A STUDY OF MICRO - ANALYTICAL PROCEDURES

APPLICABLE TO THE ESTIMATION OF BROMINE

IN BIOLOGICAL MATERIAL.

by

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CONTENTS.

INTRODUCTION.

The significance of blood - bromine for the diagnosis of the psychoses, particularly of the manic - depressive type suggested by Zondek and Bier [1], and Sacristan and Peralta [2], and also the possible relation of bromine to some of the internal secretions suggested by Kuranami [3], has resulted in much research to obtain a reliable method for bromine estimation. Especially in view of the fact that the methods used by these authors have been shown to be inaccurate.

During the last few years several methods for the estimation of small amounts of bromine have been described.   To confirm the accuracy of these it is necessary to apply them to purely synthetic mixtures of known bromine content, both organic and inorganic.

HISTORICAL.

Although most of the older methods have been discarded, it is necessary to give a chronological survey.

The oldest and best known method is by Fresenius. The silver salts of chlorine and bromine (iodine and fluorine having been removed) are subjected under heat to a stream of chlorine gas. The amount of bromine replaced by chlorine is obtained by the difference in weight of the silver salt before and after treatment. This method is accurate to 0.5 mg., and is even at present still used by some authors as a control.

Possibly the earliest physiological medical method used is that of bromine giving a brown colour with chloroform. For the separation of bromine from its combinations various methods have been used; thus Justus, in 1907, used sodium nitrite and concentrated sulphuric acid, while Authenrieth used permanganate and sulphuric acid. Instead of a comparison of colour, Gensler titrated the coloured solution with chlorine water.

Berglund [5] produced a method for the quantitative estimation of bromine. The bromine is liberated from the ashes of the substance analysed with potassium sulphate and potassium permanganate, and led into potassium hydroxide solution by means of an air current. After acidifying it is precipitated with silver nitrate, the preformed hypobromide first being decomposed by the addition of ammonia. The method was modified by Nencki and Schoumov - Simanovski [6] by passing the bromine into a potassium iodide solution and determining the amount of

liberated iodine by titration with sodium thiosulphate.

Guarischi, in 1913, found a reagent, Schiff's aldehyde reagent (fuchsin, discoloured with sulphuric acid) that is non - reactive with the other halogens, but gives a violet colour with bromine, bromate and hypobromide. Various quantitative methods were produced based on this principle. Wünsche liberated bromine with potassium permanganate and sulphuric acid; then carried it by means of special apparatus into a measured volume of the reagent. Alday - Redonnet obtained by the use of this method moderately satisfactory results, but pointed out the difficulties.

Oppenheimer allowed the liberation of bromine and its coloration of the Guarisch's indicator to go on in the same vessel. To free bromine he employed chlorine - water, i.e. a solution of calcium hypochlorite. His principle was to set a comparison between the mixture under investigation and a standard bromine solution. For this he used a complicated method of titration. Harwich, Damiens and also Benigés and Ghelle used the above with slight modifications. It is interesting to note that Damiens used potassium chromate, concentrated hydrochloric and sulphuric acids to set free the bromine.

Bernhardt and Ucko [7] heated the substance in a nickel crucible with potassium or sodium hydroxide to a grey mass, thus changing the halogens to potassium or sodium halides, which they extracted with water. As a standard for the particular

indicator used, they found that the presence of 0.05 mg. of
bromine showed the first violet colour, while in the presence
of less the colour slowly changed to a yellow, reddish - yellow
or brownish - yellow. Thus by using various amounts of filtrate
they were able to determine the bromine content in the original
substance.

Recently various methods have been produced, mostly based
on the Nencki and Schoumov - Simanovski modified Berglund's
method. Pincussen and Roman [8] heated the dried substance with
five times its weight of solid potassium hydroxide in nickel
crucibles, thus changing the halogens to potassium halides.
They claim that a loss of halogen is not to be expected due
to the large excess of the alkali. The residue is extracted with
water, and the potassium iodide removed with alcohol. Bromine
is liberated with hydrogen peroxide and concentrated nitric acid,
and extracted with chloroform. The chloroform is washed with ice-
cold solution of sodium chloride until all oxidation products
are removed. The amount of bromine present is determined by
titration with thiosulphate.

The accuracy of this method depends mainly on two points :
(I) By oxidizing a mixture containing a few mg. of bromine
with nitric acid and hydrogen peroxide, one can transfer the
bromide quantitatively as bromine into chloroform, and (2) by
shaking this chloroform mixture with sodium chloride at - 3°
all oxidation products are removed and only bromine remains.
Hahn [9] claims that even when the greatest care is taken only

about 89 % of the bromine can be extracted with chloroform, while the remainder is found present in the sodium chloride solution. Therefore by several extractions large amounts of bromine will be lost. At the same time he points out that during extraction, and also in the chloroform mixture, a variety of oxidations are proceeding. Although Bier and Roman slightly modified the procedure, Fleischhacker and Scheiderer [10] point out that, as shown by Treadwell [11], potassium bromide is oxidized by hydrogen peroxide if one uses a concentrated mixture, but in dilution oxidation in the cold is not possible (different from the iodide). Holtz and Roggenbau [12] in a critical discussion on the Roman method state that in their work with standard mixtures no satisfactory results were obtained.

In the methods given it is certain that a definite amount of chloride is oxidized along with the bromide. This produces high results. When large amounts of chloride are present and only small amounts of bromide, the error becomes great. In the methods where the substance is heated to a dry ash, as in all older methods, quite large amounts of bromine can be lost. How large this error can be is seen in a work of Nyss [13]. Only when ashing is done under constant observation and a definite temperature can this loss be minimized.

Toxopeus and Eswald [4] based their method so as to overcome the above defects. By wet ashing with nitric acid and potassium permanganate in the presence of silver nitrate, one cannot

expect to have a loss of bromine. To minimize the amount of chloride oxidized, they use a thiosulphate solution to take up the bromine and the small quantities of chlorine set free. Thus chlorine and bromine are changed to chloride and bromide. The thiosulphate is then reduced to a small bulk. This is oxidized a second time and the bromine set free is introduced into a potassium iodide solution and titrated. In the first oxidation some chlorine passes over into the thiosulphate, but in the second the amount of chlorine liberated is so small that it can be neglected. Møller [14] describes a method for the estimation of bromine in blood and urine, based on this principle. For the oxidizing mixture he uses a permanganate - pyrosulphate mixture.

Behr, Palmer and Clark [16] found that bromides can be almost quantitatively oxidized by permanganate in phosphoric acid solution; the resulting bromine can be quantitatively transferred to carbon tetrachloride. If much chlorine is present a small proportion of it is taken up by the tetrachloride. The separation can, however, be made practically complete by reducing the liberated halogens with sodium sulphite, repeating the process two or three times. Under the conditions adopted any iodide which may originally have been present is oxidized to iodate, thus escaping extraction by the organic solvent. In the ashing process they found that no appreciable loss of bromide occurs when the incineration is conducted in platinum vessels at a temperature of 460 - 475° in the presence of potassium

hydroxide. Use of nickel leads not only to notable losses, but also to profound corrosion of the metal.

Evans [17] found that in 8 - 9 N sulphuric acid solution chromic acid oxidizes bromide to bromine rapidly and completely without acting upon the chloride. He produced a method based on this principle.

A method for the estimation of bromide solution by a single oxidation must not be sensitive to variations of the concentration of other salts present. It must allow for the catalytic effect of metallic ions, if any. It must be of a sufficient hydrogen - ion concentration to make it insensitive to changes of hydrogen-ion of the same order as that of bromide estimated. The only available method of this character appears to be that of Hartner [15], which Yates [18] has found to be unsatisfactory. The chromic acid mixture described by Yates 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 such small amounts of iron, manganese and magnesium as are present in blood. Nickel salts do not appear to interfere.

Bertram [19] has produced a method for the oxidation of bromide whereby bromine to the extent of 5 - 10ɣ may be deter - mined. The principle is again the oxidation of bromide to bromine by a boiling solution of permanganate, acidified with

potassium hydrogen sulphate and buffered with potassium sulphate. He states that in the mixture used, practically no chloride is oxidized, while the iodide is changed to iodate. Thus the oxidation is influenced, and therefore controlled by potassium hydrogen sulphate, potassium sulphate and permanganate.

Francis and Harvey [20] in their work on bromine determina-tion in blood, suggested a method for destroying organic matter involving ignition with alkali under controlled conditions. In the oxidation they used a micro - modification of the chromic acid aeration process of Evans. As the amounts of bromine to be determined in a micro - method are small, 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. A chromic - phosphoric acid mixture was used for liberating bromine. They found that the conditions could be so adjusted that satisfactory results were obtained by one aeration, in the presence of amounts of chloride approximating that of 2 cc. of blood.

The problem of the estimation of bromine, as seen in all these methods, consists of two parts. (I) The quantitative separation of bromine from chlorine and iodine, and (2) the destruction of organic matter. The separation of bromide and iodide does not constitute great difficulties. It can be pro-duced by a variety of oxidizing substances, the iodide changing to iodate, the bromide to bromine. It is more difficult to separate bromide from chloride. Probably this is due to their

oxidation potentials being close together. The methods described are based on the ease with which hydrogen bromide is oxidized compared to hydrogen chloride, by oxidizing substances like permanganate and chromic acid at a definite acidity.

Leipert and Watzlawek [21] destroyed the organic substance with a chromic - sulphuric acid mixture in a closed apparatus. The chlorine and bromine set free are introduced into a sodium hydroxide solution. Here the bromide and hypobromide are oxidized by hypochlorite to bromate, and estimated iodo - metrically.

EXPERIMENTAL.

The investigation concerning the accuracy of the methods described falls into three groups.

1. Methods using the wet ashing procedure, as given by Møller [14], and Leipert and Watzlawek [21].

2. Determining the accuracy of the oxidizing mixtures described.

3. Investigating the accuracy of the ashing procedures.

The synthetic mixtures used in the analyses consist of lactose and sucrose for the organic substances, chloride as sodium and potassium chlorides, iodide as sodium and potassium iodide and di - iodo - tyrosine, and bromine as sodium and potassium bromide, di - brom - o - cresol and di - brom - tyrosine.

The materials used in making up these synthetic mixtures are, a B. D. H. analytical reagent of lactose, recrystallised sucrose, Kahlbaum " reagents for analyses " of the sodium and potassium halides, a pure " Roche " sample of di- iodo - tyrosine, and a sample of 3 : 5 - di - brom - o - cresol from the research laboratory of the Eastman Kodak Company.

The writer prepared the di - brom - tyrosine from tyrosine, according to the method outlined by Zenek [33]. The product on being recrystallised from a 5 % hydrochloric acid solution, forms rhombic plates. These on recrystallisation from hot water form fine white needles, that melt with decomposition

at 242 - 243° . The micro - Kjeldahl determination gives a nitrogen content of 4.115 % (theory 4.13 %).

The reagents used in the subsequent work are the purest available, mainly analytical reagents.

I. THE WET ASHING METHODS.

(I). The Møller method.

Briefly stated, the method consists of separating the halogens as silver halides, reducing them, removing the silver by filtration, and oxidizing the mixture containing the free halogens with a permanganate - pyrosulphate mixture.

The oxidation apparatus used consists (Figure I) of two flasks fused together, the oxidation flask with a volume of 250 cc., and the absorption flask of 125 cc.. The absorption flask is connected by a short rubber tube with a second absorption flask consisting of a large 50 cc. test tube.

Reagents: I. A 10 % solution of potassium pyrosulphate, one - fifth neutralised with sodium carbonate (1665 cc. of a 12 % solution of pyrosulphate with 170 cc. of a 2 N sodium carbonate solution diluted to 2000 cc.).

2. Thiosulphate for absorption of bromine: 1.6 g. of anhydrous sodium carbonate and 12.6 g. of sodium thiosulphate diluted to 2000 cc. (a concentration of 0.4 % of thiosulphate and 0.08 % of sodium carbonate).

Ashing: 1.00 cc. of the mixture under investigation is introduced into a 50.00 cc. measuring flask; 1 cc. of 2 N nitric acid and 2 or 3 cc. of N / 15 silver nitrate are added. Then 10 cc. of concentrated nitric acid and 10 cc. of 30 % hydrogen peroxide are added. The flask is placed in a water - bath, which is slowly brought to a boil. Heating is continued for several hours until the solution is clear, and the formation
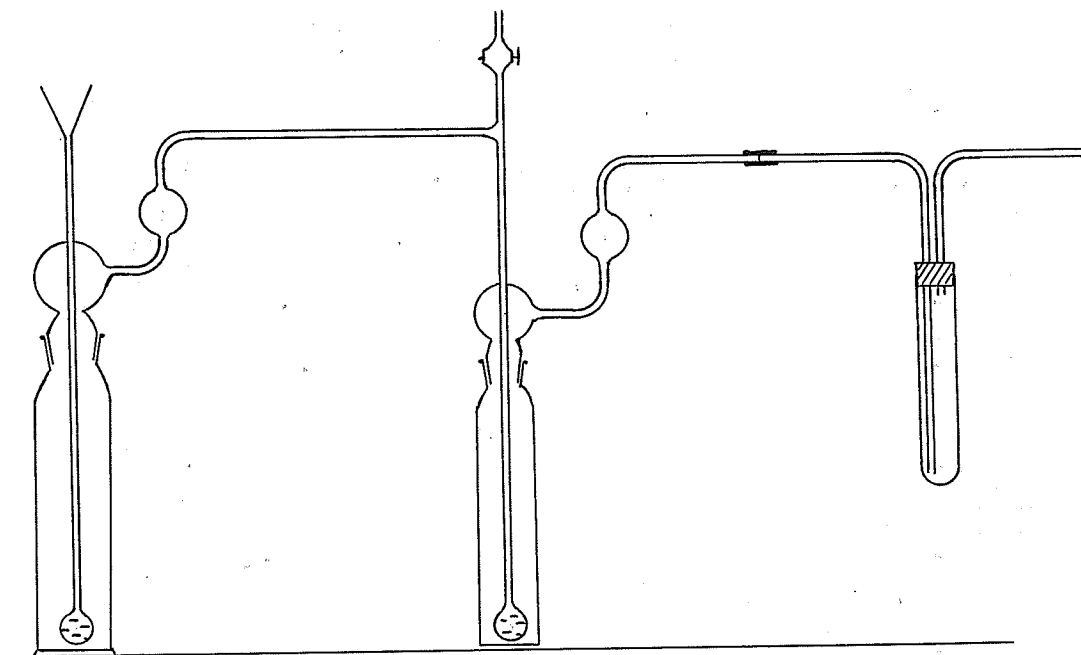
Figure I.   The oxidation apparatus as recommended
by Møller.

of gases has stopped. The silver halides have separated at the
bottom of the flask. After cooling the flask is filled to the
mark with water and filtered through a small filter funnel.

25.00 cc. of the filtrate are introduced into a 50 cc.
Erlenmeyer flask; I cc. of N ferric alum and a little ether are
added. This is titrated with a N / 30 solution of sodium thio -
cyanate from a 5.00 cc. microburette. The flask is closed with
a rubber stopper and shaken, the silver thiocyanate collects in
the ether layer leaving the lower part clear. Thereby it is
possible to determine the colour change within a 0.02 cc. drop.
From this the total halogen content of the mixture is determined.

Reduction of the silver halides: The silver halides are
washed on the filter, first with dilute nitric acid, and finally
with water. 0.10 to 0.15 g. of sodium hyposulphite are intro -
duced into the measuring flask with 5 cc. of water. The flask
is shaken and the contents poured into a small beaker. The
filter paper is also placed in the beaker and the contents are
mixed. Thus the halides are reduced to bromine, chlorine and
free silver. The contents of the beaker are again filtered into
a dry beaker. Then the flask and beaker are washed three times
with 5 cc. portions of a I % solution of sodium sulphate and
filtered.

Oxidation: Into the first absorption flask 50 cc. of the
thiosulphate reagent are introduced, into the second 25 cc.. Then
3 g. of potassium permanganate are introduced into the oxidation

flask, the stopper inserted, and the apparatus connected. The ground glass joints are lubricated with water. The contents of the beaker are now washed into the oxidation flask through the funnel with warm water. The oxidation flask is placed in a water - bath at 50 - 55°, and a slow stream of air is passed through the apparatus (0.5 - 0.6 l. per minute). Then 40 cc. of the pyrosulphate reagent are introduced into the oxidation flask, heated to about 50°. Møller states that in 2 hours all the bromine is introduced into the absorption flasks as sodium bromide.

The contents of the absorption flasks are washed into a thin - walled glass basin and evaporated on a water - bath to 10 - 15 cc.. This is oxidized a second time with 3 g. of permanganate and 40 cc. of pyrosulphate reagent. Into the absorption flasks are placed 50 cc. and 25 cc. of a 3 % solution of potassium iodide. Møller's results show that all the bromide is oxidized inside of 3 hours. The potassium iodide solution is titrated in the flasks with a N / 50 sodium thio - sulphate solution, using a 1 % solution of starch as the indicator.

No satisfactory results were obtained in my own analyses. As the most probable source of error is in the oxidation procedure, the time of aeration was lengthened. Some results obtained by the method are given in Table I. These form a complete series of results and are not selected figures.

Table I.

No.	Bromine present.	Total halogen as Br$_2$.	Oxidation.		Ash-ing	Thio-sulphate used N / 50.	Bromine found.	Dif-ference.	Error.
			1st.	2nd.					
	mg.	mg.	hrs.	hrs.	hrs.	cc.	mg.	mg.	%
I	2.050	2.250	2	2	4	0.624	0.992	-1.058	-51.71
2	2.050	2.740	3	2	6	0.830	1.328	-0.722	-35.12
3	2.050	2.140	2	2	6	0.650	1.040	-1.010	-49.27
4	1.989	1.780	3	2	6	0.902	1.443	-0.546	-27.45
5	0.401	0.397	2	3	6	0.178	0.285	-0.116	-28.94
6	0.208	0.214	2½	3½	6	0.112	0.179	-0.029	-13.69
7	2.074	2.090	3½	4	7	1.700	1.870	-0.204	- 9.83
8	2.074	2.140	3	3½	7	1.300	1.808	-0.266	-12.82

Remarks: The results show that the yield obtained depends on the time of aeration, i.e. the oxidation. As one cannot expect to have a loss of bromine in the wet ashing procedure used, one can conclude that in using a potassium permanganate - pyrosulphate mixture for oxidizing the halides, the bromide cannot be quantitatively transferred as bromine, even on pro - longed aeration.

(2). The Leipert and Watzlawek method.

The oxidation with a silver sulphate - chromic - sulphuric acid mixture for the estimation of bromine after Baubigny and Chavane, has been favorably criticized as a macro - method by Emde [22] and Vorländer [23]; it has also been introduced into micro- analytical methods by Dieterle [24], Vierböck [25], and Zacherl and Krainick [26]. Leipert [27] has used it in over a 100 cases of biological material for iodine determinations with satisfactory results.

The iodine present is held back as hydrogen iodide in the ashing mixture; chlorine and bromine escape as gases and are changed to the corresponding halides or hypohalides in the sodium hydroxide solution. The presence of chlorine has no bad influence on the bromine estimation, in fact, the presence of hypochlorite is highly desired, since then the bromide and hypo - bromide are changed to bromate:

$$Br^- + 3 H O Cl \rightarrow Br O_3^- + 3 H^+ + 3 Cl^-$$

This type of oxidation was first applied by Weszleszky [28]

for the estimation of small amounts of bromine in mineral water. He oxidized the bromide by ashing a carbonate - bicarbonate alkali mixture with chlorine water. Meulen [29] was the first to do away with ashing in a quantitative oxidation. In a mixture containing large amounts of sodium chloride, bromide was quantitatively oxidized to bromate with hypochlorite. The hypochlorite was set free with boric acid or carbon dioxide, and the mixture warmed for a short time. The hypochlorite left was destroyed with formate:

$$H O Cl + H C O O^- \rightarrow Cl^- + H_2O + C O_2$$

The bromate formed was estimated iodometrically:

$$Br O_3^- + 6 I^- + 6 H^+ \rightarrow Br^- + 3 H_2O + 3I_2.$$
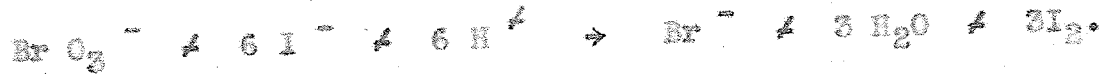
The speed of the reaction is controlled by the acidity. Even when small amounts of bromate are present, the reaction is quantitative on adding a little ammonium molybdate as a catalyser, Kolthoff [30].

Apparatus: The apparatus, my modification of that used by Leipert and Watzlawek, consists of a 250 cc. Wolff's bottle (Figure 2). One ground glass stopper carries a funnel for the ashing material, into which is fused a 1.5 cm. wide tube with an outlet tube R I. The end of the funnel reaches to about 5 cm. above the level of the liquid. By this means every drop of the mixture analysed passes the bottom of the flask and so through the chromic acid layer, whereby the iodine present is held back as hydrogen iodide. This would not happen if the mixture is dropped on the surface. Thus the formation of moisture is also

removed. A second tube, R^2, assists in producing thorough aera-
tion. The chromyl chloride destroying tube, T, is a 9 cm. long
glass tube with the sides slightly drawn in in the middle portion.
Glass beads, about 2 cm. high, are held in here, moistened with
diluted (1 : 3) sulphuric acid. Although no chromyl chloride
is formed when solid sodium chloride and a silver sulphate -
chromic - sulphuric acid mixture are heated, yet some is formed
when a sodium chloride solution is run into the hot acid mixture
while aerating. Since the warm gas stream slowly concentrates
the sulphuric acid, the solubility of bromine is lowered, and a
retention is not to be expected. The absorption tube holds 10 cc.
in the lower end. Tube R^3 is connected to the pump.

Air is first passed through a flask filled with a 10 %
solution of sodium hydroxide; a T - tube connects this with the
two inlets R^1 and R^2. The ashing bottle is immersed in a
paraffin bath held in a large beaker. Thus its contents can be
observed throughout the procedure. The whole apparatus is dried
before use by wetting with acetone, and then aerating it.

This apparatus differs from that of Leipert and Watzlawek,
in that the ashing tube used by the latter consists of a Van Slyke
tube; the funnel, aeration and outlet tubes are fused into the
ground glass stopper.

Reagents: 1. Chromic acid: 250 g. of chromic trioxide are
dissolved in 150 cc. of distilled water. This is aerated for
2 hours by passing the air first through a 10 % sodium hydroxide
solution. 1 cc. - 1 g. chromic acid.

2. Silver sulphate - sulphuric acid mixture: In a 2 litre
Kjeldahl flask 20 g. of silver sulphate are dissolved in 1 litre
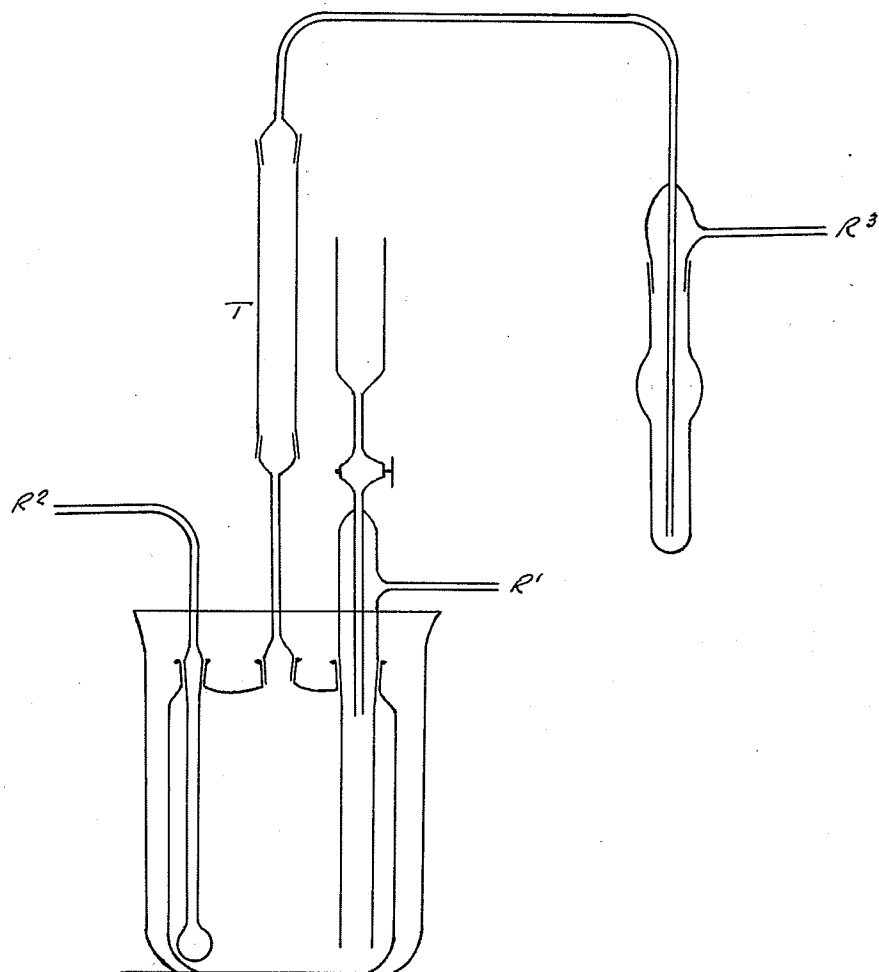of concentrated sulphuric acid, 3 cc. of the chromic acid

Figure 2.   Apparatus for the micro - determinations
of bromine as recommended by Leipert and Watzlawek, and
modified by the writer.

solution are added, a thermometer inserted, heated to 120 - 130°
and then aerated as above for 2 hours.

    3.   Recrystallized boric acid.

    4.   Concentrated sodium chloride solution: Purified by
saturating a saturated solution of sodium chloride with chlorine
gas. The salt separated is washed on the Buchner with a little
water, dried sharply and pulverized.

    5.   Hypochlorite solution: It is used only in controlling
the purity of the reagents. It is left out in analytical work.
About 7 g. of chlorine gas are introduced into a solution of
12 g. of sodium hydroxide in 100 cc. of water.

    Ashing: 100 cc. of the silver sulphate - sulphuric acid
mixture are introduced into the ashing bottle and warmed for
a short time on the water-bath. 8 - 10 cc. of chromic acid are
added, the stoppers moistened with concentrated sulphuric acid
and the flask placed in the paraffin bath at 100°. Into the
intermediate tube are placed 2 cm. of glass beads and 0.2 cc.
(5 - 6 drops) of a 1 : 3 diluted sulphuric acid solution.
This is connected with the ashing tube without further moistening
of the stopper. Into the receiver are introduced 3 - 5 cc. of
N sodium hydroxide diluted to 10 cc. with water. R^1 and R^2
are connected with the washbottle, with the clamp at R^2 closed.
Aeration is started at 2 bubbles per second. A measured amount
of the mixture under investigation is introduced into the funnel
and run into the ashing mixture drop by drop. No pressure must
develop in the apparatus so that the gases formed are easily

withdrawn. The funnel is washed first with a little water, and a second time with water to which 0.5 cc. of a saturated sodium chloride solution have been added.

The chromyl chloride appears as a faint yellow colour in the beads. At no time was any of the chromyl chloride carried over into the absorption tube. After the sodium chloride has been added, the aeration is set as at the start. Both inlets are now opened and regulated so that a maximum amount of aeration is obtained throughout the mixture. Aeration is continued for three hours, while the temperature of the bath is raised from $100°$ to $130°$, at which it is kept for the last hour.

Estimation of bromine in the absorption tube: When large amounts of sodium chloride are present it is necessary to estimate its purity. To 5 g. of sodium chloride in 25 cc. of water are added 0.5 cc. of hypochlorite solution and 1 g. of boric acid. This is shaken in a running water - bath for 10 minutes. Then 2 cc. of a 10 % sodium formate solution are added, boiled for 5 minutes on a wire - gauze, and cooled. One crystal of potassium iodide, about 1 cc. of a 2 % starch solution, and a drop of ammonium molybdate are added; finally 5 cc. of a 2 N hydrochloric acid solution are added; the iodine liberated is titrated with N / 50 thiosulphate.

A blue colour may be formed before acidifying, due to the incomplete destruction of the hypochlorite. This is caused by the distillate being too alkaline, and an insufficiency of boric acid. The analysis, however, can be saved by titrating

the solution with thiosulphate until the colour disappears, and then acidifying. This has not been observed during the analytical work recorded in this paper.

For the estimation of bromine in the absorption tube the addition of hypochlorite is unnecessary, since washing the funnel with sodium chloride solution produces an excess of hypochlorite. The stopper and tube are carefully washed; the oxidation is carried on in the absorption flask. To the tube are added 0.5 g. of sodium chloride and 0.7 - 1. g. of boric acid (depending on the amount of hydroxide present). This is placed in a running water - bath for 10 minutes, washed into a 50 cc. Erlenmeyer flask, reduced with 2 cc. of formate, and continued as stated above. Then it is acidified with 5 cc. of 2 N hydrochloric acid, and titrated with N / 50 thiosulphate from a micro - burette.

It was found that the bromine content of 5 g. of the sodium chloride used is 0.0005 mg.. This can be neglected in using 0.5 - 1. g. of it. A series of results obtained are given in Table 2.

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Table 2.

No.	Bromine present inorgan. mg.	Bromine present organic. mg.	Chlorine present conc. sol'n. cc.	Iodine present. mg.	Thiosulphate used N/50. cc.	Bromine found. mg.	Difference. mg.	Error. %
I	2.065	None	0.50	None	7.38	1.966	-0.099	-4.79
2	2.065	"	0.50	"	7.47	1.990	-0.075	-3.63
3	1.328	"	0.50	"	4.86	1.296	-0.032	-2.41
4	1.328	"	0.50	"	4.96	1.321	-0.007	-0.52
5	1.328	"	0.50	"	4.93	1.313	-0.015	-1.13
6	1.328	"	0.50	"	4.702	1.253	-0.075	-5.65
7	2.015	"	0.50	"	7.31	1.947	-0.068	-3.37
8	None	0.207	0.75	"	0.786	0.209	+0.002	+0.96
9	"	0.207	0.75	"	0.783	0.208	+0.001	+0.48
IO	"	0.102	0.70	"	0.41	0.109	+0.007	+6.86
II	"	0.102	0.75	0.092	0.404	0.102	0.000	0.00
I2	"	0.051	0.70	None	0.198	0.052	+0.001	+1.96
I3	0.075	0.061	0.53	0.921	0.514	0.137	+0.001	+0.73
I4	0.075	0.061	0.53	0.921	0.515	0.137	+0.001	+0.73
I5	0.100	None	0.50	0.092	0.365	0.102	+0.002	+2.00

Remarks concerning the technique: I. Constant observation and great care are necessary when introducing the mixture under investigation into the ashing tube. The addition of the mixture and washings usually require about 20 minutes.

2. The speed of aeration must be controlled carefully. If aeration is too vigorous, some of the bromine passes through the hydroxide without being absorbed.

3. The absorption mixture is titrated immediately after the addition of hydrochloric acid. If the mixture is allowed to stand for several minutes high results are obtained.

Additional remarks: The method described is quite satisfactory for micro - determinations of bromine. Analyses can be carried out within a short time limit.

Certain disadvantages, however, are encountered, mainly the fact that large amounts of reagents are required for each determination. Several analyses were made with the same ashing mixture, as in Table 3. The yield is always low. That solid material cannot be analysed is another disadvantage.

Table 5.

No.	Bromine present.		Chlorine present conc. sol'n.	Iodine present.	Thio-sulphate used N / 50.	Bromine found.	Dif-ference.	Error.
	inorg.	organic						
	mg.	mg.	cc.	mg.	cc.	mg.	mg.	%
1	2.065	None	0.50	None	4.845	1.290	-0.775	-37.53
2	2.065	"	0.50	"	7.25	1.932	-0.133	- 6.44
3	2.676	"	0.50	"	9.365	2.495	-0.181	- 6.76
4	0.201	"	0.50	0.092	0.687	0.183	-0.018	- 9.18

2.   THE OXIDATION PROCEDURES.

As previously stated several methods, in which the material
is first fused with alkali and then oxidized, have been produced
in recent years.   Before it is possible to investigate   the
accuracy of the fusion methods, it is essential to   obtain   a
reliable oxidation procedure.   The main difficulty   in   the
oxidation procedure lies in the separation of chlorine   and
bromine.   Three oxidizing mixtures appear promising: (I)   A
chromic - phosphoric acid mixture, as recommended by   Francis
and Harvey [20];   (2)   a potassium permanganate - potassium
hydrogen sulphate - potassium sulphate mixture, by Bertram [19];
and (3)   a chromic - sulphuric acid mixture, by Yates [18].

(I).   The Francis and Harvey procedure.

The apparatus used   (Figure 3)   consists of the oxidation
tube and two bubblers, connected in a series.   No   lubricating
grease is used, the ground joints are lubricated only with water.
Before each analysis the apparatus is cleaned with   a   chromic -
sulphuric cleaning mixture, followed by thorough washing,   first
with tap water and then with distilled water.   Finally the whole
apparatus, with the exception of the two small bubblers, is rinsed
with very dilute sulphuric acid   (about   N / 5000), well drained,
and set up in a position screened from the action of direct   sun -
light.

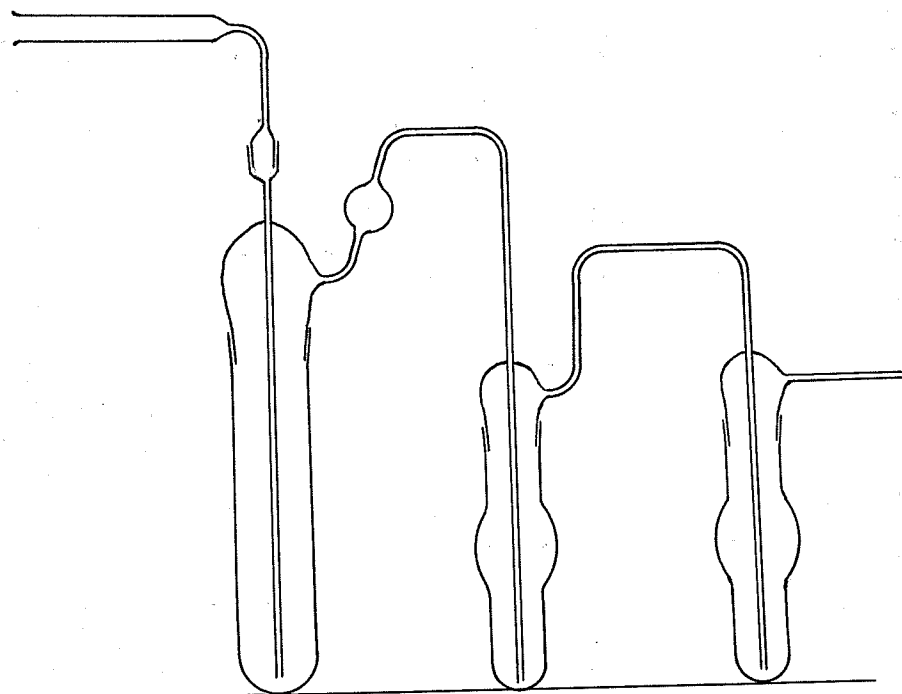Reagents: (I).   The chromic - phosphoric acid mixture:

Figure 3. The oxidation apparatus as recommended by Francis and Harvey.

Syrupy phosphoric acid (s. g. 1.70) is treated with an excess of chromic acid, and heated on a water - bath for three hours with occasional stirring. The mixture is cooled overnight and then freed from the excess chromic acid by filtration through glass wool. The mixture is again heated on the water - bath, aerating vigorously for 3 hours; the air is passed through cotton wool, then glass wool to remove all dust particles.

Diluted solution: 90 cc. of the stock solution are diluted with 5 cc. of water.

(2). A 2 % solution of potassium iodide, prepared from boiled distilled water, is kept away from light.

(3). A 0.5 % solution of arrowroot starch.

(4). A N / 500 solution of sodium thiosulphate.

Into each of the small bubblers are introduced 0.5 cc. of the potassium iodide, and 0.5 cc. of the starch solutions. The mixture containing a known amount of bromide is made up to 5.00 cc. and introduced through the funnel into the oxidation tube. A slow stream of air is drawn through the apparatus, while 5 cc. of the chromic - phosphoric acid mixture are carefully run down the funnel. The heat of the reaction causes the temperature of the solution to rise slowly. The soda - lime tube is replaced. In about 15 seconds, when effervescence has ceased, the rate of the air stream is increased. According to the authors, all the bromide is oxidized in 20 minutes.

The contents of the second bubbler are blown into the first.

This is titrated with the thiosulphate from a microburette.

In my experiments the yield is exceedingly low throughout, even when prolonging the time of aeration. No difference in yield is noticed in using the undiluted chromic - phosphoric acid mixture instead of the diluted. The first five analyses (Table 4) are made with the diluted while the last four with the undiluted mixture.

Remarks: It is evident from the results obtained that the chromic - phosphoric acid mixture used does not oxidize all the bromide present.

Table 4.

No.	Bromine present. mg.	Aeration time. min.	Thio-sulphate used N / 500. cc.	Bromine found. mg.	Differ-ence. mg.	Error. %
1	0.047	20	0.084	0.013	-0.034	-73.23
2	0.047	20	0.140	0.022	-0.025	-53.19
3	0.047	20	0.160	0.026	-0.021	-44.68
4	0.041	20	0.19	0.030	-0.011	-26.83
5	0.041	20	0.195	0.031	-0.010	-24.39
6	0.083	30	0.410	0.065	-0.018	-21.68
7	0.083	50	0.406	0.065	-0.016	-21.68
8	0.034	70	0.175	0.028	-0.006	-17.65
9	0.034	40	0.105	0.017	-0.017	-50.00

(2). <u>The Bertram procedure.</u>

The principle is the oxidation of bromide to bromine by a boiling solution of potassium permanganate, acidified with potassium hydrogen sulphate, and buffered with potassium sulphate.

The apparatus used consists of a 200 cc. Kjeldahl flask with a ground glass stopper, and a vertical condenser. They are connected by a fused glass tube which has an enlargement to serve as a trap. A 50 cc. Erlenmeyer flask (with mark at 45 cc.) is used for the collector. No lubricating grease is used, the ground joints being lubricated with water.

The mixture containing the alkali metals and halogens, but no nitrogen combinations, is brought to 50 cc. and intro -duced into the distillation flask. To the flask are added 2.5 g. of potassium hydrogen sulphate, 2.5 g. of potassium sulphate, 1.5 cc. of cold concentrated potassium permanganate solution, and several glass beads. The flask is heated with a full flame. The collector contains 3 cc. of a 0.1 % solution of potassium iodide, and 2 cc. of water acidified with one crystal of potassium hydrogen sulphate. The end of the condenser must reach below the surface. Distillation is continued until the distillate reaches the 45 cc. mark. The apparatus is then thoroughly cleaned with an oxalic - sulphuric acid mixture. The distillate is washed into the distillation flask with 5 cc. of water and redistilled, using the same amounts of reagents. Into the receiver are placed 2 drops of a 4 N sulphuric acid solution,

IO drops of a I % starch solution, and one crystal of potassium iodide in 3 cc. of water. The distillation is again continued until the distillate reaches the 45 cc. mark.

The distillate is too dilute for titration. To concentrate, it is again distilled after acidifying with 5 drops of 4 N sulphuric acid. When IO cc. have been distilled over, the collector is replaced. The first distillate is titrated with N / 500 thiosulphate; if necessary, the second is also titrated.

A series of results obtained are given in Table 5.

Remarks: The low results obtained are probably due to two factors: Either the mixture used is too weak to oxidize all the bromide present, or the loss of bromide is high in a method employing three distillations. Likely both factors come into play.

Table 5.

No.	Bromine present.	Thiosulphate used N / 500.	Bromine found.	Difference.	Error.
	mg.	cc.	mg.	mg.	%
1	0.203	1.015	0.162	-0.041	-20.20
2	0.203	1.041	0.166	-0.037	-18.22
3	0.038	0.19	0.030	-0.008	-21.05
4	0.038	0.17	0.027	-0.011	-28.95
5	0.019	0.10	0.016	-0.003	-15.79
6	0.019	0.10	0.016	-0.003	-15.79

(3). The Yates procedure.

The method is based on the partial oxidation of the halogens with a chromic - sulphuric acid mixture. At a definite pH all the bromide is oxidized in the cold and none of the chloride, while the iodide is changed to iodate - Yates.

The aeration apparatus (Figure 4) consists of a 100 cc. Erlenmeyer flask fitted with a two - holed rubber stopper. This carries a tube, drawn out slightly at the end, that reaches down to the bottom corner of the flask. The flask is inclined at an angle of 60°, thus ensuring maximum aeration. The rubber stopper carries a second glass tube bent at 60°, connected to a small bubbler. The bubbler consists of a 25 cc. test - tube, fitted with a rubber stopper, that carries an exit tube, and an inlet tube drawn out at the end. A distance of not more than 2 mm. separates the bottom of this tube and that of the test - tube.

Reagents: I. The chromic- sulphuric acid mixture. 20 g. of chromic acid and 40 cc. of concentrated sulphuric acid (s.g. 1.84) are diluted with 120 cc. of distilled water.

The solution containing the halides brought to 7.0 cc. (± 0.1 cc.), is introduced into the oxidation flask. 2.5 cc. of concentrated sulphuric acid are slowly added down the sides of the flask with shaking and constant cooling under the tap. At least 10 minutes are required for this procedure. The first
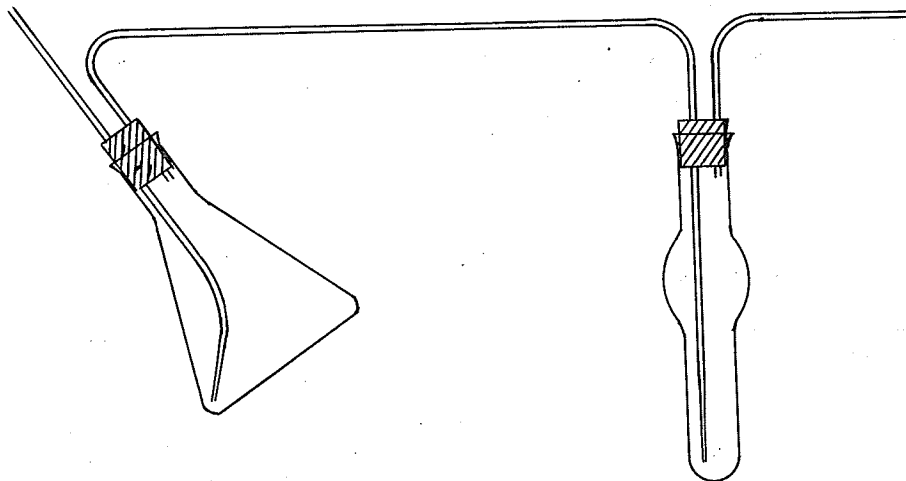
Figure 4. The oxidation apparatus as recommended by Yates.

few drops, which cause the evolution of carbon dioxide, must be carefully added in order to prevent losses of hydrogen bromide in the gases evolved.

Now 4 cc. of the chromic - sulphuric acid solution are added by allowing the solution to run all over the sides of the flask. The top of the flask is immediately wiped, and connected to the aeration apparatus. The receiver contains 1 cc. of a 10 % potassium iodide solution and 4 drops of a 1 % solution of starch. Aeration is continued for 2 hours (about 30 cc. per minute.) with a steady stream of air. At the end of this time the absorption tube is renewed and aeration continued for a further 3 hours. The iodine liberated in the two tubes is titrated with a N / 500 thiosulphate solution from a micro - burette.

A series of results obtained by this procedure are given in Table 6.

To determine the effect of light on the speed of oxidation, the writer carried out several analyses by placing the oxidation apparatus in complete darkness, ordinary daylight, and direct sunlight. The results obtained are given in Table 7. Light, therefore, has a decided influence on the speed of oxidation. In the absence of light oxidation is slow, and the yield low. In direct sunlight some of the chloride present is oxidized along with the bromide; thus the yield is high. When the oxidation is carried out in ordinary daylight, satisfactory results are obtained.

Remarks: The results obtained show satisfactory accuracy
even in the presence of excess of chloride and iodide. The results
are in close agreement with those given by Bobtelsky and Rososkaja -
Roseinskaja [31], and also Evans [17] (these authors use 5 %
chromic acid). In the procedure described 9 N sulphuric acid
and 3.5 % of chromic acid are used. The concentration of chromic
acid has little effect upon the oxidation potential of the mixture
at sulphuric acid concentrations above 4 N. The theoretical
limit of accuracy of the procedure is 0.0032 mg. of bromine.

From the results given thus far, one very interesting fact
emerges. Of the mixtures used, the only oxidizing mixture which
oxidizes bromide to bromine quantitatively is a chromic - sulphuric
acid mixture.

In the subsequent work, the oxidation method as recommended
by Yates is used throughout.

Table 6.

No.	Bromine present. mg.	Iodine present. mg.	Chlorine present. mg.	Thio-sulphate used N / 500. cc.	Bromine found. mg.	Dif-ference. mg.	Error. %
1	0.009	0.044	5.460	0.06	0.009	0.000	0.00
2	0.019	0.044	5.460	0.12	0.019	0.000	0.00
3	0.019	0.044	5.460	0.015	0.018	-0.001	-5.26
4	0.034	None	None	0.23	0.037	≠0.003	≠8.82
5	0.034	"	"	0.205	0.033	-0.001	-2.94
6	0.034	0.044	2.386	0.21	0.034	0.000	0.00
7	0.034	0.044	2.386	0.205	0.033	-0.001	-2.94
8	0.094	0.088	5.460	0.58	0.093	-0.001	-1.06

Table 7.

Bromine present. mg.		Bromine found mg.
0.079	in complete darkness.	0.022
0.020		0.013
0.020	in direct sunlight.	0.032
0.020		0.029
0.020	in ordinary daylight.	0.019
0.020		0.020

3. THE ASHING PROCEDURES.

Having obtained a reliable oxidation method, it is possible to investigate the accuracy of ashing procedures. Some controversy exists as to the use of nickel crucibles in ashing. It is more economical to use nickel crucibles instead of platinum. Claims that satisfactory results are obtained in the use of nickel crucibles have been advanced by Pincusson and Roman [8], Yates [18], and Dixon [32]. While Behr, Palmer and Clark [16] state that in the use of nickel losses occur. Francis and Harvey [20] on the other hand describe an ashing procedure, by incinerating first in nickel and finally in platinum.

In ashing the material in order to change the halogens into halides, an excess of alkali is necessary, but a large excess is undesirable in view of the oxidation procedure used. The procedure first used, as given by Francis and Harvey, consists of ashing the material under controlled conditions with various amounts of potassium hydroxide. The exact procedure follows: The material to be analysed is placed in a nickel dish with the potassium hydroxide and 0.1 cc. of a 20 % sucrose solution. The contents of the dish are well mixed with a glass rod, and the rod and sides washed with distilled water. This is evaporated to dryness on a water - bath, heated in an oven at 150 - 160° for one hour, and ignited for one hour in a muffle furnace at 475 - 485°. To the residue are added 20 cc. of water, digested on the water - bath for 2 - 3 minutes, and decanted through a

filter (previously washed with hot water) into a platinum dish; the carbon is again extracted and decanted. The filter paper, moistened with water and I cc. of N potassium hydroxide, is heated at 150 - 160° for 15 minutes and ignited at 475 - 485° for one hour, extracted and washed. The filtrates are now combined in the platinum dish, evaporated, dried at 150 - 160° for 15 minutes, and ignited at 475 - 485° for 10 minutes. The residue is dissolved in a little water and ignited twice. To remove the last traces of organic matter the residue is dissolved in a little water, treated with I cc. of a 2 % potassium di - chromate solution, evaporated and ignited for 10 minutes. The resulting alkaline residue is taken up with water and filtered cold. The filtrate is evaporated, heated at 150 - 160° for 15 minutes and ignited in the muffle furnace for 5 minutes. The residue thus obtained is taken up with 3.0 cc. of water, poured into the oxidation flask, and the crucible washed with two further 2.0 cc. portions..

The results obtained, as given in Table 8, show that the ashing procedure described gives satisfactory results. When too much alkali is present the results tend to be low, probably due to the fact that in introducing the acid into the alkaline mixture some of the bromine is lost along with the carbon di - oxide evolved. From the appearance of the ash and the results obtained, the amount of N potassium hydroxide solution to be used should be about 5 cc.

The question now arises as to whether satisfactory results

can be obtained when the incineration is conducted in nickel crucibles alone. For this purpose several analyses with nickel crucibles were made using the given procedure. The results obtained show that when the incineration and oxidation are carried out during the same day, satisfactory yields are obtained. On the other hand, if the incinerated material is allowed to stand overnight in the nickel crucible, a small but definite amount of bromine is lost, even though the crucible is kept covered.

In another series of experiments the incineration is conducted in nickel crucibles as described, washed into platinum crucibles with distilled water, heated at 150 - 160° for 15 minutes, and ignited in the muffle furnace for 5 minutes. The covered crucibles are allowed to stand overnight to be oxidized the next day. Satisfactory results are obtained. A series of results showing the above mentioned facts are given in Table 9.

Remarks: The results show definitely that the incineration can be carried out under controlled conditions in nickel crucibles. In allowing the incinerated material to stand in the crucibles for some time losses occur.

Table 8.

No.	Bromine present. mg.	Amount of N K O H used. cc.	Bromine found. mg.	Difference. mg.	Error. %
I	0.04I	25	0.036	-0.005	-I2.20
2	0.06I	I5	0.058	-0.003	- 4.9I
3	0.06I	I5	0.057	-0.004	- 6.55
4	0.04I	IO	0.039	-0.002	- 4.87
5	0.04I	IO	0.037	-0.004	- 9.75
6	0.04I	5	0.040	-0.00I	- 2.44
7	0.04I	5	0.04I	0.000	0.00

Table 9.

Bromine present. mg.		Bromine found. mg.
0.020		0.0I6
0.020	Ashed in nickel crucibles, and	0.0I7
0.04I	allowed to stand in same overnight.	0.034
0.04I		0.036
0.04I		0.034
0.04I		0.04I
0.06I	Ashed in nickel crucibles, and	0.059
0.020	oxidized the same day.	0.020
0.04I		0.040
0.06I	Ashed in nickel crucibles, and	0.060
0.020	kept overnight in platinum	0.0I9
	crucibles.	

THE METHOD ADOPTED.

The oxidation apparatus used (Figure 4) has been described. Frequent cleaning with chromic - sulphuric cleaning mixture is essential, followed by very thorough washing, first with tap water and then with distilled water.

Ashing: The substance to be analysed is introduced into a 150 cc. nickel crucible together with 5 cc. of N potassium hydroxide, 0.1 cc. of 20 % sucrose solution, and 10 cc. of distilled water. The contents are well mixed with a glass rod, the rod washed with a little water, and removed. This is evaporated to dryness on a steam - bath, heated at 150 - 160° for one hour, and ignited for one hour in a muffle furnace at 475 - 485°. 20 cc. of water are added to the residue, the carbon broken up with a glass rod, the mixture digested on a steam - bath for 2 - 3 minutes, and decanted through a filter (9 cm. Whatman paper previously washed with hot distilled water) into a clean nickel crucible; the carbon is extracted again and decanted. The filter paper is placed in the first crucible, moistened with water and 1 cc. of N potassium hydroxide, evaporated to dryness, dried at 150 - 160° for 15 minutes, and ignited in the muffle furnace for one hour. The residue again is extracted in the manner described, filtered, washed, and added to the previous filtrate. The combined filtrate is evaporated to dryness, heated at 150 - 160° for 15 minutes, and then ignited at 475 - 485° for 10 minutes. The residue is dissolved in

about 10 cc. of water, evaporated, and again ignited for 10 minutes. To remove the last traces of organic matter, the residue is dissolved in 5 cc. of water, treated with 1 cc. of 2 % potassium dichromate solution, evaporated, and ignited as before. The resulting alkaline residue is taken up with a little water, and filtered (7 cm. Whatman, previously well washed with hot distilled water) into a third crucible. The crucible and filter are washed with successive portions of water to make the filtrate up to 30 cc.. If the oxidation is carried out immediately after ignition, the contents of the crucible are evaporated, heated at 150 - 160° for 15 minutes, and ignited at 475 - 485° for 5 minutes. In case the residue has to be kept overnight, the contents are transferred to a clean platinum crucible, evaporated, and ignited as before. The crucible is covered to prevent contamination by organic matter in the form of dust from the atmosphere.

Oxidation: The contents of the crucible are dissolved in 3.0 cc. of distilled water and introduced into the oxidation flask. The crucible is washed with two further 2.0 cc. portions of water. The oxidation is carried out as previously given, using 2.5 cc. of concentrated sulphuric acid and 4 cc. of the chromic - sulphuric acid mixture.

Blanks have to be carried out from time to time. The value for the blank is found to be 0.003 mg.. The sucrose solution is added to ensure that any bromate present is reduced to bromide.

The correction necessary for bromine present in sucrose, is made automatically by adding it to both the regular analyses as well as to the blanks.

A series of results obtained by the method are given in Table IO. Thus we can see that the method is quite accurate over a wide range of bromine estimations(0.003 to 0.900 mg.).

As a final check to ascertain the accuracy of the method, it is necessary to determine whether bromine can be quantitatively recovered when added to biological material. Analyses were made with blood, urine and thymus tissues. The bromine added was recovered quantitatively in each case, as shown in Table II.

Table 10.

No.	Bromine present.		Iodine present.	Chlorine present.	Bromine found.	Dif - ference.	Error.
	inorganic.	organic.					
	mg.	mg.	mg.	mg.	mg.	mg.	%
1	0.001	0.002	0.044	2.386	0.003	0.000	0.0
2	0.001	0.002	0.044	5.460	0.004	<0.001	<33.
3	0.009	0.011	0.022	1.193	0.021	<0.001	< 5.
4	0.009	0.011	0.022	1.193	0.020	0.000	0.0
5	0.019	0.022	0.044	2.386	0.040	-0.001	- 2.4
6	0.038	0.040	0.044	5.460	0.076	-0.002	- 2.56
7	0.093	0.099	0.044	5.460	0.190	-0.002	- 1.04
8	0.186	0.203	0.044	5.460	0.387	-0.002	- 0.51
9	0.190	0.200	0.044	5.460	0.390	0.000	0.00
10	0.376	0.409	0.088	5.460	0.781	-0.004	- 0.51
11	0.428	0.467	0.088	5.460	0.877	-0.018	- 2.01
12	0.560	0.609	0.088	5.460	0.975	-0.194	-16.60
13	0.929	1.008	0.044	5.460	1.466	-0.471	-24.31

Table II.

Material analysed.	Bromine present.	Bromine added.		Total Br added.	Total Br.	Bromine found.	Bromine re-covered	Dif-ference.	Error.
		Inorg.	org.						
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	%
Beef blood	0.014	0.019	0.020	0.039	0.053	0.053	0.039	0.000	0.0
Thymus	0.006	0.019	0.020	0.039	0.045	0.044	0.038	-0.001	-2.6
Urine	0.069	0.019	0.020	0.039	0.108	0.107	0.038	-0.001	-2.6

BROMINE IN TISSUES.

Little systematic work on the bromine content of tissues has been done, making only a few comparisons possible.

Endocrine glands.

A series of bromine determinations were made on the dried materials of internal secretory glands. The results obtained are given in Table 12.

These results indicate that the amount of bromine present in the dried materials of the anterior and posterior pituitary is of the same order as that of most of the organs analysed. This is not in agreement with the claims made by Zondek et.al. that the pituitary normally contains 15 - 30 mg. / 100 g. wet weight. On the other hand the bromine content of dried thyroid material is appreciably higher.

Table 12. The bromine content of the dried materials
of endocrine glands.

Material analysed.	Amount taken for analysis.	Bromine found.	Bromine present in 100 g.
	gm.	mg.	mg.
Beef Thyroid (Armour & Co.)	0.02045	0.006	28.
	0.02995	0.010	34.
	0.1018	0.027	27.
Thyroid (Knoll & Co.)	0.0673	0.008	12.
	0.6027	0.072	11.9
Posterior pituitary (Armour & Co.)	0.1004	0.018	17.
	0.2015	0.032	16.
Anterior pituitary (Armour & Co.)	0.0608	0.007	11.
	0.12275	0.012	10.
Suprarenal - whole gland	0.1511	0.005	3.
	0.3055	0.009	3.
Thymus (Armour & Co.)	0.3021	0.006	2.

Urine and blood.

Several bromine determinations were made on urines; all were morning samples taken before breakfast. The results, though few, indicate that the amount of bromine in urine is not constant. Probably the variations are due to dietary factors.

Then two separate samples of beef blood were analysed for their bromine content. Here again variations occur. The results are of the same order as those given by Francis and Harvey [20], and Dixon [32].

To demonstrate the distribution of blood bromine between the plasma and corpuscles, one of the blood samples analysed was centrifuged (at 2500 revolutions per second), and the bromine content of the plasma determined. Thus the writer found that 100 cc. of the blood analysed (52 % plasma and 48 % corpuscles), 0.901 mg. of bromine were present in the plasma, and 0.433 mg. in the corpuscles.

The results obtained are given in Table 13.

Table I3. The bromine content of several blood and urine samples.

Material analysed.	Amount taken for analysis.	Bromine found.	Bromine present in I00 cc.
	cc.	mg.	mg.
Beef blood	2.0I5	0.0I4	0.7I
Beef blood	2.0I5	0.027	I.33
Beef plasma	2.0I5	0.035	I.73
Beef corpuscles	calculated		0.90
Human urine	4.97	0.056	I.I3
Human urine	4.97	0.089	I.8I
Human urine	4.97	0.069	I.38

Fresh tissue.

To obtain an understanding of the distribution of bromine in the animal body, a series of analyses were made on certain fresh tissues of a white rat and a dog *. The results obtained are given in Table 14.

The results show a fairly uniform distribution of bromine in most of the tissues analysed. The amount of pituitary tissue used in the analysis was too small to make a bromine determina - tion possible. This, however, possibly indicates that no excess of bromine is present. It was again noticeable that the bromine content of the thyroid is distinctly higher.

The bromine content of the tissues analysed appears to correspond somewhat to the distribution of iodine.

* The dog, weighing 32.2 lbs., was given injections of adrenalin and atropine while under a morphine - urethane anaesthetic. Then it was killed with copper sulphate and the tissues to be analysed were immediately removed.

Table 14. The bromine content of certain fresh tissues.

Material analysed.	Amount taken for analysis.	Bromine found.	Bromine present.
		mg.	mg.
Rat's organs			
blood	2.015 cc.	0.022	1.09 / 100cc.
liver	1.82505 gm.	0.007	0.4 / 100 g.
kidney	1.6293 "	0.004	0.2 "
Dog's organs			
blood	2.015 cc.	0.020	0.99 / 100cc.
pituitary	0.0494 gm.	None detected.	
thyroid	1.0545 "	0.113	10.74/ 100 g.
cerebrum	1.9854 "	0.009	0.4 "
cerebellum	2.11805 "	0.005	0.3 "
testes	1.59625 "	0.011	0.7 "
pancreas	2.2365 "	0.010	0.4 "
muscle	2.1902 "	0.010	0.5 "
spleen	1.47725 "	0.009	0.6 "
kidney	2.17265 "	0.015	0.7 "
liver	1.9398 "	0.009	0.5 "

SUMMARY.

It appears desirable to call attention at this time to the following points that have been verified by the work done.

Of the oxidizing mixtures used, only the chromic - sulphuric acid mixture oxidizes bromide to bromine quantitatively. Conditions can be so adjusted that no chloride is oxidized. The iodide changes to iodate.

Nickel vessels can be used with satisfactory results, although losses occur if the incinerated material is allowed to stand for some time in them.

The method of Leipert and Watzlawek is quite satisfactory for micro - determinations of bromine in fluids.

A new method for bromine estimation, based particularly on the method outlined by Yates and that can be used on any material to be analysed, is described. The method is fairly accurate over a wide range of bromine estimations.

A few bromine analyses of biological materials are recorded. Before a final conclusion may be drawn as to the significance of bromine, a far more extensive series of analyses is necessary.

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