FLUORIDE CONTENT AND MICRORADIOGRAPHIC FINDINGS IN SKELETAL FLUOROSIS

by

V. Freitag, W. Oelschlager and K. Loeffler
Homburg (Saar) and Hohenheim, Germany

SUMMARY: Serial F^- assays were made on ribs of 2 beef cows with clinical fluorosis, on 9 which had been given F^- supplements (68 to 75 ppm F^-/kg in dry food for 316 days) and on 4 normally-fed animals.

The ashed bones of the fluorotic animals contained between 4973 and 10,007 ppm F^-, of those with F^- supplementation of their feed 518 to 3892 ppm and of the normally fed animals, 236 to 2077 ppm. The spongiosa contained more F^- than the compacta. Within the compacta, the subperiosteal zones showed higher F^- values than those adjoining the spongiosa.

Microradiographs in the fluorotic animals showed a marked increase in the number of the Haversian canals, disseminated patchy demineralization, irregular distribution of the lacunae and periosteocytal resorption of mineral salts. In the animals receiving supplemental F^-, minor pathological changes were noted in the bone samples which contained the highest levels of F^-.

In the incipient stage of skeletal fluorosis bone changes are associated with lower F^- levels than indicated in some of the available literature. To evaluate the action of F^-, micro methods are recommended. Conventional X-ray examinations of bones are not reliable.

In 1965, Johnson et al. (1) presented a detailed description of the morphological changes of the initial and the advanced stages of skeletal fluorosis. They related their findings to the F^- content of bones.

Fluoride levels of bones are subject to wide variations which depend upon the kind of bone and upon the portion of bone from which specimens are taken. Cross sections of a rib obtained at varying distances from the ver-

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From the Universitätsklinik und Poliklinik für Zahn-, Mund-und Kieferkrankheiten, Homburg (Saar) and Universität Hohenheim, Stuttgart, Germany.

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tebral column show varying F^- levels (2). In the compacta, F^- storage varies widely in different portions of a bone (3).

In order to further elucidate this problem, we determined F^- storage at different sites in fluorotic bones and in bones which had accumulated F^- and corrolated the findings with those of microradiographic examinations.

Material and Method

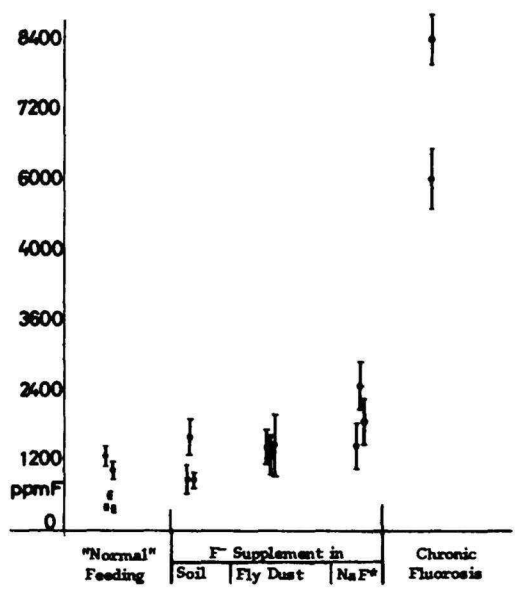
Our studies were made on beef cattle and steers. Two animals had clinical evidence of fluorosis due to feeding on a pasture near a hydrofluoric acid factory; nine animals had received F^- supplements of 68 to 75 ppm F^- in dry forage for 316 days*; four had been fed normally. Cross sections of the 7th rib, 25 centimeter distant from the costovertebral joint were studied.

Radiographs of the cross sections were made with clinical diagnostic equipment. Small segments were decalcified, prepared for thin cuts and microradiographed by means of Phillips-CMR 5. The F^- levels of the bones were determined according to Oelschlager's method (5).

Results of Fluoride Analysis

Figure 1 presents the mean F^- levels in the spongiosa and compacta. The data obtained varied widely from animal to animal, particularly when judged by the spread of individual values.

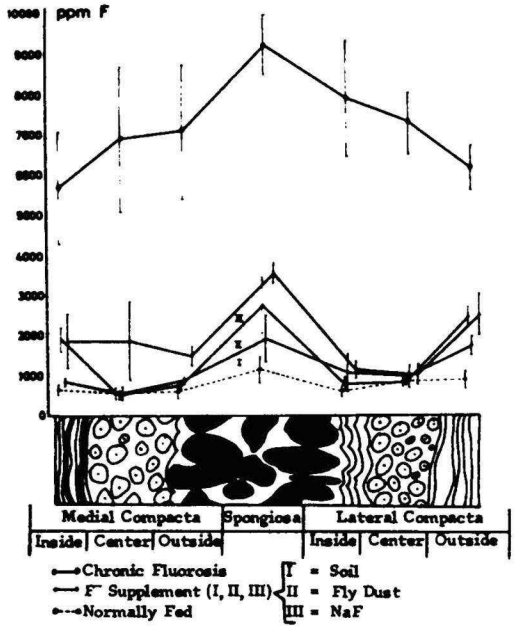
Fig. 1 Fluoride in Bone Ash (Rib Cross Sections in Beef Cattle)



*For details about management and F^- supplementation in the animals, see reference 4.

Fig. 2 reveals a characteristic pattern of F^- distribution in bone: The spongiosa shows the highest F^- values. In the F^- fed animals the superficial portions of the compacta, namely those adjoining the periosteum and endosteum, contain much more F^- than the portions adjoining the spongiosa. On the other hand, the F^- fed animals showed not only great differences in the values of individual portions of a bone, but also a much wider spread of the values which confirms the findings of Weidman and Weatherell (6). Therefore mean F^- values of a bone offer only limited information concerning F^- accumulation at a certain portion of a bone. If one considers that adjoining portions of the same bone vary markedly in their F^- content, it is clear that all mean values concerning F^- levels of bones must be viewed with much reservation.

Fig. 2 Average Fluoride Levels of Bone Constituent (Ribs of Beef Cattle)



Macroscopic X-ray Findings

Macroscopic X-rays of cross sections of the ribs show no difference between the animals with F^- supplements and the normal controls (Fig. 3). In chronic fluorosis, however, there is a marked porosification of the bones (Fig. 4).

Microradiographic Findings

In a normal animal the contact-microradiography reflects the distribution of mineral salt in hard tissue. Areas of great mineral density appear clear, portions low in minerals gray, and tissue lacking mineral black. Figure 5 represents the microradiogram of the lateral portion of the compacta

of a bovine rib. The regularly and densely mineralized ground structure of primary bone is permeated by vascular channels and by a system of Haversian lamellae (osteons) which show various degrees of mineralization. The compacta also contains a few Howslip's lacunae.

Fig. 3

X-ray of Cross Sections of Ribs

(a) (b)

Normal Feeding

F^- Supplementation
(68-75 ppm F^-/316 days)

Fig. 4

Fluorosed Animal

Similarity in (a) and (b); distinct porosity of compacta in Fig. 4 (fluorosed animal).

In comparing the section of an analogous portion of the contralateral rib (Fig. 6), it appears that the distribution and the degree of mineralization of the Haversian systems shows great variations. Upon comparing the specimens from anatomically analogous areas from different animals of equal age, the variations are even more apparent. Additional variations are age dependent (6).

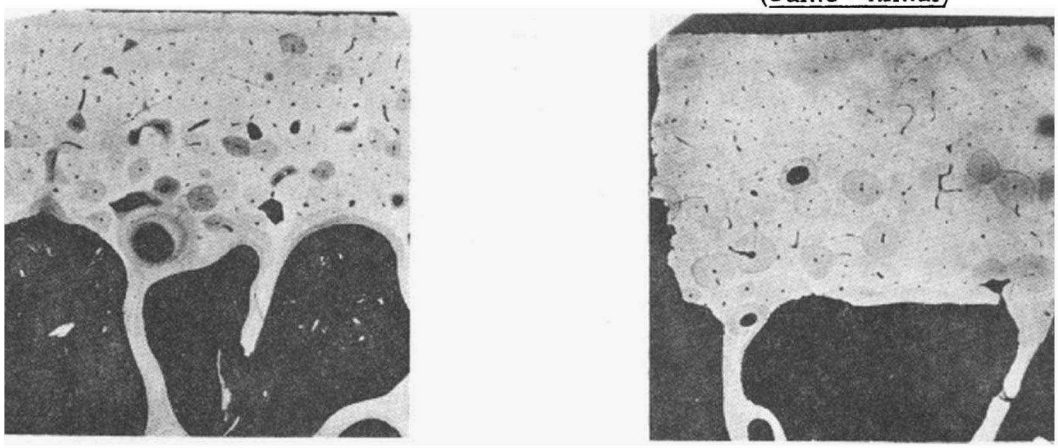
The specimens of the animals with chronic fluorosis exhibit grave structural changes (Fig. 7). The difference between the ground structure of primary bone and the Haversian system is no longer visible. Most conspicuous is the marked porosity of the bone with numerous Howslip's lacunae and with osteons showing wide central canals indicative of excessive vascularisation. The bone substance is diffusely permeated with minute spots of demineralization which, upon careful visualization, can be identified as traces of minute blood vessels and capillary excrescences, another indication of increased vascularization. The osteons show the irregularly distributed mineral salts and irregularly arranged osteocytes which tend to accumulate at the periphery of the osteon (Fig. 8).

IN NORMAL ANIMAL

Fig. 5 Fig. 6

Radioradiogram (x30)

Lateral Compacta of Left VI Rib Analogous Section of Right VI Rib (Same Animal)

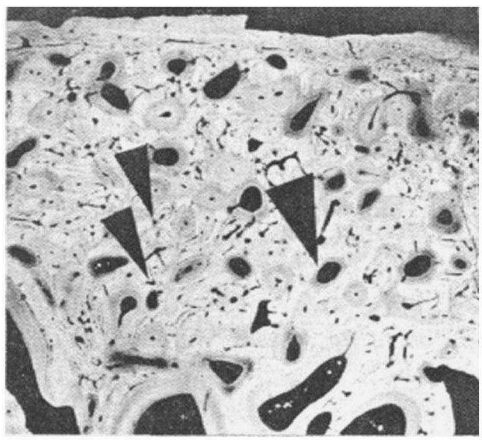


Variations in the structure of normal ribs.

IN CHRONIC FLUOROSIS

Fig. 7

Microradiogram (x30)



Excessive lacunae, widened central canals in osteons. Decreased mineral density. Disseminated minute spots of demineralization of excessive vascularization.

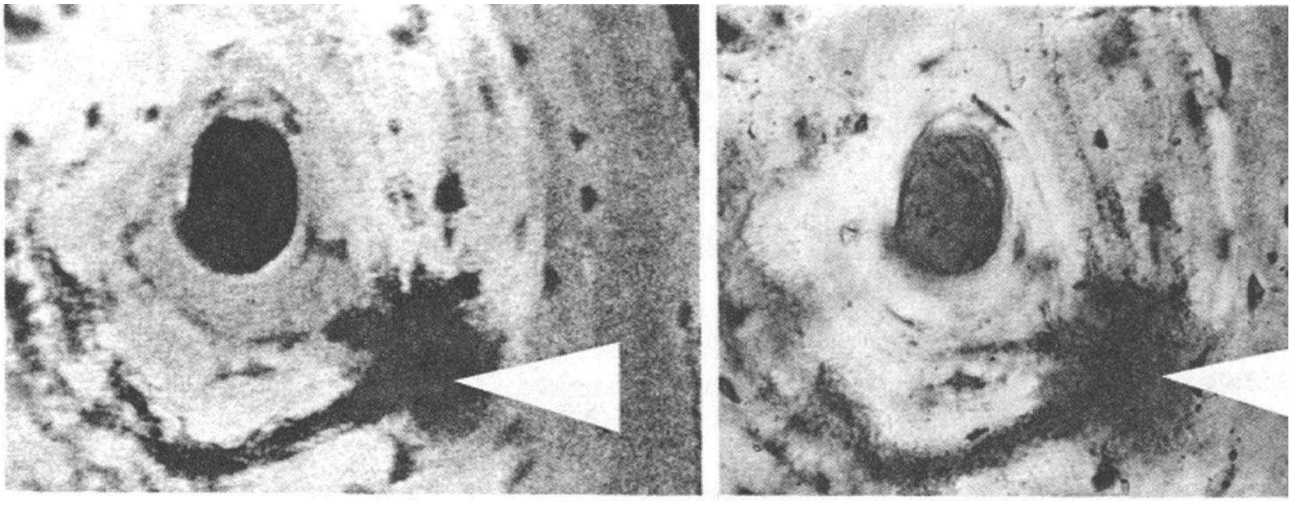
IN CHRONIC FLUOROSIS

Fig. 8

Microradiogram of Osteon (Undecalcified Rib x 450)

(a) (b)

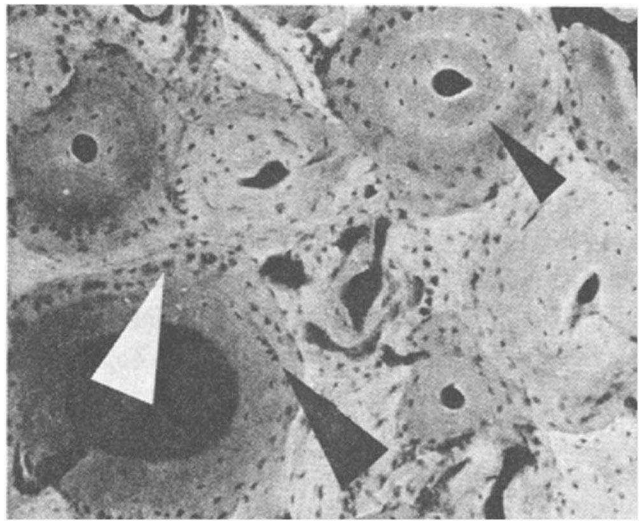
Fuchsin stain (same specimen)



Mineral defect at periphery of osteon with capillary sprout(↑).

Fig. 9

Rib x 290

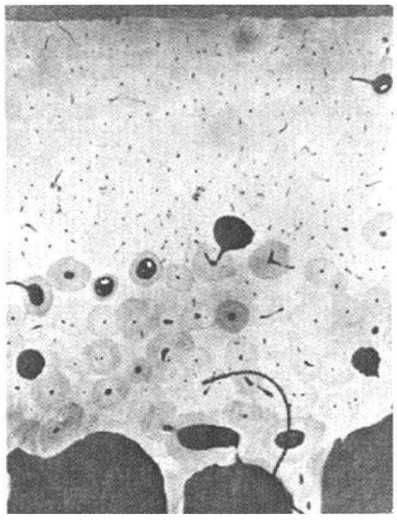


Irregular mineralization of osteons. (Accumulation of osteocytes at periphery of osteon). Spotty periosticytal mineralization.

IN ANIMALS WITH F^- SUPPLEMENTATION
(68-75 ppm F^-/316 days)

Fig. 10

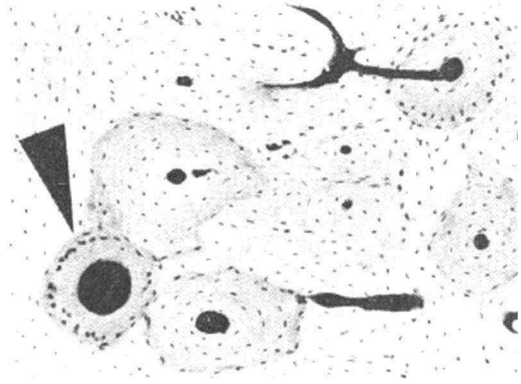
Microradiogram of Rib (x30)



No significant structural changes compared with Fig. 5 and 6.

Fig. 11

Microradiogram of Rib (x290)



Only occasional irregularities in arrangement of osteocytes. Aggregation of triangular osteocytic foci in the periphery of osteons (↑).

The osteons and lamellae exhibit strains of periostiocytic areas of mineral resorption (Fig. 9).

In the animals receiving F^- supplementation the microradiograms do not appear to show pathological changes in comparison to those of fluorotic bone (Fig. 10), particularly if one realizes the difficulties in interpreting normal conditions as outlined above. A thorough study, however, reveals in the animals with the highest F^- levels in bones - those receiving NaF supplements - accumulations of wide osteocytic lacunae at the periphery of osteons (Fig. 11) which, we believe, represent a pathological structure. The fully developed stages of these changes were designated by Johnson (7) as "mottled bone".

Discussion

Pronounced pathological changes were found in the animals with chronic F^- intoxication both in the X-ray and in the microradiographic examina-

tion. Here the F^- levels in the bone ash were of the order of 5000 to 10000 ppm, values which conform to those in the available literature.

The animals which received F^- supplements exhibited discrete microradiographic changes indicative of fluorosis which could not be detected by the macroscopic X-ray technique. In these bones the average F^- content namely 2,278 ppm (ashed) was lower than that usually recorded in the initial stage of skeletal fluorosis. Averages, however, are not a reliable indicator of F^- levels in bones.

The disturbance in the distribution of osteocytes after F^- supplementation and the pronounced spotty periostiocytic demineralization in fluorosis indicate a disturbance of the function of osteocytes by F^- as pointed out by Jowsey (8) and by Johnson (7).

Conclusion

To demonstrate or rule out incipient skeletal fluorosis, macroscopic examinations i.e. conventional X-ray studies are inadequate. Micromethods are necessary for the recognition of early skeletal pathology. Even serial macroscopic X-rays are inconclusive.

Finally, to obtain acceptable data which can be compared with that of other authors, a standardized procedure of bone biopsy for F^- analysis is desirable. This would eliminate the variations in findings which are due to the fact that areas of bone specimens are taken at different levels of bones.

Bibliography

1. Johnson, L.C., Merriman, G.M., Hobbs, C.S., Shupe, J.L., Greenwood, D.A. and Largent, E.J.: Histogenesis and Mechanisms in the Development of Osteofluorosis. In: Hodge, H.C. and Smith, F.A.: Fluorine Chemistry. Academic Press, New York/London 1965.
2. Oelschläger, W., Loeffler, K., and Opletalowa, L.: Retention von Fluor im Knochen. Zschr. Landw. Forxug im Druck.
3. Weidman, S.M. and Weatherell, J.A.: The Uptake and Retention of Fluoride on Bone. Proc. Nutr. Soc. 22:105, 1963.
4. Wöhlbier, W., Oelschlager, W., Gronbach, G. and Giebler, H.: Die Resorption von Fluor durch Ochsen aus Erde und Flugstaub einer Aluminiumhütte. In: Fluorwirkungen. Forschungsberichte Nr. 14 der DFE. Verlag: Franz Steiner, Wiesbaden 1968.
5. Oelschläger, W. and Wöhlbier, W.: Bestimmung von Fluor in pflanzlichen, tierischen und anorganischen Substanzen sowie in Wässern und Luft. In: Fluorwirkungen. Forschungsbericht Nr. 14 der DFG. Verlag: Franz Steiner, Wiesbaden 1968.
6. Sissons, H.A., Jowsey, J. and Stewart, L.: The Microradiographic Appearance of Normal Bone Tissue at Various Ages. In: X-ray Microscopy and X-ray Microanalysis. ed. Engström, A. et al. Elsevier Pub. Co., Amsterdam/London/New York/Princeton 1960.
7. Johnson, L.C.: Morphologic Analysis in Pathology. Bone Biodynamics. ed. Frost, H. J. & A. Churchill, London 1964.
8. Jowsey, J., Schenk, R.K. and Reutter, F.W.: Some Results of the Effects of Fluoride on Bone Tissue in Osteoporosis. J. Clin. Endcr. 28:869, 1968.