THE PROTECTIVE ACTION OF COPPER AGAINST TRYPANOSOMA EQUIPERDUM INFECTION IN ALBINO RATS*

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In previous work it was found that the addition of small amounts of copper to an adequate diet protected a large percentage of rats from *Bartonella muris* anemia following splenectomy (1). In subsequent experiments the effect of supplements of copper to the diet on the natural resistance of adult rats to *Trypanosoma lewisi* infection was studied. The addition to an adequate diet of copper in amounts equivalent to 0.1 mg. of elemental copper per rat per day or of iron in amounts equivalent to 1 mg. of elemental iron per day, or both, during a period of 10 days prior to an induced infection with *Trypanosoma lewisi* raised the natural resistance of the rat to the disease. The infection was completely aborted in almost 50 per cent of instances (2). Supplements of lead salts in equivalent amounts, used as control, had no effect.

In the present communication the effect of additions of copper salts to the diet on a subsequently induced infection with *Trypanosoma equiperdum* was determined. In preliminary experiments the infection was carefully standardized for our stock of rats and the pathology of the disease studied (3). The virulence of this trypanosome for the white rat is so great that it was essential first to establish the critical dose of organisms that results in a fatal infection and to determine the duration of the disease with varying numbers of parasites.

Following the standardization of the infection in normal rats the effects of copper supplements on the course of the infection was

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studied in 4 groups of rats infected with varying quantities of trypanosomes.

The rats used in these experiments have been raised in our laboratory for many years and maintained under constant environmental and dietary conditions. The diet consisted of 15 gm. per rat per day of a mixture composed of hominy 100 parts, rolled oats 25 parts, fine meat and bone scraps 25 parts, dried skimmed 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 copper content of this diet was difficult to estimate, but the food mixture was found to contain about 0.025 mg. of elemental copper per 15 gm. of food. In the experiments reported the copper was added in the form of copper sulfate in amounts equivalent to 0.2 mg. of copper per rat per day. Lactose was used as a vehicle. The iron was added in the form of iron ammonium citrate in doses equivalent to 2 mg. of elemental iron per day.[1]

The experiments were divided into 4 groups. In the first group, 20 million trypanosomes were used as the infecting dose; in the second group, 100,000 trypanosomes; in the third group, 10,000 trypanosomes; and in the fourth group 2,000 trypanosomes. In the first 3 groups the effect of additions of copper to an adequate diet on the course of the subsequently induced infection was studied. In the fourth group the effect of additions of copper or iron to the diet on the subsequently induced infection was determined.

The Effect of Copper Supplements to an Adequate Diet, on Trypanosoma equiperdum Infection Induced with Large Numbers of Parasites

The rats were divided into 2 groups. Group I consisted of 30 rats. Of these, 10 received daily supplements of copper as copper sulfate in amounts equivalent to 0.2 mg. of elemental copper per day, and 20 rats were fed on the normal diet. The supplements were commenced 10 days prior to the injection of blood of trypanosome-infected rats. Each was injected intraperitoneally with 20 million trypanosomes. Group II consisted of 16 rats. Of these, 8 received copper supplements as in Group I, during a period of 10 days prior to injection of trypanosomes, and 8 received the normal diet. Each of these rats was injected intraperitoneally with 100,000 trypanosomes.

Results.—All the rats of these 2 groups succumbed to the infection. There was no essential difference in the course of the disease, but a

[1] I am indebted to the Myron L. Walker Co., Inc., of Mt. Vernon, New York, for the copper and iron preparations.

slight prolongation of the infection in the copper-fed group was noted (see Table I). No effect on the pathology of the disease was observed.

The Effect of Copper and Iron Supplements to an Adequate Diet on Trypanosoma equiperdum Infection Induced with Small Numbers of Parasites

In these experiments 65 rats were used. These were divided into 2 groups. In the first, 8 rats received copper supplements to the diet as in previous experi-

TABLE I

*The Effect on Trypanosoma equiperdum Infection in Adult Albino Rats of Copper and Iron Supplements to an Adequate Diet**

No. of rats	Supplementary feeding	No. of trypanosomes injected (intraperitoneally)	No. of rats with abortive infection	Per cent of rats with abortive infection	Average duration of life in fatal infections
					days
10	Copper†	20 million	0	0	4.5
20	Controls	20 million	0	0	3.6
8	Copper	100,000	0	0	7.5
8	Controls	100,000	0	0	6
8	Copper	10,000	6	75	9.6
8	Controls	10,000	0	0	9.2
20	Copper	2,000	20	100	
10	Iron‡	2,000	10	100	
19	Controls	2,000	10	52	14.5

* The supplements were commenced 10 days prior to the injection of the trypanosomes.

† The copper was given as copper sulfate in amounts equivalent to 0.2 mg. of elemental copper per rat per day.

‡ The iron was given as iron ammonium citrate in amounts equivalent to 2 mg. of elemental iron per rat per day.

ments and 8 were fed on the normal diet. Each of these was injected intraperitoneally with 10,000 trypanosomes. In the second group, 20 rats received copper supplements as above, 10 received supplements of iron in amounts equivalent to 2 mg. of elemental iron per rat per day during the same period and 19 were fed on the normal diet. Each of these rats was injected with 2,000 trypanosomes intraperitoneally.

Results.—With 10,000 trypanosomes as the infecting dose all the controls succumbed to the infection. The average duration of life was 9.2 days. Of the copper-fed group, 75 per cent developed no obvious evidence of infection (abortive infection) and survived. No parasites were observed in smears of the blood. With 2,000 trypanosomes as the infecting dose, 48 per cent of the controls developed severe infections and died, but all of the rats which had received copper and iron supplements to the diet had abortive infections and survived.

From these experiments it may be observed that additions of copper or iron to an adequate diet in the rat definitely raise the natural resistance of this animal to a subsequently induced infection with *Trypanosoma equiperdum.*

DISCUSSION

The importance of copper in the resistance of albino rats to three types of infection, with *Bartonella muris*, *Trypanosoma lewisi*, and *Trypanosoma equiperdum* respectively, has been indicated by the experiments reported in previous studies and in the present one. The rat is an ideal animal in which to study the effect of copper on resistance, as the diet of the rats used in most laboratories contains a minimal amount of copper (sufficient, however, to prevent nutritional anemia).

Cunningham in a study of the relative amounts of copper in various animal and plant tissues found that the rat has less copper in its organs than any other animal studied (4). The liver of the rat contains 1/20 to 1/30 the percentage weight of copper found in the liver of the rabbit or the guinea pig. Individual variations in the resistance to *Bartonella muris*, *Trypanosoma lewisi*, and *Trypanosoma equiperdum*, may be dependent on the copper content of the food of these animals. It is of interest that the guinea pig, an animal low in resistance to many infections, is refractory to *Trypanosoma lewisi* and *Bartonella muris* and develops a chronic infection with *Trypanosoma equiperdum*. The copper content of its diet and tissues is high. The rat, highly resistant to most infections, is very susceptible to these three diseases. The copper content of its diet and tissues is low. The results of the experiments reported do not suggest, however, that

an excess of copper in a diet already adequately supplied with this element will increase the resistance of the individual above the normal level.

It is of significance that the species susceptibility of the rat to certain infections may be markedly altered by adding copper or iron to the diet prior to infection. It is probable that the protective action is dependent on the fact that copper is a catalytic oxidative agent in cellular metabolism. In rats the copper content of the tissues, while sufficient to prevent a nutritional anemia, may result in a depression of the threshold resistance of the cell to certain types of injury.

These studies on the rôle of copper in the diet in natural resistance to infections emphasize again the importance of maintaining the conditions of diet, environment, and strain of animals constant in experimental studies on infection and resistance. No doubt variations in resistance observed in apparently identical infections in different laboratories are due to subtle variations in strain, diet, and environment.

SUMMARY

The effect was studied of additions of copper to an adequate diet on the course of infection with *Trypanosoma equiperdum* in rats. Copper in amounts equivalent to 0.2 mg. of elemental copper per rat per day during a period of 10 days prior to an induced infection with small numbers of trypanosomes raised the natural resistance of the rat to the infection. The infection was aborted in all instances when the rats were infected by the injection of 2,000 trypanosomes and in 75 per cent of instances when the rats were infected by the injection of 10,000 trypanosomes.

CONCLUSION

The natural resistance of the rat to infection with *Trypanosoma equiperdum* can be markedly raised by supplements of copper to the diet prior to infection.

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