distributed striated muscle fibres alone remaining. The bulk of the transplant is composed of mesenchyme probably derived from the de-differentiation of the tail tissues. Among them are also to be found many pigmented cells. Where externally the appearance of the transplant most resembles a limb, internally nodular or elongated masses of healthy cartilage cells are found in the process of organization to form skeletal structures. In those specimens in which such organization has reached a maximum, it is possible to distinguish the appropriate parts of the pectoral girdle, stilo- and zeugopodia, carpus and digital cartilages. Surrounding the existing parts of limb skeleton are bundles of muscle fibres, almost certainly the developing limb musculature. More often, however, the girdle is absent, the skeletal components are reduced in size and number, and the musculature may be lacking.

(2) What can be said of the mechanism by which these changes are effected? As has been said previously, the progressive transformation outlined above can only occur if the graft is made into the limb field of the host. From its first appearance the limb field possesses capacity for induction, as has been shown by the present work. Probably chemical substances elaborated by the field pass into the graft. In most specimens no mesodermal cells from the host were to be found in the transplant; in the remainder they were present in extremely small numbers. Under the influence of these substances the first stages of degeneration are perhaps initiated in the donor tail, and subsequently the processes of reorganization which result in the formation of the limb from the tail. In corroboration, it should be noted that when the graft is made into a region other than the limb field, tail degeneration does not occur. Instead the tail maintains its shape and internal structure well beyond the period of metamorphosis of the Anuran host[1].

From what material is the limb formed? A study of histology directs attention to the differentiated mesenchymal cells among which are some that would naturally form the vertebral cartilages. It is probable that the limb skeleton differentiates from such cells under the inductive influence of the limb field. The caudal musculature probably redifferentiates to form the limb musculature.

(3) Recently HOLTFRETER[2] has obtained similar results to those outlined above. He has observed that tails obtained by induction (from transplants of pieces of mouse kidney into the blastocoel of *Triton alpestris*) and which find themselves in the host limb field, are transformed into limbs. He did not obtain the same results from direct transplants of the tail buds. It is possible that the explanation of the latter result is that the direct transplants were homoplastic. Xenoplastic grafts perhaps increase the inductive capacities of the limb field, as is shown by the frequent appearance of supplementary limbs.

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*Riassunto*

Bottoni caudali di Triton e di Axolotl trapiantati sul campo dell'arto di Discoglossus (allo stadio di neurula) si differenziano in code, le quali talvolta, all'epoca della metamorfosi dell'ospite, si trasformano in arti. Viene discusso il meccanismo di tale trasformazione.

[2] J. HOLTFRETER, J. exper. Zool. *129*, 623 (1955).

## On the Interpretation of the Low-Angle Scatter of X-Rays from Bone Tissue

ENGSTRÖM and FINEAN[1] demonstrated that, in addition to the wide-angle X-ray diffraction pattern, bone tissues also give a diffuse low-angle scatter. The same authors[2] assumed that the low-angle scatter could be treated as a particle scatter pertaining to the inorganic or mineral fraction. In this way they concluded that the particles are rod-shaped, the long axis 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 Å, and a length of about 210 Å.

Recently ROBINSON and WATSON[3] have criticized the conclusions of ENGSTRÖM and FINEAN, because observations with the electron microscope do not support the view that the inorganic particles are rod-shaped.

The findings which one of us published in this journal (see ASCENZI and CHIOZZOTTO)[4] on the electron microscopy of the organic bone substance, using the pseudoreplica technique, induced us to repeat and possibly to improve on the investigations made by ENGSTRÖM and FINEAN in order to find a more adequate interpretation of the low-angle diffraction pattern.

*Material and method.*— Longitudinal and cross sections (0·2 mm thick) of the femoral diaphysis of cattle were prepared by grinding. The low-angle scatter was recorded from the untreated sections as well as from similar sections from which the ossein had been removed according to GABRIEL's method (boiling in glycerol with 6% KOH). This procedure is unable to produce any change in the crystalline structure of the inorganic bone fraction[5] or increase in the size of the crystallites[6].

A low-angle scatter apparatus, somewhat similar to that employed by FINEAN[7] was used. The scatter was recorded using Ni filtered CuK$\alpha$ radiation ($\lambda = 1{\cdot}54$ Å)[8]. The maximal recorded scattering space corresponded to 250 Å. The intensity variation of the low-angle scatter was measured using a Leeds and Northrup automatic recording microphotometer.

*Results and discussion.*— The low-angle scatter of X-rays from longitudinal sections of bone shows a marked asymmetry (Fig. *a*), indicating an elongation of the elements or units responsible for the scatter along the longitudinal axis of the bone. On the contrary, the low-angle scatter obtained from cross-sections of bone (Fig. *b*) reveals no appreciable orientation.

This behaviour suggests, in agreement with ENGSTRÖM and FINEAN, that the scattering elements are well aligned and symmetrical around their long axes. Therefore the same elements appear as if they were like ellipsoids of revolution, the long axes being

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[2] J. B. FINEAN and A. ENGSTRÖM, Biochem. biophys. Acta *11*, 178 (1953); Exper. *10*, 63 (1954). – R. CARLSTRÖM and J. B. FINEAN, Biochim. biophys. Acta *13*, 183 (1954).

[3] R. A. ROBINSON and M. L. WATSON, Ann. New York Acad. Sci. *60*, 596 (1955).

[4] A. ASCENZI and A. CHIOZZOTTO, Exper. *11*, 140 (1955).

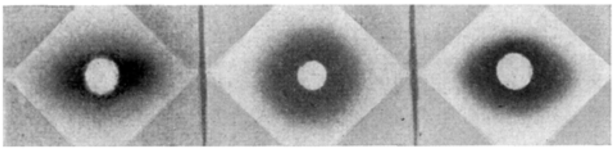
[5] M. J. DALLEMAGNE, J. Physiol. *43*, 425 (1951).

[6] R. KLEMENT and G. TRÖMEL, Hoppe-Seylers Z. physiol. Chem. *213*, 263 (1932); Klin. Wschr. *12*, 292 (1933). – E. BRANDENBERGER and H. R. SCHINZ, Helv. med. Acta, Suppl. 16, *12* (1945).

[7] J. B. FINEAN, J. sci. Instr. *30*, 60 (1953).

[8] J. B. FINEAN and A. ENGSTRÖM have observed that with a system of the type existing in the bone tissue, the use of Ni-filtered radiation (CuK$\alpha$) does not entail more substantial errors than the use of monochromatic radiation.

aligned in the direction of the longitudinal axis of the bone. The diameters of the ellipsoids calculated by applying GUINIER's theory come very close to the results obtained for the human bone by ENGSTRÖM and FINEAN. The short diameter is about 70 Å and the long one about 200 Å.



a b c

Still in agreement with the results of ENGSTRÖM and FINEAN, the low-angle scatter from the longitudinal sections of bone without ossein is much more symmetrical (Fig. c) than that obtained from untreated bone. This feature suggests that the shape and dimensions of the scattering elements are changed. In fact the short diameter of the ellipsoids is about 80 Å, while the long diameter is reduced to about 130 Å. In addition, the intensity of diffraction seems greatly increased by the removal of ossein.

The results provide the basis for the arguments in the following discussion.

The low-angle X-ray scatter might be interpreted either in terms of the *holes* irregularly placed inside a homogeneous body or in terms of a *particle scatter*, when inter-particle interference is not taken into consideration. In the general equation adopted by GUINIER in treating the scatter from such systems, the intensity of the scattered radiation appears as a function of the square of the difference of the electron densities pertaining to the single components responsible for the scatter (particles and surrounding medium or homogeneous body and holes). Such a condition does not enable us to establish, a priori, to which of the two aforesaid systems a low-angle scatter is related. In this respect the choice of the system is suggested by evidence derived from the data of other investigations.

The identity of the low-angle X-ray diffraction pattern pertaining, respectively, to the bone, collagen tissue[9] and calcified collagen[10] does not permit the acceptance of the theory that the scattering units pertain to inorganic particles, these latter being completely missing in collagen tissue. Therefore, the scattering units must be considered as related to ellipsoidal entities (200 × 70 Å) pertaining to the collagen and oriented in parallel. In bone and calcified collagen, the calcium salts, enclosing the organic ellipsoids, increase the difference in electronic density between the same organic particles of collagen and the surrounding medium.

This interpretation of the low-angle X-ray scatter from bone tissue in terms of holes (containing the organic particles) irregularly placed inside a homogeneous body (the inorganic bone fraction) is also supported by the following arguments:

(a) SCHMIDT, 20 years ago, studying the form birefringence of the bone, furnished evidence that the inorganic fraction should most likely be considered as a homogeneous body with very fine holes occupied by the micelles belonging to the ground substance[11].

(b) A bone system resulting from inorganic particles or crystallites, dispersed in the ossein, according to ENGSTRÖM and FINEAN, could not exist when ossein had been removed, since the particles would break down into an "incoherent powder". On the contrary, bone without ossein exists as a coherent structure, though its resistance is greatly decreased. In addition, the polarizing microscope and the low- and the wide-angle X-ray diffraction patterns demonstrate that the structural orientation of the inorganic fraction is unmodified. Finally, the form birefringence curve can be recorded from the bone without ossein[12].

(c) Electron microscopical investigations[13] have confirmed the results of SCHMIDT's investigations. According to ASCENZI and CHIOZZOTTO, the maximal diameter of the holes ranges from 200 to 250 Å. According to BARBOUR, the diameter of the same structures ranges from a minimum of 62 Å to a maximum of 225 Å.

(d) And lastly, it is very important to remark that the change observed in the low-angle scatter of the bone from which ossein has been removed appears as new evidence of the necessity to interpret the low-angle scatter of the bone as due to organic ellipsoidal particles enclosed in the holes circumscribed by the inorganic fraction. Indeed, such a change is neither related to the orientation nor the physical state of the inorganic crystallites, both conditions being unmodified by GABRIEL's treatment, according to the X-ray diffraction patterns. Therefore it is more plausible to maintain that in the homogeneous body pertaining to the inorganic bone fraction, the removal of the ground-substance from the holes results in an arrangement of the material delimiting the same holes. This view finds ready support in the existence of the chemical bonds between ossein and the inorganic bone fraction[14].

*Conclusions.*– This array of arguments supports the view that the inorganic bone fraction may probably be regarded as a homogeneous body with holes occupied by the micelles of the organic substance. The holes correspond to ellipsoids of revolution and are aligned with the axis of the bone. The present interpretation appears to be in agreement with the properties of the bone and not in contradiction to the knowledge derived from the wide-angle diffraction pattern. This last corresponds to the crystalline units. From their aggregation derives the homogeneous body with holes responsible for the low-angle X-ray diffraction pattern.

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*Riassunto*

Gli Autori dimostrano che lo spettro di diffrazione a basso angolo del tessuto osseo è determinato da micelle elissoidali (elissoidi di rivoluzione) delle dimensioni di 200 × 70 Å, pertinenti all'osseina e accolte in incavi delimitati dalla frazione inorganica.

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[13] A. ASCENZI and A. CHIOZZOTTO, Exper. *11*, 140 (1950). – E. P. BARBOUR, Amer. J. phys. Anthrop. *8*, 315 (1950).

[14] V. CAGLIOTI, A. ASCENZI, and M. SCROCCO, Exper. *10*, 371 (1954).