VI. THE MICRO-ESTIMATION OF IRON IN BLOOD.

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THE estimation of iron in small quantities of blood is almost universally colorimetric. The thiocyanate colour is preferable to the ferrocyanide because of its greater intensity and the unsuitability of blue solutions for colorimetric work.

The use of acetone to intensify colour and minimise the influence of interfering substances was introduced by Mariott and Wolf [1906] and used by Fowweather [1926]. It was not used by Walker [1925] and was criticised by Wong [1923] who considered that its evaporation introduced considerable errors. Personal observations strongly confirm Fowweather's contention that this error is negligible and entirely outweighed by the advantages outlined by Mariott and Wolf.

Destruction of organic matter has been accomplished by concentrated hydrobromic acid [Berman, 1918] and by potassium chlorate [Wong, 1923], and recently Fowweather has published another method using Wong's procedure, but replacing the potassium chlorate by perhydrol (100 volume H_2O_2) in order to prevent the violent bumping and frequent loss of samples with potassium chlorate.

Using Fowweather's method occasional bumping and loss of samples was still troublesome. With different samples of perhydrol, oxidation was found to be somewhat tedious. It appeared that his order of accuracy would have been attained with practice. The following has been elaborated as a shorter method for a series of estimations, entirely without risk of bumping and with probably a greater accuracy than that claimed by Fowweather.

The method depends on the rapid oxidation of blood proteins by ammonium persulphate and nitric acid, with subsequent colorimetric estimation of iron as thiocyanate in the presence of acetone, against an artificial colour standard. This enables a series of estimations to be made using the same standard whereas alternatively for accurate working it becomes necessary to make an iron standard for each sample.

PROCEDURE.

0.2 cc. of blood is delivered from a standardised pipette to the bottom of a 4½ or 5 inch Jena or Pyrex test-tube. The pipette is washed by sucking two or three drops of water up and down the pipette from a small centrifuge tube and then transferring these by the pipette to the Jena tube. The process of washing is repeated twice.

0.33 g. of a well mixed sample of powdered ammonium persulphate is added to the contents and washed down to the bottom of the tube with 2 cc. of concentrated nitric acid.

The mixture is gently heated with moderate shaking till the protein is dissolved and the solution is clear. The tube is clamped in a retort stand and heating is continued for about 1 minute, air being bubbled through the solution from a fine capillary tube in order to prevent bumping. The solution darkens slightly in shade and then grows lighter again. This may be taken as an index of complete oxidation though the slight colour change is not always noted. The flame is withdrawn and 6 cc. of distilled water are added from a burette, the stream of air ensuring mixing. The capillary tube is then withdrawn and inserted without washing into the next of the series of samples.

The first sample, which has now been boiled and diluted with 6 cc. of water, is poured into a 50 cc. flask and the test-tube washed out three times with 2 cc. of distilled water from a burette, each time adding the washings to the 50 cc. flask. The test-tube is then placed on clean blotting paper by the flask ready for the final washing with acetone.

When all the samples are dissolved and delivered into 50 cc. flasks, 25 cc. of acetone are taken in a small measuring cylinder and the first of the series of test-tubes which has already been washed with distilled water is washed out again with the acetone, the whole 25 cc. of acetone washings being delivered to the first flask which is then gently shaken and immersed in cold water for 5 minutes. 5 cc. of concentrated ammonium thiocyanate solution (24 g. per 100 cc. of water) are added and the volume is made up to the 50 cc. mark with distilled water. The iron estimation is then made colorimetrically against an artificial standard, consisting of a mixture of cochineal, methyl red, and hydrochloric acid dissolved in an equal mixture of acetone and water. The proportions are as follows:

4 cc. cochineal solution (British Drug Houses' Indicator).

3.2 cc. methyl red solution (British Drug Houses' Indicator).

4 cc. dilute hydrochloric acid (1 in 5).

This is diluted with equal parts of acetone and water to the approximate depth of colour required and a final adjustment of tint is made by adding drops of methyl red if the orange tint predominates, and of cochineal solution if the red is too clear. The colour is permanent and indistinguishable from that of the thiocyanate.

Before the ammonium thiocyanate is added to the first of a series of estimations it is best to add the acetone washings to the second of the series so that this may be cooling while the colorimetric readings are taken on the first sample.

Finally 1 cc. of a solution containing 0.1 g. of ferric iron per litre (*v. Fowweather*) is added from an Ostwald pipette to a Jena tube. 0.33 g. of ammonium persulphate and 2 cc. of concentrated nitric acid are also introduced and these are boiled down to about 2 cc. as are the samples containing blood. The solution is made up to 50 cc. as already described and compared with the artificial standard. A blood sample of equal tint to this iron sample contains 50 mg. of iron per 100 cc. of blood.

In explanation it is well to point out that ammonium persulphate as supplied by manufacturers contains minute traces of iron. Hence a well-mixed sample must be used and the error balanced by a similar addition to the iron standard. Iron-free nitric acid also gives a slight coloration with thiocyanate which is corrected by the nitric acid in the standard.

PRACTICAL PRECAUTIONS.

(1) Slight differences in the opacity of solutions always cause trouble in colorimetric work. These can be corrected by increasing the illumination on the opaque side.

(2) Bubbles of gas are set free when acetone is mixed with water. The solutions should be shaken and these allowed to escape or there will be great difficulty with the colorimeter.

(3) The colorimeter is the main source of error. When the colorimetric readings are close and the results divergent the probability is that the illumination has been unequal. The illumination is best adjusted between each reading. 15 mm. is the best level for most people when the iron content is normal, and 20 mm. for anaemic bloods. The personal factor largely governs other optimal conditions for any one observer.

RESULTS.

Results of 18 consecutive observations, made with two different samples of human blood A and B.

<i>Sample A of blood</i>	Total estimated iron in sample mg.	Known added iron mg.	Total iron minus added iron = iron in 0.2 cc. of blood taken mg.
1	0.109	0.004	0.105
2	0.106	0.000	0.106
3*	0.108	0.006	0.102
4	0.110	0.003	0.107
5	0.107	0.000	0.107
6	0.112	0.005	0.107

* In the case of this sample the acetone was delivered straight to the flask instead of using it for the final washing of the test-tube. This represented a loss of 0.004 mg. of iron;

<i>Sample B of blood</i>	Total estimated iron in sample mg.	Known added iron mg.	Total iron minus added iron = iron in 0.2 cc. of blood taken mg.
1	0.103	0.008	0.095
2	0.098	0.003	0.095
3	0.095	0.000	0.095
4	0.103	0.006	0.097
5	0.098	0.000	0.098
6	0.099	0.004	0.095
7	0.102	0.005	0.097
8	0.096	0.000	0.096
9	0.105	0.008	0.097
10	0.099	0.002	0.097
11	0.096	0.000	0.096
12	0.103	0.007	0.096

All results are stated to the nearest significant figure.

SUMMARY.

A simple and rapid method for the accurate estimation of blood-iron using 0.2 cc. of blood is described. The method depends on a colorimetric comparison of the iron as ferric thiocyanate with an artificial colour standard.

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