Acta orthop. scand. 44, 157–160, 1973

Department of Orthopaedics Budapest, Semmelweis University of Medicine,
Central Electron Microscope and X-ray Laboratory Gödöllö, Agricultural University,
and Department of Mineralogy and Petrology,
University of Technical Sciences, Budapest, Hungary

ELECTRON DIFFRACTION EXAMINATION OF THE GROWTH ZONE OF THE EPIPHYSIS

G. LÉNÁRT, I. VERES, G. BIDLÓ & L. KÉRY

Accepted 6. xii.72

The most important feature of the electron diffraction examination is the use of electron beams for the special analysis of ultrastructure. With this method it is possible to examine a few hundred Ångström thick sections of bone both from the crystallographic and morphological viewpoints.

The electron diffraction has definite advantages compared with the X-ray diffraction previously used for ultrastructural analysis of the bone. With X-ray diffraction only a greater volume of bone could be investigated, which is allways inhomogeneous and contains various phases of bone development and destruction (Lénárt et al. 1972). Electron diffraction, on the other hand, is suitable for examination of nearly homogeneous layers of bone, showing only this or the other phase of mineralogical development of the bone (Lénárt et al. 1971). This is because the ultrathin sections contain only one or a few bone crystals, mostly in the same phase of development or destruction.

The greatest difficulty in the examination of epiphyseal mineralization is also the inhomogeneity of the growth zone of the epiphysis, i.e. the most important area of the ossification. In this zone various phases of bone formation, comprising cartilaginous parts and bone tissue in the early and later stage of mineralization, can be found intermingled in a relatively small area.

In the past, the crystallographical examination of this zone was also based mainly on the X-ray diffraction method (Lénárt et al. 1971). The disadvantage of this method was that the analysis of this rather heterogeneous zone could be performed *in toto* only and so it did not

render possible a detailed examination of the various phases of the physiological mineralization.

The distinct analysis of these phases was solved by using electron diffraction as described above. By this method it is possible to examine, from the crystallographical viewpoint, strata of 400–600 Å thickness in the growth zone of the epiphysis and to observe separately in these nearly homogeneous layers, the different mineralogical phases of ossification.

MATERIAL AND METHODS

The growth zone of the proximal tibial epiphysis of 3-month-old calves was used for the examination. After excision the samples were freeze-dried and embedded in Durcupan. The transversal ultrathin sections were prepared on a Reichert ultramicrotome by using glass knives. The section thickness was about 400–600 Å. A Tesla BS-242 electron microscope was applied at an accelerating voltage of 60 kV and a maximal resolving power of 20–25 Å. Kodak Bromid Lantern L-10 plates of especially high contrast were used: they were advantageous for the detection of diffraction lines and diffraction points.

In order to evaluate diffraction patterns the diameter of the rings as well as of points of symmetrical localization (2 r) were measured. The length (L) of the tube and the wave length (λ) were reckoned from calibration photos. Gold powdered in vacuum served as calibration material. The calculation was based on Bragg's formula $d = \frac{L\lambda}{r}$ where d is the interplanar spacing and r the radius of the diffraction ring.

RESULTS

At the assessment of the results it must be taken into consideration that the main question of bone crystallography is still the decision of the problem whether bone contains hydroxylapatite ($Ca_5(PO_4)_3.F.OH$) alone, other calcium phosphates as well, or a mixture of these compounds (e.g. brushite $CaHPO_4.2H_2O$, monetite $CaHPO_4$). If a greater amount of bone is examined, as occurs during the X-ray diffraction investigation, various kinds of crystals may be examined together. In this case the hydroxylapatite, being of greater quantity, may handicap the detection of other crystals, which are possibly its precursors. With the use of electron diffraction this difficulty can be avoided.

The electron diffraction analysis of the growth zone of the proximal tibial epiphysis showed the presence of brushite (Figure 1, Table 1) and poorly developed apatite (Table 2) at the metaphyseal part of the zone.

Figure 1. Electron diffraction pattern of the metaphyseal part of the growth zone.

Table 1. The electron diffraction pattern of the metaphyseal part of the growth zone shows the presence of brushite.

dh k l Å	Intensity
4.25	2
2.75	5
1.72	2
1.52	2
1.35	2

Table 2. The electron diffraction pattern of the metaphyseal part of the growth zone shows the presence of poorly developed apatite.

dh k l Å	Intensity
2.79	3
2.05	3

DISCUSSION

The difficulties in the interpretation of the diffraction lines in bone crystallography are well known (Münzenberg 1970, 1971, Posner 1969).

The small number of diffraction lines in our cases can be explained by the fact that the growth zone is an area in the very stage of mineralization, and its crystallization has not yet reached the usual degree of the fully developed bone mineral. The mineralogical finding described above supports the assumption that in the formation of bone

apatite secondary calcium phosphates may also play a role. As in all other parts of the bone there is a constant destruction and rebuilding of the mineral, the above assumption could be most readily supported in the metaphyseal part of the growth zone of the epiphysis where crystallization has a leading role.

The results of the examination described above and of other electron diffraction examinations of developing bone tissue (Lénárt et al. 1971) show the use of this method in special problems of bone physiology. It can be stated that for the time being electron diffraction seems to be the only means for investigating separately the various phases of mixed biological calcified tissues.

SUMMARY

The growth zone of the tibial epiphysis of calves was investigated by electron diffraction. For the time being this method seems to be only suitable for a differentiated investigation of the various phases of mixed biological calcified tissues. The finding supports the assumption that in the formation of bone apatite secondary calcium phosphates may also play a role.

REFERENCES

Lénárt, G., Bidló, G. & Kéry, L. (1971) X-ray diffraction investigation on the growing zone of long bones. *Acta biochim. biopsys. Acad. Sci. hung.* **6**, 307–309.

Lénárt, G., Veres, I., Bidló, G. & Pintér, J. (1971) Bone mineralization investigated by electron diffraction. *Acta biol. Acad. Sci. hung.* **22**, 431–434.

Lénárt, G., Bidló, G. & Pintér, J. (1972) Some basic problems in the examination of the calcium hydrogen phosphates of bone. *Clin. Orthop.* **83**, 263–272.

Münzenberg, K. J. (1970) Untersuchungen zur Kristallographie des Knochenminerals. *Biomineralisation* **1**, 69–100.

Münzenberg, K. J. (1971) Die Calcifikation bei der Knochenbildung. *Arch. orthop. Unfall-Chir.* **71**, 41–54.

Posner, A. S. (1969) Crystal chemistry of bone mineral. *Physiol. Rev.* **49**, 760–792.

Correspondence to:

G. Lenart, M.D.
Orthopaediai Klinika
Budapest, XI, Karolina ut 27, Hungary