Calcif Tissue Int (2002) 70:503–511
DOI: 10.1007/s00223-001-2048-0

Calcified Tissue International

The Degree of Mineralization of Bone Tissue Measured by Computerized Quantitative Contact Microradiography

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Received: 23 April 2001 / Accepted: 30 November 2001 / Online publication: 20 May 2002

Abstract. Strength of bones depends on bone matrix volume (BMV), bone microarchitecture, but also bone mineralization, and we have recently shown in osteoporotic patients treated with alendronate that fracture risk and bone mineral density (BMD) were changed without modifications of BMV or bone microarchitecture. Mineralization of bone matrix implies two successive steps: a rapid primary mineralization on the calcification front followed by a slow process of secondary mineralization progressively adding about one-half of the mineral content on bone matrix. These two steps are clearly illustrated by microradiographs of compact and cancellous bone tissue from transiliac human biopsies. Our working hypothesis is based on the impact of changes in bone remodeling rate on the degree of mineralization of bone, i.e., on the BMD measured at the tissue level. Contact quantitative microradiography using a computerized microdensitometric method, is described and allows the measurement of the mean degree of mineralization of bone (MDMB). This parameter may be quantitatively evaluated by exposing an aluminum calibration step-wedge and a plane-parallel calcified tissue section simultaneously to the same beam of X-rays, then determining, from the resulting microradiograph, the thickness of aluminum that produces the same X-ray absorption as a given region of the bone tissue section. To be used as a control group, iliac bone samples were taken at necropsy from 43 subjects (30 women aged 48.4 ± 3.7 years and 13 men aged 66.0 ± 4.4 years) who died suddenly showing no apparent bone disease. A control MDMB, which does not change with age, and a control distribution of these values are thus established. These control values are necessary for interpreting the changes in MDMB observed in bone conditions untreated or treated.

Key words: Bone remodeling activity — Bone density — Degree of mineralization — Quantitative microradiography — Human controls

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In numerous recent papers dealing with pathophysiology and treatment of osteoporosis and other metabolic bone diseases, the terms bone mass and bone mineral density (BMD) are interchangeably used, as if they were synonymous. In fact, they represent different entities for which distinction is important for therapeutic implications, particularly for interpretation of the effects of antiresorptive therapies on bone remodeling and modeling through measurements of BMD [1, 2]. Bone mineral substance can be quantified in two different ways: microscopically, by dual X-ray absorptiometry (DXA) which gives the BMD at the organ level, or microscopically, by a microradiographic-microdensitometric method which gives the degree of mineralization at the tissue level [3, 4]. The two methods provide very different information. The former allows for a global evaluation of mineral substance of the bone as an organ including the marrow spaces, the Howship resorption lacunae, the vascular channels, and the Haversian canals. The latter method quantifies the mineral substance of the bone tissue alone, excluding the marrow and vascular spaces, and the resorption cavities.

Bone is a composite material composed of an organic phase synthesized by the osteoblasts and of an inorganic phase composed mainly of a calcium (Ca) phosphate crystallized as a nonstoichiometric apatite. The more protein solid is present, the less degree of mineralization can be reached; thus, a decreasing degree of mineralization is observed in various adult calcified tissues with enamel > dentine > calcified cartilage > woven bone > lamellar bone. Lamellar bone has the highest volume occupancy by collagen fibrils and is the least mineralized of the normal calcified tissues. During bone remodeling and after bone resorption, bone formation is a multistep process [5]. First, the organic matrix, or osteoid, is synthesized and laid down at specific sites. Following this, the new matrix begins to mineralize after about 5 to 10 days from the time of deposition, and the rate of this *primary mineral apposition* can be measured directly *in vivo* using double tetracycline labeling. After full com-

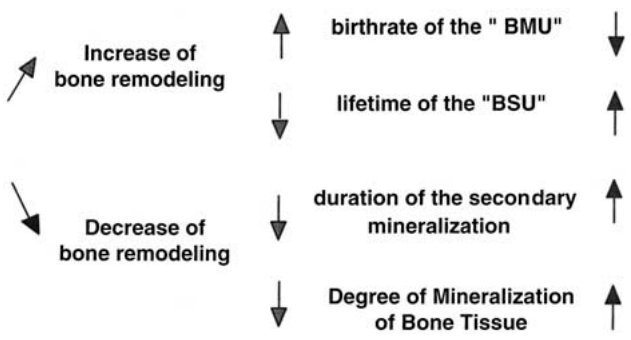

Fig. 1. Potential mechanisms by which increase and decrease of bone remodeling activity modify the degree of mineralization of bone (adapted from 2).

pletion of the basic structure units—osteons in cortical bone and trabecular packets in cancellous bone — a *secondary mineralization* begins [1, 6]. This process consists of a *slow and gradual maturation of the mineral component*, including an increase in the number of crystals, a moderate augmentation of crystal size toward their maximum and, to a greater extent, changes in the internal order of the crystals reflecting their degree of perfection [7]. During the modeling/growth period, when there is a considerable formation of new bone, the mean degree of mineralization is low.

In adult lamellar bone, the mean degree of mineralization mainly depends on the rate of remodeling. In other words, the biological determinant of mineralization is the rate of turnover [1, 8–10]. In the case of an increased bone turnover with augmentation in the activation frequency and birthrate of basic multicellular units, the lifespan of the basic structure units is shortened (Fig. 1). Consequently, they will not have enough time to reach their full degree of mineralization before being resorbed by the new osteoclasts starting a new remodeling sequence. In contrast, when bone turnover is reduced, the activation frequency and birthrate of basic multicellular units are decreased, the lifespan of the basic structure units is prolonged, and then the degree of mineralization may reach its highest level (Fig. 1).

When BMD is measured *in vivo* by single or dual X-ray absorptiometry, this measurement reflects, at the organ level or at the whole body level, the projected areal BMD (g/cm^2) and not the amount of bone matrix or bone mass. The same amount of bone matrix having either a high or a low degree of mineralization will correspond to a higher or a lower BMD. Quantitative computed tomography (QCT) can determine in three dimensions the true volumetric density (g/cm^3), but this parameter is also determined by the amount of bone tissue and its degree of mineralization. Among invasive methods, bone histomorphometry gives access to the direct measurement of the amount of bone matrix in a given volume of a biopsied bone, in particular through measurements of cancellous or total bone volume, expressed in percentage of either spongy bone tissue or core volume. It allows for a good discrimination of completely unmineralized matrix (osteoid) but in no way, however, can this method provide information on the individual degree of mineralization of each basic structure unit. The mineral content of bone is commonly measured by X-ray attenuation experiments. Most studies have used contact microradiography in which intensities are measured with photographic films: this is convenient and gives high linear resolution [11]. *Quantitative microradiography using a computerized microdensitometric method* (Fig. 2), can measure the focal degree of mineralization of each structure unit of bone tissue within the limits imposed by the thickness of the section [1, 4, 10, 12]. Microscopic mineral variations and mineral density distributions have also been evaluated by quantitative backscattered electron imaging [9, 13–26]. Finally, the structure of the mineral substance at the nanometer level (shape, typical predominant orientation, and average size of crystals) has been investigated by small-angle X-ray scattering [20, 27–30] and by Fourier transformed infrared microspectroscopy [31–33].

Microradiography has been used for the study of the structures distribution and composition of biological material, mainly bone tissue. The uneven distribution of bone mineral was revealed by this technique and quantitative information was obtained on the mineral content of different areas of bone [34]. The most important application of microradiographic techniques in the biological field has been in the study of calcified tissues. The term "microradiography" is usually used for the method rendering microscopic aspects in a tissue visible on a photographic emulsion after using soft or ultrasoft X-rays. Since the pioneering studies, numerous microradiographic methods have been described (see review in [4]). Microradiography of bone, widely used between 1950 and 1980, has been progressively abandoned in favor of absorptiometry and dynamic histomorphometry on undecalcified bone after tetracycline labeling. It may be of interest to pay attention to past observations made with this method. The present review will be limited to X-ray microscopical methods used for the study of bone tissue and the measurement of the degree of mineralization by means of computerized quantitative contact microradiography. Applications will be discussed and values measured in human controls will be described.

Preparation of Bone Sections

Undecalcified iliac bone samples were generally used mainly in humans. When studying calcified tissues, it is evident that the preservation of the mineral substance is essential, and this should be considered when choosing a

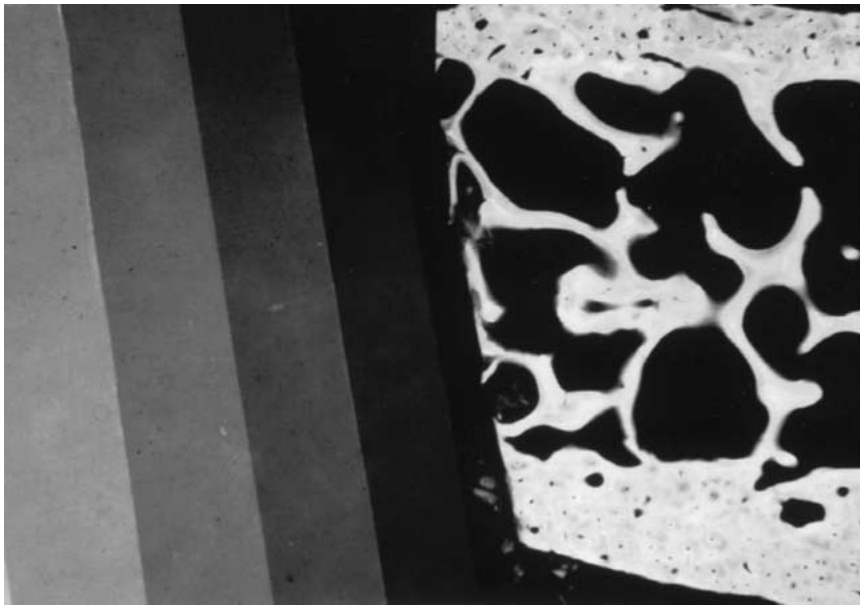

Fig. 2. To allow quantitative evaluation of microradiographs, each bone section is exposed to X-ray concomitantly with an aluminum step-wedge (× 10).

fixing solution. It is important to avoid either acidic solutions provoking complete or partial demineralization of samples, or mixtures that can react with the mineral deposits, such as phosphate buffers. In general, 80% alcohol is used as fixative and dehydrating agent. After some days or weeks, depending on the size of the samples, the specimens are placed in absolute alcohol in order to complete the dehydration. Alcohol baths are changed every day. Good dehydration is necessary because of the incompatibility of water and the embedding medium usually employed (methyl methacrylate). Finally, specimens are placed in xylene and changed daily, for 3 to 4 days, to completely defat them and to allow a good impregnation of the tissues by the embedding medium solvent [35].

Methyl methacrylate, the embedding medium generally used for undecalcified tissues, is a transparent, hard plastic having a very low X-ray absorption power. Methyl methacrylate monomer contains hydroquinone to inhibit polymerization during shipment, and this inhibitor must be removed [36]. After fixation and dehydration, the samples are kept in 2–3 different baths of methyl methacrylate monomer for several days in a refrigerator. Embedding is performed in monomer to which a catalyst has been added in order to polymerize the methyl methacrylate quickly. This catalyst, anhydrous dibenzoyl peroxide, is kept in a dessicator with a drying agent and 0.2 g is added to 100 ml of monomer. The calcified specimens are embedded in strong glass containers well closed and without air. The containers are placed in an oven (30°C) for final polymerization to obtain hard blocks.

Sections of hard tissues made by a motor-driven microtome such as polycut E (Reichert-Jung, Wien, Austria) are always fragmented and not convenient for good quality microradiography. Because of the non-uniformity in the sections, the specimens are first cut into thick slices (about 150 μm thick) and then ground manually to the required uniform thickness. Because of the difficulty in obtaining uniform sections, a low speed rotary saw (Isomet, Buehler, Limonest, France) and a precision diamond wire saw (Well model 3241, ESCIL, Chassieu, France) were used. To obtain microradiographs of good quality, the thick slices should be ground to a uniform thickness. The slices are ground by manual methods including the use of abrasive papers and diamond creams of decreasing grain, and plate glass roughened with carborundum. The sections are ground manually between two glass plates using water and a drop of wetting agent. The glass plates are frequently roughened with black silicon carbide powder (grain = 320, ESCIL, Chassieu, France). During grinding, it is advisable to check the quality of the sections under a microscope to detect any defects. The thickness of the section is also verified regularly. Microradiographs are generally made from 100 μm-thick sections mainly if compact bone is studied [1, 2, 4, 37]. Thicker sections tend to result in confusion because of overlying anatomical structures, and thinner sections decrease the difference between areas of bone. It is crucial to have sections of uniform and consistent thickness. Furthermore, the amount of bone measurable and the cutting plane of the samples (the best is perpendicular to the Haversian canals of compact bone from at least one of the cortices) were also taken into account. This cutting plan allowed for taking the measurements on the largest number of different basic structural units possible.

A Compac (Geneva, Switzerland) model 556 G comparator with a dial graduated in μm, which gives a direct reading of the thickness of small areas of the

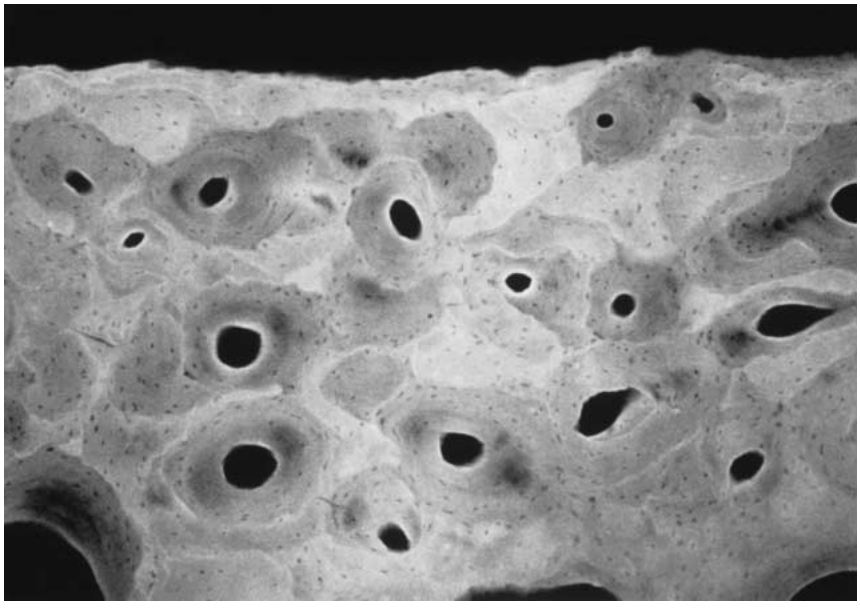

Fig. 3. Microradiograph of an iliac bone section taken from a 86-year old man. The heterogeneity of the degree of mineralization in the different basic structure units (mainly osteons), is clearly observable in compact bone tissue (×60).

section, is convenient and is used in our laboratory. This comparator is equipped with a flat-shaped sensor to reduce the penetration of the sensor into the section. The thickness of the high polished section is measured at different points of bone section with high precision (accuracy of 1 μm). When the desired thickness is obtained, the sections are cleaned in order to eliminate the abrasive grains embedded in the sample, which otherwise create radiodense spots on the microradiographs, lowering their quality. The bone sections are cleaned by sonicating in absolute alcohol. The cleanliness and quality of the sections are then verified under an optical microscope. The clean sections, ready for microradiography, may be preserved between two glass slides.

Implementation of Microradiographs

Theoretical Aspects

Three properties of X-rays are used for the elaboration of microradiographs: (1) their divergence in straight lines from the source, (2) their absorption in matter as a function of the atomic weight of the constituent elements and of the quantity of elements in the path of the beam, and (3) their production of an image on photographic emulsions.

When X-ray microscopy is applied to biological and medical research, low energy X-rays need to be used because the amounts of absorbing material per unit area are small. Furthermore, tissues commonly analyzed are composed of elements with low atomic numbers, and therefore having a low X-ray absorption power. Thus, the examination of biological specimens requires the use of soft (wavelength greater than 1 Å) or ultrasoft (wavelength greater than 10 Å) X-rays according to the terminology of Engström [38]. The hard (wavelength less than 1 Å) and ultrahard (wavelength less than 0.1 Å) X-rays are mainly used for medical and industrial radiography as well as for therapeutic and experimental X-irradiations.

When passing through material, the incident X-rays are attenuated according to the general formula: $I_t = I_o e^{-(\mu/\rho)m}$, in which I_t is the intensity of transmitted X-rays, I_o is the intensity of incident X-rays, μ/ρ represents the mass absorption coefficient and m is the mass of the absorbing material. The mass absorption coefficient depends on the material and is characteristic of the absorbing element; it is not affected by the physical and/or chemical state of this material.

Contact Microradiography

Since biological tissues have a heterogeneous elementary composition, X-rays are absorbed differently in different structures. The transmitted radiation exhibits variations in intensity that are registered on the photographic emulsion and appears as differences in photographic density on the developed microradiograph [39]. If certain experimental conditions are fulfilled, the variations in intensity can provide information concerning the quantity of the components in the tissue. More than 80% of the radiation lies then in the wavelength range of 1.5–1.6 Å. In the case under discussion (0.5–3.0 Å) the mass coefficient of apatite is about 10 times that of water or protein. Thus, the X-ray absorption in this wavelength range is mainly caused by the mineral component.

Soft X-rays may be produced and used in two kinds of apparatus: those used only for microradiography and those also used as X-ray generators for diffraction [4]. In our laboratory, a Philips (Limeil Brévannes, France) compact PW 1830/40 X-ray diffraction generator is

used. It is equipped with a diffraction tube PW 2273/20. A monochromatic X-ray beam is employed, i.e., nickel-filtered copper Kα radiation with a wavelength of 1.54 Å for which the ratio of the mass-absorption coefficients of aluminum to apatite is 0.561. The distance between the X-ray source and the specimen is about 25–30 cm.

In a dark room, a clean 100 μm-thick bone section, prepared as previously described, is placed on a photographic emulsion covered by a thin polyester (mylar) film transparent to X-rays, and placed in a specimen holder. The section is firmly pressed flat by tightening the specimen holder cap and evacuating the air situated between the mylar and the emulsion by means of a vacuum pump, thus bringing the section in direct contact with the emulsion, this being a basic principle of contact microradiography [4]. The specimen holder is placed in a camera perpendicular to the X-ray beam and locked into position during X-ray exposure. The exposure time is variable (about 20 min under 25 kV and 25 mA) depending on the thickness of the section and the operating conditions. The field of X-rays is regularly examined for homogeneity. At exposure, the specimen holder, with mainly bone section and reference system, was arranged in order to use the homogeneous direction of the X-ray field [40].

Aluminum Step-wedge Reference

Quantitative microradiography allows for the measurement of the density of mineral substance in microscopic volumes of bone tissue, i.e., its degree of mineralization. Microradiography can be made quantitative by (1) exposing an aluminum step-wedge and a bone section to the same beam of X-rays (Fig. 2); (2) determining from the resulting microradiograph the thickness of aluminum which produces the same absorption of the X-rays as a given region of the bone section; and (3) measuring the thickness of the given region of the bone section. Different methods have been described for quantitative determinations of apatite content of bone [34]. We currently use the comparison of the bone section with a reference system of known composition and thickness. A continuous X-ray spectrum is employed and aluminum (Al) has been adopted as a reference material. Al was chosen because it is a convenient material having an atomic number not far from the effective atomic number of hydroxyapatite.

As already evoked, the general equation for the absorption of X-rays is

$$I_t = I_o e^{-(\mu/\rho)m}$$

in which μ/ρ is the mass absorption coefficient (cm^2/g) and m is the mass of absorbing material per unit area (g/cm^2). In fact, $m = \rho d$, where ρ is density of absorbing material in g/cm^3 and d is the thickness of absorbing material in cm. By including an Al step-wedge of known thickness in the microradiograph with the bone section, the absorption of any area of bone is expressed in terms of an equivalent thickness of Al. By measuring the thickness of the section, the mass per unit volume is obtained [34]. It is calculated that $\rho_{apatite} = 0.561\ m_{Al}/d_{apatite}$.

In our experimental conditions, Al is used in pure form as a thin 99.5% pure foil (Strem Chemicals Inc., Bischheim, France) with d = 12–13 μm and m = 0.003366 g/cm^2 (data given by Strem Chemicals Inc.). Our reference system was constructed as a rectangular frame (staircase) with Al foils arranged in steps. This latter was used to allow a regular tracing in bone and in all steps with a regular X-ray irradiation. Thus, for the different steps of Al reference, the corresponding degrees of mineralization of bone are:

$$\text{Step1}\ \ \rho_{apatite} = 0.561 \times 0.003366/d_{apatite} = 0.0018883/d_{apatite}$$
$$\text{Step2}\ \ \rho_{apatite} = 0.561 \times 0.006732/d_{apatite} = 0.0037767/d_{apatite}$$
$$\text{Step3}\ \ \rho_{apatite} = 0.561 \times 0.010098/d_{apatite} = 0.0056650/d_{apatite}$$
$$\text{Step4}\ \ \rho_{apatite} = 0.561 \times 0.013464/d_{apatite} = 0.0075533/d_{apatite}$$
$$\text{Step5}\ \ \rho_{apatite} = 0.561 \times 0.016830/d_{apatite} = 0.0094416/d_{apatite}$$
$$\text{Step6}\ \ \rho_{apatite} = 0.561 \times 0.020196/d_{apatite} = 0.0113300/d_{apatite}$$
$$\text{Step7}\ \ \rho_{apatite} = 0.561 \times 0.023562/d_{apatite} = 0.0132183/d_{apatite}$$
$$\text{Step8}\ \ \rho_{apatite} = 0.561 \times 0.026928/d_{apatite} = 0.0151066/d_{apatite}$$

Photographic Emulsions

The most frequently used emulsion was the Kodak high resolution film (estar thick base; SO-343). To date, due to commercial difficulties to obtain Kodak film, a high resolution (more than 3000 lines/mm) VRP-M green sensible emulsion from Geola (Slavich International Wholesale Office, Vilnius, Lithuania) is used. After exposure, the film is developed for 5 min in Kodak D 19 at 20°C, rinsed, and then fixed for 5 min in Ilford Hypam. Development is carried out under a controlled temperature and constant agitation, thus minimizing photographic artifacts. The film is then washed, dried in a dust-free place, and mounted between slide and coverslip using XAM neutral medium (Gurr, BDH Laboratory, Poole, England). Finally, the microradiograph was observed under light microscopy. When possible, photon counting intensity measurements are preferred to the use of photographic film in order to obtain a larger dynamic range and greater sensitivity to small intensity changes [11]. Unfortunately, such equipment is not yet available in our laboratory.

Quantitative Evaluation of Degree of Mineralization

Evaluation of the relative content of calcium salts in various calcified structures was first used by Amprino [41] and Amprino and Engström [42]. In any microra-

diograph of bone, osteons or trabecular packets of varying degrees of mineralization are present [4, 36, 37, 43–46], those that are the least mineralized and are dark grey being the most recently formed and having a high radiotranslucency. They contrast with the fully mineralized interstitial bone adjacent to them which is white and has a low translucency. Light grey osteons (intermediary translucency) correspond to basic structure units having an intermediary density.

The degree of bone mineralization was to date quantified by a new combined contact microradiography-microdensitometry computerized method [1, 2]. Original software is used for the automatic analysis of grey levels of microradiographs with Visiolab 1000 (Biocom®, France). A true color image processing workstation operates under Microsoft® Windows and is equipped with a 800 × 600 16 M true colors framegrabber and a 32 bits video card. A 3-CCD camera (sensing chip = 2/3 inch size = 16.667 mm; true resolution 756 × 570 pixels), captures the image of the microradiograph through a microscope. Each field analyzed on the computer monitor is divided into 4350 squared measurement units of 41.4 μm side, i.e., about 100 pixels. After calibration using the Al step-wedge always microradiographed with bone section, the area measured is automatically selected, then the grey levels are measured from the computer-generated map indicating the spatial distribution of "measurement units." Due to the use of 100 μm-thick sections, projection effect errors are possible especially along the trabeculae of cancellous bone. Although difficult to totally exclude, this source of error is minimized by a very careful calibration of the grey-level done in order to measure only bone tissue totally present in the thickness of the section. Similar to the microradiographic method reported by Stein et al. [47], "measurement units" totally or not totally containing bone tissue were separated by adjusting the grey scale threshold. When the thresholding was correct, measurements were performed. The main steps of our method allowing measurement of the degree of mineralization have previously been illustrated [2]. All the results are stored in a database, transferred to a computer, and processed with an Excel 5.0 program. Data are converted from grey-level values (optical densities) in degree of mineralization with the construction of a calibration curve based on the measurements obtained from the Al reference step-wedge; the first seven steps are classically measured when bone tissue is analyzed. The degree of bone mineralization is finally expressed in g mineral/cm^3 bone adjusted according to the precise thickness of each part of the section measured before microradiography. This method reduced sampling error associated with selection of only a limited area. The coefficient of variation (intra-observer) for the quantitative microradiography technique is 3% and the inter-observer variance is 7%.

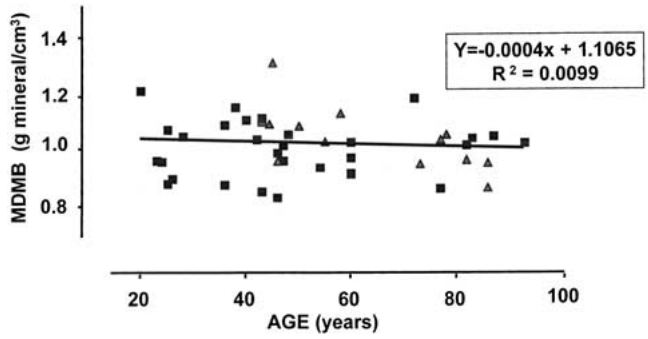

Fig. 4. Age-related change in the degree of mineralization in human total bone (compact + cancellous). There is no significant influence of age on the mean degree of mineralization of bone (MDMB) in 43 control subjects [30 women (square) and 13 men (triangle)].

Degree of Mineralization of Bone in Control Humans

To determine the abnormalities of bone turnover in bone disorders, the first necessary step was to study the pattern in normal persons. Bone samples from persons of both sexes who died as a result of cardiac arrest, suicide, or sudden accident were used. However, there is no doubt that such a group includes some who are not normal, who have bone disease or have a liver disorder or some other abnormality that will predispose them to bone disease.

Our control group was composed of iliac bone samples taken at necropsy from 30 women (aged 48.4 ± 3.7 years; range 20–93 years) and 13 men (aged 66.0 ± 4.4 years; range 43–86 years) who died suddenly showing no apparent bone disorder. In terms of mean values, distribution, and evolution with age, the degree of bone mineralization was not significantly different between the two sexes. Thus, the control group was studied as a whole, i.e. 43 persons (aged 53.7 ± 3.2 years; range 20–93 years).

In this control group, the mean degree of mineralization expressed in g mineral/cm^3 (mean ± SEM) was 1.082 ± 0.017 in compact bone (227,344 measurements), 1.099 ± 0.018 in cancellous bone (69,379 measurements), and 1.087 ± 0.017 in total bone (compact + cancellous: 296,723 measurements). In these three structures of bone, the degree of bone mineralization did not change significantly with age (Fig. 3, Fig. 4). The distributions of the degree of mineralization of bone (Fig. 5) revealed a small shift towards the high values in cancellous bone compared to compact bone. This may reflect either the presence in cancellous bone of a higher proportion of interstitial bone than in compact bone, or an "edge effect" (100 μm-thick sections) with suppression of the measurements in the lowest mineralized part of trabeculae.

From microradiographic measurements made 40 years ago, it was claimed [42, 43, 46, 48, 49] that recent

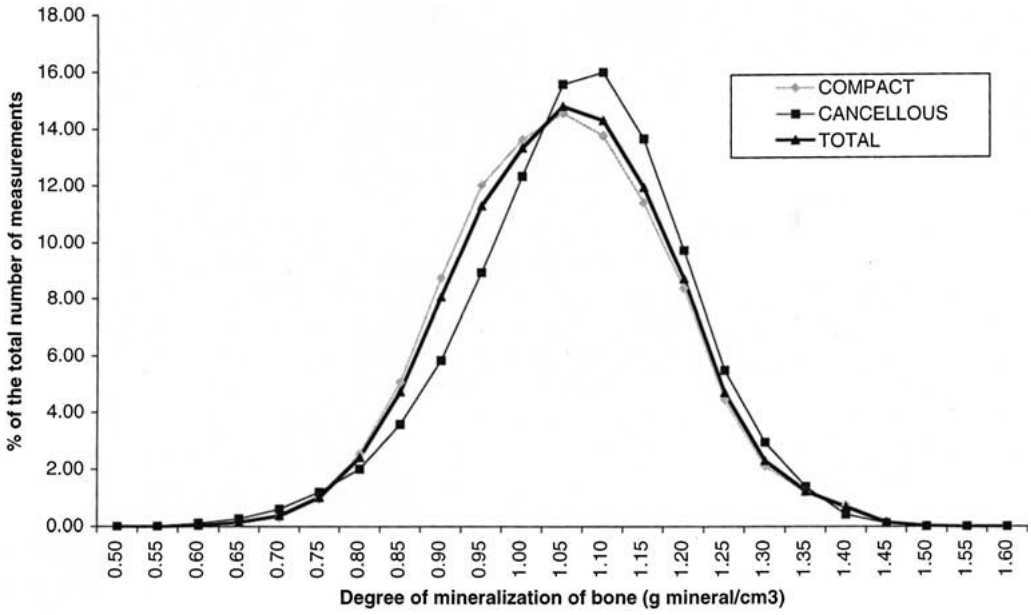

Fig. 5. The distributions of the degree of mineralization are similar in compact, cancellous and total (compact + cancellous) bone from control group.

osteons with the lowest mineral density contained approximately 70 to 75% of the mineral content of the most highly calcified bone. Recently, measurements performed in compact bone from tibia of baboons [1], as well as in iliac bone from postmenopausal osteoporotic patients [2], show that the bone area corresponding to the lowest degree of mineralization are 50 to 60% less mineralized than the area in which bone is fully if not completely mineralized. These results are confirmed by present data from human controls (Fig. 5).

Significance of Degree of Mineralization of Bone Tissue

As already mentioned, the degree of mineralization depends on how recently the bone tissue was formed, on the rate of bone growth during modeling, and in adults, on the extent of remodeling activity [1, 2, 6].

The first systematic study of the degree of mineralization of bone tissue was made on vertebral trabecular bone [50]. The density values were derived by water displacement using Archimedes' principle. In control subjects, mineralization was lowest during the rapid growth period of childhood, and reached a maximum between 35 and 50 years of age, corresponding to a quiescence of remodeling in middle age. There was a progressive, slight decrease in the 6th through the 8th decades of life. In cases of vertebral osteoporosis, 10% had density in the normal range, 20% had moderate decreases in trabecular density, and 70% had markedly increased trabecular bone density, reflecting a definite decrease in bone deposition rate. A similar evolution of the degree of mineralization as a function of age has been demonstrated by the microradiographic-microdensitometric method in Haversian systems and interstitial tissue of alveolar bone [51], in Haversian systems of the compact bone of femoral diaphyses [52], and in the iliac crests obtained from 208 (122 men and 86 women) control subjects [3].

Measurements in bone sections from man, dog, and several other species [1, 3, 43, 45, 46–48] have shown that the lowest mineral density is observed usually in newly formed secondary Haversian bone, and that the highest density corresponds to remnants of old systems between secondary Haversian systems or primary bone. Rat bone has been found to have a significantly higher maximum mineral density than human bone [46]. Values of the degree of mineralization measured in bone sections from man and several other species of animals are listed in Table 1, adapted from [46]. In humans, and similarly to data reported in the present paper, only small variations in the degree of mineralization of bone tissue as a function of age or sex, were observed in iliac crest biopsies [3, 45].

Data obtained from osteoporotic patients indicated that the mean value of degree of mineralization was variable [3, 45, 48, 53]. The low mineralization observed in certain osteoporotic patients was related to high remodeling, which results in a shortened period of secondary mineralization. Inversely, the high mineralization seen in about 50% of the cases was attributed to a decrease in remodeling activity with a longer period of secondary mineralization. This corresponds to our working hypothesis on the relationship between the degree of mineralization of bone and the remodeling activity [1, 2, 54].

Table 1. Degree of mineralization in bones from humans and animals of various ages (adapted from 46)

Men (years)	Femur Low DM[a]	Femur High DM	Clavicle Low DM	Clavicle High DM
7	0.90	1.29	0.90	1.21
29	1.20	1.44		
31	1.11	1.40	1.03	1.31
76	0.98	1.28	1.12	1.37
Dogs	Femur-Low DM		Femur-High DM	
6 weeks	1.16		1.43	
9 months	1.02		1.23	
1-2 years	1.10		1.52	
5-6 years	1.23		1.53	
11-12 years	1.27		1.54	
Animals	Low DM		High DM	
Man	0.90-1.20		1.21-1.44	
Cow	1.11		1.50	
Dog	1.02-1.28		1.23-1.54	
Mouse	1.18-1.25		1.38	
Rabbit	1.39		1.53	
Rat	1.41		1.70	

[a]DM = degree of mineralization expressed in g mineral/cm^3 bone

In fact, small changes in the structure of the mineral/collagen composite in bone may considerably affect its biomechanical properties [20, 29]. The problem now is to determine if a loss of heterogeneity in the degree of mineralization in different basic structural units could induce a partial loss of elastic properties of bone. This would require measurements in bone biopsies taken after a long-term treatment (6 years or more) with alendronate in postmenopausal osteoporotic women. Furthermore, it would be of great interest to correlate the degree of mineralization of bone with biomechanical data obtained at the tissue level. Both of these studies are in progress in our laboratory.

Acknowledgments. We express our gratitude to Prof. Charles-Albert Baud for his constant support, his fruitful advice, and the long loan of original microradiographic cameras. We also wish to thank Dr. Gideon A. Rodan (Merck Research Laboratories, West Point PA, USA) for providing bones of baboons used in the preliminary study, Professors Jacques Bejui (Laboratoire d'Anatomie, Faculté de Médecine R. Laennec, Lyon, France), Bernard Vallée (Laboratoire d'Anatomie Médico-Chirurgicale, Faculté de Médecine R. Laennec, Lyon), and Evelyne Lopez (CNRS URA 90, Museum National d'Histoire Naturelle, Paris, France) for collaboration during the preparation of bone sections and microradiographs. We also thank Catherine Simi, Delphine Farlay, Annie Buffet, and Nathalie Portero for expert technical assistance, and Christine Fourneret for typing.

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