BONE STRUCTURE IN OSTEOMALACIA, WITH SPECIAL REFERENCE TO ULTRASTRUCTURE

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Many workers have shown that vitamin D deficiency induces changes in bone ultrastructure in animals. Engfeldt and Zetterström (1955) showed that there were abnormalities in osteoid tissue and in its calcification by applying microradiography and x-ray diffraction to the study of bone in rachitic rats. Termine and Posner (1967) showed by crystallographic methods that the average size of crystals went up and crystallinity fell.

Electron microscope work on bones from rats with rickets (Robinson and Sheldon 1960, Sheldon and Robinson 1961a and b) did not reveal any important change in cells or collagen fibrils, except that fibrils appeared in slight disarray and their thickness varied in an unusual way.

Several workers have studied the structure and calcification of osteoid tissue in human osteomalacia (Frame and Frost 1965; Laval-Jeantet, Matrajt, Juster and Hioco 1967), but the fine structure of malacic bone in man has—so far as we know—been left untouched. This paper discusses four cases of osteomalacia in humans, secondary to vitamin D deficiency. It discusses the ultrastructural changes produced in osteoblasts, osteoid tissue and in calcification, and compares them with the same changes already investigated histologically by two of us (Matrajt, Bordier and Hioco 1967).

MATERIAL

The diagnosis of osteomalacia was based on three criteria (Table I): radiological (Looser pseudo-fractures), histological (increased amounts of osteoid tissue, with calcification below normal) and biochemical (alkaline phosphatase content in the serum above normal; and serum phosphate content below normal (except in one case)).

METHODS

Optical microscopy—Two methods were used for optical studies in each case. Two bone cylinders (each 14 millimetres long and 4–6 millimetres in diameter) were taken from the iliac crest (Matrajt, Bordier and Hioco 1967). One cylinder was fixed in buffered formol (pH 7·2), and included in Stratyl without being decalcified (Matrajt, Bordier, Martin and Hioco 1967). A Jung microtome was used to get a series of sections 6 to 8 μ thick. Sections were stained with toluidine blue at pH 2·8 and solochrome cyanine R (Matrajt and Hioco 1966). The other cylinder was divided in two. One part was fixed in Rossman for glycogen detection, and the other was used for electron microscopy.

Electron microscopy—Small fragments of the bone cylinders were taken. They were fixed in 2 per cent osmium tetroxide buffered to pH 7·2, then dehydrated in acetone and embedded in Araldite. A Porter-Blum microtome was used to obtain ultra-thin sections. They were left floating on water for a maximum of three minutes. This was to avoid decalcification (Boothroyd

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Untreated sections, or sections stained with uranyl acetate or lead citrate, or with phosphotungstic acid, were examined under a Siemens Elmiskop IA electron microscope.

### TABLE I

**LABORATORY FINDINGS IN FOUR CASES OF OSTEOMALACIA**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Pseudo fractures</th>
<th>Serum calcium (milligrams per litre)</th>
<th>Serum phosphate (milligrams per litre)</th>
<th>Alkaline phosphatase (uB)</th>
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<tr>
<td>1</td>
<td>Female</td>
<td>67</td>
<td>-</td>
<td>89</td>
<td>25</td>
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</tr>
<tr>
<td>2</td>
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<td>74</td>
<td>+</td>
<td>85</td>
<td>21</td>
<td>7</td>
</tr>
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<td>-</td>
<td>71</td>
<td>36</td>
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<td>60</td>
<td>-</td>
<td>90</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td>98.3</td>
<td>35.4</td>
<td>&lt;1.5</td>
</tr>
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</table>

Case 1: typical nutritional osteomalacia.
Case 2: malabsorption osteomalacia.
Case 3: nutritional osteomalacia with clinical and radiological signs, but normophosphatemia which reflects deficiency of vitamin D.
Case 4: nutritional osteomalacia, with subnormal absorption of vitamin D$_2$ ³H.

### RESULTS

**OSTEOBLASTIC CELLS**

The term "osteoblastic cells" is used instead of "osteoblasts" because the cells found in contact with the osteoid matrix varied morphologically. Obviously any attempt to classify these cells must be partly artificial, because intermediate forms can always be detected. All the same, three main groups require detailed description.

**Optical microscopy.** *Group 1.*—Cells in this group are morphologically identical with normal, active osteoblasts (Fig. 1). They are easily recognisable owing to their ovoid nucleus, poor chromatin, and at least one conspicuous nucleolus. The cytoplasm is large, but its basophilia is lower than in normal osteoblasts. Moreover, it contains, chiefly round the nucleus, granules stained by the periodic acid-Schiff method. These granules must be glycogen clusters because they disappear after ptyalin digestion.

These cells in malacic bone are sparser than are osteoblasts in normal bone. But the total number of them may be higher, because abnormally big areas of osteoid tissue are present in osteomalacia.

*Group 2.*—The histological characteristics of these cells are not very different from those in *Group 1* cells (Fig. 2). However, they can be distinguished by the characteristics of their
Part of a Group 1 osteoblastic cell and of the adjacent osteoid tissue. The cytoplasm contains many flattened, regularly ordered granular cysternae, swollen mitochondria, glycogen rosettes, and lysosome-like structures. Some cytoplasmic processes penetrate the osteoid tissue, which is formed by collagen fibrils of almost uniform thickness. Uranyl acetate-lead citrate staining. (× 22,000.)

Figure 5—Part of the cortical cytoplasm of a Group 1 osteoblastic cell, showing glycogen rosettes, lysosome-like structures, flattened granular cysternae, a mitochondrion, and very thin filaments almost parallel to the plasma membrane. Uranyl acetate-lead citrate. (× 65,000.)

Figure 6—The Golgi apparatus in a Group 1 osteoblastic cell. Note the elongated vacuoles containing very electron-dense material. Uranyl acetate-lead citrate. (× 32,000.)
nuclei, which are larger and more irregular in form than normal. Their chromatin is very pale and uniformly stained. It contains one or no visible nucleoli. Their cytoplasm has no appreciable basophilia. It always contains large amounts of glycogen.

These cells have the same kind of general appearance as young progenitor cells. Group 3—These are flat cells, morphologically similar to the "resting osteoblasts" (Pritchard 1956) often found along calcified trabeculae in normal bone (Fig. 3). Their nuclei, too, are flat and dark. Their cytoplasm is not clearly appreciable. It contains much more glycogen than do "inactive" osteoblasts in normal bone.

Electron microscopy. Group 1—The fine structure of these cells (Figs. 4 to 6) is like that of normal osteoblasts. They are large cells in contact with the osteoid tissue, but in malacic bone they do not lie so close together as usual. The cytoplasm of these cells contains a highly developed endoplasmic reticulum (Figs. 4 and 5). The granular cisternae are usually narrow, elongated and regularly ordered. They form complex structures by anastomosing each other. A few are irregularly enlarged. Polysomes are often seen where granular cisternae are cut tangentially. There is an agranular endoplasmic reticulum near the nucleus, where the Golgi area would normally be. It consists of elongated cisternae, swollen vesicles and a few elongated vacuoles containing electron-dense material (Fig. 6). There are not many mitochondria. A large proportion of them are rather swollen (Figs. 4 and 5). In this case their cristae are irregular and difficult to see.

A few lysosome-like structures—round or oval in shape and containing electron-dense material—are present in the cytoplasm. They are usually seen near the plasma membrane (Fig. 5).

Electron-dense particles, recognisable as glycogen from their shape, are scattered between the rough endoplasmic reticulum and near the plasma membrane (Figs. 4 and 5). This membrane is usually clearly recognisable. Close to it the cytoplasm contains very fine filaments—often collected in bundles—glycogen particles, a few dense vesicles and isolated ribosomes (Figs. 4 and 5). Cytoplasmic processes penetrating the osteoid matrix are sometimes seen (Fig. 4).

The nucleus is large, often eccentric and contains one or two prominent nucleoli. Group 2—These cells lie in contact with the osteoid matrix, like Group 1 cells, but their ultrastructure is different. They are nearly as big as normal osteoblasts, but rather longer (Figs. 7 and 8). The main differences between the cells in Groups 1 and 2 are found in the cytoplasm. In Group 2 there are practically no granular cisternae. The few which are present are usually small and isolated.

Most of the cytoplasm is full of free ribosomes—often organised in polysomes (Fig. 8)—and large numbers of mitochondria (Fig. 7). These mitochondria are sometimes almost in mutual contact. Some of them are swollen, but their fine structure does not differ much from normal.

Large amounts of glycogen are recognisable in the cytoplasm (Figs. 7 and 8). Although a few isolated rosettes are present, most rosettes are found in large aggregates and can be found in any part of the cytoplasm except the Golgi area. Glycogen particles are often seen in close contact with the plasma membrane. Very fine filaments are present right through the cytoplasm, but especially in the cortical cytoplasm (Fig. 8).

There is always a highly developed agranular endoplasmic reticulum (Figs. 7 and 8). It contains agranular cisternae appearing in parallel lines, and, besides these, dilated vacuoles and small vesicles. Some of these vesicles are round and are not electron-dense. Others are elongated and contain a substance which has a strong affinity for osmium (Fig. 8).

A few lysosome-like structures are present, some of them near the plasma membrane. The nucleus is large and often ovoid. It is sometimes branched and usually contains a prominent nucleolus (Fig. 7). Group 3—These cells (Fig. 9) are always elongated, and their cytoplasm has an endothelial shape.
Part of a Group 2 osteoblastic cell. The cytoplasm does not show granular endoplasmic reticulum, but many mitochondria, glycogen clusters and lysosome-like structures. The Golgi apparatus (lower right corner) is similar to that of Group 1 cells. The nucleus contains a prominent nucleolus. Uranyl acetate-lead citrate. (×18,000.)

FIG. 7

Part of a Group 2 osteoblastic cell, in which a large portion of the Golgi apparatus is shown. Note the elongated vacuoles containing very electron-dense material. Thin filaments are present in the cytoplasm, including the cortical cytoplasm. A few dilated granular cisternae and many free ribosomes are also shown. Uranyl acetate-lead citrate. (× 28,000.)

FIG. 8
There is not much granular endoplasmic reticulum. Only two or three rough cysternae are usually present in the whole cell. They are almost always swollen. Apart from these cysternae the cytoplasm contains moderate amounts of free ribosomes—often forming polysomes—and mitochondria.

Large amounts of glycogen are present. Rosettes are often gathered in large clusters. Lysosome-like particles are recognisable, often near the plasma membrane. The Golgi area is less well developed than in Groups 1 or 2, but the structures in it can still be clearly made out.

![Image](https://example.com/image.jpg)

**FIG. 9**
Part of a Group 3 osteoblastic cell and of a cell (upper right) bordering a capillary vessel. Both these cells contain clusters of glycogen and a few granular cysternae. Many thin filaments are present throughout the cytoplasm of the osteoblastic cell. Uranyl acetate-lead citrate. (×25,000.)

Large amounts of fine filaments, often collected in bundles, are present right through the cytoplasm. The nucleus is elongated, but there are no special morphologic features. A nucleolus is usually present.

**THE ORGANIC MATRIX**

**Optical microscopy**—Most of the trabecular bone surfaces are covered with uncalcified osteoid, which is frequently lamellar. Solochrome cyanine stains layers of fibrillar collagen orange and interlamellar spaces pale blue (Fig. 10). The former are birefringent in polarised light. The spaces between lamellae are bigger than normal, and the lamellae themselves much bigger than normal. Some areas of osteoid tissue are not lamellar at all. They are "woven bone." The proportion of this bone varies in different areas.

In normal bone the calcification front—the first mineral to be seen under the optical microscope—is clearly visible after staining with toluidine blue or solochrome cyanine. It consists of dark blue granules clustered in a band of uneven width at the osteoid-calcified bone interface. Between 65 and 90 per cent of normal osteoid contains this front.

In the bones investigated in this study the percentage presence of this front was incomparably less, or even zero. Variations were probably due to different degrees of vitamin D
deficiency. But some kind of calcification does take place in osteomalacic osteoid tissue. Metachromatic "cloudy" zones can be seen where small quantities of mineral are deposited (Fig. 11). These zones vary in area. Microradiography confirms that apatite has been deposited in them.

![Fig. 10](image)

**Fig. 10**
Osteoid tissue (O), showing fibrillar lamellae and interlamellar spaces, both enlarged. C—calcified matrix. (× 770.)

![Fig. 11](image)

**Fig. 11**
O—Uncalcified osteoid; Pc—cloudy zones of partially calcified osteoid; C—fully calcified bone. (× 600.)

Sometimes calcification seems to begin in one fibrillar lamella (Fig. 12), or to be concentrated very near one osteocyte, or a group of them (Fig. 13). Calcification of this type sometimes appears in most osteoid seams; it sometimes reaches the edge of the calcified bone and is sometimes separated from it by a large uncalkified area of osteoid. **Electron microscopy**—Electron microscope examination of unstained sections confirms that calcification takes place in osteomalacic bone matrix very unevenly. It sometimes involves
only a very few, small areas within large areas of matrix (Figs. 14 and 15), whereas at other times it involves nearly all the osteoid tissue.

In both these cases, and in intermediate ones, calcification means no more than the deposition of small crystallite clusters. These vary in number, size and distribution. The clusters sometimes consist simply of a few crystallites, irregularly oriented and found in
roundish, electron-dense areas (Fig. 16). In other cases more crystallites are present, and, the more there are, the more they tend to lie parallel to each other (Figs. 17 and 18). The clusters are sometimes widely separated—this usually implies they are minute (Figs. 14 to 16)—and sometimes they almost touch, with thin zones of uncalcified matrix between them (Figs. 17 and 18).

Often calcification increases with nearness to the fully calcified matrix. But in other cases well developed crystallite clusters are seen far away from calcified matrix, with large areas of completely unmineralised osteoid tissue in between.

The fine structure of the mineral is sometimes identical with that of normal bone, and sometimes absolutely different from it.

In the first case needle-shaped crystals between 20 and 45 Å thick (mean value 37 Å) are seen gathered in roundish clusters of variable size (Figs. 14 to 16), or in elongated clusters (Figs. 17 and 18), shaped like those in calcified fibrils. In this case the crystals are almost exactly parallel to the long axis of the fibrils.

The second type of calcium deposition (Fig. 19) often begins with the appearance of small roundish areas of almost amorphous, or finely granular, calcium salts in osteoid tissue (Fig. 20). These salts sublimate easily under the electron beam. With increasing calcium salt deposition these areas become bigger and take on a more or less crystalline appearance. All the same, no real needle-shaped crystals are formed—only very thin, elongated, finely granular structures, which appear rather indistinctly even in sharply focused photographs (Fig. 21). When even more calcium salts are deposited these clusters become even bigger, and often become star-like in shape (Fig. 22). Crystal-like structures, averaging 180 Å in thickness and 1,500 Å in length, radiate from the cluster like the rays of a star.

As calcification goes forward these clusters gradually coalesce and take on a normal crystalline structure. When calcification is almost complete, real needle-shaped crystals are intermixed with other crystals which tend to become needle-shaped, but still remain finely granular (Fig. 23). Both types of crystal are between 50 and 100 Å thick (average thickness 70 Å)—a good deal more than normal crystals. All the same, electron diffractographs of these star-like clusters did not look significantly different from diffractographs of normal bone.
Staining sections makes it possible to study the fine structure of collagen fibrils. Of course, the morphology of crystallites is now different. Results in still uncalcified areas are not the same as those in which calcium salt deposition has already taken place.

In the first case the osteoid tissue consists of a large number of collagen fibrils, which sometimes appear haphazardly, and sometimes form lamellar structures (Figs. 24 and 25). The second possibility is more common. In this case lamellae are not separated by any special structure. Individual lamellae are recognisable only because the fibrils in any one lamella have a fairly uniform direction, and this direction changes in the next lamella. Fibrils in one lamella do occasionally continue into the next without any interruption.

The fine structure of collagen fibrils in osteoid tissue in osteomalacic bone is the same as that of fibrils in normal uncalcified bone matrix. The characteristic 650 Å banding is always present (Fig. 25). Fibrils near osteoblastic cells—that is, new fibrils—are less thin, sometimes, than old fibrils. But this difference is usually very small, fibril thickness ranging between 600 and 675 Å. The collagen fibrils in osteoid tissue are not closely packed. Spaces of different sizes, looking like fissures, separate them (Figs. 24 and 25). They have a very low electron density. But uranyl acetate-lead citrate staining does show up a few very fine granules only 50 Å in diameter in the spaces between fibrils (Figs. 25 and 27).

In the areas where calcification has already taken place many fibrils tend to aggregate (Figs. 26 and 27), so that large fibrils appear. Their thickness ranges between about 1,500 Å and 7,000 Å.

Initially these aggregated fibrils are separated by extremely thin, apparently empty fissures. But in the biggest aggregates fibrils lose their separate identity. After aggregation, cross-sectioned fibrils appear as large, electron-dense fields. These fields are irregularly polygonal in shape (Figs. 26 and 27).

Clusters of normal and/or abnormal crystallites are scattered between and on these fibrils (Figs. 26 and 27). When examined in longitudinal section some aggregated fibrils do not display the usual periodic banding (Fig. 27).

DISCUSSION

Two topics will be discussed here: what is abnormal about osteoblastic cells; and what kind of calcification takes place in the osteoid tissue.
Area of calcification more advanced than that shown in Figures 14 and 15. Crystal clusters enlarged by addition of new crystals, which gradually become orientated in the same direction. Unstained. (× 65,000.)

Area of calcification more advanced than that shown in Figure 17. Although the process is almost complete, many areas of the organic matrix are left uncalcified. Unstained. (× 100,000.)
Abnormalities of osteoblastic cells—Of the three groups of cells found in contact with malacic osteoid tissue, those in Group 1 are not very different from normal osteoblasts. The main difference is that their cytoplasm contains glycogen.

Considering this glycogen, Schajowicz and Cabrini (1958) concluded that it was always present in cells in membranous bone, but that its volume depended on the rate of bone formation—there is little or none in active hypertrophic osteoblasts, a bit more in preosteoblasts and a lot in quiescent osteoblasts. Loss of glycogen in active osteoblasts has been confirmed by several authors (Pritchard 1956).

![Fig. 19](image1)

**Fig. 19**
Calcification of the osteoid tissue near a fully calcified area. Though this picture does not seem different from that reported in normal bone, the apatite crystals are abnormal, as shown in the following photographs. Unstained. (× 4,000.)

![Fig. 20](image2)

**Fig. 20**
Detail of the initial calcification in an area as those reported in Figure 19. Although the electron density is very high, no needle-shaped crystals are present. Compare with Figure 16. Unstained. (× 90,000.)

Bonucci (1965) reported the presence of glycogen in fibroblasts and preosteoblasts but not in osteoblasts, and suggested that the acquisition of a high rate of synthesising activity in osteoblasts may be connected with glycogen depletion. So the presence of a relatively large amount of glycogen in Group 1 osteoblastic cells—which otherwise look normal—suggests that these cells probably secrete proteins at a lower rate than normal.

This possibility is strengthened by the fine structure of their cytoplasm. The granular cysternae in it are regularly ordered, elongated and narrow. Porter (1966), discussing the different types of organisation found in relatively quiescent cells as opposed to highly synthesising ones, stated that a compact, ordered granular endoplasmic reticulum may indicate...
Figure 21—This photograph shows a stage of calcification probably more advanced than that in Figure 20. Very thin crystalline structures having a finely granular appearance begin to form in the dense areas of calcium salt deposition. Arrow points to a roundish area in which the granular appearance of this mineral is clearly visible. Unstained. (× 60,000.) Figure 22—This photograph shows a calcification stage perhaps still more advanced than that in Figures 20 and 21. Calcium salts are laid down in a characteristic radial pattern. Note the very high electron density at the centre of calcium salt cluster and the granular crystals which form the rays of the "star." Unstained. (× 90,000.) Figure 23—This photograph shows a still more advanced stage of calcification than in Figures 20 to 22. The bone mineral is formed by thick crystals which are granular and poorly defined. At the upper corners are a few needle-shaped crystals similar to those observed in normal bone. Compare with Figure 18. Unstained. (× 90,000.)

a low rate of synthesis. And Peach, Williams and Chapman (1961) described fibroblasts with granular cisternae distributed in an orderly way as "migratory," and considered those with rough cisternae of irregular size and variable shape as "secretory."

A low rate of protein synthesis in osteomalacia may be due to another factor—the presence of more non-secretory cells than normal. Group 3 cells have the same structure as "resting" osteoblasts (Pritchard 1956). These are considered non-synthesising cells (Dudley and Spiro 1961).

Group 2 cells, too, have the morphological appearance of non-synthesising cells. The main consideration here is the tiny amount of granular endoplasmic reticulum. The general characteristics of these cells are the same as those of young progenitor cells, except that young progenitor cells are not normally found in contact with osteoid tissue.

The presence of undifferentiated cells in osteomalacia seems to agree with Bélanger and Migicovsky's (1963) findings, which suggested that, in H:\-thymidine-injected rachitic chicks, cellular differentiation and matrix formation both slow up.

Changes in osteoblast morphology may be connected with two factors involved in the physiopathology of osteomalacia—vitamin D deficiency and high parathormone secretion. In the bones investigated by us histological and biochemical evidence left no doubt that both these factors were involved.

In rats treated with high doses of parathormone, Cameron, Paschall and Robinson (1967) showed that changes occurred in osteoblast ultrastructure, mitochondria became swollen, both rough- and smooth-surfaced vesicles were distended, and ribosomes lost contact with membranes.
Gaillard (1965) showed that parathormone treatment of isolated rat radii resulted in an impairment of the osteoblastic function and reported that Shreft's preliminary electron-microscopical studies on these radii showed that the endothelial reticulum of osteoblasts became less well developed. Fewer ribosomes were present, too.

In rats under parathormone treatment Young (1963) noted that osteoblasts became elongated. Their basophilia fell for the first few hours and, twenty-four hours later, all the characteristic features of osteoblasts had disappeared. The only cells then present were osteoprogenitor cells, macrophages and osteoclasts. Kroon (1958) showed that the main effect of parathormone in rodents was on osteoblasts. They dedifferentiate and proliferate.

These experimental studies cannot be directly compared with the results of this investigation, on the one hand because the doses used on animals are very high, or even
Fig. 26
This photograph shows the aggregation of fibrils in osteoid tissue in areas of calcification. Aggregated fibrils interweave with fibrils of normal thickness. Uranyl acetate-lead citrate. (× 10,000.)

Fig. 27
An area of fibrils aggregation is shown at the bottom of this photograph. At upper left a fibril without period and with lateral filaments. Arrow points to an isolated crystal in the interfibrillary space. Uranyl acetate-lead citrate. (× 65,000.)
toxic, and the hormone used is not pure; and, on the other hand, because the pathological processes involved in osteomalacia are not only due to hyperparathyroidism but also to a coexisting vitamin D deficiency.

Vitamin D deficiency may have a direct effect on osteoblasts. Kodicek (1963), after taking autoradiographs of rat bone labelled with C14-vitamin D, saw a great deal of isotope in proliferating chondrocytes, and some incorporation in osteocytes and osteoblasts. He suggested that the high concentrations of isotope in chondrocytes might indicate that vitamin D was involved in the production of proper organic matrix. Without it, matrix may be unable to calcify.

If it is true that the activity of osteoblasts is reduced or inhibited in osteomalacia, their morphology might be that of relatively quiescent osteoblasts, or incompletely differentiated ones, or dedifferentiated ones. In fact, we found that all three of these types were present. This is in agreement with Frost (1967) who found that osteons took longer to form in osteomalacia than in normal bone, and that a prolonged osteon life span meant lethargic osteoblasts.

**Calcification in osteoid tissue**—The second topic to be discussed is how osteoid tissue calcifies. The single collagen fibrils are not different from those found in normal bone. Some of them do not show any discernible periodic banding, but this may be because in all these cases fibrils have been sectioned obliquely.

It is of interest that in partly calcified areas in osteoid tissue fibrils are not as closely packed as in normal bone, where they often appear in register (Robinson and Watson 1952; Ascenzi, Bonucci and Bocciarelli 1967).

It is possible that fibrils in normal bone become more closely packed at the initial stage of calcification, and that the aggregation of fibrils is simply a form of fibril reorganisation (Cameron 1963). In malacic bone lateral aggregation of fibrils with their period in register can only be found in a few fully mineralised areas. Electron microscopy shows that this process is present—even if incomplete—even in partly mineralised areas. What happens is that it seems to stop once bundles about 7,000 Å thick have been formed.

It should be emphasised that our investigation shows that initial calcification does occur in malacic osteoid. Although no calcification front like that found in normal bone is seen—this is, by the way, in agreement with histological investigations (Matrajt and Hioco 1966; Matrajt, Bordier and Hioco 1967)—electron microscopy shows up tiny clusters of apatite crystals scattered irregularly throughout the osteoid tissue. These clusters are usually too small to be seen under the optical microscope. They are sometimes exactly the same as those found in normal bone (Cameron 1963), and sometimes completely different from them.

In the second case unusually amorphous calcium salts are deposited, and this is followed by the formation of crystals which are initially granular and, later, star-shaped. It might be thought that this is due to the formation of an abnormal bone mineral, because the calcium phosphorus product is abnormal in osteomalacia. But these unusual crystals seem to turn into normal ones if or when calcification proceeds. So they might be due simply to the slowing up of calcification.

This problem deserves further study. It is interesting in the meantime to note that the finding of amorphous calcium salt deposition and of unusually thick crystals is in agreement with spectroscopic and x-ray diffraction work showing that in rachitic bone in calcium, phosphorus or vitamin D-deficient chicks amounts of amorphous calcium phosphate are higher than usual, and apatite crystals are unusually thick (Termine and Posner 1967).

Apart from osteoid matrix containing scattered clusters of crystallites, areas of more advanced calcification can be found. This calcification is hardly ever complete, but it is a proof that a second stage of calcification can occur after the original deposition of apatite crystallites.

This second phase of osteoid calcification probably goes forward extremely slowly, so that, even when advanced, it leaves incompletely calcified trabeculae surrounded by calcified
ones. This is clearly visible under the electron microscope and has already been observed in microradiography on osteomalacic bone (Engfeldt and Zetterström 1955, Laval-Jeantet et al. 1967).

On the basis of microradiographic studies Bohatirchuk (1963, 1966) concluded that these areas of incomplete calcification were due to a process of partial demineralisation. But neither the fine structure of these areas nor the histological data so far obtained on calcification seem to substantiate this view. Histochemical data on the effects of vitamin D therapy confirm that calcification in these areas is incomplete (Matrajt, Bordier and Hioco 1967).

**SUMMARY**

1. Four cases of osteomalacia secondary to vitamin D deficiency have been investigated histologically and with the electron microscope.
2. Three main types of cells were found along the osteoid tissue. Cells of Group 1 are like normal osteoblasts, except that their cytoplasm has an ordered granular endoplasmic reticulum, without enlarged cysternae. Moreover, it contains isolated rosettes of glycogen. Cells of Group 2 are like young progenitor cells. There are almost no rough cysternae in the cytoplasm. This contains clusters of glycogen, isolated ribosomes and many mitochondria. Cells of Group 3 are structurally like "resting," flat osteoblasts in normal bone.
3. The paper discusses how the presence of the three groups may be related to vitamin D deficiency or secondary hyperparathyroidism.
4. Malacic osteoid tissue consists of apparently normal collagen fibrils. Both optical and electron microscopy show that this tissue can calcify. But calcification stops at an early stage, or proceeds much more slowly than normal. So large areas of osteoid tissue are left uncalcified.
5. Calcium salts are laid down either as needle-shaped crystals exactly like those in normal bone, or else abnormally. Where abnormal they either appear in a finely granular, almost amorphous form, or else acquire a characteristic star-like crystalline structure.
6. Where calcification takes place bundles of laterally aggregated collagen fibrils are found.

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**REFERENCES**


