oral dose of 10 mg of this steroid. Since no reports were made concerning desoxycorticosterone acetate, we thought that it would be of interest to know whether it is also able to produce artificial deciduoma.

Eighteen albino female rats weighing 150-200 g were divided into one experimental and one control group, each consisting of 9 animals. The procedure used was reported by Rothschild and Meyer[10] as giving a high percentage of positive results. The animals were spayed while in estrus, then treated with desoxycorticosterone acetate in a daily dose of 10 mg this being given in 2 subcutaneous injections of 0.2 cc of peanut oil. On the fifth day of treatment, the endometrium was traumatized with a suture needle. The hormone treatment was continued for 5 more days after which the animals were killed. The same procedure was used in the case of the animals of the control group except that cholesterol was given instead of desoxycorticosterone acetate.

At autopsy, all the experimental rats showed deciduomata (++++ reaction according to Astwood's scale except a ++ reaction in one sick animal). All the controls were negative.

Conclusions. In rats, desoxycorticosterone acetate is able to determine deciduoma formation in all 9 of the animals used, with a daily dose of 10 mg.

[10] Rothschild, I., and Meyer, R. K., *Physiol. Zool.* 1942, **15**, 216.

14365

A Tracer Study with Mn^{56} on Chicks with Perosis Produced by a Synthetic Manganese Deficient Diet.*

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A high calcium and phosphorus content in the diet of the chick results in the bone condition known as perosis.[1,2] The work of a number of investigators makes it apparent that under the above conditions, the disease is caused by an interference with the absorption of the manganese in the diet.[3,4] Administration of extra manganese, either orally or by injection, prevents the onset of perosis in the chicks reared on the high calcium and phosphorus diets.[3-6]

So far perosis has been produced only in birds reared on natural foodstuffs. It was of interest, therefore, to determine if the condition could be produced by a synthetic diet deficient in manganese, but of normal calcium and phosphorus content. In the experiments reported here, chicks were fed such a diet. The synthetic diet caused the development of the characteristic signs of perosis. The condition in the affected chicks did not differ in any particular form from the perosis caused by natural foodstuffs. It follows from this that primarily the deficiency of manganese and not any particular relationship between the manganese and calcium and phosphorus intake is responsible for the onset of perosis.

Tracer studies with Mn^{56} of the metabolism of manganese have yielded important information about the fate of this element in the body

* The synthetic chick ration used in this study was devised by Dr. H. J. Almquist of the Division of Poultry Husbandry of the University. We gratefully acknowledge our indebtedness to him for supplying us with the ration and for rearing the chicks.

[1] Titus, H. W., *Poul. Sci.*, 1932, **11**, 117.

[2] Hunter, J. E., Dutcher, R. A., and Knandel, H. C., *Poul. Sci.*, 1932, **11**, 239.

[3] Schaible, P. J., and Bandemer, S. A., *Poul. Sci.*, 1942, **21**, 8.

[4] Wilgus, H. S., Jr., Norris, L. C., and Heuser, G. F., *Science*, 1936, **84**, 252; *J. Nutr.*, 1937, **14**, 155.

[5] Lyons, M., Insko, W. M., Jr., and Martin, J. H., *Poul. Sci.*, 1938, **17**, 12, 264.

[6] Wilgus, H. S., Jr., and Patton, A. R., *J. Nutr.*, 1939, **18**, 35.

TABLE I.
Synthetic Chick Ration.*
Constituents in g per 100 g feed.

Basal Components		Salts	
Casein†	20	Calcium gluconate	8.0
Gelatin	5	$Ca_3(PO_4)_2$ precipitated	2.0
Wesson Oil	2.5	K_2HPO_4	0.5
Cod liver oil (U.S.P.)	1.0	KCl	0.3
Cellulose (Cellu-flour)	5.0	NaCl	1.0
Yeast Extract‡ equivalent to	5.0	$MgSO_4 \cdot 7H_2O$	0.3
Glucose (cerelose)	to 100	$Na_2SiO_3 \cdot 6H_2O$	0.2
Vitamin Supplements		$FeSO_4$	0.04
Cholic acid	0.1	$CuSO_4$	0.005
α-tocopherol	0.005	$ZnSO_4$	0.005
Thiaminchloride	0.001	$(Al)_2(SO_4)_3$	0.05
Riboflavin	0.001	KI	0.01
Pyridoxine	0.001	$Co(OOC\,CH_3)_2 \cdot 4H_2O$	0.002
Nicotinic acid	0.003		
Calcium pantothenate	0.004		
Biotin concentrate§	0.5		
Choline chloride	0.2		
Vitamin K‖			
Hydroquinone	0.001		

† Reprecipitated and dried with ethanol.
‡ 50% methanol extract concentrated to 1 ml = 4 g.
§ Biotin concentrate from S. M. A. Corp.
‖ 2 methyl-1,4-naphthohydroquinone-diphosphoric ester (tetra sodium salt).

of the mammal.[7-8] Because of the many differences in physiological characteristics between the mammal and the bird, a tracer study was conducted with Mn^{56} of the fate of manganese in the manganese-low chicks and their controls.

Experimental. Perosis. Newly hatched chicks were placed on the synthetic ration which had the composition shown in Table I. An analysis of this diet showed it contained 2.8 mg Mn per kilo of feed. The content of the control diet was 59.6 mg Mn per kilo.

The chicks placed on the manganese-low diet were retarded in weight and began to show evidence of perosis in about two weeks time. The leg bones of the deficient chicks were shorter in comparison to the width than the bones of the controls. A comparison of the weight and bone measurements are given in Table II. In several of the chicks the deformity due to the perosis progressed to such a degree that they were no longer able to stand up and feed.

Tracer Experiments. After the 2-week growth period, groups of 4 each of the deficient and control chicks were given the radioactive manganese. Each chick was given 1 ml of a solution containing 15.8 μg of Mn*† dissolved in 0.9% NaCl solution. The radioactive count in each ml of solution was 9200 per minute for Group I and 10,000 for Group II. Half of each group of chicks were given the Mn* orally, the other half by subcutaneous injection.‡ Each chick was placed in a separate metabolism cage and the excreta collected. They were sacrificed 72 hours after the administration of the radioactive material and the liver, kidney, and carcass as well as the excreta were analyzed for the distribution of the Mn*. The gastrointestinal tract was dissected out and added to the excreta of each chick. The results are given in Table III. The radioactivity of the samples was measured as described in previous publications.[7,8] Total manganese was determined by the colorimetric method of Wiese and Johnson.[9]

Discussion. The total radioactive man-

[7] Greenberg, D. M., and Campbell, W. W., *Proc. Nat. Acad. Sci.*, 1940, **26**, 448.

[8] Greenberg, D. M., Copp, D. H., and Cuthbertson, E. M., *J. Biol. Chem.*, 1943, **147**, 749.

† The asterisk is used to designate a labeled element.

‡ One animal of Group I died when injected intraperitoneally with the manganese-containing solution.

[9] Wiese, A. C., and Johnson, B. C., *J. Biol. Chem.*, 1939, **127**, 203.

TABLE II.
Bone Measurements of Manganese-Deficient and Control Chicks.

	Tibia		Metatarsus		Weight of 2 tibia + 2 metatarsals (g)
	Length (cm)	Joint width (cm)	Length (cm)	Joint width (cm)	
		Deficient.			
Group I	3.77 ± 0.15 (6)	0.575 ± 0.03 (6)	2.77 ± 0.17 (6)	0.62 ± 0.03 (6)	0.81 ± 0.15 (3)
Group II	4.0 ± 0.12 (8)	0.77 ± 0.6 (8)	3.0 ± 0.07 (8)	0.73 ± 0.04 (8)	1.325 ± 0.30 (3)
		Controls.			
Group I	4.5 ± 0.20 (8)	0.60 ± 0.04 (8)	3.5 ± 0.15 (8)	0.60 ± 0.04 (8)	1.165 ± 0.24 (4)
Group II	4.9 ± 0.10 (8)	0.65 ± 0.04 (8)	3.45 ± 0.13 (8)	0.70 ± 0.03 (8)	1.505 ± 0.9 (3)

Measure of variability is mean deviation of the mean. Numbers in parentheses represent the number of samples.

TABLE III.
Partition of Labeled and Total Manganese in Manganese-Deficient and Control Chicks.*

	Excreta	Liver	Kidney	Carcass
	Labeled manganese in per cent of total dose.			
Deficient, Oral	96.3 ± 0.85 (4)	1.0 ± 0.14 (4)	0 (3)	2.4 ± 0.7 (4)
" Injected	65.0 ± 0.6 (3)	16.0 ± 0.33 (3)	2.3 ± 1.3 (3)	10.3 ± 0.7 (3)
Control, Oral	99.0 ± 0.05 (4)	0.3 ± 0.3 (4)	0 (3)	0.7 ± 0.17 (4)
" Injected	75.6 ± 3.1 (4)	3.5 ± 0.36 (4)	2.35 ± 0.2 (4)	18.2 ± 1.4 (4)
	Total manganese (mg per 100 g fresh tissue).			
Deficient		0.053 ± 0.012 (7)	0.05 ± 0.06 (6)	0.023 ± 0.03 (7)
Control		0.20 ± 0.026 (8)	0.22 ± 0.006 (7)	0.07 ± 0.015 (8)

* The figures represent mean values ± mean deviation. Numbers in parentheses are the number of chicks analyzed.

ganese recovered from each animal was computed from the measured activities of the excreta, carcass and the tissues analyzed separately. The recovery varied between 90 and 101% of the total radio-counts. To give a uniform basis of comparison, the data have been corrected in each case to a total value of 100.

Examination of the data of Table III shows that the major part of the administered dose of Mn*, little as it was, was excreted by both the manganese-low and the control chicks. This was true of the injected as well as the orally administered manganese. In the case of both routes of administration, however, the deficient chicks retained a greater proportion of the Mn*, than did the controls. Nearly all the tissues of the manganese-deficient birds showed a considerable retention of the administered Mn*, liver being the most conspicuous. Bone showed little participation in the turnover of the administered manganese. Measurable amounts of Mn* appeared only in the bone of the injected deficient chicks. No Mn* was demonstrable in the bone of either the manganese-low or control birds which were given the dose orally. The manganese requirement of bone must be extremely minute.

The greater accumulation of Mn* in the livers of the deficient over that of the control chicks signifies that manganese has an important role in liver function. It has been surmised that in the mammalian liver, manganese is required for the activation of the enzyme arginase. The liver of the chick does not contain any arginase. Evidently manganese is required by the liver for other processes besides arginase activation.

There was no differentiation in the uptake of Mn* by the kidney between the deficient and control chicks. The Mn* that appeared in the kidney presumably was intended for excretion since no Mn* was found in this organ when the element was administered orally.

Summary. Perosis has been produced in chicks reared on a synthetic manganese-deficient diet. Tracer experiments with labeled manganese show that the major part of the manganese, whether administered orally or by injection, is excreted by both the deficient and control chicks. The liver is the

most active participant in the body in the metabolism of manganese. The uptake of labeled manganese by bone was not measurable when the dose was administered orally.

14366

Relaxation of the Pelvic Ligaments of Castrate Hysterectomized Guinea Pigs Induced by Progesterone.*

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Haterius and Fugo[1] showed that in castrate female guinea pigs relaxation of the pelvic ligaments could be elicited by treatment with crystalline progesterone after preliminary conditioning with estrogens. These findings cast some doubt upon the existence of a separate relaxation hormone, or at least its indispensability in the process of pelvic relaxation.

More recently Hisaw *et al.*[2] confirmed the ability of crystalline progesterone to relax the pelvic ligaments in the spayed and estrogenized guinea pig. But they reported that in castrates which were hysterectomized the estrogen-progesterone treatment did not induce relaxation. The authors concluded therefore that under the conditions of their experiments "the uterus is indispensable for the formation of relaxin."

It would seem from this interesting report that the presence of the uterus in some unexplained way facilitates relaxation but is not a necessary factor in the development of this reaction. Similarly progesterone is not indispensable since relaxin alone produces relaxation in the estrogenized hysterectomized castrate. Surprisingly, however, the combination of two factors (uterus and progesterone) each shown to be nonessential, together give a positive reaction.

It was not the intention of this study to analyze the complicated nature of this reaction. Rather it was thought necessary to reinvestigate our earlier conclusion, namely, that progesterone alone was sufficient to produce relaxation when given in combination with estrogen.

Furthermore it occurred to us that perhaps the failure of Hisaw *et al.* to obtain relaxation in castrate hysterectomized guinea pigs might

TABLE I.
Summary of Cases of Relaxation Following Estrogen-progesterone Treatment in Castrated-hysterectomized Guinea Pigs.

Guinea pig No.	1	2	3	4	5	6	7	8	9*	10*	11*	12*
0.5 mg Est. Diprop. daily (days)	7	7	7	7	7	7	7	7	0	0	0	0
0.5 mg Est. Diprop. + 0.5 mg Prog. daily (days)	7	7	7	7	7	7	7	7	0	0	0	0
1.0 mg Est. Diprop. + 1.0 mg Prog. daily (days)	4	2	2	3	0	1	3	0	0	0	0	0
Total mg Est. Diprop.	11.0	9.0	9.0	10.0	7.0	8.0	10.0	7.0	0	0	0	0
Total mg Prog.	7.5	5.5	5.5	6.5	3.5	4.5	6.5	3.5	0	0	0	0
Day Relax. occurred after init. inject.	19	17	17	18	15	16	18	15	N.R.	N.R.	N.R.	N.R.

N.R. = No relaxation.
*Castrated and hysterectomized.

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1 Haterius, H. O., and Fugo, N. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 155.

2 Hisaw, F. L., Zarrow, M. X., Talmage, R. V. N., Money, W. L., and Abramowitz, A. A., *Anat. Rec.*, 1942, **84**, 457.