THE ACTION OF COPPER IN IRON METABOLISM*

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The importance of copper as a supplement to iron for hemo-
globin regeneration in anemic rats was first demonstrated 4 years
ago by Hart, Steenbock, Waddell, and Elvehjem (1). This work
showed definitely that in the presence of copper, soluble inorganic
iron salts can be used directly for hemoglobin formation. Since
that time a number of workers have studied factors affecting hemo-
globin production in rats rendered anemic by whole milk diets.
Most of this work has verified the original conclusions concerning
the importance of copper, and today, although there are some who
still feel that copper is not the only element in addition to iron
which possesses hematopoietic properties, practically all workers
agree that copper is an active agent in hemoglobin synthesis.

In most of these studies the activity of copper has been measured
by comparing the increase in the hemoglobin content of the blood
of anemic rats when pure iron alone is fed with the improvement
obtained when the iron is accompanied by traces of copper. Aside
from the fact that the hemoglobin molecule does not contain
copper (2), nothing is known about the action of this element. In
other words, we know that copper is necessary for the production
of the finished blood pigment, but we have no understanding of
how it functions in this interesting synthesis.

In this paper we wish to present the results obtained when
special emphasis was placed on the action of copper on the storage
and utilization of iron in the animal body. Since the liver and

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cultural Experiment Station.
Spleen are known to be storage centers for iron a study has been made of the changes brought about in the iron content of these organs when copper was added to the diet of anemic animals and the iron thereby made available for hemoglobin formation.

**EXPERIMENTAL**

Rats were used throughout the entire investigation and were rendered anemic according to the technique described by Elvehjem and Kemmerer (3). The essential points in this method are: the young together with the mothers are placed on screens at birth, the mother is fed milk in the same cage but removed to another cage daily to receive the stock ration, and the young are weaned at 21 days of age when they are placed on whole cow's milk.

The young raised under these conditions become anemic much earlier than rats raised to weaning in an ordinary stock colony. In order to determine whether this difference is due to a reduced iron storage during the suckling period, the total iron content of a number of young was determined at short intervals between birth and weaning. For the purpose of comparison a similar study was made on a group of young raised in the ordinary way. A total of six litters was used; three were raised on screens and three on shavings. One rat in each litter was killed at birth, and one when the litter was 6, 14, 19, and 28 days old. The animals were dried and analyzed for iron.

Curves showing the variations in the total iron per rat and the mg. of Fe per gm. of dry material during the nursing period for the two groups are given in Chart I. The curve representing the iron content of rats reared on shavings and in the presence of the mother's ration is very similar to the curve given by Smythe and Miller (4) for normal young. It also substantiates their conclusions, that the absolute amount of iron in each rat increases with age, although the percentage of iron decreases during the same period.

The curves for the rats raised on shavings and on screens are quite similar until the animals are 14 days old. Even the rats raised on screens show an increase in total iron during the nursing period. When the rats are 10 days old the total iron content has increased to about twice the amount present at birth. The average increase for a number of rats during this time was found
to be 0.25 mg. of Fe. Since there is no other source of iron, this amount must come from the mother's milk. This indicates that the young rats must consume more than 100 cc. of milk in 10 days or that the iron content of rat milk is considerably higher than that of cow's milk. An attempt was made to determine the iron content of rat milk by removing the milk from the stomachs of day old rats directly after nursing. This method cannot be considered very accurate, but the individual results were found to be fairly constant; namely, 50 mg. of Fe per kilo of dry milk. This is about 3 times that of cow's milk.

The latter part of the curve for the rats reared on screens readily explains the early development of anemia in these animals.
The rats raised under the restricted condition store much less iron than the others and are unable therefore to maintain the hemoglobin content of the blood for as long a period after weaning. If the rats are weaned at 21 days those raised by the old method contain almost twice as much iron as the others. Some workers do not wean their rats until they are 28 days old; in this case the difference would be still greater. The curves for the percentage of iron do not differ to such a large extent because the rats raised on screens become anemic much earlier and therefore do not grow as rapidly. The decreased weight causes an increase in the percentage of iron.

The difference which has been demonstrated for the total iron content of the entire rat also holds in the case of the liver. The livers from rats raised in contact with the mother's ration, weaned at 21 days, and kept on milk alone for 2 weeks contained 0.25 mg. of Fe, while the livers from rats raised on screens and treated in the same manner contained only 0.10 mg. of Fe. The rats used in the following investigation were therefore well suited for our studies, because the store of iron present in the livers at birth and available for hemoglobin formation was entirely depleted before additional iron was added to the diet.

The following procedure was used for studying the action of copper. Litters of six rats each were weaned and placed on whole cow's milk. When the hemoglobin had decreased to 2 to 3 gm. per 100 cc. of blood, usually 14 days after weaning, two rats in the litter were killed by decapitation and the liver and spleen removed carefully. The remaining four rats were given 0.5 mg. of Fe as purified FeCl₃ daily. After 2 weeks of iron feeding two of these rats were killed and the livers and spleens removed. The iron was taken away from the two remaining rats and they were fed 0.05 mg. of Cu as CuSO₄ daily. After 2 weeks these rats were killed for the removal of the liver and spleen. The organs were dried, weighed, and analyzed for iron. The hemoglobin content of the blood of the rats was determined weekly. A total of five litters was used for this study. The results are given in Table I.

When pure iron is fed to anemic rats for a period of 2 weeks there is no increase in the amount of hemoglobin in the blood, but there is a decided increase in the total iron content of the liver and spleen. The total iron in the liver increased from an average
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of 0.12 to 0.46 mg. of Fe and that of the spleen from 0.024 to 0.068 mg. of Fe. The increase when calculated as percentage of iron is not quite so large because the average weight of the liver and spleen increased during the 2 week period.

When the iron was taken away and 0.05 mg. of copper substituted, the average increase in hemoglobin for the 2 weeks was 2 gm. as compared with 0.1 gm. when the iron was fed. Most of the increase in the hemoglobin took place during the 1st week of copper feeding, and in many cases the hemoglobin regeneration had reached a maximum at 1 week and decreased thereafter, due to lack of sufficient iron. After 2 weeks of copper feeding the iron content of the liver had dropped to approximately the same level as was present before any iron was fed. The total iron content of the spleen decreased but very little. The percentage of iron decreased due to a decided increase in the weight of the spleen. A pronounced increase in the size of the spleen was noted in all rats when they were changed from a diet of milk and iron to one of milk, iron, and copper. This increase in the size of the spleen with copper feeding is very interesting and further study is needed to elucidate the significance of the change.

From these results it is evident that in the absence of copper inorganic iron is readily assimilated and stored in the liver and spleen. The iron so stored cannot be used for hemoglobin formation until copper is supplied, when the greater part of the iron in

### TABLE I

**Effect of Copper on Iron Content of Livers and Spleens from Rats**

<table>
<thead>
<tr>
<th>Change in Hb</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight</td>
<td>Fe per gm. dry liver</td>
</tr>
<tr>
<td>Anemic rats</td>
<td>0.694</td>
<td>0.126</td>
</tr>
<tr>
<td>Rats fed 0.5 mg. Fe for 14 days</td>
<td>+0.10</td>
<td>1.030</td>
</tr>
<tr>
<td>Rats fed 0.5 mg. Fe for 14 days then 0.05 mg. Cu alone for 14 days</td>
<td>+2.00</td>
<td>0.970</td>
</tr>
</tbody>
</table>
the liver is removed and built into hemoglobin of the blood. Copper is not necessary for iron assimilation but it is necessary for the synthesis of hemoglobin.

In the work which has just been described iron was fed for 2 weeks, then discontinued and followed by copper feeding immediately. A few experiments were conducted, therefore, to show what happens when the rats are continued on milk alone after the iron feeding. The results obtained with six typical animals are given in Table II. All the rats received 0.5 mg. of Fe for 2 weeks.

### Table II

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Treatment</th>
<th>Hemoglobin</th>
<th>Total Fe in liver</th>
<th>Fe per gm. dry liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14 days</td>
<td>21 days</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.5 mg. Fe daily for 14 days</td>
<td>2.96</td>
<td>2.87</td>
<td>0.630</td>
</tr>
<tr>
<td>612</td>
<td>0.5 mg. Fe daily for 14 days followed by milk alone for 7 days</td>
<td>3.47</td>
<td>3.85</td>
<td>0.270</td>
</tr>
<tr>
<td>401</td>
<td>0.5 mg. Fe daily for 14 days followed by milk + 0.05 mg. Cu daily for 7 days</td>
<td>2.70</td>
<td>2.13</td>
<td>0.121</td>
</tr>
<tr>
<td>613</td>
<td>0.5 mg. Fe daily for 14 days followed by milk + 0.05 mg. Cu daily for 7 days</td>
<td>5.91</td>
<td>6.66</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

Two of the animals were killed, two were continued on milk alone for 1 week, and two received 0.05 mg. of copper for 1 week before the organs were removed. It is readily seen that although there is some loss of iron in the liver of rats continued on milk alone, it is not nearly as great as when copper is fed. The loss observed in the milk-fed animals is undoubtedly due to a slow filtration of the iron into the channels of excretion, because there is no increase in the hemoglobin of the blood. The increase in the hemoglobin of the blood of the animals fed copper for 1 week is higher than the average given in Table I but as has already been stated, the figure is generally higher at the end of the 1st week than after 2 weeks of copper feeding.
Since inorganic iron was used in these experiments, the question of the possibility of rats assimilating and storing organic forms of iron naturally arises. Elvehjem (5) has shown recently that organic iron, in the absence of copper, is equally as ineffective as inorganic iron for hemoglobin formation, while in the presence of copper the regeneration with organic iron is much inferior to that with inorganic iron. The analysis of the livers from a few animals led to the conclusion that the decreased activity of the organic iron is due to the inability of the animal to assimilate iron in the form of hematin. This conclusion is amply substantiated by a few typical results given in Table III. Two of the animals received 0.5 mg. of Fe per day as hematin and two the same amount of iron as FeCl₃ for 7 days. The livers from those receiving ferric chloride contained 5 times as much iron as those from rats given hematin. This method may be used for testing the availability of any iron compound which can be prepared free from copper.

A second experiment was conducted in order to determine how much iron must be fed to allow hemoglobin regeneration and iron storage to take place at the same time. Graded levels of inorganic iron were fed with and without copper to anemic rats for 14 days, after which the rats were killed and the livers removed for iron analysis. The hemoglobin was determined weekly. The results are given in Chart II.

When the iron was fed alone the hemoglobin of the blood remained unchanged but the amount of iron stored in the liver was proportional to the amount of iron fed. When the iron was fed with copper the rate of hemoglobin formation was dependent upon
the iron intake, but the iron in the liver did not increase until 0.3 mg. of Fe was fed daily. In other words, when copper is present the inorganic iron is built directly into hemoglobin, and the amount in excess of what is needed for hemoglobin production is stored in the liver until the optimum level of iron storage in this organ is reached. It is impossible to increase the iron content of rat livers much above 1 mg. per gm. of dry matter by prolonged iron feeding when fed with or without copper.

Copper must therefore be active in the synthesis of hemoglobin from inorganic iron or in some process which affects this transformation.
DISCUSSION

It is interesting to compare the results presented in this paper with those published by Williamson and coworkers (6-8) a few years ago. They used animals which were not depleted in their original iron reserves and did their work before the importance of copper was known; nevertheless, the conclusions made from the two studies are comparable in many ways.

Williamson and Ets (6) demonstrated that inorganic iron, whether given by mouth, subcutaneously, or intravenously, increased the store of iron in the liver and spleen, but had no effect upon the rate of hemoglobin regeneration. They (7) also showed that rats fed ferric citrate in addition to a standard diet for some time did not recover any faster from anemia induced by bleeding than the controls not given any iron. The stored iron could not be used for hemoglobin formation. Williamson and Ewing (8) concluded, "the fault lies not with the absorption of iron, as was supposed by Bunge and his followers, but with its ability to be converted to hemoglobin."

The results presented in this paper show that the iron content of the livers of rats can be reduced to 0.1 mg. of Fe per gm. of dry matter by feeding a milk diet and increased without altering the hemoglobin of the blood to about 1 mg. of Fe by feeding inorganic iron. The iron so stored is readily converted to hemoglobin when copper is fed. Williamson's animals probably lacked sufficient copper to bring about this change. When he and Ewing (8) fed liver, a reserve of iron was built up which could be used for hemoglobin regeneration. In this case the iron was available because the liver also supplied copper.

A few workers have studied the assimilation and utilization of iron after the importance of copper was discovered. Cook and Spilles (9) showed that the addition of copper to an iron-low diet resulted in a depletion of the reserve iron in the spleen. When copper was added to a dietary supplied with sufficient iron, the amount of splenic iron was greatly increased, but iron alone did not bring about this augmentation. The results in this paper show that the iron content of the spleen is increased with iron feeding when the original reserve of iron is first depleted.

Cunningham (10) studied the effect of copper feeding on the iron content of the livers and the bodies of rats. His results are...
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far from conclusive, but he suggests that the inclusion of copper in the diet has the effect of lowering the iron content of the liver and increasing that of the body.

In a more recent paper Josephs (11) concludes that copper has no influence on the retention of iron but does influence the proportion of the retained iron found in the hemoglobin. He presents figures to show that when rats are receiving a plentiful supply of iron 80 per cent of the retained iron goes into hemoglobin formation and that this amount is increased to 100 per cent under the influence of copper. This clearly indicates that his rats were not depleted in their copper stores. In the absence of copper none of the retained iron can be built into blood hemoglobin. He concludes that copper decreases the non-hemoglobin iron of the tissues (total body iron minus blood hemoglobin iron). This is not strictly true because the tissues contain hemoglobin as well as the blood. He later suggests that copper influences mainly the mobile portion of the non-hemoglobin iron and that the majority of this iron is found in the liver. This conclusion is in agreement with our results. He emphasizes the fact that the fixed iron in the liver does not fall below a certain concentration which is maintained at all cost. We have also found that the iron content of the liver rarely falls below 0.1 mg. per gm. of dry tissue. When this level is reached the animal fails to grow.

All these workers reached the same general conclusion, namely, that copper mobilizes the iron in the liver, but the results in this paper give a definite account of the retention and utilization of inorganic iron in the absence and presence of copper.

SUMMARY AND CONCLUSIONS

1. Chart I is presented to show the effect of postnatal care on the iron content of young rats.

2. The addition of pure iron to the milk diet of anemic rats, which had been well depleted in their reserve of iron, had no effect on the hemoglobin content of the blood, but increased the total iron content of the liver and spleen to a large extent. When the iron was replaced by copper, the store of iron in the liver was used directly for building blood hemoglobin. The copper caused only a slight decrease in the iron content of the spleen but produced a definite increase in the size of this organ.
3. Inorganic iron (FeCl₃) was found to be much more readily assimilated and stored in the liver than organic iron (hematin).

4. When graded levels of inorganic iron were fed in the absence of copper, the hemoglobin content of the blood remained unchanged and the amount of iron stored in the liver was proportional to the amount of iron fed. In the presence of copper, the rate of hemoglobin formation was dependent upon the iron intake and the liver showed no iron storage until 0.3 mg. or more of iron was fed.

5. Copper does not affect the assimilation of iron but does function in the conversion of inorganic iron into hemoglobin.

BIBLIOGRAPHY

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