CXCVIII. THE DETERMINATION OF SMALL QUANTITIES OF IODINE IN BLOOD.

BY ERIC NEWMARCH ALLOTT, JAMES ARNOLD DAUPHINEE
AND WILLIAM HOLDSWORTH HURTLEY.

*From the Dunn Laboratories and Chemical Department,
St Bartholomew's Hospital.*

(Received August 23rd, 1932.)

SINCE the publication of von Fellenberg's method [1923] for the determination of small quantities of iodine, a very large number of new methods and modifications have been described, some of which claim a surprising degree of accuracy. The multiplicity of methods is sufficient to show that none of them is really satisfactory.

The von Fellenberg method includes two methods for the final determination, colorimetric and titrimetric. We have attempted to produce a method which is free from some of the objections which can be raised against the older ones. While it can be said that the colorimetric method here described, which was devised by one of us (W. H. H.), is satisfactory within the limits of such a method, the titrimetric method has not been quite completed; owing, however, to the impossibility of further collaboration it seemed desirable to record such observations as we have made.

Colorimetric method.

Ashing. Most of the previous workers have not laid down any very strict regulations as to the control of temperature necessary during ashing of the blood, but it became apparent early in the work that this would require careful attention. At temperatures above 500° potassium and still more sodium iodide is appreciably volatile, and even at 500° small quantities of the order of 10 γ volatilise very rapidly, though the loss is very much slower in the presence of alkali (see Fig. 1). Scheffer [1930] regards 500° as too high, but our results show that in the presence of sufficient alkali, heating can be carried out at 500° for prolonged periods without loss of iodine. Thus, in one experiment where 5 γ iodine in the form of potassium iodide were added to blood together with 2 cc. of 80 % potassium carbonate, 5 γ were recovered even after 2 hours' heating at 500°.

The blood was ashed by adding 1.5 cc. of approximately 10N potassium hydroxide to 10 cc. of blood in a nickel basin, drying thoroughly in an electric oven at 150°, and then heating at 500° for 1 hour in an electric muffle furnace

which had been calibrated by means of a pyrometer. If the preliminary drying at 150° is omitted, and the blood merely evaporated to dryness on a water-bath, there is great risk of loss through sputtering.

Extraction of iodide. The separation of the iodide from the other components of the ash is carried out as follows. The ash is very thoroughly extracted 4 times, each time with 4 cc. of distilled water, and the extracts filtered through a 3 G 4 Jena glass filter. The combined aqueous extracts are saturated with CO_2 to convert the hydroxide into the carbonate, and evaporated to dryness on the water-bath in a platinum or nickel basin. The carbonate

Fig. 1.

Fig. 1. 1 cc. of a solution containing 10γ iodine in the form of potassium iodide was placed in a platinum dish, and varying amounts of alkali added, as indicated on the curves. The diagram shows the amount of iodine recovered after heating in a muffle furnace at 500° for varying lengths of time.

Fig. 2.

residue is then extracted 4 times with 3 cc. of alcohol, the first extraction being carried out with 97 %, the second with absolute, and the last two again with 97 %. By this means, the carbonate becomes pasty, then rather granular, and finally quite liquid. The alcoholic extracts are carefully decanted from the carbonate residue and again filtered through a Jena glass filter. The combined alcoholic extracts, which contain all the iodide and very little of the other salts, are very carefully evaporated to dryness on the water-bath in a small platinum basin, the greatest care being taken, by covering the basin with an inverted funnel, to avoid any possibility of dust falling in, as this interferes seriously with the final colorimetric determination.

Liberation and extraction of iodine. The basin with its contents is then heated for 2 mins. in the muffle furnace at 500° to drive off traces of solvent

and any other organic matter, and the contents extracted repeatedly with small volumes of water to a total volume of 1 cc., the aqueous solution being poured off into one of the special stoppered tubes with a graduation at 1 cc. (Fig. 2). The iodine is then liberated by adding 1 drop of nitrosulphonic acid from a very fine pipette, and extracted by shaking very thoroughly with 0.05 cc. of pure carbon disulphide.

The use of carbon disulphide for this purpose is preferable to that of chloroform or carbon tetrachloride, as the mean partition coefficient for iodine between CS_2 and water is about 620, for CHCl_3 and water 130, and CCl_4 and water 87. The carbon disulphide was purified by adding about 3 % of pure iodine to remove iodine-binding substances, the iodine being removed next day by shaking with sodium hydroxide, and the carbon disulphide washed with water, dried over calcium chloride and distilled. It should be kept in a dark cupboard when not in use.

Colorimetric comparison. The tube is then compared with a set of standards freshly prepared by treating appropriate quantities of a standard potassium iodide solution with nitrosulphonic acid and carbon disulphide in an identical manner. The comparison should be carried out by daylight, or by the use of a daylight lamp.

A blank determination with the reagents used must always be carried out and the result subtracted from the result found. A fresh blank must be determined whenever any new reagent is taken into use.

Using this method it was found possible to recover quantitatively potassium iodide added to blood, and *p*-iodobenzoic acid was oxidised satisfactorily as is shown by the recovery of 2.0, 3.7 and 5.6 γ of iodine when amounts of *p*-iodobenzoic acid containing 1.85, 3.7 and 5.6 γ of iodine were added to blood.

The difficulty found in detecting small differences, in a total quantity of the order of 1 γ (and this is the type of result to be expected working with 10 cc. of blood with an iodine content of about 10 γ per 100 cc.) led us to try to produce a satisfactory titration method, where it might be expected that such quantities could be more accurately estimated.

Titration of small quantities of iodine, using starch as indicator.

Sensitivity of starch-iodine reaction. In attempting to apply this reaction to the determination of very small quantities, the first point requiring consideration is the sensitivity of the starch-iodine reaction, *i.e.* the visibility of the blue colour produced by small amounts of iodine in the presence of starch.

Table I shows our findings when various amounts of iodine in the form of potassium iodate were treated with varying amounts of iodide, 0.2 cc. *N*/5 sulphuric acid and 1 drop of 1 % soluble starch in small tubes, the total volume in each case being made up to 1.5 cc. with distilled water.

From these figures it is seen that something of the order of 0.08 γ of "iodate-iodine" can be detected by this reaction. It might therefore be expected that titration results would be more or less consistently 0.1 γ low on

Table I.

I as KIO_3 (γ)	KI			Free I present (γ)
	1000 γ	2000 γ	5000 γ	
0.05	0	0	0	0.30
0.07	0	?	Faint trace	0.42
0.09	Faint trace	Faint trace	Trace	0.54
0.11	Trace	Trace	Pale blue	0.66

this account, but this was not found to occur, the error being at any rate roughly neutralised by the inevitable over-titration. Further, the KOH and K_2CO_3 used were not absolutely iodine-free, but had a blank of about 0.3 γ in the quantities used for a determination, and even if one is not titrating to a correct end-point this blank correction is a constant which includes the titration error, and the latter will not therefore interfere with obtaining a true value for the unknown iodine.

Effect of iodide and acid concentration. The next point requiring consideration, and one which has been neglected by many workers, who add "a small crystal" of potassium iodide, is the effect of potassium iodide and acid concentrations on the titration value.

Our findings for the effects of potassium iodide concentration are given in Table II. The results were obtained by titrating a known amount of iodate-iodine (2 γ) in presence of varying amounts of iodide with $N/1000$ thiosulphate from a microburette constructed from capillary tubing, with a delivery of 1.2 mm.³ per mm. of scale.

Table II.

Exp.	I as KIO_3 (γ)	I as KI (γ)	I found (γ)
1	2	20	1.31
2	2	50	1.4
3	2	100	1.6
4	2	200	1.6
5	2	500	1.73
6	2	1,000	2.02
7	2	2,000	1.98
8	2	5,000	1.93
9	2	10,000	2.02
10	2	20,000	2.08
11	2	50,000	2.85
12	2	100,000	4.73

In each experiment 2 γ I (as KIO_3), the varying amounts of KI, and sufficient water to make a volume of 1.5 cc. were added to small titration vessels. 0.2 cc. $N/10$ H_2SO_4 and 1 drop of 1 % soluble starch were added, and the iodine liberated was then titrated with $N/1000$ thiosulphate which had immediately previously been standardised by macro-titration against potassium iodate.

From these results it is seen that reasonably accurate figures are obtained with a KI content between 1000 γ and 2000 γ , smaller quantities giving low results, and larger very high results. The end-point with the larger quantities of iodide is very unsatisfactory, the starch-iodine complex being of a dirty purple colour instead of a clear blue. If such a purple solution is allowed to stand, the complex separates as a deep purple precipitate with a colourless supernatant fluid.

It was found that substantially correct titration results were obtained when the acidity of the solution varied between 0.002 *N* and 0.05 *N*, but that lower concentrations of acid led to somewhat low results. Reith [1929] has found that correct results were obtained when the p_H of the solution varied between 1 and 6. If too strong acid is used, iodine may be liberated from KI even in the absence of iodate. Thus a mixture of 0.65 cc. water, 0.2 cc. 1 % KI, and 1.10 cc. of 10 % H_2SO_4 , became blue immediately on adding starch. The concentration in this mixture is 1000 γ KI per cc., and the acidity roughly *N*.

We can therefore, by choice of suitable conditions, titrate 2 γ of iodate-iodine in a volume of 1.5 cc. with an error of, at most, ± 4 %.

Oxidation of HI to HIO₃. For this oxidation, chlorine water was first tried, as recommended by Veil and Sturm [1925], but as shown by various workers, especially Höjer [1928], even fresh chlorine water is unreliable and tends to give high results. Our work confirming this unsatisfactoriness will not be detailed.

Bromine water is much more satisfactory, and provided careful attention is paid to its freshness, quantitative oxidation of HI to HIO₃ can be carried out.

5 γ iodine (as KI) in 1 cc. and 2 drops of *N*/5 H_2SO_4 were treated in a small pyrex tube, $2\frac{1}{2}'' \times \frac{3}{4}''$, through the bottom of which a piece of platinum wire had been fused to prevent bumping¹, with 3 drops of fresh bromine water. The tubes were then heated in a deep sand-bath and boiled vigorously for varying lengths of time. They were then cooled in ice, 2000 γ KI (0.2 cc. 1 % KI) and 1 drop of 1 % soluble starch² added, and the contents titrated as before. It was found that satisfactory results were obtained when the solution was boiled for periods between 1 and 2 mins. Shorter boiling failed to remove all the bromine, and boiling for longer periods led to loss of iodine. If the solution were evaporated to dryness, no iodic acid at all was recovered.

A consecutive series of results obtained by this method, varying quantities of iodine being used, and a constant boiling time of 1 min. being employed, is given below (Table III). With the exception of No. 17, these results can be claimed as satisfactory, at any rate for quantities greater than 0.1 γ .

Efficiency of extraction of carbonate residue. To test the efficiency of the extraction of the iodide from the carbonate residue, 10 γ iodine (as KI) were added to 2 cc. of 80 % potassium carbonate in an 8 cm. nickel basin and evaporated to dryness in an air-oven at 150°. The dry carbonate was extracted four times with 3 cc. of alcohol, by grinding up very thoroughly with a nickel spatula, and decanting the separate alcoholic extracts into the small titration vessel. The alcohol was then evaporated off on the sand-bath and the dry residue heated $1\frac{1}{2}$ min. on the sand-bath to drive off the last traces of alcohol.

¹ We were using this device for some months before the publication of Aitken [1930] on the determination of iodine.

² The starch should not be too old, but we found it to keep unimpaired in the ice-chest for at least a week.

Table III.

Exp.	I as KI oxidised (γ)	I as HIO_3 found (γ)	Exp.	I as KI oxidised (γ)	I as HIO_3 found (γ)
1	5.0	5.07	11	1.0	1.01
2	"	5.07	12	0.5	0.55
3	"	5.12	13	"	0.52
4	"	5.07	14	"	0.52
5	2.5	2.58	15	0.25	0.25
6	"	2.49	16	"	0.25
7	"	2.49	17	"	0.42
8	"	2.56	18	"	0.22
9	1.0	1.04	19	0.10	0.08
10	"	0.96	20	"	0.06

The iodine content of each fraction was determined as follows. The residue was dissolved in 1 cc. water, neutralised to Congo red (as external indicator) with $N/5 \text{ H}_2\text{SO}_4$, 2 drops of acid were added in excess and the oxidation with bromine was carried out as previously described. Two such experiments were carried out, one with absolute, the other with about 90 % alcohol.

Table IV.

Solvent	Absolute alcohol		90 % alcohol	
	I found (γ)	% of total I	I found (γ)	% of Total I
First extraction	4.4	43	8.4	81
Second "	2.0	19	1.4	14
Third "	3.2*	31	0.9	9
Fourth "	0.9	9	0.2	2
Total	10.5		10.9	

(Total I content, including K_2CO_3 blank, 10.3 γ .)

* Sufficient water was added here to make the carbonate layer a liquid paste.

The figures (Table IV) clearly show that the extraction with the more dilute alcohol is very much more rapid and complete than that with the absolute, in accord with the findings of Scheffer [1930], and, in practice, the rather liquid residue is more easy to deal with than the sticky solid obtained with the absolute alcohol. The only disadvantage is that rather more alkali is taken up by the weaker alcohol. In subsequent work, absolute and 90 % alcohol were used as required to keep the carbonate pasty, without becoming either granular or liquid.

To avoid the necessity of filtering and carbonating the aqueous extract of the ash, as described in the colorimetric section, it seemed desirable to try ashing the blood with carbonate instead of potassium hydroxide, and extracting the ash after ignition for 1 hour at 500° directly with four quantities of 3 cc. alcohol. The alcoholic extracts were then evaporated to dryness in the water-bath in 5 cm. nickel basins, and the dry residue again extracted with alcohol, 1 cc. followed by three amounts of 0.5 cc. being used. The extracts were poured into the little glass vessels, evaporated to dryness on the sand-bath, heated a further 90 secs. and acidified and oxidised with bromine as before.

The results of experiments on mixtures of potassium carbonate and iodide (without blood) showed that the whole process, including the two separate alcoholic extractions could be carried out satisfactorily, the recoveries in four successive experiments with 2.5 γ iodine as KI being 2.46, 2.46, 2.76, 2.66 γ , and potassium iodide added to blood could also be recovered fairly satisfactorily, though results were sometimes a little low. It was found that when the blood was not carefully burned, and a large amount of carbon residue was left, direct alcohol extraction failed to remove all the iodine, though that missing could be recovered by re-ashing the residue and again extracting, or better by a first aqueous extraction. Unfortunately, when the whole procedure was carried out, using carbonate as the alkali for ashing, and thyroxine or *p*-iodobenzoic acid were added to blood, the recoveries were usually low, values of the order of 85–95 % of the added iodine being recovered. When the work had reached this stage further experiments had, unfortunately, to be abandoned. It would therefore seem that under these conditions, potassium carbonate is not entirely satisfactory as an alkali for ashing the blood, though further work might enable suitable conditions to be worked out.

It is suggested that a suitable method would probably be found by ashing the blood with potassium hydroxide as described in the colorimetric method, filtering and carbonating the aqueous extract of the ash, and then working up the dried carbonate containing the iodine by two alcoholic extractions and bromine oxidation as described above.

Our thanks are due to Prof. F. R. Fraser for the interest he has shown throughout.

One of us (E. N. A.) is indebted to the Medical Research Council for a part-time grant, and one (J. A. D.) to the University of Toronto for an Ellen Mickle Fellowship.

REFERENCES.

- Aitken (1930). *Biochem. J.* **24**, 1456.
von Fellenberg (1923). *Biochem. Z.* **139**, 391.
Höjer (1928). *Biochem. Z.* **205**, 273.
Reith (1929). *Biochem. Z.* **216**, 249.
Scheffer (1930). *Biochem. Z.* **228**, 426.
Veil and Sturm (1925). *Deutsch. Arch. klin. Med.* **147**, 166.

(The above papers contain fairly complete references to the extensive literature of the subject.)