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Measurement of Bone Mineral in vivo: An Improved Method

Abstract. *The mineral content of bone can be determined by measuring the absorption by bone of a monochromatic, low-energy photon beam which originates in a radioactive source (iodine-125 at 27.3 kev or americium-241 at 59.6 kev). The intensity of the beam transmitted by the bone is measured by counting with a scintillation detector. Since the photon source and detector are well collimated, errors resulting from scattered radiation are reduced. From measurements of the intensity of the transmitted beam, made at intervals across the bone, the total mineral content of the bone can be determined. The results are accurate and reproducible to within about 3 percent.*

An improved method for measuring the mineral content of bone in vivo by photon absorption techniques has been developed. The methods previously described in the literature (*1, 2*) are based on measurement of the transmission through bone of photon beams which are generated by standard x-ray tubes. The transmission is usually determined by densitometric measurements of x-ray films. The method described here differs from each of these earlier methods in one or more ways: in this method (i) the transmission of the photon beam is measured directly by counting techniques, by means of a scintillation detector system; (ii) the photon beam used is essentially monochromatic; (iii) the photon beam and detector are well collimated; and (iv) the effects of the tissue around the bone are taken into account. These factors eliminate errors resulting from the variability of x-ray films and film development techniques, reduce uncertainties in absorption coefficients, reduce the effects of scattered radiation, and reduce errors arising from the presence of tissue.

Figure 1 is a schematic diagram of the equipment used. The radioactive photon source used at present is iodine-125 (5 mc) contained in a thin-walled stainless steel tube 1 cm long, 3 mm in diameter. [An americium-241 source (1 mc) in tubing of similar dimensions is also used.] The tube is placed in a hole 3 mm in diameter in a small lead cube. The hole is drilled in such a way that the end of the tube is 5 mm below the surface of the lead, for purposes of collimating the photon beam. The tube is viewed end-on by the crystal detector system, which is also collimated, as shown schematically in Fig. 1. Two holes, each 3 mm in diameter, drilled in a pair of lead plates, each 5 mm thick, serve as collimating apertures. The plates are 4 cm apart, and the two holes are aligned on a common axis with the photon source.

The source and the detector system are rigidly coupled by mechanical means and are driven simultaneously in 1-mm steps, in a direction transverse to the bone, by the motor-drive system. Measurements of the transmission of the photon beam through the bone are made for a 10-second interval after each step. The starting and stopping of the drive motor and scaler and the resetting of the scaler are automatically performed by the timer unit, and the operator need only record the scaler reading after each step. In the course of the scan, measurements of the transmission of the photon beam through tissue alone, on either side of the bone, are also made. From the data obtained in these measurements, an equivalent thickness of bone mineral can be computed, as follows.

Let I_o be the intensity of the unobstructed photon beam, (as measured at *a* of Fig. 1); I_o^* the intensity after passage of the beam through a thickness T of tissue, (as measured through *b* of Fig. 1); and I the intensity after passage of the beam through an equal thickness of bone mineral plus tissue $T_b + T_m$ (as measured through *c* of Fig. 1). If μ_b and μ_m are the mass absorption coefficients of bone mineral and of tissue, respectively, then:

$$I_o^* = I_o \exp(-\mu_m \rho_m T)$$

$$\begin{aligned} I &= I_o \exp(-\mu_m \rho_m T_m - \mu_b \rho_b T_b) \\ &= I_o \exp(-\mu_m \rho_m (T - T_b) - \mu_b \rho_b T_b) \\ &= I_o \exp(-\mu_m \rho_m T) \exp(-\mu_b \rho_b T_b + \mu_m \rho_m T_b) \end{aligned}$$

$$I = I_o^* \exp(-\mu_b \rho_b T_b + \mu_m \rho_m T_b)$$

$$T_b = [\log_e (I_o^*/I)] \,/\, (\mu_b \rho_b - \mu_m \rho_m) \qquad (1)$$

Equation 1 gives an equivalent thickness of compact bone mineral of density ρ_b for the point at which the intensity of the transmitted photon beam is I. Measuring I at closely spaced intervals across the bone gives a series of equivalent thicknesses which, when summed, give the equivalent cross-sectional area of compact bone mineral in the bone. A standard composition of bone mineral is assumed in these calculations (*3*). The absorption coefficient μ_b can be determined on the basis of this assumption from tabulated atomic absorption coefficients (*4*). It is also assumed that all non-bone-mineral substances absorb radiation to the degree that striated muscle tissue does. The absorption coefficient μ_m can be calculated in a similar manner, or it can be experimentally determined for the particular tissue under study.

As shown in Fig. 1, the bone and tissue under observation are placed between form-fitting pieces of tissue-equivalent material [for example, Mix-

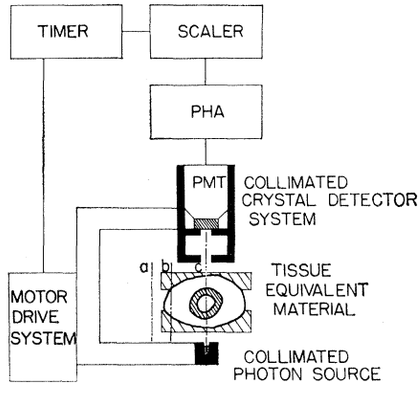

Fig. 1. Schematic diagram of equipment for measuring the mineral content of bone.

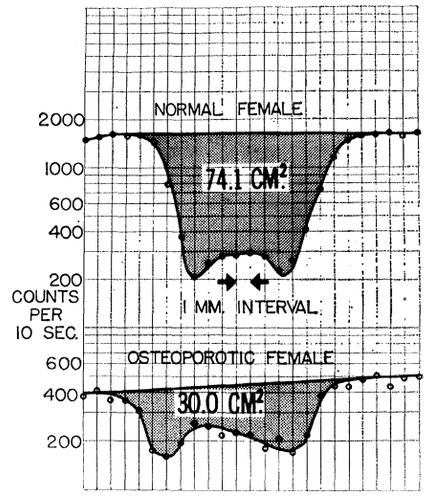

Fig. 2. Scanning records for a 28-year-old normal female and for a 75-year-old female with osteoporosis.

D (*5*)] with parallel opposite faces. This is done to make sure that the quantities I_o^* and I are measured for equal thicknesses of "tissue" or of tissue plus bone; equal thicknesses are necessary if the mathematical relationships given in Eq. 1 are to be true.

The method has been used to determine the mineral content of bone in over 200 persons. At present, the 10-second transmission counts are recorded from the scaler at each of the 1-mm intervals across the bone. These transmission measurements are plotted at equal intervals on semilogarithmic graph paper, and a smooth curve is drawn through them. The extended I_o^* curve is also drawn in, as though the bone were replaced with tissue. Examples of such graphs are shown in Fig. 2; these are graphs for scans, made with I^{125} as a radiation source, on a typical normal female and a typical osteoporotic female. The distance between the two curves at any point is directly proportional to $\log_e(I_o^*/I)$, and hence, in accordance with Eq. 1, to T_b. The total area between the curves, which can be accurately measured with a planimeter, is thus proportional to the cross-sectional area of bone mineral in the path scanned. This area of bone mineral is numerically equal to the volume of bone mineral per unit length of bone. From the known density (*2*) one can determine the mass of bone mineral in a unit length of bone.

The scans recorded in Fig. 2 were made on the radius of the left arm, about 10 cm from the distal end. Figure 3 is a plot of the mineral content of bone relative to age for 137 female subjects; the scans were made on the left radius. The content of bone mineral is given in grams of hydroxyapatite per 1-cm length of bone. Data points for patients for whom a clinical diagnosis of osteoporosis had been made are indicated by a star. The phenomenon of "postmenopausal osteoporosis" after age 50 is clearly noticeable.

Repeat measurements have been made for most subjects, and in general, results have been found to be reproducible to within 3 percent. A common source of error in the reproducibility data is movement on the part of the subject while the scan is in progress. This error appears in the results as a change in the apparent width of the bone. The measured area can be adjusted to minimize this error by comparing the apparent width as determined from the scan with the width measured from an x-ray of the bone.

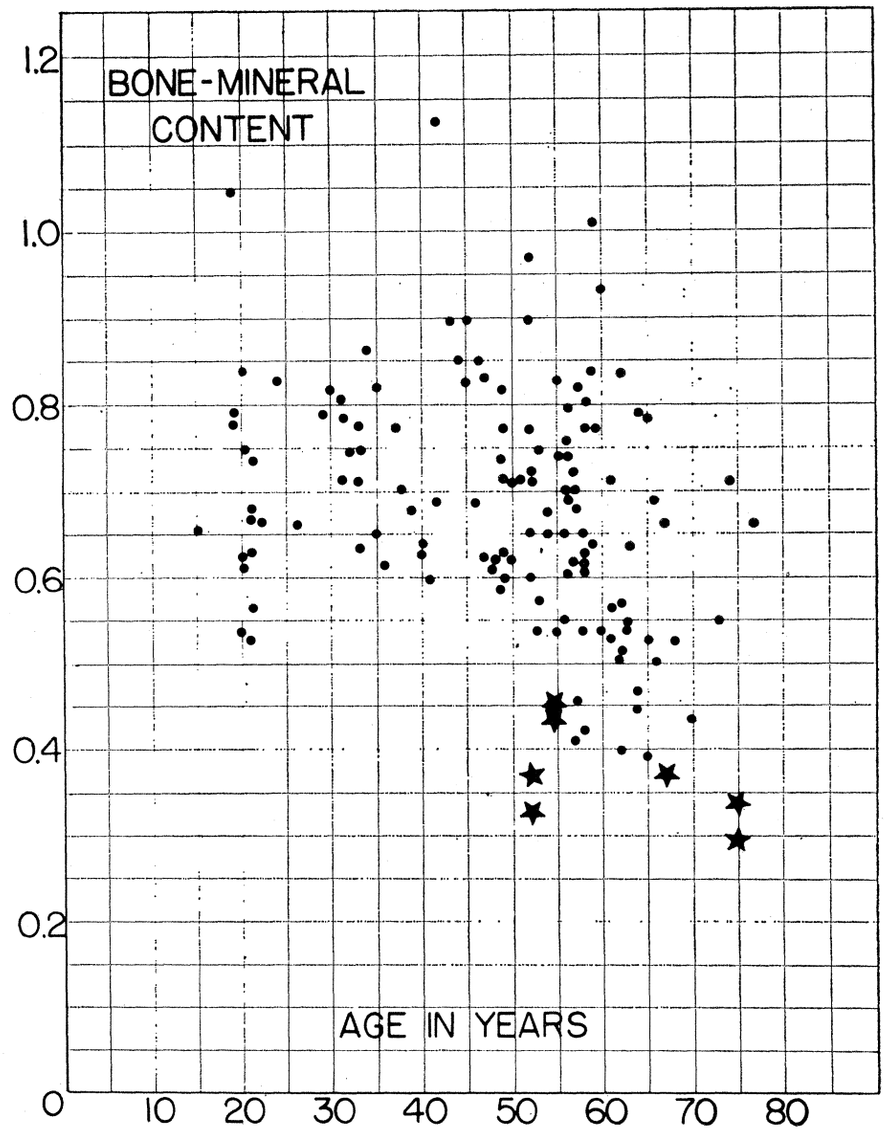

Fig. 3. Plot of mineral content of bone versus age distribution for 137 female subjects. The mineral content is given in grams of calcium hydroxyapatite per centimeter length of bone.

The results of this method of determining the content of bone mineral are accurate to within about 3 percent, as determined by studies on bone phantoms.

It is possible to extend this method to obtain information about the composition of the bone mineral. The mass absorption coefficient of a homogenous combination of n elements, such as bone mineral, can be expressed as

$$\mu_b = f_1\mu_1 + f_2\mu_2 + \ldots + f_n\mu_n \qquad (2)$$

where f_1, f_2, . . . f_n are the fractions (by weight) of the elements present and μ_1, μ_2, . . . μ_n are the mass absorption coefficients of these elements. These absorption coefficients are known (*4*), so that when this expression for μ_b is substituted in Eq. 1, an equation in $n + 1$ unknowns results, f_1, f_2, . . . f_n, and T_b. If I_o^*/I is measured at n different photon energies, a set of n equations of the form of Eq. 1 is obtained. These n equations, along with the equation

$$f_1 + f_2 + \ldots + f_n = 1, \qquad (3)$$

form a system of $n + 1$ equations in $n + 1$ unknowns which may be solved by algebraic methods for f_1, f_2, . . . f_n and T_b. The primary mineral elements of bone are calcium and phosphorus, so, in principle, the Ca/P ratio for a particular bone could be determined by measuring I_o^*/I over the same point on the bone at two different photon energies.

Equation 1 may be transformed to read

$$A_b = C \times G/(\mu_b\rho_b - \mu_m\rho_m) \qquad (4)$$

where A_b is the amount of bone mineral in a cross section of bone and G is the graph area obtained by plotting and measuring the area between the I and I_o* curves on semilogarithmic graph paper, as described previously. C is a constant which depends on the physical dimensions of the graph paper used.

If G is measured at two different energies, the Ca/P ratio for the cross section of bone under observation could be obtained from Eq. 3 and two equations of the form of Eq. 4. This method could also be used to determine the relative amount of fat in tissue.

This technique has been tested on a two-phase material with I^{125} (27.3 kev) and Am^{241} (59.6 kev) as radioactive sources. The materials used were blocks of paraffin and $CaCO_3$, homogeneously mixed in known proportions. The measurements resulted in determination of the percentage, by weight, of $CaCO_3$ present in the blocks, over a wide range of compositions, to an average accuracy of within 3 percent.

The principal errors at the present time are the result of uncertainties in the absorption coefficients and the lack of complete monochromaticity in the photon sources. These errors can be reduced through further study. If the only errors involved were statistical ones, an accuracy to within less than 2 percent could be expected in determining the mineral content of bone by this method.

The radiation exposure per scan with the techniques described is of the order of 0.15 rem with an I^{125} (27.3 kev) source of 5-mc activity. The exposure is about 0.05 rem when a source of Am^{241} (59.6 kev) of 5-mc activity is used. This exposure is limited to a small area of the forearm and should be compared with the maximum permissible dose to the forearms of children of 7.5 rem per year (*6*, *7*).

Note added in proof. We have found a simple method for making "point" sources of I^{125}. Iodine is removed from solutions by an ion-exchange resin, Dowex 1 × 4, 20–50 mesh (*8*). Single grains of the resin, allowed to stand for periods of about 48 hours in freshly prepared carrier-free radioactive iodine solutions, will take up 5 mc of iodine. Grain diameters are less than 1 millimeter.

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Aragonite and Calcite as Constituents of Adult Oyster Shells

Abstract. *Adult oyster shells are composed mainly of calcite, but there are five small areas of aragonite: the resilium, the two pads at which the adductor muscle is inserted, and the two pads at which Quenstedt's muscles are inserted. Quenstedt's muscles are proposed as a name for the small modified, one-time pedal muscles of unknown function, discovered by Quenstedt.*

Nearly all the bivalve mollusks have shells composed of conchiolin and aragonite, the orthorhombic allomorph of calcium carbonate. Calcite, the more stable, rhombohedral allomorph, is not found in their shells. In contrast, the adult shell of the oysters of the family Ostreidae is composed mainly of conchiolin and calcite. Only five small, distinct, well-defined areas of the adult oyster shell are composed of aragonite. These areas are: the resilium between the two valves; the pads, one on each valve, at which the large adductor muscle is inserted; and the pads, one on each valve, at which Quenstedt's muscles are inserted (Fig. 1).

The resilium is one of the three portions of the ligament, which connects the two valves and allows the animal to open or shut its shell. The ligament is made of two kinds of conchiolin and, in the oysters, is divided lengthwise into three portions. The mid-portion is the resilium and consists of one kind of conchiolin, while both flanking portions consist of another kind. The resilium is strong under compression, which is built up when the animal shuts its shell, but weak under tension; it is fibrous, brown to whitish gray, and semitranslucent and has lighter and darker parallel layers, normal to its fibers. The layers are produced during the intermittent growth of the ligament. The very fine fibers are subparallel but diverge like a fan toward the ventral border of the ligament; they are nearly normal to the ventral or growing border of the ligament, which in life is covered by the epithelium of the ligamentous crest of the animal's soft parts. The resilium fibers are composed of conchiolin and of aragonite; the aragonite fibers are the whitish ones. An x-ray diffraction pattern of many small fragments of the resilium from several oysters (*Crassostrea virginica*), ground to a powder, showed the curve of the mineral aragonite (*1*).

The large and powerful adductor muscle is inserted on the two valves on special pads, commonly called muscle imprints, which are located on the posterior half of each valve. Each pad is a smooth and very thin calcareous film, glossier and slightly more translucent than the rest of the valve. In *Crassostrea virginica* the pads appear dark purple in color, because the calcitic shell material directly beneath the semitranslucent pads is purple and can be seen through them.

With the aid of an electric engraving tool (Burgess vibro graver) many small fragments were broken from the pads of several valves of *Crassostrea virginica*. With this method it is not possible to obtain a pure sample from the pads alone, because they are too thin, and the calcitic shell material beneath the pads was dug into inadvertently. The x-ray diffraction pattern of the sample showed a curve indicative of calcite and aragonite. In addition, staining with Feigl's solution (*2*) demonstrated on several different species of oysters that the adductor muscle pads are composed of aragonite.

Quenstedt (*3*) discovered tiny places of insertion of muscles on the valves of the Early Jurassic oyster *Gryphaea arcuata* Lamarck, 1801, and surmised that they were vestiges of the anterior adductor muscle. Herdman and Boyce (*4*) were the first to give a histological description of these muscles based on *Crassostrea virginica*. Their work is still