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CRYSTAL ARRANGEMENT IN ANORGANIC BONE*

by

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Since *Losee & Hurley* in 1956 reported the first very promising results on the use of anorganic bone as an implant material, there has been a rather extensive use of this material in many surgical procedures involving treatment of osseous lesions. Anorganic bone is obtained by treating whole bone with ethylenediamine, after the method of *Williams & Irvine* (1954). The end result is the approximately pure inorganic framework of whole bones.

For early reports with rather short observation periods, the host response to anorganic bone was reported unusually good, but when longer follow-up studies were published (*Boyne & Lyon,* 1959, and *Bell,* 1960, 1961), it was noted that resorption of anorganic bone was very slow; in many cases no sign of resorption was noticed at all *(Hjørting-Hansen).*

Macroscopically, anorganic bone is very porous and should permit a rather extensive capillary ingrowth, but microscopically the areas which show capillary ingrowth are very small compared with the total area of the implanted bone particles *(Hjørting-*

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Hansen). It was felt worthwhile to evaluate the ultrastructure of anorganic bone to see if there might be some reason for the failure of complete utilization of such implants by the host.

MATERIALS AND METHODS

Pure anorganic bone,* MESH 10/20 made from bovine femur head according to the method of *William & Irvine* (1954), was embedded in equal parts of butyl and methyl methacrylate, and sectioned with a diamond knife mounted in Porter-Blum microtome. The sections were from 200—300 Å thick, and were placed directly onto formvar grids. No shadowing or staining techniques were used. The specimens were examined in a Phillips EM-75B at 50 KV. Electron diffraction was also carried out.

RESULTS

The sectioning proved easier than was expected, and in many instances fairly long ribbons were obtained. Fig. 1 shows at relatively low magnification several lamellae showing what is recognized in light microscopy as alternating punctuate and striate lamellae, representing the different orientation of collagen. Where the plane of section was parallel to the long axis of the position which the collagen had occupied (Figs. 1—2), the crystals of apatite had retained a position which reflects the periodicity of collagen. These periods are approximately 700 Å apart. Fig. 2 shows more detail of Fig. 1.

Fig. 3 is a high magnification of a lamella in which the collagen had previously been parallel to the plane of sections. The negative image of the collagen can still be seen, and those periods are almost, but not quite, in register across the whole field. Electron diffraction patterns showed typical apatite.

DISCUSSION

The 700 Å spacing seen in these sections is greater than usually described in native collagen. However, *Bear* (1942 and 1944) has shown that the 640 Å periodicity seen in electron microscopy is

* The anorganic bone was kindly supplied by Armour Company, Chicago, U. S. A. under the trade name "OSSAR".

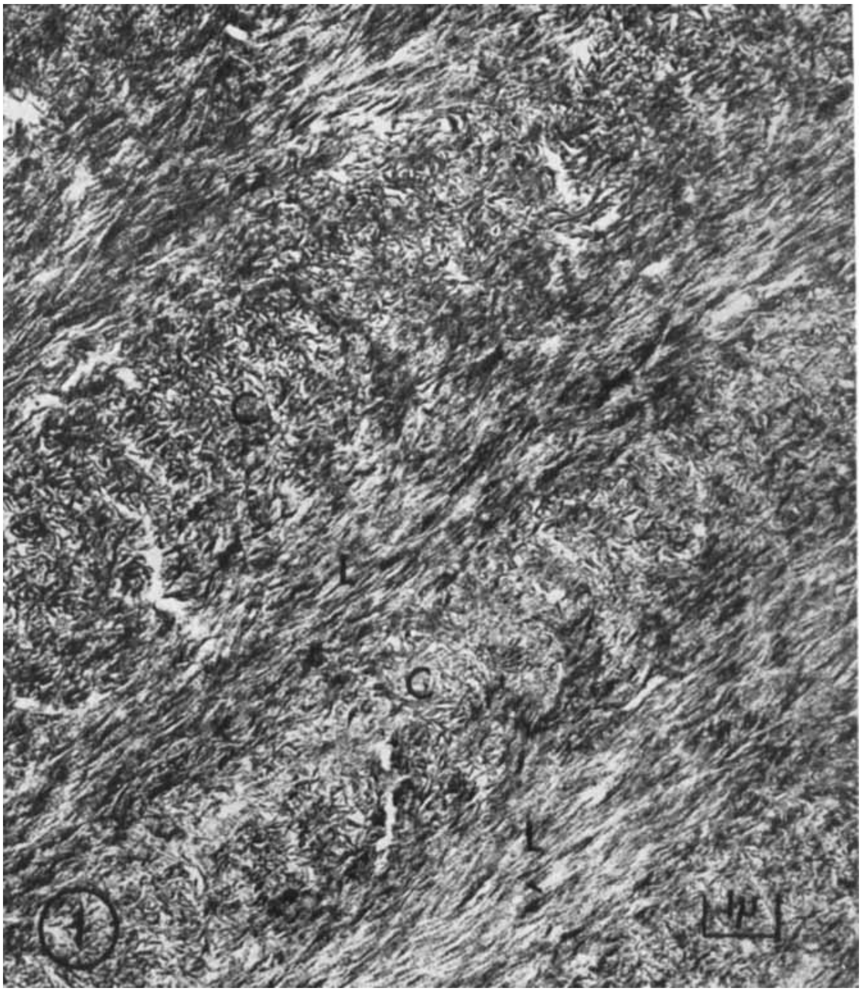

Fig. 1.

Electron micrograph of a thin section of anorganic bone. Alternating lamellae can be seen. (C) Lamellae in which the collagen fibers had been perpendicular to plane of section. (L) Lamellae in which the collagen had been parallel to the plane of section. The negative image of collagen can be seen.

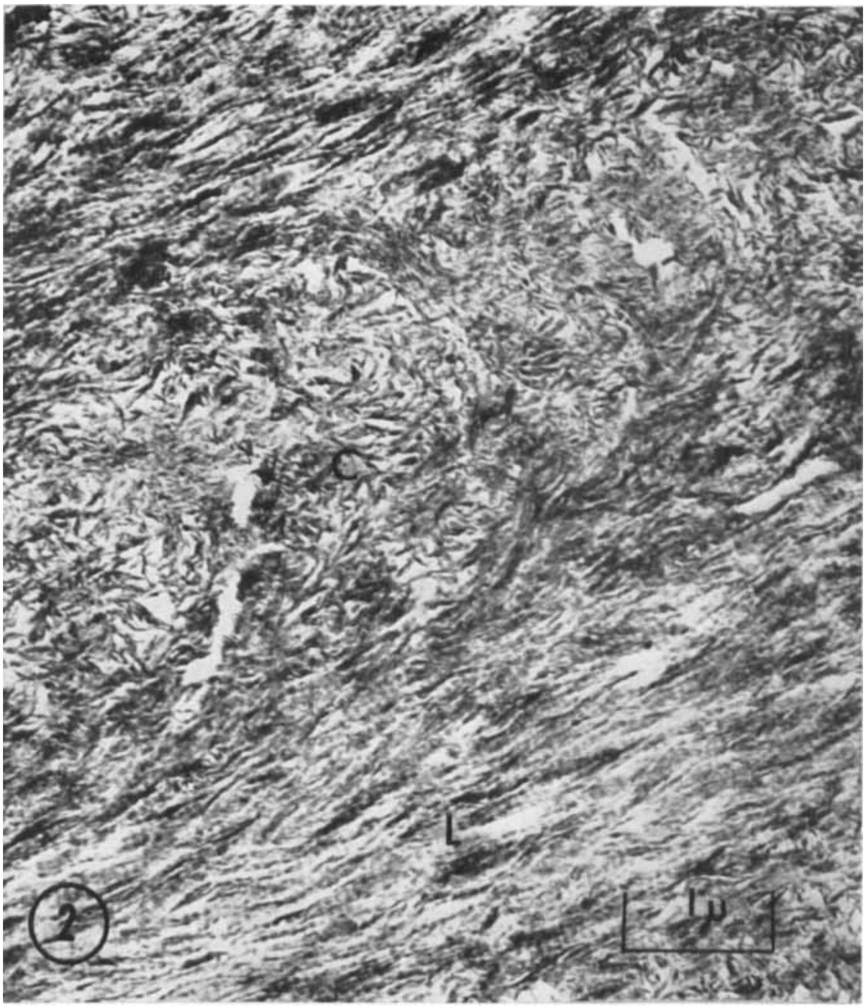

Fig. 2.

Higher magnification of Fig. 1.

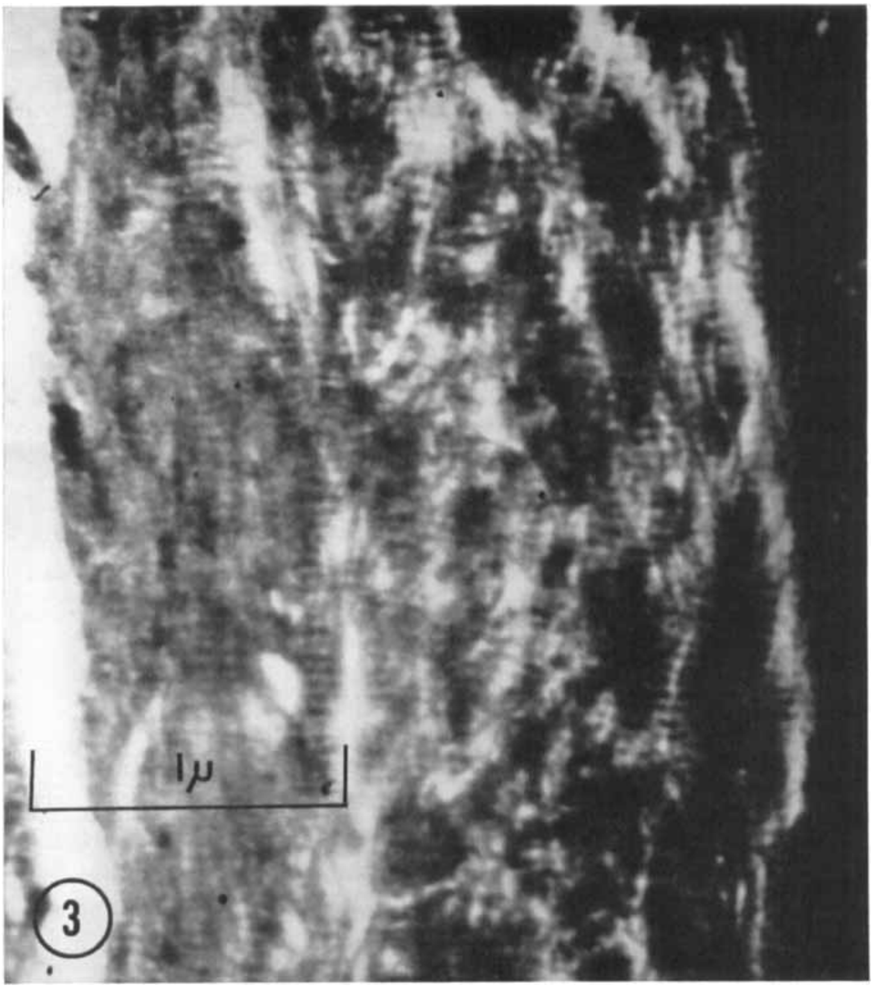

Fig. 3.

Very high magnification of anorganic bone. The almost perfect coincidence of the striations across the field can be seen.

of dried collagen and that moist collagen periods are close to 700 Å. It is suggested in the present study that, although the preparations were dried, the rigidity of the tissue due to the presence of apatite prevented shrinkage from occurring. From the chemical analysis of the anorganic bone (nitrogen 0.4 %), it was felt that no collagen remained, and that therefore the periodicity was due solely to the arrangement of the crystallites. The almost perfect alignment of the crystals across a considerable distance agrees with the findings of *Scott & Nylen* (1959) in the collagen of the mineralizing turkey tendon.

The failure of anorganic bone to be utilized in healing is perhaps due to the high degree of organization of the apatite. This study offers no explanation of why this organization persists. It can be speculated that the crystals are bound tightly to each other, and therefore would be relatively unavailable to the host. This study will be continued to determine if there are any changes in the ultrastructure of anorganic bone after varying periods of implantation in an experimental animal. Electron diffraction of these crystals showed typical apatite patterns, and it is thought that there was no change in the mineral phase.

SUMMARY

Electron micrographs of commercially prepared anorganic bone revealed that the crystallites retain their relationship as if the collagen was still present. The periodicity of the crystals was 700 Å approximately, reflecting the periodicity of moist collagen. This periodicity was almost, but not quite, in register across the lamella. It is suggested that the high degree of orientation of the apatite may account for the reported failure of the utilization of anorganic bone by the host.

RÉSUMÉ

DISPOSITION DES CRISTAUX DANS L'OS INORGANIQUE

Des microphotographies électroniques d'os inorganique préparé commercialement ont révélé que les particules cristallines conservaient leurs rapports comme si le collagène était encore présent. La périodicité des cristaux était d'environ 700 Å, ce qui

correspond à la périodicité du collagène humide. Cette périodicité se trouvait presque — pas tout à fait cependant — à un même niveau au travers de la lamelle. Les auteurs émettent l'hypothèse que l'échec rapporté en ce qui concerne u'utilisation de l'os inorganique par l'organisme-hôte puisse être mis sur le compte du degré élevé d'orientation de l'apatite.

ZUSAMMENFASSUNG

ANORDNUNG DER KRISTALLE IN ANORGANISCHER KNOCHENSUBSTANZ

Durch elektronenmikroskopische Aufnahmen von kommerziell hergestellter anorganischer Knochensubstanz wurde festgestellt, dass die Kristallite ihre gegenseitige Anordnungsweise so bewahrten, als wäre tatsächlich Kollagen vorhanden. Die Periodizität der Kristalle betrug ca. 700 Å, der Periodizität ungetrockneten Kollagens entsprechend. Quer über die Lamellen lag die Periodizität fast im Register, aber doch nicht ganz genau.

Es wird die Hypothese aufgestellt, dass sich das beschriebene Versagen der Ausnützung der anorganischen Knochensubstanz beim Wirtsorganismus durch die sehr feste Orientierung des Apatits erklären lasse.

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