As can be seen when iron is administered to the anemic animal for 14 days there is an increase in the iron content of the liver as well as an increase in the blood hemoglobin and erythrocytes. With discontinuing the iron at the end of 14 days and giving milk only the liver iron decreases as does the blood hemoglobin and erythrocytes. When copper is given at the end of 14 days, and no iron, the liver iron markedly decreases, while the blood hemoglobin and erythrocytes increase. Copper given to anemic animals reduces the liver iron to a minimum level with a coincident increase in blood hemoglobin and erythrocytes.

It may be concluded that copper when given to anemic rats can mobilize the iron stored in the liver to produce hemoglobin and increase the red blood cells.

6702

Determination of Iron in Biological Material.

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A method is proposed for the determination of iron in biological material which makes use of the reaction between iron and thioglycollic acid in an alkaline solution with the production of a purple color.

Method for blood. Measure 0.2 cc. of blood into a pipette accurately calibrated to contain 0.2 cc. and deliver it into a Pyrex test tube, 20x180 mm., calibrated at 10, 15 and 20 cc. Add 1 cc. of 10 N sulfuric acid and introduce a small piece of silica or a glass bead to prevent bumping. Heat, preferably over a hot plate to evaporate the water. As soon as the material chars, allow to cool for 45 seconds and add 4 drops of 30% hydrogen peroxide drop by drop. Continue the heating for 5 minutes after the solution becomes colorless. Allow it to cool and add 10 cc. water. Into a similarly calibrated Pyrex test tube accurately measure 1 cc. of standard iron solution containing 0.1 mg. iron and dilute with distilled water to about the 15 cc. mark. Add one drop (0.05 cc.) of thioglycollic acid to both standard and unknown, mix by lateral shaking. Run in concentrated ammonium hydroxide from a burette until the permanent purple color makes its appearance, pH of 8 to 10. Mix con-

tents of both tubes, make up to volume of 20 cc. with distilled water and mix again by inversion. Comparison is then made in the colorimeter. The simplified calculation is 1000/R = mg. iron per 100 cc. blood. This is converted to volumes per cent of oxygen by multiplying the mg. iron by 0.4. To convert this value to grams hemoglobin, divide the volume per cent of oxygen by 1.34.

Method for urine, feces, milk, etc. A portion of material to be analyzed is evaporated to dryness, ashed in an electric muffle furnace, and the ash dissolved in 6 N hydrochloric acid. The insoluble residue is filtered off, washed, and filtrate made up to volume of 50 or 100 cc. To separate the iron from all possible interfering substances use is made of cupferron, the ammonium salt of nitrosophenylhydroxylamine, which precipitates ferric iron quantitatively in an acid solution. Measure an aliquot, 25 cc., of the acid solution of ashed material into the Pyrex test tubes used in the case of blood, add a dilute solution of $KMnO_4$ drop by drop until a permanent pink color appears (to oxidize all the iron to the ferric state). Add 5 drops freshly prepared 9% solution of cupferron. Mix well and centrifuge at a relatively high speed for 4 minutes. Decant the supernatant liquid. In case the iron concentration is very low, add another aliquot portion of the acid solution of ashed material, oxidize with $KMnO_4$ as before, add more cupferron, mix and centrifuge. The final precipitate is digested by adding 1 cc. of 10 N sulfuric acid, and heating over a microburner. When the material is well charred, add 30% hydrogen peroxide drop by drop and continue heating until a clear solution is obtained. Allow tube and contents to cool, add 10 cc. of water, one drop of thioglycollic acid, make alkaline with concentrated ammonium hydroxide, make up to volume of 15, 20, or 25 cc. The standard may consist of 0.05 or 0.10 mg. iron, depending upon the quantity of iron in the unknown. The color is developed here as in the case of blood and comparison made in the colorimeter.

The recovery of added iron to biological material is quantitative (Table I).

The use of cupferron enables one to make a quantitative separation of iron from substances which might interfere if present in high concentration. However, ortho- and pyro-phosphates do not interfere if present even in such quantities as 750 and 500 mg. respectively per each 0.1 mg. of iron.

The proportionality of color development over a wide range, 0.04 mg. to 0.50 mg. is practically perfect. The fading of the purple

TABLE I.
Recovery of Added Iron to Biological Material.

Material Analyzed	Iron per Specimen	Iron Added	Total Iron Recovered
	mg.	mg.	mg.
300 cc urine	0.074	0.040	0.119
300 " "	0.052	0.040	0.091
1 gm liver	5.630	1.000	6.611
2 " "	11.260	0.700	11.964
11 " feces	1.670	0.500	2.180
25 " "	6.327	3.000	9.235
3 " milk powder	0.056	0.050	0.108
4 " " "	0.074	0.050	0.119

color reported[1, 2] does not occur when one drop of the thioglycollic acid is used in the presence of these low concentrations of iron. The color is permanent for 30 minutes. In high concentrations of iron, 1 mg. or more, there is fading with the subsequent return of purple color on shaking with air.

Thioglycollic acid is specific for both ferrous and ferric iron, as is shown by standard solutions of ferrous iron, which yield the usual red color with ammonium thiocyanate only after oxidation with $KMnO_4$ but yield quantitative results with thioglycollic acid both before and after oxidation.

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Effect of Testicular Hormone on Hypophysectomized Rats.

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The purpose of these experiments was to analyze further the factors necessary for the maintenance of the prostate. It is well known that prostatic atrophy follows either hypophysectomy or castration. The atrophy which follows castration can be prevented by the use of testicular hormone. Moore and Price[1] believe that the prostatic atrophy which follows hypophysectomy is entirely secondary to the changes which occur in the testes. Moore has quoted unpublished

[1] Michaelis, L., and Guzman Barron, E. S., *J. Biol. Chem.*, 1929, **83**, 191.

[2] Cannan, R. K., and Richardson, G. M., *Biochem. J.*, 1929, **23**, 1242.

[1] Moore, C. R., and Price, Dorothy, *Am. J. Anat.*, 1932, **50**, 13.