahn (1933). *Klin. Woch.* **12**, 390.
Hartner (1933). *Z. physiol. Chem.* **214**, 179.
Hastings and Van Dyke (1931). *J. Biol. Chem.* **92**, 13.
Hibbard (1926). *Ind. Eng. Chem.* **18**, 57.
Holtz and Roggenbau (1933). *Klin. Woch.* **12**, 1410.
Lang (1925). *Z. anorg. Chem.* **144**, 75.
Lewis (1931). *Chem. Rev.* **8**, 102.
Møller (1932). *Biochem. Z.* **245**, 282.
Nichols (1929). *Ind. Eng. Chem. Anal. Ed.* **1**, 215.
Orr and Leitch (1931). *Med. Res. Coun. Spec. Rep.* No. 154.
Pincussen (1929). *Biochem. Z.* **216**, 336.
—— (1932). *Klin. Woch.* **11**, 1550.
—— and Roman (1929). *Biochem. Z.* **207**, 416.
Reith (1930). *Biochem. Z.* **224**, 232.
Sacristà and Péraila (1933). *Klin. Woch.* **12**, 469.
Schwaibold and Harder (1931). *Biochem. Z.* **224**, 441.
Tezler (1926). *Z. ges. exp. Med.* **50**, 714.
Toxopéus and Van Esvelt (1930). *Arch. exp. Path. Pharm.* **149**, 263.
Turner (1930). *J. Biol. Chem.* **88**, 497.
Widmann (1932). *Biochem. Z.* **254**, 223.
Woodhouse and Pickworth (1932). *Biochem. J.* **26**, 309.
Zondek and Bier (1932). *Klin. Woch.* **11**, 633.
—— — (1933). *Klin. Woch.* **12**, 55.