

**Factors Involved in the Regeneration of Hemoglobin.**

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All of the experiments were performed on rats. The animals were made anemic by feeding whole milk, collected in glass containers. The materials studied for hemoglobin regeneration were all tested for copper and used only when this element was absent. Hemoglobin was determined by the Newcomer method. The animals were bled by the tail.

There was no effect on hemoglobin when anemic rats were fed 1, 5, and 10 mg. Fe as  $\text{FeCl}_3$  together with whole milk. We then studied the effect of tyrosine, tryptophane, glutamic acid, aspartic acid, and arginine on hemoglobin formation. The rats were fed on milk when 30 days old and after 4 weeks were given 100 mg. of amino acid plus 0.5 mg. of Fe as  $\text{FeCl}_3$  daily. Regeneration of hemoglobin did not occur on any of the amino acids employed. The conflicting results in the literature on the potency of various elements in hemoglobin building may be due to different amounts being absorbed; to test this point we injected various salts intraperitoneally. The following elements were tested: As, Mn, V, Ge, Ni, Zn, Cr, Ti, Se, Hg, and Rb. Most of the metals were injected at levels from 0.05 mg. to 0.1 mg. daily. Arsenic was injected at 0.01 to 0.2 mg. daily. None of these elements caused regeneration of hemoglobin; in fact, the only element we have thus far found which has a positive effect is copper. Although we found that Fe as  $\text{FeCl}_3$  when administered orally did not stimulate hemoglobin regeneration, we were anxious to ascertain if the same results would be obtained by intraperitoneal injection of the Fe. Three mg. of Fe as  $\text{FeCl}_3$  injected every other day into anemic rats increased the hemoglobin from initial levels of 3.0 to 5.5 gm. to final values of 9.5 to 12.1 gm. per 100 cc. of blood. Injections of  $\text{FeCl}_3$  are severe on the animal resulting in necrosis of the surrounding tissue at the point of injection. Intraperitoneal injection of aqueous ferric citrate or  $\text{FeCl}_3$  dissolved in glycerine were found not to have the deleterious effect of aqueous  $\text{FeCl}_3$  and to stimulate hemoglobin regeneration. The utilization of the iron under these conditions appears to be temporary. It does not mean a utilization of Fe to the exclusion of copper, but may denote a failure of absorption of iron in nutritional anemia. Five rats were made anemic by feeding milk. They

ranged in hemoglobin from 3.1 to 5.0 gm. per 100 cc. of blood. They were then given orally 0.05 mg. Cu as  $\text{CuSO}_4$  daily and were injected every other day with 1 mg. of Fe as a suspension of  $\text{Fe}(\text{OH})_3$ . The collodion dialysate of the  $\text{Fe}(\text{OH})_3$  showed no test for Fe by  $\text{KCN}$ S. After 11 weeks the final HHB values ranged from 13.2 to 16.3 gm. per 100 cc. of blood.

Detre<sup>1</sup> showed that hemorrhagic anemia responds to treatment with acids. This led us to a study of the effect of HCl on hemoglobin regeneration in nutritional anemia. The acid was prepared by decomposing  $\text{MgCl}_2$  with  $\text{H}_2\text{SO}_4$  and passing the HCl so formed into Cu free water through a drying train to dry the gas and prevent the carrying over mechanically of traces of Cu.

Four rats were made anemic with milk and had initial hemoglobin values of 6.5, 8.0, 7.4, and 8.2 gm. per 100 cc. of blood. They were then injected intraperitoneally with 1 cc. of 0.0333 N HCl every other day. After 5 weeks the hemoglobin values were: 6.8, 10.2, 8.7, and 11.0. A second lot of 5 rats were treated in the same way, except 1 cc. of 0.092 N HCl was used for the injection. These rats had initial hemoglobin values of 4.1, 4.0, 6.5, 4.3, and 7.2. The maximum values found in about 3 weeks were 8.7, 7.5, 8.9, 6.7, and 9.8 gm. per 100 cc. of blood. After this time the hemoglobin fell so that in 7 weeks the values were 6.0, 5.0, 8.6, 6.1, and 5.8. The acid injection was then discontinued and 0.01 mg. Cu as  $\text{CuSO}_4$  was injected every other day. After 4 weeks the hemoglobin values were 12.0, 15.4, 16.2, 14.5, and 15.1. A third group of 4 animals were injected with 0.5 cc. of 0.092 N HCl every other day. The initial readings were: 6.1, 6.0, 5.7, and 5.7. The maximum hemoglobin levels reached in about 14 weeks were: 10.3, 11.5, 12.0, and 13.5. The last 2 animals died shortly after the final readings of HHB were made. At the end of 18 weeks, the first 2 animals had HHB values of 9.1 and 8.2. The first lot of rats on acid received  $\text{FeCl}_3$  in the milk and the last 2 lots  $\text{Fe}(\text{OH})_3$ , equivalent to 0.50 mg. of Fe daily. Acid apparently causes a temporary rise in hemoglobin, but later the level of hemoglobin falls.

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<sup>1</sup> Detre, L., *Klin. Wochenschr.*, 1929, **8**, 1312.