Effect of Dietary Magnesium and Fluoride on Citric Acid Content of Chick Bones.*† (29322)

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Chicks maintained on diets high in both magnseium and fluoride have been shown to have high plasma magnseium, low bone ash, poor growth and leg weakness when compared with chicks maintained on normal diets or with those maintained on diets high in either magnesium or fluoride(1,2). It was also found that the fluoride content of bones from chicks maintained on diets supplemented with 0.25% magnesium and 0.08% fluoride was lower than that of chicks maintained on diets which contained 0.08% supplementary fluoride alone(2). Zipkin *et al*(3) found that human bone samples containing up to 0.4% fluoride had normal calcium, phosphorus and ash although citric acid content was reduced. Menczel *et al*(4) reported that fluoride increased the "crystallinity" of bone as indicated by X-ray diffraction. Zipkin *et al*(5,6) and Schraer *et al*(7) suggested that the effective crystal surface area of the bone was reduced as the result of larger crystals and/or fewer imperfections in the presence of fluoride, thus excluding citric acid from the bone surface.

Two experiments were conducted to determine the effects of dietary supplements of magnesium and fluoride, individually and in combination, on the citric acid content of chick bones.

Materials and methods. In the first experiment, day-old White Cross cockerels were randomly assigned to 4 groups of 25 chicks each, banded, weighed and placed in electrically heated, wire-floored batteries. The basal diet consisted of the following in percentage of the total: ground yellow corn, 47.30; dehulled soybean meal, 36.00; fish solubles, 4.00; NaCl (iodized), 0.25; $MnSO^4$, 0.05; dicalcium phosphate, 1.80; ground limestone, 1.10; soybean oil, 5.00; vitamin premix, 1.25.§ Treatment diets were obtained by supplementing the basal with magnesium ($(MgCO_3)_4\ Mg(OH)_2 \cdot 4\ H_2O$) and with fluoride (NaF) as shown in Table I. Dietary sodium was held constant by addition of sodium carbonate to the low fluoride diets and all diets were adjusted to 100% with glucose. Feed and water were provided *ad libitum.*

TABLE I. Influence of Dietary Magnesium and Fluoride on Citric Acid Content of Chick Bones.*

Exp		Supp. F (% of diet)	Supp. Mg (% of diet) .00	.25
1	Femur†	.00	.45 ±.01	.43 ±.02
		.08	.26 ±.02	.16 ±.04
2	Tibia‡	.00	.41 ±.02	.39 ±.02
		.08	.32 ±.03	.15 ±.01

* All values are expressed as % citric acid in the dry, fat-free bone.
† Avg of 10 samples per group ± S.E.
‡ Avg of 8 samples per group ± S.E.

On the fourteenth day, 10 chicks were randomly selected from each group and killed. The right femora were removed and stored at —15°C until analyzed. The bones were stripped of all flesh, extracted with ethanol and then ether in a Soxhlet apparatus and dried under vacuum at 90°C for 12 hours. After weighing, they were extracted with 1 N HCl over gentle heat. The filtrate from the extraction mixture was analyzed for citric acid by the method of Taylor(8).

The diet for the second experiment contained 40% dehulled soybean meal and the fish solubles product was omitted. Tibiae

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§ The vitamin premix supplied the following amounts per 100 g of diet: vit. A, 1000 IU; vit. D_3, 150 ICU; vit. E acetate, 0.56 IU; choline chloride, 150 mg; niacin, 3.17 mg; D-calcium pantothenate, 1.41 mg; riboflavin, 0.71 mg; menadione sodium bisulfite, 0.07 mg; procaine penicillin, 0.44 mg; vit. B_{12}, 0.66 μg.

from 8 chicks per group were analyzed for citric acid content. All other conditions were the same as in the first experiment.

Results and discussion. The addition or both magnesium and fluoride to the diets of growing chicks caused a greater reduction in growth rate than the addition of either element alone and the leg weakness previously described(1,2) was observed only in chicks fed diets containing elevated levels of both magnesium and fluoride. A greater reduction in citric acid content of bone was observed when both magnesium and fluoride were added to the diet than when the dietary level of only one of the elements was increased (Table I). The chicks that were maintained on the high fluoride diets without added magnesium had 42% and 22% less bone citric acid than the chicks fed the basal diet in the first and second experiments, respectively. Addition of 0.25% magnesium to the basal diet had no effect on bone citrate in either experiment. When the two elements were supplemented in combination, however, bone citric acid was reduced 64% in each experiment when compared with the bones of chicks maintained on the basal diet. This was a 39% and 53% reduction in the first and second experiments, respectively, when compared with the diets containing fluoride alone.

The reduced citric acid content of bones from chicks fed the low magnesium-high fluoride diets agrees with the reports of Zipkin *et al*(3,5,6), Menczel *et al*(4) and Schraer *et al*(7). However, the fact that chicks maintained on high magnesium-high fluoride diets had even lower bone citric acid suggests that factors other than fluoride content influenced citric acid since it has been shown that chicks grown on low magnesium-high fluoride diets have higher bone fluoride than those grown on high magnesium-high fluoride diets(2). The possibility of the reduced citrate being associated with increased "crystallinity" still exists, however, since the magnesium content of bone increases with high fluoride intake(9), and the influence of bone magnesium on crystal structure is not known. It has been shown that bone ash is reduced by high magnesium-high fluoride diets(1,2); thus there would be less crystal surface for absorption of citrate.

Low citric acid could also result from the inhibition of a synthesizing enzyme or the acceleration of a catabolizing enzyme. The former seems more likely. A magnesium-fluorophosphate-protein complex similar to the one described by Warburg and Christian(10) may be involved in the reduction of citrate. Although it has been shown that the conversion of 3-phosphoglycerate to phosphoenolpyruvate is not affected by high magnesium-high fluoride intake(2), there are, of course, many other possible sites of inhibition.

Summary. Addition of both magnesium and fluoride to the diet of growing chicks caused a greater reduction in the citric acid content of bone than that caused by addition of either element alone. Bones from chicks maintained on either the basal diet or diets supplemented with 0.25% magnesium contained approximately 0.42% citric acid and those from chicks maintained on high fluoride diets contained approximately 0.29% citrate. Bones from chicks fed diets high in both magnesium and fluoride contained only 0.16% citric acid.

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