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Blood Iron and Copper in Hemochromatosis.

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Hemochromatosis is a rare disease of disturbed iron metabolism usually associated with diabetes and accompanied by cirrhosis of the liver, bronzed pigmentation of the skin, and by the presence of an iron-containing pigment, hemosiderin, and a non iron-reacting pigment, hemofuscin, in the skin, liver, pancreas and various other organs.

Copper has been proposed as an etiological factor in hemochromatosis by Mallory and his coworkers. Mallory, Parker and Nye[1] announced that it was possible to produce pigmentation and cirrhosis of the liver in rabbits and sheep by the administration of copper salts or metallic copper in powdered form. Mallory[2] made a careful study of a series of cases of hemochromatosis among human beings. He pointed out 2 definite factors which he believed had a bearing on the production of the disease, one, the excessive indulgence in alcohol, and the other, occupational contact with copper. In some cases both factors operated. Mallory pointed out that samples of alcohol used in the prohibition era were considerably contaminated by copper. Mallory and Parker[3] repeated their experiments on chronic poisoning with copper and again reached the same conclusions, namely, that as a result of repeated injections of copper over a long period of time a form of pigmentation cirrhosis of the liver was produced.

The results of Mallory and his associates were confirmed by Hall and Butt,[4] but have been denied by Flinn and Von Glahn,[5] by Polson,[6] by Oshima and Siebert,[7] and by Herkel.[8] Mills,[9] after a statistical study of hemochromatosis and diabetes in Koreans who

1 Mallory, F. B., Parker, F., Jr., and Nye, R. N., *J. Med. Res.*, 1921, **42**, 461.
2 Mallory, F. B., *Am. J. Path.*, 1925, **1**, 117.
3 Mallory, F. B., and Parker, F., Jr., *Am. J. Path.*, 1931, **7**, 351.
4 Hall, E. M., and Butt, E. M., *Arch. Path.*, 1928, **6**, 1.
5 Flinn, F. B., and Von Glahn, W. C., *J. Exp. Med.*, 1929, **49**, 5.
6 Polson, C. J., *Brit. J. Exp. Path.*, 1929, **10**, 241.
7 Oshima, F., and Siebert, P., *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **84**, 106.
8 Herkel, W., *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **85**, 513.
9 Mills, R. G., *J. A. M. A.*, 1925, **84**, 1326.

use copper and brass utensils almost exclusively, stated that the rate of incidence of these diseases was less than in the United States. Ramage and Sheldon[10] in a number of chemical and spectrographic analyses of tissues from patients with hemochromatosis have shown the iron content to be tremendously increased over the normal. They have also reported substantial increases in the copper and calcium content of these tissues.

In the light of Mallory's work, we studied the blood copper of 2 male adult patients with hemochromatosis and one male adult patient with a doubtful diagnosis of hemochromatosis. We also determined the blood iron in these 3 patients.

A review of the literature discloses a few scattered figures for the iron content of the blood in cases of hemochromatosis. Garrod and his coworkers[11] reported that in a case of theirs the iron was found to be 48 mg. per 100 cc. of blood. Taking 42 mg. per 100 cc. as the normal blood iron content, they concluded that their figures indicated iron retention in this case of hemochromatosis. Fowell,[12] however, working in the same hospital, had the previous year published figures for the iron content in normal blood ranging from 51 to 55 mg. per 100 cc. with an average of 54.5 mg. His figures for the blood iron content of a case of hemochromatosis were 46 mg. and 52 mg. per 100 cc. of blood. In other reports, Jeanselme[13] recorded a blood iron content of 54.2 mg. per 100 cc. in one case, Howard and Stevens[14] found 45 mg. of iron per 100 cc. of blood in their case, and Cruickshank[15] stated that in a case observed by him there was no increase in the blood iron.

All the investigations cited have been made earlier than 1921. The methods for the determination of iron and the technics employed in these cases may therefore be justifiably questioned. Another factor which makes the data difficult to evaluate is the lack of representative normal blood iron figures as a basis for comparison and the failure to recognize the difference in the blood iron content of adult males and females. With the exception of Fowell, who had a series of 13 determinations on normal individuals, none of these investigators had a normal or control group with which to compare their blood iron findings in hemochromatosis.

10 Ramage, H., and Sheldon, J. H., *Quart. J. Med.*, 1935, **4**, 121.

11 Garrod, A. E., Gaskell, J. F., Sladden, A. F., Wallis, R. L. M., and Vaile, P. T., *Quart. J. Med.*, 1914, **7**, 129.

12 Fowell, P. H. C., *Quart. J. Med.*, 1913, **6**, 179.

13 Jeanselme, *Bull. et Mem. Soc. med. d. Hop. de Paris*, 1897, **14**, 179.

14 Howard, C. P., and Stevens, F. A., *Arch. Int. Med.*, 1917, **20**, 896.

15 Cruickshank, J., *Brit. Med. J.*, 1921, **2**, 783.

Most of them compared a single determination with a normal reported by another investigator, which may have been estimated by an entirely different quantitative method. On this basis a blood iron determination of 48 mg. per 100 cc. would appear to be high by comparison with the standard of 42 mg. which Garrod adopted, while it would appear low if compared to Fowell's standard of 54.5 mg. For these reasons we feel that our results compared with our normal averages based on determinations in 200 normal adult males are much more significant.

Our findings are presented in Table I.

TABLE I.

Patient	Date Sample Drawn	Time Sample Drawn	Red Cells per Cu. mm.	Hemoglobin,* gm. per 100 cc.	Iron, mg. per 100 cc.	Copper, mg. per 100 cc.
H.	5/26/36	8 AM	4,380,000	11.21	37.56	.148
56 yrs.		4 PM	4,380,000	11.10	37.20	.149
	6/4/36	8 AM	——	12.11	40.56	.145
S.	6/4/36	8 AM	4,100,000	13.26	44.44	.148
48 yrs.		4 PM	4,100,000	12.61	42.24	.130
M.†	5/26/36	8 AM	4,010,000	11.94	40.00	.152
44 yrs.		4 PM	4,010,000	12.44	41.68	.156

*Hemoglobin calculated from blood iron by factor: Mg. of Fe divided by 3.35 = gm. of hemoglobin per 100 cc. Hemoglobin contains 0.335% Fe.[16]

†Doubtful case.

Determinations of copper were made on 5 cc. samples of whole blood by an iron precipitation modification of McFarlane's method[17] using sodium diethyldithiocarbamate. Iron determinations were made by a dry ashing method on 5 cc. samples using potassium thiocyanate reagent. Details of the copper and iron[18, 19] methods have been described previously.

The normal adult male whole blood iron content has been found to average 50 mg. per 100 cc. in a series of 200 determinations previously made in our laboratory.[18, 20] We have found the normal adult whole blood copper to be 0.132 mg. per 100 cc.[18] On the basis of these normal averages, there is no marked increase in blood iron or copper in the cases of hemochromatosis reported here. The blood iron values run from 88.9% to 74.4% of the normal average, and the red cell count from 87.6 to 80.2% of the normal, secondary

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

17 McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

18 Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

19 Fabian, A. A., Sachs, A., and Levine, V. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 662.

20 Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366.

anemia being present in all 3 cases. The low iron values in the blood may be due to the secondary anemia present or may be due to the fact that the deposition of iron in the tissues would tend to reduce the quantity of this element available for the production of hemoglobin. According to Ramage and Sheldon[10] there is also increased copper deposition in certain tissues in hemochromatosis. The absence of a high blood copper content in our cases may be the result of retention in the tissues of this element, but it more likely points to the fact that clinical reports indicate the presence of a mild anemia in hemochromatosis. We have found in general that the more severe the anemia the higher is the blood copper content, especially when there is a marked deficiency both in red cells and in hemoglobin.[18]

The blood copper is slightly higher than the normal average by 9.8 to 18.2%, but yet within the range of normal variation. One determination, however gave a blood copper figure corresponding to the average normal. In the small number of cases reported here the increase in the blood copper above the average figure is not high enough to warrant any relationship in hemochromatosis between the copper in the blood and the abnormal iron metabolism in the tissues.

* We wish to thank Dr. Russell M. Wilder of the Mayo Clinic for permitting us to make copper and iron determinations on blood samples from the three patients mentioned in this article.

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Enzyme for Decomposition of Creatinine and its Action on the "Apparent Creatinine" of Blood.

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The nature of the substance in filtrates of whole blood and plasma which gives the color with alkaline picrate (Jaffe's reaction) has been for many years a subject of controversy. One group of investigators believe that this material is true creatinine—others deny that creatinine exists in normal blood. Because of the non-specific methods employed for the identification of creatinine, and the very minute quantities of the chromogenic material available in normal blood, it has been difficult for either group to present convincing evidence.

To obtain a definitive answer regarding the nature of the Jaffe-