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CHANGES IN QUALITY OF BONE MINERAL ON AGING AND IN DISEASE

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Abstract

This paper reviews the changes in the quality of bone mineral with age and in disease. After a brief review of morphological changes with aging in mammalian bones, microradiography is compared to backscattered electron imaging and their use in bringing out subtle changes in bone mineralization outlined.

Changes in the quality of bone with disease is described using osteoporosis as an example. Chemical changes in the skeleton are then discussed and related to morphological changes. Finally, some examples of localized and generalized changes in bone mineral are given. This paper emphasizes that understanding the nature of the mineral phase in bone as well as its heterogeneity and its changes with age and in disease is essential to the elucidation of skeletal physiology and pathology.

Key Words: Bone quality, Aging, Microradiography, Backscattered electron imaging, Bone disease, Chemical changes in bone, localized versus generalized bone loss.

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Introduction

Bone, like other tissues, undergoes changes with aging, not only quantitative changes (bone loss), but also qualitative changes. Bone is heterogeneous on many levels. Morphologically there are many types of bones: woven and lamellar which can be trabecular or cortical. In any macroscopic piece of bone there are local areas which have just mineralized next to local areas which have been mineralized for longer periods of time. This fact renders the analysis of mineralization in whole bone very misleading. To understand the chemical and structural changes which occur in bone mineral and matrix in order to distinguish between normal aging and disease process, chemical methods have been devised which separate bone into fractions of increasing density, hence increasing the degree of mineralization and therefore tissue age (Richelle and Onkelinx,1969). These methods have been used in aging studies (Bonar et al,1983) and in studies on bone diseases (Russell and Avioli,1972; Dickson and Kodicek,1979; Grynpas et al,1986a). Methods have also been devised to look at the distribution of mineral within a given piece of bone. Microradiography, which is based on the differential absorption of X-ray through bone slices of different degrees of mineralization, was first developed as a quantitative technique by Amprino and Engstrom (1952). This technique demonstrated that inhomogeneities in mineralization could be visualized and quantitated. The correlation of the chemical techniques and microradiography as well as newer techniques which have just appeared will be the subject of this review which is intended to illustrate the qualitative as well as the quantitative changes which occur in bone tissues with age and in disease states.

Aging Changes in Bone

Using histological techniques many investigators have shown an increase in intracortical porosity with aging in human cortical bones. This increase seems to be secondary to an increase in the number and mean diameter of Haversian systems. The inner cortex is more affected than the outer cortex (Martin et al,1980) and Wolff' s law was invoked to explain this effect. Laval-Jeantet et al (1983) hypothesized that an increase in porosity would lead to decrease in macroscopic mineral density, but others have found an increase with age in males (Lindahl and Lindgren,1967). Decrease in cross sectional area of long bone with age has also been documented (Thompson,1980; Dequeker,1975) but remains controversial (Laval-Jeantet,1983). Microradiographic studies by Sharpe (1979) and Jowsey et al (1974) confirmed the histological findings of more variably mineralized bone, with hyper and hypo-mineralized osteons and more fragmented osteons with plugged caniculi, with aging. Barbos et al (1983) developed a classification of osteons based on the degree of mineralization which includes an initial stage, two intermediate stages and a final stage of full mineralization. These findings correspond to the concept of bone mineral being laid down very quickly to reach 70% of full mineralization followed by a period of maturation during which the remaining 30% of the mineral is gradually deposited over a period of months. Microradiography has also been used to try and determine the rate of intra-osteonal remodelling (Pankovich et al,1974; Trotter and Dixon (1974); Lacroix and Dhem,1967) by comparing single and double osteons. Double osteons are the result of intra osteonal remodelling; their number was found to increase with age while the number of single osteons decreased at a rate of 2.5% per decade after the age of 30. An increase in the number of incomplete osteons with age was also found. Coupled with the fact that the outermost lamellae in double osteons were nearly always highly mineralized, this suggests that remodelling favours older more highly mineralized osteons. In most studies it is also suggested that bone loss begins in normal aging only after the third or fourth decade of life and takes place at different rates in trabecular and cortical bone (Atkinson,1969; Riggs et al,1982; Mazess,1982).

Microradiography Versus Newer Techniques

The main problem with microradiography is that it requires that the thickness of the sections be absolutely even because the differences in gray levels are related both to section density and thickness. Another major drawback is that it is very time consuming and until recently was not truly quantitative. With the advent of computerization, attempts have been made to automate microradiography. Phillips et al (1978) first described a computerized system for the analysis of mineral densities but no studies using this system have been published. More recently Pugliese and Anderson (1986) have used a Bioquant system for assessing the relative concentration and distribution of mineral in undecalcified bone section. When this system was used for study on human bone biopsies and in experimental animal studies, it was the first time that quantitative microradiography was attempted on trabecular bone. Finally, in our own laboratory we have used an IBAS image analysis system (Zeiss) to automatically analyze microradiographs of human male femoral shaft sections from four age groups (20-25,40-45,60-65,80-85). In figure 1 the process is illustrated by showing a microradiograph (A), its video-image (B), and its processed image(C); finally, samples of the other age groups are shown in figures 1D (20-25y), 1E (60-65y) and 1F (80-85y). These changes correlate well with findings from our laboratory showing an increase in bone specific gravity from the 20-25y group to the 40-45y group, a subsequent decrease in the 60-65y group due to endosteal trabecularization and a final increase in the 80-85y group (Simmons et al,1985).
An alternative to classical microradiography has recently been developed by Boyde and Jones (1983) Reid and Boyde (1987) based on backscattered electron imaging (BSE) in the SEM. Using this technique, they have analyzed a series of human ribs, from neonates to 59 years old, transversely sectioned and embedded in PMMA, and they have shown that this method was capable of

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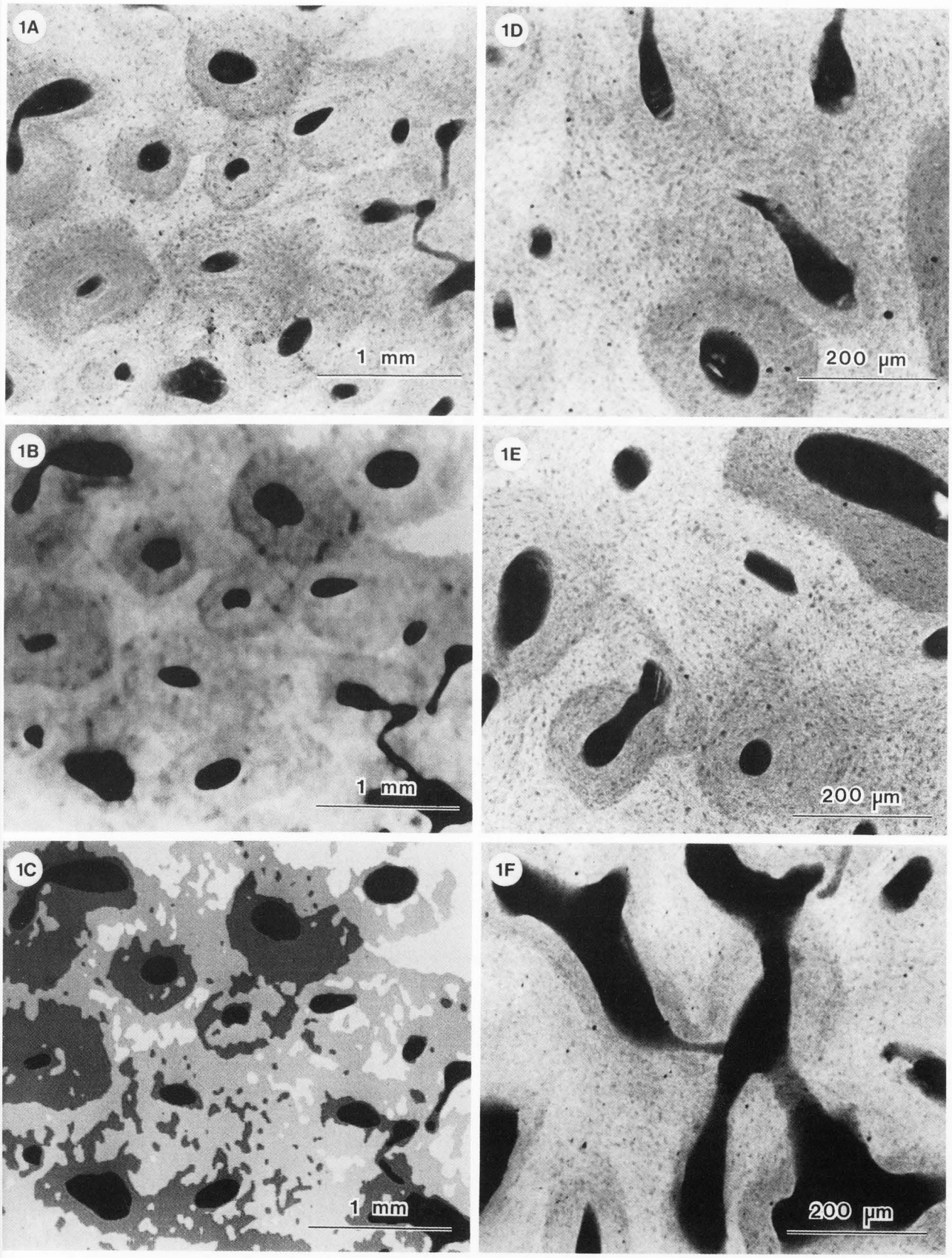
Fig.1: Microradiography of sections of 100 µm cortical bone (human male femoral shaft): **A)** from a specimen of the 40-45 year old group; **B)** video image of "a"; **C)** processed video image; **D)** from a specimen of the 20-25 year old group; **E)** from a specimen of the 60-65 year old group; **F)** from a specimen of the 80-85 year old group.


1A
1 mm
1D
200 µm
1B
1 mm
1E
200 µm
1C
1 mm
1F
200 µm

distinguishing between various mineral levels in bone. In our laboratory we have analyzed monkey bones using this method, as shown in figures 2A, 2B and 2C and we are in the process of quantitating the results using an image analysis system similar to the one we have used for our microradiographs. Figure 2D illustrates the difference between secondary imaging in the SEM and BSE imaging on the same sample (figure 2C).

Changes in the Quality of Bone with Diseases

Many human diseases and pathological conditions result in changes in bone tissue which affect the rate of bone deposition, resorption or turnover. This will affect bone mineral and the distribution of bone particles containing various amounts of mineral within the tissue. Changes in bone tissue are usually studied by histology, histomorphometry and bone density measurements. However, subtle changes in mineralization patterns are usually not detected by these methods. Age related loss is universal in most mammalian species and as early as 1824 Sir Astley Cooper stated that " regular decay of nature which is called old age, is attended with changes which are easily detected in the dead body, and one of the principal of these is found in the bone , for they become thin in their shell and spongy in their texture ". However it has always been difficult to draw a very firm line between normal age related bone loss and osteoporosis. Osteoporosis is most commonly a disease of the elderly (senile osteoporosis) and of postmenopausal women (postmenopausal osteoporosis) which is characterized by an abnormal loss of bone mass (osteopenia) accompanied by pain, spinal deformity, loss of height and fractures. Its onset is earlier and it progress faster in women than in men and in Caucasians and Asians than in Blacks (Avioli,1977). The risk of fracture is very high and is accompanied by a high degree of morbidity. The traditional view of osteoporosis expressed by Albright and Reifenstein (1948) is that it is a disease where bone is lost in an abnormal amount but where the bone which remains behind is normal. A more modern view has been expressed by Frost (1985) which stated that osteoporosis is a manifestation of osteopenia and mechanical incompetence. The osteopenia can follow insufficient bone accumulation during growth secondary to abnormalities in cortical bone modeling and/or remodeling of trabecular bone. Alternatively, it can follow pathological bone losses due to altered activation of bone remodeling units and any combination of the above can occur. Mechanical incompetence (fracture and/or bone pain during normal mechanical usage) is due partly to osteopenia which reduces bone strength by up to 40% of normal, which is still sufficient to support many times the maximum normal mechanical load. Further weakening of osteoporotic bone is due to accumulation of mechanical microdamages due to disuse or malfunction of the remodeling mechanism which occur consistently in most osteoporoses. Using the concept of bone multicellular units (BMU) it has been shown that the normal cycle of quiescence, resorption, reversal, early and late bone formation can be altered in at least two different ways. In high turnover osteoporosis, resorption cavities of excessive depth remain incompletely refilled even though the osteoblasts produce normal amounts of new bone, while in low turnover osteoporosis, resorption cavities of normal depth remain incompletely refilled because the osteoblasts make a subnormal amount of new bone (Parfitt,1979). Because no mammalian species other than man loses sufficient amounts of bone to produce fractures there is no recognized animal model of spontaneous osteoporosis. Therefore various manipulations have been used in experimental animals to induce bone losses similar to human osteoporosis. There are five different mechanisms of inducing profound and sustained bone losses: a) immobilization by plaster casting, nerve or tendon sectioning or bed rest (Uthoff and Jaworski,1978; Burkhart and Jowsey,1967); b) ovariectomy and castration will induce bone loss in the rat in an age dependent manner (Wink and Felts,1980) and in the dog (Shin et al,1976); c) various dietary manipulations: calcium deficiency, high protein diet, high phosphate diet, acid loading can induce severe bone losses (Jowsey et al,1974; Whiting and Draper,1980; Barzel, 1975); d) some drugs can cause excessive loss of bone such as

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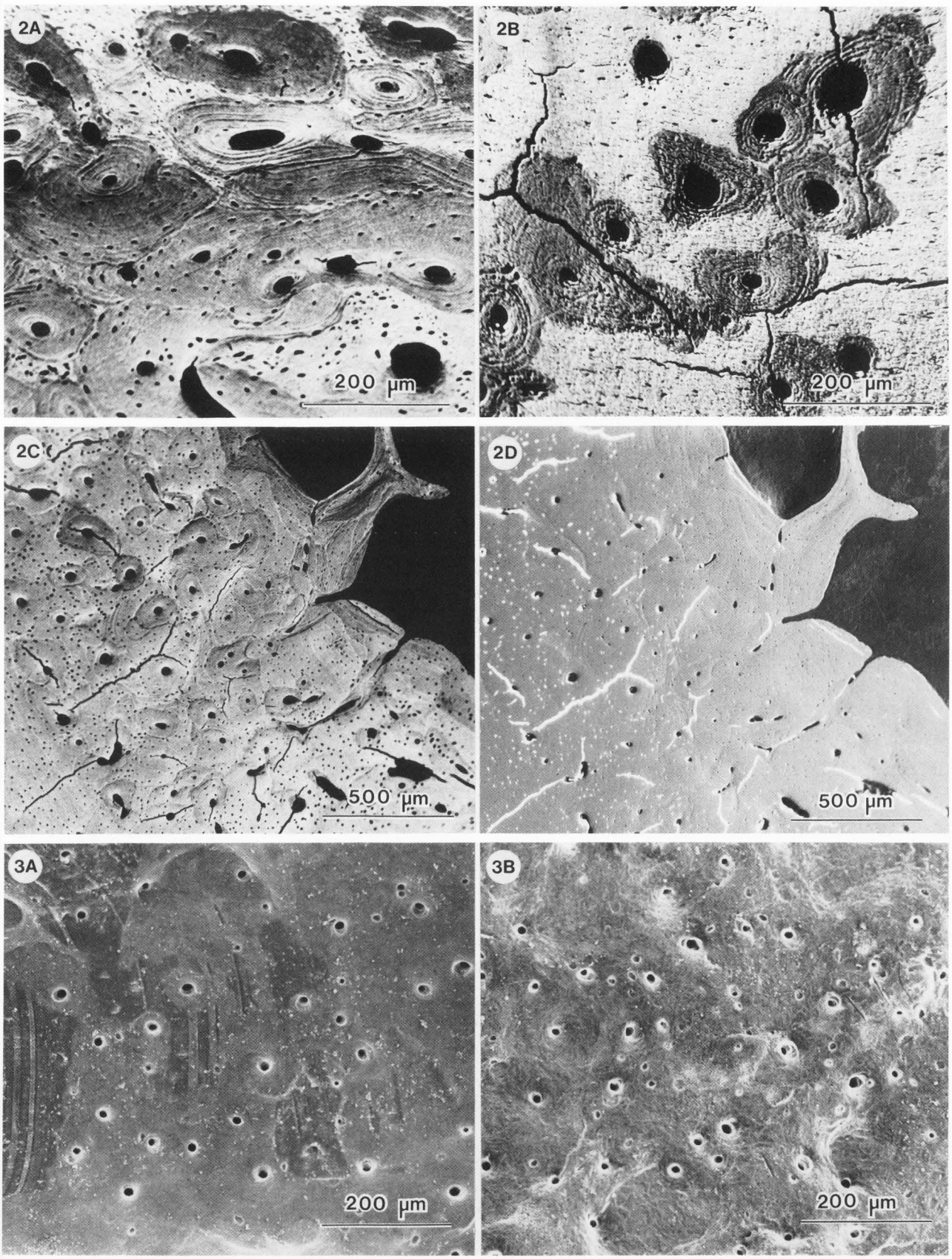
Fig.2: Backscattered electron imaging (BSE) in the SEM showing variations in mineral density: **A)** femoral cross-section of a 6 year old female rhesus monkey; **B)** femoral cross-section of a 24 year old male rhesus monkey; **C)** BSE image from a femoral cross section of a 6 year old female monkey; **D)** secondary electron image of the section shown in 2C.

Fig.3: SEM of deproteinized endosteal surface of rat cortical bone: **A)** Control rat (resting surface); **B)** Rat on a high fluoride diet for 3 months (active surface).


2A
200 µm
2B
200 µm
2C
500 µm
2D
500 µm
3A
200 µm
3B
200 µm

corticosteroid (Bressot et al,1976) or heparin (Thompson,1973); e) finally inflammation can cause localized or generalized bone loss (Bauss et al,1985).

Changes in Chemistry

Chemical changes with age in the skeleton is a subject which has received scant attention. A study conducted on primates (Lei and Young,1978) shows a decrease in Ca/Zn and Mg/Zn ratios in older macaques which agree with Aitken's (1976) findings of reduced Ca/Zn ratio in the cortical bone of human femur with increasing age. It has also been recently reported (Burnell et al,1986) that calcium deficiency was present in 25% of a group of osteoporotic patients. This was shown to be inversely correlated to the Na/Ca ratio in bone of these patients. The main problem with chemical analyses is that they are traditionally done using destructive methods such as atomic absorption or emission. Some recent attempts have been made to use the non destructive technique of instrumental neutron activation analysis INAA (Gatschke et al,1980; Grynpas et al,1987). Despite problems with phosphorus and some trace elements this technique (INAA) has shown that in the case of fluoride the element accumulated in the most heavily mineralized fractions (Grynpas et al,1986b) and that this accumulation was a function of time and concentration. There seems to be no change in crystal size and therefore the increase in density is related to greater packing of crystals in a given volume of bone. In figure 3 we show the difference between the deproteinized endosteal surface of rat cortical bone which have been fed a high fluoride diet (3B) and control rats (3A). One can see that the smooth appearance of the bone in the control has given rise to uneven and rough surfaces. Other trace elements such as strontium (Marie et al,1985), copper (Underwood,1977), and manganese (Strause et al,1984) have also been shown to have a profound influence on bone structure and chemistry.

Examples of Localized Versus Generalized Changes in Bone

In human diseases bone disorders can be localized to a particular anatomical site or generalized to the whole skeleton or parts of it (spine, limbs). In osteoarthritis, there is remodeling of the subchondral bone leading to thickening and osteosclerosis. Although the focus of research has been on cartilage in order to identify the earliest changes in osteoarthritis, subchondral bone changes have been the object of recent studies. Radin et al (1970) proposed that subchondral bone stiffening, due to healing microfractures, might be the primary pathogenic event. Other studies of osteoarthritic human hips suggest that the bony changes (trabecular bone volume, ash content, alkaline and acid phosphatase activity) were greater in the weight-bearing region (Cameron and Fornasier,1979; Reimann and Christensen,1979). Sokoloff (1969) showed in human hip joint that cartilage fibrillation could not be dissociated from bony changes, even in the earliest stages of osteoarthritis. In preliminary studies in our own laboratory we have shown (Alpert et al,1985) that even though the subchondral bone in osteoarthritis is thicker than age matched or young control in hip of human males, the mineralization profile of the arthritic bone shows a decreased mineralization with respect to the young and age-matched controls. But this is not true for deep cancellous bone from the same femoral heads, showing that the changes are limited to the subchondral area. Figure 4 shows deproteinized scanning electron micrographs of subchondral bone of young(A), old(B), and osteoarthritic(C) human femoral heads illustrating the changes in bone architecture. In another study on femoral heads removed at surgery for osteoporotic fractures compared to young and age-matched postmenopausal controls preliminary results show (Katz et al,1986) that again the mineralization profile of the osteoporotics show a decreased mineralization compared to young and age-matched controls, which could be due to ongoing healing of trabecular microfractures. Contrary to the osteoarthritic situation these changes persist in the deep cancellous bone of the femoral heads. Figure 5 shows deproteinized SEM illustrating the

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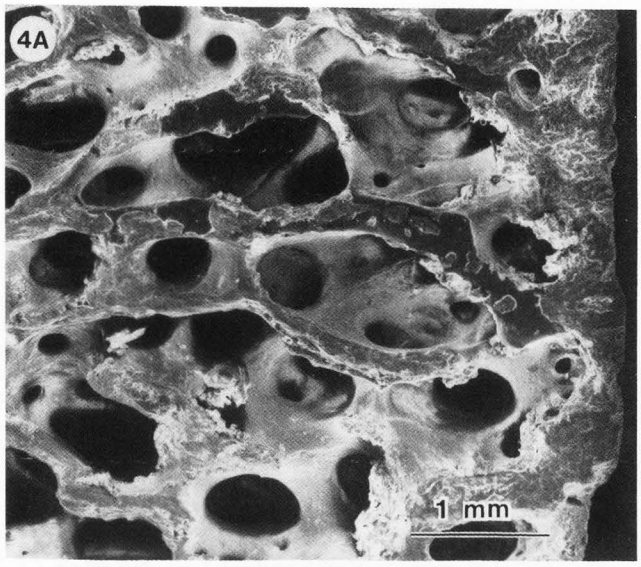
Fig.4: SEM of deproteinized subchondral bone of human male femoral heads showing osteoarthritic changes (the bone surfaces are shown on the right hand side of the pictures): **A)** young control; **B)** old control showing age-related changes; **C)** osteoarthritic bone showing subchondral thickening.

Fig.5: SEM of deproteinized cancellous bone from human female femoral heads illustrating the loss of bone in osteoporosis: **A)** premenopausal control; **B)** postmenopausal control showing age related bone loss; **C)** osteoporotic fracture showing severe bone loss.


4A
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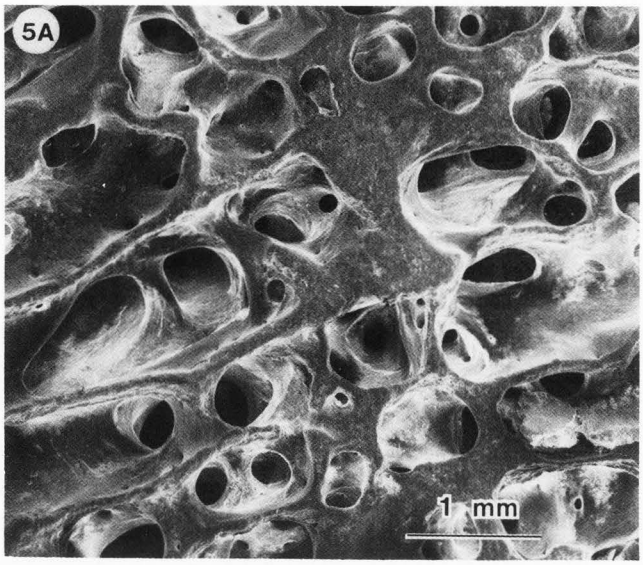
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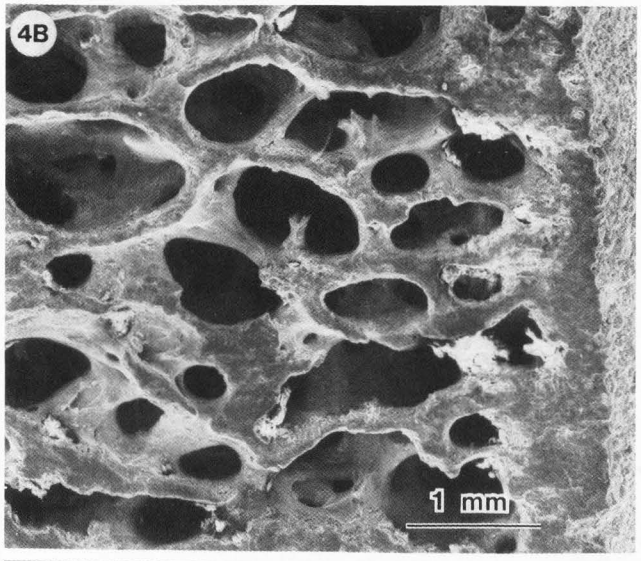
4B
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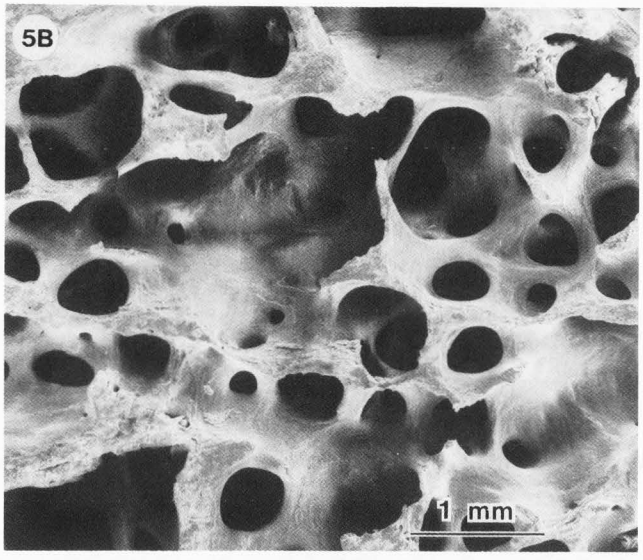
5B
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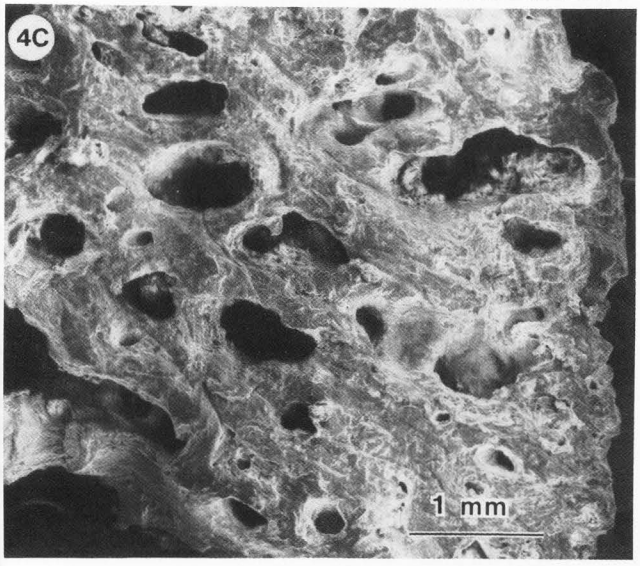
4C
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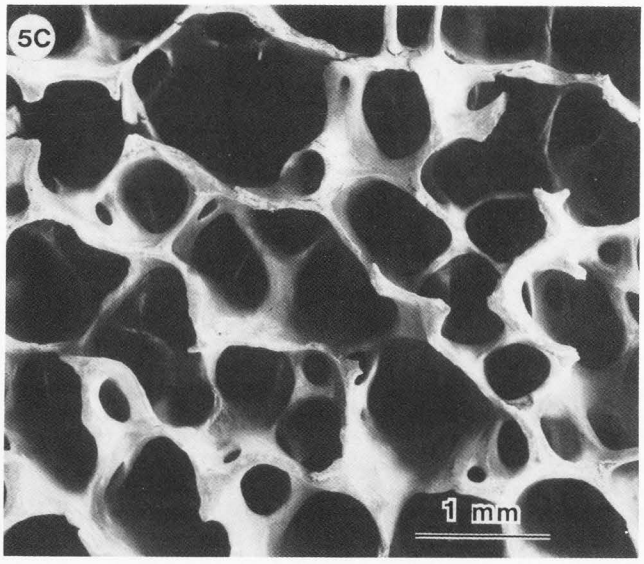
5C
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loss of cancellous bone in premenopausal(A) and postmenopausal(B) controls and osteoporotic bone(C). While this illustrates very well the dramatic changes in bone architecture, it does not show the changes in mineralization which have occurred.

Conclusion

It is becoming increasingly clear that both of the major functions of the skeletal system (ion reservoir and mechanical support) are significantly dependent on the chemical nature, size, shape, and orientation of the mineral components and on the interactions between these components and the organic matrix. Because of the rates at which calcified tissues are turned over, there is a population of mineral particles of different ages and with different properties in every sample. Therefore the changes in the chemical and structural characteristics of the mineral components and their interaction with the organic matrix at the various stages need to be understood in order to correctly interpret changes which occur in the qualities of bone fabric as a tissue and as an organ, as a function of age, in both normal and pathological conditions such as osteoporosis, osteoarthritis, osteomalacia and rickets.

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Discussion with Reviewers

E. Bonnuci: Although "changes in chemistry" are considered, it is not discussed to what extent they may reflect on the structure and quality proper of bone mineral. Can it perhaps be done?
Authors: Changes in the chemistry of the mineral and the matrix as well as changes in crystal size/strain and crystal packing are all reflected in shifts of the mineralization profiles and therefore are all parameters of "bone quality", but their individual contribution to this bone quality is difficult to assess.
S.B. Doty: 1) Do you have the same problems with BSE generated images as with electron beam generation of x-rays; ie, the beam spreads out as it penetrates specimen so that regions of different densities may generate electrons from variable volumes of tissues? 2) What happens to non-weight bearing bones during the aging process? Does it go through similar changes in density and architecture as the weight bearing bones? 3) Have the authors ever investigated bone from osteopetrotic skeletons? Does this bone have "normal" characteristics but exist in too much quantity; ie, the opposite of osteoporotic bone?
Authors: 1) Beam spreading from BSE generated images comes from a much smaller volume of sample than X-rays generated from the electron beam therefore the beam spreading is lower than its equivalent in X-ray microanalysis but there is still a small dependence on energy, tilt angle, spatial distribution, and atomic number. However BSE images are not subjected to changes in grey level due to specimen thickness as in microradiography because it is basically a surface technique. 2) To my knowledge no analysis of bone quality changes with aging has been done on non-weight bearing bones using the techniques mentioned in this paper. 3) We have not investigated osteopetrotic bones but Boskey and Marks (Calc. Tissue Int.,1985,37:287-292) have used density fractionation to study the bones of osteopetrotic rats and have shown an increase in mineralization, a lack of growth of the bone crystals and an increased Ca/P ratio in osteopetrotic bones.
A.M. Parfitt: 1) How many classes of mineralization can be reliably identified by the video image method? What changes in the frequency distribution of these classes can be detected with age? 2) What is the evidence that any of the qualitative changes described contribute to fracture risk?
Authors: 1) From our own data (unpublished) and those of Pugliese and Anderson (1986) the useful number of mineralization classes vary between 3 and 6 in the video image method. In our own study of male in the age groups 20-25y, 40-45y, 60-65y, and 80-85y there is a shift towards higher mineralization with increasing age which agrees with data obtained from density fractionation of the same bones. 2) It is very difficult to relate microscopic properties like degree of mineralization, crystal size/strain, changes in chemistry of the mineral and the matrix, to mechanical properties like the ultimate tensile or compressive strength which are the direct cause of fracture. Nevertheless because in equivalent circumstances not all people with equivalent bone mass will fracture, there must be qualitative differences in their bone which explain why some bones fracture while others do not. What is extremely difficult to quantitate is the effect of bone quality and to measure its contribution to fracture risks.