CXI. A METHOD FOR THE ESTIMATION OF IRON IN SMALL QUANTITIES IN BIOLOGICAL SUBSTANCES.

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THE general principles of the method are not dissimilar to those in general use, but certain defects in the KMnO_4 method can be overcome by the use of a "reductor" instead of the more usual Bunsen valve, previous to titration with permanganate, and titration in an atmosphere of hydrogen makes the method reliable and accurate. The method has been worked out, using blood.

Incineration. 3 cc. of fully oxygenated whole blood are run into a weighed "vitreosil" crucible and accurately weighed. The crucible is then placed without lid in a steam oven for half an hour, or, if time will not permit of this, the blood is dried carefully with a small flame. When dry, the crucible is heated more strongly in order to ash the contents. Small crystals of ammonium nitrate are added from time to time to help combustion.

Strong heating is to be avoided, otherwise iron will be lost, as pointed out by Peters [1912], because if strongly heated the iron residue becomes insoluble. The burning mass should not be allowed to froth up round the sides of the crucible but be kept as compact as possible.

Solution of the iron residue. When combustion is complete the crucible is allowed to cool; about ten drops of pure concentrated hydrochloric acid added and the whole gently heated to dissolve the iron residue, further heating is applied and the contents of the crucible evaporated to dryness.

In order to remove chlorides, since these vitiate the result as pointed out by Barcroft [1914], approximately 1 cc. of pure concentrated sulphuric acid is added to the chloride residue, which is again heated till white fumes are no longer evolved. The contents of the crucible are finally taken up in four successive portions of 3 to 4 cc. of 10 % sulphuric acid.

Reduction of the iron and titration with permanganate. It is in this stage of the proceedings that improvements on former permanganate methods have been introduced. For conversion of the iron to the ferrous state a modified Jones' reductor is used.

The reductor. A white glass filter flask is fitted with a two-holed rubber cork to take the reductor and a burette. The reductor itself is a tube 75 cm.

by 2.5 cm. packed to a length of 30 cm. with "cadmiumised" zinc, that is, zinc which has been soaked in 2 % cadmium sulphate solution for five minutes and then well washed.

Pure zinc is too slow in its action to reduce the iron properly. No trace of zinc must be allowed to pass into the flask; to prevent this happening the narrow part of the tube just above the tap is packed with "melting point" tubing and capped with a plug of glass wool. This type of filter is used because if a close filter is used the process is too slow.

The end of the reductor below the tap is cranked before passing through the cork. The burette is filled with standard permanganate and inserted before reduction begins.

Reduction. The flask is connected to a pump and the solution containing the iron in 10 % sulphuric acid is poured in a steady stream down the reductor, the tap opened and the solution allowed to escape at a slightly slower rate into the flask. The reductor is washed with about 20 cc. of 5 % sulphuric acid. During these proceedings the tap must not be allowed to become unsealed.

Titration. With the pump still going the standard permanganate is run in from the burette and the flask well shaken till the solution takes on a pink tinge. The colour is permanent even though it is faint; the end point is therefore very distinct.

A blank titration is made by running through the reductor 12 cc. of 10 % sulphuric acid and 20 cc. of 5 % acid and titrating. This solution never required more than 0.05 cc. of permanganate, and this amount was deducted from the iron titrations.

Since very small quantities of permanganate make a great difference to the quantity of iron estimated, a 10 cc. burette graduated in $\frac{1}{50}$ ths was used. The permanganate employed was tried at various strengths, $N/60$, $N/80$, $N/100$; the end point was quite definite even with the $N/100$ solution. All these solutions were made up immediately before use from a stronger solution which was always standardised. The $N/80$ strength permanganate seemed to be the most convenient.

Peters [1912], in his work on the constancy of the proportion of iron to oxygen in oxyhaemoglobin, used the titanium method and Barcroft [1914] considered that the application of this method made the estimation simpler and more accurate than was possible with the permanganate method. It is doubtful to me whether the method is simpler; it is certainly not a student's method.

The use of permanganate for the estimation of iron in blood was deprecated by Barcroft and Peters because it is vitiated by the presence of chlorides, whereas the titanium method is not: if chlorides, however, are first removed, this criticism does not apply.

Many investigators decry methods which involve titrations with dilute permanganate; no doubt the greatest source of error in these is the indefiniteness of the end point, but titrations done under the conditions described give a perfectly clear-cut end point.

RESULTS.

Series 1.

Cat's blood	Weight of blood, g.	cc. N/80	Fe % in blood	Fe % computed from O ₂ capacity
<i>A</i>	3.1412	2.00	0.0443	—
<i>B</i>	3.1481	2.05	0.0454	—
<i>C</i>	3.1403	2.00	0.0445	—
		Mean	0.0447	0.0446

Series 2.

Cat's blood	Weight of blood, g.	cc. N/100	Fe % in blood	Fe % computed from O ₂ capacity
<i>A</i>	3.1295	2.85	0.0510	—
<i>B</i>	3.1819	2.88	0.0506	—
<i>C</i>	3.0536	2.78	0.0510	—
<i>D</i>	3.0752	2.82	0.0513	—
		Mean	0.0510	0.050

Series 3.

Cat's blood	Weight of blood, g.	cc. N/60	Fe % in blood	Fe % computed from O ₂ capacity
<i>A</i>	3.0124	1.46	0.0452	—
<i>B</i>	3.0812	1.49	0.0451	—
<i>C</i>	2.7587	1.30	0.0439	—
		Mean	0.0447	0.0439*

* The discrepancy here is probably due to the fact that a less accurate burette and a stronger solution of KMnO_4 were used.

CONCLUSIONS AND SUMMARY.

1. Iron is estimated in an ashed biological product by means of $\text{N}/80 \text{ KMnO}_4$.
2. By using the "reductor" described and titrating in the absence of air a very definite end point is obtained.
3. The method is probably a simpler one than that involving the use of titanium chloride.

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REFERENCES.

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 Peters (1912). *J. Physiol.* **44**, 131.