

Rats of carrier stock with the spleen intact, between the ages of 6 and 8 weeks, suffer from a severe infection of *Bartonella muris* with little or no anemia. This is manifested by the occasional appearance of *Bartonella muris* bodies in the blood cells and the marked hyperplasia and congestion of the spleen. Protection in such instances against anemia following splenectomy by an extract of spleen is obviously, therefore less effective. In the adult, the infection is latent and the spleen shows little evidence of hyperplasia and congestion. In these rats protection was obtained in a large percentage of instances.

A "lipoid" extract of the spleen was prepared which protects adult albino rats of carrier stock in a large percentage of instances against *Bartonella muris* anemia following splenectomy. One cc. of extract (corresponding to 100 gm. of fresh spleen) is injected daily intraperitoneally in divided doses for a month. It is suggested that the extract contains a specific hormonal substance.

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Protective Action of Copper and Iron against *Bartonella Muris* Anemia.\*

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\* The copper and iron were obtained through the courtesy of the Myron L. Walker Company, Mt. Vernon, N. Y.

<sup>1</sup> Hart, E. B., Steenbock, H., Elvehjem, C. A., and Waddell, J., *J. Biol. Chem.*, 1925, **65**, 67.

<sup>2</sup> Hart, E. B., Elvehjem, C. A., Waddell, J., Herrin, R. C., *J. Biol. Chem.*, 1927, **72**, 299.

<sup>3</sup> Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 777.

<sup>4</sup> Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

<sup>5</sup> Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

<sup>6</sup> Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

<sup>7</sup> Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

<sup>8</sup> Beard, H. H., Rafferty, C., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 111.

<sup>9</sup> Myers, V. C., Beard, H. H., and Barnes, B. O., *J. Biol. Chem.*, 1931, **94**, 117.

<sup>10</sup> Beard, H. H., Baker, R. W., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 123.

<sup>11</sup> Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 135.

and others have established the importance of copper and iron in hemoglobin formation. Young rats fed on a diet of milk deficient in copper develop an anemia within 6 to 8 weeks, that can readily be prevented by the administration of small quantities of copper to the diet. The minimal requirement was 0.025 mg. copper per rat per day. Since albino rats are subject to *Bartonella muris* infection spontaneously during the early weeks following weaning it was thought that the "milk" anemia of the rat may be complicated by infection with *Bartonella muris*. The beneficial prophylactic effect of copper in "milk" anemia suggested its trial in *Bartonella muris* anemia of splenectomized rats.

The rats in these experiments are all carriers of *Bartonella muris* infection. They have been raised in our laboratory under constant environmental and dietary conditions. The diet for the past 10 years has consisted of 15 gm. per rat per day of a mixture composed of hominy 100 parts, rolled oats 25 parts, fine meat and bone 25 parts, dry skim milk 16 parts and salt 1½ parts. Twice a week the rats received whole milk and bread *ad lib.*, and greens (lettuce leaves). The exact quantity of copper in this diet was difficult to estimate but the food mixture was found to contain about 0.025 mg. per 15 gm. of food.† The copper was added in the form of copper sulphate in doses equivalent to 0.1 mg. of elemental copper per day. Lactose was used as a vehicle. The iron was added in the form of iron ammonium citrate. The daily addition of iron was 1 mg. as elemental iron.

Both mature and immature rats were used. The experiments were divided into 2 groups. In one, additions of copper alone, of iron alone and of copper and iron to the basic adequate diet were made during a period of 9-12 days prior to the removal of the spleen, and continued for one month thereafter. In the second group the copper and iron supplements were added to the diet for only 2 days prior to splenectomy and then continued thereafter. Daily hemoglobin estimations and smears were made on all the rats and the red cell counts made every second day.

In these experiments 94 rats were used. The addition of copper or iron to the diet of albino rats in the amounts indicated for 2 days prior to splenectomy and continued after splenectomy, failed to protect in any case adult albino rats from *Bartonella muris* anemia.

Complete protection against *Bartonella muris* infection and anemia, however, was obtained by the addition of copper alone or cop-

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† The determinations of the copper content of the diet were made by Dr. Marta Sandberg.

per and iron to an adequate diet when the supplements were given for a period of 10 days prior to splenectomy and continued after splenectomy. Seventy-five percent of 12 adult rats fed copper and 75% of 8 adult rats fed copper and iron were protected. The period of observation is one month following the operation. Copper was definitely more effective than iron, and copper plus iron slightly more effective than copper alone. Fifty percent of 10 rats fed iron were protected. The adult rats were protected in twice as many instances as were the immature rats. This is due to the fact that the severity of the infection with *Bartonella muris* (without anemia) is much greater in the immature rat with intact spleen than in the adult rat in which the infection is entirely latent. Copper protects 75% of the rats against this anemia if it is added as a supplement to an adequate diet for a period of 10 days prior to splenectomy.

These experiments suggest that the utilization of copper and of iron in the body is intimately concerned with the function of the spleen. Copper is involved in some way in the mechanism of resistance in the body as well as in the production of hemoglobin. We have been able to demonstrate an active substance in the spleen<sup>12</sup> which when injected into albino rats from the day of splenectomy protects in a large percentage of instances against *Bartonella muris* anemia. This extract contains neither copper nor iron. The relation of copper to the spleen may be somewhat analogous to the relation of calcium to the parathyroid gland. The small amount of copper in the adequate diet is insufficient for the needs of the rat in the absence of the splenic hormone. An excess of the metallic element may compensate for a deficiency of the hormone. Further, the copper as administered cannot be utilized at once but must be converted into a form that is more readily utilizable by the body in the mechanism of resistance to *Bartonella muris* anemia.

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## Fever in Certain Cases of Heart Failure.

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The great frequency with which fever occurs during the course of heart disease is well known and has been frequently commented

<sup>12</sup> Perla, D., and Marmorston-Gottesman, J., in press.