CCXII. THE MICRO-DETERMINATION OF BROMINE IN BLOOD.

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For certain biochemical work it is necessary to determine the bromine content of blood in circumstances which preclude the use of samples of greater volume than 1 or 2 cc.

Experience gained during an investigation carried out for the Medical Research Council on methods for the determination of iodine in blood suggested that a method of destroying organic matter involving ignition with alkali under controlled conditions might prove to be satisfactory, and that the bromine might subsequently be determined by a micro-modification of the chromic acid aeration process [Baughman and Skinner, 1919; Evans, 1931].

In the chromic acid aeration processes described by these workers, a double aeration is necessary when chlorides are present, to eliminate errors due to a small amount of chlorine evolved during the first aeration.

In attempting to evolve a micro-method of aeration, a considerable amount of work has been done, the earlier results being promising but erratic at times. When a double aeration was employed, the recovery of bromine varied, and was sometimes as low as 60 %.

As the amounts of bromine to be determined are small (about 0.02 mg.) some variation in the recovery is to be expected, but it appeared that, with a method employing double aeration, the losses would be higher than desirable.

In the later experiments a chromic-phosphoric acid mixture was used for liberating the bromine, and it was found that the conditions could be so adjusted that satisfactory figures were obtained by one aeration in the presence of an amount of sodium chloride approximating to that present in 2 cc. of blood (Table I). The recovery of bromine is within ± 10 % of the theoretical, which is satisfactory for work of this kind.

Table I. *Determination of potassium bromide added to sodium chloride.*

	Bromine added mg.	Bromine found mg.
Control, 20 mg. of bromide-free NaCl	Nil	Nil
Ditto plus 0.005 mg. of bromine (KBr)	0.005	0.0049
" 0.010 "	0.010	0.0099
" 0.020 "	0.020	0.0214
" 0.030 "	0.030	0.0327
" 0.050 "	0.050	0.0515

Preparation of reagents.

Distilled water. The water should be treated with a mixture of potassium carbonate and potassium permanganate and distilled to remove bromine compounds and organic matter.

N *potassium hydroxide*. Prepared from specially selected material or freed from bromine by electrolysis.

20 % *solution of sucrose*. This solution is added to ensure that in the "blank" determinations, any bromate present is reduced to bromide. In the actual blood determinations, any bromate will be reduced during the incineration of the blood. The sucrose solution should be substantially free from bromine compounds, but it is added to the blood determinations as well as to the "blanks" so that any correction necessary for bromine present in the sucrose is made automatically.

Chromic-phosphoric acid mixture. (1) Stock solution. Pure syrupy phosphoric acid, sp. gr. 1.75, is treated with sufficient chromic acid to produce a saturated solution and leave an excess of chromic acid undissolved, when the mixture is heated in a boiling water-bath for 3 hours, with occasional stirring. This procedure also destroys organic matter and decomposes any chloride or bromide.

The mixture is allowed to cool overnight, and is then freed from excess of chromic acid by filtration through glass wool. The solution is now again heated in a boiling water-bath and is aerated for 3 hours with a vigorous stream of air to remove chlorine or bromine. The air should be filtered through a plug of cotton wool to remove dust and then through a plug of glass wool to remove cotton wool fibres. After cooling, the solution is ready for dilution with the special distilled water.

(2) Diluted solution. Before being used under the conditions of aeration to be described later, the stock chromic-phosphoric solution must be diluted slightly so that, with 20 mg. of bromide-free sodium chloride, under the standard conditions of aeration, no iodine is liberated in the potassium iodide bubblers, and the recovery of bromine from potassium bromide in the presence of the sodium chloride is satisfactory (see Table I). Dilution of 90 cc. of the stock solution with 5.0 cc. of water usually provides a solution of the required concentration, but it is necessary to examine every batch of stock solution to ascertain whether it functions properly when so diluted.

2 % *aqueous solution of potassium dichromate*.

2 % *solution of potassium iodide*. This solution should be prepared from distilled water freed from dissolved gases by recent boiling. When protected from the action of daylight and oxidising vapours, the solution is quite stable, and a fresh solution need only be prepared every 2 or 3 weeks.

0.5 % *solution of soluble arrowroot starch*. This solution, which must be freshly prepared, is obtained by rubbing up the starch with a little water, and pouring the mixture into boiling water. The total time of boiling should not exceed 2 minutes.

Standard N/500 solution of sodium thiosulphate. This solution is made with distilled water freed from dissolved gases by boiling and containing 1 % by volume of pure amyl alcohol, as recommended by Aitken [1930]. It should be stored in a container fitted with a soda lime tube and a siphon made entirely of glass terminating in a glass tap. When shielded from direct daylight, the solution is quite stable, but should be restandardised at least once a week. The solution may be standardised against a solution of potassium bi-iodate, N/100 in oxidising power.

Destruction of the organic matter.

1–2 cc. of blood, carefully measured or weighed, are placed in a round-bottomed nickel dish, 9 cm. in diameter, and treated with 20 cc. of water, 3.0 cc. of N KOH, and 0.1 cc. of the sucrose solution. The mixture is well stirred with a glass rod, the rod being rinsed with a little water and removed.

After taking the solution to dryness on the steam-bath, the dish and its contents are placed in an oven at 150–160° for 1 hour and then ignited for 1 hour in a muffle furnace at 480–500°.

The material is now moistened with about 20 cc. of water, the carbon is thoroughly broken up with a glass rod, and the mixture is allowed to digest on the steam-bath for 2–3 minutes while being stirred with the rod.

The hot solution is now decanted through a filter (9 cm. Whatman No. 5 paper previously thoroughly washed with hot distilled water before use) into a 100 cc. beaker, the carbon in the dish being again extracted by digestion with water on the steam-bath and finally extracted with water until the filtrate has a volume of about 40 cc. The filtrate is evaporated on the steam-bath in a recently ignited platinum dish, about 8 cm. in diameter.

The filter-paper is placed in the nickel dish, and the contents of the dish are moistened with water and treated with 1.0 cc. of *N* KOH. After being mixed thoroughly, the mixture is evaporated to dryness on the steam-bath, dried for 15 minutes at 150–160° and ignited in the muffle furnace at 480–500° for 1 hour.

The residue, which now contains only a little carbon, is extracted by digestion with water on the steam-bath in the manner already described, the filtrate being added to the previous filtrate which has been evaporating in the platinum dish.

(*Note.* The nickel dishes should be freed from carbon after use by igniting them in the muffle furnace.)

The combined filtrates in the platinum dish are evaporated to dryness, the dish is placed in the oven at 150–160° for 10–15 minutes and is then ignited for 10 minutes in the muffle at 480–500°.

The residue is dissolved in a little water, the solution is evaporated, and the dish is placed in the oven at 150–160° for 10–15 minutes and ignited for 10 minutes in the furnace at 480–500°.

The moistening, evaporation, drying and ignition for 10 minutes are repeated once again.

To assist the final removal of traces of organic matter, the residue is now dissolved in a little water, treated with 1 cc. of 2 % potassium dichromate solution, and the evaporation, drying and ignition for 10 minutes at 480–500° are repeated.

The alkaline residue is dissolved in about 15 cc. of cold water and the solution is passed through a filter (7 cm. Whatman No. 42, previously well washed with hot water), washing with cold water until the filtrate has a volume of 30–40 cc.

The filtrate is evaporated in the platinum dish; the dish is placed in the oven at 150–160° for 10–15 minutes and is finally ignited for 5 minutes at 480–500°.

The dish is now covered to prevent contamination by organic matter in the form of dust from the atmosphere.

Aeration and titration of the liberated iodine.

The apparatus (Fig. 1) must be kept scrupulously clean and free from minute traces of organic matter. On no account must lubricating grease or rubber joints be used, the ground joints being lubricated with distilled water only.

Frequent cleansing by soaking in chromic-sulphuric cleaning mixture is essential, and this should be followed by very thorough washing, first with tap water, and then with distilled water. Finally, the entire apparatus, with the exception of the two small potassium iodide-starch containers, is rinsed with

very dilute sulphuric acid (approx. $N/5000$), well drained and set up in a position where it is screened from the action of direct sunlight.

(Note. The action of daylight encourages the reaction,

which reaction, should it take place in the tubes D and E , will prevent the bromine from being completely evolved. Similarly, if the tubes are not kept slightly acid, but tend to be alkaline, evolution of the bromine will be incomplete.)

Fig. 1.

Each of the small bubblers B and C contains 0.5 cc. of the potassium iodide solution and 0.5 cc. of the starch solution¹.

The residue in the platinum dish is dissolved in exactly 3.0 cc. of water, and the solution is carefully poured down the funnel into the large bubbler A , the dish being twice rinsed with exactly 1.0 cc. of water, the total volume of water used being 5.0 cc.

The water-pump is now turned on, and a slow stream of air at the rate of about 3 litres per hour² is passed through the apparatus, while 5.0 cc. of the diluted chromic-phosphoric mixture are carefully run down the funnel into the large bubbler. The heat of reaction causes the temperature of the solution to rise slightly.

The soda-lime tube is replaced, and, in about 15 secs. when the effervescence ceases, the rate of the air stream is increased to 8–10 litres per hour and maintained at this rate for 20 minutes (laboratory temperature 15–20°).

The contents of the second small bubbler C , which should contain little or no liberated iodine, are now blown back into B , and the end of the tube D is

¹ The addition of starch to the bubblers is very beneficial in assisting the retention of the liberated iodine [Thomas and Cross, 1928; Zepf and Vetter, 1930].

² If the air stream is too rapid, small drops of the alkaline solution may be splashed into the upper part of the large bubbler and may absorb some of the evolved bromine.

rinsed with a little water, whereby the total volume of liquid in the container of bubbler *B* is brought up to 3–4 cc.

The iodine is now titrated with *N*/500 thiosulphate, using a micro-burette. In the work for the Medical Research Council previously mentioned it was found necessary to make a small correction for the end-point error. In terms of bromine, for small volumes, this correction is +0.0002 *V* mg., where *V* = the volume of the solution after titration (*i.e.* one adds to the figure for bromine obtained by titration, 0.0002 mg. for every cc. of solution).

Some results obtained by the method are given in Table II. These form a complete series of results and are not selected figures.

Table II.

Recovery of bromine compounds added to 2.0 cc. of horse blood in each case.

Results obtained by deducting the figures found for the bromine naturally present in the blood.

Form in which the bromine was added to the blood	mg. of bromine added	mg. of bromine found
Potassium bromide	0.010	0.011
"	0.020	0.022
"	0.030	0.030
<i>o</i> -Bromobenzoic acid	0.010	0.012
"	0.020	0.019
"	0.016	0.017
"	0.024	0.027
"	0.030	0.031
"	0.024	0.027
"	0.030	0.031
"	0.040	0.040
Bromodiethylacetylurea	0.010	0.010
"	0.016	0.015
"	0.026	0.026
"	0.030	0.030

Bromine found in samples of blood kindly supplied by Dr J. B. Orr.

		Total mg. of bromine found	mg. of bromine per 2.0 cc. of blood corrected for "blank"
"Blank"		0.0007	—
"		0.0009	—
*1. Cow	0.98 cc.	0.0171	0.033
"	1.94 "	0.0349	0.035
2. Guinea-pig (F).	0.97 "	0.0217	0.043
"	1.92 "	0.0492	0.050
3. Fowl (M).	0.98 "	0.0075	0.014
"	1.94 "	0.0134	0.013
4. Sheep (F.)	0.94 "	0.0092	0.018
"	1.86 "	0.0171	0.018
5. Rat (M).	0.93 "	0.0116	0.023
"	1.84 "	0.0198	0.021

* This animal had been receiving a diet containing potassium iodide. The figure obtained for bromine may therefore be slightly higher than the true value.

Frequent "blanks" should be carried out to ensure that bromine is not being picked up from the atmosphere of the laboratory or other sources. If the blood is oxalated, then the "blanks" must contain a corresponding amount of the oxalate.

Satisfactory results will only be obtained when strict attention is paid to details. The aerations must be carried out in precisely the manner described, and constant vigilance must be exercised to ensure that errors are not creeping in from unexpected sources. It appears to be desirable to call attention to the following points.

1. The presence of minute traces of organic matter in the apparatus may cause low results as also may the presence of foreign organic matter in the starch solution.

2. If the potassium iodide or starch solutions are alkaline, the results will be low.

3. It is not permissible to use chromic-phosphoric mixture which evolves a small amount of halogen on aeration and to correct for this halogen by means of a "blank." Such a "blank" may be due to elemental chlorine in the chromic-phosphoric solution, which, while being evolved in the "blank," may not be evolved in the actual determination owing to its interaction with the bromide present.

4. High results may be obtained with materials containing iodine. Normally, blood does not contain sufficient iodine to affect the bromine figures.

5. For the recovery of volatile bromine compounds, such as bromoform, the blood would have to be evaporated and burnt in a closed system. The figures obtained (Table II) indicate that the method of alkaline ignition in open dishes is satisfactory with bromine compounds of the nature of those likely to be present in blood.

6. If colloidal silica were present in the large bubbler during aeration, the removal of the bromine would probably be incomplete. Such silica might be derived from the caustic potash or from the material undergoing examination, but no trouble of this kind has been experienced while working with the samples of blood.

7. Examination of the results embodied in Tables I and II shows that, although no chlorine is evolved when working with pure sodium chloride, the figures for bromine tend to be high rather than low, although it was found that, in the absence of chloride, the chromic-phosphoric aeration process always gave low results for the recovery of bromine (about 85 % recovery). As this error tends to increase with increased bromine concentration it appears that, when carrying out a series of comparative determinations of blood-bromine, the results will be more strictly comparable if the volumes of blood taken for analysis are such that approximately the same quantity of bromine is recovered in each case.

SUMMARY.

A method is described for the determination of bromine in small volumes of blood with an accuracy of approximately 10 %.

In conclusion, we wish to thank Sir Robert Robertson for granting us permission to publish this paper, and Dr J. B. Orr for arranging for the supply of samples of blood.

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