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SECTION MEETINGS

DISTRICT OF COLUMBIA	
Georgetown Univ. School of Medicine	December 7, 1961
IOWA	
State University of Iowa	November 26, 1961
NORTHWEST	
Hanford Labs., Richland, Wash.	November 17-18, 1961
SOUTHEASTERN	
Emory Univ. and Communicable Disease Center, Atlanta, Ga.	November 17-18, 1961
SOUTHERN	
Louisiana State Univ. School of Medicine	December 5, 1961

Radiographic Studies on Skeletal Parts of Zinc Deficient Pullets.* (27165)

THOMAS R. ZEIGLER, MILTON L. SCOTT, RICHARD K. MCEVOY, ROBERT H. GREENLAW, FELIX HUEGIN AND WILLIAM H. STRAIN

Department of Poultry Husbandry and Graduate School of Nutrition, Cornell University, Ithaca, N. Y., and School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

In experiments originally designed to explore whether zinc deficiency favors deposition of cholesterol in the blood vessels and/or gall bladders, radiographs were made of pullets grown for 3 weeks on various levels of zinc supplementation, and on a group grown for 10 weeks with 2% of cholesterol added to the rations after the 4-week initial growth period.

Although radiographs are not commonly used in experimental studies on poultry, they are particularly suitable for graphic portrayal of differences in skeletal development. In the present study the effectiveness of radiographic comparisons is illustrated by the roentgenograms of pullets raised on varying levels of available zinc. Radiographs of the hocks at 3 weeks of growth show that the deficiency of zinc affects primarily the cartilaginous structures. In contrast, the roentgenograms made after 10 weeks of growth show extensive differences in calcification of the joints and long bones as well as alteration in size and shape of the skeletal structures.

Materials and methods. The basal diet used in this study was diet C described previously by Zeigler *et al.*(1). This diet was supplemented with 5, 10, 15 or 60 ppm of zinc as zinc sulfate. At the end of 4 weeks, 2% of USP cholesterol was mixed into each diet, and the pullets maintained on these diets

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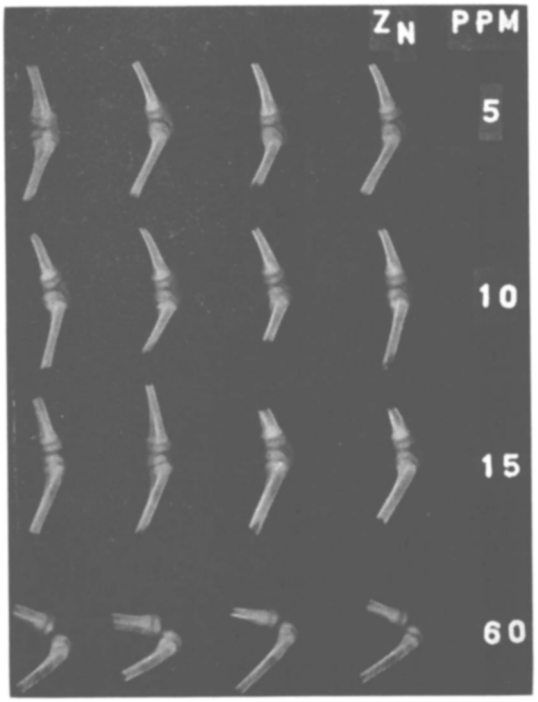

FIG. 1. Radiographs of hocks from 3-wk-old pullets showing that elaboration and ossification of the epiphyseal cartilages are directly related to level of zinc supplementation. Caliber of the shaft and over-all size of the metaphyseal-epiphyseal section is smaller and there is less prominent evidence of endosteal osteoclastic activity as zinc intake is restricted. Note absence of osteoporosis.

until the end of the study. Feed was supplied *ad libitum* in stainless steel trays, and distilled water was available from plastic and glass fountains.

Twenty female, hatch-mate, day-old White Cornish × White Rock crossbred chicks produced by hens receiving a commercial breeder ration were placed on each of the diets. The chicks were housed in lacquered, electrically-heated batteries.

Pullets from each group were killed at 3, 8 and 10 weeks, usually after various supplementary studies, such as radiozinc-65 uptake, had been carried out. Radiographs were made of the whole pullets and of various dismembered structures; the latter were placed with the medial side down on the X-ray film holder. Fixed factors used in making the radiographs were as follows: KV. P. – 30; MA – 50; distance – 72″; F cone; no filtration; and screen film in cardboard holders. Time of exposure was adjusted to the thickness of the part and the quality of radiograph desired. Most of the radiographs were made at 4 or 6 seconds by exposing 2 or 3 times for 2 seconds each so as to protect the X-ray tube.

Results. Radiographs of the hocks, alone, from the 3-week-old pullets are shown in Fig. 1. The hocks were placed in formalin prior to radiography so that sections could be prepared, if desired, for microradiography. Accordingly, the radiographs show only bone on either side of the hock and none of the skin, since the latter was removed before the exposures were made.

Photographs of the 10-week-old pullets, together with their weights, are given in Fig. 2A. The radiographs of the left leg from each of these pullets are reproduced in Fig. 2B, of the left wing with nearly all feathers removed in Fig. 2C, and of the necks with the skin removed in Fig. 2D.

Discussion. Differences in radiographic density of the structures of the hocks shown in Fig. 1 are slight, yet there is a definite trend toward higher calcification with increased zinc content of the diet. The radiographs suggest that the hock formation of these 3-week-old pullets may have been considerably influenced by the zinc supplied from the egg, as well as by the metal available from the diet.

The radiographs reproduced in Fig. 2B-2D of the legs, wings and necks of the 10-week-old pullets show that with extension of growing time the level of calcification does reflect the available zinc in the diet. The differences in calcification are seen both in the long bones and in the cartilaginous structures of the

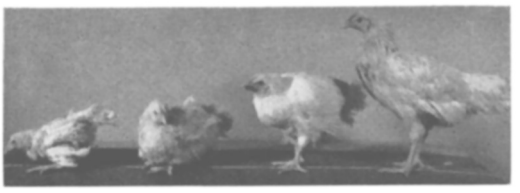

FIG. 2A. Effect of zinc supplements on growth of pullets 10 wk after hatching. Externally, zinc-deficient birds show poorer growth, poorer feathering, and enlarged hock joints. Zinc-deficient pullets exhibit extreme weakness and standing erect appears difficult or painful.

joints. The relative thickening and shortening of the long bones of the pullets on the zinc deficient diets is as evident in the wing bones as in the leg. The radiographs of the

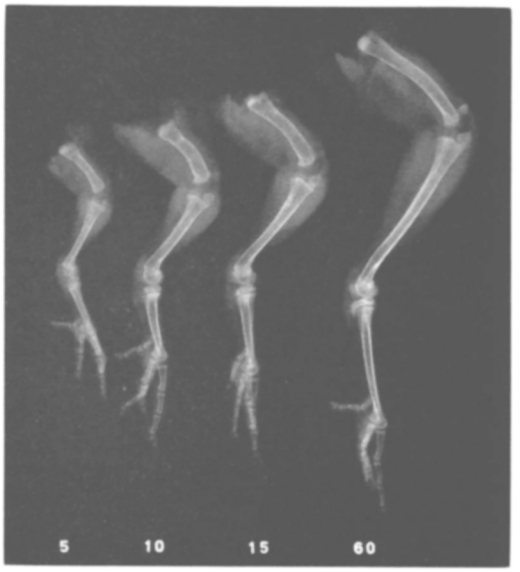

FIG. 2B. Radiographs of legs from pullets at 10 wk showing effects of zinc deficiency on calcium deposition, and on shortening and thickening of long bones. Comparing lengths of femora, with 60 ppm as the normal, 5 ppm is less than half, 10 ppm slightly more than half, and 15 ppm approximately two-thirds that of normal. Difference in caliber of the long bones is not proportionately similar to difference in length. Only 5 ppm is significantly smaller in diameter. The striking difference is centered in the area of epiphysis where marked retardation in development and ossification is present in the most deficient bird and progressively less marked as level of supplementation is increased. Cortical bone is thinner in deficient birds and there is less evidence of endosteal reconstruction in the cancellous portion.

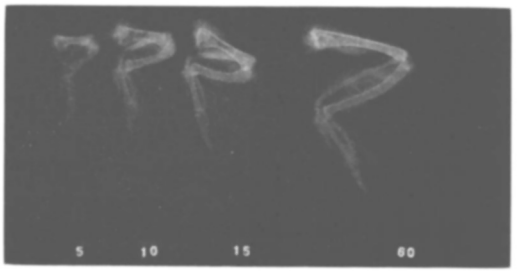

FIG. 2C. Radiographs of wings from pullets at 10 wk of age. Comparison of length of humeri, with 60 ppm as normal, shows the same relative shortening with decrease in zinc intake as was obtained in femora (Fig. 2B). Caliber of bones is only significantly smaller in pullet on 5 ppm of zinc. Increased calcification with increased zinc supplementation is seen most clearly in carpal joints.

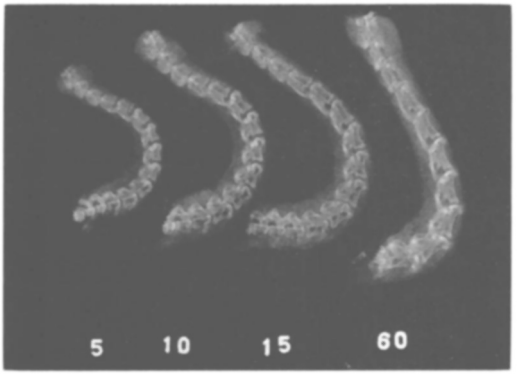

FIG. 2D. Radiographs of cervical vertebrae from pullets at 10 wk of age. Restriction of zinc intake results in the same relative retardation in bone growth, primarily affecting the length, as noted in legs and wings. Also, there is relative widening of intervertebral space. Increased bone trabeculation with increasing zinc supplementation is seen more clearly in cervical vertebrae than in legs or wings.

cervical vertebrae suggest most definitely that zinc deficiency produces a state of suspended growth.

These radiographic studies supplement earlier work on the pathology of zinc deficiency. The initial descriptions (Morrison *et al.*(2); Morrison and Sarett(3)) focused on the abnormality of the hock. Chemical analyses of the ash from joints taken from chicks on varying levels of zinc showed that enlargement and elongation of the joints took place without any change in percentage composition of the bone ash. O'Dell, Newberne and Savage(4) pointed out that severe zinc deficiency produced poor calcification of the bone without apparent failure of cartilage cell development in the epiphyseal plate region, and decreased osteoblastic activity in the thin bony collars. The radiographic studies presented here confirm these observations.

Summary. Radiographs made of the long bones, joints and necks of pullets raised on varying levels of zinc supplementation show graphically how zinc availability affects the level of calcium deposition. These comparisons demonstrate the advantages of the roentgenographic method for portraying skeletal differences in poultry studies.

1. Zeigler, T. R., Leach, R. M., Jr., Norris, L. C., Scott, M. L., *Poultry Sci.*, 1961, v40, 1584.

2. Morrison, A. B., Dam, R., Norris, L. C., Scott,

M. L., *J. Nutrition,* 1956, v60, 283.

3. Morrison, A. B., Sarett, H. P., *ibid.,* 1958, v65, 267.

4. O'Dell, B. L., Newberne, P. M., Savage, J. E., *ibid.,* 1958, v65, 503.

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Metabolism of Tritium-Labeled Pyridoxine in Rats.* (27166)

S. H. COX, A. MURRAY, AND IRENE U. BOONE
Los Alamos Scientific Laboratory, University of California, Los Alamos, N. Mex.

Earlier investigations on the metabolism of pyridoxine in animals have involved the study of degradation products by chemical methods and microbiological assay procedures (1-5). The present knowledge of the metabolism of Vit. B_6 (pyridoxine, pyridoxamine, and pyridoxal) in man and other animals, obtained by using these procedures, has been summarized by Snell(6). Low recoveries of ingested or injected pyridoxine in previous metabolic studies in animals have led to the speculation that a portion of the "missing" pyridoxine is excreted in conjugated forms as yet unidentified. An alternate hypothesis for the low recoveries would be that the ingested drug was stored in the tissues. This latter explanation was discounted by Rabinowitz and Snell(5) on the basis that no great storage of the vitamin would be expected in normal animals, and on the fact that excretion levels rapidly returned to base levels in human subjects. The present study of the turnover times and urinary and fecal excretion of H^3-pyridoxine was undertaken to investigate these hypotheses further.

Methods. Tritium- (H^3) labeled pyridoxine hydrochloride was obtained by exchange of pyridoxine borate in tritium water over platinum catalyst(7). Chemical and radioactive purity was established by paper chromatography. Specific activity of the H^3-pyridoxine hydrochloride was 308 μc/mg. The solution used for injection contained 2 mg (616 μc) of labeled and 18 mg of unlabeled pyridoxine hydrochloride/ml. All injections were intravenous *via* the jugular sinus route. The rats weighed between 250 and 350 g. Rat A received 184.8 μc H^3-pyridoxine · HCl. Rats B and C received 369.6 μc, while rats D through K received 616 μc H^3-pyridoxine · HCl. Following injection of the drug, the rats were placed individually in standard metabolism cages.

Rats A, B, and C had blood and urine samples taken at various time intervals post injection. Urine and fecal samples were collected from rats D to K for detailed urinary and fecal excretion and chromatographic studies.

Blood and urine specimens were measured for tritium using the liquid scintillation-dioxane system as described previously by Richmond *et al.*(8). The feces were homogenized in distilled water, decolorized with charcoal, and counted as above. The counting efficiency of each sample was determined with the use of an internal standard. All calculations were in absolute counting rates (dpm) and results expressed as % of injected dose.

All urine specimens obtained up to 5½ hours after injection were chromatographed by the descending method(9). One-tenth ml of urine was placed directly on No. 1 Whatman filter paper strips. In all instances, this was at least 5×10^6 dpm of H^3 activity. Eighty per cent propanol was the solvent system used. It was necessary to expose the chromatogram strips to X-ray film for at least 4 weeks to get satisfactory exposures on the film.

Results. Concentrations of H^3 activity in blood as a function of time after administration of labeled pyridoxine are shown in Fig. 1. The log of the % injected dose is plotted

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