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A Method for the Estimation of Serum Iron.*

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The drawing of blood under conditions aiming to avoid hemolysis, as described by Fowweather,[1] has yielded in our hands serum that invariably gives a positive benzidine test and shows the absorption bands of oxyhemoglobin if a sufficient thickness of solution is examined. The present method for the estimation of the non-hemoglobin iron of blood serum consists of 2 steps, the analysis of the serum for total iron and for hemoglobin iron.

The total iron is determined by ashing 2 cc. of serum with 2 cc. of concentrated sulfuric acid, with the aid of 30% H_2O_2. The ashed sample is diluted to 15 cc. with water, enough potassium permanganate is added to give a permanent pink color, 5 cc. of ethyl acetate are layered over the solution and, finally, 5 cc. of 20% ammonium thiocyanate solution are added and the mixture is shaken. The color in the ethyl acetate layer is compared in a micro-colorimeter with a standard containing 0.005 mg. of iron similarly treated. The traces of iron in the reagents are determined by blank analyses.

The quantitative benzidine method[2] is employed for the determination of the hemoglobin, but allowance must be made for the effect of serum proteins on the reaction. Proteins and certain salts cause a diminution in the color produced in the benzidine acetate-

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[1] Fowweather, F. S., *Biochem. J.*, 1934, **28**, 1160.

[2] Bing, F. C., *J. Biol. Chem.*, 1932, **95**, 387.

hemoglobin-H_2O_2 system. The method may be summarized briefly. To 2 cc. of the benzidine reagent 0.5 cc. of blood serum and 0.5 cc. of water are added, followed by 1 cc. of 0.6% H_2O_2 solution. In another test tube the same procedure is carried out, except that 0.5 cc. of the standard solution of blood, containing 0.05 mg. of hemoglobin per cc., is used in place of 0.5 cc. of water. The colors are allowed to develop and compared with a standard in the usual way.[2] If 40% of the hemoglobin added is recovered (the recovery varies from about 30 to 50% depending on the age of the reagents) the figure for the apparent hemoglobin content of the serum is multiplied by 2.5 to obtain the true value. Finally, the mg. of hemoglobin per 100 cc. of serum are computed as micrograms of iron by multiplying by the factor 3.35.

The validity of the hemoglobin method has been checked by spectroscopic examination of serum. The thickness of solution at which the α band of oxyhemoglobin just disappeared showed that the hemoglobin actually present was approximately 2 or 3 times more than the apparent concentration shown by the benzidine reaction. In addition, serum was treated with 30% H_2O_2 at room temperature until all the hemoglobin had been destroyed. The recovery of added hemoglobin was the same as the recovery of hemoglobin added to untreated serum.

TABLE I.
Iron Content of Blood Serum.

Specimen No.	Total Fe	Hb Fe	Non-Hb Fe	Inorganic Fe
1	330	44	286	270
2	333	33	300	330
3	375	47	328	320
4	365	17	348	350

All figures are reported in terms of micrograms of Fe per 100 cc. of serum.

Table I shows the results of analyses of 4 different samples of dog serum, drawn several hours after the oral administration of iron salts. Only 3 cc. of serum are required for a single determination of both total iron and hemoglobin iron. For comparison, estimations of the inorganic serum iron were made by the method of Tompsett,[3] which determines the ferrous iron in the protein-free filtrate of serum that first is treated with a reducing agent. This method requires 10 cc. of serum. The satisfactory agreement of the 2 sets of results indicates that the non-hemoglobin iron of blood serum is inorganic iron.

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[3] Tompsett, S. L., *Biochem. J.*, 1934, **28**, 1536.