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Comparison of Wet and Dry Ashing Methods for Determining Blood Iron.

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Iron represents 0.335% by weight of the hemoglobin molecule.[1] Using this factor, blood hemoglobin may be estimated in grams per 100 cc. by the blood iron method as accurately as by the accepted oxygen capacity method.[2] Different standards representing 100% hemoglobin must be adopted for men,[3] women[3] and children.[4] The figure for the milligrams of iron per 100 cc. of blood may be converted into percentage hemoglobin by multiplying by 2 for men, by 2.25 for women, and by 2.5 for children.

Calibration of clinical hemoglobinometers such as the Dare and the Newcomer instruments is facilitated by using the blood iron method in preference to more complicated standardized methods. Simplification of procedures for determining blood iron even makes possible their use routinely in clinical laboratories. In view of these facts it is of utmost importance to have definite assurance of the accuracy of the method employed for the determination of blood iron.

We wish to report a comparative study of 2 different methods we have employed for blood iron in an effort to determine the normal range of this element in the blood of men, women, and children. One method involved the original Wong wet ashing procedure and the other method involved a dry ashing process.

Fowweather,[5] Smirk,[6] Kennedy,[7] Wong,[8] and others have reported slightly different wet ashing methods for determining blood iron in which the organic material is oxidized by an acid in combination with a strong oxidizing agent. The value of these methods lies in their simplicity and the speed with which the determinations may be made.

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1 Butterfield, E. E., *Z. f. physiol. Chem.*, 1909, **62**, 173.

2 Karshan, M., and Freeman, R. G., Jr., *J. Lab. and Clin. Med.*, 1929, **15**, 74.

3 Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366; Sachs, A., Levine, V. E., and Fabian, A. A., *Ibid.*, in press.

4 Sachs, A., Levine, V. E., and Fabian, A. A., unpublished communication.

5 Fowweather, F. S., *Biochem. J.*, 1926, **20**, 93.

6 Smirk, F. H., *Biochem. J.*, 1927, **21**, 36.

7 Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

8 Wong, S. Y., *J. Biol. Chem.*, 1928, **77**, 409.

In the original Wong procedure oxidation is carried on without the aid of external heat. We have modified the procedure by the application of heat to a flask containing blood to which have been added sulphuric acid and a saturated solution of potassium persulphate. Heating on the water-bath at 80°C. for 10 minutes causes a complete release of iron from the protein components of the red cell and insures more accurate results. Our figures for blood iron have been confirmed by other investigators who have used the Wong method.[9] Where other wet ashing methods have been used the figures did not agree with those which we obtained by the Wong procedure.[9, 10]

The other method involves dry ashing of the blood. Five cc. of oxalated blood were transferred to a vitreosil dish. The blood was dried on a hot plate with constant stirring to prevent spattering, and then ashed in an electric muffle for 8 hours at low red heat. To the ash was added 1 cc. of concentrated nitric acid, and the acid evaporated off on the hot plate. The procedure varies from this point if (a) blood copper and iron are to be estimated on the same sample and if (b) blood iron alone is to be determined.

(a) The ash is taken up with 3 cc. of 6N HCl and transferred to a centrifuge tube. The iron is precipitated as the hydroxide with 2 cc. of strong ammonia water. The tube is centrifugalized and the supernatant fluid containing the copper is poured off. For the determination of blood copper we employed the McFarlane[11] method. The iron determination is then continued as in (b).

(b) 4 cc. of concentrated sulphuric acid are added to 50 cc. of distilled water. Using this solution, the nitric acid treated ash or the ferric hydroxide obtained in (a) is dissolved and transferred to a 100 cc. flask. Two cc. of a saturated solution of potassium persulphate are added to the flask and the contents made up to the mark with distilled water. Five cc. of the solution in the flask are transferred to a 25 cc. graduated cylinder and 0.6 cc. of concentrated sulphuric acid added. The cylinder is cooled and made up to the 20 cc. mark with distilled water. A standard containing 0.1 mg. of iron and 0.08 cc. of concentrated sulphuric acid is made up to the 20 cc. mark in another cylinder. To the standard and unknown are added 1 cc. of the persulphate solution and 4 cc. of a 3N potassium thiocyanate solution, and comparison made colorimetrically.

Elvehjem[12] has reported a dry ashing method for determining

[9] Helmer, O. M., Emerson, C. P., Jr., *J. Biol. Chem.*, 1934, **104**, 157.

[10] Murphy, W. P., Lynch, R., and Howard, I. M., *Arch. Int. Med.*, 1931, **47**, 883.

[11] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[12] Elvehjem, C. A., *J. Biol. Chem.*, 1930, **86**, 466.

iron in biological materials in which the ash is boiled for an hour with strong alkali to convert to orthophosphates the pyrophosphates, which form at the temperature required for ashing. This step is necessary because pyrophosphates bind the iron in a complex molecule and thus prevent the union of ferric and thiocyanate ions. With some materials very rich in phosphates the color development is entirely inhibited unless measures are taken to eliminate or convert the phosphates. In blood, however, the problem of phosphate interference is of no great moment, since blood is relatively poor in phosphorus. If any pyrophosphate is present in the ash it tends to be converted to orthophosphate by the hot nitric acid which is added. Hot nitric acid converts metaphosphates and pyrophosphates to orthophosphates in experiments which we have performed.

We have made a series of 50 comparative determinations of blood iron by the modified Wong procedure and by our dry ashing method. We have found close agreement in the figures obtained when both methods were applied to the analysis of specimens of the same blood. The percentage deviation is about 1% for the whole series. This agreement justifies the use of our modification of the wet ashing procedure of Wong, which we have employed in the determination of the iron content of small samples of blood. It also justifies the use of the dry ashing method in a series of iron determinations we have just completed.

The recent reports[13] on copper in the blood with its special relation to anemia make it imperative at times to determine both the iron and copper content on the same blood. The wet ashing method is not suitable for the determination of both elements on the same sample of whole blood. The dry ashing method we have adopted has a great advantage, because it can be applied with convenience and accuracy to the simultaneous determination of iron and copper on the same blood sample. Using the dry ashing method for the determination of both iron and copper, we have made a study of bloods brought by one of us (L) from the Arctic in an effort to establish the incidence of anemia among Alaskan Eskimos.[14]

[13] Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, in press.

[14] Sachs, A., Levine, V. E., and Fabian, A. A., unpublished communication.