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Protective Effect of Iron and Copper in Infectious Anemia of the Rat.†

HOWARD H. BEARD AND E. VON HAAM.

*From the Department of Biochemistry and the Institute of Pathology and Bacteriology, Louisiana State University Medical Center, New Orleans.*

Pure iron, alone or in combination with a small optimum dose of copper or other catalytic metals, or combined with ultraviolet rays, is able to prevent and cure nutritional anemia in the weanling rat when fed upon a diet of whole milk for a period of 6-9 weeks.<sup>1, 2</sup> A similar preventive effect of both iron and copper has recently been advanced by Perla and Marmorston-Gottesman<sup>3</sup> against the anemia of the splenectomized rat produced by Bartonella infection. It was further suggested by these investigators that the so-called nutritional anemia of the white rat may be complicated or influenced by a spontaneous infection of the animal with Bartonella.

Perla observed that the addition of copper, either when fed or

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\* P represents a preliminary, C a complete manuscript.

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<sup>1</sup> Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 89.

<sup>2</sup> Beard, H. H., Johnson, A. G., and Andes, E. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **31**, 23.

<sup>3</sup> Perla, D., and Marmorston-Gottesman, *J. Exp. Med.*, 1932, **56**, 783.

injected, to an adequate diet for a period of 10 days prior to splenectomy and 1 month thereafter protected 75% of their animals against the anemia, while iron, under similar conditions, protected against anemia in 50% of the animals. It was stated that copper is essential with iron in hemoglobin regeneration in rats suffering from nutritional anemia and also that an excess of copper plays a rôle in the mechanism of resistance to *Bartonella muris* anemia in the rat after splenectomy.

The following work was undertaken to compare the effect of both iron and copper in this infectious anemia with their action in nutritional anemia.

Eighty animals were kept in large galvanized iron cages recently painted with aluminum paint and fed with Purina Dog Chow‡ which represents the standard food for our stock animals in this study. Groups of 10 animals were usually kept together in one cage so as better to expose them to the possibility of infection. Four cages with 20 adult and 20 young rats served as control. In order to avoid mistakes due to irregular intake of metals by the animals, the colloidal copper and iron preparations were administered by intraperitoneal injection. To omit trauma the injections were given every fifth day commencing 10 days previous to splenectomy and subsequent thereto for one month. 2.5 mg. of the iron were given to the first group; 2.5 mg. of iron-copper solution were given to the second group; 0.25 mg. of the copper solution were given to the third group which corresponds to a daily dose of 0.5 mg. iron and 0.05 mg. copper, doses which proved optimal in the treatment of nutritional anemia. These doses are one-half those used by Perla. One group of 10 rats was exposed daily for 30 minutes to the rays of the General Electric Sunlight Mazda Lamp, Type S-I, (at a distance of 4 feet) which has been used in previous experiments.<sup>2</sup>

The animals were splenectomized with the usual technic and red cell counts and blood smears were examined every third day. The splenectomized animals were under observation for 2 months when this report was made. The results are shown in Table I.

It is clearly seen that the animals on Purina Dog Chow (groups A and B) containing sufficient iron and copper, were heavily in-

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‡ The composition of this product was as follows: meat, wheat germ meal, dried skim milk, cod liver oil, barley malt, blackstrap molasses, oat cereal, corn cereal, wheat bran and iodized salt. Analysis: Protein 20.0%; fat 3.0%; fibre 6.0%; nitrogen-free-extract 50.0%; carbohydrates 56.0%; ash 6.0%; having a caloric value of 1,500 calories per pound. This diet contains ample copper and iron. (Popular Bull. No. 4, Research Department, Purina Mills, St. Louis.)

TABLE I.

| Group | Supplement  | No. of animals used | No. of animals infected with <i>Bartonella M.</i> |           |       | No. of animals protected against infection |
|-------|---|---------------------|---|-----------|-------|--|
|       |   |                     | Died  | Recovered | Total |  |
| A     | Normal adult rats fed on Purina Dog Chow  | 20                  | 6   | 13        | 19    | 1  |
| B     | Normal young rats fed on Purina Dog Chow  | 20                  | 18  | 1         | 19    | 1  |
| C     | Intraperitoneal administration of Copper in form of CuS (Kupferdiasporal)                                       | 10                  | 3   | 4         | 7     | 3  |
| D     | Intraperitoneal administration of Iron in form of colloidal highly dispersed ferrous hydroxide (Eisendiasporal) | 10                  | 7   | 3         | 10    | 0  |
| E     | Intraperitoneal administration of colloidal-highly dispersed Iron-Copper solution                               | 10                  | 5   | 5         | 10    | 0  |
| F     | Administration of Ultra-violet Rays   | 10                  | 8   | 1         | 9     | 1  |

ected with *Bartonella*, which was more fatal in the young than it was in the adult animals. In group C, receiving copper alone only 3 out of 10 rats were protected from the anemia. These results resemble somewhat those of Perla, who, however, observed a much better protective effect with copper. Intraperitoneal injection of iron, iron and copper, and iron plus ultraviolet rays, to groups D, E, and F, respectively, did not afford protection to any of the animals.

We conclude from these observations that iron and copper, in doses which prevent and cure nutritional anemia in the albino rat, do not protect the animal against the anemia of *Bartonella* infection following splenectomy.

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## Pathological Changes in Liver and Spleen in Nutritional Anemia in Rat.

E. VON HAAM AND HOWARD H. BEARD.

*From the Institute of Pathology and Bacteriology and the Department of Biochemistry, Louisiana State University Medical Center, New Orleans.*

Pure milk fed to young rats produces severe anemia which finally results in death in the majority of cases. In our studies of the