These comparative measurements by interference microscopy and chemical analysis prove that at least four-fifths of the myosin in these fibrils is present as the *A* substance, and the results are in excellent agreement with the hypothesis that *all* the myosin is concentrated in the *A* bands.

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# ELECTRON MICROSCOPY AND X-RAY DIFFRACTION OF BONE

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Recently, the apatite-containing structures in the organism have attracted considerable attention because of their marked ionic exchange properties. The capacity of the apatites in biological systems to bind and release various types of ions has a special physiological significance. Special interest attaches to these phenomena in connection with infection with "bone-seeking" radioactive substances.

The adsorptive properties of the bone apatite depend upon the extremely small size of the individual hydroxyapatite particles, and hence the elucidation of the submicroscopic organization of bone tissue is of fundamental importance.

Polarized light studies[1] have revealed that the collagen fibres, constituting the bulk of the organic fraction of bone, are capable of "adsorbing" the apatite particles in a highly ordered way. The hydroxyapatite belongs to the hexagonal system and since in X-ray diagrams the *oo*l-reflections are less broadened than the *h*oo-lines it is clear that the crystallites are elongated with their *c*-axis parallel with the long axis of the crystallite. Moreover, the wide-angle X-ray diffraction diagrams of oriented bone specimens indicated that the *c*-axes of the crystallites are arranged parallel with the collagen fibres[2,3]. Bone tissues gives a diffuse low-angle X-ray scattering[4] most probably attributable to the apatite particles. The low-angle X-ray diagrams, also, indicated that the particles were elongated and aligned along the collagen fibre axis. From these studies the dimensions of the apatite particles were estimated to be 40–75 A

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wide and about 200 A long. The long dimension agrees well with the value 230 ± 20 A calculated from the integral line width of the 002-reflection[3]. Additional evidence derived from the low-angle studies suggested that the apatite particles were accurately adjusted at certain sites of the collagen fibres[3,5].

In contrast to the X-ray diffraction studies the first comprehensive electron microscope studies of bone tissue[6] described plate-like structures, 350–400 A long, with a width of about the same value, and a thickness of only 25–50 A. Ovaloid particles that can reach a length of up to 1300 A have also been described[7].

The discrepancy between the X-ray findings and the published electron microscope studies suggested a reinvestigation of bone tissue with preparation techniques adequate for high resolution electron microscopy.

## METHODS

The development of an ultramicrotome equipped with a diamond knife[8] now makes it possible to obtain undistorted serial ultra-thin sections of extremely hard and brittle solids like certain metals and bone[9]. These techniques are suitable for obtaining a large number of sections of hard materials, which is important for a systematic study of bone.

After dehydration in ethyl alcohol, undecalcified bone from fish, hen, rat and human were embedded in methacrylate. Specimens were also fixed in buffered 1% osmium tetroxide or stained with phosphotungstic acid (2%). Several thousand ultra-thin serial sections were prepared using the Moran Ultramicrotome equipped with a standard diamond knife[9]. The average sections were 100 to 200 A thick. However, for most of the high resolution work presented here even thinner sections of the order of 50 A were prepared by a special technique using this equipment. These sections, which are hardly visible under the electron microscope, are particularly suitable for a study of the macromolecular structure of bone. The sections were examined with an RCA EMU-3B electron microscope working at 50 and mostly at 100 kV. This instrument, provided with a specially regulated power supply, has an objective aperture of 15–20 microns, which can be compensated for electrostatically. The micrographs were taken at electron optical magnifications of 12,000 to 51,000 diameters and the very high voltage stability of this instrument made it possible to use special Kodak high resolution plates (KP 41394) with exposure times upto 10 seconds at the highest electron optical magnifications. These extremely fine grain plates can be subsequently enlarged up to 100 times, thus making the achievement of final magnifications of 1 to 1.5 million diameters possible, a necessary step in evaluating details of the order of 20 A.

The specimen films for this high resolution work were made of formvar with holes of 0.1 to 1 micron, which were covered subsequently with a very thin carbon film. In some cases the thin sections were lying free of any background structure over the holes in the film. Since the specimen blocks were 0.2–0.4 mm, extensive areas of the sample could be examined in serial sections.

## RESULTS

Examination of longitudinal sections through compact bone reveals compact bundles of dense filamentous components with a characteristic fine structure. In suitable, thin sections and at higher magnifications (Fig. 1) it is seen that these dense filaments are formed by needle-shaped particles, about 40 A in diameter and approximately 200 A long. The particles are aligned longitudinally on a fibrous substrate, having a typical collagen periodicity of 660 A (Fig. 2). The rod- or needle-like elements (Fig. 3), have indications of a fine structure with a periodicity of about 50–60 A (Fig. 4). In addition to this highly ordered arrangement many areas are seen with random orientation of the unit particles, which here reach lengths of over 1000 A (Fig. 3). That we are really dealing with rod-shaped elements is clearly shown in cross sections (Figs. 6–9). Here a regular pattern consisting of circular or hexagonal dense points of approximately 30–40 A in diameter is seen. The dense points are regularly distributed around lighter

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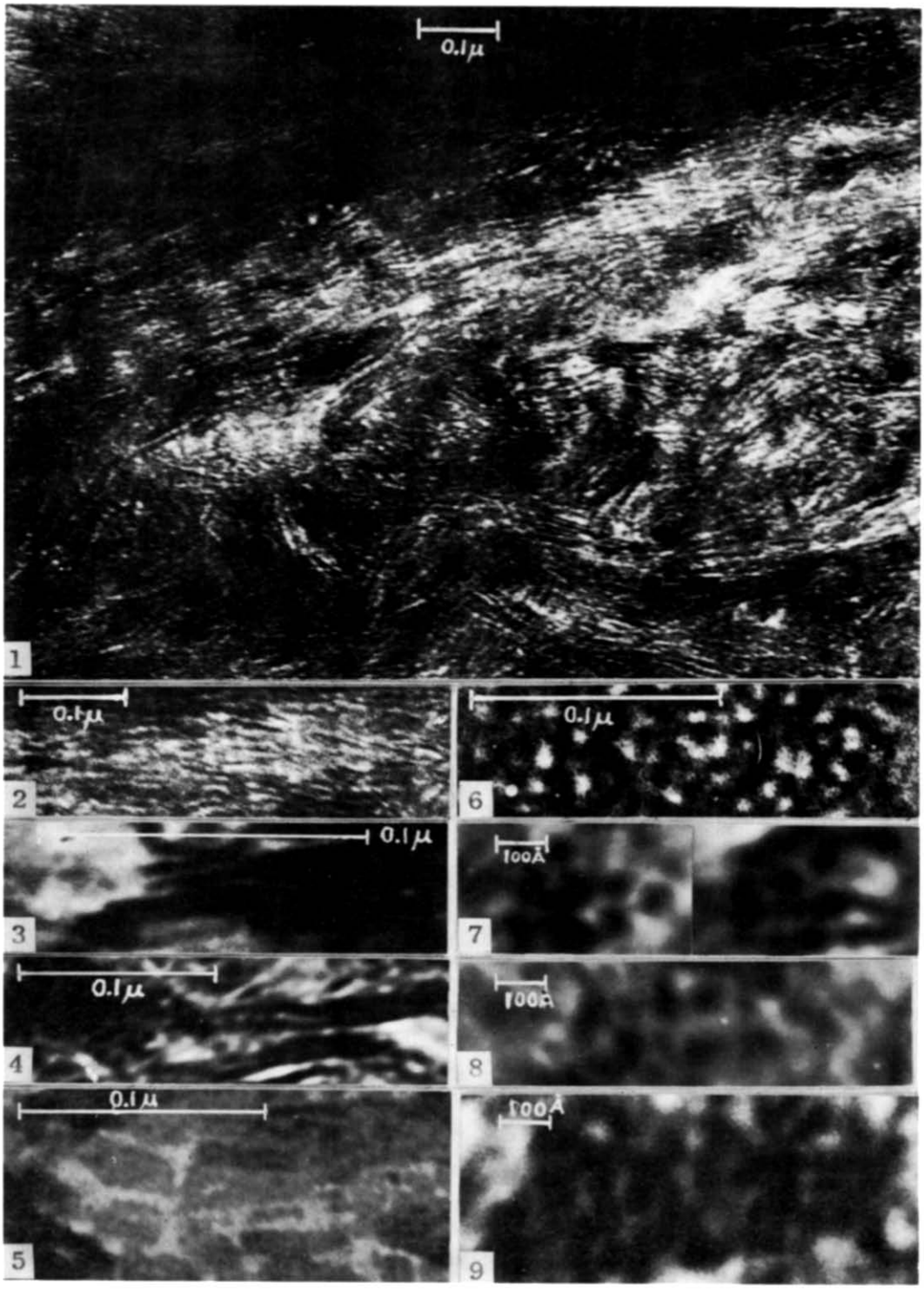
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areas of various widths, (Figs. 7–9). These intermediate spaces show various degrees of density and have not been clearly resolved, but presumably they correspond to the dehydrated organic matrix. In all sections of various types of bone examined up till now, the predominant structure encountered is a system of rod or needle-shaped apatite particles aligned along the collagen fibres of various diameters. Electron diffraction diagrams made of the sections show apatite-like patterns.

In certain areas, however, very thin flakes of 20 to 50 A thickness and 300 to 500 A in diameter, with rounded or angular contours, are encountered irregularly distributed among the fibrous elements. These flakes show a fine internal structure represented by regular striations of 60 to 100 A, indicating that they are possibly formed by lateral aggregation of elongated elements. Flakes with distinct holes or spots forming a regular pattern of approximately 60 A diameter are also found (Fig. 5).

Thus the evidence derived from electron microscopy conforms with the results of polarized light and X-ray diffraction studies and lends support to the view that we are dealing with rod- or needle-shaped crystallites, 30–60 A in diameter and with an average length of about 200 A, oriented in an orderly way on the fibrous elements of the organic matrix of bone. Furthermore, electron microscopy visualizes directly the highly regular arrangements of these elements, and their relationship with the cellular components of bone. Thus in osmium-fixed specimens, osteocytes in the lacunae[9] and the structural relationships between the bone marrow cellular elements and the compact osseous tissue are distinctly shown. In the latter areas extremely small rod-shaped particles, 20–30 A long, appear to aggregate linearly to form longer elements. These details, and the wealth of structure now opened to investigations by means of high resolution electron microscopy of bone, will be reported in detail in a later publication.

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## SUMMARY

Evidence derived from X-ray crystallographic and polarized light studies point to the existence of rod-or needle-shaped apatite particles in bone. From low-angle X-ray scattering studies and from measurements of the profiles of the wide-angle X-ray lines a particle diameter of about 50 A was derived. The application of the diamond knife to the sectioning of intact bone has made it possible to obtain ultra-thin sections of bone tissue, suitable for electron microscopy. The electron micrography indicated that the apatite particles are needle-shaped, 30–60 A in diameter and aligned along the collagen matrix.

Fig. 1. Section of undecalcified hen bone showing alignment of apatite particles along collagen fibres (120,000 ×).
Fig. 2. Needle-shaped apatite particles precisely aligned on the collagen periodicity (160,000 ×).
Fig. 3. High resolution micrograph of long needle-shaped apatite particles (500,000 ×).
Fig. 4. Indications of axial periodicity of about 60 A in apatite needles (300,000 ×).
Fig. 5. Flakes with internal granular structure associated with rod-shaped particles (370,000 ×).
Fig. 6. Ultra-thin section of bone showing cross-sections of apatite particles separated by holes or areas of low density (370,000 ×).
Figs. 7, 8, 9. High-resolution micrographs of 50 A thick sections showing the hexagonal or circular cross-sections (30–40 A) of individual apatite particles. Taken with 100 kV on Kodak high-resolution plates (750,000 ×).

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# SUR QUELQUES ESSAIS DE PURIFICATION DE LA LIPASE PANCRÉATIQUE

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La purification de la lipase pancréatique mérite d'être entreprise car: (a) cet enzyme joue un rôle important pendant la digestion intestinale des graisses; (b) doué apparemment d'une certaine solubilité dans l'eau mais capable d'hydrolyser des substrats insolubles à une vitesse considérable, il doit posséder des propriétés et une structure bien particulières; (c) les travaux récemment consacrés à l'étude du mode d'action de la lipase sur les triglycérides *in vitro*[1–3] auraient intérêt à être répétés avec des préparations enzymatiques pures.

Plusieurs tentatives sérieuses[4–6] ont d'ailleurs été faites en vue de cette purification. Mais les résultats obtenus jusqu'ici se prêtent mal à une discussion objective car deux erreurs fondamentales ont été commises. La première est d'avoir considéré *a priori* la lipase comme un enzyme instable et d'avoir voulu la stabiliser par diverses substances qui gênent ultérieurement la purification[4]. En fait, la lipase jouit d'une stabilité satisfaisante dès que les enzymes protéolytiques qui l'accompagnent dans les extraits pancréatiques sont inhibés ou éliminés[7]. La deuxième erreur est d'avoir utilisé des techniques incorrectes pour la mesure de l'activité lipasique. Les critiques formulées au sujet de ces techniques sont déjà connues[7–9]. Tantôt, le substrat est choisi de façon si malheureuse[5] que la nature même de l'enzyme en cours de purification paraît incertaine. Tantôt, les conditions dans lesquelles s'effectuent les mesures sont si peu conformes aux règles élémentaires de l'enzymologie[4] qu'il est difficile d'attribuer aux résultats une signification précise. Le rendement des opérations et le degré de purification réellement obtenu restent donc douteux. Toutefois, malgré tant de défauts qui en limitent singulièrement la portée, les travaux antérieurs sur la lipase suggèrent que cet enzyme passe aisément en solution quand on traite de la pancréatine bien dégraissée par de l'eau ou divers liquides aqueux et qu'il précipite comme une protéine ordinaire par addition de sels neutres, d'acétone ou d'alcool.

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