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Original Articles

Value of the Bone Biopsy in the Diagnosis of Industrial Fluorosis

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Summary. Iliac crest biopsies taken from 43 men with industrial fluorosis were compared with control bone samples. The bone fluoride content was determined, histological examinations were made on stained sections and microradiographs, and morphometric analysis performed on the microradiographs alone.

In the subjects with fluorosis, the bone fluoride content (5617 ± 2143 ppm) was found to be significantly higher ($P < 0.00005$) than in control subjects (1036 ± 627 ppm). It decreased slowly, however, after exposure had ceased (to about 50% in 20 years). The histological changes consisted of a non-specific remodeling activity (resulting in increased trabecular bone volume and cortical porosity, as well as hypervascularization and linear formation defects) and modifications of the perilacunar walls (i.e., presence of mottled lacunae and enlarged lacunae). These histological changes were found more likely to occur when the bone fluoride content was high but no correlation between the two parameters was observed.

Although certain clinical and radiological data associated with a high urine fluoride content can sometimes establish a diagnosis of skeletal fluorosis, many cases require the use of bone biopsy, which also provides a direct evaluation of the bone fluoride content and can establish the absence of any other bone disease.

Key words: Bone biopsy – Skeletal fluorosis – Occupational medicine – Morphometry – Microradiography.

Since prolonged exposure to fluoride is known to result in impregnation of the calcified tissues and more or less pronounced bone structure alterations, bone biopsy could be very useful in establishing the diagnosis of skeletal fluorosis. The purpose of this article, therefore, is to discuss the advantages and

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limitations of bone biopsy as compared with other methods of diagnosing fluorosis when an industrial origin is suspected.

Subjects and Methods

1. Subjects

Forty-three men who had worked in, or were still working in, electrolysis rooms with open furnaces of two aluminium factories located in the same Swiss valley, were subjects of a study requested by the "Caisse Nationale Suisse d'Assurances en cas d'Accidents". The average period of exposure to fluoride was 27 years (SD = 9 years) and the average age of the subjects was 62 years (SD = 6 years). This study on bone biopsies was part of a broader one, including clinical and radiological observations, various laboratory determinations (namely fluoride concentration of the urine, PTH level of the blood) (Boillat et al., 1978).

In 33 of these subjects, one bone biopsy was taken from each and fluoride content determinations and histological examinations were made. In nine of the subjects, two biopsies were taken at intervals of 2 to 5 years; in seven of these nine, fluoride levels were determined and histological examinations were made of both biopsies while for the remaining two, no histological examination was performed on the first biopsy. In the remaining subject, three bone biopsies were taken, yielding a total of 54 biopsies, in which 52 had both fluoride content determinations and histological examinations made and two, fluoride determinations alone. Of the 54 biopsies, 14 were taken while the subjects were still being exposed to fluoride and 40, from 1 to 20 years after the exposure had ceased. In 38 of the latter group, both fluoride determinations and histological examinations were made; in two, only the fluoride determinations were made. All bone biopsies were performed under local anesthesia with a Bordier's trephine (Bordier et al., 1964), 2 cm behind the anterior-superior iliac spine and 2 cm below the superior edge of the iliac crest. The bone material comprised the external and internal cortices as well as the spongy bone between them.

Male control subjects of the same average age as the fluorotic subjects but having had no history of bone disease, were used in this study. They had all worked at jobs requiring similar physical effort as those of the subjects with fluorosis but had not worked in aluminium factories. Half of them had lived in the same region as the fluorotic subjects and the other half in Geneva and vincinity. Control samples were obtained at autopsy from the same area of the iliac crest as were the biopsies. Thirty-one control samples (average age of subjects, 61 years, SD = 10 years) were used for bone fluoride content determinations and eleven (average age of subjects, 62 years, SD = 8 years) were used for quantitative histological examinations.

2. Methods

a. Bone Fluoride Content and Distribution. Fluoride content was determined on calcinated compact bone of the external cortex by the specific electrode method (precision: 1 SD = 3%) described by McCann (1968).

Topographical distribution of fluoride in bone samples was determined by means of an electron probe X-ray microanalyzer (Baud and Bang, 1972). In order to determine the topographical distribution of fluoride with the highest possible degree of accuracy, a large scale mosaic (about 1 cm^2) was created by reassembling a great number of high resolution (about 1 μm) F Kα X-ray images made by scanning a surface of 320 × 400 μm (Bang and Baud, 1977).

b. Qualitative Histological Observations. Conventional histological studies were performed on undecalcified thin sections obtained by grinding and polishing and stained with basic fuchsin or hematoxylin-eosin. Microradiographic studies were made on thick sections (100 μm) cut with a diamond wheel saw using the method described by Baud (1957) as well as on thin sections ground manually to 10 μm. The histological examination was at first directed toward detecting the usual histopathological changes of bone, i.e., Haversian cortex and endosteal-trabecular remodeling, either active (resorption by osteoclasts and formation by osteoblasts) or old (lamellar 'breccia' structure, best seen in polarized light), porosity of compact cortical bone, presence of newly-formed periosteal bone and state of vascularization. Examinations were then made in an attempt to detect other modifications more specifically suggestive of fluorosis that have been reported in the literature. Small areas of hypomineralization (dark on the microradiographs) and hypoplasia (decreased birefringence of the collagen fibers) have been described in cows (Johnson, 1965; Freitag et al., 1970) and in osteoporotic patients treated with fluoride (Jowsey et al., 1968; Thiebaud et al., 1970). These areas were found to correspond to that part of the bone containing large amounts of fluoride (Baud and Bang, 1971, studying human patients; Baud and Bang, 1972, studying cows). These changes which have been observed primarily at the periphery of periosteocytic lacunae result in a mottled appearance of the lacuna and sometimes, when they are very pronounced, in linear formation defects as well. It is important that these mottled lacunae be carefully distinguished from enlarged lacunae as has been reported in a previous paper (Baud and Boivin, 1978) in which detailed characteristics of these two aspects of periosteocytic lacunae have been described.

c. Quantitative Histological Observations. Microradiographs of sections of undecalcified iliac crest biopsies embedded in methyl-methacrylate were analyzed by means of an automatic texture analyzer system (TAS, Leitz) and the following parameters of this bone tissue were quantified: trabecular bone volume (TBV) and trabecular thickness (TT) for cancellous bone, cortical porosity (CP), periosteocytic lacunar surface (PLS) and osteocytic population (OP) for compact bone. The OP was quantified under the same methodological conditions as the PLS and the results are expressed as the number of osteocytes per mm^2 of compact bone tissue. The detailed procedures for these morphometric studies have been described and discussed in previous papers (Boivin, 1978; Boivin and Baud, 1978). Since the cutting plane was found to have an effect on the evaluation of certain parameters of cortical compact bone, only samples having the same cutting plane, i.e., perpendicular to the Haversian canals, were used for those measurements.

Results

1. Fluoride Content of Compact Bone

This was found to be significantly higher in samples from contaminated subjects (5617 ppm, SD = 2143) than in the control samples (1036 ppm, SD = 627). There was no significant difference in the results obtained for the two control groups. The distribution of values obtained for the 54 biopsies and the 31 controls is illustrated in Figure 1. No correlation was found between bone fluoride content and duration of exposure. There was, however, a correlation between bone fluoride content and the length of time without exposure; as can be seen in Figure 2, the fluoride content decreased linearly, by approximately one half,

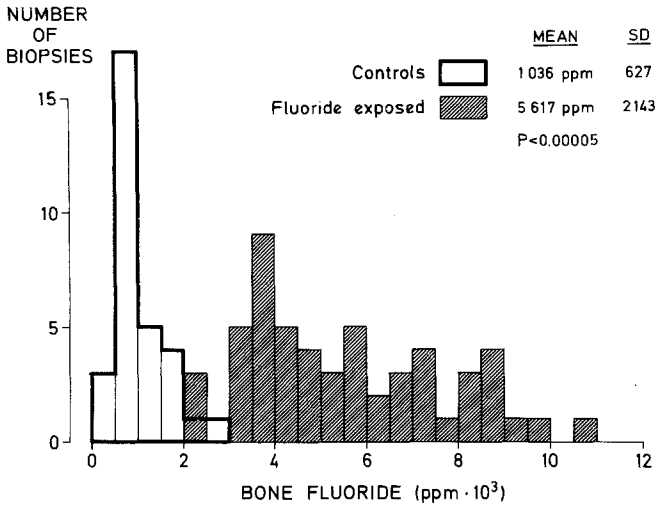


Fig. 1. Histogram showing the distribution of bone fluoride content in control and fluorotic groups

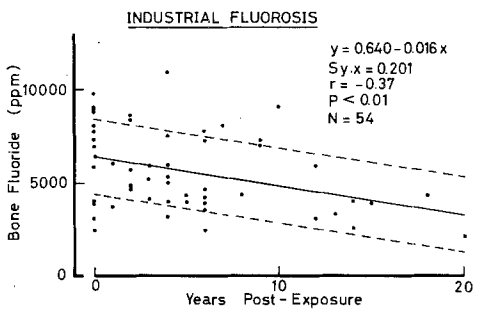


Fig. 2. Evolution of bone fluoride content in fluorotic group, as a function of the length of the time period between the end of the fluoride exposure and the biopsy

in a twenty year period. This linearity indicates a constant rate of decrease of fluoride rather than a more rapid elimination during the first years following cessation of exposure. In three cases, the bone fluoride content was found to be less than 2500 ppm; one case biopsied immediately after 32 years of exposure and two cases biopsied at 6 and 20 years respectively after 12 and 14 years of exposure, illustrate the dispersion of individual values that was observed.

Electron probe X-ray microanalyzer images showed that the fluoride distribution of fluorotic bone tissue was not homogeneous (Fig. 3). The histograms of the distribution of the various fluoride contents, as a percentage of bone volume, show the presence of zones of high fluoride content (more than 4400 ppm) not found in the control samples (Fig. 4). This observation is particularly important since the presence of such zones in subjects who have ceased to be in contact with fluoride for 10 to 20 years and whose average bone fluoride content is therefore very low (about 2000 ppm), can confirm a diagnosis of fluorosis.

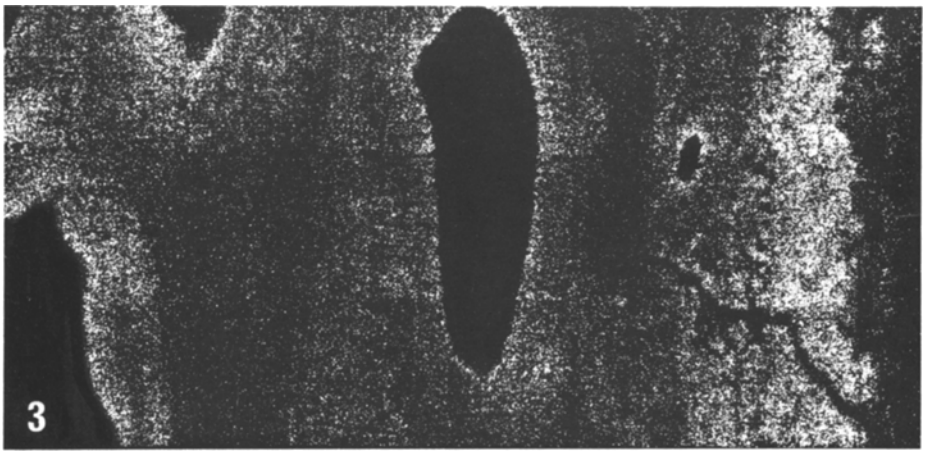


Fig. 3. F Kα X-ray images of an iliac crest biopsy taken from a fluorotic subject, It can be seen that the fluoride is not evenly distributed. × 70

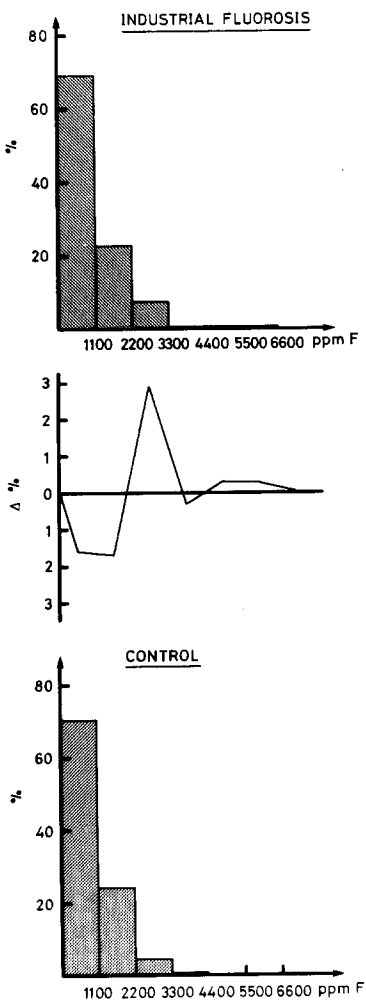


Fig. 4. Histograms showing the distribution of fluoride content as a percentage of compact bone volume, in fluorotic subject (biopsy taken 20 years after the end of an exposure period of 14 years) and control subject with the same age and comparable average bone fluoride content (fluorotic subject, 2100 ppm; control, 1700 ppm). The differential histogram (*middle part*) clearly demonstrates the presence in the fluorotic subject of small zones of high fluoride content

Table 1. Morphometric data – Male human iliac crests

	TBV %	TT μm	CP %	PLS μm^2	OP nb/mm^2
Industrial Fluorosis	19.6±5.5 (33)	123.7±21.9 (33)	13.8±4.2 (13)	45.8±3.7 (20)	450±59 (20)
Controls	12.8±2.5 (11)	130.9±9.4 (11)	6.8±3.1 (5)	23.4±2.9 (6)	455±26 (6)

Mean value±SD, (N)

TBV	= Trabecular Bone Volume	$P<0.0005$
TT	= Trabecular Thickness	not significant
CP	= Cortical Porosity	$P<0.005$
PLS	= Periosteocytic Lacunar Surface	$P<0.00005$
OP	= Osteocytic Population	not significant

2. *Histological Aspects of Fluorotic Bone Tissue*

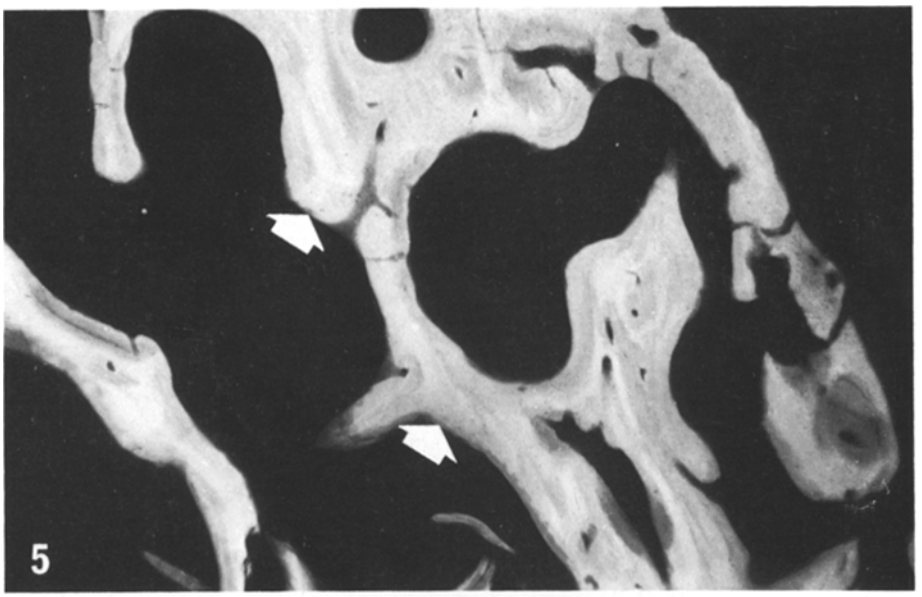
Examination of the stained sections revealed more marked bone remodeling activity in the fluorotic subjects than in the controls, which appeared to be in direct correlation with the bone fluoride content. This remodeling was not very active as indicated by the rarity of osteoclastic resorption and osteoblastic apposition. Most of the observed remodeling was not recent as shown by the polarized light images of 'breccia' in both the compact and cancellous lamellar structures as well as by the presence of cementing lines which were sometimes quite wide and strongly basophilic. Occasionally, basophilic periosteocytic halos with decreased birefringence were observed (Fig. 10). The hemopoietic marrow was well preserved.

The effects of fluoride on this remodeling could be precisely defined by means of qualitative and quantitative studies (Table 1) of the microradiographs. Results showed that the TBV values obtained for fluorotic cancellous bone tissue samples were significantly higher than those obtained for the control samples, the difference between the TT values for the two groups was not significant (Table 1). The increase in porosity of cortical compact bone that was frequently observed in fluorotic bone tissue (Fig. 5) was quantified and the values are shown in Table 1. It can be seen that they are significantly higher for fluorotic tissue than for control samples; this indicates extensive spongiosis of fluorotic cortices.

Hypervascularization of the bone tissue is demonstrated in Figure 6 by an unusually large number of vascular channels, many of which are abnormal in appearance. These were observed at relatively low fluoride contents (2100 to 4000 ppm) and were found to increase in number as a direct function of increasing fluoride content.

Linear formation defects (Fig. 7) of the bone tissue were frequently observed.

Newly formed periosteal bone (Fig. 8) was rarely observed.



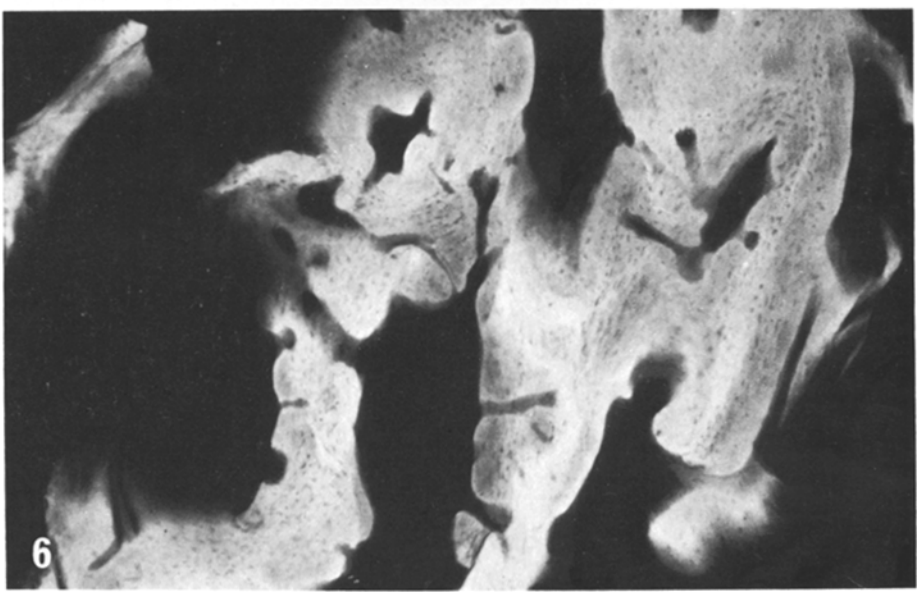


Fig. 5. Microradiograph of compact bone tissue showing the considerable cortical porosity in fluorotic subjects (the periosteal border is seen on the right and the endosteal border is shown by arrows). × 35

Fig. 6. Microradiograph illustrating the hypervascularization of fluorotic bone tissue. × 45

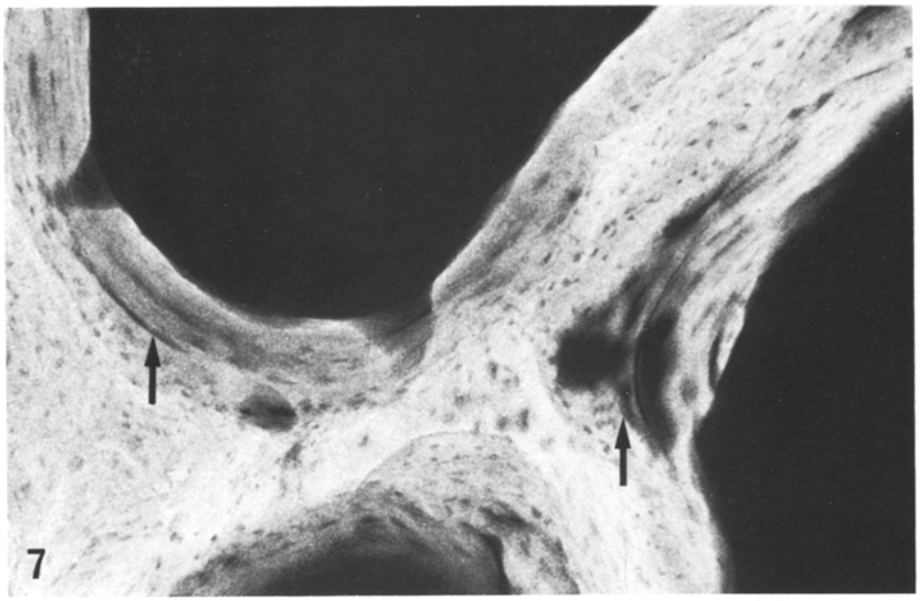
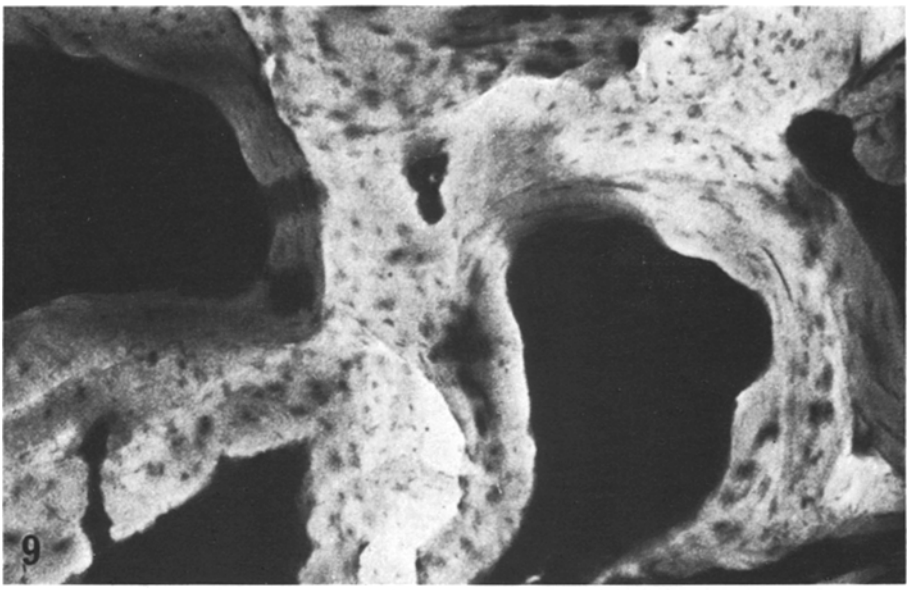


Fig. 7. Microradiograph of fluorotic bone tissue showing hypomineralized linear formation defects (*arrows*). ×110

Fig. 8. Microradiograph of fluorotic compact bone tissue showing both newly-formed hypermineralized periosteal bone tissue and a large number of enlarged periosteocytic lacunae (*arrow*). ×85



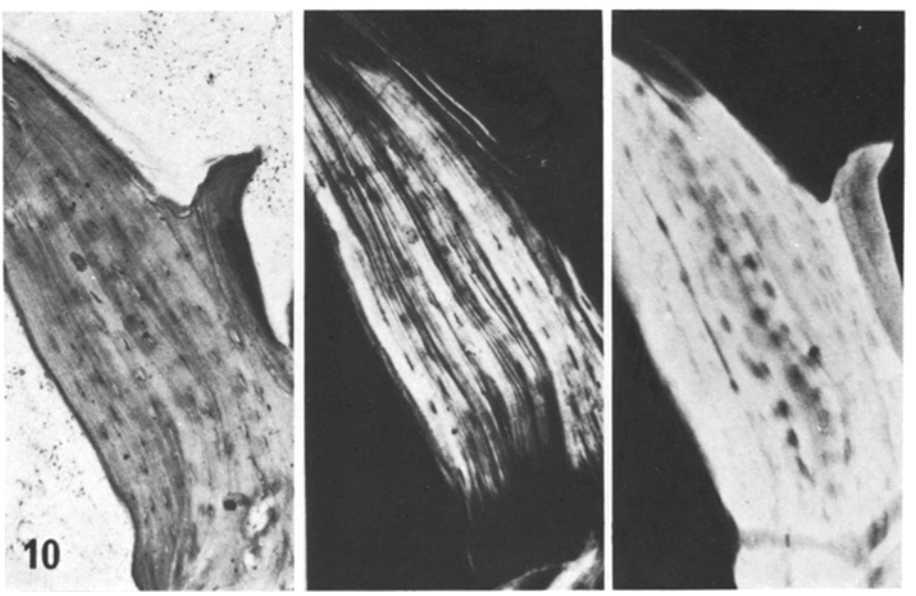


Fig. 9. Microradiograph of fluorotic bone tissue illustrating the mottled appearance of certain periosteocytic lacunae. × 110

Fig. 10. A section of fluorotic bone tissue can be seen after hematoxylin-eosin staning (*left part*), in polarized light (*middle part*) and in a microradiograph (*right part*). Mottled lacunae and linear defect of bone formation are visible in the microradiograph and appear as zones of low birefringence in polarized light and as basophilic zones after staining. × 80

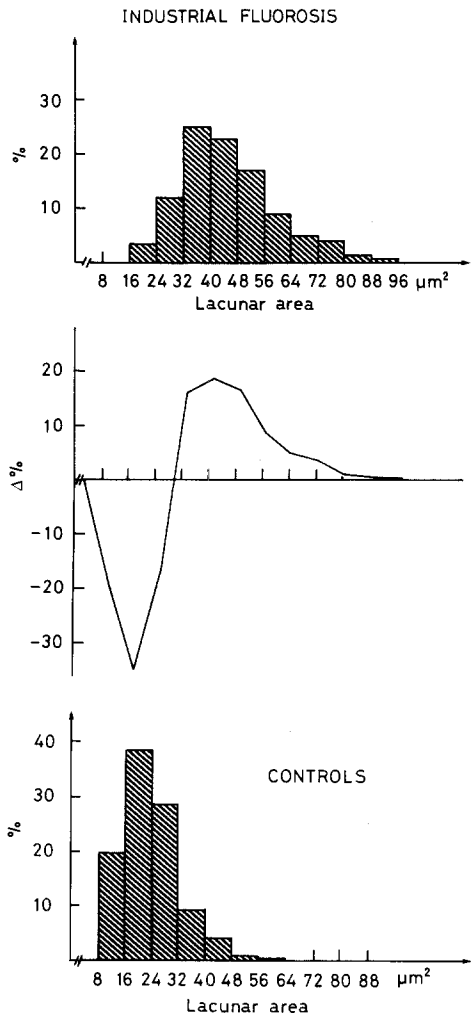


Fig. 11. Histograms showing the distribution of the periosteocytic lacunar surfaces in fluorotic and control groups. They reveal the predominance of low values for the controls and high values for the fluorotic subjects. The differential histogram (*middle part*) confirms this difference

Two different marked changes in the wall of the periosteocytic lacunae i.e., the presence of mottled lacunae and the greatly increased number of enlarged lacunae were distinguished. The principal characteristic of the mottled lacuna, as observed in microradiographs, is that it is surrounded by an eccentrically disposed hypomineralized zone (Figs. 9, 10) which appears, in the stained sections, as a basophilic halo (Fig. 10) and, in polarized light, as a zone of low birefringence (Fig. 10). As for the enlarged lacunae (Fig. 8), the increase in their size was found to be caused by perilacunar resorption (periosteocytic osteolysis) and was confirmed by the sharp increase in PLS (Table 1). In some cases, the osteocytic population of the fluorotic samples seemed to be higher than in control samples, but quantification did not confirm this impression which was quite possibly due to the enlargement of the periosteocytic lacunae

(Table 1). The histograms showing the distribution of lacunar surfaces (Fig. 11), illustrate the evolution of the size of the periosteocytic lacunae. The composite histograms, which show average values for fluorotic and control samples, confirm the sharp increase in fluorotic PLS values and the differential histogram underlines the differences in values obtained for the fluorotic and the control samples. It was noted that the individual histograms for the fluorotic samples were either flattened and enlarged or concentrated around the high PLS values. Neither of these forms seemed to be related either to bone fluoride content, degree of mineralization or to the time interval between the end of exposure to fluoride and the biopsy. Although the mottled lacunae were generally scarce in compact bone, in about 20% of the biopsies, they were abundant and accompanied by high bone fluoride content (average: 7400 ppm) and corresponded to prolonged exposure periods (average: 20 years).

Discussion

1. Bone Fluoride Content as the Main Criterion

Although the fluoride content of normal bone tissue in known to increase with age (Zipkin et al., 1958; Franke and Auermann, 1972; Parkins et al., 1974), it is clear from the results of the present study that the bone fluoride content of subjects who had been submitted to fluoride contamination, was significantly higher than that observed for control subjects. Since exposure conditions varied with the individuals, even in any given factory, it is understandable that the values would be more dispersed in the fluorotic group, and that they are not correlated with the length of exposure. In patients examined sometime after fluoride exposure had ceased, it was found that the fluoride liberated during the resorption phase of bone remodeling was only partially reused; which would explain the decrease in fluoride content over the years (about 50% in 20 years). In cases where this decreased fluoride bone content of fluorotic subjects is within the control range, the electron probe X-ray microanalyzer may demonstrate fluoride contamination by revealing small zones rich in fluoride.

Bone fluoride content thus appears to be a direct and objective criterion for the diagnosis of bone fluorosis. However, since this valuable information can be obtained only by means of biopsy, it is important to carefully weigh the relative reliability of a diagnosis without biopsy, with the contribution that the histological examination could make.

According to Boillat (1978) and Rey et al. (1978), a diagnosis of high probability (96.8% of subjects correctly classified) can be established without biopsy by the simultaneous presence of: (a) urine fluoride content value in excess of 1.5 mg/g creatinine, as determined after at least 6 days without contamination; (b) articular pain involving at least 8 of 24 possible areas; (c) hyperostosis at insertion sites, as revealed by X-ray, in at least two different places, i.e., knee and heel. The inclusion of other biological and radiological parameters did not increase the accuracy of the classification.

2. The Histological Changes in Skeletal Fluorosis

The histological study was useful not only in establishing the presence of skeletal fluorosis but also the absence of any other bone disease.

Study of the stained sections by light microscopy revealed, for example, the presence of only slight osteosclerosis with thick basophilic cementing lines and occasional basophilic periosteocytic zones. These findings are classic in endemic fluorosis but rare in industrial fluorosis (Lagier, 1978). Light microscopy also enabled an evaluation of some aspects of bone remodeling. A more precise evaluation was subsequently achieved by morphometry using microradiographs (Bang et al., 1978; Boivin, 1978). Although TBV and CP values obtained for the fluorotic samples were found to be significantly higher than those for control samples, the increase was not proportional to bone fluoride content nor was it correlated with the length of the exposure period or the length of the period between the termination of exposure and the biopsy. A direct linear correlation between TBV and TT was, however, observed in a previous study of cancellous trabecules of thirty fluorotic subjects (Boivin, 1978).

The microradiographs revealed the localized mineralization defects usually described in fluorosis, linear formation defects and mottled periosteocytic lacunae. These defects correspond to alterations in the organic matrix since they appear as low birefringence zones in polarized light and as basophilic zones in stained sections. The mottled lacunae are found in areas revealed by the electron probe X-ray microanalyzer to be rich in fluoride; they appear more numerous when the bone fluoride content is higher. The mottled lacunae are the result of impaired bone formation (Baud and Boivin, 1978) due, possibly, to topical calcium deficiency, increased appositional growth rate, some perturbation preventing normal evolution of the osteoblast into osteocyte, an alteration of osteocytic activity or, perhaps, some combination of these factors. Although mottled lacunae are not specific to skeletal fluorosis since they are also observed in hypophosphatemic vitamin-D resistant rickets (Steendijk et al., 1967; Steendijk, 1976), after parathyroidectomy in dogs (Burkhart and Jowsey, 1966; Jowsey, 1977) and in renal osteodystrophy (Bonucci et al., 1976; Bonucci and Gherardi, 1977), they are nevertheless highly suggestive of fluorosis in bone with high fluoride content and in a certain clinical context.

It is important that mottled lacunae should not be confused with lacunae enlarged by periosteocytic lysis (Baud and Boivin, 1978). The latter, which were easily detectable by morphometric analysis of the microradiographs, were significantly more numerous in fluorotic bone tissue. This was not due to an increased parathyroid hormone level which appeared to be in the normal range when determined by immunological methods (Boillat et al., 1978). It would seem, therefore, that, contrary to what has been concluded in numerous papers, this modification can not be considered characteristic of hyperparathyroidism.

The hypervascularization of the fluorotic bone tissue observed in this study is evidently related to bone remodeling and therefore not a primary manifestation. It appears remarkably similar to that of Paget's disease, thus indirectly supporting the view that the vascular changes in Paget's disease are a secondary manifestation rather than an etiological factor (Lagier and Baud, 1976). Hyper-

vascularization which cannot be considered characteristic of fluorotic bone tissue, appears to be proportional to the rate of bone deposition (Enlow, 1966).

3. Industrial Fluorosis as an Entity

It can thus be established, based on diverse but closely related data, a physiognomy of the nosological entity 'skeletal fluorosis', considered 'industrial' if the disease was caused by prolonged fluoride contamination in a factory.

The fluoride impregnation effects the bone remodeling which explains the changed radiological aspects (occasional intrinsic condensation, particularly hyperostosis at insertion sites) as well as the clinical symptoms.

The pains reported by fluorotic patients can be explained by hypervascularization of the bone tissue, which has also been observed by Shupe and Alther (1966) and Freitag et al. (1970). The decreased amplitude of the articular movements is probably due to these pains, since it appears to be clearly proportional to them (Boillat et al., 1978) rather than to osteoarthritis or a mechanical restriction caused by hyperostosis (Boillat et al., 1978).

In the diagnosis of industrial fluorosis, bone biopsy can be a conclusive element and should be requested if the other data are insufficient for a definitive diagnosis. It has the further advantage of permiting earlier detection than radiological observations (Vischer et al., 1970). It also allows for the exclusion of other diseases and can, depending on the clinical content, not only permit the diagnosis but also help to evaluate the stage of the disease. Bone biopsy can also reveal evidence of previously existing fluorosis that is presently disappearing and allows an evaluation of the degree of fluoride impregnation which would be impossible by other means. Finally, it can furnish extremely important data for fundamental research on skeletal fluorosis. It should be noted, however, that for the detection of early fluoride impregnation, urine analysis is indicated since bone biopsy would be too extreme a measure.

Conclusions

In practice, an iliac crest biopsy performed with an 8 mm inner diameter trephine is recommanded although biopsies in other places are sometimes justified to verify a doubtful X-ray or when skeletal surgery is being performed for some other reason. The bone samples should be placed in a non-acid fixative solution which will not modify the bone mineral substance. Alcohol, which is often used for this purpose, is very convenient for preserving the samples to be used for fluoride determination and microradiographs but is not suitable for those intended for cellular study. The specific electrode method of McCann (1968) seems to be the most reliable method for determination of bone fluoride content.

Based on the data already published (Johnson, 1965; Franke and Auermann, 1972) as well as the results of the present study, it can be concluded that a bone fluoride content higher than 4000 ppm would confirm a diagnosis of

bone fluorosis and eliminate the need for a histological examination. The latter is useful, however, to exclude the simultaneous presence of any other bone disease. Bone fluoride content of between 2000 ppm and 4000 ppm would, however, require complementary histological data. Although pronounced bone remodeling observed on stained decalcified sections would be suggestive, the observation of mottled bone on the microradiographs of undecalcified sections, and of zones revealed by the electron probe X-ray microanalyzer to have a high fluoride content, is necessary to confirm a diagnosis of bone fluorosis. If the bone fluoride content is below 2000 ppm and if zones with high fluoride content are absent, however, a diagnosis of bone fluorosis must be excluded.

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