

BIOLOGICAL AVAILABILITY TO THE RAT OF IRON AND COPPER FROM VARIOUS COMPOUNDS

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INTRODUCTION

Trace minerals for livestock feeding have become an accepted part of modern feeds. Availability of these nutrients to the animal from various sources has been studied in our laboratory for many years. Elmslie *et al.* (1942) published on the availability of manganese, cobalt and copper in new sources of these mineral elements.

Among the various problems facing the feed nutritionist is the stability of certain fat soluble vitamins, particularly vitamins A and D. It has been well established by a number of investigators that the stability of these vitamins is affected by the trace elements added to the diet.

Fritz *et al.* (1942) found mineral mixtures to be destructive to vitamin D. Halverson and Hart (1950) demonstrated a rapid destruction of vitamin A induced by adding iron, copper, cobalt and manganese to an unsealed white corn sample containing added cod liver oil. Destruction was largely prevented when the minerals were covered by a gelatin mixture. Halverson and Hendrick (1955) demonstrated an even greater loss of vitamin A when the trace minerals were added at higher levels as in a feed concentrate. Kamstra *et al.* (1953) found that carotene stability was also affected by trace minerals.

In these cases cited, the trace minerals were added in the form of either the sulfate or the chloride

which are water-soluble. In an attempt to prevent this destruction, some commercial manufacturers of trace mineral premixes have utilized the water-insoluble forms. In this series of experiments we attempted to measure the relative biological availability of some of the water-insoluble forms of trace elements claimed to improve vitamin stability.

EXPERIMENTAL PROCEDURE—IRON AVAILABILITY STUDIES

Two experiments using albino rats were conducted to study the relative availability of iron from ferrous carbonate, ferrous sulphate and metallic iron. In both trials essentially the same experimental procedure was followed. Pregnant females were placed in clean, freshly painted litter cages shortly before parturition. The cages were equipped with a wire floor and a board covering one-half of the wire with cotton for bedding.

Milk was used as the basic ration for the anemia studies, since it is almost devoid of iron, which is essential to the production of the hemoglobin molecule. Only fresh milk was offered to the female in the cage in addition to water. Therefore, once a day she was removed to a separate cage for dry food. After being allowed to eat for approximately one hour, she was brushed thoroughly to remove excess dry feed and returned to the litter cage.

At 21 days the young were weaned and placed in individual cages. Each

rat was fed daily 1 g. of mineralized dried milk containing 0.05 mg. of copper and 0.04 mg. of manganese. After the dried milk supplement was consumed, fresh cow's milk was supplied *ad lib.* Hemoglobin and red blood cell counts were obtained according to standard procedures at weekly intervals. Blood samples were obtained from the tail of the rat by splitting the vein with a sharp scalpel. Blood flow could be easily stopped by pressure placed over the wound. After three weeks on the milk diet the rats were sufficiently anemic to be assigned the various treatment procedures for repletion feeding. The animals were distributed among the various treatments according to litter, weight, sex and blood picture. Supplementary iron was supplied in the mineralized dried milk supplement at the rate of 0.2 mg. of iron per rat per day.

RESULTS—IRON AVAILABILITY STUDIES

In Experiment 614 ferrous sulphate and ferrous carbonate were compared for hemoglobin and red blood cell regeneration in the rat. Iron deficiency anemia is described as microcytic hypochromic in nature. The normal rat three weeks post-weaning should have a hemoglobin level in the neighborhood of 13 g. per 100 cc of blood and a red blood cell count of from 8 to 10 million cells per cubic millimeter. At three weeks post-weaning these rats exhibited a typical blood picture of iron deficiency anemia. After 12 days of repletion feeding at the rate of 0.2 mg. of iron per rat per day, the rats receiving their iron in the form of ferrous sulphate increased

hemoglobin 129% over their initial level while the rats supplemented with ferrous carbonate increased only 26%. The increases in red blood cells were in the same direction (Table 1).

In Experiment 716 ferrous sulphate, ferrous carbonate and finely divided metallic iron were compared. Again ferrous sulphate increased the hemoglobin 121% while ferrous carbonate increased it only 35% and metallic iron 67% in two weeks of repletion feeding. The red blood cells follow the same pattern except that the metallic iron in this case was equal to ferrous sulphate (Table 2).

EXPERIMENTAL PROCEDURE— COPPER AVAILABILITY STUDIES

In the copper work a different technique was employed instead of the direct approach used in previous studies (Elmslie *et al.*, 1942) which had been based upon the requirement of the rat. The present method involved high level supplementation, with storage of copper in the liver as the criterion of availability. Boyden *et al.* (1938) observed considerable liver storage of copper when rats were fed a diet containing 500 ppm of copper with no substantial reduction in food consumption. This finding served as a guide in the design of our experiment.

The regular colony ration was used as the basal diet. This diet was formulated from natural feed ingredients and contained some copper from these sources. To this basal ration supplementary levels of copper were added. Copper sulphate (25½% Cu) to furnish 125, 250 and 500 mg. of copper per kilogram of

TABLE 1.—Experiment 614. Ferrous sulphate vs. ferrous carbonate for hemoglobin and red blood cell regeneration in the iron deficient rat.

| | Ferrous Sulphate | Ferrous Carbonate |
|--|------------------|-------------------|
| Number of rats | 6 | 6 |
| Blood picture, 3 weeks depletion post-weaning | | |
| Ave. red blood cells/mm. ³ | 4,510,000 | 5,090,000 |
| Ave. hemoglobin g./100 cc blood..... | 4.25 | 4.24 |
| Blood picture, 12 days repletion (0.2 mg Fe/rat/day) | | |
| Ave. red blood cells/mm. ³ | 8,070,000 | 6,000,000 |
| % Increase | 78.9 | 17.8 |
| Ave. hemoglobin g./100 cc blood..... | 9.74 | 5.35 |
| % Increase | 129.1 | 26.1 |

TABLE 2.—Experiment 716. Comparison of ferrous sulphate, ferrous carbonate and metallic iron for hemoglobin and red blood cell regeneration in the iron deficient rat.

| | Ferrous Sulphate | Ferrous** Carbonate | Metallic Iron |
|---|------------------|---------------------|---------------|
| Number of Rats | 6 | 5* | 6 |
| Blood picture, 3 weeks depletion post-weaning | | | |
| Ave. red blood cells/mm. ³ | 4,320,000 | 4,000,000 | 4,060,000 |
| Ave. hemoglobin g./100 cc blood..... | 5.96 | 6.23 | 5.87 |
| Blood picture 2 weeks repletion (0.2 mg Fe/rat/day) | | | |
| Ave. red blood cells/mm. ³ | 6,540,000 | 5,180,000 | 6,180,000 |
| % Increase | 51.3 | 29.5 | 52.2 |
| Ave. hemoglobin g./100 cc blood..... | 13.18 | 8.43 | 9.84 |
| % Increase | 121.1 | 35.3 | 67.6 |

* One animal developed pneumonia, removed from test.
 ** Different source of supply from Experiment 614.

diet served as the control. Copper oxide (83.7% Cu), copper pyrophosphate (16.2% Cu) and copper concentrate (74.1% Cu) were added to furnish copper levels of 250 and 500 mg. per kilogram of diet. After four weeks of feeding, the animals were sacrificed and the liver removed, weighed and analyzed for copper.

RESULTS—COPPER AVAILABILITY STUDIES

In the copper studies, Experiment 732, liver copper levels ranged from 75 µg. per liver on the basal diet, which may be considered a "normal" ration, to over 1500 µg. per

liver at the 500 mg. of copper level furnished by copper sulphate. At the 500 mg. level from copper pyrophosphate, copper oxide and the copper concentrate, the livers contained on the average only 400, 180 and 260 µg. of copper, respectively (Table 3). It is obvious that these compounds are much less available than copper sulphate as measured by the liver storage technique.

Since there was some variation in the actual copper intakes, availability was calculated on the basis of intake rather than the concentration in the diet. Using the graphical method of estimation and assuming copper sulphate to be 100%

TABLE 3.—Experiment 732. Liver copper levels in the rat fed copper from various sources and relative availability of copper.

| | Copper Added (mg./kg. diet) | Ave. Total Gain Per Rat (g.) | Suppl. Copper Intake/Rat (mg.) | *Total Copper Per Liver (μ g.) | % Avail- ability |
|---------------------------|--------------------------------|---------------------------------------|---|--|------------------------|
| Basal Control..... | None | 118.7 | None | 74.5 | |
| Copper sulphate..... | 125 | 113.5 | 44.0 | 215.7 | } 100 |
| Copper sulphate..... | 250 | 120.7 | 95.5 | 304.0 | |
| Copper sulphate..... | 500 | 118.7 | 178.0 | 1,517.7 | |
| Copper pyrophosphate..... | 250 | 117.0 | 94.5 | 221.5 | } 49 |
| Copper pyrophosphate..... | 500 | 121.0 | 191.0 | 404.0 | |
| Copper oxide..... | 250 | 113.5 | 95.6 | 111.5 | } 27 |
| Copper oxide..... | 500 | 124.2 | 204.0 | 181.7 | |
| Copper concentrate.... | 250 | 127.0 | 120.0 | 185.7 | } 34 |
| Copper concentrate.... | 500 | 126.0 | 204.0 | 261.0 | |

* Average of 4 rats on each treatment. Livers were analyzed individually.

available, copper pyrophosphate appears to be 49%, copper oxide 27% and the copper concentrate 34% available under the conditions of this experiment. As in most biological tests, considerable variation and uncertainties exist; therefore, the availability percentages assigned serve merely as a relative ranking with respect to copper sulphate, rather than as exact percentages.

DISCUSSION

Under our conditions with the particular basal diets used, the carbonate form of iron is relatively unavailable for the iron-deficient rat. For maximum rate of hemoglobin and red blood cell regeneration in the rat it appears that ferrous sulphate, a water-soluble form of iron, is more efficient than the other sources of iron used in this study.

This may not necessarily be true for other species, such as poultry.

It is quite possible that copper availability may be influenced by the kind of diet used or the species of animals concerned. Unpublished work from our laboratory indicates that poultry may utilize copper oxide more efficiently than does the rat. Under certain conditions in practical livestock feeding water-insoluble sources of copper may be as satisfactory or more so than the readily soluble salts. Becker *et al.* (1953) reported that 5 g. of copper sulphate fed daily to a 500 lb. steer was toxic with death occurring after 122 days of feeding. An equivalent amount of copper from copper oxide was fed to two 500 lb. steers daily for 15 months without observing toxicity. Our data suggest that the relative toxicity of copper oxide and copper

sulphate may be due to their relative availability of copper but does not exclude the possibility that the rate at which copper may be absorbed into the body is also a factor.

SUMMARY

1. Based on hemoglobin and red blood cell regeneration in the anemic rat, ferrous carbonate was relatively unavailable when compared to ferrous sulphate. Metallic iron was intermediate between ferrous carbonate and ferrous sulphate in its ability to regenerate hemoglobin and red blood cells.

2. Using liver copper storage in the rat as the criterion, copper oxide copper concentrate and copper pyrophosphate were approximately one-fourth, one-third and one-half as available, respectively, as was copper sulphate.

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