# Effect of Fluoride on Bone Formation and Strength in Japanese Quail[1]

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ABSTRACT The effect of fluoride on bone metabolism was studied using Japanese quail fed diets containing 1.2% calcium, 1.2% calcium + 0.075% fluoride, 0.4% calcium, and 0.4% calcium + 0.075% fluoride. In the first experiments, quail were fed the diets immediately after hatching. Low calcium intake (0.4%) resulted in a 23% reduction in body weight, a 38% decrease in bone ash and a twofold elevation in bone pyrophosphatase levels compared with controls (1.2% calcium) after 11 days of treatment. Supplementation of fluoride to the low calcium diet, however, resulted in increased calcium retention, growth rates, and bone ash. The bone calcium/phosphorus ratio did not vary significantly and did not appear to be affected by the experimental diets. An elevation of bone magnesium, however, was observed in both of the fluoride-supplemented groups as well as the low calcium group compared with the control. In further experiments, groups of quail were fed the control diet (1.2% calcium) for 10 days and then one of the other diets for the following 35 days. Under these conditions, the birds fed the diet containing only 0.4% calcium did not develop any severe calcium deficiency signs and for the most part appeared normal. Tetracycline labeling studies indicated a significant increase in periosteal bone formation in the fluoride-supplemented groups. Von Kossa-stained bone sections indicated adequate mineralization of this new bone. An increase in the number of osteons appeared to be present in bone sections from fluoride-treated birds. These changes in bone, however, were not accompanied by an increase in bone strength. Dietary fluoride supplementation resulted in a 30% decrease in bone torsional strength. The results demonstrate that fluoride supplementation increases calcium retention, but at a high level has little effect on bone integrity and strength. J. Nutr. *103:* 1431–1440, 1973.

INDEXING KEY WORDS fluoride · calcium · bone · Japanese quail

Supplementation with fluoride has been reported to restore calcium balance and increase bone density in several animal species (1–7). Fluoride is known to increase bone crystal size. It also appears to play a role in modifying a variety of enzymatic processes known to be related to normal bone formation and resorption (8).

The studies reported here are related to this problem, i.e., the relationship between bone calcium retention and integrity as affected by elevated dietary fluoride. Japanese quail (*Coturnix japonica*) were fed diets in which the calcium and fluoride contents were altered. Japanese quail appeared well suited for the studies, because of their rapid growth rate and relatively high tolerance to fluoride. Our observations corroborate the findings that, in general, elevated dietary fluoride results in an acceleration of bone mineralization. Uniquely, however, the increase in mineralization was accompanied by a decrease in bone strength.

## MATERIALS AND METHODS

*Quail and diets.* In the first two experiments, four groups of 1-day-old quail were fed diets from the day of hatching in which

Received for publication March 22, 1973.
[1] Supported by NIH Grant AM-14403 and a grant from the Nutrition Foundation.
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TABLE 1

*Composition of the basal and experimental diets*

| Ingredients in basal diet | Percentage |
|---|---|
| Isolated soybean protein | 32.0 |
| Glycine | 0.3 |
| DL-Methionine | 0.5 |
| Corn oil | 5.0 |
| Cellulose | 3.0 |
| Vitamin premix[1] | 1.0 |
| Choline chloride | 0.2 |
| Mineral premix[2] | 1.5 |
| $K_2HPO_4$ | 1.46 |
| $Na_2HPO_4$ | 2.47 |
| Glucose | Added to total 100% |

| Groups | Experimental diets[3] Amount added to the basal diet | |
|---|---|---|
| | $CaCO_3$ (%) | NaF (%) |
| I (1.2% Ca) | 3.0 | — |
| II (1.2% Ca + 0.075% F) | 3.0 | 0.177 |
| III (0.4% Ca) | 1.0 | — |
| IV (0.4% Ca + 0.075% F) | 1.0 | 0.177 |

[1] The diet contained per kg the following vitamins: (mg) niacin, 264; Ca pantothenate, 92; *p*-aminobenzoic acid, 44; riboflavin, 29; pyridoxine·HCl, 29; thiamin·HCl, 18; folic acid, 55; inositol, 220; biotin, 0.9; DL-α-tocopheryl acetate, 110; menadione, 11; cyanocobalamin 0.1; retinyl acetate, 50,000 (IU); vitamin $D_3$, 5,000 (IU). [2] Minerals were added so that each kg of diet contained: (mg) $MnSO_4 \cdot H_2O$, 500; $FeSO_4 \cdot 7H_2O$, 400; $ZnCO_3$, 9.2; $CoSO_4 \cdot 7H_2O$, 4.7; $CuSO_4 \cdot 5H_2O$, 18; KI, 12; $Na_2MoO_4 \cdot 2H_2O$, 1.1; $MgSO_4$, 3,000; KCl, 4,000; NaCl, 6,000. [3] Dietary phosphorus constant at 0.6% in all diets.

the calcium and fluoride contents were varied as indicated in table 1. The combined data from these studies will be referred to in the text as experiment 1. The birds were housed in conventional electrically heated brooders with small-meshed screen floors. Feed and deionized water were given ad libitum.

In a further experiment (designated as experiment 2) all birds were fed the basal diet supplemented with 1.2% calcium for 10 days before they were randomly fed the experimental diets for 35 days.

*Roentgenographic and mineral analysis of bone.* Radiographs of the femur and tibia of quail were obtained by means of a low voltage radiographic inspection system.[3] Representative bones from each of the four groups were developed in one exposure to facilitate comparative examination.

Bone specific gravity was measured by the Archimedean principle. The femurs and tibia were first cleansed of adhering tissue and extracted with absolute alcohol followed by diethyl ether. The fat-free bones were then hydrated by immersion in distilled water for 5 hours. The bones were weighed in air and in distilled water to the nearest tenth milligram. Care was taken so that the medullary canal was drained of water when weighed in air and filled with water when weighed in water. For bone mineral determinations (fluoride, calcium, magnesium, and phosphorus) the bones were ashed at 550°. Fluoride was measured with a fluoride electrode[4] as described by Singer and Armstrong (9). Following appropriate dilutions, calcium, magnesium, and phosphorus were determined by means of an AutoAnalyzer system (10–12).[5]

*Pyrophosphatase assays.* Bones (right femur and tibia) were first cleansed and the marrow removed by a stream of cold Tris–acetate buffer (pH 7.5, 0.05 M). Using the same buffer, the bones were thoroughly homogenized and the volume adjusted to give a 1% suspension. This suspension was centrifuged at 25,000 × *g* for 20 minutes (4°). The clear supernatant fraction was used for the pyrophosphatase (EC 3.6.1.1, pyrophosphate phosphohydrolase) assay according to the method of Woltgens et al. (13). The final reaction mixture contained 0.33 mM $MgCl_2$, 0.01 M Tris–acetate buffer (pH 8.0), and 0.33 mM $Na_2P_4O_7$, and the supernatant fraction. The reaction mixtures were incubated for 30 minutes at 37°. Activity is expressed as μmoles inorganic pyrophosphate liberated per milligram protein per minute. The activity was proportional with time and the amount of supernatant fraction added. Protein was measured by the method of Lowry et al. (14). A more detailed description of this assay and comments regarding various properties of quail bone pyrophosphatase have been published (15).

*Calcium retention studies.* Groups of 1-day-old quail were fed one of four experimental diets from the day of hatching. After 2 days of growth, the birds were injected intraperitoneally with 10 μCi of $^{45}Ca$ (250 mCi/mole).[6] The quail were killed at 2, 4 or 6 days after the injection.

[3] Faxitron, model 805, Field Emission Corp., McMinnville, Ore.
[4] Orion Research Inc., Cambridge, Mass.
[5] Technicon Instruments Corp., Terrytown, N. Y. 10591.
[6] New England Nuclear, Boston, Mass.

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The whole carcass was then ashed and dissolved in 0.1 N HCl. Radioactivity was determined by means of a scintillation counter.

*Tetracycline labeling and histology.* Quail from experiment 2 were injected with tetracycline (5 mg/100 g body weight) after 25 days of feeding one of the four experimental diets. A second dose was administered 7 days after the first dose. The birds were then killed 48 hours after the second injection. The right tibia and humerus were removed, cleansed, and fixed in neutral formalin. After embedding in methylmethacrylate, transverse sections of 50 to 100 $\mu$ thickness were cut from the tibia mid-shaft using a thin sectioning device.[7] Two sections from each bone were first mounted unstained, examined, and photographed under reflected ultraviolet light.[8] The negative from each photograph was examined by projection on a large screen. The distance between the edges of the two tetracyline bands found in the cortical bone near the periosteal surface was measured at ten different points in each section. The average distance between the bands was taken as an index of transverse bone formation for the 7-day period (16). In addition, some sections from the tibia and humerus were stained for mineral using the Von Kossa technique (17) in order to determine if any unmineralized osteoid was present. Standard hematoxylin- and eosin-stained (H and E) sections of demineralized bone were also prepared. The bone for all of the histological examinations was obtained at the termination of experiment 2.

*Bone breaking and cortical thickness.* The bone-breaking strengths of femurs obtained from birds at the termination of experiment 2 were also determined. The ends of the bones were first potted with a dental acrylic[9] in small molds in order to fit grips on the bone-breaking apparatus.[10] During the period of potting, the bones were kept moist with saline. After the dental acrylic had dried, one end of the bone was fastened to a reaction torque sensor and the other to a freely rotating grip. At the distal end of this rotating grip were metal dogs which contacted a large pendulum near the bottom of its arc, which when released, applied a rotary force (torque) to the bone shaft. Attached to the rotating grip was an angle position transducer that measured the resistance from the bone with respect to the applied torque. The results were displayed on a storage oscilloscope and measurements were recorded. Values for torque were obtained at the point of fracture. Cortical thickness and the diameter of the femurs were measured by means of a micrometer.[11] Values represent the average of two measurements for each bone.

## RESULTS

The data in tables 2 and 3 summarize the values for growth, mortality, and bone mineral composition obtained from quail in experiment 1. By day 11, the addition of fluoride to diets improved the growth rate of quail. Quail fed diets containing only 0.4% calcium never survived longer than 12 days. Addition of fluoride to the low calcium diet dramatically increased survival. Three to four days were required before the quail appeared to adapt to their surroundings. In the control group of 70 birds, 11 of the 12 deaths occurred during this period. Although all birds were given free access to water, some of the quail that had received fluoride initially appeared dehydrated. This usually resulted in an additional 13 to 14 deaths. Although not as severe, similar effects have been observed in young chicks fed fluoride (7).

The addition of fluoride to the low calcium diet also caused a significant increase in the amount of ash and the specific gravity of bone (table 3). The values approached those of quail fed the diet containing 1.2% calcium. Likewise, fluoride addition tended to enhance bone ash and specific gravity values of bone from quail fed diets containing calcium at 1.2%. With respect to the composition of the ash, the ratio of calcium to phosphorus in the bone was not varied significantly. Lower ratios, however, were often observed in the bones

[7] Bronwill Scientific, Inc., Rochester, N. Y.
[8] A Zeiss photomicroscope with BG3 excitation filter and a #50 barrier filter, Oberkocher, Wuerttemberg, Germany.
[9] Nu Weld, L. D. Clark Co., Milford, Del. 19963.
[10] A complete description of the bone breaking apparatus may be obtained from A. H. Burstein and V. H. Frankel, A standard test for laboratory animals bone biomechanics, Division of Orthopaedic Surgery, Case Western Reserve University, Cleveland, Ohio.
[11] Multianvil micrometer, #220 AFL, L. S. Starrett Co., Athol, Mass.

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TABLE 2

*The effect of dietary calcium and fluoride levels on growth and mortality (experiment 1)*[1]

| Diet | Days | Wt[2] | Mortality |
|---|---|---|---|
| | | *g* | % |
| 1.2% Ca | 6 | 14.8±0.7[a] | 16 |
| | 11 | 24.1±1.9[a] | 17 |
| | 20 | 57.4±4.2[a] | 17 |
| 1.2% Ca + 0.075% F | 6 | 11.4±1.5[b] | 36 |
| | 11 | 29.3±1.9[b] | 38 |
| | 20 | 57.8±3.5[a] | 38 |
| 0.4% Ca | 6 | 13.1±1.2[a] | 28 |
| | 11 | 20.0±3.7[a] | 54 |
| | 20 | — | 100 |
| 0.4% Ca + 0.075% F | 6 | 13.0±0.8[a] | 40 |
| | 11 | 26.0±1.9[a] | 40 |
| | 20 | 49.0±3.6[a] | 40 |

[1] Mean ± SEM. Each group initially contained 70 birds. [2] For a given time period, a difference in superscript indicates a significant difference in value at $P < 0.05$.

from quail supplemented with fluoride or fed the 0.4% calcium diet. The calcium appeared to be displaced in part by magnesium. The magnesium level in ash was significantly elevated ($P < 0.05$) in groups fed diets supplemented with fluoride. As expected, the fluoride content in ash was also elevated markedly in the groups receiving fluoride.

The radiographic observations shown in figure 1 corroborate and are consistent with the observation of reduced bone ash. There was a reduction in the radiographic density of bone from quail fed the diet containing 0.4% calcium. An increase in the bone radiographic density was evident when fluoride was added to diets.

When 2-day-old quail were injected with a single dose of $^{45}Ca$, calcium retention appeared to be increased in both of the groups receiving fluoride and in the group fed calcium at 0.4% (table 4). Six days after the injection of $^{45}Ca$, the group fed 1.2% calcium retained 67% of the injected dose compared to 83% to 86% in the other three groups.

A factor that may be important in the metabolism of bone calcium is the level of enzymatic bone pyrophosphatase activity (see Discussion). The levels of pyrophosphatase activity in bone extracts were evaluated in quail bone supplemented with fluoride after 10 and 20 days of growth (fig. 2). At 10 days of growth, pyrophos-

TABLE 3

*The effect of dietary calcium and fluoride levels on bone composition*[1, 2]

| Diets | Days | Ash | Ca/P ratio | F | Mg in ash | Specific gravity |
|---|---|---|---|---|---|---|
| | | % | | *ppm* | % | |
| 1.2% Ca | 6 | 44±1.0[a] | 2.04 | 19±7[a] | 2.56±0.10[a] | 1.106±0.002[a] |
| | 11 | 47±1.4[a] | 2.05 | 12±2[a] | 2.33±0.10[a] | 1.121±0.004[b] |
| | 20 | 51±0.7[b] | 1.97 | 12±2[a] | 2.50±0.19[a] | — |
| 1.2% Ca + 0.075% F | 6 | 43±1.1[a] | 1.99 | 1448±74[b] | 3.40±0.16[b] | 1.151±0.038[b] |
| | 11 | 51±0.7[b] | 2.06 | 1448±101[b] | 3.13±0.18[b] | 1.143±0.004[b] |
| | 20 | 53±0.6[b] | 1.89 | 1235±111[b] | 3.50±0.23[b] | — |
| 0.4% Ca | 6 | 29±0.7[c] | 1.91 | 61±10[c] | 3.37±0.14[b] | 1.058±0.004[c] |
| | 11 | 29±2.6[c] | 2.01 | 64±8[c] | 2.56±0.27[a] | 1.052±0.013[c] |
| | 20 | — | — | — | — | — |
| 0.4% Ca + 0.075% F | 6 | 39±1.0[d] | 1.91 | 1777±162[b] | 4.27±0.29[c] | 1.084±0.011[d] |
| | 11 | 45±0.7[a] | 1.93 | 1492±112[b] | 4.18±0.22[c] | 1.077±0.004[d] |
| | 20 | 46±1.1[a] | 1.93 | 1685±154[b] | 4.53±0.14[c] | — |

[1] Values represent mean ± SEM of groups of seven or eight quail fed experimental diets with the exception of 0.4% Ca at 11 days, which represents the mean ± SEM of four determinations. [2] Within a column, a difference in superscript indicates a significant difference in value at $P < 0.05$.

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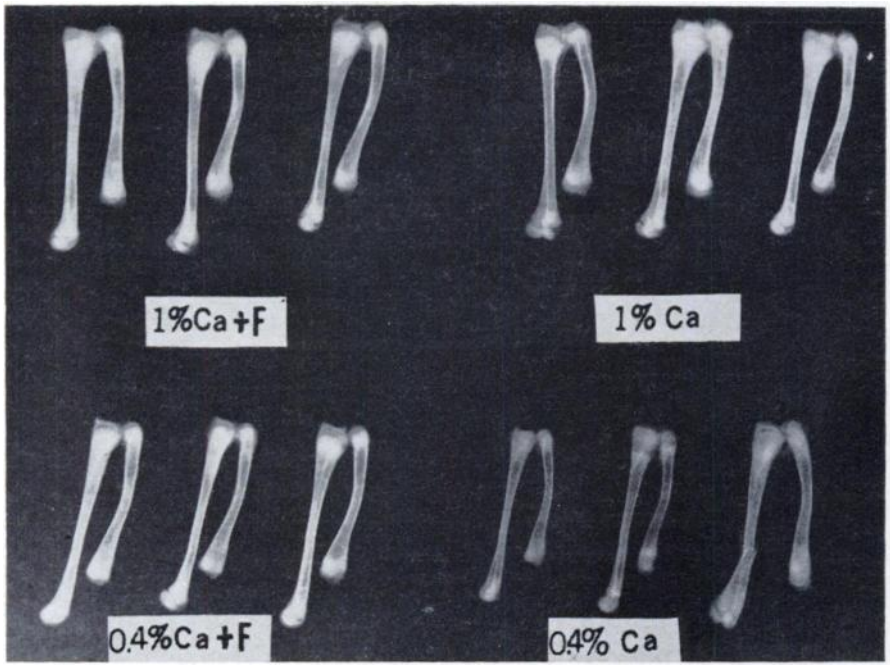


Fig. 1 Radiograms of left tibia and femur from 11-day-old quail fed diets at different levels of calcium and fluoride. Bones from quail fed 0.4% calcium show a marked decrease in bone density compared to the other three groups. Supplementation of 0.4% calcium diet with 0.075% fluoride resulted in increased bone density.

phatase activity was greatly increased in the group fed the diet containing 0.4% calcium. After 20 days of growth, pyrophosphatase values were significantly higher in bone extracts from quail fed fluoride ($P < 0.05$) than those from quail fed the control diet (1.2%).

The values of growth and mineral analysis for quail in experiment 2 are presented in table 5. In contrast to the observations in experiment 1, in which 1- to 20-day-old quail were studied, the overall effects of fluoride addition to diets were somewhat less dramatic. No deaths in any of the groups were observed when diets were fed to 10-day-old quail for the 5-week experimental period. Growth rate was not affected by fluoride or the reduction in the calcium content of diets. The presence of fluoride in diets, however, did produce a slight but significant increase in bone ash (table 5). Similar to the results of experiment 1, the fluoride content in the ash was markedly elevated. Likewise, the magnesium content was significantly increased in the two groups fed the diets containing fluoride (table 5).

Histological examination of the bones removed from quail fed the fluoride-supple-

TABLE 4

*The effect of dietary calcium and fluoride levels on calcium-45 retention*

| Diets | Days after injection | % of $^{45}Ca$ retention[1, 2] |
|---|---|---|
| 1.2% Ca | 2 | 87±6 (5)[a] |
| | 4 | 78±9 (5)[a] |
| | 6 | 67±6 (8)[a] |
| 1.2% Ca + 0.075% F | 2 | 94±3 (5)[a] |
| | 4 | 90±3 (6)[a] |
| | 6 | 86±13 (2)[a, b] |
| 0.4% Ca | 2 | — |
| | 4 | 86±3 (6)[a] |
| | 6 | 85±4 (9)[b] |
| 0.4% Ca + 0.075% F | 2 | 93±3 (6)[a] |
| | 4 | 93±4 (6)[a] |
| | 6 | 83+3 (7)[b] |

[1] Percentage of the initial dose that was injected (i.p.) into 2-day-old quail. Birds were killed at 2, 4, or 6 days after injection. The values represent the mean % ± SEM; number of observations in parentheses. The entire carcass was ashed for determinations. [2] For a given time period, a difference in superscript indicates a significant difference in value at $P < 0.05$.

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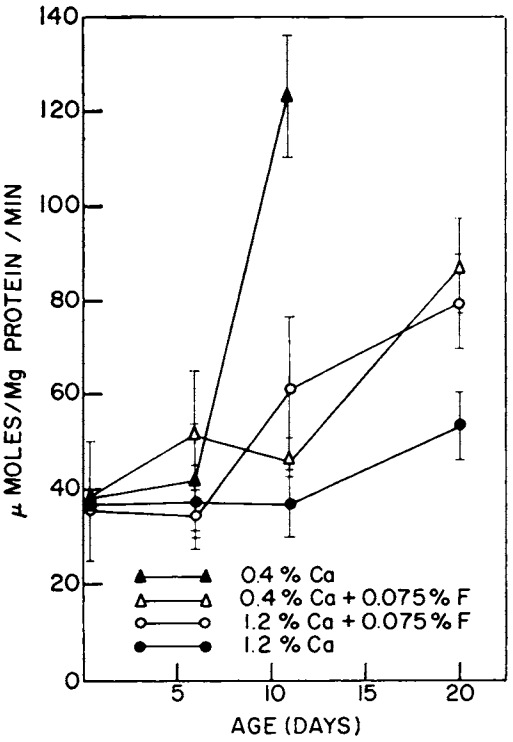


Fig. 2 The effect of dietary calcium and fluoride levels on bone pyrophosphatase activity. The enzyme activity is expressed as μmoles inorganic phosphate liberated per milligram of extractable protein per minute. Values represent the means ± SEM. Quail were fed the diets as outlined for experiment 1. Each point represent at least seven determinations with the exception of 0.4% calcium at 11 days, which represents three determinations.

mented diets (experiment 2) demonstrated excessive periosteal bone formation in some birds (fig. 3). Generally, the activity along the periosteal surface was orderly. The increased bone formation along this surface could also be demonstrated by the increase in the distance between tetracycline labels as shown in figure 4 and reported in table 6. Interestingly, no tetracycline labeling appeared near the surface of the bones facing the medullary cavity, and because no increase in cortical thickness was observed in fluoride-supplemented birds (table 6), it was assumed that resorption of endosteal bone was occurring. The new bone formed under the influence of dietary fluoride apparently almost equaled the amount of old bone that was resorbed. Areas of active bone turnover, such as those shown in figure 5, were most often found in cortical bone of fluoride-supplemented quail. In calcified sections, Von Kossa stains of these areas showed them to be mineralized, and tetracycline was heavily deposited in these areas in labeled animals.

## DISCUSSION

The data presented here indicate that, in early stages of growth, dietary fluoride markedly affects bone calcium retention. An increase in calcium retention could be demonstrated in studies using radioactive calcium. Growth, survival, bone density, and ash were all increased when fluoride was added to a diet containing insufficient calcium to maintain normal development.

It has been proposed that pyrophosphate and the regulation of pyrophosphate levels by the action of pyrophosphatase are involved in calcium homeostasis (18). The presence of pyrophosphate as low as 2 μM in plasma is known to inhibit the deposition of calcium phosphate salts (19, 20). Classical inorganic pyrophosphatase and pyro-

TABLE 5

*The effect of dietary calcium and fluoride levels on body weight gain and tibia composition (experiment 2)*[1, 2]

| Diets | Wt of bird | Bone ash | Ca/P ratio | Bone fluoride | Bone magnesium |
|---|---|---|---|---|---|
| | *g* | % | | *ppm* | % |
| 1.2% Ca | 110±10[a] | 57±0.4[a] | 2.08±0.01[a] | 13±1[a] | 2.32±0.13[a] |
| 1.2% Ca + 0.075% F | 103±11[a] | 62±0.7[b] | 2.14±0.02[a] | 1963±138[b] | 3.48±0.23[b] |
| 0.4% Ca | 102±9[a] | 58±0.6[a] | 2.09±0.01[a] | 14±2[a] | 2.47±0.07[a] |
| 0.4% Ca + 0.075% F | 99±14[a] | 61±0.6[b] | 2.11±0.02[a] | 2223±171[b] | 4.65±0.29[c] |

[1] Values represent mean ± SEM of eight quail fed the control diet for 10 days and the experimental diets for 35 days. [2] Within a column, a difference in superscript indicates a significant difference in value at $P < 0.05$.

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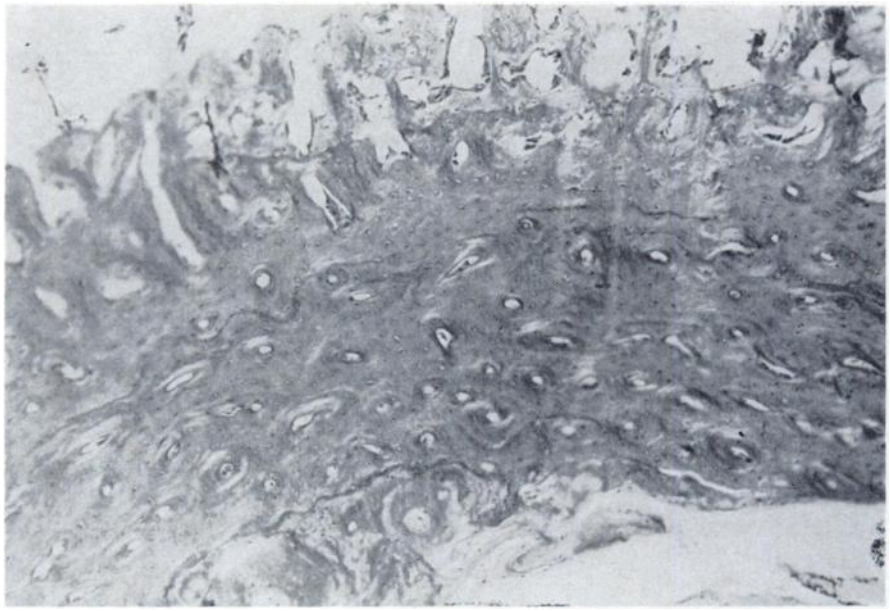

Fig. 3 Cortical bone (humerus) from a 45-day-old quail fed a diet containing 1.2% calcium and 0.075% fluoride for 35 days (experiment 2). A large amount of bone formed at the periosteal surface appears as trabeculae on the lower portions of the photomicrograph. (Demineralized sections, stained with H and E, magnification ×90.)

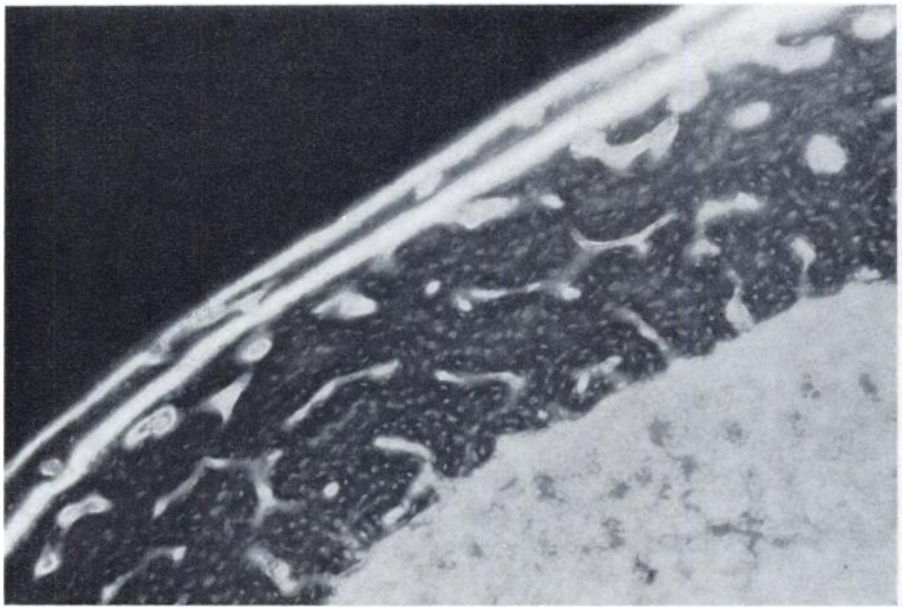

Fig. 4 Photomicrograph of cortical bone (tibia) from a quail fed the 1.2% calcium diet (experiment 2) showing the tetracycline labels at the periosteal surface. From the time at which each of the tetracycline labels were given and the distance between the two labels (white lines) the net increase in periosteal bone formed was calculated (see table 6). Note that there was no tetracycline deposited in endosteal bone. (Calcified and unstained sections photographed under reflected ultraviolet illumination, magnification ×90.)

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TABLE 6

*The effect of dietary calcium and fluoride levels on femur growth and breaking strength (experiment 2)*[1, 2]

| Diets | Distance between two tetracycline labels (cm × 10³) | Femur diameter (cm × 10²) | Cortical thickness (cm × 10²) | Breaking strength Newton-meters × 10² |
|---|---|---|---|---|
| 1.2% Ca | 4.9±0.2[a] | 10.3±0.2[a] | 3.0±0.7[a] | 18.6±3.4[a] |
| 1.2% Ca + 0.075% F | 6.6±0.5[b] | 10.0±0.5[a] | 3.0±0.5[a] | 13.4±1.8[b] |
| 0.4% Ca | 4.9±0.4[a] | 9.9±0.6[a] | 3.0±0.2[a] | 18.5±3.4[a] |
| 0.4% Ca + 0.075% | 6.0±0.2[b] | 10.1±0.2[a] | 3.3±0.2[a] | 11.9±1.3[b] |

[1] Values represent mean ± SEM of groups of seven or eight quail fed experimental diet for 34 days. [2] Within a column, a difference in superscript indicates a significant difference in value at $P < 0.05$. [3] Represents periosteal growth.

phosphate levels have been investigated in bone (15, 18, 21–23). The analysis of pyrophosphatase activity in quail bone homogenates reported here indicated an elevated activity of this enzyme in the fluoride-supplemented groups. Such an elevation is consistent with the observations of increased bone formation (18). The stimulation of pyrophosphatase activity in bone from quail fed 0.4% calcium could represent a compensatory effect, i.e., an attempt by the tissue to optimize conditions for mineralization when dietary calcium is low. Fluoride appears to induce pyrophosphatase in vivo rather than inhibit it, as is the case in vitro (15, 23).

The relationship between dietary fluoride, calcium, and magnesium, and chick bone formation have been studied extensively by Parker, Rogler and co-workers (7, 23–26). Similar to their studies, when quail were fed diets containing elevated fluoride, the magnesium content of bone was increased. Although the calcium level in the diets appeared to have little effect on the amount of bone magnesium in the absence of fluoride, addition of fluoride to diets significantly elevated magnesium particularly

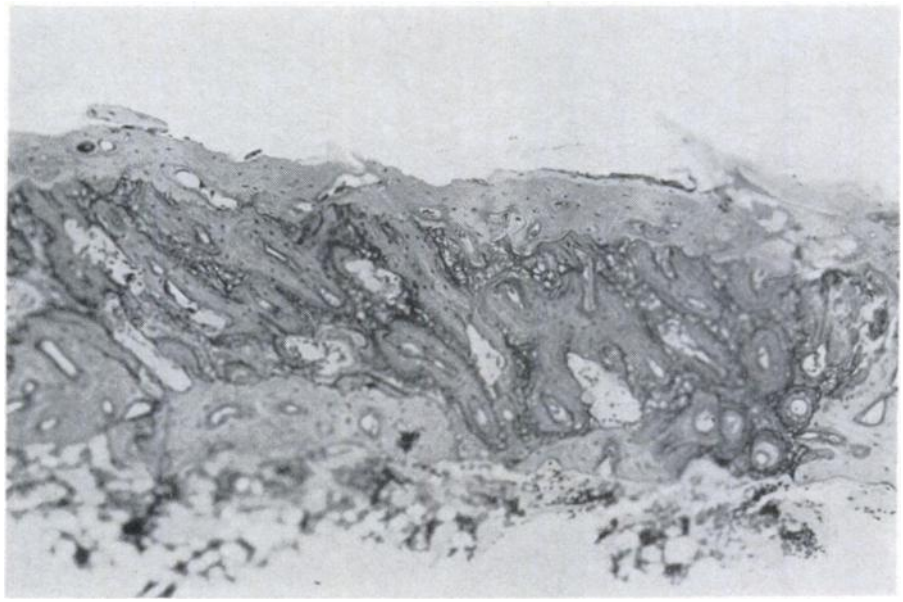

Fig. 5 Photograph of cortical bone (tibia) from a 45-day-old quail supplemented with fluoride in the diet containing 0.4% calcium. An area of very cellular immature bone is shown (stained with H and E, magnification ×90).

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when the calcium level was low. As expected, the fluoride content of bone reflected the high dietary level of fluoride (1.2 to 2.2 mg of fluoride/g of ash). It should be noted also that calcium at 0.4% in the diet of quail appeared marginally adequate, if for the first 10 days of growth the birds were fed a diet adequate in calcium. Reducing the calcium level of diets as in experiment 2 did not result in a reduction of ash, reduction of bone breaking strength, reduced bone cortical thickness, or reduced weight gain.

Fluoride addition, however, to the diets containing the two levels of calcium had an effect (experiment 2). The bone from birds fed fluoride appeared to be in a state of accelerated remodeling. The distance between tetracycline bands of periosteal bone was increased with fluoride supplementation. Grossly, an increased number of osteons was usually present in sections taken from bone of fluoride-treated quail. It was felt that more of these areas were found in bone from fluoride-supplemented quail, but it was difficult to quantify precisely the number of osteons or the amount of bone in areas undergoing rapid turnover. Such areas were not found uniformly in all sections of fluoride-treated birds, and being normal events of bone growth they were found in sections of control bone. The number of osteons is related to the tensile strength and the elastic modulus of bone (27). Within a fixed area, an increase in the number of osteons tends to reduce both the tensile strength and elastic modulus. The most probable reason is the relatively greater number of cement lines, which may act as sites of weakness where fracture can occur. Evans and Bang (27) have pointed out that the percentage increase or decrease in the number of osteons does not have to be great to markedly affect bone strength. With respect to the action of fluoride, the presence of a greater number of osteons and related changes may account for the decrease in tensile strength.

For the most part, bone from the fluoride-supplemented quail resembled that from the two corresponding unsupplemented groups with the exception of signs of increased remodeling. Our work would agree with most of the other observations related to fluoride's effect on calcium retention (1–7). It is important to add, however, that the changes in bone that occur with prolonged and excessive fluoride ingestion may result in a reduction of bone strength.

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