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STUDIES ON REGENERATION OF HEMOGLOBIN

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Many investigators in the past few years have been interested in the problem of the relation of iron to anemia. This problem originated in connection with studies involving a comparison of inorganic versus organic iron, which later led to studies on the relation of solubility of iron compounds to regeneration of hemoglobin and the treatment of anemia. Following this, studies of ferric as compared to ferrous compounds in iron therapy were made and then, in the last few years, the effect of copper in hemoglobin building has attracted the attention of a considerable number of workers in laboratories throughout this country and in foreign universities as well. Hart, Steenbock, and co-workers (1) were the first to call attention to the fact that pure iron salts were ineffective in the cure of nutritional anemia and that the addition of a very small quantity of copper salt to the iron salt resulted in hemoglobin synthesis. Evvard, Nelson, and Sewell (2) conducted some investigations on the nutritional role of copper simultaneously with Hart, Steenbock, and co-workers and noted that the greater part of the stored copper was in the liver. They observed, furthermore, that rats and swine make better gains and exhibit a higher food utilization per unit of weight increase when small amounts of copper sulphate are incorporated in the ration. They stated at this time, "It is possible that the medicinal and nutritive value of liver and its proper functioning may be somehow related to this element." A considerable number of investigators have not supported the work of Hart and his fellow workers. It is not necessary to cite all the evidence for or against copper in hematopoiesis. However, the data by Drabkin and co-workers (3) and Beard and Myers (4) are at such variance with the results obtained by the Wisconsin workers that it became evident further work on this problem was necesary before definite conclusions could be made. Drabkin and Miller (3) showed that on the basis of daily consumption the anemia produced in rats on a diet of cow's milk can be cured by synthetic diets containing less copper than is present in milk. They observed that hemoglobin regeneration was very rapid upon a synthetic diet which was deficient in vitamins and

salts and which contained only a small amount of iron. The quality of the protein was different in the synthetic diet as compared to milk, and the level of protein fed per day in the synthetic diet was higher than in milk; consequently, they were led to believe that certain amino acids play a part in hematopoiesis. They claim that arginine and glutamic acid were very effective in hemoglobin building, whereas certain other amino acids were less potent or had no effect at all. Beard and Myers (4) state, "The view that inorganic Fe cannot be utilized by anemic young rats for blood regeneration is not supported by the experiments recorded in this paper —. These findings are quite in agreement with the observations of Mitchell and Schmidt, Drabkin and Waggoner, and Keil and Nelson on the rat and Whipple and Robscheit-Robbins, Riecker, Riecker and Winters, and Steiger on the dog —." Furthermore Myers and Beard (5) say that other elements are effective in hemoglobin regeneration, especially so the elements Ni, Ge, Mn, As, Ti, Zn, Rb, Cr, V, Se, and Hg.

The experiments recorded in this paper were planned to answer certain questions; first, do amino acids have any effect on regeneration of hemoglobin? Secondly, does iron alone, especially in high doses, have the capacity to regenerate the respiratory pigment? Thirdly, do other elements behave like copper?

Rats were used in all of the experiments. Anemia was produced by an exclusive milk diet. The milk was obtained from pure bred Holstein cows, and special care was taken to collect the milk directly into glass containers. All of the animals were housed in individual cages of galvanized netting, and the bottoms of the cages were composed of the same material to avoid coprophagy. Copper free water was used for washing the cages and utensils.

The amino acids were isolated in the laboratory by the ordinary methods of preparation. It is not necesary to record these procedures. They were pure and conformed to the various criteria of purity employed. Furthermore, purchased amino acids gave the same results as those prepared in the laboratory. The following amino acids were studied; tyrosine, tryptophane, glutamic acid, aspartic acid, and arginine. The animals were placed on milk when thirty days old, and, after one month on this diet, were given 100 mg. of the different amino acids in the milk daily plus 0.50 mg. of Fe as $FeCl_3$. None of the amino acids caused regeneration of hemoglobin. The blood contained between 5.80 and 9.50 per cent of hemoglobin at the start of the experiment, and after four to six weeks the hemoglobin values ranged from five to nine percent.

The next point to be investigated was whether iron in large amounts would stimulate hemoglobin building. Three groups of anemic rats were fed one, five and ten mg. Fe as $FeCl_3$ daily in addition to milk. The hemoglobin values of these rats at the beginning of the experiment averaged nine percent and after eight weeks on the different iron levels the hemoglobin ranged from 4.20 to seven percent. In other words as much as ten mg. of Fe daily failed in hematopoiesis. This is forty times the minimum employed by Beard and Myers and on which they say recovery was obtained in six weeks. Ten mg. of Fe as $FeCl_3$ was the maximum we were able to add and still have the animals consume the diet.

The following elements were next injected intraperitoneally in order to see if they had any effect on hemoglobin regeneration: — Ni, Zn, Ge, Mn, V, As, Ti, Se, Hg, Rb, Cr, and Cu. Ni, Zn, Mn, Rb, and Cr were made up as chlorides. Ge and As were used as oxides. V_2O_5 in HCl was a source of vanadium. TiO_2 was dissolved in aqua regia. Selenic acid was a source of selenium. Mercuric acetate was a source of mercury and $CuSO_4$ was used as a source of copper. Of all these elements copper was the only one to cause regeneration of hemoglobin. The rats received a diet of milk collected in glass together with 0.50 mg. of Fe as $FeCl_3$ daily. The elements were injected at a level of 0.05 to 0.10 mg. daily. The hemoglobin curves fell in all cases except for the animals receiving Cu as $CuSO_4$. As little as 0.005 mg. Cu as $CuSO_4$ caused marked effect so that in less than four weeks the hemoglobin was normal.

We have been interested in the mechanism of copper in hematopoiesis and in some of our work injected iron salts intraperitoneally. Some of the animals from the cobalt and germanium experiments above were used for this work. The hemoglobin values of the blood of these animals were 5.50, 4.50, 4.20, 3.00, 4.00, 3.50, and 4.50 gms. per 100 cc. of blood. Three mgs. of Fe as $FeCl_3$ were injected intraperitoneally, the rats receiving only milk collected in glass, and four weeks later the hemoglobin rose to 11.8, 12.0, 9.50, 12.40, 14.50, 9.70, and 13.10 gm. per 100 cc. of blood respectively. This same $FeCl_3$ failed to stimulate formation of hemoglobin when fed orally at a level of ten mg. daily. Aqueous solutions of $FeCl_3$, when injected intraperitoneally, cause lesions, necrosis, and sloughing off of the hair and skin where applied. $FeCl_3$ dissolved in glycerol does not have this effect. One mg. of Fe as $Fecl_3$ in glycerol was injected intraperitoneally every other day to rats made anemic by milk feeding. The initial readings of

hemoglobin were 7.00, 7.10, 4.50, 9.10, 6.50, 8.90, 4.80, 6.60, 6.50, 8.70, 6.00, 5.20, 6.00, 5.50, 4.30, and 5.50 gm. per 100 cc. blood. The final amounts of hemoglobin in the same order were: — 9.20, 9.30, 8.00, 10.2, 9.40, 11.5, 10.6, 6.00, 10.0, 9.50, 10.5, 7.50, 8.30, 11.4, 12.5, and 10.0 gms. per 100 cc. of blood. Ferric citrate when injected intraperitoneally gives results comparable to $FeCl_3$. No irritation or necrosis results, however. Four rats fed milk until anemic were given one mg. of Fe as citrate intraperitoneally every other day. The initial hemoglobin values were, 6.50, 5.80, 6.70, and 7.70 gms. per 100 cc. of blood. The maximum hemoglobin values were 12.5, 12.0, 14.0, and 12.7 gms. per 100 cc. of blood. The ferric citrate was prepared from purified materials and when fed orally at a level of 3.30 mg. daily failed in hematopoiesis. Ferric hydroxide when administered orally can be used as a source of iron. We were interested to ascertain if it could be utilized when injected intraperitoneally. Five rats were made anemic by feeding milk. The hemoglobin varied from 3.10 to 5.00 gm. per 100 cc. of blood. They were given 0.05 mg. Cu as $CuSO_4$ in the milk daily and one mg. of Fe as Fe $(OH)_3$ was injected every other day intraperitoneally. The collodion dialysate of the Fe-$(OH)_3$ suspension gave no test for Fe with KCnS. After eleven weeks the final hemoglobin values ranged from 13.2 to 16.3. Injections of $Fe(OH)_3$ either subcutaneously or intraperitoneally did not stimulate hematopoiesis in anemic rats fed milk without copper. Ferric hydroxide does not cause necrosis like $FeCl_3$.

SUMMARY

Pure iron, in the form of $FeCl_3$, does not stimulate regeneration of hemoglobin when fed to anemic rats at a level as high as ten mg. daily. This is the highest amount of iron that could be fed and still have the animals consume the food.

Tryptophane, tyrosine, aspartic acid, glutamic acid, and arginine failed in hematopoiesis when fed at a level of 100 mg. daily to anemic rats.

Of all the elements studied Cu was found to be the only one which had a positive effect on hemoglobin building. Intraperitoneal injections of salts of Ni, Zn, Ge, Mn, V. As, Ti, Se, Hg, Rb, and Cr failed to increase the amount of hemoglobin in anemic rats.

Intraperitoneal injection of $FeCl_3$ or ferric citrate into rats with nutritional anemia caused an increase in hemoglobin.

Pure $Fe(OH)_3$ stimulated hemoglobin regeneration when administered intraperitoneally to anemic rats on milk and Cu. When injected either subcutaneously or intraperitoneally to rats not receiving Cu regeneration did not occur.

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