ANALYSIS OF THE DEGREE OF MINERALIZATION OF BONE TISSUE AND CALLUS

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Diffraction patterns of the scatter of x rays from samples of human bone tissue and callus were obtained. The degree of mineralization of callus is less than that of normal bone tissue, evidence of the immaturity of the callus.

Certain functional properties of bone tissue are largely determined by its crystalline structure. This is characterized by a definite quantitative ratio between its mineral and organic components. During reparative regeneration of bone quantitative changes take place in the content of the mineral component of the bone tissue, i.e., of hydroxyapatite.

The object of the present investigation was to study differences in the hydroxyapatite content of bone tissue and callus.

EXPERIMENTAL METHOD

The ordinary methods of determining the degree of mineralization of bone tissue, as well as the method of microroentgenography which has recently been used [2, 4], are very tedious. Photometric estimation of microroentgenograms likewise introduces an additional error into the determination. In the present investigation the bone tissue was studied by the x-ray diffractometry method.

The diffractogram is a curve showing the intensity of the scattered x-ray beam as a function of the angle of scatter. It provides information on the nature of a crystalline substance and on its quantity. The results of x-ray diffractometry of bone tissue, crushed and pressed into pellets, have been published. As regards the diffractometric study of the quantitative content of hydroxyapatite in immature bone callus compared with normal bone tissue, very little information has been obtained. The experimental material consisted of samples of bone tissue and callus obtained from the diaphyses of human long bones. The samples of bone tissue were taken from cadavers of persons dying accidentally.

In some cases samples of normal bone tissue were obtained during operations, so that the structure of normal bone tissue and callus could be studied in the same person. The samples were cut in the form of rectangular parallelepipeds. One edge of the parallelepiped was parallel to the axis of the medullary channel (direction 1 in Fig. 1). Two other edges were perpendicular to this axis. Direction 2 coincided with the tangent to the surface of the diaphyses, and direction 3 with the radius of the medullary channel. In each case the beam of x rays was directed parallel to one edge, and was designated 1, 2, or 3 depending on its direction relative to the sample.

The samples of bone callus and bone tissue were investigated with the URS-50IM diffractometer with a BSV-2 tube and copper anticathode. The intensity of the scattered x-rays was monitored by means of a type MSRT counter and recording system. The diffractogram was automatically recorded on the automatic writer of the diffractometer. Altogether 60 diffractograms were analyzed.

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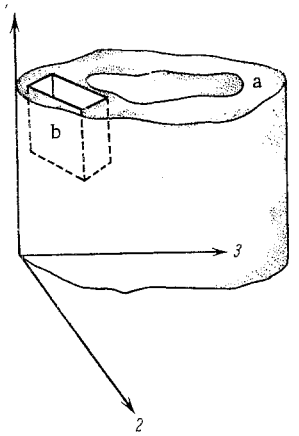

Fig. 1

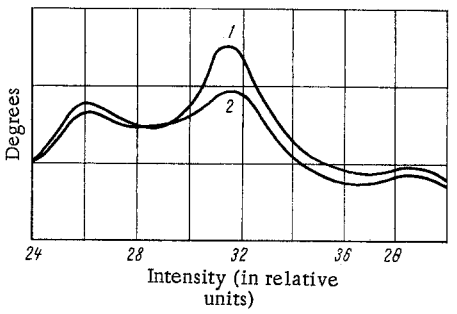

Fig. 2

Fig. 1. Diagram showing position of primary x-ray beam relative to sample of bone tissue [a) fragment of long bone; b) specimen]; 1, 2, 3) directions parallel to which the primary x-ray beam is aimed.

Fig. 2. Diffractograms of samples of bone tissue (1) and bone callus (2).

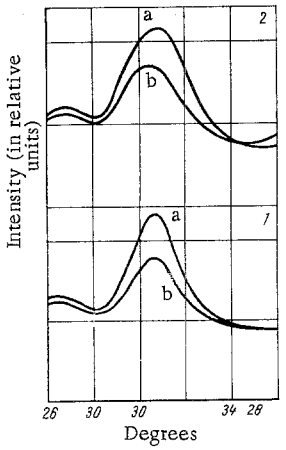

Fig. 3. Diffractograms for normal bone tissue (a) and bone callus (b) in directions 1 and 2.

EXPERIMENTAL RESULTS

Analysis of the diffractometric scatter curves shows that maxima of scatter are due entirely to hydroxyapatite. The location of the characteristic maxima of hydroxyapatite were identical on diffractograms of bone tissue and bone callus, but the height of the maxima on the curves differed. The roentgenogram of a system consisting of a mixture of substances is known to be the result of superposition of the roentgenograms of the individual substances, and the intensities of the lines of the roentgenogram are proportional to the quantity of the substance in the mixture. The content of hydroxyapatite in the bone tissue callus could therefore be estimated from the intensity of the characteristic maxima on the diffractograms.

Diffractograms of specimens obtained at operations on the same patient are shown in Fig. 2. Analysis of these diffractograms reveals significant differences between the degrees of mineralization of the bone tissue and bone callus. Similar differences were also observed between samples of bone tissue and callus obtained from different people. On analysis of the diffractograms, the degree of mineralization was found to be independent of sex and age (between 20 and 50 years).

Particularly important results are obtained by comparing diffractograms for the same pair of samples: a sample of bone callus and a sample of bone tissue placed in different positions relative to the direction of the x-ray beam. Diffractograms for normal bone tissue (a) and for bone callus (b) for two identical orientations of the sample relative to the primary x-ray beam (along directions 1 and 2) are given in Fig. 3. With samples of exactly the same dimensions (13.5 × 13.5 × 5.6 mm) a characteristic difference in the degree of mineralization of the normal bone tissue and bone callus was found.

In addition, the distribution of the hydroxyapatite crystals is evidently more regular in normal bone tissue.

These results indicate that the diffractometric method can be used to verify the degree of minerialization of bone tissue and bone callus. The degree of mineralization of bone callus was shown to be less than that of normal bone tissue, and this may possibly indicate immaturity of the callus. In order to obtain a quantitative estimate of the hydroxyapatite content as it varies with the degree of maturity of the callus by analysis of curves of the diffractometric investigation of bone tissue, appropriate experiments are being conducted on animals.

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