DOES LIVER SUPPLY FACTORS IN ADDITION TO IRON AND COPPER FOR HEMOGLOBIN REGENERATION IN NUTRITIONAL ANEMIA?*

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According to our present knowledge, the nutrients needed for maximum hemoglobin regeneration in uncomplicated secondary anemia are iron, copper, and amino acids for the construction of the protein part of the hemoglobin molecule. Since very rapid hemoglobin regeneration has been obtained in both rats and children on diets of whole milk plus iron and copper, we may conclude that milk is low only in these two elements as far as hemoglobin formation is concerned, and that the proteins in milk supply the amino acids essential for the production of the hemoglobin molecule. We should mention that milk may be low in manganese and an unknown organic factor, both of which are necessary for normal growth, but independent of hemoglobin formation.

In spite of these facts and probably because liver is such a valuable material for the treatment of pernicious anemia, great emphasis has been placed on the use of liver and liver products in secondary anemia. Minot and Castle (1) pointed out in 1931 that whole liver was not highly active in various types of human secondary anemia. Elvehjem (2), 1932, suggested that the limitations of liver in treatment of secondary anemia were probably due to the relatively low level of available iron supplied by most samples of liver.

However, Whipple, Robscheit-Robbins, and Walden (3) have described a secondary anemia fraction for liver which is active in hemoglobin regeneration in dogs suffering from hemorrhagic anemia. Sturgis and Farrar (4), using the technique described by Whipple and

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associates, found that liver promoted a definitely greater regeneration of hemoglobin than did the addition of an amount of inorganic iron equivalent to that contained in the added liver. They also showed that the response was no greater with iron plus casein than with iron alone which eliminates the possible protein effect of the liver. In spite of these facts high levels of inorganic iron have proved very effective in the treatment of a variety of hypochromic anemias. There is still some question about the reason for the need of such large amounts. Brock and Hunter (5) have shown that large doses are not needed for assimilation, and that when large amounts are fed the percentage utilization is very low. It is probable that the high intake of iron salts supplies added amounts of copper as a contamination, but this has not been demonstrated beyond question. Similarly in the experiments involving hemorrhagic anemia in dogs, no definite comparison has been made between the results obtained when whole liver is fed and both iron and copper are fed in amounts equivalent to that found in the liver. Whipple's diet contains some copper but according to the figures of Sturgis and Farrar the copper intake on the basal ration alone is quite low. The intake varies from 0.06 to 0.07 mg. per kilo of body weight per day. The requirement for a rat is at least 0.2 mg. per kilo. When liver was fed the intake of copper was raised to about 0.5 mg., which would be very close to the optimum intake. This may explain why Sturgis and Farrar obtained the increased hemoglobin regeneration with liver as contrasted with iron alone in hemorrhagic anemia.

We must also recognize that by bleeding many blood constituents are removed, the regeneration of which may require factors other than those needed for hemoglobin production.

In this paper we wish to emphasize that liver contains no factors in addition to iron and copper which can function as a supplement to milk for hemoglobin regeneration in rats.

EXPERIMENTAL

The usual technique was used for the production of the anemia, for feeding the animals, and for making the blood tests. The anemia was produced by restricting the young rats kept on galvanized wire screens to cow's milk. The records in most cases are the average of a number of results obtained over a period of several years. Since records have been chosen from rats showing very similar growth responses, the growth curves have been eliminated and only the hemoglobin curves are presented. These curves are the composite record of four animals.

In Chart 1 results are given for rats receiving liver in addition to adequate amounts of iron and copper, for rats receiving liver as a

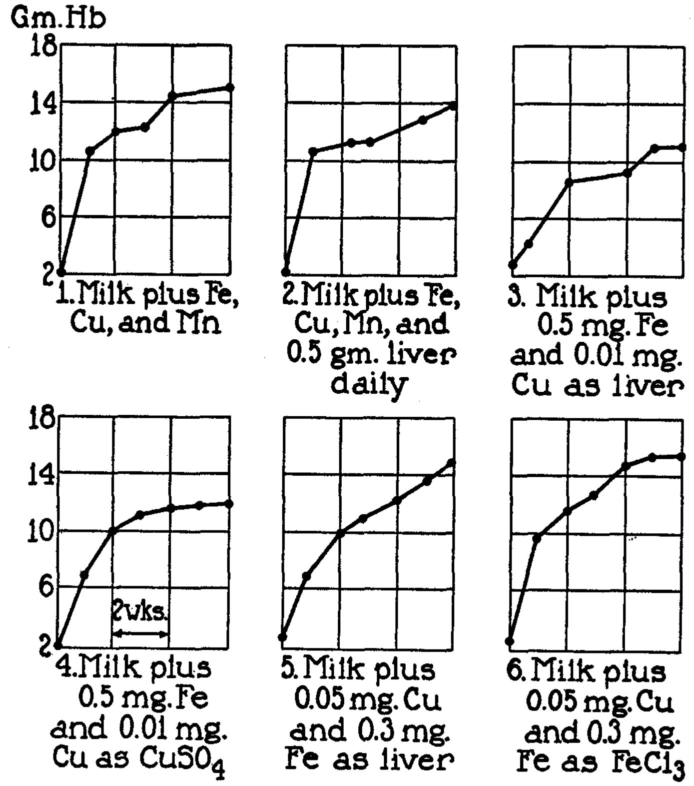

CHART 1. Curves showing the hemoglobin response in anemic rats on a milk diet when given liver in addition to iron, copper, and manganese, when given liver as a source of copper, and when given liver as a source of iron.

supply of copper, and for rats receiving liver as a supply of iron. In all cases the liver was obtained directly from the packing plant and dried at 65°C. Both beef and hog liver have been used. Curve 1 shows the typical response of rats receiving 0.5 mg. Fe, 0.05 mg. Cu, and 0.05 mg. Mn daily in addition to whole milk. Hundreds of curves of this type have been obtained during the past 7 years. Curve 2 illustrates the average response of ten animals which received the same mineral supplement plus 0.5 gm. dry beef liver per day. The results are identical. Thus the liver produced no change in the course of regeneration. The remaining curves show that liver can serve as a source of both iron and copper when the intake of these elements from the basal diet is limited. Curves 3 and 4 are taken from a recent paper by Schultze, Elvehjem, and Hart (6). The rats in both groups received 0.5 mg. Fe daily. Curve 3 shows the response in the rats receiving 0.01 mg. Cu in the form of hog liver and Curve 4 shows the response in those receiving 0.01 mg. as $CuSO_4$. The results are very similar and show that when copper is the limiting factor the effectiveness of liver is directly proportional to its copper content. The response in either case is not optimum because of the low level of copper used. Curves 5 and 6 show similar results in the case of iron. These rats received 0.05 mg. Cu per day. Curve 5 illustrates the response obtained in rats receiving 0.3 mg. total iron from beef liver (1.15 gm. dry basis) and curve 6 the response when 0.3 mg. of $FeCl_3$ was fed daily. Again the response is very similar although the regeneration in the rats receiving the liver was somewhat retarded the first 2 weeks. This can be accounted for by the fact that not all the iron in liver is available to the rat. This sample of liver contained 70 per cent of the total iron in available form. When iron is the limiting factor in the basal ration, the value of liver depends upon its available iron content.

A very large number of results of this type could be presented. They all show that the value of liver in the treatment of nutritional anemia depends directly upon the amount of available iron and copper which the liver can supply. Very recently several products have been placed on the market in which iron or iron and copper preparations have been combined with whole liver, liver extract, or other liver preparations. Liver extract is, of course, very low in iron but does contain some copper. In fact liver extract was one of the products used in the early experiments which demonstrated the essential nature of copper. In order to determine if these combinations have any virtue in simple hemoglobin regeneration beyond their copper and iron content, several preparations were purchased on the market, analyzed for iron and copper, and fed to rats at levels sufficient to supply 0.5 mg. Fe daily. Typical results for four of these preparations are given in Chart 2. Results for control rats receiving 0.5 mg. Fe and 0.05 mg. Cu daily are included for comparison. Preparations A and C gave responses very similar to the controls. In both cases the daily dose necessary to supply 0.5 mg. Fe contained about 0.02 mg. Cu, a level which is sufficient to give good regeneration. Sample B gave a fairly good response and supplied 0.008 mg. copper daily, which is below the needed amount. The response with sample D was

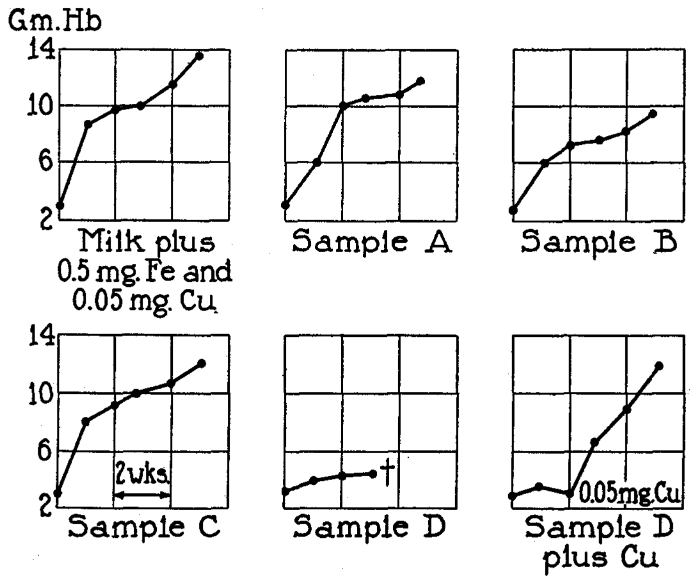

CHART 2. Hemoglobin response in anemic rats on milk diet supplemented with various commercial liver preparations when fed at levels which supplied 0.5 mg. Fe daily.

practically negative due to the low copper intake of 0.0005 mg. daily. Further evidence that this is a true copper deficiency is shown in the next curve, where 0.05 mg. copper is added after 2 weeks on the preparation alone. An immediate response resulted when the copper was added. Here again the potency of these products for the cure of nutritional anemia in rats is directly related to the iron and copper content and the ratio of one element to the other. The presence of the added liver products adds nothing to their value for this particular purpose.

These facts do not detract from the nutritional value of liver extract, for there is ample evidence in the literature that this product is a most excellent source of most of the factors in the B complex, but they do emphasize the importance of recognizing the value and limitations of each ingredient in such mixtures. In the case of anemia due to a simple deficiency of iron and copper, which is the case in many children, small doses of iron and copper work very efficiently with no other additions. When milk constitutes a large part of the diet it supplies ample amounts of the other nutrients needed for hemoglobin formation. In more complicated deficiencies the anemia should be separated from the other disturbances. If the additional disturbances are due to a lack of certain of the B vitamins, the pernicious anemia factor, or protein, liver extract may be used very efficiently. Each material should stand on its own merits and be used only when there is a need of the nutrients which it supplies. False association or the so called shotgun therapy should be discouraged.

SUMMARY

1. Our data indicate that the effectiveness of whole liver in the treatment of nutritional anemia in rats induced by a milk diet is directly proportional to its available iron and copper content. The other constituents in liver are not needed for maximum hemoglobin regeneration on a diet of milk, iron, copper, and manganese.

2. Commercial preparations of liver products with iron or iron and copper vary greatly in their hemoglobin-regenerating efficiency in rats with nutritional anemia. The variation is correlated directly with the iron and copper content of the preparation. When the copper-iron ratio was too wide hemoglobin regeneration was checked, although the iron supply was sufficient for optimum regeneration.

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