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The Need for Manganese in Bone Development by the Rat.*

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Shils and McCollum,[1] by feeding a high calcium-phosphorus diet low in manganese, obtained results indicating that the surviving young of deficient females developed skeletal abnormalities. Wachtel, Elvehjem, and Hart[2] reported a reduction in the ash content, density, and length of bones of manganese-deficient rats, fed a high calcium-phosphorus diet. Whether these results were caused by the retarding effect of manganese deficiency on growth in general or by a specific effect upon bone formation could not be determined from the evidence. Studies were undertaken, therefore, to determine if, by feeding a similar diet, evidence could be obtained of abnormal bone formation in rats in which the gains in weight of pairmates were equalized throughout the experimental period.

Rats of the Sprague-Dawley strain were used in these studies. All mothers were placed on the low-manganese diet 2 weeks after the birth of the litters to prevent excessive storage of manganese by the young prior to the experimental period. At 4 weeks of age the rats were paired according to weight and placed on the experimental diets. Each rat was weighed daily and the feed consumption of the rat on the high-manganese diet was limited so that it gained in weight at the same rate as its pairmate. The rats on the low-manganese diet were fed *ad libitum*.

The basal diet was a modification of low-manganese diet 21 devised by Shils and McCollum.[1] Additional $Ca_3(PO_4)_2$ was added so that the diet contained approximately 2% calcium and 1% phosphorus. The high-manganese diet contained a supplement of 0.06% manganese as $MnCl_2 \cdot 4H_2O$.

The rats were killed at 76-87 days of age and the left and right femur, tibia, and humerus dissected out and cleaned. Length, density, breaking strength, and bone phosphatase[3] were determinted on the fresh bone. The tibiae were used for the determination of bone ash. The ash samples were dissolved in 1:4 HCl and aliquots of these solutions were used for analyses of calcium,[4] phosphorus,[5] and magnesium.[6]

The results obtained are presented in Table I. The bones of the rats on the high-manganese diet were slightly longer than those on the low-manganese diet. When the lengths of the tibiae of pairmates of the same weight were compared and analyzed statistically by Student's method for paired data, the odds that the difference was significant were 185:1.

The density of the fresh bones of manganese-deficient rats was somewhat lower than that of their pairmates on the high-manganese diet. The odds that the difference was real were 70:1, showing that the difference, though slight, was significant. The

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TABLE I.
Summary of Data.

Manganese content of diet	Females		Males	
	Low	High	Low	High
No. of rats	13	13	5	5
Final weight, g	190.8	191.1	237.0	237.6
Length of fresh tibia, cm*	3.38	3.43	3.44	3.49
Density of fresh bone, g/cc†	1.536	1.570	1.461	1.479
Breaking strength of femur, kg	7.90	9.35	7.89	9.91
Phosphatase, units/g fresh bone	12.69	14.46	17.02	17.43

* Avg of 2 bones.
† Avg of all bones.

lower density of the bones of the manganese-deficient rats observed in this study cannot be explained by a reduction in ash content of the bones since the percentage ash was the same for both groups. Caskey, Norris, and Heuser (unpublished results) found that the bones of manganese-deficient chicks contained slightly more fat and less water than those of normal chicks. If the same is true of the rat, it could explain the lower density without corresponding decrease in ash content.

The percentage of calcium and phosphorus in the fat-free dry bone was not lowered in the manganese-deficient animals. These results agree with those of Wachtel *et al.*[2] The percentage of magnesium was also the same for both groups.

The breaking strength of the bones of manganese-deficient rats was much less than those of the controls. The odds that the difference was significant were greater than 999:1. These results are in agreement with those reported by Smith, Medlicott, and Ellis[7] for the rabbit. The difference may be due in part to the lower density of the bones of deficient rats or to structural changes in the matrix of the bone, not affecting density and mineral content. The possibility also exists that there may be changes in the crystal structure of the bone salts.

The amount of phosphatase activity of the fresh bone was less in the manganese-deficient rats. The difference was found to be highly significant with odds of 830:1. These results are not in agreement with those of Wachtel *et al.*[2]

Summary. The length, density, breaking strength, and phosphatase content of the bones of manganese-deficient rats were lower than in pairmates of the same weight receiving adequate manganese. There was no difference between the two groups in percentage of ash, calcium, phosphorus, and magnesium in the fat-free dry bone.

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A Method for Determination of Streptomycin in Body Fluids.

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The introduction of streptomycin as a chemotherapeutic agent for the treatment of bacterial infections[1-4] has emphasized the need for a quantitative method for the determination of the drug in body fluids.

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