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Alkaline Phosphatase, Feathering and Bone Ash in Chicks As Affected by Hemin.* (28081)

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Previous work(1,2) showed that parenteral injections of hemin, protoporphyrin and hematoporphyrin produced severe growth depression and varying degrees of mortality in chicks. Also, injections of hemin and protoporphyrin resulted in moderate to severe bowing of legs in a large percentage of chicks similar to nutrient deficiencies. Incidence of bowing was directly related to dose.

Present experiments were designed to correlate certain physical and biochemical aspects of the deformity with those of known nutrient deficiencies. Among these nutrients are zinc(3), manganese(4), choline(5), biotin(6,7), niacin(8) and folic acid(9).

Materials and methods. Day-old White Rock male chicks (Arbor Acres) were used in all experiments. Weighing, distributing, housing and feeding of chicks and preparation and administration of solutions were essentially as previously reported(2). Solutions were prepared fresh daily. In these trials, 5 mg level of hemin (Eastman Kodak Co.) was used and treatment was started on 8th day.

Serum alkaline phosphatase activity (SAP) and bone alkaline phosphatase activity (BAP) were determned by the method of Bessey, Lowry and Brock(10). SAP determinations were made at 2, 6 and 10 days after treatment in Trial 1. SAP and BAP were determined at 2 and 6 days in Trial 2. Bone enzyme was extracted from cleaned, weighed tibiae by grinding in cleaned sea sand and adding sufficient distilled water to make a 2% solution (w/v). This mixture was incubated for 3 days at 34°F, filtered, and a 0.1 cc sample used for activity determination.

Per cent bone ash was determined on fat-free, dry femurs according to the method described by the Assn. of Official Agricultural Chemists(11).

Results. The effect of hemin on SAP is shown in Table I. In Trial 1, SAP is significantly depressed for 6 days following the last injection and for 2 days in Trial 2. At 10 days in Trial 1 and at 6 days in Trial 2, average activity and range of activity are still below those of the controls; however, the dif-

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TABLE I. Serum Alkaline Phosphatase Activity of White Rock Males After Treatment with 5 mg Hemin Daily for 5 Days (mmoles nitrophenol/hr/0.1 cc).

Day after treatment	Treatment	Controls
	Trial 1	
2 (20)	67.2 ± 11.9*‡	186.1
6 "	59.0 ± 12.3*	103.2
10 "	51.4 ± 15.4†	74.6
	Trial 2	
2 (20)	112.6 ± 9.5*	149.3
6 "	100.6 ± 13.1†	119.1

* $p = .001$ (t test). § $p = .010$ (t test).
† $p = .200$ (t test).
‡ Mean ± S.E. of difference from control.
( ) Number indicates No. of birds/determination.

ference is not statistically significant.

BAP determined on some birds in Trial 2 is shown in Table II. Treatment significantly depressed activity for 2 days following last treatment. Analysis showed 6 day data approached significance at the 5% level.

Table III presents data obtained on 2 trials for the effect of hemin on percentage of bone ash. There is virtually no difference between treatment and control groups.

TABLE II. Bone Alkaline Phosphatase Activity of White Rock Males After Treatment with 5 mg Hemin Daily for 5 Days (mmoles nitrophenol/hr/g wet bone).

Day after treatment	Treatment	Controls
2 (5)	1960 ± 307.25*‡	3500
6 "	2520 ± 334.7§	3220

See Table I for symbols.

TABLE III. Per Cent Bone Ash of Left Femur of White Rock Males After Treatment with 5 mg Hemin Daily for 5 Days (Expressed on Fat-Free, Dry Basis).

Day after treatment	Treatment	Controls
	Trial 1	
2 (19)	41.87	(20) 43.61
6 "	43.96	(19) 45.16
10 (20)	44.80	(20) "
	Trial 2	
2 (20)	43.28	(20) 43.67
6 "	45.58	" 45.17
10 "	45.39	" 44.99

See Table I for symbols.

Feathering is affected grossly by hemin treatment (Fig. 1). There appears to be an absence of barbs and barbules on some feathers of the treated chicks. However, no abnormal feathering was noted in birds which survived treatment for 10 or more days, even though their legs might be badly bowed.

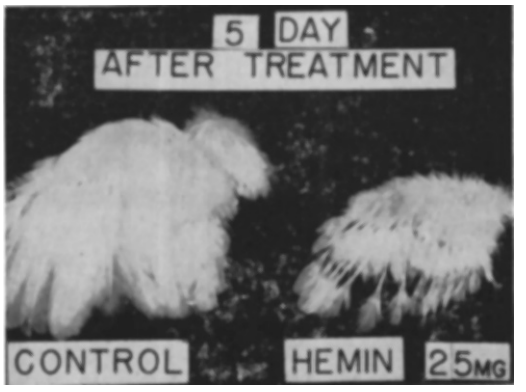

FIG. 1. Abnormal feathering noted in chicks following treatment with hemin, 5 mg daily, for 5 days. Compared with control chick of same age.

Discussion. The exact mechanism for production of osteodystrophy in chicks by hemin treatment is not known. Observations presented here are similar in some respects to observations of others in cases of nutrient deficiency perosis. SAP and BAP are decreased in manganese deficiency(12,13). The effect of choline on these enzymes is unclear. SAP and BAP have been reported by one laboratory(14) to be unrelated to choline perosis; however in another(15) bones from choline deficient birds had lower BAP. This latter group(15) found no decrease in BAP in zinc or folic acid deficient birds. However, SAP is very sensitive to dietary zinc in swine(16). Manganese, zinc, choline and folic acid deficiencies cause reduction in bone length, but no significant difference in thickness, breaking strength or ash content(15). Extremely poor feathering has been described in zinc deficient chicks(17,18). Gross symptoms in hatched chicks and embryos made porphyric by injections of allylisopropylacetylcarbamide (Sedormid)(19) closely resemble those in embryos and hatched chicks from zinc(20) and manganese(21) deficient dams.

Appearance of bowed legs, feather abnormalities, decreased SAP and BAP and lack of differences in bone ash following hemin treatment are similar to observations of oth-

ers in perosis due to the above nutrient deficiencies. Thus it is possible that osteodystrophy in chicks following hemin administration may be associated with interruption of some biochemical pathway(s) in common with the anomalies produced by these deficiencies and Sedormid.

Summary. An investigation of certain physical and biochemical aspects of osteodystrophy in chicks treated with hemin revealed a significant decrease in serum and bone alkaline phosphatase activity. In addition, feathering was abnormal. No change in per cent bone ash was observed. Possible relationship to known nutrient deficiency symptoms is discussed.

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Histology of Osteodystrophy in Hemin Treated Chicks.* (28082)

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Abnormal bone development, poor growth and high mortality in chicks after porphyrin administration has been demonstrated in this laboratory(1,2). Certain physical and biochemical changes associated with hemin treatment have also been reported(3). The present study was designed to describe the histology of these dystrophic bones and possibly relate these findings to bone pathology reported in zinc(4,5), manganese and choline (6) deficiency perosis.

Materials and methods. Day-old White Rock male chicks (Arbor Acres) were used. Housing and feeding of chicks and preparation and administration of solution were essentially as previously reported(2). In this study 5 mg level of hemin (Eastman Kodak Co.) was used and treatment was started on 2nd day. The distal epiphysis of the tibiotarsus and the proximal epiphysis of the tarsometatarsus of 20 chicks (10 control, 10

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