XL. AN IODIMETRIC METHOD FOR THE DETERMINATION OF IRON IN BLOOD.

By JANET HENDERSON BLACKWOOD

(*Carnegie Research Scholar*)

AND

JOHN DEMPSTER STIRLING.

From the Hannah Dairy Research Institute, Ayr.

(Received February 13th, 1932.)

IN the course of certain investigations, at present being carried out at this Institute, it became necessary to employ a routine but accurate method for the estimation of iron in small quantities of blood.

The well-known reaction of ferric ions with thiocyanate is the basis of colorimetric procedures used by Wong [1923], Walker [1925] and others, but the disadvantages attending the colorimetric comparison inclined us against the adoption of these methods.

Jahn [1911] has described a procedure in which the blood is submitted to dry ashing and the iron residue titrated with titanous chloride in the presence of potassium thiocyanate. We have found dry ashing of the blood to be unsatisfactory on account of the difficulty of obtaining the iron residue in a form in which it is completely soluble in concentrated hydrochloric acid. Moreover it is necessary to carry out titanous chloride titrations in an atmosphere of carbon dioxide.

Murray [1924] has employed dry ashing of the blood, followed by reduction of the iron, which is then titrated with standard permanganate in an atmosphere of hydrogen. Using dilutions commensurate with the small quantities of iron present in blood the permanganate end-point is difficult to judge and is therefore unreliable.

Accordingly we have developed an iodimetric method which, employing the sensitive starch iodide indicator, gives results accurate to about 2 % on 2 cc. portions of blood.

The reaction between ferric and iodide ions proceeds according to the reversible equation

We have been unable to find any information on the state of equilibrium of this system at the dilutions which must be employed in the analysis of small quantities of blood. Our first efforts were accordingly directed to finding conditions suitable for the employment of the above reaction in the estimation of the amount of iron in 2 cc. of blood (about 0.7 mg.).

It was found that in neutral solution the equilibrium of the iron-iodide

system lies only a short distance from the left and is therefore useless for quantitative purposes. With increasing hydrogen ion concentration, however, the equilibrium moves towards the right, until in sufficiently acid solution quantitative reduction takes place. The liberated iodine may then be removed by titration with $N/200$ thiosulphate solution.

For our present purpose it will be sufficient to refer to Table I which summarises the results of a series of titrations carried out by us. The figures were obtained by titrating with $N/200$ thiosulphate the iodine liberated by the interaction of a known quantity of ferric iron with potassium iodide.

Table I.

		Initial concentration of iodide, 50 mM per litre. Concentration of hydrochloric acid, 5 M.									
Initial concentration of ferric salt (mM)		1	2	3	4	5	6	7	8	9	10
Thiosulphate titration of 10 cc. of solution	Found at equilibrium	1.97	3.97	2.96	3.94	2.505	2.96	3.48	3.99	4.45	4.95
	Calculated for complete reduction	2.00	4.00	3.00	4.00	2.50	3.00	3.50	4.00	4.50	5.00
Normality of thiosulphate		0.005	0.005	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
Ferrous salt at equilibrium (%)		98.5	99.2	98.6	98.5	100.2	98.6	99.4	99.7	98.8	99.0

It will be seen that in solutions which are 5 M with respect to HCl and 50 mM with respect to potassium iodide it is possible to estimate quantitatively amounts of iron representing concentrations of from 1–5 millimols per litre. Under these conditions the end-point is sharp and there is no return of colour within 10 minutes.

It remains therefore to develop a preliminary treatment of 2 cc. of blood which will allow of the above conditions in the final titration. In practice it was found that the following method gave satisfactory results.

METHOD.

The following reagents, which should be free from iron, are required.

Sulphuric, nitric and hydrochloric acids.

Potassium iodide (4 % solution—free from iodate: this is made up fresh each day).

Starch indicator (1 % solution of starch in sat. sodium chloride).

$N/200$ sodium thiosulphate.

Merck's perhydrol.

Potassium dichromate.

Preparation of thiosulphate. $N/200$ sodium thiosulphate is best prepared according to the instructions given by Pregl [1930]. $N/10$ sodium thiosulphate, which has been ripened to constant strength, is diluted exactly to $N/200$ strength by means of boiled out distilled water. The diluted solution contains 5 % by volume of amyl alcohol as a preservative and we have found that, when kept in amber-coloured bottles attached to automatic burettes protected by soda-lime tubes, the reagent retains its strength over long periods.

Standardisation of thiosulphate. 5 cc. of conc. hydrochloric acid are placed in an $8 \times 1\frac{1}{4}$ in. pyrex test-tube, and boiled gently for 1 minute, a glass bead being added to prevent bumping. The tube is then cooled under the tap and 3 cc. of standard potassium dichromate solution (25 mg. $\text{K}_2\text{Cr}_2\text{O}_7$ in 100 cc.) and 2 cc. of 4 % potassium iodide solution are added. A tightly fitting rubber stopper is then inserted and the tube is allowed to stand for exactly 10 minutes. The thiosulphate solution is then added drop by drop with shaking until the yellow colour of the solution almost disappears. At this point 10 cc. of distilled water are added followed by 5 drops of the starch indicator when the characteristic blue colour of the starch iodide complex develops. After shaking, thiosulphate is added until the blue colour disappears. The last few drops should be added very slowly, drop by drop, and with constant shaking.

A blank titration is carried out exactly as above except that 3 cc. of distilled water take the place of the dichromate solution. The value of the blank is subtracted from that of the first thiosulphate titration.

$$\text{Normality of thiosulphate} = \frac{3x}{4 \cdot 9y}.$$

Where $x = \text{g. } \text{K}_2\text{Cr}_2\text{O}_7 \text{ in 100 cc. of solution}$ and $y = \text{cc. of thiosulphate required.}$

The estimation of iron in blood.

2 cc. of blood are drained under the surface of 2 cc. of distilled water contained in a pyrex test-tube, the pipette (which is graduated to contain and not to deliver) being rinsed twice with the supernatant water.

5 cc. of conc. HNO_3 are then added and the tube is placed in a bath of boiling water until only 2 cc. of pale yellow solution are left. In this way the blood-proteins are digested smoothly and most of the organic matter is oxidised.

The latter part of the combustion is best carried out by hand. 0.5 cc. of conc. H_2SO_4 is added to the tube along with a glass bead to prevent bumping and the liquid is boiled gently over the flame of a small burner until charring is complete. The tube is allowed to cool a little, 1 cc. of conc. HNO_3 is added and the liquid is again boiled. If any organic matter still remains a few more drops of HNO_3 are usually sufficient to complete the destruction. 1 cc. of distilled water and 5 drops of perhydrol are added to the cooled liquid which is again boiled until white fumes appear. Two successive quantities of 1 cc. of distilled water are now added and after each addition the liquid is evaporated down in order to remove any remaining HNO_3 or perhydrol.

5 cc. of conc. HCl are introduced into the cooled tube and the liquid is boiled gently until a clear yellow solution is obtained. The tube is cooled under the tap, 3 cc. of distilled water and 2 cc. of 4 % KI solution are added immediately and the tube is stoppered tightly.

The titration is then carried out, as before, at the end of exactly 10 minutes.

A blank estimation is carried out using 2 cc. of water instead of blood. The blank value is subtracted from the titration figure. In practice we have found

that the blank value remains constant and need only be redetermined when any change is made in the reagents.

Mg. iron per 100 cc. blood = $2792 xy$.

Where x = cc. of thiosulphate required and y = normality factor of thio-sulphate.

RESULTS.

The following table gives the results of estimations in which a solution of ferrous iron of known strength was used instead of blood. The complete procedure outlined above was followed.

Table II.

mg. Fe present	mg. Fe found	Percentage error
0.6055	0.6149	1.4
0.8074	0.8088	0.17
1.0093	0.9954	-1.3
1.2111	1.1920	-1.6

The following experiment, using cow's blood, illustrates the recovery of iron added to the blood before ashing.

Table III.

Fe in 2 cc. of blood mg.	Fe added mg.	Total Fe mg.	Fe found mg.	Fe recovered %
0.6829	—	0.6829	0.6829	—
0.6829	0.2830	0.9659	0.9576	99.2
0.6829	0.4000	1.0829	1.067	98.5
0.6829	0.4800	1.1629	1.143	98.2
0.6829	0.5600	1.2429	1.214	97.8

We have now carried out analyses on over 50 samples of cow's blood and have obtained excellent agreement between duplicates.

SUMMARY.

An iodimetric method is described for the estimation of iron in 2 cc. of blood.

Our thanks are due to Dr N. C. Wright for his interest in this work and to Mr L. Jordan who obtained blood samples for us.

One of us (J. H. B.) is indebted to the Trustees of the Carnegie Research Fund for a Scholarship during the tenure of which this work was carried out.

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