THE ROLE OF MANGANESE AND COBALT IN NUTRITION

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By

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PREFACE

Mineral metabolism has commanded the attention of investigators for many years, but especially within the last decade. In the tissues of plants and animals the values of certain minerals have been found to be quite constant. This consistency has led many to believe that their presence is not incidental, but that they are essential for normal growth and development. Minerals in the body of mammals function in at least three ways, which have been listed by Henry C. Sherman in Chemistry of Food and Nutrition:

(1). As constituents of the bones and teeth, giving rigidity and relative permanence to skeletal tissues.

(2). As essential elements of the organic compounds which are the chief solid constituents of the soft tissues (muscles, blood cells, etc.).

(3). As soluble salts (electrolytes) held in solution in the fluids of the body, giving these fluids their characteristic influence upon elasticity and irritability of muscle and nerve, supplying the material for the acidity or alkalinity of the digestive juices and other secretions, and yet maintaining the approximate neutrality of the body's fluids as well as their osmotic pressure and solvent power.

The function of calcium, phosphorus, sodium, potassium, magnesium, sulphur, iron, copper, and iodine in preventing certain deformity diseases has been well demonstrated.

The role of calcium and phosphorus may be considered together, being constituents of the bones and teeth, and playing a leading part

in contraction and relaxation of the muscle tissues. Calcium above the normal causes a toxic condition of tonic contraction, commonly known as tetany.

Sodium salts take the chief part in the maintenance of normal osmotic pressure and also have a specific influence. Contractibility and irritability disappear if sodium salts are absent; but if present alone they produce relaxation of muscle tissue.

Potassium salts occur to a greater extent in the soft tissues; the corpuscles of the blood, the protoplasm of the muscles and other organs, and also the highly specialized fluids. The absence of potassium brings about a neurosis of the tissues of the abdominal cavity. A high intake of potassium tends to increase sodium elimination. A balance must be maintained between calcium on one hand and sodium and potassium on the other.

According to Graham Lusk 71 per cent of the magnesium of the body is found in the bone structure. The muscles contain more magnesium than calcium. In a deficient magnesium diet experimental animals begin to show mild symptoms of hyperirritability as early as the first week. The irritability becomes greater as the experiment is continued. There are also changes in the skin hyperenia. Death occurs in about 34 days with a loss in weight.

The compounds containing sulphur that have been studied to the greatest extent are the amino acids. Recently other compounds containing sulphur have grown in interest, such as insulin, vitamin B, and heparin. It seems that only the organic compounds of sulphur are used by the body.

Iodine has been known to be an element in the thyroid gland since 1895. Later experiments have shown that iodine is an element in the compound thyroxin. Whether thyroxin, the excretion of the thyroid gland, circulates and functions in the body in its free state or in combination with protein, as in thyroglobin, or in both ways is still a subject of discussion. It is now generally agreed that a deficiency of iodine is the cause of common goiter.

The influence of bromine in the body has been connected with sleep and sedative powers. Some investigators feel that bromine is the secretion of the pituitary gland. The ingestion of bromine brings about an increased excretion of chlorine. There is always a balance maintained by the halogens. If there is more iodine ingested, some of the bromine and chlorine will be lost; likewise, fluorine, when included in the diet, brings about a loss of the other halogens. In a report, however, by E. W. McCollum at the Biochemical Meeting in Baltimore, Maryland, March, 1938, bromine was considered not to be needed in normal metabolism.

Iron and copper are now considered together for hemoglobin formation and regeneration. There has been much discussion of late as to whether copper is the specific activator for iron in hemoglobin regeneration, or whether other trace elements also play a part. The weight of the evidence seems to point to the fact that copper is the specific activator.

Manganese and cobalt have been studied from this point of view. Some investigators feel that copper and iron in the presence of manganese is more effective in the development of hemoglobin. They also

feel that manganese is needed for reproduction and lactation.

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INTRODUCTION

Perosis, a name given to a deformity of the tibial-metatarsal joint in chickens, is frequently found in a large percentage of battery-fed chickens. The ration fed is apparently well supplied with all known nutritive requirements for growth and skeleton formation. Experiments conducted in many laboratories indicate that the condition can be prevented by including small amounts of manganese.[1,2,3] This fact was suggested when an experiment with the use of rice bran extract resulted in an avoidance of the malformation. It was further proved that this was not due to any known vitamin. It was suggested by H. S. Wilgus, L. C. Norris, and G. F. Heuser that manganese or other trace elements were preventives. The analysis of the rice bran[2] extract showed that manganese was present and, therefore, might be the solution of the problem.

Although the exact function of this element or other trace elements is still unknown, these experiments indicate that manganese is needed in bone formation in the species aves.

It is also known that manganese is found in the bones of mammals, and possibly its need will be proved. It is now postulated that it is in some way connected with bone development. Most recent reports indicate that additions of traces of manganese to low rations produce as much as 15 per cent increase in the length of leg bones. The best

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[1] H. S. Wilgus, Jr., L. C. Norris, and G. F. Heuser. Science, 84: 252, 1936.

[2] V. G. Heller and R. Penquite. Poultry Science, 16: 243-246, 1937.

[3] W. D. Gallup and L. C. Norris. Jour. Biol. Chem., 117, xxxvi-xxxvii, 1937.

chemical analysis fails to show that manganese has increased in the chemical structure of bone, but shows that it is accumulated in the bones when fed in large amounts and in some way catalizes bone metabolism.[4]

The fact that its deficiency has not been noted may be caused by the difference in the anatomical structure of the skeleton, which may be due to a small need or possibly to the fact that the ration fed is not unbalanced in certain minerals as the perosis-producing ration.

E. E. Orent and E. W. McCollum found that rats fed on a manganese-deficient diet failed in 58 to 59 cases to suckle their young, and that when these females were given foster young from the stock litters, in 8 cases out of 10, they failed to suckle them. They also found that the stock females did not show interest in and did not suckle the young of the manganese-free mothers, that 7 out of 100 of the manganese-free young were reared by stock-foster mothers, and that these were undersized and of inferior appearance.[5]

Male rats showed no abnormality other than testicular degeneration with complete sterility resulting. The addition of small amounts of manganese in the form of chlorides kept the males in sexual potency.

Manganese is in some way connected or concerned with the production of a hormone by the interior lobe of the hypophysis. This

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[4] Reported at the Biochemical Meeting in Baltimore, Maryland, March, 1938.

[5] E. E. Orent and E. W. McCollum. Jour. Biol. Chem., 92: 651, 1931.

hormone is essential for the functioning of the testes and for the proper development and functioning of the mammary tissue.

Amy L. Daniels and Gladys J. Everson[6] have also stated that rations containing less than minimum amounts results, possibly, in a failure of mammary gland development in the parturient female. But apart from the fact that diets lacking in manganese result in testicular atrophy, the specific role that manganese plays in the well being of the organism as well as the relative amounts needed are unknown.

The purpose of the investigation reported in the body of this thesis is to study the function of related trace elements upon the growth, reproduction, and general well being of mammals. The results of the incidence of perosis in chickens has been studied. The basic perosis ration as well as the supplement used is indicated in Table 1. The effect of these rations on the chickens can be judged by the per cent of birds affected as presented in the table.

The observation of these data will indicate that a large per cent of the birds on the basal diet are affected, that the addition of a trace of manganese reduces the incidence of the malformation, that cobalt and iron do not function in a similar manner, and that the water extract of rice bran proved to be the best preventive. The difficulty is reduced when bone meal is removed from the diet.

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[6] Amy L. Daniels and Gladys J. Everson. Jour. of Nutr., 9: 191-203, 1935.

Table No. 1

THE BASIC PEROSIS RATION PLUS THE SUPPLEMENTS
AND THEIR EFFECT ON CHICKENS

(Expressed in Per Cent)

Constituents of Ration	Rations							
	I	II	III	IV	V	VI	VII	VIII
Yellow corn	67.0	66.0	66.0	66.0	71.0	67.0	67.0	67.0
Wheat middlings	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Alfalfa	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Buttermilk	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Cod liver oil	.25	.25	.25	.25	.25	.25	.25	.25
NaCl	.75	.75	.75	.75	.75	.75	.75	.75
Meat scraps	5.0	5.0	5.0	5.0		5.0	5.0	5.0
Dehydrated meat					5.0			
Bone meal	5.0	5.0	5.0	5.0				
$MnCO_3$		.01				.01		
$CoCl_3$			.02					
Fe lactate				.25				
Casein						5.0	5.0	5.0
Water extract of rice bran						Ad. Lib.	Ad. Lib.	
Water extract of rice bran ashed								Ad. Lib.
Per cent of birds affected	100.0	4.0	54.0	64.5	75.0	10.0	14.0	53.9

An X-ray study of the bones of these chickens, Plates 1 and 2, shows that the malady does not resemble rickets in that there is not a lack of calcification, but that the shaft of the bone is bent, and in many chickens the distal end of the tibia is misshaped; this has permitted a slipping of the tendons from the hock articular. This condition has caused the malformation to be known as slipped tendons, the result of which is an enlarged articular often filled with serum. The bones shown in Plate 1 are normal in shape, while Plate 2 illustrates a typical structure in a perosis bird; Plate 3 is a picture of a chicken so affected.

It has been stated that manganese and rice bran extract aid in bone formation and in the prevention of the malformation found in perosis. The effect that these supplements might have on the animals affected with rickets has not been investigated. Although rickets is a bone malformation of another type, an attempt has been made in this investigation to determine the effect of manganese and rice bran extract and also cobalt on rickets.

Plate 1

Skiagram of the tibia
of a normal chicken.

Plate 2

Skiagram of the tibia
of a chicken with perosis.

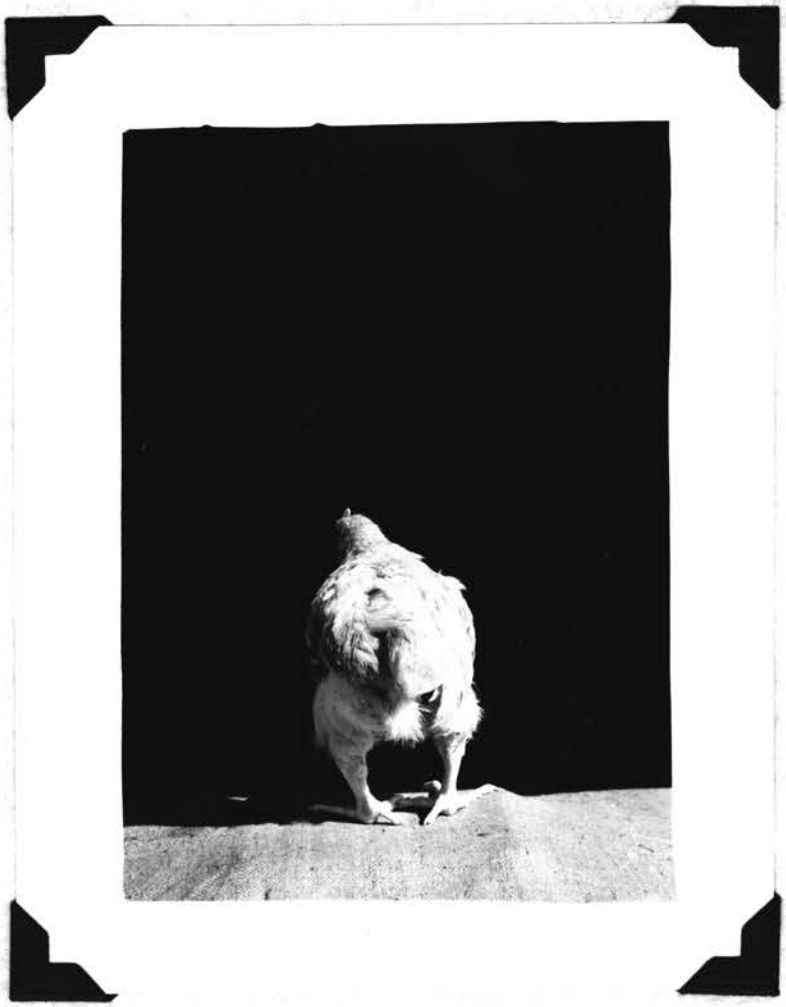

Plate 3

A chicken affected with perosis.

EXPERIMENTAL

In order to determine whether or not a similar condition would result in mammals, the basic perosis ration and the supplements as indicated in Table 2 were fed.

The rat was used as the experimental animal, because it has become the usual laboratory animal, because it is easily grown, and because its life cycle is rapid. The rats were taken from the stock litters at 4 weeks of age and placed on the different rations. They were observed and their weights recorded weekly. Their average weekly gain in weight and their total gains in weight are given in Table 3, 4, and 5. Skiagrams were made at the end of a 3-week period. These can be seen on Plate 4.

The Steenbock ricket-producing ration and the added supplements as indicated in Table 6 were fed. The average rate of growth was recorded weekly. The results are found in Tables 7, 8, and 9. Skiagrams were made at the end of three weeks after the rats were placed on the diet. These can be seen on Plate 5.

In many instances the rats were observed through five series of trials. The average weights of these rats appear in the tables. Two of these series were carried through reproduction.

Table No. 2

THE BASIC PEROSIS RATION PLUS THE SUPPLEMENTS USED IN FEEDING RATS

(Expressed in Per Cent)

Constituents of Ration	Rations								
	I	II	III	IV	V	VI	VII	VIII	IX
Yellow corn	67.0	67.0	67.0	72.0	67.0	67.0	67.0	57.0	67.0
Wheat middlings	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0	5.0
Alfalfa	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Buttermilk	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Cod liver oil	.25	.25	.25	.25	.25	.25	.25	.25	.25
NaCl	.75	.75	.75	.75	.75	.75	.75	.75	.75
Dehydrated meat	5.0	5.0	5.0	5.0					
Casein					5.0	5.0	5.0	5.0	5.0
Bone meal	5.0								
$CaCO_3$		5.0							
Na_3PO_4			5.0						
$MnCO_3$						.007			
$CoCl_3$							.01		
Rice bran								15.0	
Extract of rice bran									Ad. Lib.

Table No. 3

THE EFFECT OF MANGANESE SALTS WHEN ADDED
TO THE BASIC PEROSIS RATION ON GROWTH IN RATS

(Expressed in Grams)

Ration	Initial Weight	Gains Per Week								Total Gains
		1	2	3	4	5	6	7	8	
Males										
Basic ration	48.5	14.5	9.0	21.4	21.9	20.8	15.8	14.0	8.0	125.4
Basic ration plus .25% $MnCO_3$	45.0	21.0	10.5	22.0	23.0	2.5	25.5	23.0	27.5	155.6
Basic ration plus .05% $MnCO_3$	35.5	11.0	14.0	17.0	16.0	19.5				77.5
Basic ration plus .007% $MnCO_3$	57.0	31.5	30.0	36.5	42.5	26.0	0	17.7	7.5	191.7
Females										
Basic ration	44.2	12.2	14.7	17.0	19.3	11.4	17.4	14.0	15.2	121.2
Basic ration plus .25% $MnCO_3$	44.5	18.3	8.8	18.3	17.0	12.3	18.5	10.0	15.0	118.2
Basic ration plus .05% $MnCO_3$	43.5	12.5	23.0	16.0	14.5	17.5	5.0			88.5
Basic ration plus .007% $MnCO_3$	65.0	22.0	18.0	15.0	20.0	9.0	6.0	0	7.0	97.0

Table No. 4

THE EFFECT OF COBALT SALTS WHEN ADDED TO THE BASIC PEROSIS RATION ON GROWTH IN RATS

(Expressed in Grams)

Ration	Initial Weight	Gains Per Week								Total Gains
		1	2	3	4	5	6	7	8	
Males										
Basic ration	48.5	14.5	9.0	21.4	21.9	20.8	15.8	14.0	8.0	125.4
Basic ration plus .05% $CoCl_3$	38.5	7.5	8.5	9.2	6.7	12.0	13.5	10.0	9.5	76.9
Basic ration plus .01% $CoCl_3$	62.5	3.5	5.0	41.0	40.0	20.5	6.0	9.0	15.0	140.0
Females										
Basic ration	44.2	12.2	14.7	17.0	19.3	11.4	17.4	14.0	15.2	121.2
Basic ration plus .05% $CoCl_3$	65.0	5.0	3.5	10.0	7.5	-.5	5.5	2.0	5.5	38.5
Basic ration plus .01% $CoCl_3$	56.0	-4.0	0	33.0	35.0	17.0	8.0	-3.0	10.0	96.0

Table No. 5

THE EFFECT OF VARIOUS SUPPLEMENTS WHEN ADDED
TO THE BASIC PEROSIS RATION ON GROWTH IN THE RAT

(Expressed in Grams)

Ration	Initial Weight	Gains Per Week 1	2	3	4	5	6	7	8	Total Gains
Males										
Basic ration	48.5	14.5	9.0	21.4	21.9	20.8	15.8	14.0	8.0	125.4
Basic ration plus $CaCO_3$	73.0	7.0	12.5	5.5	17.0	15.0	15.0	11.0	.5	83.5
Basic ration plus Na_3PO_4	70.0	22.0	30.0	33.0	35.0	2.0	-2.0	20.0	10.0	150.0
Basic ration minus bone meal	65.0	21.0	24.0	30.0	27.0	7.5	-7.5	17.5	0	119.5
Basic ration plus casein	63.0	37.0	20.0	30.0	40.0	35.0	-10.0	20.0	10.0	182.0
Basic ration plus rice bran	66.5	33.5	26.0	32.0	24.0	9.0	17.5	8.5	16.5	167.0
Basic ration plus rice bran extract	68.0	19.0	24.0	29.0	32.0	17.0	12.5	9.0	17.5	160.0
Females										
Basic ration	44.2	12.2	14.7	17.0	19.3	11.4	17.4	14.0	15.2	121.2
Basic ration plus $CaCO_3$	61.5	4.5	16.5	10.0	12.5	6.0	10.0*	8.0	0*	67.5
Basic ration plus Na_3PO_4	57.5	21.5	16.0	16.0	16.0	7.5	22.5*	7.5	32.0*	139.0
Basic ration minus bone meal	58.0	15.0	19.0	17.0	21.0	9.0	10.0	12.5	-10.0	93.5
Basic ration plus casein	56.0	20.0	12.0	17.0	20.5	15.0	13.5	30.0	8.0	136.0
Basic ration plus rice bran	60.0	20.0	15.0	15.0	20.0	-3.0	20.0	-7.0	5.0	85.0
Basic ration plus rice bran extract	63.0	22.0	22.5	15.0	22.5	7.0	10.0	-5.0	5.0	99.0

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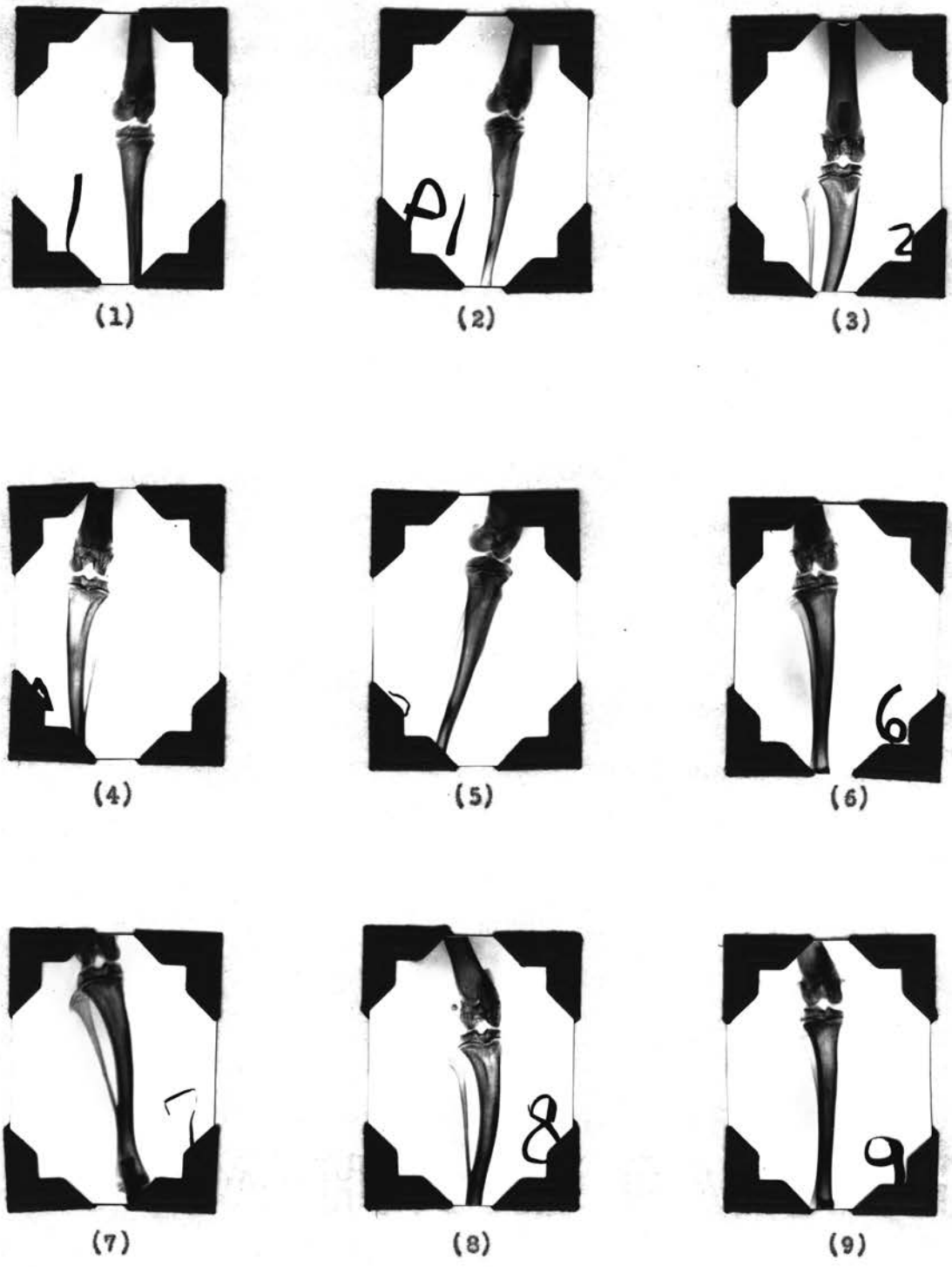


Plate 4

Skiagrams of the tibia of rats on the basic perosis ration and supplements. (1) Basic ration, (2) basic ration plus $CaCO_3$, (3) basic ration plus Na_3PO_4, (4) basic ration without bone meal, (5) basic ration plus casein, (6) basic ration plus $MnCO_3$, (7) basic ration plus $CoCl_3$, (8) basic ration plus rice bran, and (9) basic ration plus water extract of rice bran.

Table No. 6

THE STEENBOCK-RACHITIC PRODUCING RATION
AND THE SUPPLEMENTS USED IN FEEDING RATS

(Expressed in Per Cent)

Constituents of Ration	Rations								
	I	II	III	IV	V	VI	VII	VIII	IX
Yellow corn	76.0	76.0	76.0	76.0	76.0	76.0	76.0	61.0	76.0
Wheat gluten	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$CaCO_3$	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
NaCl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$MnCO_3$		.5	.25	.05	.007				
$CoCl_3$						.1			
Cod liver oil							.1		
Rice bran								15.0	
Rice bran extract									Ad. Lib.

Table No. 7

THE EFFECT OF MANGANESE SALTS WHEN ADDED TO THE STEENBOCK-RACHITIC RATION ON GROWTH IN RATS

(Expressed in Grams)

Ration	Initial Weight	Gains Per Week						Total Gains
		1	2	3	4	5	6	
Males								
Basic ration	45.7	9.0	4.9	14.1	5.5	6.1	10.1	49.7
Basic ration plus .5% Mn	46.0	8.5	6.5	6.5	8.5	5.5	10.0	45.5
Basic ration plus .25% Mn	42.5	8.5	4.5	6.8	4.5	-4.5	14.5	34.3
Basic ration plus .05% Mn	45.0	9.5	9.0	7.0	8.5	7.5	1.0	42.5
Basic ration plus .007% Mn	43.0	4.6	6.0	8.0	3.3	1.6	9.0	32.5
Basic ration plus cod liver oil	46.6	6.6	6.0	11.3	6.0	2.0	14.6	46.5
Females								
Basic ration	46.7	8.0	8.0	9.8	4.9	3.4	5.8	39.9
Basic ration plus .5% Mn	42.5	8.0	3.4	5.9	8.1	-5.0	10.5	30.9
Basic ration plus .25% Mn	49.0	11.0	10.0	11.0	7.5	5.0	9.0	53.5
Basic ration plus .05% Mn	53.0	6.5	8.0	7.0	5.5	5.0	0	32.0
Basic ration plus .007% Mn	42.0	5.0	8.0	12.0	3.0	7.0	10.0	45.0
Basic ration plus cod liver oil	42.5	7.5	3.0	12.0	5.0	2.0	9.0	38.5

Table No. 8

THE EFFECT OF COBALT SALTS WHEN ADDED TO THE STEENBOCK-RACHITIC RATION ON GROWTH IN RATS

(Expressed in Grams)

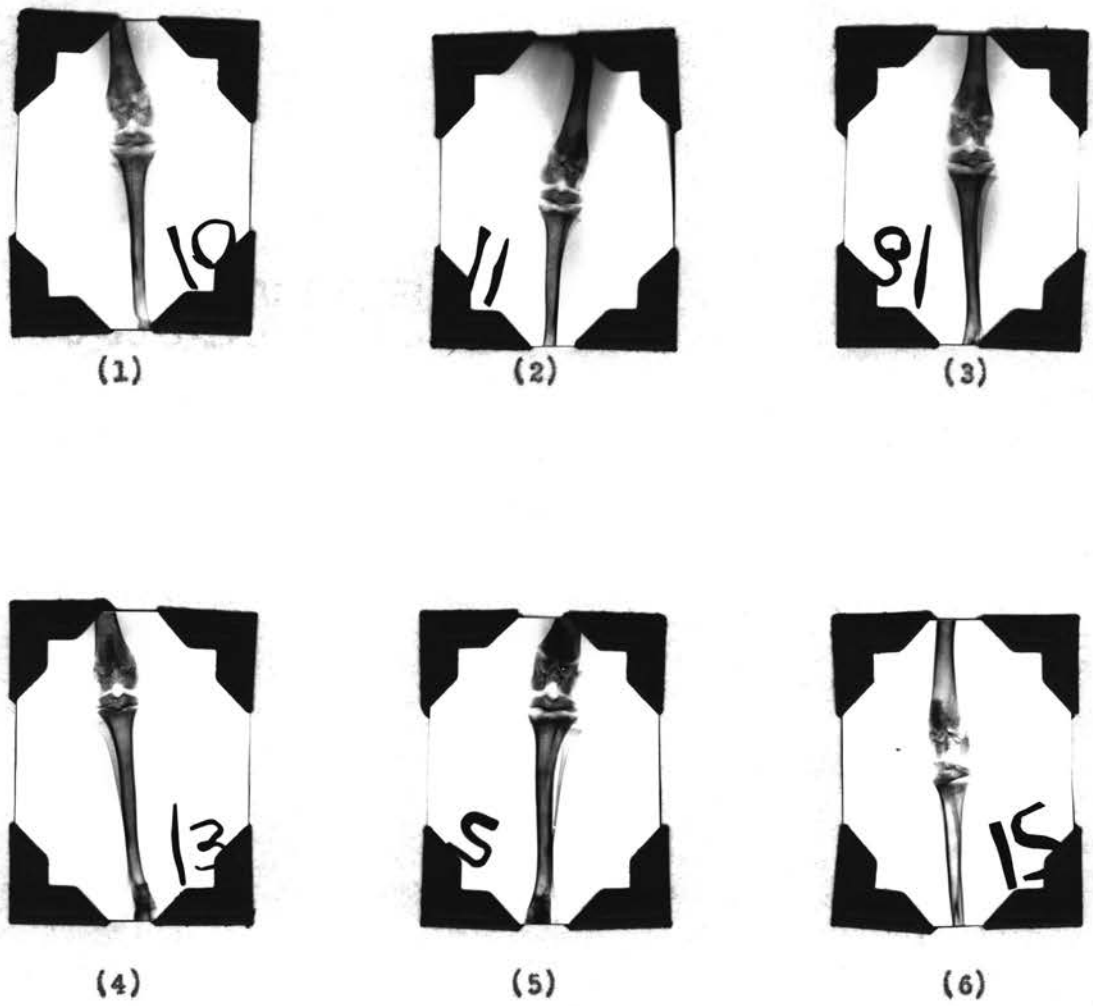
Ration	Initial Weight	Gains Per Week						Total Gains
		1	2	3	4	5	6	
Males								
Basic ration	45.7	9.0	4.9	14.1	5.5	6.1	10.1	49.7
Basic ration plus .1% $CoCl_3$	50.0	7.0	3.0	5.0	5.0	0	2.0	22.0
Basic ration plus .01% $CoCl_3$	41.0	8.0	5.3	9.7	2.6	10.6	6.6	42.8
Basic ration plus cod liver oil	46.6	6.6	6.0	11.3	6.0	2.0	14.6	46.5
Females								
Basic ration	46.7	8.0	8.0	9.8	4.9	3.4	5.8	39.9
Basic ration plus .1% $CoCl_3$	44.0	11.0	5.0	7.5	7.5	2.0	2.0	35.0
Basic ration plus .01% $CoCl_3$	46.0	10.0	5.0	11.0	3.0	5.0	8.0	42.0
Basic ration plus cod liver oil	42.5	7.5	3.0	12.0	5.0	2.0	9.0	38.5

Table No. 9

THE EFFECT OF RICE BRAN AND RICE BRAN EXTRACT
WHEN ADDED TO STEENBOCK-RACHITIC RATION ON GROWTH IN THE RAT

(Expressed in Grams)

Ration	Initial Weight	Gains Per Week						Total Gains
		1	2	3	4	5	6	
Males								
Basic ration	45.7	9.1	4.9	14.1	5.5	6.1	10.1	49.7
Basic ration plus rice bran	48.3	12.3	6.3	16.0	11.6	14.3	7.3	67.8
Basic ration plus extract of rice bran	43.0	11.2	4.3	15.7	3.6	10.3	15.0	60.1
Basic ration plus cod liver oil	46.6	6.6	6.0	11.3	6.0	2.0	14.6	46.5
Females								
Basic ration	46.7	8.0	8.0	9.8	4.9	3.4	5.8	39.9
Basic ration plus rice bran	45.0	10.0	10.0	25.0	7.0	8.0	7.0	67.0
Basic ration plus extract of rice bran	35.0	9.0	3.0	8.0	5.0	2.0	10.0	37.0
Basic ration plus cod liver oil	42.5	7.5	3.0	12.0	5.0	2.0	9.0	38.5



(1) (2) (3)

(4) (5) (6)

Plate 5

Skiagrams of the tibia of rats on Steenbock-ricket producing ration and the Steenbock ration plus the supplements. (1) Basic ration, (2) basic ration plus $MnCO_3$, (3) basic ration plus $CoCl_3$, (4) basic ration plus cod liver oil, (5) basic ration plus rice bran, and (6) basic ration plus the water extract of rice bran.

RESULTS

It can be seen from the tables that at the end of eight weeks the rats fed the basic-perosis diet had an average total gain in weight of 125.4 grams for the males and 121.2 grams for the females. This indicates that the males are somewhat slower in growth than the males fed on a normal-stock ration. The normal males at the end of this period had an average gain of about 155 grams and the females of about 100 grams. So it can be said that the basic-perosis diet does not produce quite so good a growth as the normal-stock ration.

If the growth of the rats on the basic stock-ration is contrasted with the growth after the addition of the different per cents of manganese carbonate, it can be seen that whereas the basic males had an increased growth of 125.4 grams, the males with a .25 per cent addition of manganese had an increased growth of 155.6 grams, and those with a .007 per cent addition had an increased growth of 191.7 grams. Although this indicates that manganese increases the growth rate of the males, the greatest increase came when the smallest amounts were added; this shows that manganese is a needed element, but that a trace is more effective than a larger amount.

If the females of the basic ration are contrasted with those on the basic ration plus the manganese supplement, it can be seen that the females on the basal diet had a total gain in weight of 121.2 grams; those with the .25 per cent manganese addition, 118.2 grams; and those with the .007 per cent addition, 97.0 grams. It seems that in the case of the female, manganese does not aid in

increasing the rate of growth as it does in the case of males but rather has a retarding effect.

With the addition of different percentages of cobalt to the basic ration, the largest per cent, which is .05, gave an increased growth of 76.9 grams for males as contrasted with the increased growth of the basic ration of 125.4 grams, and the smaller addition of cobalt, which is .01 per cent, gave an increased growth of 140.0 grams. Here we find that cobalt has an effect of either increasing or decreasing the rate of growth of the males.

The females showed a slower growth with the addition of cobalt in both concentrations used; 121.2 grams increase for the basic, 38.5 grams for the largest per cent, and 96.0 grams increase for the smallest per cent of cobalt fed.

Upon the addition of calcium carbonate to the basic ration the rate of growth for males was decreased, 125.4 grams for basic and 83.5 grams for those with the calcium carbonate supplement. Likewise, with the females the rate of growth was much smaller, 121.2 grams for the basic ration as contrasted with 67.5 grams for the calcium carbonate supplement.

With the addition of sodium phosphate to the basic ration there was an increased rate of growth for the males of 125.4 grams for the basic rations and 150.0 grams increase for the sodium phosphate; for females the same was true, 121.2 grams increase for the basic females and 139.0 grams for the sodium phosphate.

When bone meal was removed from the ration both the males and females had a decreased rate of growth. In the males the basic ra-

tion supported an increase of 125.4 grams as compared to 119.5 grams when bone meal was removed. In the females the basic ration gave an increase of 121.2 grams as compared to 93.5 grams for the basic ration without the bone meal.

With the addition of rice bran and the water extract of rice bran the male rats grew with an increased rate. The basal diet gave an increase of 125.4 grams, while the rice bran gave an increase of 167.0 grams and the water extract of rice bran an increased gain of 160.0 grams. The females on these same rations were 121.0 grams for the basal, 85.0 grams for the rice bran, and 99.0 grams for the water extract of rice bran.

REPRODUCTION

The basic perosis ration used was an adequate diet for reproduction, for the rats on this ration were carried through two generations. Those on the diets with the manganese supplement were also able to reproduce. Those on the ration with the addition of 0.05 per cent of cobalt, however, did not reproduce although they were carried through 14 weeks after being placed on the ration. The females were then placed with stock males and the males with stock females of known potency. Neither of them reproduced after 4 more weeks. The animals on the lower concentration of .01 per cent cobalt reproduced at the end of 9 weeks after being placed on the diet.

The rats with the supplements of casein and sodium phosphate reproduced at 7 weeks after being placed on the diet, and also those on the rice bran and rice bran extract.

BONE MALFORMATION

The X-rays showed no signs of bone malformation.

RESULTS OF THE STEENBOCK RATION

The rate of growth on the Steenbock diet was, of course, much slower than that on the normal diet; the rats were not held over for reproduction as in the case of those fed the perosis diets.

The rats fed the Steenbock diet and the Steenbock diet plus the cod liver oil showed very little difference in gains in weight.

Male rats on the basic Steenbock ration had an average increase in weight of 49.7 grams during the 6-week period. Those on the .5 per cent, .25 per cent, .05 per cent, and .007 per cent manganese supplements had an average increase of 45.5 grams, 34.3 grams, 42.5 grams, and 32.5 grams, respectively. The female rats on the basic Steenbock ration had an average increase in weight of 39.9 grams. Those on the manganese supplements showed an increase in weight of 30.9 grams on .5 per cent, 53.5 grams on .25 per cent, 32.0 grams on a .05 per cent, and 45.0 grams on a .007 per cent.

The male rats on the cobalt-supplement rations as contrasted with the basic ration in regard to average gains in weight showed an increased weight of 49.7 grams for the basic, 22.0 grams for the .1 per cent cobalt chloride, and 42.8 grams for the .01 per cent cobalt. The females fed the cobalt supplement as contrasted with those fed the basic in regard to average gains in weight in-

creased 39.9 grams for the basic, 35.0 grams for the .1 per cent cobalt, and 42.0 grams for the .01 per cent cobalt.

The addition of rice bran and the water extract of rice bran aided in the rate of growth. The basic ration for the males gave an average gain in weight of 49.7 grams. The rats fed the basic ration plus the rice bran had an average gain in weight of 67.8 grams, and those given the water extract of rice bran 60.1 grams. The average gains in weight for the females on the basic ration, the rice bran addition, and the water extract of rice bran were 39.9 grams, 67.0 grams, and 37.0 grams, respectively.

The skiagrams of the tibia of the rats consuming the Steenbock ration and the Steenbock ration plus the supplements show that the rats on the basic Steenbock, the manganese, the cobalt, and the rice bran had rickets. Those consuming rations supplemented with the water extract of rice bran and the cod liver oil did not.

DISCUSSION

From the results of rats consuming the basic Steenbock ration as compared to those receiving the same ration supplemented with manganese, it appears that the need of the males is greater than that of the females as judged by their growth and development. A need for that element is apparently very small in the female, and the amount present within the basic ration is sufficient. Higher percentages or additional amounts have a detrimental effect, and the growth is retarded.

Cobalt can be said to have a toxic effect when added in concentrations of .05 per cent, for the rate of growth of the males is decreased, but when added in per cents of .01 it seems to speed up the growth. From this it is seen that although cobalt is an essential element needed for the growth and development of the male rat, the amount needed is very small.

The retarding effect that cobalt seems to have on the females means either that the amount they need for normal growth and development is so small that the trace which is found in the ration is sufficient or that they do not need cobalt for normal growth and development.

It seems that when large amounts of calcium carbonate are added to the basic ration the rate of growth for both male and female is retarded. This may indicate that the calcium carbonate addition unbalances the ration by having an excess of calcium in the diet.

With the sodium-phosphate addition to the diet the increased rate of growth for both male and female suggests that sodium phosphate brings about a better balance between calcium and phosphorus within the ration, and therefore a better growth is found.

The removal of bone meal from the basic ration of rats and of chickens has different effects. In the case of chickens the rate of growth increased, while in the case of the rats, both male and female, the rate is decreased. This can be interpreted to mean that the difference in the anatomical structure of the two species brings about a different need in the relation of the elements found in the bone meal.

From the effect of the addition of rice bran and the water extract of rice bran on the rate of growth of the male rats it can readily be seen that both the rice bran and the extract of rice bran give an increased growth. It has been stated that rice bran and the water extract of rice bran contain trace elements and that these are responsible for the differences noted when chickens are fed the rice bran and the extract. It can be assumed that the same result will be produced in the case of rats. The females fed the basic diet plus the rice bran and the rice bran extract, do not show so great an increase as did the females on the basic ration. It is suggested from this that perhaps the factors responsible for the increase in the males are not needed in as high concentrations for the females, and that these factors are present in large enough quantities within the basic ration for normal growth and development.

The increased growth of males shows that manganese carbonate in the concentrations of .007 per cent, cobalt chloride in .01 per cent, and the rice bran and rice bran extract seem to have a beneficial effect. It has been stated that perhaps the beneficial effect of the rice bran and rice bran extract is due to the trace elements present, namely, manganese and cobalt, or to those other, as yet unknown, factors, which are present and which are superior to the trace elements when added alone. The evidence found here can be interpreted in this light.

It can be also said that the basic perosis ration will support reproduction and also the manganese additions. The addition

of cobalt in the concentration of .05 per cent seems to be toxic to both male and female in that the rats are unable to reproduce even after being placed with stock animals of known potency. Reproduction can be attributed to the fact that the added protein, casein, supplies the needed essentials.

Cobalt chloride and calcium carbonate, however, when added to the basic ration will not support reproduction.

The X-ray skiagrams show no sign of bone malformation. It is evident that rats are not affected with a malformation of the bone that is similar to that of perosis in chickens or that their need of the essential factors in its prevention is so small that the basic ration fed is adequate in that respect.

The effect of the Steenbock diet on the rate of growth of rats is retarding, the diet being a deficient one. With the addition of manganese carbonate and cobalt chloride the rate of growth is still further retarded.

The addition of the rice bran and water extract of rice bran increases the rate of growth as the rice bran adds to the nutritive value of the diet, and thus there is an increase in growth.

The X-ray pictures show that manganese cobalt and rice bran do not prevent rickets. The addition of the water extract of rice bran, however, prevents the occurrence of rickets when added to a deficient diet. This is very hard to explain. In the prevention of rickets three factors are known to be necessary; namely, calcium, phosphorus, and vitamin D.

Vitamin D is a fat soluble vitamin and cannot be present in the water extract used. The ration fed is deficient in phosphorus and without vitamin D. What the exact nature of the factor that is present in the water extract of rice bran is cannot be definitely stated. As the result of experiments with the prevention of perosis in chickens, using the water extract of rice bran, the investigators believe that the prevention of that malformation is due to the presence of trace elements, namely, manganese. The manganese, in the case of rickets, had no effect so it cannot be said that manganese present in the water extract of rice bran is the factor.

Before a definite conclusion can be reached further investigations will be necessary.

SUMMARY

1. Rats do not develop a malformation of the bone known as perosis as is found in chickens because there may be a difference either in the anatomical structure or because their needs for those factors preventing the disease are so small that they are present in sufficient quantities in perosis-producing rations fed.

2. Manganese added to the basic ration will increase the rate of growth when added in very small amounts.

3. Cobalt is toxic when fed in amounts of .05 per cent of the ration or over, for the rats are unable to reproduce and the rate of growth is retarded.

4. The addition of rice bran and the water extract of rice bran increases the rate of growth when fed to rats consuming a basic perosis or Steenbock ration.

5. Manganese and cobalt do not aid in the prevention of rickets.

6. The water extract of rice bran contains some factor or factors that aid in the prevention of rickets.

7. More work needs to be done with the water extract of rice bran to determine the exact constituent that prevents the occurrence of rickets.

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