X-ray diffraction and bone structure in reindeer

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Abstract: Bone consists of an organic matrix and mineral calcium phosphate. Bone mineral consists of a crystalline phase similar to hydroxyapatite (CHA) and an amorphous calcium phosphate phase. The proportion of this non-crystalline fraction has been found to be related to the age, diet and the degree of tissue mineralization.

The relative weight fractions of crystalline hydroxyapatite and amorphous calcium phosphate and the relative crystal sizes of the diaphyseal and epiphyseal metacarpus bone, spongy and compact rib bone, calvarium, pedicle and the middle and distal parts of antlers of 27 male and female reindeer calves and adult hinds were studied by x-ray diffraction techniques. The samples were collected in autumn and stored at — 20°C for less than three weeks.

No significant sex-differences were noticeable in calves. Significant differences were found between calves and adult hinds. Crystal size and crystalline hydroxyapatite content in hinds were larger than that of calves but the relative fractions were equal. The differences were greatest in compact rib bone and in epiphyse. The results indicate that the mineralization of epiphyseal tissue in calves has not been completed.

The structure of antlers in reindeer calves and hinds was similar: from the pedicle to the top the crystalline hydroxyapatite content and the crystal size decreased. The structure was typical to young tissue.

X-ray diffraction techniques are very suitable for studying the bone calcium phosphate metabolism of the reindeer and they indicate also the age and the growth phase of the bones.

Introduction

The determination of crystal size by x-ray diffraction technique

The average crystal size is inversely proportional to the width at half-maximum intensity of the x-ray diffraction peak:

$$D = \frac{K\lambda}{B\cos\Theta}$$

D = average crystal size
K = numerical constant (=0.9)
λ = wave length of x-rays used
B = half-width of the x-ray diffraction peak
Θ = diffraction angle

The quantitative phase analysis of a multi-component system by x-ray diffraction technique

The method was developed by Alexander and Klug (1948).

The intensity (area) of the diffraction maxima associated with one component is dependent on the weight fraction of that component within a multi-phase system:

$$I_1 = \frac{K_1 x_1}{P_1 (x_1 (\mu_1 - \mu_m) + \mu_m)}$$

I_1 = the diffraction intensity of component 1
K_1 = material constant for the component 1
x_1 = weight fraction of component 1
P_1 = density of component 1
μ_1 = mass absorption coefficient of component 1
μ_m = average mass absorption coefficient of the rest of the multiphase system

The quantitative phase analysis of the bone tissue

Bone consists of an organic matrix and mineral calcium phosphate. Bone mineral consists of a crystalline phase similar to hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and an amorphous calcium phosphate phase. The proportion of this non-crystalline fraction has been found to be related to the age, diet and the degree of tissue mineralization of the animal (Termine and Posner 1967). It is suggested that the amorphous bone mineral fraction is a metastable precursor of crystalline bone apatite.

The x-ray diffraction method, which has been utilized to quantitate the amorphous/crystalline mineral compositions of bone powder specimens, has been developed by Harper and Posner (1966). It is an adaptation of the technique developed by Alexander and Klug (1948).

The intensity (area) of certain discrete bone apatite x-ray diffraction maxima is directly proportional to the amount of crystalline apatite within the tissue, and the intensities of different samples are directly comparable if the amount of water and protein in tissues are approximately the same and the mass absorption coefficients of amorphous calcium phosphate and hydroxyapatite are equal. These assumptions are usually valid except in very poorly mineralized samples.

Material and methods

The tissues studied were the diaphyseal and epiphyseal metacarpus bone, spongy and compact rib bone, calvarium, pedicle and the middle and distal parts of antlers of 27 male and female reindeer calves and adult hinds. The samples were collected in autumn and stored at —20°C less than three weeks. After the storage period the bones were dried in ethanol-trichlor-ethylene and ground to 75 μm before use.

The samples were examined by the x-ray diffraction technique described by Harper and Posner (1966) using a Philips diffractometer with scintillation counter (copper target, nickel filter, 36 kV, 24 mA). The x-ray data for each sample were obtained in two regions: (002)-reflection between 24,50° and 27,25° (20°-scale) and (hk0)-reflections between 37,50° and 41,25° (20°-scale). The intensities of reflections were determined gravimetrically. The bone intensity used for weight fraction measurements was the intensity of (002)-reflection plus half of the intensity of (hk0)-reflections. For crystal size measurements the half-width of the (002)-reflection was used.

Results

The diffraction intensities which describe the proportion of crystallinen hydroxyapatite in the various bone tissues are presented in Fig. 1. The corresponding half widths of the (002) reflection peak of the same tissues which describe the inverse of the crystal size are presented in Fig. 2.

No significant sex-difference was noticeable in calves. This is understandable because the hormonal activity has not started yet. Significant differences were found between calves and hinds: crystal size and crystalline hydroxyapatite content in hinds were greater than in calves. The difference was largest in compact rib bone and in epiphyse. The results indicate that the mineralization of epihyseal tissue in calves has not been completed (Figs 1 and 2). In antlers the crystalline hydroxyapatite content and the

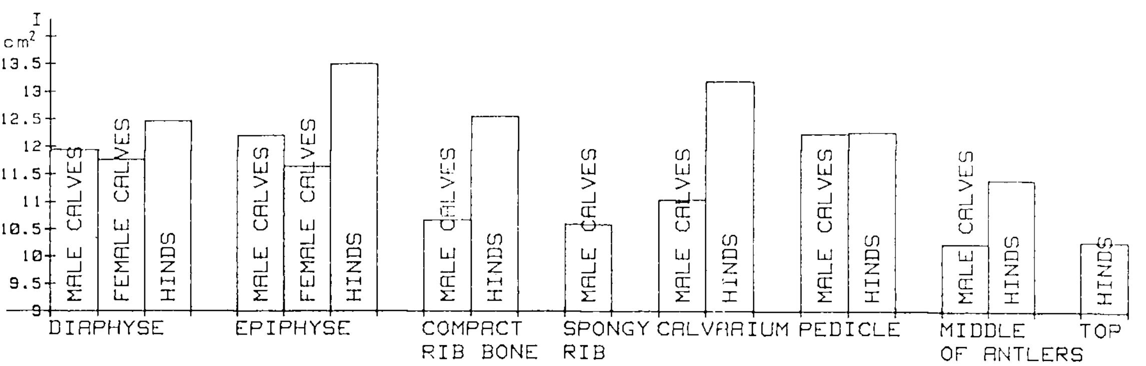

Fig. 1. The diffraction intensities of different samples (directly proportional to the weight fraction of hydroxyapatite).

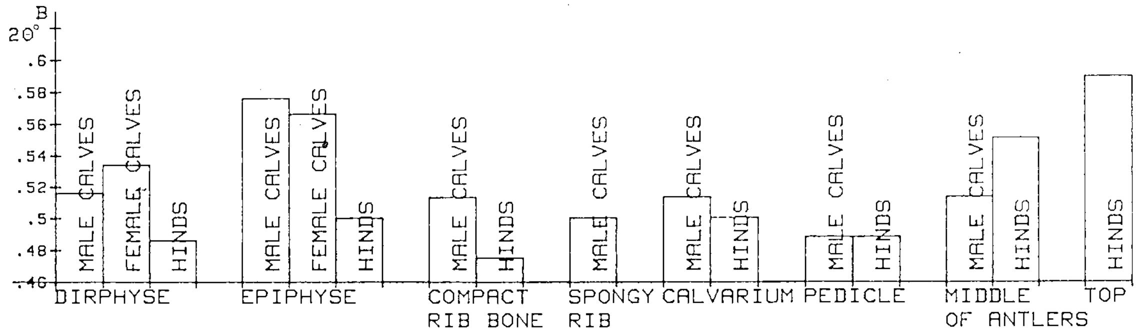

Fig. 2. The half-widths of different samples (inversely proportional to crystal size).

crystal size decreased from the pedicle to the top. The structure is typical to young tissue.

The diffraction intensity in February of the spongy rib of one starved female calf was only 7.03, whereas the corresponding value of normal calves was 10.7 (Fig. 1). The half width of the (002)-peak was 0.488°. This shows that the degree of mineralization of the rib has been strongly reduced, but the crystal size of the crystals remaining was equal to normal animals (Fig. 2).

Discussion

The diffraction technique is a reliable method for following the degree of mineralization of bone tissues (Termine and Posner 1967). Also the treatment of specimen before measurements have no influence on the results (Eskelinen and Nieminen 1976). The large amount of specimen which is necessary for the powdering of the tissue reduces the applicability of the method in the case of small animals, but is not important in the case of reindeer.

The result obtained with the starved calf clearly demonstrated the significance of bone tissues as a calcium pool when the nutrition of the animal is poor. Another interesting problem where the diffraction method might be useful is, how the growth of antlers will stress the animal and change the degree of mineralization of other bone tissues.

References

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