# THE LOW-ANGLE SCATTER OF X-RAYS FROM BONE TISSUE

by

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Bone tissues in general give characteristic X-ray diffraction patterns that contain numerous well-defined wide angle rings. These patterns are derived from the crystalline apatite component, and the observation that in the patterns of longitudinal bone sections several rings show definite orientation suggests that the apatite crystallites are well orientated in the bone structure. In addition to this wide angle diffraction pattern, bone tissues also give a diffuse low-angle scatter. In longitudinal sections of bone this scatter is also orientated, and the diffuse scatter may in fact be a particle scatter from the apatite crystallites1. In this work, the possibility of deducing the shape and size of particles or crystallites in bone from the low-angle diffuse scatter has been investigated.

The crystallite size in this system has previously been studied by examination of the line-broadening effects in the wide angle X-ray diffraction patterns, and by electron microscopy. From a study of the widths of diffraction lines, STÜHLER<sup>2</sup> has estimated the average diameter of the crystallites to be in the range 31 A to 200 A. "Für die mittleren linearen Abmessungen der Apatitkristallite des unbehandelten Knochens ergeben sich demnach einige 10<sup>-7</sup> cm." From measurements made on crystalline particles seen in electron micrographs of fragmented bone preparations, ROBINSON<sup>3</sup> has suggested that the crystallites of bone approximate to a tabular form of hexagonal outline, the average dimensions being 500 A in length, by 250 A in width, by 100 A in thickness, and the long axis lying roughly parallel to the collagen fibres. Crystal dimensions varying from 1500 A to 20 A were measured. Other authors4 have obtained electron micrographs of replicas of bone surfaces, and estimated the particle dimensions to be less than 100 A. The distinction between particle size and crystallite size is based solely on the experimental method used in the investigation. In the studies of diffuse lowangle scatter, it is a particle size which is being considered. The relation between this and the crystallite size will be discussed later.

The principles of the method for the estimation of particle sizes from diffuse lowangle scatter of X-rays has been clearly outlined by Guinier<sup>5,6</sup>. The treatment of experimental results suggested by him has been modified by numerous other workers<sup>7-21</sup> in applying the method to a wide range of systems.

It has been shown that in the case of disperse systems containing particles of uniform size and random orientation, the logarithm of the intensity of scattered radiation is a linear function of  $\varepsilon^2$ , where  $\varepsilon$  is the scattering angle measured in radians.

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The general expression adopted by Guinier<sup>5,6</sup> in treating the scatter from such systems was of the form:

$$\log I = \log K - \frac{4\pi^2}{3\lambda^2} \log e R^2 \epsilon^2$$
 (1)

From this was derived the simple relation (when  $\lambda$  (CuKa) = 1.54 A):

$$R = 0.644 \sqrt{a} \tag{2}$$

between R, the radius of gyration of the particle (as defined in the usual mechanical case), and a, the slope of the curve relating  $\log I$  and  $\varepsilon^2$ . It the particle shape is known, the dimensions can be calculated from this R value (equation 2). However, in the case of asymmetrical particles of unknown shape the relation between the radius of gyration and the actual dimensions of the particles is not easily derived, and other workers in this field have preferred to use an expression which gives a value for the radius of the particle in terms of the slope of the curve relating  $\log I$  and  $\varepsilon^2$ , or  $r^2$ , where r is the distance from the scattering centre.

The expression given by Jellinek and Fankuchen<sup>14</sup> for scattering at low angles where  $\tan 2\theta$  is approximately equal to  $2\sin\theta$  is:

$$\ln I = \ln K - \left(\frac{2\pi R}{\sqrt{5}S\lambda}\right)^2 r^2 \tag{3}$$

From this can be derived:

$$R = 0.83 \, S\sqrt{a} \tag{4}$$

where R is the radius of the scattering particle, S the sample to film distance, and  $\alpha$  the slope of the curve relating  $\log I$  and  $r^2$ . CuK $\alpha$  radiation is assumed.

In the case of systems in which the particle size is not uniform, the plot of  $\log I$  against  $r^2$  does not give a straight line, and the particle sizes cannot be determined directly. The method generally used in treating such systems is to devise theoretical curves for different possible distributions of particle sizes, and to match these with the experimental curves.

When a well-orientated system of elongated particles is examined, the different particle axes can be treated independantly. In the case of an ellipsoidal shaped particle with axes a, a, and v.a. which is arranged with the v.a axis perpendicular to a symmetrically collimated X-ray beam, the resulting shape of the low-angle scatter will be similar to that of the particle but turned through 90°. If the axes of the scatter corresponding to the axes a and v a of the particle are v and v respectively, then the expression for the intensity of scattered radiation from one particle at any point on the film can be written:

$$\log I = \log K - \frac{4\pi^2}{5\lambda^2 S^2} \log e \left( a^2 x^2 + (v.a)^2 y^2 \right) \tag{5}$$

From this it is evident that if the intensity is examined along a main axis of the scatter only one dimension of the particle is involved. For practical purposes, using CuKa-radiation ( $\lambda = 1.54$  A), the expression:

$$R = 0.83 \, S \sqrt{a} \tag{6}$$

where a is the slope of the curve relating  $\log I$  and  $r^2$  along a main axis of the scatter, References p. 189.

R the corresponding radius of the particle, and S the sample to film distance, can be applied.

Preliminary low-angle diffraction pictures<sup>1</sup>, obtained from longitudinal sections of bone using a symmetrical beam collimation, showed marked asymmetry, indicating a pronounced elongation of the bone particles in the direction of the longitudinal axis of the bone. It therefore seemed possible that the scatter could be treated according to the method outlined above for orientated ellipsoids of revolution in order to obtain values for the axial dimensions. However, the above expressions are derived assuming that each particle scatters independently, and in close-packed systems the possibility of particle-particle interference must be considered. Guinier<sup>6</sup> states that if the apparent density,  $d_1$ , of the system as a whole is appreciably less than the real density, d, of the particles, then the absolute intensity of the scattered radiation may be diminished by inter-particle interference, but the slope of the curve of intensity as a function of the scattering angle is not appreciably modified. However, this statement has been criticized by Lund and Vineyard<sup>22</sup>, whose calculations for fine powders show that inter-particle interference may not be safely neglected unless  $d_1/d$  is much less than 1.

In the case of so-called compact human bone, the mineral content is about 65% by weight (for dried, de-fatted bone), the remainder being organic matter, mainly collagen. The density of apatite is of the order of 3 whilst the maximum density of the dried compact bone is about 2. This means that the volume of organic matter is greater than that of the mineral component. In the case of fibrous bone of hen and frog, and in that of calcified tendon, the mineral content is even lower. If, therefore, the apatite particles are distributed evenly through the bone, there is little likelihood of inter-particle interference, though the intermediate organic phase may affect the intensity of scatter to some extent. However, from optical and electron microscope studies of bone under various conditions, there seems to be a definite possibility that there is a clumping of the particles in certain regions. In such a case, inter-particle interference would again become a possibility. Nevertheless, in the case of the low-angle scatter of X-rays from sections of bone there are no indications of peaks corresponding to inter-particle distances. The intensity of the scattered radiation shows a continuous decrease with increase in scattering angle such as would be expected from a system in which there is no particle-particle interference. The extreme case discussed by Guinier<sup>6</sup> is one in which the system becomes so compact that it can be regarded as a homogeneous body with occasional irregularly placed holes. In this case the low-angle scatter is to be interpreted in terms of the holes. It is very unlikely that such a system is present in bone. The electron microscope studies, and the general pattern of crystallite formation and mineral salt metabolism in bone point to a system of apatite particles.

From these preliminary considerations it is evident that the low-angle scatter from normal compact bone can be treated as a particle scatter, and it is interpreted according to the expressions derived by previous workers for orientated asymmetrical particles, neglecting inter-particle interference.

## MATERIAL AND METHODS

Longitudinal and cross-sections of compact bone from human, cow, hen, and frog were prepared by grinding. The thickness of these sections was of the order of 0.2 mm.

The low-angle scatter was recorded photographically using Ni filtered CuKa radiation, and a pin-hole collimating system, obtained by arranging two similar sets of simple slit apertures at right References p. 189.

angles to each other  $^{23}$ . The width of the undiffracted X-ray beam at the plane of the film was calculated to be 0.53 mm.

A comparison was made between low-angle scatter recorded using a Guinier camera (providing monochromatic CuKa radiation), and that obtained using simple slit collimating apertures and Ni filtered Cu radiation. In this comparison an unsymmetrical beam (a slit) had to be used, the length being arranged parallel to the longitudinal direction of the bone section. With such an arrangement it was not possible to use the results directly to calculate a value for the short dimension of the particle (the relation between log I and r² was not linear), but the comparison served to show that the difference in the intensity of scatter for the two types of radiation used became appreciable only at very low angles. Shull and Roess¹6 estimated that in the determination of the average particle size in a randomely orientated system, the error introduced by the use of nickel filtered radiation rather than crystal reflected monochromatic radiation could be as high as 25%. However, the determination of particle size in such systems involves the matching of theoretical and experimental intensity curves, and here the exact curve shape is very important. The direct treatment of the scatter from the bone samples was such that the deviations in this very low-angle region would not affect the results appreciably.

The variation of intensity of low-angle scatter of X-rays, as recorded on photographic film, was examined using either a Hilger microphotometer or an automatically recording densitometer  $^{24}$  (Aperture corresponding to area of 0.1 mm  $\times$  0.2 mm on film). The relation between X-ray intensity and film blackening in these experiments was checked and found to be linear. The air scatter was found to be small in comparison with the diffuse scatter from the bone specimens.

Wide-angle diffraction patterns and low-angle scatter were recorded simultaneously on separate films placed at different sample-to-film distances. The undiffracted X-ray beam and low-angle scatter passed through an aperture in the centre of a filmholder placed close to the specimen and were recorded at a much greater distance from the sample. More detailed wide-angle diffraction patterns were recorded separately.

The low-angle scatter of X-rays associated with the short dimensions of the particles in bone tissues could be conveniently examined at 10 cm to 20 cm sample-to-film distance using a 1 mm beam stop of circular cross-section to absorb the undiffracted X-rays. The scatter from the long particle dimension of normal bone samples was at much lower angles, and difficult to record satisfactorily. Furthermore, a faint coherent diffraction pattern, probably due to collagen, was found to be superimposed on the diffuse particle scatter in the direction corresponding to the longitudinal axis of the bone. This made a direct determination of the particle dimension in this direction impossible. However, a value for this dimension was obtained from a consideration of the overall form of the particle scatter from a longitudinal section of bone using a pin-hole collimating system.

In addition to the examination of sections of normal bone, one longitudinal section of cow femur which had been heated to  $400^{\circ}$  C to  $500^{\circ}$  C in order to remove organic constituents was studied, and also an autoclaved longitudinal section of human bone.

A number of electron micrographs were obtained from fragmented human bone. The E.M. preparations were made by collecting bone dust (produced by sawing a bone sample) on formvar-covered specimen grids, and examined in an RCA microscope, type EMU. The magnification of this instrument was calibrated using a replica of a line grating, and with Dow latex particles.

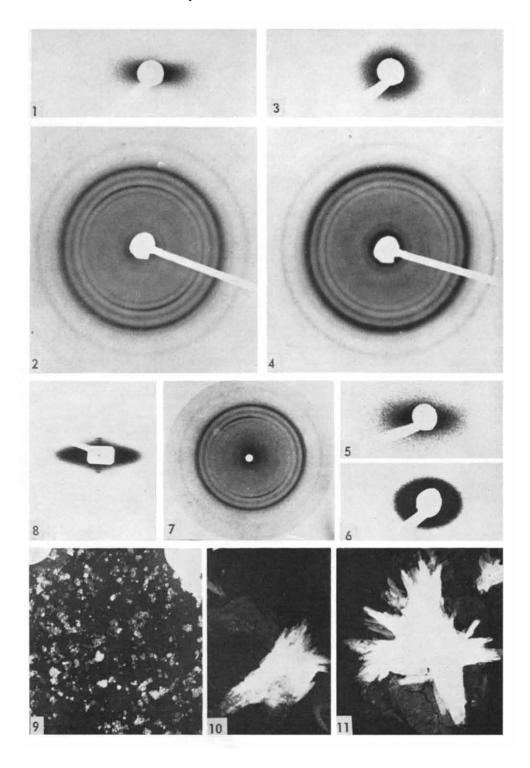
# RESULTS

The low-angle scatter of X-rays from longitudinal sections of each type of normal bone showed marked asymmetry (Fig. 1 and Fig. 5), indicating in every case an elongation of the particles in the direction of the longitudinal axis of the bone. In the corresponding wide-angle pattern (Fig. 2), the oo2 reflection<sup>2, 25</sup> showed intensifications in the direction at right angles to the long axis of the low-angle scatter.

The autoclaved longitudinal section of human bone gave diffraction diagrams which did not differ appreciably from those of the normal material.

The low-angle scatter from cross-sections of bone (Fig. 3) showed no appreciable orientation, and neither did the reflections in the corresponding wide-angle pattern (Fig. 4). The ooz ring was much less intense than in the patterns from longitudinal sections, and several other reflections showed similar reductions of intensity.

The low-angle scatter from the longitudinal section of cow femur which had been heated to remove organic matter was much more symmetrical (Fig. 6) than that from References p. 189.



the normal section (Fig. 5), but the wide-angle pattern (Fig. 7) still showed marked orientation of the oo2 and other reflections. The intensity of diffraction from this specimen seemed to have been greatly increased by the heat treatment.

In most of the low-angle patterns only the scatter corresponding to the short dimension of the particles was resolved. The variation of intensity along the long axis, LM (Fig. 12) of the scatter from longitudinal sections was measured, and the values (I) plotted against distance (Fig. 13). From the symmetry of the curve it was possible to choose the diffraction centre, and the radii (r) were measured from this point. A plot was then made of log I against  $r^2$ . When the intensity variations were recorded automatically, this plot could be made directly from the automatic recording. In many cases (notably those in which human bone was used) the points fell on a straight line (Fig. 14), but in others, a line through the points gave a slight curve. The deviations were, however, small, and in general it was possible to obtain a reliable value for the slope of the curve (a), and to use this in the simple expression  $R = 0.83 \, S\sqrt{a}$  (for ellipsoidal-shaped particles) to determine  $R_s$ , the radius of the short dimension of the scattering particles.

The  $R_s$  values calculated from longitudinal sections showed good agreement with those from cross-sections (Table I).

It was not possible to treat the long axis of bone particles using this direct method. Exposures made using very fine collimating systems resolved the scatter from the long dimensions, but a faint coherent diffraction pattern was also recorded in this direction. This was almost certainly a collagen pattern, the third and fourth order diffractions being clearly shown. In compact bone, the collagen diffraction is weak and difficult to reproduce, but Fig. 8 shows the diffraction pattern from a sample of ossified tendon which contains relatively more collagen, and this also has the collagen pattern orientated in the same direction as the low-angle diffuse scatter associated with the long axes of the mineral particles (AB. Fig. 12). The superimposition of the collagen pattern prevents a direct treatment of the variation of the intensity along the short axis (AB) of the low-angle scatter. An attempt was made to obtain an R value for the long dimension of the particles indirectly by examining the intensity variations along a number of regularly spaced lines (CD, EF etc. Fig. 12) parallel to the short axis of the scatter but at some distance from it where the collagen diffraction did not interfere. In this way a number of curves of intensity against distance were obtained, examples of which

Fig. 1. Low-angle scatter of X-rays from a longitudinal section of human femur.

Fig. 2. Wide-angle diffraction pattern from a longitudinal section of human femur.

Fig. 3. Low-angle scatter from a cross-section of human femur.

Fig. 4. Wide-angle diffraction pattern from a cross-section of human femur.

Fig. 5. Low-angle scatter from a longitudinal section of cow femur.

Fig. 6. Low-angle scatter from a longitudinal section of cow femur which had been heated to about 400° to 500° C for one hour in order to remove organic material.

Fig. 7. Wide-angle diffraction pattern from the heated section (as Fig. 7).

Fig. 8. Low-angle diffraction from a specimen of ossified tendon, showing diffuse, low-angle scatter and the 9th and 11th order diffractions of collagen long spacing (reproduced by kind permission of Prof. R. Bear, Dept. of Biology, Massachusets Inst. of Technology, with whose equipment this diffraction pattern was obtained).

Figs. 9, 10, 11. Electron micrographs of fragmented human bone. Magnification: 9, 60,000 ×; 10 and 11, 30,000 ×. (Electron micrographs obtained in collaboration with Prof. H. Fernández-Morán). References p. 189.

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	$_{\rm BL}$		

Bone specimen		$R_{_{S}}\left( A ight) -$	$R_L^*$ (A)		
			(x)	(2)	(3)
Human (Normal femur)	Longitudinal section	37	112	106	98
	Cross-section	39			
Cow (Normal femur)	Longitudinal section	32	93	91	87
	Cross-section	35		_	
Cow (Specimen previously heated to 400° to 500° C)	Longitudinal section	50		80	-
Hen (Normal femur)	Longitudinal section	27	98	95	93
	Cross-section	29		_	
Frog (Normal femur)	Longitudinal section	34			

<sup>\*</sup> R<sub>L</sub> values (1) Calculated from a value obtained by simple extrapolation of experimental curves to beam centre (Fig. 17).

- (2) Calculated from a value obtained after correction for finite beam size.
- (3) Calculated from highest  $\alpha$  value measured outside beam stop.

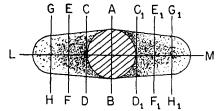


Fig. 12. Diagram showing the shape of the orientated low-angle scatter of X-rays from longitudinal sections of normal bone, and indicating the lines along which the variations of intensity were measured in order to deduce the dimensions of the scattering particles.

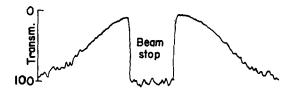


Fig. 13. Graph showing variations in intensity along the long axis (LM) of scatter from a longitudinal section of human femur.

are shown in Fig. 15. From each curve a plot of  $\log I$  against  $r^2$  was made (Fig. 16) and values for the slopes (a) obtained. When these were plotted against distance (x) from the central axis (AB) of the scatter they were found to give an approximately straight line relationship. As shown in Fig. 17, a small extrapolation of the two lines representing the relationship between a and x on either side of the beam stop produced an intersection at the scattering centre. The value of a at this point was used to calculate an approximate value for  $R_L$  (along the long axis of the particle) as quoted in Table I.

A similar procedure was applied in the perpendicular direction, a number of curves being obtained along lines parallel to LM (Fig. 12). Accurate values for a were obtained, and when these were plotted against distance from LM the curve shown in Fig. 18 was obtained. In this case the a to x relationship is shown to be strictly linear over most of the range covered but the peak is flattened over a distance of about 0.5 mm. This is almost certainly the effect of the finite size of the incident X-ray beam, and as the beam is symmetrical the a/x curve for the short axis of scatter should probably show a similar flattening. If the latter curve is treated accordingly (Fig. 17), the second  $R_L$  References p. 189.

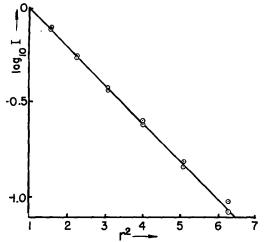


Fig. 14. Graph showing relationship between  $\log_{10}I$  and  $r^2$  along the long axis of low-angle scatter from the longitudinal section of human femur.

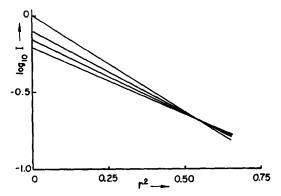
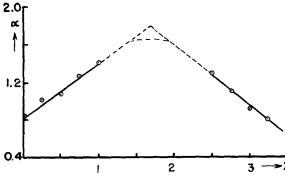


Fig. 16. Graph showing  $\log_{10}I$  against  $r^2$  relationships corresponding to intensity curves in Fig. 15.



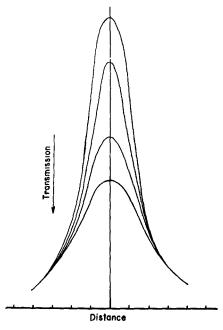


Fig. 15. Graph showing the variations of intensity along a number of lines (CD, EF, etc. Fig. 12) across the low-angle scatter from a longitudinal section of human bone.

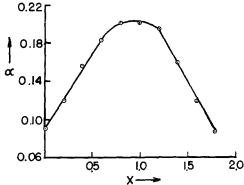


Fig. 17. Graph showing the a against x relationship for the short axis of the particles.

Fig. 18. Graph showing variation of a with distance (x) from diffraction centre (corresponding to long axis of particle).

value given in Table I is obtained. The third value given is calculated from the scatter close to the beam stop (i.e. along CD and  $C_1D_1$ , Fig. 12). These values will be discussed later. When the longitudinal section was tilted with respect to the incident X-ray beam, the asymmetry of the low-angle scatter was appreciably reduced. The effect was noticeable at angles of 30° or less, and at 45° the two axes of scatter were difficult to distinguish. At this angle, however, there was still a small difference in the intensity curves corresponding to the two directions.

In the case of the heated bone specimen in which there was no longer any interference from collagen diffraction, both axes of the low-angle scatter could be treated directly. If the scatter is assumed to come from ellipsoidal shaped particles, and equation 6 applied, the  $R_s$  and  $R_L$  values shown in Table I are obtained.

As illustrated in Fig. 10, some of the electron micrographs showed bundles of well-aligned, rod-shaped particles, the minimum observed diameters being of the order of 100 A. However, in other preparations in which a much finer dispersion of material was obtained, a very wide range of particle sizes and shapes could be seen (Fig. 11). In both cases, electron diffraction patterns of the preparations were of the apatite type.

## DISCUSSION

The general form of the low angle scatter of X-rays from longitudinal sections of normal bone indicates that the particles within the structure are elongated in the direction of the longitudinal axis of the bone. The orientation of some of the reflections in the wide-angle diffraction pattern, particularly that of the ooz reflection which has been reliably indexed, shows that the c-axis of the crystallites is orientated approximately parallel to the direction of elongation of the particles. Also, the direction of orientation of the collagen diffraction pattern demonstrates that this direction of elongation of the particles coincides with the fibre axis of the collagen fibers.

The absence of appreciable orientation in the low-angle scatter from cross-sections of bone suggests that the particles are well aligned, and are probably symmetrical about their long axes. The straight line relationship between  $\log I$  and  $r^2$  in different direction across this scatter tends to confirm these deductions, and the agreement between values for the particle diameters derived from cross-sections and those from the corresponding longitudinal sections, lends further support. The general form of the low-angle scatter would appear to be in keeping with an ellipsoidal or rod-shaped scattering particle.

In the case of the sample of human bone, where the relationship between  $\log I$  and  $r^2$  for the short axis of the particle is strictly linear, a value for  $R_s$ , the radius along the short axis, can readily be obtained from equation 6 for ellipsoidal shaped particles. The accuracy with which the diameter  $(2R_s)$  can be estimated depends mainly on the accuracy of the constant factor (0.83) in equation 6 as applied to this system. The approximation of the particle shape to an ellipsoid of revolution must be fairly close, as is indicated by the shape of the particle scatter. Even if the particles are cylindrical the adjustment necessary in this factor would cause only a relatively small change in the  $R_s$  value. The effect of inter-particle interference on the result is more difficult to assess accurately, but the fact that the form of the scattering curve does not appear to be affected appreciably is taken as an indication that the error introduced by neglecting such interference in this system will not be serious. The small deviations from the linear  $\log I/r^2$  relationship shown by some specimens may reflect either a limited References p. 189.

variation in particle diameters, or a relatively poor alignment of particles in the section. As the deviations are not sufficiently pronounced to interfere seriously with estimation of the slope, the accuracy of the calculated diameters should not be affected appreciably.

The R value obtained for the long axis of the particles may be as reliable as that for the short axis. The linear relationship between a and x for an incident beam of negligible cross-section seems to be faily well established in practice by the experimental curves shown in Figs. 17 and 18. The correction for the finite beam size appears to be given precisely by the complete curve relating a and x across the short axis of scatter (Fig. 18). The flattening off of the peak of the curve is over a distance of about 0.5 mm which corresponds almost exactly to the calculated width (0.53 mm) of the undiffracted X-ray beam at the plane of the film in these experiments. The X-ray beam used was symmetrical and therefore this correction factor for finite beam size can probably be applied directly to the curve for the long axis of scatter (Fig. 17). The middle value given for  $R_L$  in Table I is therefore probably the most accurate.  $2R_L$  is the estimated length of the particle. One possible source of error in obtaining the  $R_L$  value arises from the fact that the curves relating log I and  $r^2$  across the scatter are not strictly linear, particularly close to the beam stop. However, the fact that the estimated values of the slopes give a linear relationship with x indicates that this error is probably small.

Thus, this consideration of the low-angle scatter of X-rays leads to the suggestion that the particles in bone are rod-shaped, the long axes of the rods being aligned in the direction of the longitudinal axis of the bone, and parallel to the collagen fibres. In the intact human bone these particles appear to have a diameter of about 73 A and a length of about 210 A. The axial ratio of the particles is thus between 2:1 and 3:1 which is in general agreement with the overall shape of the low-angle scatter from longitudinal bone sections. The asymmetry of the scatter indicates a difinite elongation of the particles, but the fact that the asymmetry is quickly reduced when the specimen is tilted with respect to the incident X-ray beam suggests that the axial ratio is not very high.

In the small number of normal bone samples examined the lengths of the particles do not appear to vary appreciably, but in the case of the diameters the figure for hen bone seems significantly lower than for human. When the organic component is removed from compact bone by heating to  $400^{\circ}$  to  $500^{\circ}$  C, the composition of the system is changed, and this in itself may have an effect on the form of the low-angle scatter. The change may be reflected in the increased intensity of scatter. In addition, there is probably also a change in the physical state of the apatite, and if the scatter is considered as a simple particle scatter from ellipsoidal shaped particles, the dimensions differ considerably from those obtained for the normal bone. Further interpretation of these changes will be considered elsewhere.

Simple autoclaving appears to have little effect on the low-angle scatter.

Comparing these conclusions concerning the sizes and shapes of particles in normal intact bone with those of previous workers, it is seen that the dimensions deduced from low-angle X-ray scatter fall within the limits for crystallite size set by STÜHLER<sup>2</sup> from a consideration of the broadening of diffraction lines in the wide angle X-ray pattern. The particles considered in this paper may in fact be the crystallites, and in this case a comparison of low-angle and wide-angle X-ray patterns shows that the crystallites are elongated in the direction of the c-axis. The results are not however, readily related to the idealised crystal dimensions proposed by Robinson. In a modification of his References p. 189.

original suggestion (Addenda to 3) Robinson indicates the c-axis of the crystallographic unit cell as being parallel to the long dimension (500 A) of the crystals. The possibility can be considered that the particles examined in the study of the low-angle X-ray scatter are sub-units of the crystals seen by Robinson, in which case the crystal would include perhaps four such cylindrical sub-units. That this is the case in intact bone seems to us most unlikely, for the very nature of low-angle particle scatter indicates that there are definite dicontinuities or changes in structure at the boundaries of these particles such as would not be expected in single crystals.

Our own electron micrographs were of two types. One type showed bundles of well-aligned, rod-shaped particles such as would be expected from the low-angle X-ray data, the minimum measured diameters of the rods being of the order of 100 A. The other (Fig. 11), showed a much finer dispersion of particles with no consistent sizes or shapes. Tabular particles of the type reported by Robinson were observed, but in many cases these appeared to be breaking down into rods and even into granules of very small dimensions (circa 50 A). In general, no reliable conclusions could be drawn from such pictures as to the sizes and shapes of elementary particles.

This preliminary work shows the possibilities of the application of the low angle X-ray scatter method to an extensive examination of particle sizes in bone tissues, and the problems associated with mineral salt metabolism in which the particle size appears to be important. Further work along these lines is being carried out. The effects of heat treatment on the particle shape are also being considered in greater detail.

#### SUMMARY

A study has been made of the diffuse low-angle X-ray scatter from a number of longitudinal and cross-sections of normal intact bone. From considerations of the variations of intensity of scatter from well-orientated specimens, the size and shape of the scattering particles have been deduced.

In intact human bone, the scattering particles appear to be of uniform size and to approximate to rods which have a diameter of about 75 A and are about 210 A long. The particles appear to be well-aligned, with their long axes in the direction of the longitudinal axis of the bone and parallel to the collagen fibre axis.

Particles from other types of normal bone seem to be of a similar shape and size.

# RÉSUMÉ

La diffraction diffuse sous angle faible des rayons X a été étudiée sur un certain nombre de sections longitudinales et transversales d'os normal intact. Les variations de l'intensité de la diffraction sur des échantillons bien orientés permettent de déterminer les dimensions et la forme des particules diffractantes.

Dans l'os humain intact, les particules diffractantes sont de taille uniforme et se rapprochent de bâtonnets d'environ 75 A de diamètre sur 210 A de longueur. Ces particules sont bien alignées, leur grand axe dirigé selon l'axe longitudinal de l'os et parallèle à l'axe de la fibre de collagène.

Les particules d'autres types d'os normaux paraissent de forme et de taille semblables.

## ZUSAMMENFASSUNG

Es wurde die diffuse Kleinwinkelstreuung von Röntgenstrahlen bei einer Anzahl von Längsund Querschnitten normaler intakter Knochen untersucht. Aus Betrachtungen der Abhängigkeit der Intensität der Streuung gut orientierter Proben wurde die Grösse und die Form der streuenden Teilchen abgeleitet.

In intakten menschlichen Knochen scheinen die streuenden Teilchen von einheitlicher Grösse zu sein und sich der Form eines Stabes mit einem Durchmesser von ca. 75 A und einer Länge von ca. 210 A zu nähern. Die Teilchen scheinen mit ihren langen Achsen zur Richtung der Längsachse der Knochen und parallel zur Achse der Kollagenfaser ausgerichtet zu sein.

Teilchen von anderen Typen normaler Knochen scheinen ähnliche Form und Grösse zu besitzen.

References p. 189.

## REFERENCES

- 1 A. ENGSTRÖM AND J. B. FINEAN, Nature, 171 (1953) 564.
- <sup>2</sup> R. Stühler, Fortschr. Gebiete Röntgenstrahlen, 57 (1938) 231.
- <sup>3</sup> R. A. Robinson, J. Bone and Joint Surg., 34-A (1952) 389.
- 4 CH. ROUILLIER, L. HUBER, E. KELLENBERGER AND E. RUTISHAUSER, Acta Anat., 14 (1952) 9.
- <sup>5</sup> A. Guinier, Thesis, Series A, No. 1854, University of Paris, 1939.
- <sup>6</sup> A. GUINIER, X-ray Crystallographic Technology, English translation by T. L. TIPPELL. Edited by K. Lonsdale, F.R.S. Hilger and Watts Ltd. London, 1952.
- 7 O. KRATKY, A. SEKORA AND R. TREER, Z. Elektrochem., 48 (1942) 587.
- 8 O. Kratky and G. Porod, Acta Phys. Austriaca, 2 (1948) 134.
- O. Kratky, J. Polymer. Sci., 3 (1948) 195.
   O. Kratky, G. Porod and L. Kahovec, Z. Elektrochem., 55 (1951) 53.
- <sup>11</sup> R. Hosemann, Z. Elektrochem., 46 (1940) 535.
- 12 R. HOSEMANN, Kolloid-Z., 116-117 (1950) 13.
- 13 J. BISCOE AND B. E. WARREN, J. Appl. Phys., 13 (1942) 364.
- 14 M. H. JELLINEK AND I. FANKUCHEN, Ind. Eng. Chem., 37 (1945) 158.
- 15 M. H. JELLINEK, E. SOLOMON AND I. FANKUCHEN, Ind. Eng. Chem., 18 (1946) 172.
- 16 C. G. SHULL AND L. C. ROESS, J. Appl. Phys., 18 (1947) 295.
- 17 L. C. ROESS AND C. G. SHULL, J. Appl. Phys., 18 (1947) 308.
- 18 K. L. YUDOWITCH, J. Appl. Phys., 20 (1949) 174.
- 19 G. POROD, Kolloid-Z., 124 (1951) 83.
- 20 G. POROD, Kolloid-Z., 124 (1952) 51. <sup>21</sup> G. POROD, Kolloid-Z., 125 (1952) 108.
- 22 L. H. LUND AND G. H. VINEYARD, J. Appl. Phys., 20 (1949) 393.
- 23 J. B. FINEAN, J. Sci. Instr., 30 (1953) 60.
- <sup>24</sup> M. SIEGBAHN, Phil. Mag., 48 (1924) 217.
- 25 A. ENGSTRÖM, B. ENGFELDT AND R. ZETTERSTRÖM, Experientia, 8 (1952) 259.

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