CXXVII. METHOD OF ESTIMATING MINUTE QUANTITIES OF IODINE IN BIOLOGICAL MATERIAL.

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THE importance of iodine to the animal organism is becoming increasingly apparent, and at the outset of an investigation into this question we found it necessary to review the existing methods for estimating minute quantities of iodine in organic substances, primarily with regard to thyroid gland studies and also with a view to ascertaining if possible the iodine content of pastures and other foodstuffs.

A number of methods have been described for such estimations, and a survey of the literature on the subject reveals many figures for foodstuffs and like material which appear to us to need some investigation. For 100 g. of dried material some methods claim an accuracy of 0.03 mg. %, others 0.005 mg. % and figures even much smaller than these are quoted.

A short discussion of each of the methods investigated by us will be made, finishing with a description of the Kendall method which in its broad outlines we have adopted.

Hunter's method [1910]. The organic matter is fused with a mixture consisting of potassium carbonate, sodium carbonate and potassium nitrate. The sodium iodide thus fixed is converted to iodic acid by oxidation with sodium hypochlorite, and, after boiling off the excess of chlorine, the iodine is liberated from the iodic acid by means of an excess of potassium iodide, according to the equation

and titrated with a solution of approximately 0.005 *N* sodium thiosulphate. The outstanding advantage of this method is the titration of six times the amount of "original" iodine.

In analysing samples of seaweed, however, we found that considerable loss of iodine took place during the fusion process and further, owing to the formation of oxy-chlorine compounds [Kendall, 1912], which subsequently interfered with the titration, the results obtained were never reliable.

Hanzlik's method [1910]. This method consists in (a) desiccation in sodium hydroxide, (b) fusion and oxidation with sodium nitrate and sodium carbonate, (c) acidification of the extracted melt with sulphuric acid and the extraction

of the free iodine with chloroform. The chloroform is separated from the rest of the solution, washed, and the iodine titrated direct with $N/10$ sodium thiosulphate until the chloroform becomes colourless.

This method has been used by us with some success where the quantity of iodine present is fairly large, e.g. 0.1 g., but for amounts less than this we find it difficult to achieve complete extraction of the iodine by the chloroform.

During neutralisation with sulphuric acid free iodine is very liable to escape, and to obviate this risk we found it advisable, by means of a simple contrivance, to pass the gases evolved during neutralisation through chloroform which was subsequently used for extracting the rest of the iodine present.

The washing of the chloroform completely free from acid was also found difficult, and considerable loss of iodine may occur here.

When minute quantities of iodine are present, it is almost impossible to see the iodine colour in the chloroform and the titration becomes increasingly difficult.

Krauss' method [1915]. This is a colorimetric method based on the fact that palladous chloride precipitates iodine from dilute solutions as palladous iodide. This gives a deep brown coloration which can be compared colorimetrically with standards of known strength. We were unable to get satisfactory results with this method, largely on account of the difficulties experienced in completely extracting the iodine with the acetone-alcohol mixture and in differentiating between the colorations given when minute quantities of iodine were present.

Some of the earlier methods used are reviewed by Cameron [1914], notably those of Baumann, and Bourcet. The latter employed large quantities of material, and his method seems impracticable for general application where rapidity and convenience combined with accuracy are aimed at.

Kendall's method [1912, 1914, 1920]. The method which (with slight modifications) was found by us to be the most satisfactory is that of Kendall, and our results place its absolute limit of accuracy at 0.0001 g. %. The maximum amount of material which can conveniently be used is 5 g. The minimum amount of $N/200$ sodium thiosulphate with which a change of colour can be observed is 0.05 cc. which is equivalent to 0.0000317 g. iodine (i.e. 0.0000053 g. of "original" iodine). Should an excess of one drop be used in the titration, which is within the reasonable limits of experimental error, then it is obvious that the error in the percentage is materially increased, if such a minute quantity is present in the material to be analysed. The colour given by starch when such small quantities of iodine are present is also exceedingly faint, and we feel that figures quoted even below 0.0002 % are unreliable.

As has been stated, many workers claim by their methods an accuracy far more delicate than this, and one finds in the literature figures for foodstuffs with as low an iodine content as 0.0000005 % [Fellenberg, 1923].

We are in complete agreement with the findings of Bohn [1916], who, it appears, experienced the same difficulties as ourselves and whose results are

tabulated like those of Forbes [1916], viz. in a qualitative rather than a quantitative manner.

We have adopted the Kendall method in its broad outlines, but would make the following suggestions.

(1) We found it unnecessary to control the acidity during the boiling with bromine. Our experience has been that when neutralising the dissolved fusion melt, an excess of 3 cc. of syrupy phosphoric acid gives the necessary acidity for boiling off the nitrous acid. No further addition of phosphoric acid is needed before final titration. (2) In the initial fusion of pastures a considerable quantity of potassium nitrate is required completely to oxidise the large amount of carbonaceous matter (5 g. samples taken).

On acidification with phosphoric acid, nitrous acid is evolved in fair quantity owing to the reduction of the nitrate during fusion. The final titration would be impossible were this not boiled off, and an excess of 3 cc. of phosphoric acid is found sufficient for this purpose.

The pink colour of the just acid solution (methyl orange as indicator) will persist for a few moments, but is quickly destroyed by the liberation of the nitrous acid on the addition of 3 cc. excess of phosphoric acid.

The solution is now boiled for 15–20 minutes and disappearance of the last traces of nitrous acid may be watched by means of potassium iodide starch paper. We find that 2 cc. of sodium bisulphite added at this point is sufficient to reduce any oxidising agents which would interfere with the iodide-iodate reaction, and the solution is reboiled for 10 minutes or so to expel the excess of sulphur dioxide.

(3) To prevent bumping during boiling, small pieces of unglazed porcelain, obtained from broken triangles were found by us to be as convenient and satisfactory an agent as any. This should be removed before the titration is carried out and a long glass rod with a flattened end serves for this purpose.

(4) The method adopted by us for adding the potassium iodide is to dissolve a small crystal (0.25 g.) in about 20 cc. of water in a small beaker and add this immediately to the solution to be titrated. This is more satisfactory than adding a crystal direct as advised by Kendall, which causes a pink coloration with starch instead of a blue.

(5) In certain cases, notably pastures, owing to the large amount of sodium hydroxide used in the fusion process and the quantity of acid needed to neutralise this, the resulting solution becomes rather concentrated. This factor to some extent controls the speed of the iodide-iodate reaction which is sometimes slow. Five minutes are adequate to ensure the complete liberation of the iodine before the end point is finally determined. Forbes [1916] recommends the use of a little sodium chloride to accelerate the reaction and no doubt this is an advantage. The solution should remain clear for at least half an hour after titration, no blue colour reappearing.

Kendall's method as used by us is as follows:

Outline of Method.

(1) Fusion with sodium hydroxide and destruction of organic matter by potassium nitrate, with fixation of iodine as sodium iodide.

(2) Oxidation of iodine to iodic acid by bromine

(3) Liberation of iodine from iodic acid by potassium iodide, and titration with sodium thiosulphate, using starch as indicator.

Reagents Required.

Sodium thiosulphate <i>N</i> /200 solution	Salicylic acid (pure)
Potassium bi-iodate standard solution	Potassium iodide crystals
Sodium bisulphite 20 % solution	Phosphoric acid (sp. gr. 1.75)
Soluble starch 1 % solution	Bromine

Details of Method.

Fusion. In the case of thyroid glands about 0.5 g. of fresh material and for pastures 5 g. previously dried and ground are taken.

The sample is accurately weighed into a nickel crucible of 5.9 c. in diameter, covered with a convenient amount of 40 % sodium hydroxide solution (10 cc. for thyroids, 35 cc. for pastures) and heated in an oven for about half an hour.

The nickel crucible is then placed in another of slightly larger capacity half filled with sand, and the whole is placed in a crucible furnace and heated gently over a moderate flame.

It will be found that the substance saponifies and froths considerably, and gentle stirring with a nickel rod is necessary to keep the melt from creeping over the sides of the crucible. In about 10 minutes the frothing ceases, most of the carbonaceous matter becomes burned off and the melt settles to the bottom.

At this point about 5 g. of solid sodium hydroxide (powdered or in flake) are added and the melt becomes liquid and more easily worked. Small quantities of potassium nitrate are added repeatedly until all the carbon is completely oxidised and further additions cause no bubbles or frothing. The crucible is removed from the sand-bath and allowed to cool. When cool it is placed in a beaker of 500 cc. capacity and the melt extracted with boiling distilled water.

In some cases a certain amount of insoluble residue is inevitable and the solution, which should amount to about 400 cc., is transferred to a conical flask of 700 cc. capacity, filtering if necessary. The cooled solution is neutralised with syrupy phosphoric acid, run in carefully from a burette using methyl orange as an indicator. From 2–3 cc. excess of acid should be used.

The solution is boiled for about 15–20 minutes to get rid of the liberated nitrous acid. 2 cc. of 20 % sodium bisulphite are added and the solution boiled for about 10 minutes to expel the excess of sulphur dioxide, fragments of broken triangles being added to prevent bumping.

Oxidation to iodic acid. On cooling, from 5–10 drops of bromine are added, and the flask and contents well shaken until the bromine imparts a distinctly brown colour to the solution. Care should be taken not to let the volume get too low during the previous boilings.

The solution is again boiled for about 15 minutes to expel the excess of bromine. Great care must be taken to get rid of the last traces of bromine, and to ensure this a pinch of salicylic acid is added before the solution is cooled down, previous to titration. The unglazed porcelain is removed from the flask and the solution cooled.

Titration. The potassium iodide is added in the manner already described. The liberated iodine is then titrated with a solution of approximately $N/200$ sodium thiosulphate, using a 1 % solution of soluble starch as indicator.

$$\frac{\text{Thiosulphate used} \times \text{iodine equivalent of thiosulphate solution}}{6}$$

= total iodine in the amount of substance taken for estimation.

The strength of the thiosulphate solution alters from day to day and its iodine equivalent must be determined before each set of titrations. This is conveniently done by titrating it against a solution of potassium bi-iodate (KIO_3HIO_3) which is a stable solution, the iodine equivalent of which has previously been determined [Kendall, 1920].

The accuracy of the Kendall method is illustrated by the following figures. To 5 g. of a dried pasture in which the complete absence of even a trace of iodine had previously been determined, definite amounts of iodine were added.

Estimations were then carried out and the iodine recovered is given in column 2.

g. Iodine added as KI to 5 g. of pasture	Iodine recovered, g.
·00005	·000049
·0001	·000106
·0002	·000200
·0003	·000302

Amounts of iodine as shown were added to 5 g. samples of another pasture taken from land which had been manured with seaweed. More iodine was recovered than was added and it is concluded that this pasture contains about 0·001 % of iodine.

Iodine added, g.	Iodine recovered, g.	Iodine in 5 g. sample of dried grass, g.
·00005	·000098	·000048
·0001	·00015	·00005
·0002	·000248	·000048

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