CXXIII. THE ESTIMATION OF IODINE IN FOODSTUFFS AND BODY FLUIDS.

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THE study of iodine metabolism has been retarded by the technical difficulty of estimating accurately the small amounts of iodine which occur in foodstuffs and in tissues and body fluids.

Kendall's method [1914, 1920], which is that most widely used at the present time, is admirably suited for the analysis of thyroid tissue, which contains roughly from 0.01 g. % to 0.5 g. % of iodine, and, as modified by Kelly and Husband [1924], it is in routine use at this Institute for that purpose. The method has, however, a lower limit of detectable iodine of 5 γ ($\gamma = 0.000001$ g.). For the estimation of iodine in human blood, therefore, Kendall required 100 cc., since the content is only 13 γ %. The method is accordingly of little use for routine clinical purposes. The iodine content of milk is still lower than that of blood, and Forbes [1916], using Kendall's method, failed to detect iodine in any of 18 samples of milk.

Taking up the problem at the suggestion of the Swiss Goitre Commission, Fellenberg [1923, 1924] described a more delicate method, of which the best account is given by Veil and Sturm [1925] who have applied it to clinical work on blood. This method has a lower limit of detectable iodine of 0.1 γ . Thus the iodine content of milk can be conveniently estimated in 10 cc., and that of blood in even less.

Fellenberg's method involves both a colorimetric and a titrimetric estimation. The material is ashed with potassium hydroxide and the ash extracted with alcohol. From this extract the iodine is liberated by sulphuric acid plus potassium nitrite. The liberated iodine is taken up in a drop of chloroform and estimated colorimetrically. Thereafter the iodine is oxidised to iodate by chlorine water and the solution evaporated to about 2 cc. The "iodide plus iodate" reaction is used as in Kendall's method and the liberated iodine titrated with $N/500$ thiosulphate, using starch as indicator.

With regard to the relative accuracy of the two estimations, it is claimed that the colorimetric is more accurate at the lowest levels. But the amount estimated by titration is six times the amount originally present and estimated colorimetrically. Hence, to be less accurate than the colorimetric estimation, the error in titration would have to be more than six times as great as the error

in the colorimetric estimation. This did not appear at all likely, and therefore, since the colorimetric estimation involves additional apparatus and a great deal more work, we decided to investigate the accuracy of the titrimetric estimation at the lowest levels, with a view to the possible elimination of the colorimetric part of the method.

We have made the following alterations in the procedure described by Veil and Sturm.

(1) Nickel crucibles are used instead of iron in view of the catalytic effect of iron and the consequent risk of loss of iodine in ashing.

(2) The colorimetric estimation is omitted: the residue after evaporation of the alcoholic extract is glowd and transferred at once to a 50 cc. flask for titrimetric estimation.

(3) Since the titrimetric estimation does not require the liberation of iodine, potassium nitrite is omitted. 2*N* sulphuric acid is used for acidification. We consider that the end-point is better with sulphuric than with other acids tried, *e.g.* hydrochloric or phosphoric.

(4) We use bromine water in place of chlorine water used by Fellenberg and Veil and Sturm. It is much more easily prepared and there is no difficulty in removing the last traces by boiling. We found the addition of salicylic acid, as recommended by Kendall, unnecessary.

As regards the delicacy of the titration, we found it possible to detect the difference produced by 0.002 cc. *N*/500 thiosulphate. This corresponds to 0.08 γ actual iodine.

Fig. 1. Showing degree of accuracy of titration.

Accuracy of titration. The accompanying graph illustrates the relationship between actual and estimated iodine (after oxidation with bromine, etc.) when quantities varying from 0.125 to 2.5 γ are used. With quantities under 0.5 γ the error may be as much as 15 % of the total. It is less than 10 % between 0.5 γ and 1 γ , and above that it is negligible. We find that more than half of these titration errors with quantities below 1 γ are on the high side. This is due to the fact that an over-titration by as little as 0.001 (which does not give a detectable difference) means a positive error of 8 % at 0.5 γ . This error is, however, of little or no practical significance.

Recovery of iodine. To test the reliability of the method as a whole, we estimated known quantities of iodine added, as potassium iodide, to starch and to milk. Blanks of starch paste gave no iodine coloration when well ashed. A slight pink coloration occurred in two cases where the ashing was not good. Some representative figures for the recovery of added iodine are given in the following table:

Table I.

Substance used	Iodine present γ	Iodine added γ	Iodine estimated γ	Difference γ
1 g. starch	0	0.5	0.57	+0.07
1 "	0	0.5	0.53	+0.03
0.5 "	0	0.57	0.44	-0.13
1 "	0	1.0	0.9	-0.1
1 "	0	1.0	1.0	0
1 "	0	2.0	2.2	+0.2
1 "	0	2.0	2.3	+0.3
10 cc. goat's milk	1.2	0.5	1.9	+0.2
10 "	1.2	0.5	1.7	0
10 cc. cow's milk	1.0	1.0	1.9	-0.1
10 "	1.0	1.0	1.9	-0.1
10 "	1.0	1.0	1.8	-0.2
10 cc. colostrum	0.5	1.0	1.25	-0.25

These figures indicate that the error in the method as a whole is about ± 10 % when the iodine is present in such minute quantities as 0.5 γ to 2 γ . Veil and Sturm [1925] state that there is an error of 10–15 % in the colorimetric method, and therefore, since the error in the titrimetric estimation is certainly not greater than this, even at the lowest levels, we have decided that we are justified in eliminating the colorimetric, and confining ourselves to the titrimetric estimation. The advantages gained are the saving of apparatus and time, and a very much simpler technique.

We have used the method with satisfactory results for work on blood, milk and pasture grasses. Papers on these subjects will follow.

METHOD.

The method as we use it is as follows.

Reagents and apparatus required.

- (1) Potassium hydroxide sticks: iodine-free.
- (2) Alcohol for extraction: 95 cc. absolute alcohol, 5 cc. distilled water.
- (3) Methyl orange: 0.05 % *watery* solution.

- (4) Sulphuric acid: approximately $2N$.
- (5) Bromine water: to be prepared as required.
- (6) Sodium thiosulphate: approximately $N/500$. This solution should be made up periodically from $N/10$ stock solution. $N/500$ thiosulphate does not keep well and must be standardised daily by means of potassium bi-iodate as described by Kendall [1920].
- (7) A solution of potassium iodide made up just before the final titration. A small crystal is dissolved in about 20 cc. of water.
- (8) Starch solution: to 50 cc. distilled water add a pinch of soluble starch. Boil for 1 to 2 minutes and cool. A fresh solution should be prepared every third day.
- (9) Small chips of pumice stone, about the size of rice grains. These chips, after removing dust through a fine sieve, are boiled in dilute nitric acid, washed with distilled water, dried, and then strongly heated in the crucible furnace. After the estimation they are recovered. From time to time they are retreated and used over again.
- (10) Davis combined crucible furnaces with Teclu burners. Vitreosil basins for the sand are most suitable.
- (11) Nickel crucibles (6 cm. and 5 cm. in diameter). Nickel stirring rods.
- (12) Flasks (50 cc. capacity).
- (13) A serum pipette, 0.1 cc. capacity graduated in thousandths of a cc.

Procedure.

Ashing. A suitable quantity of the substance to be analysed is measured into a nickel crucible (6 cm. diam.), and 1 g. of potassium hydroxide stick added, dissolved in water in the case of dry substances. The contents of the crucible are stirred and then heated gently over a Bunsen burner till all bubbling ceases, and thereafter more strongly till no more fumes come off. Heating is continued on the furnace to a dark grey ash. The crucible must not be allowed to glow. When cool the ash is moistened with distilled water and carefully heated over a low flame till dry. The crucible is then returned to the crucible furnace and heated till no further change appears to be taking place (say 10 minutes). After cooling, about 5 cc. distilled water are added and the contents of the crucible filtered through a No. 40 Whatman ash-free filter-paper. (In all pouring operations it is necessary to vaselin the lip of the crucible with iodine-free vaselin.) The filter-paper is washed three times with about 5 cc. water. The filtrate, collected in a small beaker, is set aside. It should be clear, or at most, only very faintly yellow.

The filter-paper with its charred contents is returned to the nickel crucible, dried and then heated in the crucible furnace till a clean ash is obtained. The crucible is cooled, the filtrate returned to it and evaporated to dryness. This operation requires the greatest caution towards its completion, since spurting and crackling may cause loss. The crucible is again heated in the furnace for a minute or so, then cooled and about 3 cc. of water are added. This thick

solution is gently evaporated over a low Bunsen flame until a skin begins to form on the surface. Then the contents, after cooling, are ready for extraction.

Extraction. The ash is extracted three times with 3 cc. of the alcohol solution, the extract being filtered through a No. 40 Whatman paper into a 5 cm. crucible. The ash should form a smooth paste. If it is gritty one or two drops of water should be added, but excess should be avoided. After the third extraction the paste is poured on to the filter and the last of the alcohol allowed to drain. The filter paper is rejected, the funnel washed with a little distilled water and the filtrate evaporated to dryness on the water-bath.

The thin film of salt on the bottom of the crucible should be practically free from carbon. To get rid of any small particles which may be present, the bottom of the crucible is glowed gently for a few seconds, the salt film being just allowed to melt.

When the crucible has cooled the contents are dissolved in distilled water, and transferred to a 50 cc. flask.

Titration. Two drops of the methyl orange solution are added. The solution is rendered neutral by the addition, drop by drop, of 2*N* sulphuric acid. One drop is added after the neutral point. In all about three drops are generally required. Thereafter a few drops of bromine water are added. The red colour is immediately discharged. About 1 cc. bromine water should be added in excess of this, until the solution is distinctly yellow.

Three or four pieces of pumice stone are added and the solution is boiled over a medium flame, until the volume is reduced to between 1 and 2 cc., and then cooled. Two drops of the potassium iodide solution and two drops of the starch indicator are added and the titration carried out with *N*/500 thio-sulphate from the 0.1 cc. pipette. By holding the flask at an angle it is possible by tapping the bottom of the flask with the point of the pipette to run in as little as 0.001 cc. at a time. If due precautions have been taken, the end-point is perfectly definite and after a little practice may be determined to about 0.002 cc.

Calculation: $\frac{\text{Titration figure} \times \text{iodine equivalent of thiosulphate}}{6}$ gives the amount of iodine in the sample.

SUMMARY.

Fellenberg's method of estimating iodine is modified by the elimination of the colorimetric part and concentration on the titrimetric estimation. In this form the method is much simpler and has been found suitable for routine work on blood, milk and pastures.

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