3. During the investigation, it became necessary to improve the methods employed in the preparation of cyclopropane-monocarboxylic acid and its ammonium salt, and also, to synthesize the following new compounds: bi-dibenzylmethyl urea and tribenzylmethyl chloride. The failure of tribenzylmethyl chloride to react with magnesium to form a Grignard compound is discussed.

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A NEW QUANTITATIVE METHOD FOR THE DETERMINATION OF IRON IN THE BLOOD.

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A new method for the determination of small amounts of iron in the blood which has been worked out in this laboratory offers possibilities for use in pathological laboratories and clinical diagnosis.

Principle.—A known amount of blood after laking in water is treated with conc. hydrochloric acid and a very small amount of potassium chlorate. By this method the proteins are precipitated and the iron rendered soluble. After heating in boiling water, the material is filtered and filtrate made up to a known volume. An aliquot portion of the filtrate is then compared colorimetrically with a standard iron solution.

Method.—To 4 cc. of distilled water contained in a test-tube 0.5 cc. of blood sample is added. After laking, 1 cc. of conc. hydrochloric acid and a very small amount of potassium chlorate (about 0.01 g.) are added. The test-tube and contents are then placed in boiling water and heated for about 15 minutes, until the liquid becomes white or light yellow and the proteins are entirely precipitated and have become white. The material is then allowed to cool and the residue to settle. The latter is then filtered and washed with water until 15 cc. of filtrate is obtained. After thoroughly shaking, 1.5 cc. of the filtrate is placed in one side of the test-tube colorimeter. To the other side of the colorimeter 0.25 cc. of the standard iron solution is added. To both tubes is added enough 0.1 N potassium permanganate solution to oxidize all the iron completely and until a permanent pink color remains. Then 5 cc. of 5 N ammonium thiocyanate solution is added and the standard diluted to 25 cc. with distilled water. The sample is then diluted until the two colors match. Should a brown residue form after the addition of ammonium thiocyanate solution, several drops of a 10% solution of hydrochloric acid are added to dissolve the precipitate.

Solutions.

(1) **Standard Iron Solution.**—This solution is made by dissolving 0.7 g. of crystallized ferrous ammonium sulfate in 50 cc. of distilled water and adding 20 cc. of dil. sulfuric acid. The solution is warmed slightly and potassium permanganate added until the iron is completely oxidized, after which it is diluted to 1 liter. One cc. of this solution is equal to 0.1 mg. of iron.

(2) **Potassium Permanganate.**—This solution does not have to be standardized exactly but should be made approximately 0.1 *N*. It is prepared by dissolving 0.316 g. of potassium permanganate in 1000 cc. of distilled water.

(3) **Ammonium Thiocyanate.**—This is made approximately 5 *N* by dissolving 380 g. of iron-free ammonium thiocyanate in 100 cc. of distilled water.

Calculation.

0.25 cc. of standard iron solution as above prepared is equivalent to 0.000025 g. of iron which has been diluted to 25 cc. Thus, when 1.5 cc. of the filtrate is used and the colors matched upon dilution to 17.5 cc. we obtain the ratio 25 : .000025::17.5 : x. Therefore x equals 0.0000175 g. of iron in 1.5 cc. of the filtrate and in 15 cc. we obtain 0.0000175 times 10 or 0.000175 g. of iron which is contained in 0.5 cc. of blood; in 100 cc. we obtain 200 times the above figure or 0.036 g. of iron or 36 mg. per 100 cc. of blood.

When the iron solution is made as above described and the amount of blood and reagents are used as directed, the calculations given above are not necessary and the iron can be calculated directly from the reading of the diluted sample by multiplying the reading by 2. Thus above where the reading of the sample was 17.5 it would become 35 after multiplying by 2. The above method has been checked very carefully against a method by Louis Berman[1] and the results agree very closely, as will be seen from the following data.

Brown	Berman
Fe per 100 cc. of blood	
Mg.	Mg.
30	28
39	35
32	30
36	37
37	36
—	—
35 Av.	33 Av.

The above work was done on several different rabbits and at different times.

This method has been further checked by weighing a known amount of ferrous ammonium sulfate and adding a large excess of several other salts together with egg albumin. By using the method described above practically the same amount of iron was obtained as was theoretically present.

In order to ascertain whether all the iron present in the blood is dissolved by the treatments of concd. hydrochloric acid and potassium chlorate several samples of blood were ashed in a platinum crucible, treated with hot hydrochloric acid, washed with water and made up to a definite volume.

[1] Berman, *J. Biol. Chem.*, **35**, 231 (1918); *C. A.*, **12**, 1977 (1918).

An aliquot portion was then taken for analysis by the method as given above and the results obtained were as follows.

Whole blood	Ash
Iron per 100 cc. of blood	
Mg.	Mg.
38.0	39.0
30.0	32.0
42.0	42.5

The iron content of rabbit's blood varies from 27 to 42 mg. per 100 cc., while dog's blood varies from 40 to 48 mg. per 100 cc. and human blood from 45 to 52 mg. per 100 cc.

Summary.

The method may be summarized as follows: 0.5 cc. of blood after laking in 4 cc. of water is treated with conc. hydrochloric acid and a very small amount of potassium chlorate placed in boiling water until white or light yellow, cooled and filtered, and the residue washed with water until 15 cc. of filtrate is obtained; an aliquot portion is then compared colorimetrically with standard iron solution using potassium thiocyanate as the indicator.

The advantages are: (1) A small amount of blood sample needed; (2) the analysis is rapid, not over 20 minutes being required; (3) greater accuracy is insured because the contrast in colors is more marked; (4) since it is a colorimetric method the results are more accurate with minute amounts than by either gravimetric or volumetric analysis.

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NOTES.

The Preparation of Phenylacetylene.—Phenylacetylene has been prepared by a number of methods, the two outstanding ones being those of Glaser[1] and Holleman,[2] in which phenylpropiolic acid is used, and the method of Nef,[3] which employs ω-bromostyrene. In Nef's experiments, the bromostyrene was heated with potassium hydroxide and a small quantity of absolute alcohol. The products obtained were phenylacetylene and phenylvinyl-ether, $C_6H_5CH=CHOC_2H_5$, the yield of phenylacetylene being 60% of that calculated. Owing to the considerable conversion of starting material into by-product by Nef's method, the writer tried the use of molten potassium hydroxide instead of alcoholic potash.

The potassium hydroxide (80 g.) was placed in a distilling flask provided

[1] Glaser, *Ann.*, **154**, 151 (1870).

[2] Holleman, *Ber.*, **20**, 3080 (1887); *Rec. trav. chim.*, **15**, 157 (1896).

[3] Nef, *Ann.*, **308**, 264 (1899).