IX. THE DETERMINATION OF BROMINE IN NORMAL BLOOD.

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DURING an investigation into the halogen constituents of the blood of psychotics, who, it is stated by Zondek and Bier [1932], have a greatly lowered blood-bromine, it was found that none of the methods available for blood-bromine determination was satisfactory. In particular that of Pincussen and Roman [1929], on which Zondek bases his conclusions, was shown to be subject to gross errors.

In all the methods the blood is first ashed with excess of alkali whereby the bromine is fixed as alkali bromide. The bromine is then liberated by oxidation with KMnO_4 and H_3PO_4 [Behr *et al.*, 1931] or HNO_3 [Pincussen and Roman, 1929] and collected in chloroform or carbon tetrachloride, or it is distilled over into potassium iodide solution. In any case it is difficult to avoid oxidising chloride and also to get rid of all traces of oxidising agents even if aeration is resorted to. In the method of Roman the melt is subjected to an alcoholic extraction which is claimed to remove potassium iodide only. The present author has shown, however, that although potassium bromide is only slightly soluble in alcohol the amount (100 γ) present in the potassium carbonate melt from 10 ml. normal blood can be quantitatively extracted with 90–95 % alcohol, the bromide and iodide being thus extracted together. Fleischhacker and Scheiderer [1933] have shown that the oxidation with HNO_3 and H_2O_2 is not quantitative. In the Roman method excess of oxidising agent is removed by shaking the CHCl_3 layer with ice-cold NaCl solution. Hahn [1933] has shown that NaCl dissolves out some Br as well as the oxidising agents. In any case iodine titration in a water-chloroform mixture is a difficult procedure and not one capable of great accuracy.

According to van der Meulen [1931] bromides can be quantitatively oxidised to bromates by $2\frac{1}{2}$ times the theoretical amount of hypochlorite in the presence of borate buffer and half saturation with NaCl according to the equation

The completion of the reaction from left to right is ensured if the oxidation is carried out at 85° and excess hypochlorite is removed by addition of H_2O_2 , which in turn is removed by boiling. Addition of bromates to iodides in acid solution results in the liberation of iodine equivalent to 6 times original amount of Br according to the equation

It was found that the above procedure could be modified to deal with quantities of the order of 50 γ such as are likely to be encountered in 10 ml. normal blood.

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It has the advantage that the oxidation and titration are carried out in the same vessel and all the oxidising agent is removed. A small amount of chlorate is unavoidably formed but this is allowed for by using controls which have been subjected to the same procedure from the start. Using this method of oxidising bromide and determining it by the iodine liberated it was found possible to evolve a method for determining bromine in 10 ml. normal blood.

Reagents required.

Water distilled from alkali in an all glass apparatus.

10 *N* KOH and 10 *N* K₂CO₃ purified so as to contain no bromide by von Fellenberg's [1926] method.

Pure ethyl alcohol distilled in all glass apparatus from KOH.

N KClO in *N*/10 KOH. Made by passing 71 g. chlorine gas, cooled and dried, into 1.8 litres of KOH containing 2.2 g. mols. or 123.2 g. KOH, finally making up to 2 litres with water. The KOH is contained in a flask cooled in ice while the chlorine is passing through to minimise formation of KClO₃.

N KI, preferably made fresh, contained in a brown glass bottle.

N HCl.

0.5 % starch; acid-washed soluble starch freshly made up each time required.

N H₂O₂.

N/500 sodium thiosulphate solution.

The proposed method for blood is as follows. 10 ml. of oxalated blood are measured into a 300 ml. flask. 0.8 ml. 10 *N* KOH, 0.8 ml. 10 *N* K₂CO₃ and 30 ml. water are added, and the whole is heated on the water-bath for 4 hours. The mixture is then transferred to a nickel basin of 10 cm. diameter and evaporated to dryness on the water-bath. The basin is dried at 150–160° for 60 mins. and then heated at 420° in the muffle furnace for 2 hours and finally at 500° for ½ hour. The contents are ground up with water by means of a glass rod, evaporated to dryness on the water-bath, dried at 150–160° for 30 mins., and then ignited for ½ hour at 500°. The grinding with water, drying at 160° and igniting at 500° for ½ hour are repeated. During ignition in the muffle the contents of the basin swell up considerably and there is danger of loss unless the surface of the melt is pricked with a finely pointed glass rod, thus allowing tarry vapours to escape. 20 ml. of water are then added to the basin, which is heated for a few minutes on the water-bath. The contents are ground up with a glass rod and then filtered through a 9 cm. Whatman's No. 5 filter-paper previously well washed, into a 9 cm. porcelain basin. The residue is repeatedly extracted with hot and finally cold water until there are about 50 ml. in the porcelain basin which are then evaporated on the water-bath. The porcelain basin is heated on the water-bath to dryness, carefully dried first at 120° for 15 mins., then at 150–160° for 15 mins., and finally ignited in the muffle for 10 mins. at 500°. The contents are dissolved in 15 ml. water and filtered through a 7 cm. Whatman's No. 42 filter-paper into another porcelain basin, the filter being washed several times with *N*/10 K₂CO₃. The solution is evaporated on the water-bath and the residue dried at 150–160° and ignited in the muffle for 5 mins. at 500°. It is then completely dissolved in 1.8 ml. water and alcohol added 5 ml. at a time with continuous stirring, the mixture being well rubbed with a round-ended glass rod, until 15 ml. alcohol have been added. The mixture is stirred until the K₂CO₃ passes into a pasty condition when the alcoholic layer is decanted through a Jena glass sintered filter No. 3 into a 30 or 40 ml. flask. The residue is extracted with two more successive lots of 5 ml. of alcohol. To the potassium carbonate from which the alcohol has been drained

is then added 0.6 ml. water and the above alcoholic extraction is repeated. The contents of the flask are evaporated on the water-bath to dryness, with addition of a couple of pumice granules to facilitate smooth boiling and, if the flask looks very full, under a stream of nitrogen. The last traces of alcohol are removed by heating at 150–160° for 10 mins. To the residue are added 2 ml. water, 1 ml. KClO and 2.5 ml. saturated NaCl, and the mixture is warmed to about 85° on the water-bath and treated with 1 ml. saturated H_3BO_3 . The flask is removed after 20 mins. and cooled and then 1 ml. $N H_2O_2$ added, after which the contents are gently boiled for 5 mins. The flask is cooled and 1.5 ml. $N HCl$, 5 drops of starch and 1 ml. $N KI$ are added, and the solution is titrated against $N/500$ thiosulphate using a 3 or 5 ml. micro-burette

$$1 \text{ ml. } N/500 = 26.7\gamma \text{ bromine.}$$

The oxidation of the bromide in the presence of chloride is very conveniently carried out in the same flask as the titration so that there is very little chance of loss.

The preliminary heating on the water-bath with water and alkali causes partial if not nearly complete hydrolysis of the blood-proteins, and as the author has reason to think some of the bromine in the blood is bound to protein, there is less likelihood of loss and at the same time the use of huge quantities of alkali with consequent corrosion of dishes and basins is avoided. It also permits the use of nickel and porcelain basins for the ashing.

The chief aim in the ashing process is to evaporate most of the tarry matter at 400° and then to reduce the ash so that there is very little carbon left by heating at 500°. Grinding up the ash with water and igniting three successive times at 500° seems to remove any possibility of organic matter coming through to the hypochlorite stage. If organic matter is present it will lead to low figures. The method of ashing will not recover substances like bromobenzene or ethyl bromide which are immiscible and evaporate before fixation, but there is no reason to believe that such substances exist in blood.

The contents of the flask after the addition of the KClO must not be boiled as there is danger of excessive KClO₃ formation. The titration end-point is quite sharp, but owing to the large salt content of the solution the colour change is from blue-purple to colourless. The blank determination on the 10 ml. sucrose solution control which is put through exactly the same process is of the order of 0.4 ml. of $N/500$ thiosulphate and is due to a small amount of chlorate forming during the heating at 85° on the water-bath and includes the inevitable over-titration so that these both cancel out.

Iodides are also quantitatively oxidised by hypochlorite to iodates and are thus estimated together with bromide.

Normal blood, however, contains 500–1000 γ /100 ml. bromine and 10–15 γ /100 ml. iodine, and thus variations in the latter unless very large, as for instance after iodine therapy, do not appreciably affect the figures for bromine.

Table I. *Showing amounts of bromine as γ Br found in 10 ml. samples of normal human blood together with amounts found after bromide had been added.*

	Normal blood	+ 100 γ Br as KBr	Difference
A	89	184	95
B	120	217	97
C	173	277	104

SUMMARY.

A method is described for the determination of the bromine content of 10 ml. of normal blood, whether it contains 0.1 mg. or 100 mg./100 ml.

Through the courtesy of Sir Robert Robertson and the late Sir Walter Fletcher the author has had access to an unpublished report of work on the determination of iodine carried out for the Medical Research Council at the Government Laboratory by Mr C. O. Harvey, and he has been guided by it in choosing the conditions for the preliminary treatment and incineration of the blood and for the alcoholic extraction of the potassium bromide. He wishes to express grateful thanks for this privilege.

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