CXVIII. A METHOD FOR THE ESTIMATION OF IODINE IN THYROID GLAND.

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AN examination of the published methods for the estimation of iodine in tissues has shown certain objections to their use for the special purpose of determining the iodine in thyroid gland.

Iodine is very reactive and is volatile so that its estimation is not easy. Many methods for its estimation have been put forward, the most important of which are briefly criticised below.

In comparing them however with the method to be given later, attention is drawn to the desirability of avoiding all processes involving unknown factors, and those subject to a large personal equation such as the colorimetric.

Baumann's method and its large number of modifications consist in the fusion of the gland with sodium hydroxide and potassium nitrate, followed by acidification and the extraction and estimation of the liberated iodine. The objection to all modifications of this method is that the effervescence which occurs on acidification causes an indefinite loss of iodine.

Hunter's method destroys the organic compounds by heating with a mixture of potassium and sodium carbonates and nitrates; the dissolved melt is treated while still alkaline with sodium hypochlorite; the solution is acidified and boiled to expel excess of chlorine, potassium iodide added and the iodine titrated with thiosulphate. The objections are the possible formation of oxychlorine compounds (Kendall) which would invalidate the result; and also the fact that the fusion process is inadequate with organic iodine compounds such as are met with in thyroid. Kelly and Husband also find that the process gives low results with seaweed.

Kelly and Husband's [1924] modification of Kendall's process was published while the present work was in progress. It consists of alkali fusion with sodium hydroxide and completion of the oxidation with potassium nitrate as in the Baumann method; acidification with phosphoric acid, and prolonged boiling to get rid of the nitrous acid. Sulphite is added and the SO_2 boiled off and then bromine is added, excess of which is also got rid of by prolonged boiling. Potassium iodide is then added and the liberated iodine is titrated.

One objection, as in the Baumann method, is the possible loss of iodine in the neutralisation of the mixture with acid, which liberates nitrous acid and much carbon dioxide.

Another objection is the fact, easily demonstrable in a test-tube, that bromides liberate iodine from potassium iodate in the presence of acid; an indefinite quantity of bromide is necessarily formed in the process of oxidation by the bromine.

The prolonged boiling and bromine vapour also present certain practical objections.

In Fellenberg's process [1924] the fusion process differs from the foregoing in that no potassium nitrate is added; but iodine is estimated colorimetrically. It is not intended by this author to be exactly quantitative since the results are multiplied by $\frac{5}{4}$ to allow for loss. It is a good method for the detection of minute amounts of iodine.

The method put forward depends upon the destruction of organic matter by fusion with alkali and oxidation of the resulting iodide to iodate with permanganate solution; excess of the latter is removed with animal charcoal, and after adding iodide the liberated iodine is titrated with thiosulphate.

The complete process is as follows:

0.25 g. of finely powdered dry thyroid⁽¹⁾ is weighed and introduced into a nickel crucible of 150 cc. capacity; 10 cc. 50 % sodium hydroxide⁽²⁾ is added and mixed by rotating the crucible; it is covered with a lid having a hole $\frac{1}{8}$ " in the centre and heated slowly till the water is evaporated; it is next heated in an oven considerably below red heat but hot enough to give a clear fusion product in about an hour⁽³⁾; the crucible is next heated over a large free flame protected so that the crucible and the lid can be rapidly raised to a dull red heat, which is maintained $\frac{1}{2}$ —1 minute⁽⁴⁾.

Allow the crucible to cool, add about 70 cc. water and place on a warm plate for about an hour, when gentle stirring will bring the whole into solution⁽⁵⁾; transfer to a 250 cc. Erlenmeyer flask, add three drops 10 % sodium sulphite solution⁽⁶⁾; acidify with 50 % sulphuric acid⁽⁷⁾ from a burette, about 13 cc. being required (test acidity with a narrow strip of litmus paper mounted on a holder); add 3 cc. in excess, cool and add 5 cc. approx. *N*/10 permanganate; after 3 minutes add a suspension of charcoal⁽⁸⁾; when decolorisation is complete filter through a suction filter⁽⁹⁾ washing with a little water, or through a pleated filter paper⁽¹⁰⁾; add a crystal of potassium iodide and titrate immediately with freshly prepared *N*/100 thiosulphate, adding starch only towards the end of the titration.

Each 1 cc. thiosulphate $\times 85$ gives the milligrams of iodine in 100 g. of dried gland when 0.25 g. is taken for the analysis. It is found convenient to work with batches of six thyroids.

NOTES ON THE METHOD.

(1) The thyroid should pass a 60 sieve.

(2) The soda should be free from nitrate and from chloride, the commercial "Soda by alcohol" answers well.

(3) Potassium nitrate must not be added.

(4) Prolonged heating at red heat causes a slight loss.

(5) A few carbon particles remain which do not affect the result. See Exp. 7 below in which a small amount of charcoal is added at the initial stage.

(6) This does not apparently affect the result but is added as a precaution against the possibility of iodine being liberated at this stage by traces of impurity such as iron.

(7) The sulphuric acid must be chemically pure.

(8) Shake 20 g. animal charcoal with 1000 cc. water and 2 cc. sulphuric acid. The British Drug Houses "Animal charcoal purified by acid" is satisfactory. Use a pipette with a very wide jet for measuring rapidly before sedimentation takes place. The amount required is determined by that which will reduce 5 cc. acidified $N/10$ permanganate in not less than 5 or more than 20 minutes: this is usually 6 cc.

(9) The filtrate must be inspected very carefully to see that it is absolutely free from colour and from minute particles of carbon.

(10) If a pleated filter is used it is found more convenient, instead of washing, to weigh against another paper as a counterpoise and make the small correction for the amount of fluid on the paper and in the flask, e.g. if the weight on the paper is 3 g. and that in the flask 0.5 g. the corrected figure will be 113.5/110 with an amount of fluid of 110 cc.

Suitable suction filters are the Jena Sintered Glass 17G 4/< 7-filters.

The permanganate may be added to the alkaline solution instead of after acidification if desired, in which case addition of sulphite is unnecessary.

Iron should be removed from the solution if the thyroids contain a large excess of blood by adding to the solution after acidification enough soda to make just alkaline and after standing some hours filtering off the precipitated iron, acidifying, adding 3 cc. of 50 % acid and proceeding as above.

Test experiments with experimental mixtures.

Solutions used. Potassium iodide $N/200$. Thiosulphate $N/100$ made fresh each day by diluting 10 cc. of $N/10$ to 100 cc. The $N/10$ thiosulphate was preserved with a crystal of thymol and 25 cc. were equivalent to 0.1226 g. $K_2Cr_2O_7$.

In tests 1–11, 1 cc. of $N/200$ KI was measured by means of a 1 cc. D and R pipette the standardisation of which was checked by weighing. The theoretical titration figure in these experiments (1–11) was therefore 3.00 cc.

Exps. 1–9 consisted of the latter half of the process; i.e. without addition of alkali and the fusion process. Variations in amount of reagents and other factors were introduced into the test processes below to show the flexibility of the process in allowing such variation:

(1) *The amount of acid* (instead of 3 cc.) was varied as follows:

Acid (cc.)	Titre
0.5	2.60
1.0	2.86
2.0	3.02
4.0	2.99
5.0	3.03
Blank 12.0	0.00

The amount of acid must therefore be at least 2 cc.

(2) *The amount of permanganate* (instead of 5 cc.) was varied as follows:

KMnO ₄ (cc.)	Titre
0.5	2.93
1.0	3.02
2.0	2.97
5.0	3.01
10.0	2.97

The amount may vary considerably.

(3) *Temperature of action of KMnO₄:*

	Titre
Ordinary	3.01
Hot 60–70°	2.95
Hot 60–70°	2.97

The temperature is not important.

(4) *Time of action of KMnO_4 :*

	Titre
Immediate	2.99
1 min.	3.02
2 mins.	3.04
5 "	3.07
90 "	3.07
90 "	3.06

The permanganate oxidises the iodide immediately.

(5) *The amount of charcoal (instead of 6 cc.) was varied as follows:*

cc.	Titre
5	3.05
7	3.02
9	3.00

The amount of charcoal may vary within reasonable limits.

(6) *The amount of iodine:*

cc.	Titre
1	3.01
2	5.97

Also see No. 10.

(7) *Charcoal (0.5 cc. suspension) present at start:*

Titre 3.03

A small quantity of carbon present in the process will not affect the result.

(8) *Time in contact with the charcoal after the decoloration is completed:*

mins.	Titre
30	2.97
120	2.98

The charcoal does not reduce the iodate.

(9) *Effect of salts:*

	Titre
10 cc. NaOH neutralised, 2.5 cc. excess acid	3.01
10 cc. NaOH neutralised, 2.5 cc. excess acid (blank)	0.00
10 cc. NaOH neutralised after previously adding 5 drops of 10 % sulphite...	3.02

The salts present in the actual experiment do not affect the result.

(10) The following were experimental mixtures of the iodide solution and 0.25 g. of meat mixed and treated by the complete process as above:

cc.	Titre
1	2.96
2	5.93

The amount of iodine may vary widely.

(11) *Temperature and amount of heating varied:*

30 mins. slow heating till clear, then as follows:		Titre
1.	Melt, not red heat	2.90
2.	Red hot 1 min., quick heating	3.00
3.	Red hot 3 mins.	2.98
4.	Red hot 10 mins.	2.91
9.	Blank with starch	0.01
10.	Blank with meat	0.04

The amount of heating is important. Also see Exp. 12.

(12) *Thyroid gland*:

	Titre
Just red hot	1.65
Red hot 1 min.	1.62
Red hot 5 mins.	1.62
Red hot 10 mins.	1.61
Red hot 30 mins.	1.61
Well heated but always under red heat	1.64

There is a slight loss by prolonged heating at red heat.

The above show that there is considerable liberty allowed in the amount of reagents and other factors of the method.

Duplicate experiments give results which are correct to one drop of titrating solution. All duplicate results were obtained at different times.

Over 100 human thyroid glands have been analysed by the method and results obtained ranging from 5-520 mg. per 100 g. of dried tissue; and 0.4-52.5 mg. per whole gland obtained, details of which are being published in the *Journal of Mental Science*.

The above work was carried out in the laboratories of the Joint Board of Research for Mental Diseases, City and University of Birmingham.

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