DETERMINATION OF TRACES OF FERRIC IRON, AS IN BLOOD SERUM.1)

By

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Within the past few years, the role of non-hemoglobin iron in blood has assumed considerable importance, largely due to the work of G. BARKAN 2, O. WARBURG 3, and others 4, 5, 6, 7. This iron has always been found in the ferric state. Also, iron is so common a contaminant of chemical and biological systems, that the determination of traces of this element is of considerable importance.

With the exception of the catalytic method used by WARBURG 3, and the titanous chloride titration with thiocyanate indicator as used by LANGER 4, nearly all the available data on the direct determination of non-hemoglobin iron in blood have been obtained by use of the colorimetric thiocyanate method. Only a portion of the non-hemoglobin iron is found in the serum. For the minute quantities of iron present in serum, LANGER's simple titanous chloride titration has been found of no value due to the inadequate protection from air, and the very indistinct endpoint obtained when the iron is of the magnitude of about 1 part per mil-

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2 Ztschr. physiol. Chem. 171, 179 (1927); 171, 194 (1927); 216, 1 (1933); 221, 241 (1933). — Klin. Woch. 11, 598 (1932); 11, 1050 (1932).
3 Biochem. Ztschr. 187, 255 (1927); 190, 143 (1927).
7 GUTHMANN, H., BRUCKNER, M. A., EHRENSTEIN, and WAGNER, H., Arch. Gynäkol. 147, 469 (1931).
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Evidence has been obtained in this laboratory and elsewhere that phosphates and certain other materials interfered with the production of maximum color in known iron solutions when the colorimetric thiocyanate method was used. This fact was supported by the findings of C. V. Smythe and C. L. A. Schmidt 9 and by the evidence of W. D. McFarlane 8 who found the titration method with titanous salts in many respects preferable to the colorimetric method. It will be shown in this paper that McFarlane underestimated the possibilities of the titanous titration, though his conclusions in other respects are confirmed by this investigation. As will be shown later, another hitherto unnoticed difficulty is inherent in the thiocyanate method when it is applied to minute concentrations of iron.

The thioglycollic acid colorimetric method, originally introduced by E. Lyons 10 has been found to be insufficiently sensitive for studies of serum iron or iron solutions of similar concentrations. J. F. King and F. H Howard 11 determined total blood iron by use of potentiometric titration with titanous salt, as did McFarlane 9. L. Pincusen and W. Roman 12 titrated similarly with titanous solution but determined the endpoint by the disappearance of the color with added thiocyanate. Many other procedures have been used for total blood iron, but are not sufficiently sensitive or accurate for serum iron.

From these considerations and various preliminary studies that were carried out, it appeared that a differential electrometric titration using titanous chloride offered the best possibility for the determination of minute traces of iron, such as are found in blood serum. The results of a study of this method are reported here and shown to have certain advantages over other methods.

**Experimental.**

Since this method was developed with a view to the determination of serum iron which, according to Barkan and others, has

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