Of considerable interest were the findings in animals infected with the protozoa, *Trypanosoma equiperdum.* Although these animals maintained a moderately high level of eosinophile cells, there were few or no Kurloff bodies in their blood. This may be explained by the fact that in this infection there is a lymphocytosis and the monocytes are few. This relationship between the level of the monocytes and Kurloff bodies was not constant in all of the preceding animals mentioned.

These observations not only confirm the work of Benedict and De Witt, but also demonstrate that an acute bacterial infection in guinea pigs can reduce eosinophile cells but not the number of Kurloff bodies. However, if an infection results in reducing the number of monocytes as in Trypanosomiasis, the Kurloff bodies are also reduced and may become absent. In conclusion, although there appears to be a numerical relationship between the eosinophile cells and Kurloff bodies in guinea pigs' blood, the absolute number of Kurloff bodies is dependent upon the level of the monocytes.

8834 C

Reciprocal Relationship of Copper and Iron in Blood. Polycythemia Vera.

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We have already reported the blood iron content of the adult to be an average of 50 mg. per 100 cc. for men and 45 mg. per 100 cc. for women, and the blood copper 0.132 mg. or 132 micrograms per 100 cc. for men and women.[1, 2] In determining the blood iron and copper in various pathological conditions accompanied by anemia, we observed an inverse relationship between the copper and the iron in the blood. As the blood iron fell, the blood copper rose. Hypercupremia was the usual response to hypoferronemia.[1, 2]

We have already reported the blood findings in a white woman, age 60, suffering from polycythemia vera.[2] We now report another case which verifies our first findings. The aim of the treatment in vogue in this condition, which is manifested by an increase in the red cell count beyond the normal, is to cut down the number of red cells

1 Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366.
2 Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

to the physiological level. In this type of therapy it is often advisable to diminish the red cell count even below the normal in order to postpone the rapid return of the polycythemic state. The drug used, phenylhydrazine hydrochloride, is a cumulative poison, so that its destructive effect upon the erythrocytes goes on after its discontinuance. A polycythemic individual therefore in the course of the treatment now prevailing may be converted to an anemic individual with a low red cell count. In periods of non-treatment the red cells have a tendency to increase to the polycythemic stage. The periods of treatment alternated by periods of non-treatment offer a chance to study the reciprocal relationship between blood iron and blood copper.

The second case is a white woman, 65 years of age. She was under treatment a short time before being referred to us for observation and further treatment. Her red cell count was well over 7,000,000, the blood volume was increased, her spleen was enlarged, she had vertigo, the characteristic facies and other findings of a true polycythemia vera.

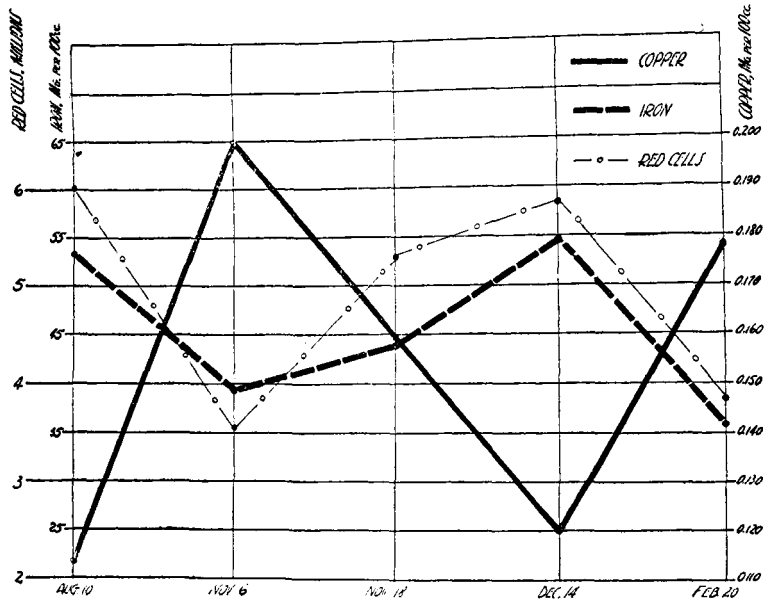
On August 10, 1935, the red cell count was 6,080,000, the blood iron was 53.16 and the blood copper 0.113 mg. or 113.5 micrograms per 100 cc., a figure below normal. She was placed on phenylhydrazine treatment, which was carried out irregularly and intermittently, since the patient showed a marked intolerance for the drug. Difficulty was experienced in finding the weekly dose of phenylhydrazine that would maintain the red cell count at the normal level. On November 6 her red cell count had dropped to 3,520,000 and her blood iron had gone down to 39.36 mg., while her blood copper had risen to 0.200 mg. or 200 micrograms per 100 cc. On November 18 after a period of non-treatment, the red cells went up to 5,376,000, the blood iron to 44 mg. per 100 cc. and the copper fell to 0.158 mg., or 158 micrograms per 100 cc. Treatment was temporarily discontinued.

On December 14 the red count was 5,760,000, the blood iron 54.79 mg. and the blood copper 0.120 mg. or 120 micrograms per 100 cc. Further treatment with phenylhydrazine being considered inadvisable due to the sensitivity of the patient to the drug, radiation with X-ray over the long bones was attempted for a short time in an effort to inhibit the bone-marrow in its production of red cells.

The patient disliked the X-ray treatments, and hence she did not receive sufficient radiation substantially to lower the red cell count. On December 30, 1935, venesection was performed and about 800 cc. of blood removed. It was now found that by decreasing the dose of phenylhydrazine, the patient could take the drug with greater toler-

ance. On February 20, 1936, the red cell count was 3,776,000, the blood iron 35.88 mg. per 100 cc. and the blood copper, 0.178 mg., or 178 micrograms per 100 cc. She is now on a weekly ration of phenylhydrazine and is progressing very well.

We have plotted graphs to show the hypoferronemia and the corresponding hypercupremia observed during treatment with phenylhydrazine and the increase in the red cells and in the blood iron during periods of non-treatment accompanied by a fall in blood copper. We have published elsewhere the graph accompanying the findings in the first case.[2] The graph accompanying the findings in the second case reported here is given below.



Fluctuations in Erythrocytes, Blood Iron, and Blood Copper in Female Patient, 65 years of age, suffering with Polycythemia Vera, in periods of treatment and non-treatment. Aug. 10, 1935, treatment with phenylhydrazine begun; Nov. 6, 1935, treatment stopped; Dec. 30, 1935, venesection performed.

Iron was determined by the Wong[3] method and the results were checked with the dry ashing method recommended by us.[5] The copper was determined by McFarlane's method.[4] Slight modifications were introduced in both procedures.[1, 2, 5]

Copper and iron are stored in the liver. It may be argued that

3 Wong, S. Y., *J. Biol. Chem.*, 1928, **77**, 409.

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

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

phenylhydrazine damages the liver and renders it unable to retain copper. The copper that is forced to leave the liver mobilizes into the circulating fluid, and as a result an increase in blood copper ensues. If this reason holds true for the rise in blood copper, we are at a loss to understand why under the same circumstances the blood iron should fall considerably.

Iron and copper may be considered catalysts of oxidation. As a matter of fact the enzymes concerned in biologic oxidation, such as peroxidase and catalase, are organic complexes containing iron.[6]

Copper is a much more active catalyst than iron. Warburg[7] cites Meyerhof, who observed that copper accelerated the oxidation of fructose to the extent of 140%, while iron only increased the oxidation by 70%. According to Voegtlin, Johnson and Rosenthal,[8] the oxidation of glutathione is easily accomplished in the presence of copper, whereas iron fails in this reaction as a catalyst. Mawson[9] has reported that ascorbic acid is relatively stable in glass distilled water, which is free from copper and iron. Copper, and to a lesser extent iron, and especially a mixture of these two elements, act as positive catalysts in the aerobic oxidation of ascorbic acid. Jones and Smedley-MacLean[10] have shown that the oxidation of phenyl derivatives of fatty acids with hydrogen peroxide is greatly accelerated by the presence of copper. Cunningham[11] has observed that copper markedly accelerated the oxidation of dopa (1,3,4 dihydroxyphenyl alanine) by dopa oxidase. Even in the absence of this enzyme the oxidation of the reagent was to some extent catalyzed by copper alone.

Keil and Nelson[12] have reported what they term "a hitherto undescribed property of copper" in connection with carbohydrate metabolism. They showed that by oral administration of copper alone to experimental animals made anemic, they could bring the abnormal anemic glucose tolerance curve for the blood back to normal values even before any hemoglobin regeneration became evident. They assigned this phenomenon to the activity of copper with relation to the physiologic oxidative processes involving carbohydrate.

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6 Kuhn, W., Hand, D. B., and Florkin, M., *Z. Physiol. Chem.*, 1931, **202**, 255.

7 Warburg, O., *Science*, 1925, **61**, 575.

8 Voegtlin, C., Johnson, J. M., and Rosenthal, S. M., *Pub. Health Rep.*, 1931, **46**, 2234.

9 Mawson, C. O., *Biochem. J.*, 1935, **29**, 569.

10 Jones, R. O., and Smedley-MacLean, I., *Biochem. J.*, 1935, **29**, 1877.

11 Cunningham, I. J., *Biochem. J.*, 1931, **25**, 1267.

12 Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1934, **106**, 343.

It seems that when the quantity of the catalyst, iron, falls below the normal, the biologic system attempts to compensate for the dangers of lessened oxidative activity by an increase in the content of the more vigorous and the more highly efficient catalyst, copper. The body needs little copper under normal conditions and the rise under abnormal conditions need not be very high, since a small quantity of this element goes a long way in the mechanism of catalysis.

In the event of hypoferronemia the observed rise in the copper content of the blood leads to the conclusion that the biologic organism mobilizes copper, which is poured into the blood from the storehouses. The copper thus mobilized not only acts in the capacity of a stimulator of hematopoietic activity, but also in the capacity of an emergency oxidative catalyst.

8835 P

Visualization of Preparalytic Lesions of Poliomyelitis by Intravital Staining.*

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The gradual descent of poliomyelitic infection, after intranasal inoculation, through the olfactory tract, the basal ganglia, midbrain, pons and medulla to the spinal cord was demonstrated in experimental animals (monkeys) by Faber and Gebhardt,[1] using the method of recovering virus from bits of nervous tissue excised on successive days of the preparalytic period. Evidence was presented by Faber[2] that the infection in man may follow a similar pathway. Since this method of recovering virus permits only a rough localization, other means were sought to obtain a more precise definition. Evans and MacCurdy[3] reported successful vital staining of poliomyelitic lesions in monkeys, but failed to state the dye used or the period of infection when the animals were examined. McClellan and Goodpasture[4] were able, by means of intravital staining with

* This study was supported by a grant from the Rockefeller Fluid Research Fund.

1 Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

2 Faber, H. K., *Medicine*, 1933, **12**, 83.

3 Evans, H. M., and MacCurdy, J. T., *Berl. klin. Wchnschr.*, 1912, **49**, 1695.

4 McClellan, R. H., and Goodpasture, E. W., *J. Med. Res.*, 1924, **44**, 201.