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Relative Effectiveness of Ingested and Injected Manganese in Preventing Perosis.*

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Manganese was shown by Wilgus, Norris and Heuser[1] to be markedly effective in preventing the development of the osteodystrophy of chicks, named perosis by Titus and Ginn.[2] Prior to this discovery it was found by Hunter, *et al.*,[3, 4] and others that diets high in calcium and phosphorus are in general more perosis-producing than diets containing less of these constituents. Gallup and Norris[5] reported that extremely high levels of manganese in high calcium-phosphorus diets are ineffective in preventing a small residual amount of perosis in Rhode Island Red and New Hampshire chicks. They[6] also reported that under such a dietary regime little manganese is retained in the body of the chick.

In view of these results a study was undertaken to determine if the need for dietary manganese is increased by an increase in the calcium and phosphorus content of the diet. A further purpose was to determine if the causal relation between high calcium-phosphorus diets and perosis is due to the effect of an excess amount of these mineral elements upon the availability of manganese in the intestinal tract.

Rhode Island Red chicks were used as the experimental subjects in this study. They were quartered in battery brooders with wire-mesh floors so as to reduce coprophagy as much as possible. Water was supplied in non-metallic drinking vessels. The experimental period was of 6 weeks' duration.

The basal diet was composed of 40% ground yellow corn, 25.25%

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1 Wilgus, H. S., Norris, L. C., and Heuser, G. F., *J. Nutr.*, 1937, **14**, 155.

2 Titus, H. W., and Ginn, W. M., *Science*, 1931, **74**, 249.

3 Hunter, J. E., and Funk, E. M., *Proc. Poul. Sci. Assn.*, 1930, 22nd ann. mtg., 45.

4 Hunter, J. E., Dutcher, R. A., and Knandel, H. C., *Poul. Sci.*, 1931, **10**, 392.

5 Gallup, W. D., and Norris, L. C., *Poul. Sci.*, 1939, **18**, 76.

6 Gallup, W. D., and Norris, L. C., *Poul. Sci.*, 1939, **18**, 99.

degerminated yellow corn meal, 15% dried skim milk, 5% dehydrated alfalfa meal, 8% commercial casein, 3% meat scrap, 2% liver meal, 1% calcium carbonate, 0.5% iodized salt and 0.25% cod liver oil, containing 250 U.S.P. units of vitamin D per gram.

This diet contained approximately 20% protein, 1% calcium, and 0.5% phosphorus and was designated the low calcium-phosphorus diet. By substituting 7% of bone meal for an equal amount of ground yellow corn in this diet a high calcium-phosphorus diet, containing approximately 3% calcium and 1.5% phosphorus, was obtained.

Manganese was supplied both by incorporation in these diets and by intraperitoneal injection. In the diets c.p. manganous carbonate was used as the source of manganese and in preparing the solutions for injection c.p. manganous chloride was used. A sufficient quantity of sodium chloride was added to each solution so as to make it isotonic with blood.

All the injections were made at 3-day intervals starting when the chicks were 3 days of age. The quantity of solution injected the first time was 0.5 cc. Thereafter it was increased at every second injection by 0.5 cc. A total of 28 cc was injected during the experimental period.

Four lots of 25 chicks each were fed the low calcium-phosphorus diet, supplemented with sufficient manganese to give levels of 0.5 mg, 1.5 mg, 2.5 mg, and 3.5 mg per 100 g of diet respectively. Four lots of chicks were fed the high calcium-phosphorus diet, supplemented with 0.5 mg, 3.5 mg, 7 mg and 14 mg per 100 g of diet respectively. Three lots of 10 chicks each were fed the high calcium-phosphorus diet accompanied by injection of 10 mg, 20 mg, and 60 mg of manganese respectively as previously indicated.

The results of supplying manganese in the diet (Table I) show that, as the manganese level in either the low calcium-phosphorus diet or the high calcium-phosphorus diet was increased, the final average weight of the chicks increased and the incidence of perosis decreased. The apparent exception in the high calcium-phosphorus lot receiving 0.5 mg of manganese per 100 g of diet was due probably to the severe retardation of growth in this lot which according to Gallup and Norris[7] lowers the incidence of perosis. The results also show that a level of 1.5 mg of manganese per 100 g of a diet containing 1% of calcium and 0.5% of phosphorus was just as effective in preventing the development of perosis as a level of 14 mg of manganese per 100 g of a diet containing 3% of calcium and 1.5%

[7] Gallup, W. D., and Norris, L. C., *Poul. Sci.*, 1937, **16**, 351.

TABLE I.
Relationship Between Level of Dietary Calcium and Phosphorus and Level of Dietary Manganese Needed to Prevent Perosis.

Exp.	Chicks per lot	Mg Mn. per 100 g diet	Mg Mn. ingested in 6 wks	Avg wt in g at 6 wks	% of perosis	Severity[1] index
		Calcium 1.0%, phosphorus 0.5%.				
1	25	0.5	4.4	410	61	26.4
	25	1.5	15.5	500	27	10.7
	25	2.5	26.9	515	0	0
	25	3.5	38.0	525	5	2.7
		Calcium 3.0%, phosphorus 1.5%.				
1	25	0.5	3.6	270	42.0	20.5
	25	3.5	28.6	310	66.6	33.9
	25	7.0	60.9	400	44.4	16.6
	25	14.0	141.7	500	26.0	8.0

[1] See reference 1.

of phosphorus. Hence, it is evident that high levels of calcium and phosphorus by increasing the need for dietary manganese interfere in some manner with the effectiveness of this mineral element.

The results of supplying manganese by intraperitoneal injection (Table II), show that 10 mg of manganese per chick during the experimental period completely prevented the development of perosis in spite of the fact that the chicks received a diet abnormally high in calcium and phosphorus whereas the oral intake of 141.7 mg of manganese was only partially effective. The injection of 20 mg and 60 mg of manganese during the experimental period retarded growth considerably. In previous work Gallup and Norris[5] found that the oral intake of approximately 1100 mg of manganese per chick during the same period of time by means of a diet containing 100 mg of manganese per 100 g had no detrimental effect upon growth.

The fact that small amounts of manganese when injected intraperitoneally are completely effective in preventing the development of perosis in chicks fed a high calcium-phosphorus diet was confirmed by the results of a second experiment. In this experiment a lot of chicks injected with an isotonic saline solution containing no manganese served as a control.

From these results it is evident that calcium and phosphorus when present in the diet in excess amounts greatly reduces the availability of manganese in the intestinal tract. This conclusion is supported by the work of Becker and McCollum,[8] who found that diets high in soluble manganese are not toxic to rats provided sufficient phosphorus is present. It is also supported by the work of Blumberg,

[8] Becker, J. E., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1938, 38, 740.

TABLE II.
Effect of Intraperitoneal Injection of Manganese in Preventing Perosis.

Exp.	Chicks per lot	Injection treatment	Mg Mn. injected in 6 wks	Avg wt in g at 6 wks	% of perosis	Severity index
		Calcium 3%, phosphorus 1.5%.				
1	25	None	0	270	42	20.5
	10	Isotonic $MnCl_2$-NaCl soln	10	444	0	0
	10	" $MnCl_2$-NaCl "	20	357	0	0
	10	" $MnCl_2$-NaCl "	60	277	0	0
		Calcium 2.5%, phosphorus 1.25%				
2	20	Isotonic NaCl soln	0	320	40	14
	20	" $MnCl_2$-NaCl soln	14	360	0	0

Shelling and Jackson,[9] who obtained rickets in rats by including large amounts of either manganous chloride or manganous carbonate in diets in which the calcium-phosphorus ratio was 1.1 to 1. Still further support is provided by the results of Wilgus and Patton,[10] who found that the amount of dializable manganese in the intestinal tract of chicks decreases with an increase in the calcium and phosphorus content of the diet.

Summary. The relative effectiveness of ingested and injected manganese in preventing the development of perosis in chicks fed a high calcium-phosphorus diet was studied. The results obtained showed that excess calcium and phosphorus in the diet greatly reduces the availability of manganese in the intestinal tract.

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Experimental Production of Leprosy in the Rabbit with Chrome Acid-Fast Culture.

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It has been noted[1] that the rabbit is susceptible to inoculations with culture of Duval's chromogenic acid-fast bacillus from leprosy. Observations on its behavior to infection by an old and recent chrome acid-fast culture from the human leprous lesion are, therefore, described here. *B. smegmatis* and *B. phlei* were used as controls.

9 Blumberg, H., Shelling, D. H., and Jackson, D. A., *J. Nutr.*, 1938, **16**, 317.

10 Patton, A. R., and Wilgus, H. S., Jr., *J. Nutr.*, 1939, in press.

1 Kriz, J. R., *Am. J. Trop. Med.*, 1938, **18**, 213.