The study of bones by neutron diffraction. By G. E. Bacon, Department of Physics, The University, Sheffield, England, P. J. Bacon, Edward Grey Institute, Department of Zoology, The University, Oxford, England and R. K. Griffiths, Department of Anatomy, The University, Birmingham, England

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High-intensity neutron beams provide a means of measuring the integrated orientation of apatite crystals in bulk samples of bone. With a resolution of 2 mm² in compact bone and 12 mm² in trabeculae this promises a tool for the study of the load-bearing function of bone in a variety of anatomical sites.

We report an application of neutron diffraction with a biological interest well removed from the macromolecular studies which have been greatly developed in recent years. We take advantage of the penetrating power of neutrons to examine the preferred orientation of the apatite crystals in bones, being able to determine this orientation over distances of the order of 1 cm in bulk samples, in contrast to the study of thin sections and the surface and near-surface examination which is achieved by electron microscopy and X-ray diffraction [see for example, Steve-Bocciarelli (1973) and Selvig (1975)]. Our measurements so far have been made with a variety of neutron instruments and suggest a fruitful field of investigation bearing in mind that the distribution of the apatite crystals can provide an index of the stress distribution in both compact and trabecular bone. We emphasize that we are only dealing with the orientation of the mineral crystals and are not concerned with the use of neutrons to unravel further their structural crystallography and crystal chemistry in the manner pursued by Young (1975) and his co-workers in their studies of dental enamel.

Fig. 1. Variation of intensity of 0002 apatite reflexion, recorded in the horizontal plane, with the inclination of the shaft of an ox femur to the horizontal.

Many authors have drawn attention to the way in which the trabecular pattern of bone, as seen in radiographs and cross sections, seems to follow the lines of stress and Oxnard (1973) has described an optical method of assessing overall the geometrical disposition of the trabeculae seen on the surface of bone slices. It can readily be demonstrated using X-rays that within individual trabeculae the c axes of the apatite crystals are preferentially aligned in the trabecular fibre direction, though the nodular cross-over regions in the spongiosa are largely isotropic. On the other hand in the shafts of long bones, where the stress lines may be largely unidirectional – albeit for tension or compression – the crystallite orientation might be expected to be considerable. Because of the low absorption of neutrons their diffraction pattern will in principle readily provide an integrated picture of the orientation and in practice the scale of distance over which integration is made can easily range from 1 mm to 1 cm. In our first exploratory measurements on the medium-flux reactors at the AERE Harwell we have established both the very high crystallite orientation in bond shafts and the essential correlation of crystallite orientation with trabeculae direction. The curve in Fig. 1 shows quantitatively the preferred orientation in the shaft of an ox femur for a section of thickness 1 cm and a cross-sectional area of about 1 cm². The spatial density of the hexagonal c axes of the apatite
crystals has fallen to half its maximum at 15° away from the line of the shaft. This curve was obtained by measuring the angular variation of intensity with inclination of the shaft for the 0002 apatite reflexion in the neutron diffraction pattern. We emphasize that the neutrons permeate the whole thickness of such a sample so that the results are fully representative of the three-dimensional situation. A practical problem in this type of measurement is to avoid the intense incoherent scattering from the hydrogen atoms in the collagen of the bone. For the present we have overcome this by the effective, but destructive, process of first heating the samples at about 550°C. We have established in subsidiary experiments that this treatment satisfies our requirement of not disturbing the essential fabric of the material and, in particular, of not affecting significantly the c axes of the mineral component. We have assured ourselves of this first by showing that the orientation of individual trabeculae fibres assessed by X-ray diffraction is little affected and, secondly, by making some bulk measurements of untreated compact bone using neutrons. The latter measurements, though without the precision which we can achieve when we avoid the incoherent scattering, are adequate to assure us that we have scarcely disturbed the mineral orientation.

As a second example we have looked at the specimen of bone trabeculae, which is photographed in Fig. 2, the lower end of which shows a rather well-defined case of two orthogonal directions for the trabeculae. For this sample the intensity of the 0002 reflexion, recorded in a horizontal plane, shows only a rather slow fall as the sample is rotated, as indicated by the crosses in Fig. 3. For the orthogonal arrangement in the region examined this is what would be expected, since the 0002 reflexion would peak for either a horizontal or vertical position of the sample. Quantitatively, as shown by the curves drawn in Fig. 3, the observed variation is in agreement with the assumption of a ratio of about 2:1 in the quantities of vertical and horizontal fibres, which is consistent with visual inspection. Improved accuracy of measurement which we achieve as described in the following paragraph, would give a proper quantitative assessment.

![Diagram](image)

Fig. 3. Curve (i), determined with X-rays, indicates the preferred orientation in an individual trabecular fibre mounted horizontally and curve (ii) is for the same trabecula mounted vertically. Curve (iii) is then deduced for two orthogonal directions of trabeculae present in equal quantity. Curve (iv) is deduced for a 2:1 ratio in the two directions. The crosses indicate neutron measurements over the lower section of the trabeculae photographed in Fig. 2 utilizing a circular area of about 14 mm diameter.

![Graph](image)

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Fig. 4. Neutron diffraction patterns for the solid bone in the shaft of an ox femur recorded in 20 min at λ = 2.4 Å on the instrument D1B at ILL Grenoble. For the upper curve the bone shaft is vertical: in the lower curve the shaft is horizontal. Sample area, 20 mm²; thickness of section, 8 mm. The reflexions which are notably enhanced in each pattern are indexed in heavy type. The redundant third Miller index is omitted.
The prospects of developing these observations to provide a practical tool for studying the orientation of crystallites in bone in a topographical way are revolutionized by carrying them out at very high-flux reactors and with much more sophisticated measuring equipment. The instrument D1B at the Institut Laue–Langevin, Grenoble, has 400 counters arranged at intervals of 0.2° of scattering angle and all of these record the arrival of diffracted neutrons simultaneously and continuously. Fig. 4 shows patterns secured with this instrument in a time of only 20 min, for a mid-shaft section of an ox femur for which an area of only 20 mm² was irradiated. The upper and lower patterns were obtained with the shaft of the bone vertical and horizontal respectively. Using a neutron wavelength \( \lambda \) of 2.4 Å both the intensity and angular resolution of the diffraction pattern are very adequate. Thus for the horizontal sample the greatly-enhanced basal resolution of the diffraction pattern are very adequate. For the vertical sample we find that for any individual reflection the ratio of the intensities in the two patterns is in good agreement with calculations based on an orientation function similar to that in Fig. 1. We conclude from these results that we could reduce the irradiated area by a further factor of ten without detriment, at the worst requiring an increased counting time of one hour, enabling us to measure with accuracy the direction and the degree of the orientation for units of area of 2 mm². Our technique may be especially useful where the stress load in bone is complex, such as in the areas close to muscle attachment points. For the trabecular bone the reduced density lowers the diffracted neutron intensity but we have obtained patterns of similar precision to Fig. 4 in a time of two hours for the same irradiated area of 20 mm², indicating an effective reduction of density of about six times. We conclude that adequate patterns would be obtained in one hour for an area of 12 mm², i.e. a square of side 3.5 mm: an area of this size is indicated in Fig. 2. This would allow study of several anatomical sites of predominantly trabecular bone whose functional mechanisms are still in doubt; for instance the apparent dome-like orientation of the trabeculae in the arches of the foot; the vertebral, and other areas where the complex dynamic loads of the moving limbs are transmitted to the axial skeleton. The method may also provide a tool whereby the reaction of bone to abnormal stresses can be assessed and the success of the healing and remodelling of the fracture process following fracture can be investigated.

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References


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Plastic deformation accompanying unsteady cleavage-crack propagation was studied in NaCl and LiF single crystals by X-ray topography. It was found that, for slowly propagating cracks, in both materials the main activated systems are the transverse systems, particularly at the stopped crack fronts. They are remarkably intense in NaCl crystals.

Plastic deformation in LiF single crystals has been intensively studied by X-ray topography (Newkirk, 1959; Yoshimatsu & Kohra, 1960; Burns & Webb, 1966; Forwood & Lawn, 1966) but some specific experimental problems arise when working with NaCl crystals. Even single crystals grown under very carefully controlled conditions exhibit subgrain boundaries which make it very difficult to observe the traces of activated slip planes simultaneously in large areas of the crystal. Besides, NaCl is highly hygroscopic and humid air dissolves the crystal surface after a short exposure.

Recently Strunk (1973) has studied, by X-ray topography, NaCl crystals plasticly deformed under compression.

An X-ray topography study of cleavage surfaces of NaCl single crystals, carried out to determine the slip systems that comprise plastic deformation accompanying cleavage-crack propagation, is presented in this communication. It is a part of a study on the plastic deformation accompanying unsteady cleavage-crack propagation.

To compare the behaviour of both LiF and NaCl crystals under the same experimental conditions, an X-ray topography study of LiF cleavage surfaces was carried out.

Experimental

Specimens of NaCl and LiF, 15 mm long by 5 mm wide by 3 mm thick, were cleaved from large blocks of high-purity single crystals from Hilger & Watts. Cleavage cracks were introduced in these prisms with a razor blade and a hammer, propagated and stopped several times before completion of cleavage.

A Rigaku Denki X-ray generator and Lang camera A3 were used to obtain the topographs. The Lang reflection technique was used. Reflecting planes were chosen so that the penetration depth was adequate to observe the defect structure close to and at the surface. Cu Kα radiation was used for NaCl with a diffraction vector \( g = [044] \) and pene-