Calc. Tiss. Res. 4, 180—184 (1969)

# Effect of High Dietary Strontium Levels on Bone and Egg Shell Calcium and Strontium

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Received November 12, 1968/revised May 5, 1969

The results of feeding high dietary levels of stable strontium to hens are reported. Dietary levels of strontium from 3,000 p.p.m. to 50,000 p.p.m. showed a significant increase in strontium content of the tibia bone and essentially no change in the calcium content. Plasma calcium concentration was shown to decrease with increasing dietary strontium treatment.

Egg shell calcium showed a progressive decrease with increasing dietary strontium treatment, whereas the strontium content has a corresponding increase.

X-ray diffraction analyses of bones and shells containing large amounts of strontium were unsuccessful in evaluating the form in which strontium was deposited.

*Key words:* Strontium — Calcium — Bone — Shell.

Des poules ont été nourries avec une alimentation riche en strontium stable. Des concentrations de strontium de 3000 p.p.m. à 50000 p.p.m. montre une augmentation nette en strontium du tibia, alors que le contenu en calcium n'est pas modifié. Les concentrations en calcium sérique diminue, lorsque le strontium alimentaire augmente.

Le calcium des coquilles d'oeufs diminue progressivement avec l'augmentation du strontium alimentaire, alors que le contenu en strontium des coquilles présente une augmentation correspondante.

Des analyses de diffraction par rayons X des os et des coquilles ne permettent pas de déterminer sous quelle forme le strontium est déposé.

Die Resultate einer Verfütterung von hohen stabilen Strontiumdosen an Hühnern werden erläutert. Strontiumzusätze zur Nahrung in der Höhe von 3000—50000 p.p.m. führten zu einer signifikanten Zunahme des Strontiumgehaltes der Tibia und verursachten keine wesentlichen Änderungen des Calciumgehaltes. Die Calciumkonzentration im Plasma verminderte sich, wenn der Nahrung ansteigende Strontiummengen zugegeben wurden. Mit zunehmendem Strontiumzusatz zur Nahrung zeigte der Calciumgehalt der Eischale eine fortlaufende Abnahme, während sich der Strontiumgehalt entsprechend erhöhte.

Durch Röntgendiffraktionsanalysen der stark Strontium-haltigen Knochen und Eischalen konnte nicht festgestellt werden, in welcher Form das Strontium abgelagert wurde.

## Introduction

Increased interest in the metabolism of stable strontium has resulted from its possible use in reducing the uptake of radioactive strontium by calcified tissue (Cohn *et al.*, 1961). The administration of stable strontium decreases the uptake of radioactive strontium by saturating the bone tissue (Teree *et al.*, 1965). Neuman and Neuman (1958), in reviewing the literature on the accumulation of ions by calcified tissue, state that growing and calcifying rat bones show a greater uptake of $^{90}Sr$.

For reprints: Dr. B. L. Reid, Dept. of Poultry Science, University of Arizona, Tucson, Arizona 85721. Arizona Ag. Exper. Sta. Journal Art No. 1491.

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Young chicks fed 12,000 p.p.m. strontium accumulated 10% of this element in the tibia ash (Weber *et al.*, 1968). These workers showed also that the strontium was uniformly deposited throughout the bone. Accordingly, it seemed important to supplement these studies with observations on bones and shells from mature hens fed high dietary levels of stable strontium in order to determine the amounts of strontium deposited in bone and egg shell, to ascertain the effect of high strontium diets on plasma elements (Ca, P, Na, K), and to determine the form of strontium deposited in bone and egg shell.

## Methods

Individually-housed, $^1/_2$-year-old Kimber No. 137 hens were used. Feed and water were provided *ad libitum* during a four-week period. A standard laying diet, which supplied all known dietary requirements and had Ca, P and Sr levels of 2.93%, 0.79% and 0.09%, respectively, was given to the controls. Other groups were fed this diet supplemented with 3,000 to 50,000 p.p.m. of strontium (as carbonate). Birds were weighed at the beginning and end of the experiments and eggs were collected on five consecutive days during the fourth week. The number and weights of eggs, their general appearance and shell thicknesses (Crowley *et al.*, 1963) were noted. Strontium was determined by X-ray fluorescence spectrometry (Weber *et al.*, 1968) on ashed egg shells, and on ashed tibial (epiphysis and diaphysis) samples. The shells and tibiae (epiphyses) were also examined by X-ray diffraction to identify the minerals composing them. Calcium and phosphorus were determined on the ash samples by means of flame photometry for calcium (Coleman Instruments[1]) and by a colorimetric method for phosphorus (Koenig and Johnson, 1942). In addition to calcium and phosphorus, potassium and sodium (Coleman Instruments[1]) levels were determined in blood at the time the animals were sacrificed.

## Results and Discussion

Calcium and strontium contents of epiphysis and diaphysis of tibiae from hens fed 20,000 to 50,000 p.p.m. strontium are shown in Table 1. When the dietary strontium level was increased, the calcium content of the epiphysis and diaphysis was statistically unaltered, while a significant increase in the amounts of strontium was found (Table 1).

Table 1. *Strontium and calcium analyses of ashed tibial bone*

| Dietary treatment (p.p.m. Sr) | Bone section | Calcium[a] (%) | Strontium[a] (%) | Diaphysis/ Epiphysis Sr ratio |
|---|---|---|---|---|
| 0 | Epiphysis | 37.80 (5) ±1.42 | 0.08 (6) ±0.01 | |
| | Diaphysis | 37.18 (5) ±0.79 | 0.07 (8) ±0.01 | 0.88 |
| 20,000 | Epiphysis | 36.60 (5) ±0.66 | 2.10 (5) ±0.32 | |
| | Diaphysis | 36.30 (5) ±0.45 | 1.17 (6) ±0.21 | 0.56 |
| 30,000 | Epiphysis | 33.90 (5) ±1.16 | 2.59 (6) ±0.50 | |
| | Diaphysis | 37.36 (4) ±0.81 | 1.48 (6) ±0.26 | 0.57 |
| 40,000 | Epiphysis | 35.33 (3) ±0.96 | 2.54 (3) ±0.46 | |
| | Diaphysis | 34.93 (3) ±1.30 | 1.57 (3) ±0.32 | 0.62 |
| 50,000 | Epiphysis | 37.16 (8) ±0.99 | 2.73 (8) ±0.22 | |
| | Diaphysis | 35.94 (9) ±0.67 | 1.65 (8) ±0.16 | 0.60 |

[a] Mean ± standard error; number of samples in parentheses.

1 Coleman Model 21 Flame Photometer, operating instructions, 1958.

X-ray diffraction analyses of tibial samples for each dietary treatment showed only poorly-resolved apatite patterns. No change in cell dimensions was noted in any of the bones examined. MacDonald *et al.* (1951) reported that rat bone rich in strontium gave a pattern of apatite with altered cell dimensions, indicating the incorporation of strontium in the apatite structure. Recently Baud *et al.* (1968) confirmed these observations of a lengthening of the parameters of the unit cell and they suggested that this was due to an isomorphous substitution of strontium for calcium in the apatite.

The diaphysis/epiphysis strontium ratio (Table 1) reflects a preferential increase in strontium in the epiphysis compared with diaphysis. Much of the work done on the bone-seeking properties of strontium (Neuman and Neuman, 1958; Comar and Wasserman, 1964) has demonstrated concentration in the growing areas of calcified tissues in amounts related to the species and age of the animal. Uptake may be expected to be greatest in young bones and in those parts of mature bones (epiphysis) in which metabolism is most active. The amounts reported here are distinctly less than in the tibiae of young chicks (Weber *et al.*, 1968).

No statistically-significant change in tibial ash weight (average 44.75% ash) was noted during the four-week experimental period, when levels of 50,000 p.p.m. or less dietary strontium were fed.

Table 2. *Analyses of plasma calcium and phosphorus*

| Dietary treatment (p.p.m. Sr) | Plasma[a] calcium (mg. %) | Plasma[a] phosphorus (mg. %) |
|---|---|---|
| 0 | 26.41 (6) ± 1.21 | 4.47 (4) ± 0.29 |
| 12,000 | 26.12 (6) ± 1.19 | 5.32 (6) ± 0.38 |
| 20,000 | 25.55 (3) ± 2.09 | 4.70 (6) ± 0.80 |
| 30,000 | 22.87 (4) ± 1.01 | 4.70 (6) ± 0.45 |
| 40,000 | 19.62 (3) ± 1.84 | 5.68 (3) ± 0.70 |
| 50,000 | 17.50 (7) ± 2.39 | 4.73 (7) ± 0.46 |

[a] Mean ± standard error; number of samples in parentheses.

The results of blood analyses of hens fed 12,000 to 50,000 p.p.m. supplemental strontium are shown in Table 2. A decrease in plasma calcium with increasing dietary levels of strontium was found. Neither significant change in plasma phosphorus with dietary treatment, nor changes in plasma sodium, potassium or whole blood phosphorus levels, was noted. Since plasma strontium levels were not determined, it is not possible to determine if strontium replaced plasma calcium on a molar basis.

Large amounts of dietary calcium are used in shell formation; it was, therefore, to be expected that strontium added to the feed would appear in the shell (Comar and Wasserman, 1964). Earlier work by Banks and Odum (1957) suggested that egg shells may accumulate as much as 25% strontium carbonate (14.7% strontium). Our data indicate an increase in the strontium content of egg shells which was directly related to the amount of this element in the diet (Table 3).

Table 3. *Strontium and calcium analyses of egg shells*

| Dietary treatment (p.p.m. Sr) | Shell[a] calcium (%) | Shell[a] strontium (%) | Molar Sr/Ca ratio |
|---|---|---|---|
| 0 | 33.12 (8) ±0.50 | 0.09 (6) ±0.02 | 0.001 |
| 3,000 | n.d. | 2.1 (2) ±0.30 | — |
| 6,000 | n.d. | 4.6 (2) ±0.01 | — |
| 12,000 | 26.34 (3) ±0.69 | 6.4 (2) ±0.01 | 0.11 |
| 20,000 | 24.20 (2) ±1.35 | 7.2 (2) ±0.35 | 0.14 |
| 30,000 | 22.56 (9) ±0.36 | 11.0 (4) ±0.65 | 0.22 |
| 50,000 | 20.62 (11) ±0.81 | 13.4 (4) ±0.96 | 0.30 |

[a] Mean ± standard error; number of samples in parentheses.

It is possible that the upper limit of strontium deposition in egg shells was not reached at the levels fed in the present experiments.

The 900 p.p.m. strontium level found in shells from control animals resulted mainly from the bentonite filler used in the feed. The strontium calcium molar ratio changed progressively as more strontium was incorporated into the shell.

A significant decrease in shell thickness was noted only when a diet containing 30,000 p.p.m., or greater, of strontium was fed. Egg shells from hens fed up to 20,000 p.p.m. strontium averaged 0.360 mm thick, whereas shells from hens receiving 30,000 and 50,000 p.p.m. strontium were 0.298 and 0.267 mm thick, respectively. No change in shell phosphorus (average 0.119%) was noted with dietary treatment.

X-ray diffraction photographs of shells from hens receiving the higher dietary strontium levels contained strong lines of calcite, which is the normal inorganic phase of egg shells. With the exception of three unknown lines, all reflections could be accounted for as $CaCO_3$. We have been unable so far to determine the nature of these unknown reflections, which most likely represent the strontium phase. X-ray diffraction and chemical analysis suggest that strontium was not incorporated as either $SrCO_3$ or $Sr_3(PO_4)_2$ but further work is necessary to identify the chemical composition of strontium in egg shells.

Since mature hens can tolerate considerable amounts of strontium in the diet, egg weight (64.1 g), egg production (60.1%), feed consumed/bird/day (0.23 lb), and body weights (1963 g), were unaffected by dietary levels of strontium up to 30,000 p.p.m. during the four-week experimental period. At the dietary level of 50,000 p.p.m. strontium, egg weight, egg production and feed consumed/bird/day were 59.4 g, 9.92%, 0.18 lb respectively.

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