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Protective Action of Copper against Infection with *Mycobacterium tuberculosis* (Bovine) in Albino Rats.

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The protective action of supplements of copper to the diets of rats against infections with *Bartonella muris, Trypanosoma lewisi* and *Trypanosoma equiperdum* has been reported.[1, 2, 3] The present communication deals with the effect of additions of copper salts to the diet on a subsequently induced infection with *Mycobacterium tuberculosis* (bovine strain) in albino rats. It was observed[4] that the adult rat of our stock, though highly resistant to tuberculous infection, could be infected with 0.1 mg. of a virulent bovine strain of tubercle bacilli and revealed gross lesions of tuberculosis in the lungs within a period of 3 to 5 months.

The rats are from the stock raised in our laboratory for many years and maintained under constant environmental and dietary conditions. The diet has been described[2] and was found to contain the equivalent of about 0.025 mg. of elemental copper per 15 gm. of food. Supplements of copper were given in the form of copper sulphate in amounts equivalent to 0.2 mg. of copper per rat per day.*

Fifty-six rats were divided into 4 groups; 12 received daily supplements of copper in the amounts indicated above; 12 received supplements of iron; 12 received supplements of copper and iron. All supplements were commenced 14 days prior to the injection of the tubercle bacilli, and continued during the entire experimental period. Twenty rats were fed on the normal diet. Each of the rats was injected intraäbdominally with 0.1 mg. of bovine tubercle bacilli ("Ravenel" strain). All the animals were killed 5 months after the injection of the bacteria.

From the table it may be seen that of the rats receiving copper-supplements, 6 showed no evidence of tuberculous lesions at autopsy and 6 had a few scattered tubercles in the lungs (indicated in the

1 Perla, David, and Marmorston-Gottesman, J., *J. Exp. Med.*, 1932, **56**, 783.

2 Perla, David, *Am. J. Hyg.*, 1934, **19**, 514.

3 Perla, David, *J. Exp. Med.*, 1934, **60**, 541.

4 Perla, D., and Marmorston, J., *Arch. Path.*, 1933, **8**.

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

table as "slight infections"). Of the rats receiving iron-supplements alone, none escaped infection; 7 showed large conglomerate lesions in the lungs (indicated as "severe infection" in the table) and marked involvement of the retroperitoneal lymph nodes; 3 showed evidence of a slight infection and 2 of a moderately severe involvement of lungs and lymph nodes. Of those rats which were fed both copper and iron supplements, one escaped infection; 5 had only slight tuberculosis and 6 moderately advanced infections. Of the 20 controls, none escaped infection; 3 showed numerous lesions in the lungs and lymph nodes; 9 moderately advanced lesions in the lungs, and 8 slight infection as manifested by an occasional scattered tubercle in the lungs and little or no lymph-node involvement.

TABLE I.

Effect on Infection with *Mycobacterium tuberculosis* (Bovine-"Ravenel") in Adult Albino Rats of Copper and Iron Supplements to an Adequate Diet.

All the rats were killed 5 months after the intraäbdominal injection of 0.1 mg. of tubercle bacilli.

No. Rats	Supplementary feeding*	Type of infection			
		No evidence	Slight	Mod.	Severe
12	Copper†	6	6	0	0
12	Copper and iron‡	1	5	6	0
12	Iron	0	3	2	7
20	Controls	0	8	9	3

*The supplements were commenced 14 days prior to the injection of the tubercle bacilli.

†The copper was given as copper sulphate 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.

The natural resistance of the rat to infection with *Mycobacterium tuberculosis* (bovine strain) can be raised with supplements of copper to the diet. This effect is consistent with previous findings on the influence of copper on infections in the rat. The beneficial effect of copper is probably due to its physiological importance as a catalytic agent in oxidative processes in cellular metabolism.

In these experiments iron-supplements had a deleterious effect on the course of the tuberculous infection and the lesions were more extensive than in the control rats. It may be that an excess of iron over a long period of time results in cellular injury due to excessive storage of the metal and may interfere with the mechanism of resistance to tuberculosis.

The iron is found deposited in large amounts in the macrophagic tissue of the spleen, the Kupffer cells of the liver and in large macrophages within the tuberculous lesions of the lungs.

Menkin[5] has shown that following repeated injections of a dilute solution of ferric chloride in rabbits the iron is deposited in large amounts within and about the tuberculous lesions and its presence tends to favor fibrosis and prolong the life of the animal.

Menkin administered 3 injections a week intravenously each of 6 cc. of a 0.25% solution of ferric chloride, during a period of 4 months. This represents a total amount of approximately 220 to 250 mg. of elemental iron for the entire experimental period. Though this is approximately the same quantity as that used in the experiments here reported, it is probable that the differenes in the animals used may account for the difference in the results.†

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Relation of Cholane Nucleus to the Female Bitterling Test for Male Hormone.*

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After demonstrating that the female bitterling test was not a test for pregnancy,[1] we have stated[2] that the fraction of male urine containing the male hormones is responsible for the ovipositor elongating reaction. A positive reaction was taken as a lengthening of the ovipositor from a quiescent stage to a length equal to that of the anterior edge of the anal fin, *i. e.*, the ovipositor must reach the end of the fin.

Crystalline theelin and theelol were also tested and although they

5 Menkin, Valy, *J. Exp. Med.*, 1932, **55**, 101; 1934, **60**, 463.

† The literature[6-10] on the therapeutic use of copper is reviewed in a previous article.[3]

6 Bevan, cited by Corper, H. L., DeWitt, L., and Wells, H. G., *J. Am. Med. Assn.*, 1913, **60**, 887.

7 Von Linden, G., *Beitr. Klin. Tuberk.*, 1912, **23**, 201.

8 Meissen, E., *Beitr. Klin. Tuberk.*, 1912, **23**, 215.

9 Strauss, A., *Beitr. Klin. Tuberk.*, 1912, **23**, 223.

10 Corper, H. J., *J. Infect. Dis.*, 1914, **15**, 518.

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1 Kleiner, I. S., Weisman, A. I., and Barowsky, H., *J.A.M.A.*, 1935, **104**, 1318.

2 Kleiner, I. S., Weisman, A. I., and Mishkind, D. I., *J.A.M.A.*, in press.