

Efficacy of Intraperitoneal Injection of Iron in the Nutritional Anemia of Rats.

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Hart and his collaborators¹ reported that anemic rats did not recover when pure iron salts were administered unless small amounts of copper were also given. These observations have been confirmed in several laboratories, but Myers and his collaborators² have always been able to secure some response with iron alone.

The present series of experiments continues work previously reported designed to gain some insight into the reasons for the conflicting results. It is well-known that many factors may affect intestinal absorption of iron. We have therefore studied the effect of parenterally administered solutions of ferric chloride. Rats were made anemic by restriction to a diet of certified milk, using both the technic described by Waddell *et al*³ and the method of Elvejhem and Kemmerer.⁴ No difference in the response of the anemic animals to treatment was observed with either procedure. The animals, which were severely anemic, were injected intraperitoneally with ferric chloride solutions prepared from electrolytic iron. The stock solution of iron was diluted with warm, sterile saline solution (prepared from copper-free sodium chloride) and injected immediately after dilution, using a tuberculin syringe and chromium plated needles. The dilutions were so arranged that the amount of fluid injected into each animal was always 0.50 cc. regardless of the dosage. The acidity of the original stock solution was also adjusted so that the reaction of the material injected was between pH 2.0 and pH 2.5. To avoid excessive irritation the animals received injections only on alternate days. At regular weekly intervals hemoglobin determinations and red cell counts were made upon blood obtained by clipping the tail.

The rats consumed 20 to 30 cc. of milk daily at the beginning of

¹ Hart, E. B., Steenbock, H., Waddell, J., and Elvejhem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

² Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

³ Waddell, J., Steenbock, H., Elvejhem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 769.

⁴ Elvejhem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

the experiment, increased the intake with the instigation of iron therapy, and drank as much as 90 to 100 cc. per day after attaining a weight of about 125 gm. The rats approximately tripled their original weights, ultimately weighing over 200 gm., indicating that the milk used was of satisfactory vitamin content. The rate of growth was independent of the blood response, and practically identical in the different groups irrespective of the iron dosage. All the rats, in addition to the increase in total hemoglobin that must accompany growth, were able to raise the hemoglobin concentration of the blood to an extent depending upon the dosage of iron. With 0.5 mg., or more, of iron the hemoglobin reached the normal level of 12 to 15 gm. per 100 cc. of blood in 7 weeks or less; with 0.2 mg., the rate of regeneration was slower, but was complete in about 14 weeks. Lower dosages did not result in complete restoration within 22 weeks, at which time the experiment was discontinued. These animals attained hemoglobin concentrations of 8 to 10 gm. per 100 cc. of blood. The erythrocytes of the rats in this group, however, increased from an average initial level of 2.3 to 3.3 million per cubic millimeter of blood to 11 million and over. This peculiar condition of *polycythemia accompanied by low hemoglobin values* was manifest after 11 weeks on the curative regimen and was seen in all the animals in this group until the end of the experiment 3 months later. Although no data were obtained on the blood volume we believe this chronic condition was due to the production of red cells outstripping the rate of hemoglobin formation, which, in turn, was limited by the amount of iron administered to the animals.

Further experiments were performed to ascertain the effect of added copper on the rate of regeneration observed with injected iron. Anemic rats received injections of iron, as before, the dosages ranging from 0.025 to 0.50 mg. of iron every other day. Some animals received in addition 0.025 mg. of copper in the form of copper sulfate solution, which was either injected with the iron or fed by mouth on alternate days. The total number of rats receiving iron supplements was 33; of those receiving supplements of iron and copper, the number was 18. Within an observation period of 6 weeks the rate of growth was identical in both groups, irrespective of the presence or absence of copper, or the dosage of iron. The red cells and hemoglobin of the blood also increased, the extent of hemoglobin regeneration depending only on the dosage of iron. All previous workers who have found the oral administration of pure iron salts to be effective in curing anemic rats have noted that the addition of copper causes a marked acceleration of the cure. The failure of copper

to speed up the rate of hemoglobin production, when the iron is administered parenterally, indicates to us that the rôle of copper in hemoglobin production must now be evaluated in terms of iron absorption.

The milk used contained an average of 0.34 mg. of copper per liter, as estimated by the Biazzo procedure described by Ansbacher, Remington and Culp.⁵ Keil and Nelson^{6, 7} have reported the failure of pure iron to cure nutritional anemia, when the milk contained 0.24 mg. of copper per liter. However, in a few rats receiving the same source of milk they found that intraperitoneally injected iron was curative. Our results confirm and extend theirs, although the copper content was somewhat higher in the milk used by us. Preliminary experiments performed in this laboratory indicate that the administration of injected iron solutions also cures rats made anemic on milk containing only 0.14 mg. of copper per liter.

6706

Variations in Serum Calcium and Phosphorus During Pregnancy.

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The serum calcium and phosphorus were followed in a series of 579 dispensary patients throughout the course of their pregnancy. 3501 determinations were made in the interval between 28 weeks before delivery and 7 weeks postpartum.

Two factors were found to influence the calcium, the development of the pregnancy and the season. As the pregnancy progressed there was a gradual decline which reached a maximum a few weeks before delivery. This decline was not so sharp, from 9.86 mg. to 9.64 mg., during the first 4 months of the year, a season of low values, as during the remaining months, 10.46 mg. to 9.90 mg., but the drop is still significant. The last few weeks preceding delivery show a slight rise in both cases, succeeded immediately after the delivery by a marked increase, to 10.20 mg. and 10.40 mg. respect-

⁵ Ansbacher, S., Remington, R. E., and Culp, F. B., *J. Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 314.

⁶ Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1931, **93**, 49.

⁷ Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.