

# FLUORIDE EFFECTS ON BONE MORPHOLOGY AND CALCIUM KINETICS

by

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**SUMMARY:** Undelcalcified sections of several bones were obtained from  $F^-$  treated and control calves after in vivo tetracycline labelling. Microradiographically and histologically, the trabeculae appeared coarser, thicker and separated by longitudinal lines which gave a stratified appearance to the bone of  $F^-$  treated calves. Taken in conjunction with previously published studies of calcium kinetics in these calves, the morphologic findings suggest that a transient phase of hypermineralization of bone was followed by a suppression of gastrointestinal absorption of calcium, compensated for by increased bone resorption, with a subsequent loss of bone mineral in the  $F^-$  treated calves.

In addition to the hyperostosis which is associated with chronic fluorosis in dairy cattle, there is frequently evidence of increased resorption of bone mineral. Shupe has suggested that the osteopenia is associated with prolonged high  $F^-$  intakes whereas lower intakes of  $F^-$  lead to increased mineralization of bone (1). The ability of high calcium diets to prevent the development of  $F^-$  induced osteopenia in other species (2,3) has led to the postulation of an influence of  $F^-$  on calcium absorption from the gastrointestinal tract. In a previous report (4) we showed that absorption of calcium was inhibited in calves which had been given 100 ppm  $F^-$  in their drinking water for a period of 11 months. The present paper reports microradiographic and histologic observations on bone from these calves and compares these observations with previously published radiographs and kinetic findings (4).

## Method

Three pairs of Holstein calves were fed ad libitum a commercial diet which contained 1.18% calcium and 0.29% phosphorus. One of each pair was given water which contained 100 ppm  $F^-$  as sodium fluoride. The control calves were provided with water without addition of  $F^-$ . Ten months later, the calves were placed in metabolism crates designed for the separate quantitative collection of urine and feces. After a two week adjustment period,

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a ten day combined nutritional balance and  $^{45}\text{Ca}$  kinetic study was performed on each animal. The experimental technique and detailed results of multi-compartmental analysis of the data have been described previously (4).

After the nutritional balance study, the calves were given tetracycline intravenously (15 mg/kg) for the purpose of labelling bone apposition. They were killed 48 hours later. The third and fourth right metacarpal bones, the first phalanx of the third digit and several coccygeal vertebrae were dissected free of soft tissues, radiographed on non-screen films and fixed in a 10% solution of neutral formalin. The specimens were then freed from water in a series of ethanol baths of increasing concentration. Embedding, sawing, grinding and mounting of the specimens followed according to the technique described by Olsson and Reitz (5). Prior to mounting, the sections were microradiographed. The mounted specimens were viewed under the microscope, both in ultra-violet and regular transmitted light.

### Results

**Bone Morphology:** The metacarpal bones of the  $\text{F}^-$  treated calves appeared somewhat thicker, having a greater diameter in the midshaft region than those of the control animals. Longitudinal and transverse sections of the metacarpal bones of the  $\text{F}^-$  treated calves appeared radiographically to be of lower mineral density than the controls, and prominent resorption spaces were evident near the medullary surfaces in the midshaft region (4). The macroscopic and radiographic appearance of the other specimens from the  $\text{F}^-$  treated calves did not differ from those of the controls.

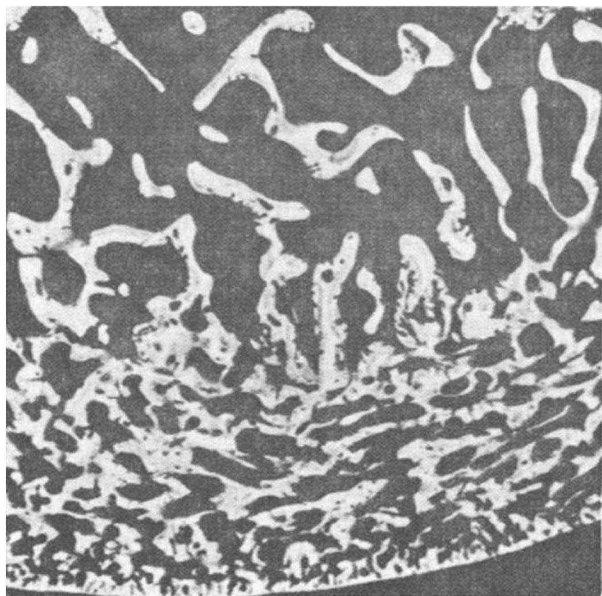
The microradiographic appearance of the bones from the  $\text{F}^-$  treated calves was definitely pathological in all bones except the vertebrae. The trabeculae of the test animals were coarser and plumper than those of the controls indicating an increased bone mass (Fig. 1a and b). The trabeculae were stratified, with marked cement lines separating layers of bone. The surface of the bone trabeculae was usually rough with a large number of excavations indicating resorption. The osteocyte lacunae were larger than normal and in some areas these large lacunae were confluent.

In the control calves the number of smooth surfaces was equal to or greater than the number of rough surfaces, while in the  $\text{F}^-$  treated calves the rough surfaces greatly outnumbered the smooth surfaces. The ground sections viewed under ultra-violet light showed fewer surfaces labelled by tetracycline in the  $\text{F}^-$  treated calves than in the controls. This difference was especially striking when the surfaces of the excavations and vascular channels were taken into account.

**Calcium Kinetics:** The major differences in calcium kinetics between the control and  $\text{F}^-$  treated calves are summarized in Table 1. Further details are presented elsewhere (4). Endogenous fecal calcium excretion was lower in the  $\text{F}^-$  treated calves, but it was correlated with body weight ( $r = 0.91$ ,  $p < 0.01$ ). Since this has been observed previously in growing calves (6), it may not have been a direct effect of  $\text{F}^-$ .

Fig. 1. FLUOROSSED CALFMicroradiograph, Distal End of First Phalanx

(a)



Densely packed, thick and plump trabeculae with prominent vascular channels. Rough surfaces indicating active bone resorption ( $\times 12$ ).

(b)



Higher magnification, same specimen. Large - sometimes confluent osteocyte lacunae; stratification of bone with longitudinal separating lines ( $\times 40$ ).

(c)



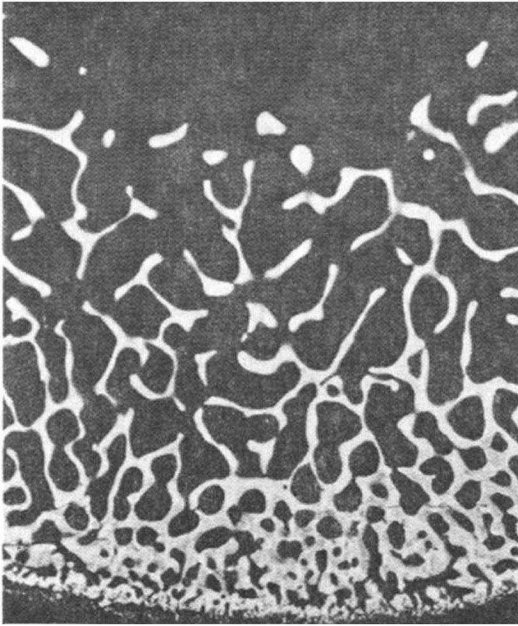
Ground section, same area viewed in ultra-violet light.

Only a few well labelled apposition surfaces (small  $\uparrow$ ); most surfaces undergoing resorption (medium  $\uparrow$ ); or resting (long  $\uparrow$ ) ( $\times 40$ ).

Fig. 2. CONTROL CALF

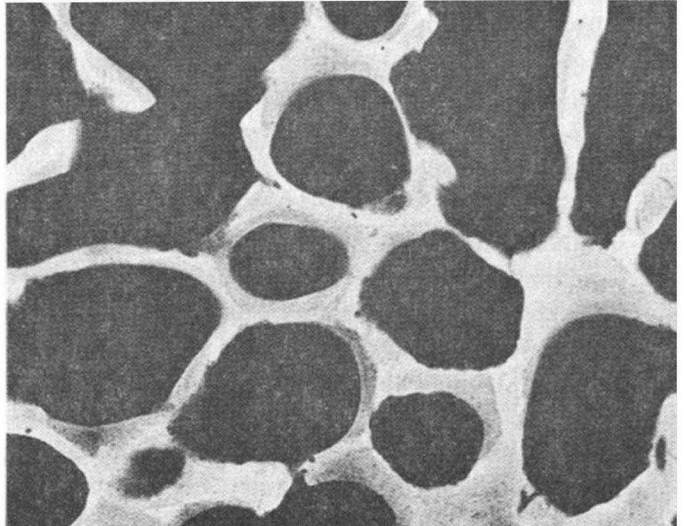
Microradiograph, Distal End of First Phalanx

(a)



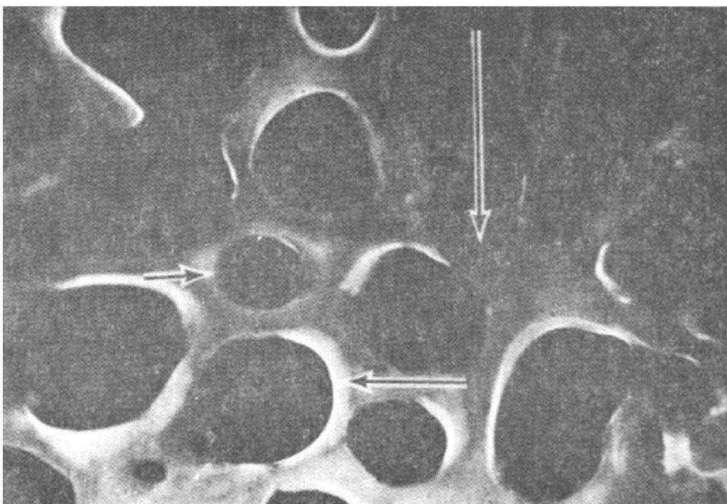
Less bone than in Fig. 1a; trabeculae slender ( $\times 12$ ).

(b)



Same specimen as 2a ( $\times 40$ ). Even proportion of smooth-surfaces (resting or in apposition) and rough surfaces (resorption). Different structure and size of osteocyte lacunae and different bone density than in Fig. 1b.

(c)



Same area as in 2b; Ground section viewed by ultra-violet light ( $\times 40$ ).

More than half of total surface resting (small  $\uparrow$ ) or in apposition (medium  $\uparrow$ ); some evident resorption surfaces (long  $\uparrow$ ).

TABLE 1Rates of Calcium Transport in Control and F<sup>-</sup> Treated Calves

	Control Calves	F <sup>-</sup> Treated Calves
Body weight, kg	353 ± 62*	234 ± 27 **
F <sup>-</sup> intake, mg/kg/day	---	6.2 ± 0.67**
Mass of exchangeable calcium, g	237 ± 30	243 ± 47
Dietary calcium intake, g/day	97.5 ± 3.2	62.7 ± 0 **
Fecal calcium, g/day	73.5 ± 2.9	60.1 ± 5.4 **
Endogenous fecal calcium, g/day	3.30 ± 0.32	2.88 ± 0.19**
Urinary calcium, g/day	0.030 ± 0.014	0.054 ± 0.019
Calcium deposition into bone, g/day	32.2 ± 2.8	30.0 ± 4.9
Gastrointestinal calcium absorption, g/day	27.3 ± 1.5	5.50 ± 5.5 **
Calcium removal from bone, g/day	± 2.1	27.4 ± 10 **
Calcium balance, g/day	± 1.6	2.56 ± 3.8 **

\*mean ± SD

\*\*Significantly different from controls (p &lt; 0.01).

In the control calves, gastrointestinal absorption was the major source of calcium supply, while in the F<sup>-</sup> treated calves calcium absorption from the gut was suppressed. Most of the calcium supplied to the exchangeable pool was derived from bone (Table 1). Consequently, calcium balance was severely depressed in the F<sup>-</sup> treated animals. The depression in calcium absorption in the F<sup>-</sup> treated calves was far greater than could be accounted for by their lower food intake. The efficiency of calcium absorption (100 x absorbed calcium/dietary calcium) averaged only 8.9% in the F<sup>-</sup> treated calves while the control calves absorbed 28% of their dietary calcium.

Discussion

The morphological appearance of bone may vary depending upon the sampling site and the observational level of biological organization (7). This is illustrated by the apparently decreased bone mass in gross radiographs of sections of the metacarpals and the increased bone mass in microradiographs and ground sections of the phalanges. The many possible sources of errors preclude the reliable quantitation of overall rates of resorption and formation solely by measuring apposition and resorption surfaces on microradiographs and intravitaly labelled ground sections. On the other hand, the kinetic technique measures gross rates of movement of calcium into and out of bone, without indicating the mechanism by which this is accomplished. A portion of the kinetic estimates of calcium deposition into and removal from bone may be comprised of slow exchange while some rapid phases of bone formation and resorption may be viewed in the kinetic analysis as intercompartmental exchange. The kinetic and morphological



techniques are complementary, however. When used together, they indicate the current state and suggest the recent history of bone metabolism at various organizational levels ranging from the whole animal to the cell.

The morphologic findings reported here support the conclusions derived from the calcium kinetic study, i. e. that the observed suppression of calcium absorption from the gastrointestinal tract, and the compensatory increase in calcium removal from bone in the  $F^-$  treated calves may be the mechanism for development of the osteopenic lesions which accompany severe skeletal fluorosis in dairy cattle. The elevated calcium removal rate from bone in the calves given  $F^-$  was reflected in morphologic signs of increased resorption of bone. There were enough signs of increased resorption in all the  $F^-$  treated animals to make it possible to distinguish them from the controls by viewing anonymous slides. In addition, the microradiographic appearance of densely packed, thick trabeculae, with longitudinal stratifications suggested abnormal mineralization. The failure to observe an increased amount of labelling by tetracycline, and the similar rates of calcium deposition into bone in the  $F^-$  treated calves compared to the controls indicated that bone mineralization was not proceeding at an abnormally high rate at the time that this study was performed. The morphologic evidence of an increased mineral mass in certain bones suggests that a phase of hypermineralization may have occurred at some time prior to the initiation of the kinetic study. It is apparent that the extremely low calcium balance observed in the  $F^-$  treated calves could not have predominated for the entire time they were receiving  $F^-$ ; otherwise, the bones would have been almost devoid of mineral.

Taken together, the morphological and kinetic data suggest that exposure to high levels of  $F^-$  in the drinking water results in a chronological sequence of a transient phase of hypermineralization, followed by a suppression of gastro-intestinal absorption of calcium, and functional adaptation of bone resorption with subsequent loss of bone mineral.

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## TOXICOLOGY OF CHLOROTRIFLUOROETHYLENE

by

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SUMMARY: Chlorotrifluoroethylene (CTFE) an intermediate product in the manufacturing of plastics, fire extinguishing agents and such pharmaceuticals as halothane is an extremely toxic agent of the "c times t" type. It is 40 times as toxic as tetrafluoroethylene.

Mice were exposed for 3 to 24 hours to air containing CTFE in concentrations of 0.1 to 0.8%. Biochemical, optical, electron-microscopic and enzyme-histochemical examinations were made.

Exposure to CTFE leads to immediate, generalized damage of mitochondria which particularly affects the kidneys. Simultaneous changes occur in the mitochondria of the liver and the myocardium which appear to induce "immediate" and follow-up" lethality. The "delayed" lethality is the result due to prolonged and serious damage to kidneys.

Chlorotrifluoroethylene is a basic material or an intermediate product for certain plastics and fire extinguishing agents and such pharmaceuticals as halothane. The reported data concerning this agent which shows marked discrepancies and uncertainties regarding its damaging effect upon the lungs, brain, liver and kidneys suggested a reinvestigation of its toxic properties (1, 2).

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