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THE ROLE OF COPPER IN HEMOGLOBIN REGENERATION

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During the past few years a considerable amount of investigation has been devoted to the part played by copper in hemoglobin formation. Hart, Steenbock, Waddell, and Elvehjem (1) were the first to show that in nutritional anemia regeneration of hemoglobin does not occur when pure iron salts are administered, but if a small amount of copper as copper sulphate be added along with the iron salt regeneration is very rapid. McHargue, Healy, and Hill (2) came to the same conclusion shortly after the announcement by Hart and co-workers. Evvard, Nelson, and Sewell (3) found that rats and swine make better gains and show a higher food utilization per unit of weight increase when small amounts of copper sulphate are mixed in the ration. When the rats fed copper sulphate were ashed it was observed that the greater part of the stored copper was confined to the liver. The authors stated at this early date; "It is possible that the medicinal and nutritive value of liver and its proper functioning may be somehow related to this element."

Since the above work was done a large number of investigators have brought forth a considerable amount of data both for and against the necessity of copper in hemoglobin building. Some workers contend that iron alone in the form of certain salts, such as the chloride and sulphate, is sufficient for hematopoiesis when administered to anemic animals in appropriate quantity. Other investigators claim that other elements besides copper have the capacity of stimulating hemoglobin regeneration in anemic animals, and that the effect is not specific for copper. It is not necessary to review all of the work pertaining to this particular subject since the above references are sufficient for the purpose at hand. Further evidence for or against the necessity of copper in hemoglobin regeneration has been presented by Lewis, Weichselbaum, and McGhee (4), Krauss (5), Elden, Sperry, Robscheit-Robbins, and Whipple (6), Drabkin and Waggoner (7), Myers and Beard (8), Mitchell and co-workers (9), Keil and Nelson (10), Titus, Cave, and Hughes (11), Underhill, Orten, and Lewis (12), and Williamson and Ewing (13).

The object of the work reported in this paper is to determine if copper is unique in its capacity to stimulate the formation of hemoglobin or if other elements have this same property. The results reported here are the outcome of researches in progress for the past two years.

EXPERIMENTAL

The clear cut results of Hart and co-workers prompted us to duplicate their methods whenever possible. A group of female and male rats were selected at thirty days of age from the Iowa State College Stock Colony and were fed the Wisconsin ration consisting of: ground yellow corn 76, linseed oil meal 16, crude casein 5, alfalfa meal 2, calcium carbonate 0.5, and sodium chloride 0.5. The young when weaned were placed in galvanized iron wire cages and fed upon milk which was collected from two pure bred Holstein cows. The rats on this milk became anemic within five to seven weeks. They were then transferred to individual cages of hardware cloth which were supported on glass rods over galvanized pans containing shavings for litter. These cages were kept in a special room in order to minimize contamination due to dust and air-borne particles of feed. All feeding utensils were washed with copper free water.

The ferric chloride was made from either Baker's analyzed iron wire or from electrolytic iron and purified by the method of Hart, Steenbock, and co-workers (14). All of the iron solutions were analyzed by the KCnS method so that the amount administered might be known quantitatively. A series of elements were investigated to determine whether these substances could replace copper in hematopoiesis. The elements studied were: V, Ti, Mn, Cu, Ni, As, Ge, Zn, Cr, Co, Sn, and Hg. Salts of the above elements were dissolved in copper free water. Condensed spark spectrograms were made upon all solutions by means of a Hilger quartz prism spectrograph employing carbon electrodes, the lower one being hollowed out so that it might contain the solution at all times during the exposure. This technique proved sufficient to bring out the ultimate lines in a known copper standard where the concentration was 0.0005 mg. Cu. per cc. Those solutions showing Cu lines were discarded. The substances showing Cu lines were further purified and only used when the spectrographic examination failed to show the presence of Cu. The different solutions were accurately measured in separate graduated pipettes to insure quantitative administration.

Each curve in chart I represents data from five to ten rats. It is evident from the downward trend of the curves that, of all the elements tested, copper is the only one which can make iron available for Hemoglobin regeneration in anemic rats. These results are in harmony with the work of Hart and co-workers but do not agree with the data of Myers and Beard (15) or Drabkin and Waggoner (7). The animals used in the experiments for Chart I received milk collected directly in glass vessels from pure bred Holstein cows.

It is possible that the conflicting results obtained by many investigators who have studied this problem may be due to contamination of the milk with copper. Under such conditions the addition of pure iron will cause regeneration and unwarranted conclusions regarding Fe and other elements will be drawn. Ordinary market milk will lead to such results. This is illustrated in

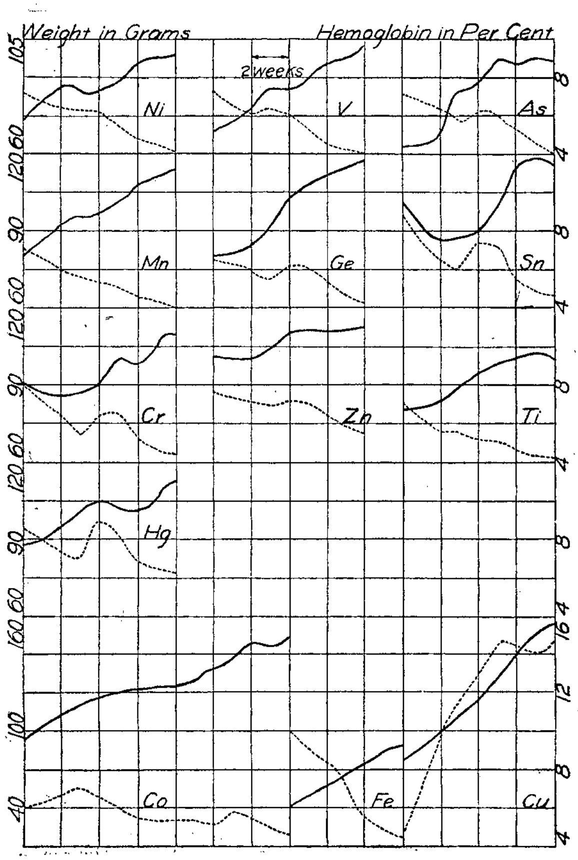

Chart I

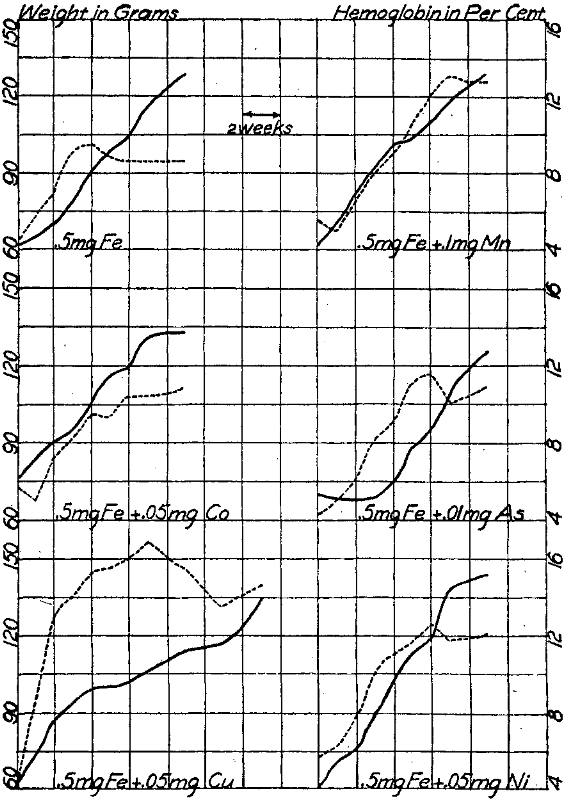

Chart II

Chart II. It is evident from the data that pure iron caused some regeneration with market milk, but that it failed to do so when added to milk collected in glass. It is also to be noted that when market milk was used, Mn as chloride gave better results than iron alone. We have no explanation for this. It is obvious from the data in Chart I that Mn cannot replace Cu in hematopoieses. Arsenic and nickel also gave better results when market milk was used than did Fe alone, although the results were not as pronounced as in the case of Mn. Although copper is specific in its action on hematopoiesis, it is possible that elements such as Mn, As, and Ni may enhance the effect of the copper alone when used along with copper. Further work is in progress on this point. Analyses of the milk collected in glass showed a copper content between 0.20 mg. and 0.25 mg. per liter. The market milk had a copper value between 0.35 mg. and 0.44 mg. per liter. The copper was determined by the carbamate method.

We desire to express our appreciation to the Dairy Husbandry Department, Iowa State College, for their kindness in supplying milk for these experiments.

SUMMARY

1. Rats on a whole milk diet readily develop anemia.

2. Pure iron as ferric chloride when added to milk collected in glass does not cause regeneration of the respiratory pigment.

3. Copper was the only element of those tested which had a positive effect on hemoglobin formation when fed to anemic rats receiving milk collected in glass plus pure $FeCl_3$.

4. Salts of V, Ti, Mn, Ni, As, Ge, Zn, Cr, Co, Sn, and Hg did not stimulate hemoglobin regeneration when added to milk, collected in glass, and fed together with pure iron as the chloride.

5. Hemoglobin regeneration is possible with pure iron and market milk because the latter contains some copper.

6. Manganese, arsenic, and nickel together with $FeCl_3$ gave better results than iron alone when added to market milk.

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