CLIX. AN IMPROVED METHOD FOR THE DETERMINATION OF IODINE IN BLOOD.

BY HARRY ARTHUR ASHWELL AITKEN.

From the Medical School, Otago University, New Zealand.

(Received August 28th, 1930.)

SINCE the development by Fellenberg [1923] of Chatin's method for the detection of minute amounts of iodine, numerous further improvements in the technique of micro-analysis for iodine have been introduced. In particular, a titrimetric estimation based on the iodide-iodate reaction of Winkler [1915] has been found to have advantages over Fellenberg's colorimetric procedure, and a method on this basis devised by Leitch and Henderson [1926] has proved to be highly convenient for all ordinary purposes. It is noteworthy, however, that while the colorimetric method has been pushed to its limit of sensitivity, the iodine in the sample being finally extracted with one drop of chloroform, users of the titrimetric method have been content to carry out the titration in a final volume of 1 or 2 cc. While this is in general satisfactory it has the result, where materials of low iodine content such as blood are being examined, that the liberated iodine is in too low a concentration for the starch-iodide indicator to yield a sharp end-point. In consequence, the error involved in the case of bloods is $\pm 10\%$ [Leitch and Henderson, 1926] being much the same as that in the colorimetric method. The urgent need of greater accuracy than this for the investigation of problems in iodine metabolism has led the author to devise a further modification of the titrimetric method in which the titration is performed in a volume of about 0.2 cc. without loss of either accuracy or convenience. The increased concentration ensures a deep blue colour with starch-iodide and a definite end-point. As a result the error involved is about $\pm 1\%$.

Titration vessels.

In the procedure of Leitch and Henderson the potassium iodide extracted by alcohol from the combustion residue is transferred in aqueous solution to a 50 cc. Erlenmeyer flask, acidified, oxidised with bromine water and concentrated to a volume of 1 to 2 cc. Since lower volumes than this cannot be conveniently attained in such flasks, the author has devised titration vessels in the form of short tapering tubes (Fig. 1, actual size) in which the solution can be brought with ease to a volume of 0.2 cc. The tubes can be readily made by cutting off the upper cylindrical portion of centrifuge test-tubes. Regular boiling is ensured either by standing in the liquid a length of capillary tubing

in which the capillary is closed by a constriction about 0.5 cm. from the lower end, or, more conveniently, by sealing a short platinum wire into the tube as shown in Fig. 1.

The tubes provided with platinum wires were tested as follows. 2 cc. of a solution of potassium iodide containing about 0.5 γ of iodine per cc. were added to each of two tubes and 2 cc. of water to a third. The contents of each tube were acidified with three drops of 1 % sulphuric acid and brought to the boil, whereupon three drops of saturated bromine water were added. The boiling was continued until the tubes contained about 0.2 cc. They were then cooled and a minute crystal of potassium iodide was added to each, followed by a small drop of starch solution. No colour developed in the control tube, but a strong blue appeared in the others, each of which on titration required 0.0485 cc. *N*/1000 thiosulphate solution. In an estimation on the larger scale with ten times as much (20 cc.) of the same solution in an Erlenmeyer flask the titre was 0.485 cc. The accuracy of the small scale method is thus established.

Fig. 1.

The titration is performed with a 0.1 cc. pipette which has been drawn off to a fine tip. This has the additional advantage of making it slower in emptying and more readily controlled. The author uses a pipette in which divisions representing 0.01 cc. are 2 cm. apart. A short graduated sleeve made from glass tubing and provided with a simple clip to hold it any desired position enables quantities of 0.0005 cc. to be readily measured. In titrating, the pipette is kept in the liquid and used to stir it during each addition of the standard solution. It is rarely necessary to refill the pipette during a titration, though this can be done if required from a small tube to which 0.1 cc. of standard solution has been added. The error due to the trace of solution removed on the tip of the pipette then becomes negligible.

Since certain steps in the earlier processes of combustion and extraction are important when the final volume for titration is going to be small, the whole course of the analysis is now described.

Method of analysis.

5 cc. of blood are transferred to a 6 cm. nickel crucible and 4 cc. of 50 % potassium hydroxide are added. The contents of the crucible are evaporated to dryness on a sand-bath and then heated below red heat at about 500°. This is best achieved by placing the crucible inside a slightly larger one containing a few chips of pipe-clay to prevent actual contact; the larger crucible, which can be conveniently held in an iron ring, is heated by a large non-luminous flame. A trial with the inner crucible empty and shaded from direct light will show how strong a flame can be used without bringing the inner crucible to a glow.

When all fuming has ceased (about 7 minutes) the crucible is allowed to

cool and the residue is thoroughly crushed with a nickel spatula, taken up with water and again evaporated to dryness on the sand-bath. After further combustion for about 5 minutes, the residue is taken up with water and filtered hot into a beaker, a fast pipette (such as can be made from a discarded 50 cc. pipette) being used to transfer the liquid from the crucible to the filter. The pipette is rinsed into the empty crucible and this liquid is used to wash the precipitate. The precipitate is washed three times with hot water. The filtrate which should be almost colourless is then transferred to a clean crucible and evaporated to dryness on the sand-bath, the flame being turned down towards the end of the operation to avoid sputtering or decrepitating. It is advantageous to place the lids on the crucibles, the projection on the lid being bent underneath so as to engage with the edge of the crucible and leave a narrow opening for the escape of steam.

The dry residue is heated as before for about 3 minutes and finally cooled. Just sufficient saturated potassium carbonate solution is now added from a small dropping tube to give a stiff smooth paste when rubbed into the residue with the aid of two fairly stout glass rods about 5 inches long and flattened at the ends. A few cc. of 95 % alcohol are then added and worked thoroughly into the paste. The extract is decanted into a shallow 5 cm. platinum dish with the aid of a thin glass rod. The extraction is repeated two or three times until the paste begins to crumble into fine particles which have to be pressed together before the alcohol can be effectively decanted.

The alcoholic extract with the addition of four drops of saturated potassium carbonate solution and a few cc. of water is gently evaporated to dryness. The residue is then heated for a brief interval in a colourless Bunsen flame to destroy any remaining traces of organic matter. The platinum vessel must be kept moving during this operation so that no portion comes to a glow. The time during which the crucible can be kept in the flame without overheating depends on the thickness of the platinum and is best judged by making a trial with the vessel empty. After cooling, the residue is brought into solution by the addition of two or three drops of water, evaporated to dryness and again heated in the flame as described.

A drop of saturated potassium carbonate solution is then added to form a paste with the residue and the extraction is carried out as before, using first 1 cc. of alcohol and then three successive amounts of about 0.5 cc. The extracts are decanted into the titration tube described above with the aid of a thin glass rod, a little water is added and the contents are evaporated to dryness on the sand-bath. Some provision is necessary for supporting the somewhat unstable tubes on the sand-bath; the author places them inside a glass cylinder made by cutting a beaker in half. It is also convenient to have a small vessel of sand in which the tubes can be firmly planted when being carried about the laboratory.

To the tube which now contains a very small residue of salt is added about 1 cc. of water and the resulting solution is acidified by adding three drops

of 1 % sulphuric acid from a dropping bottle. The tube is then replaced on the sand-bath and, when the contents begin to boil, three drops of saturated bromine water are slowly added. The solution is evaporated to a volume of about 0.2 cc. and then cooled; a minute crystal of potassium iodide is added followed by a small amount of fresh starch solution. The starch solution is added from a dropper drawn out to a capillary tip; a small drop is produced on the tip which is then touched against the inside of the tube just above the surface of the liquid. Finally, the solution is titrated as described above with $N/1000$ thiosulphate solution. The thiosulphate solution should be made up in large volume with water containing 1 % of amyl alcohol [Meyr and Kerschbaum, 1928] and preserved in a large container of brown glass fitted with a soda-lime tube at the air inlet. Such a solution keeps its strength for months and frequent standardisation is not necessary.

The following results illustrate triplicate analyses for iodine in two samples of blood:

I 106, 108, 108 γ /litre.

II 266, 262, 266 γ /litre.

In conclusion the author wishes to thank Dr C. E. Hercus for permission to make the investigation which was carried out in connection with the Goitre Research at the Medical School, Otago University.

REFERENCES.

- Fellenberg (1923). *Biochem. Z.* **139**, 371.
Leitch and Henderson (1926). *Biochem. J.* **20**, 1003.
Meyr and Kerschbaum (1928). *Z. anal. Chem.* **73**, 321.
Winkler (1915). *Z. angew. Chem.* **28**, 477, 494.