ELECTRON MICROSCOPIC STUDY OF PERIOSTEAL HYPEROSTOSIS IN RATS WITH LATHYRISM INDUCED BY AMINOACETONITRILE

BY

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ABSTRACT

In various mesenchymal tissue alterations in lathyrism, as the lesions of cartilage and connective tissue and dissecting aneurysm of the aorta, a defect in formation of collagen fibers and mucopolysaccharide has been considered as an essential basic change. The present electron microscopic study was made on the ultrastructures of the bone matrix forming cells and the newly formed bone matrices in the aminoacetonitrile induced lathyrism, in order to see if the defect mentioned above is also found in the periosteal hyperostosis. The present investigation revealed following points: (1) The basic changes in the lathyric periosteal hyperostosis consist of a failure in development of the cytoplasmic organellae of the bone matrix forming cells, and a defect in formation of the collagen fibers as well as the ground substance of the newly formed bone matrix, resulting in characteristic mineralization disturbance in mottled pattern. (2) Of these basic changes the defect in formation of the collagen fibers as well as the ground substance, probably mucopolysaccharide, seems to be the same as those in the other mesenchymal tissue. (3) The failure in development of the cytoplasmic organellae, especially the granular endoplasmic reticulum of the bone matrix forming cells may be related to the defect in formation of the collagen fibers and the ground substance. (4) The defect in formation of the bone matrix may be responsible for the characteristic disturbance of mineralization in the newly formed bone matrix.

INTRODUCTION

It is well known that lathyrism, intoxication with Lathyrus odoratus seeds[1–17], their active principle β-aminopropionitrile[7,16–36], or some chemicals such as aminoacetonitrile[7,16,20,37–47], β-mercaptoethylamine[18,25,48,49], etc.[16,25,48,50–52], cause various alterations of mesenchymal tissues, such as damage of epiphyseal cartilage[1,11,15,20,25,28,30,38,41,44,45,48,49], hyperostosis at the sites of muscle attachment[1,6,9–11,13–15,20,25,27,28,31,33,34,41,44,46–48,51], transformation of the periodontal membrane into osteoid tissue[12,42], dis-

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secting aneurysm of the aorta[3,8,17,21,36], inguinal hernia, and so on. These alterations have been studied histopathologically[8,9,12,15,40,42,49], histochemically[7,11,16,27,31,32,41,47], or chemically[29,38,39,45] by many investigators and it has been clarified that the essential changes in these various mesenchymal tissue alterations are probably due to the failure in maturation as well as formation of collagen fibers and in formation of mucopolysaccharide. These essential changes are thought to be caused by the lathyrogenic factors.

Hartmann (1963)[30] reported that disturbed function of the ground substance of the costochondral cartilage in lathyric rats and subsequent failure in maturation of collagen fibers are probably attributed to inhibition of the mucopolysaccharide synthesis by chondrocytes.

The hyperostoses at the sites of muscle attachment are frequently so striking that they appear to be a proliferative change, in contrast to the damage of epiphyseal cartilage, dissecting aneurysm, inguinal hernia, etc. The problems remain unsolved as to whether the hyperostoses are quite different from other lathyric alterations or also ascribable to the same essential change as that in the other lathyric lesions.

The present author made electron microscopic study to gain information on the ultrastructural change of the lathyric periosteal hyperostosis and to clarify its essential changes.

Materials and Methods

Wistar strain young rats were intraperitoneally given 2.5% aqueous solution of aminoacetonitrile hydrogen sulfate in dosage of 200 mg/kg daily. The experimental animals were sacrificed at intervals of 8 to 10 days after the beginning of aminoacetonitrile administration. Small pieces of the subperiosteal bone matrix in the lathyric hyperostosis were removed and fixed immediately in 2% osmium tetroxide buffered at pH 7.4 with the veronal acetate buffer for 1 to 2 hours. After fixation, the materials were dehydrated in graded ethanol and embedded in styrene and *n*-butyl methacrylate mixture. Ultrathin sections were cut with Fernández-Morán ultramicrotome using diamond knives and stained with lead hydroxide (Millonig)[53] or 1% aqueous solution of phosphotungstic acid. Some sections were not stained. The sections were examined with Hitachi HU-10 or HU-11A electron microscope.

As a control, metaphyseal bone of femur in the normal young rats and fracture callus of experimentally fractured tibia in young rats were also examined.

Observation

I. Histological findings of the periosteal hyperostosis of the femur in the lathyric rats

At the sites of muscle attachment of the femur in the rats receiving aminoacetonitrile, rather prominent periosteal hyperostosis was seen, although there were some differences in the degree of hyperostosis among the animals. The outermost zone of the hyperostosis consisted almost exclusively of proliferating cells with scanty elongated cytoplasm (Fig. 1). There were almost no intercellular substances. The more interior zone of the hyperostosis was composed of proliferating cells with ample cytoplasm. Some of the cells showed eccentric location of nuclei and juxtanuclear vacuole as in osteoblast. Among these cells a small quantity of hyaline-like intercellular material was found. In the midzone of the hyperostosis spindle-shaped cells with profuse cytoplasm were dispersed in the hyaline-like intercellular material. Many of the cells resembled osteoblasts (Fig. 2). The intercellular material increased in density inwards and came into contact with the cortex of the femur diaphysis. In the deepest zone of hyperostosis the intercellular material had undergone mineralization. Some of the cells enclosed in newly formed mineralized bone matrix showed pyknosis of the nuclei and decreased in the size of cytoplasm.

It seems most probable that the hyaline-like intercellular material may be considered as unmineralized bone matrix or osteoid, because of its tendency to undergo mineralization. Consequently, the cells lying in the hyaline-like intercellular material are probably bone matrix forming cells, i.e. osteoblasts or osteoid osteocytes, while the cells in the newly formed mineralized intercellular material may be considered as osteocytes.

II. Electron microscopic findings of the periosteal hyperostosis of the femur in the lathyric rats

The cells with elongated scanty cytoplasms in the outermost zone of the periosteal hyperostosis contained poorly developed organellae, i.e. a small number of free ribosomes, granular endoplasmic reticulum (GER), and mitochondria (Fig. 3). The cells lying inside the above-mentioned cells had somewhat more cytoplasms with rather developed organellae. The free ribosomes and GER increased in number. The Golgi complex was rather well organized and predominantly consisted of microvesicles as well as vacuoles. The nuclei were elongated and the chromatin was accumulated slightly along the nuclear membrane. The nucleoli were rather prominent.

Among these cells there were narrow intercellular spaces, in which

occasionally a small amount of flocculent or filamentous substances were present.

The cells in the more interior zone of the hyperostosis had elongated ample cytoplasms with well developed organellae (Fig. 4). The organellae consisted of abundant granular as well as agranular endoplasmic reticula (AER), many mitochondria, and highly organized Golgi complex. The nuclei were clear and irregular in shape. The chromatin showed slight accumulation along the nuclear membrane. There were broader intercellular spaces in which irregular deposition of rather abundant filamentous substances without distinct period occurred. The cells in the midzone of the hyperostosis had elongated rather profuse cytoplasms with well developed organellae (Figs. 5 and 6), and, in the vicinity of the nucleus, rather well organized Golgi complex was seen. The nuclei were frequently located eccentrically in the cytoplasm. These cells were surrounded by a larger quantity of intercellular material. The intercellular material contained numerous fine collagen fibers with periods (Fig. 7) but their distribution was irregular and in places the intercellular material was free from collagen fibers. The intercellular material was mineralized in the deepest zone of the hyperostosis (Fig. 8). In the newly mineralized intercellular matrix the cells with well developed organellae were present (Fig. 9). Some of them resembled active osteoblasts.

Electron microscopy can clearly exhibit the collagen fibers, one of the organic bone matrix, but the mucopolysaccharide, the other constituent of the organic bone matrix is difficult to demonstrate. Therefore, it is not easy to identify an intercellular material as osteoid by electron microscopy alone.

With respect to identification of osteoid, therefore, light microscopy may be more helpful than electron microscopy, because it is easy to demonstrate the ground substance of osteoid on the specimen stained with hematoxylin-eosin.

As stated earlier, histological observation of the lathyric periosteal hyperostosis revealed that the intercellular material in the hyperostotic area consisted almost exclusively of osteoid, except in the outermost zone. Consequently, in the hyperostotic area the intercellular material containing collagen fibers can probably be considered as osteoid. By taking that into account, it will become comparatively easy to identify the osteoid in the electron micrographs of the periosteal hyperostosis. In electron microscopy the cells in the outermost zone had slightly developed GER and were surrounded by a small amount of flocculent or filamentous intercellular material. Therefore, these cells are probably pre-osteoblasts and the cells which had rather well developed organellae and were surrounded by the intercellular material with filamentous substance or collagen fibers are most

probably osteoblasts. The cells surrounded by the intercellular matrix containing collagen fibers are considered as osteoid osteocytes (Dudley and Spiro, 1961)[54].

III. Comparison of the periosteal hyperostosis in the lathyric rats with osteogenesis of the metaphyseal trabeculae and fracture callus in the normal young rats

The comparison was made to elucidate the ultrastructural differences of the bone matrix forming cells, and unmineralized and mineralized bone matrices in the lathyric hyperostosis from those in the non-lathyric osteogenesis.

A. Differences in the ultrastructures of the osteoblasts

Osteoblasts on the metaphyseal trabeculae in the normal 10 day old rats: The osteoblasts had ample polygonal cytoplasm containing abundant, well developed organellae, especially the great bulk of the GER and AER (Fig. 10). The cisternae of the GER generally showed flattened tubular structure and only occasional dilatation. The dilated cisternae contained granular or flocculent somewhat dense material. The highly organized Golgi complex occupied the juxtanuclear region, the counterpart of the juxtanuclear vacuole in light micrograph (Figs. 10 and 11). Small groups of vesicles were scattered throughout the area that was largely occupied by the GER. Mitochondria were dispersed among the GER. The cortical cytoplasm without GER was occasionally found. The nuclei were situated eccentrically in the cytoplasms and rather clear. The nucleoli were prominent. The small cytoplasmic processes extended into the newly formed unmineralized bone matrix.

Osteoblasts of the fracture callus of the tibia in the normal young rats: The osteoblasts in general indicated ultrastructures similar to the osteoblasts of the metaphyseal trabeculae (Fig. 12) but the dilated cisternae of the GER were more frequently seen and prominent than in the osteoblasts on the metaphyseal trabeculae. The cortical cytoplasm without the GER was wider. The cytoplasmic processes were irregular in thickness.

On comparison of the ultrastructures of the osteoblasts in the normal metaphyseal osteogenesis and fracture healing with those of the osteoblasts in the lathyric hyperostosis, it is evident that considerable decrease in the number of GER is the most striking alteration of the osteoblasts in the lathyric periosteal hyperostosis.

B. Differences in the ultrastructures of the osteoid osteocytes and osteocytes

Some of the osteoblasts become enclosed in the newly formed unminer-

alized bone matrix and are then usually called osteocytes. However, according to Dudley and Spiro (1961)[54], the present author termed the cells enclosed in the unmineralized bone matrix osteoid osteocytes and the cells surrounded with the mineralized bone matrix, osteocytes.

1. Osteoid osteocytes

The osteoid osteocytes in the newly formed osteoid of the metaphyseal trabeculae in the normal young rats: The osteoid osteocytes had undergone more or less advanced involution (Fig. 13). Their cytoplasmic organellae generally decreased in number. The cisternae of the GER often showed dilatation but such cisternae did not contain any granular dense material as in the osteoblasts. Lipid droplets were frequently found. The nuclei became smaller.

The osteoid osteocytes of the fracture callus in the normal young rats: The osteoid osteocytes had rather abundant well developed organellae (Fig. 12). Consequently, it seems that involution of the cytoplasmic organellae is of a slight degree.

The osteoid osteocytes in the lathyric periosteal hyperostosis: The rather well developed cytoplasmic organellae remained almost unchanged as in the osteoid osteocytes of the fracture callus (Figs. 5 and 6).

2. Osteocytes

The osteocytes in the newly formed mineralized bone matrix of the metaphyseal trabeculae in the normal young rats: The cytoplasmic organellae had undergone advanced involution (Fig. 14). The GER considerably decreased in number and showed varying dilatation. Mitochondria decreased also in number and were swollen. The Golgi complex became indistinct. The chromatin was more condensed in clumps along the nuclear membrane. The nuclei became small.

The osteocytes of the fracture callus in the normal young rats: Contrary to the above mentioned osteocytes, most of the well developed organellae remained almost unchanged.

The osteocytes in the lathyric periosteal hyperostosis: The involution of the cytoplasmic organellae were of rather slight degree (Fig. 15). Consequently, involution of cytoplasmic organellae seems to occur very slightly in the osteoid osteocytes and osteocytes in the lathyric periosteal hyperostosis.

C. Differences in the ultrastructures of the unmineralized bone matrix

The unmineralized bone matrix of the metaphyseal trabeculae in the normal young rats: As stated earlier, it is not always easy to differentiate the unmineralized bone matrix from the other intercellular matrix, especially that of the fibrous connective tissue in electron micrograph, because electron microscopy can demonstrate the collagen fibers of bone matrix,

but not the mucopolysaccharide. The degree of masking of the collagen fibers probably suggests indirectly the density of the ground substance, mucopolysaccharide. In the newly formed unmineralized bone matrix occupying the space between the osteoblasts and the mineralized bone matrix, the collagen fibers were haphazardly arranged (Figs. 10 and 11). The collagen fibers tended to increase in thickness and number with increasing distance from the osteoblasts. In electron micrographs stained with lead hydroxide the contour of the collagen fibers were somewhat indistinct. It seems to suggest masking of the fibers with the ground substance.

In the unmineralized bone matrix surrounding the osteoid osteocytes, the collagen fibers ran in various directions, but were almost equal in thickness and distribution (Fig. 13). Masking of the collagen fibers increased in degree with increasing distance from osteoblasts.

In the unmineralized bone matrix surrounding the osteocytes, the situation was almost the same as in the osteoid around the osteoid osteocytes (Fig. 14).

The unmineralized bone matrix of the fracture callus in the normal young rats: In the newly formed unmineralized bone matrix occupying the space between the osteoblasts and the mineralized bone matrix the mode of deposition of the collagen fibers were similar to that of the metaphyseal trabeculae (Fig. 12).

The unmineralized bone matrix of the hyperostotic area in the lathyric rats: In the newly formed unmineralized bone matrix the collagen fibers were occasionally rather irregular in distribution and did not increase in thickness (Figs. 8 and 9). In places masking of the collagen fibers was so slight that the contour of the collagen fibers in haphazard arrangement was clearly demonstrable. Consequently, in the unmineralized bone matrix of the lathyric periosteal hyperostosis, the collagen fibers occasionally did not increase in number or thickness. Moreover, masking of the collagen fibers occasionally seemed insufficient.

D. Differences in the ultrastructures of the mineralized bone matrix

The mineralized bone matrix of the metaphyseal trabeculae in the normal young rats: The periphery of the mineralized bone matrix, calcification front (Robinson and Cameron, 1958)[55], showed fairly uneven outline with many band-like projections (Figs. 10, and 14). These projections were closely related to the pre-existing collagen fibers and frequently showed crossing. The mineral crystals were mostly slightly bent and ran parallel to the long axis of the collagen fibers (Fig. 16). The periods of the collagen fibers were occasionally recognized even in the fully mineralized bone matrix. In electron micrographs stained with 1% aqueous solution of

phosphotungstic acid the mineralized bone matrix contained the great bulk of the collagen fibers and showed fairly regular intervals of the period of the collagen fibers (Fig. 17).

The mineralized bone matrix of the fracture callus: The situation seemed to be almost the same as in the above mentioned bone matrix.

The mineralized bone matrix of the periosteal hyperostosis in the lathyric rats: The projections of the calcification front were rather thin and not so numerous as in the front in normal rats (Figs. 8 and 9). Consequently, the calcification front in the lathyric hyperostosis appears not so uneven. In the mineralized bone matrix the period of the collagen fibers was almost not discernible and mottled pattern was present (Figs. 8, 15, and 18). The mottles indicated high mineralization at the periphery and low mineralization in the central portions (Fig. 18). In the electron micrographs stained with phosphotungstic acid increase in the number of collagen fibers was not so prominent and the mottles did not contain any collagen fibers (Fig. 19).

On the basis of these findings, the mineralized bone matrix of the periosteal hyperostosis in the lathyric rats shows characteristic alterations, such as rather even calcification front and mottled pattern. It seems most probable that these alterations mostly are related to the failure in the formation of collagen fibers in the mineralization.

Discussion

With respect to electron microscopic, histochemical and chemical studies on the alterations of matrix of the cartilage in lathyrism, there have been several reports. Follis and Tousimis (1958)[39] made electron microscopic observation as well as chemical analysis of homogenized fraction of cartilage in aminoacetonitrile-treated rats and clarified that the mature collagen fiber in cartilage matrix decreased in number as well as in width. At the same time, they carried out hydroxyproline determination in homogenized fraction of epiphyseal cartilage in experimental animals and demonstrated that there was little difference in collagen content compared with that of the control animal. On the basis of these findings, Follis and Tousimis assumed that the defect in fibrogenesis of epiphyseal cartilage in aminoacetonitrile-induced lathyrism is ascribed to the failure of tropocollagen molecules to form collagen fibers. On the other hand, Kennedy and Kennedy (1962)[16] performed autoradiographic distribution studies with radio-sulfate and labeled amino acids on epiphyseal cartilage in lathyrism caused by sweet pea seeds, β-aminopropionitrile, and aminoacetonitrile. They suggested that partial or complete defect in fibrogenesis in

epiphyseal cartilage in lathyrism is due to blocking of complex formation between protein and chondroitinsulfate A and C by the lathyrogenic factors. Hartmann (1963)[30] made electron microscopic and histochemical studies on costochondral cartilage in rats with lathyrism induced by β-aminopropionitrile. The electron microscopy revealed alteration of cartilage matrix and considerable decrease in the number as well as in the size of the cisternae of the endoplasmic reticulum in the cartilage cells which is related to production of mucopolysaccharide-protein complex. On the other hand, it was demonstrated that acid mucopolysaccharide in cartilage matrix reduced histochemically and hexosamine content of the matrix also decreased. From these findings Hartmann assumed that impairment in maturation of the collagen fibers is attributed to defect in the mucopolysaccharide synthesis by chondrocytes. Moreover, failure of inducing action of mature paracrystalloid collagen fibers resulted in mineralization disturbance of the cartilage.

The present electron microscopic study on the periosteal hyperostosis in the lathyric rats revealed occasional defect in formation of collagen fibers as well as the ground substance of the newly formed bone matrix and failure in development of the cytoplasmic organellae of the osteoblasts, especially granular endoplasmic reticulum. These findings suggest that the essential changes in the lathyric hyperostosis consist of defect in formation of collagen fibers as well as the ground substances as in other mesenchymal tissues, and these changes are thought to be related to poor development of the granular endoplasmic reticulum of the bone matrix forming cells, including osteoblasts and osteoid osteocytes.

In the periosteal hyperostosis in the aminoacetonitrile-induced lathyrism and the fracture healing the osteoid osteocytes and osteocytes generally exhibited less involution of the cytoplasmic organellae, such as the granular endoplasmic reticulum, Golgi complex, etc., than in the normal osteogenesis of the metaphysis. These findings suggest that the osteoid osteocytes and osteocytes in the lathyric periosteal hyperostosis are active longer in the bone matrix formation than in the normal metaphyseal osteogenesis.

In the mineralization of the osteoid of the normal metaphyseal osteogenesis and fracture healing, the collagen fiber of the unmineralized bone matrix rapidly increased in number as mineralization proceeds and the deposition of mineral crystals occurs in close relation with the preformed collagen fibers of the newly formed unmineralized bone matrix. However, in the lathyric periosteal hyperostosis mineralization of the osteoid occurs without marked increase in the number of the collagen fibers, followed by occasional loss of the fibers. The latter may be responsible for the mottled pattern in the mineralized bone matrix. The even calcification front and the

mottled pattern in the mineralized bone matrix are considered to be characteristic findings of mineralization in the lathyric periosteal hyperostosis.

The present study suggests that aminoacetonitrile causes both alteration of the bone matrix and disturbance in development of the bone matrix forming cells, and the latter is thought to be related to the former.

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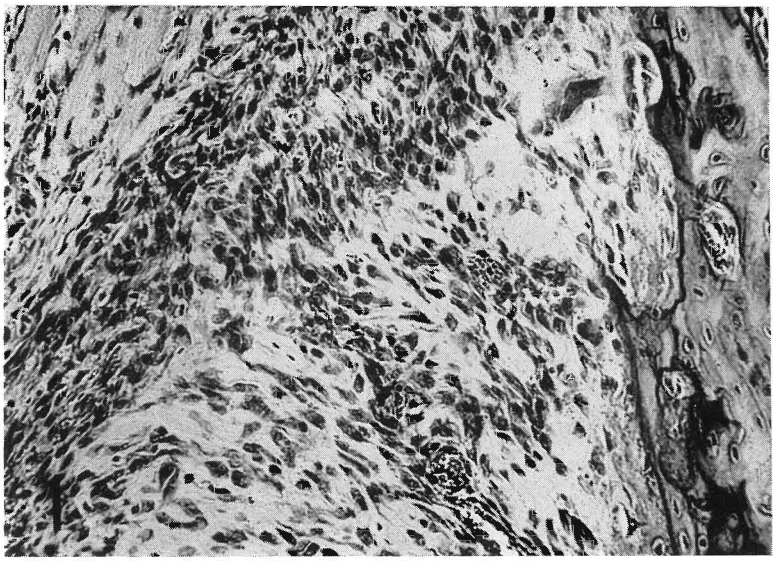

Fig. 1. Periosteal hyperostosis at muscle attachment of the femur in lathyric rat. The outermost zone consists of proliferating cells with scanty cytoplasm. The interior zone consists of proliferating cells with profuse cytoplasms. The midzone is composed of a large quantity of osteoid and scattered osteoid osteocytes. The deepest zone shows beginning of mineralization of the osteoid. H.E. ×180.

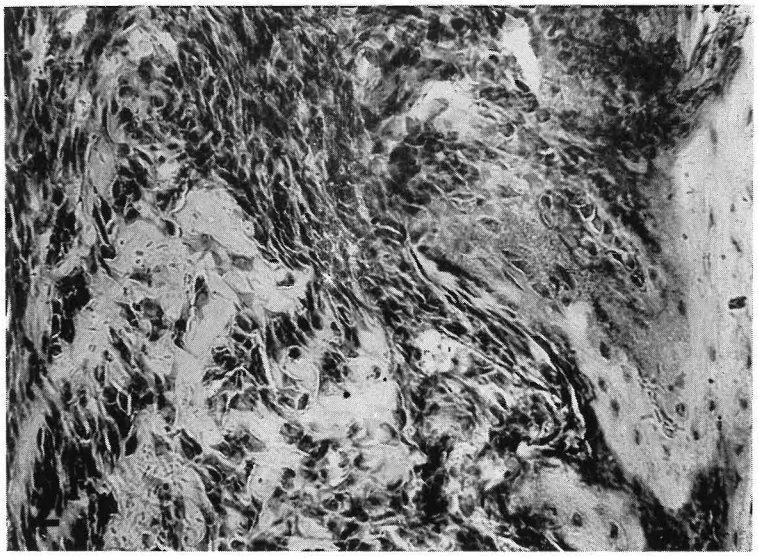

Fig. 2. Periosteal hyperostosis at muscle attachment of the femur in lathyric rat. The osteoid osteocytes enclosed in osteoid show a characteristic feature of osteoblast. H.E. ×180.

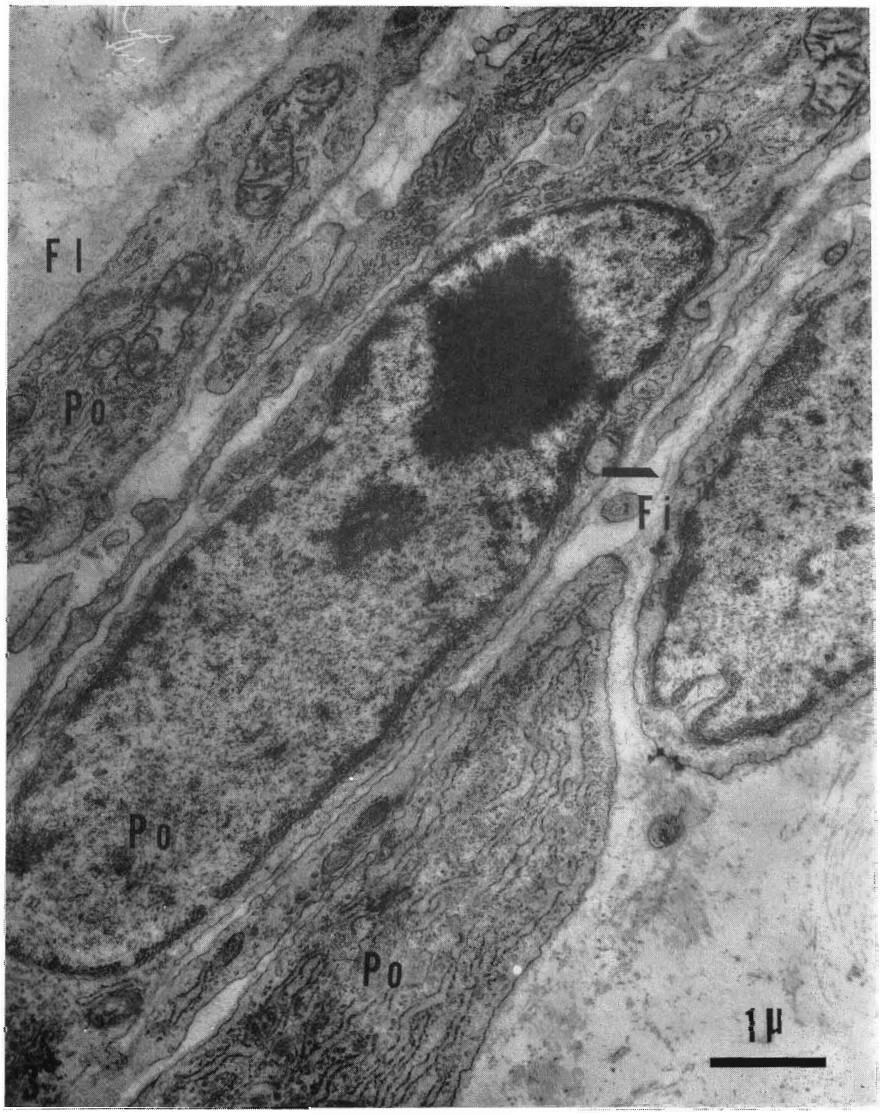

Fig. 3. The peripheral zone of the lathyric periosteal hyperostosis. The cells in the outermost zone are pre-osteoblasts with poorly developed organellae (Po). Flocculent intercellular material (Fl). Filamentous intercellular material (Fi). Lead hydroxide.

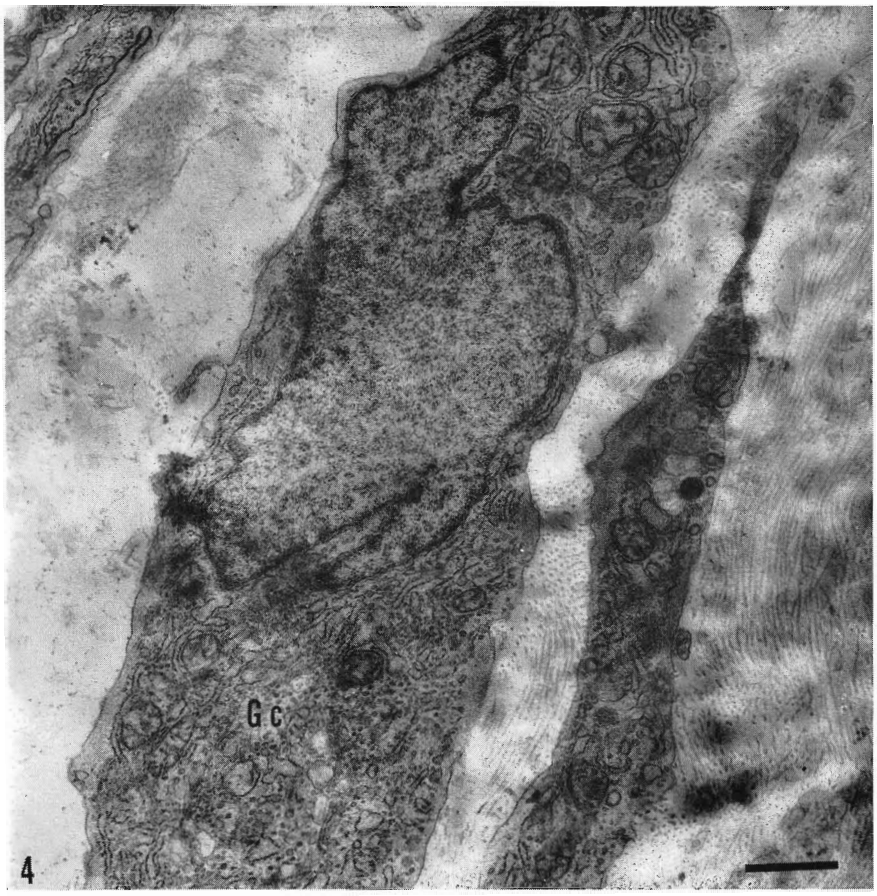

Fig. 4. A more interior zone of the lathyric periosteal hyperostosis. Osteoblast with rather well developed organellae. Development of GER is not considerable. Intercellular space is wide and consists of a material with filamentous structure and matrix with collagen fibers. Golgi complex (Gc). Lead hydroxide.

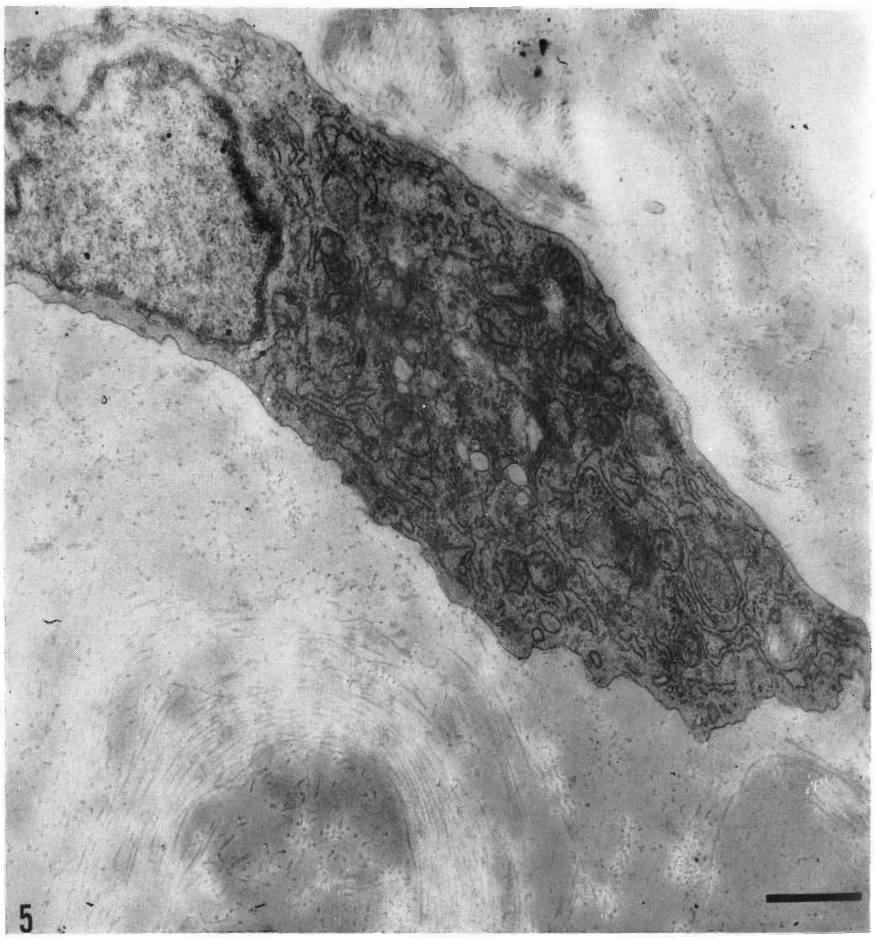

Fig. 5. Osteoid osteocyte in the midzone of the lathyric periosteal hyperostosis. The cytoplasmic organellae are rather well developed, but GER is not so profuse. Extracellular material consists of unmineralized bone matrix with moderate number of collagen fibers in irregular distribution. Lead hydroxide.

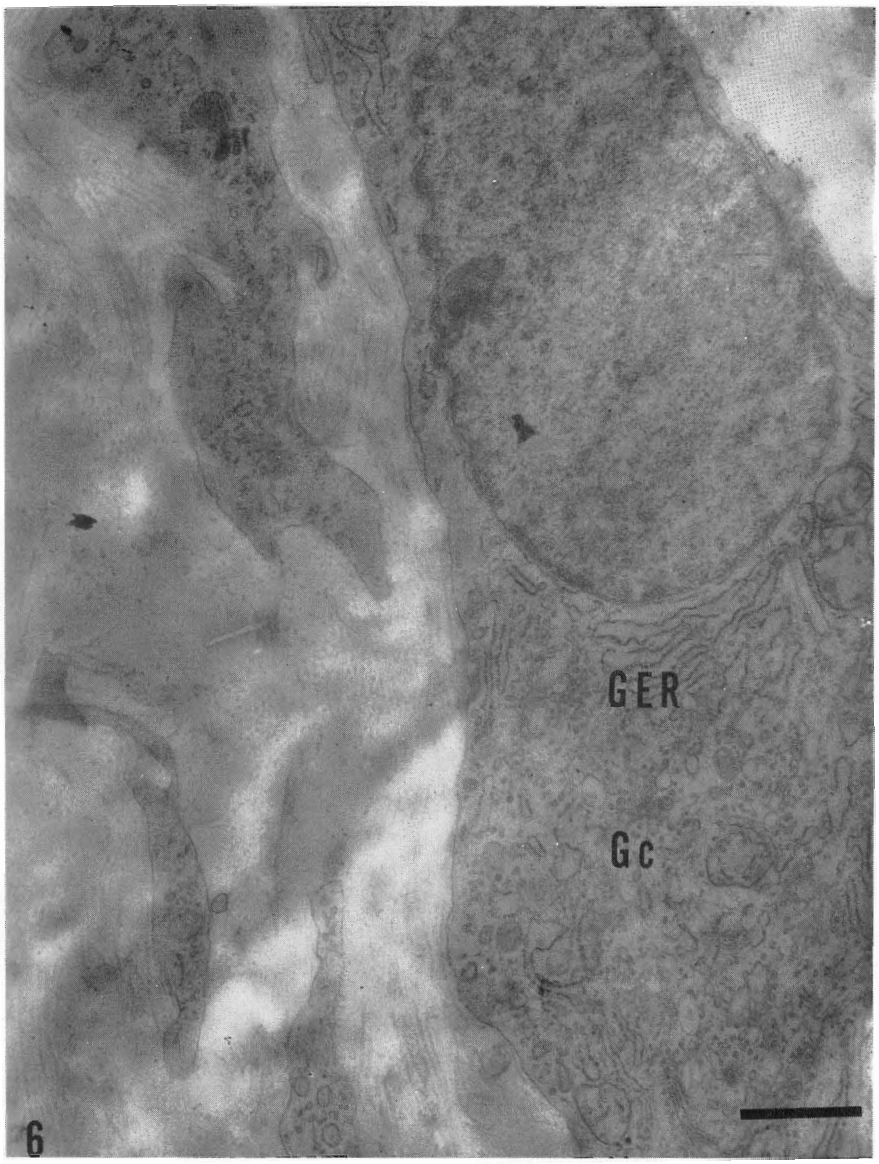

Fig. 6. Osteoid osteocyte in the midzone of the lathyric periosteal hyperostosis. Granular endoplasmic reticulum (GER). Golgi complex (Gc). Irregular deposition of collagen fibers in unmineralized bone matrix. Lead hydroxide.

Fig. 7. Unmineralized bone matrix in the midzone of the lathyric hyperostosis contains abundant collagen fibers with period. Distribution of collagen fibers is irregular. Phosphotungstic acid.

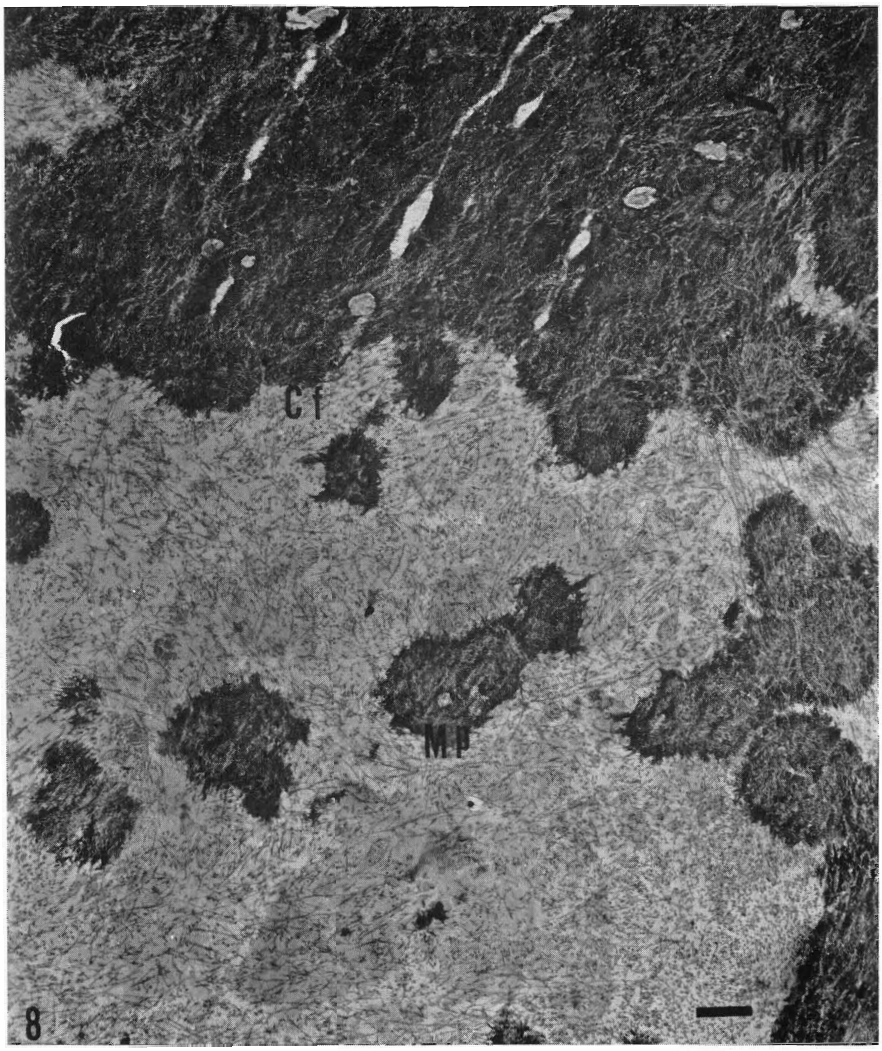

Fig. 8. Unmineralized and mineralized bone matrix of the lathyric hyperostosis. No increase in the thickness of collagen fibers. Even calcification front (Cf). Mottled pattern of mineralization (Mp). Lead hydroxide.

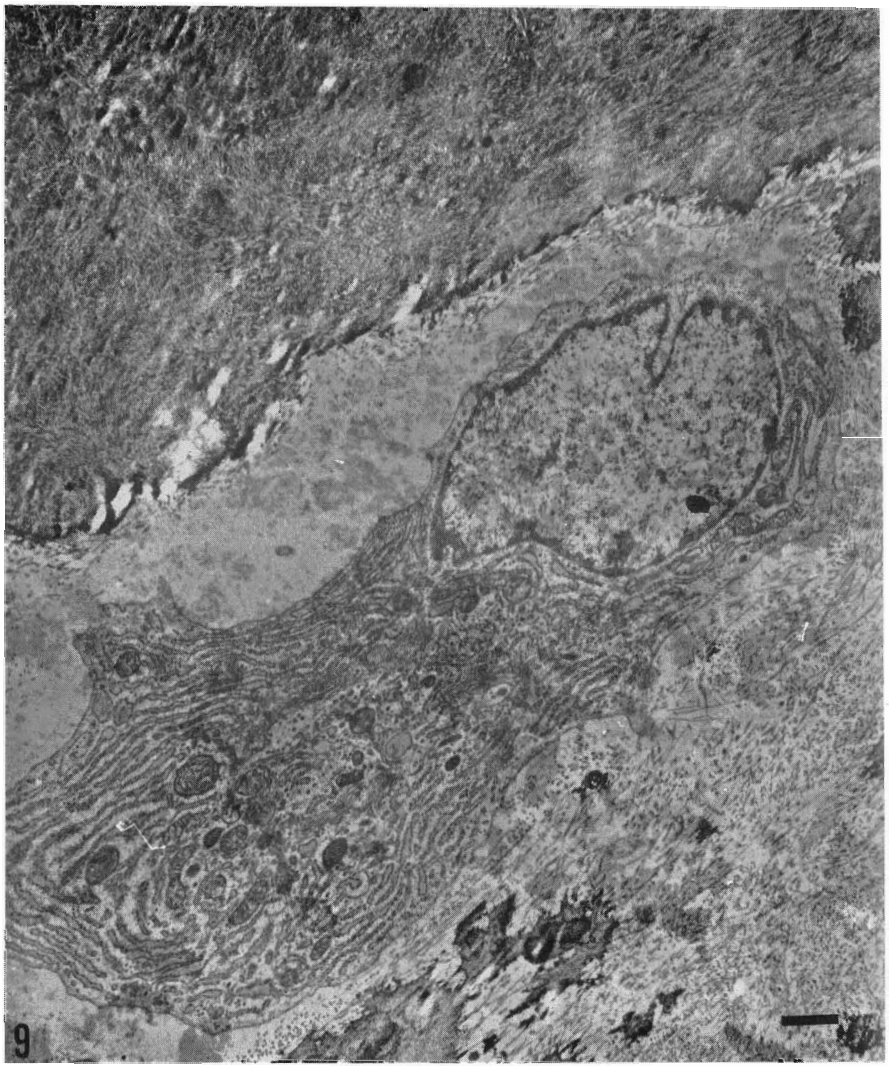

Fig. 9. Osteocyte in newly formed mineralized bone matrix in lathyric periosteal hyperostosis. The cytoplasmic organellae are rather well preserved. Occasionally no deposition of collagen fibers in unmineralized bone matrix. Calcification front with occasional thin projections. Lead hydroxide.

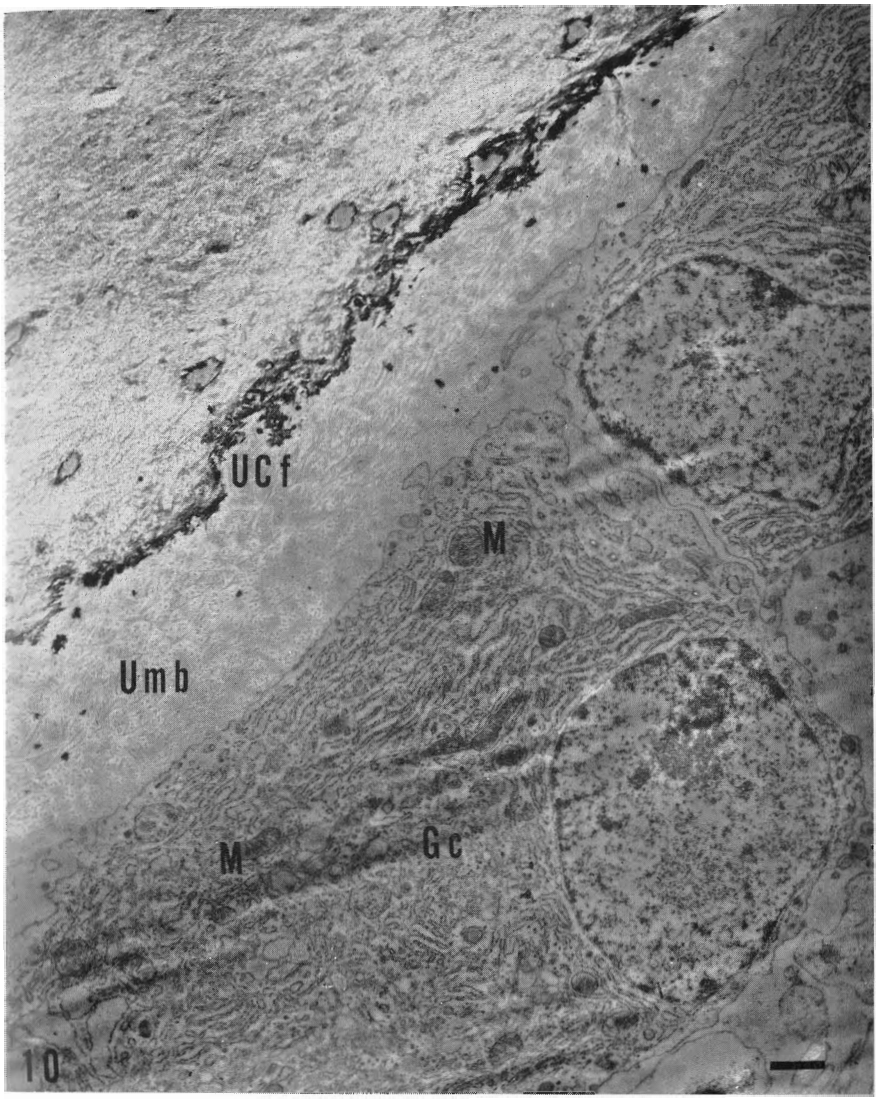

Fig. 10. Typical active osteoblasts on metaphyseal trabecula in normal young rat. The cytoplasmic organellae are highly developed. Abundant GER. Eccentric location of nucleus. Newly formed unmineralized bone matrix (Umb) with collagen fibers. Uneven calcification front (UCf). Highly organized Golgi complex (Gc). Mitochondria (M). Lead hydroxide.

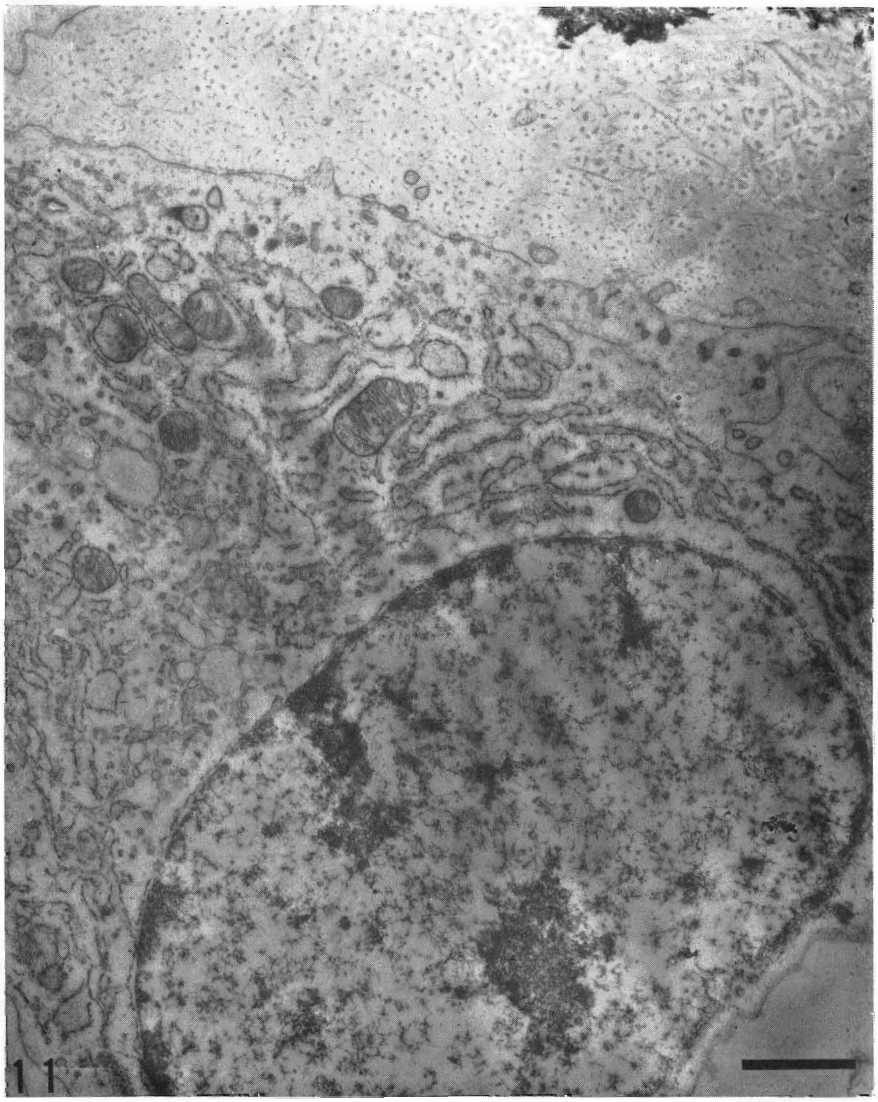

Fig. 11. Typical active osteoblast on metaphyseal trabecula in normal young rat. Increase in the thickness and number of collagen fibers in newly formed osteoid. Cortical cytoplasm with a few vesicles. Highly organized Golgi complex consists of microvesicles and vacuoles. Prominent nucleolus. Lead hydroxide.

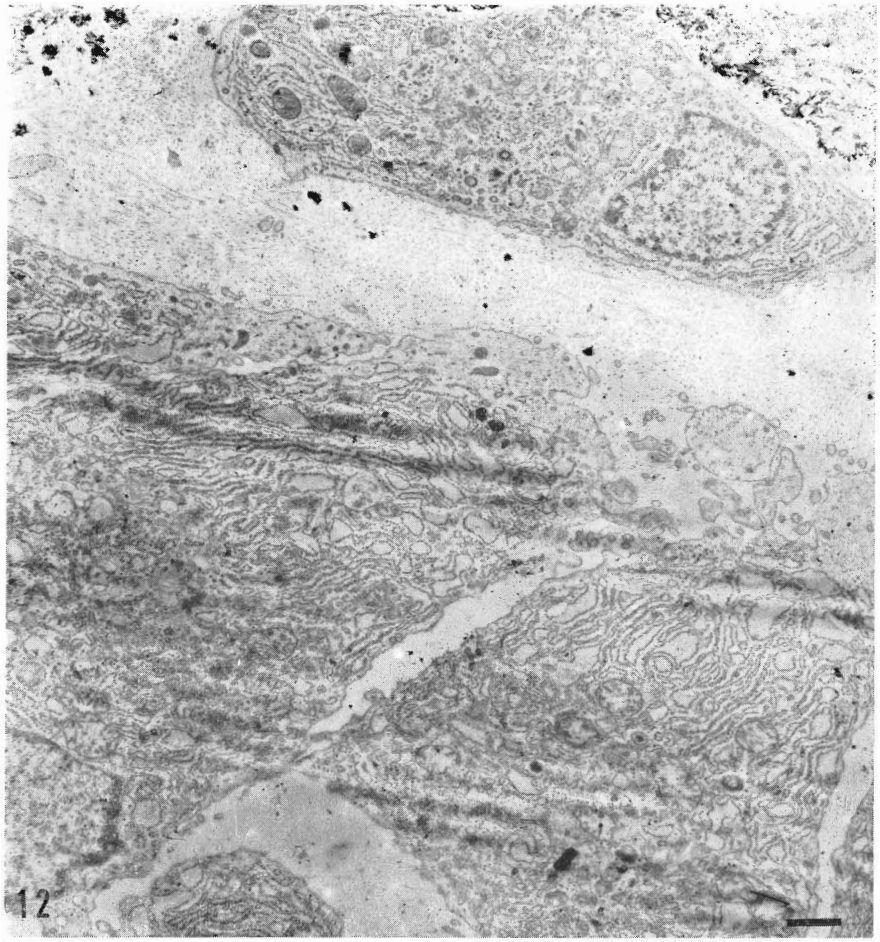

Fig. 12. Active osteoblasts and osteoid osteocyte of the fracture callus in normal young rat. The cytoplasmic organellae are well developed in osteoblasts and preserved rather well in osteoid osteocyte. The cisternae of GER of osteoblasts frequently show dilatation. Newly formed osteoid with abundant collagen fibers. Lead hydroxide.

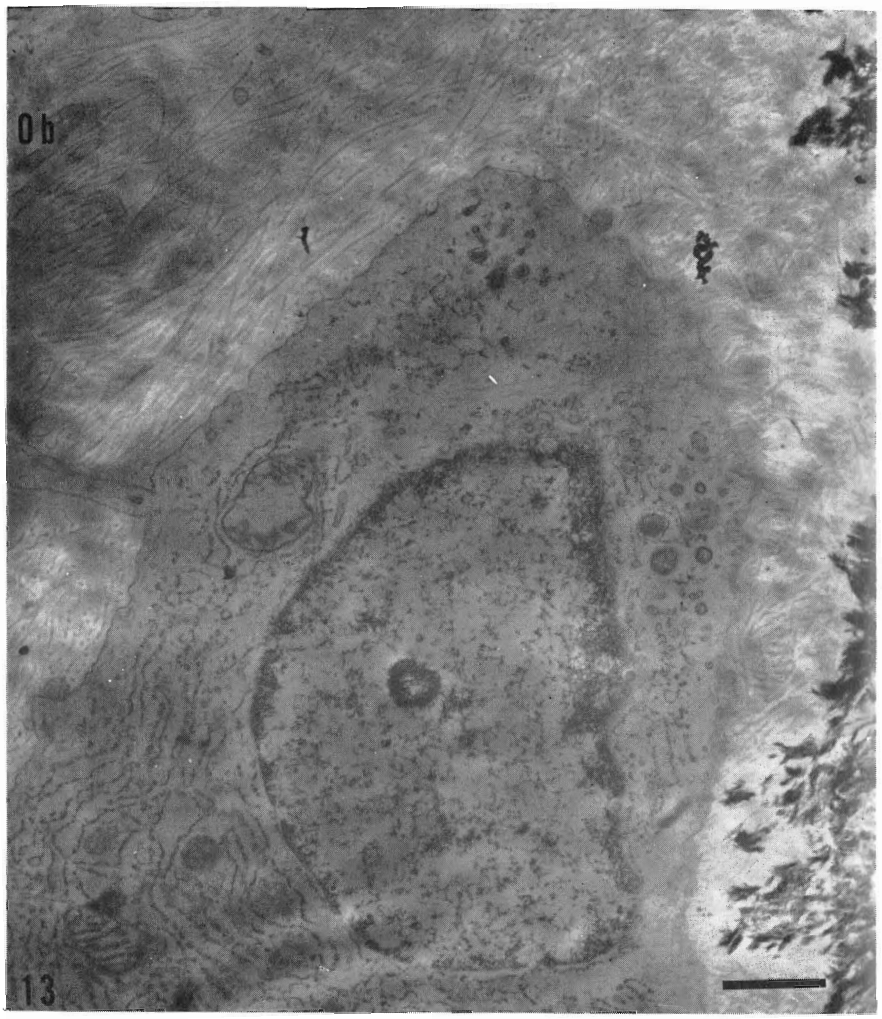

Fig. 13. Osteoid osteocyte with involuted organellae of metaphyseal trabecula in normal young rat. Small nucleolus. Osteoid with abundant collagen fibers. Masking of collagen fiber increases with increasing distance from bone matrix forming cell (Ob). Lead hydroxide.

Fig. 14. Osteocyte of metaphyseal trabecula in normal young rat. Cytoplasmic organellae showed advanced involution. Uneven calcification front. Mineralization is in relation with pre-existing collagen fiber in unmineralized bone matrix. Lead hydroxide.

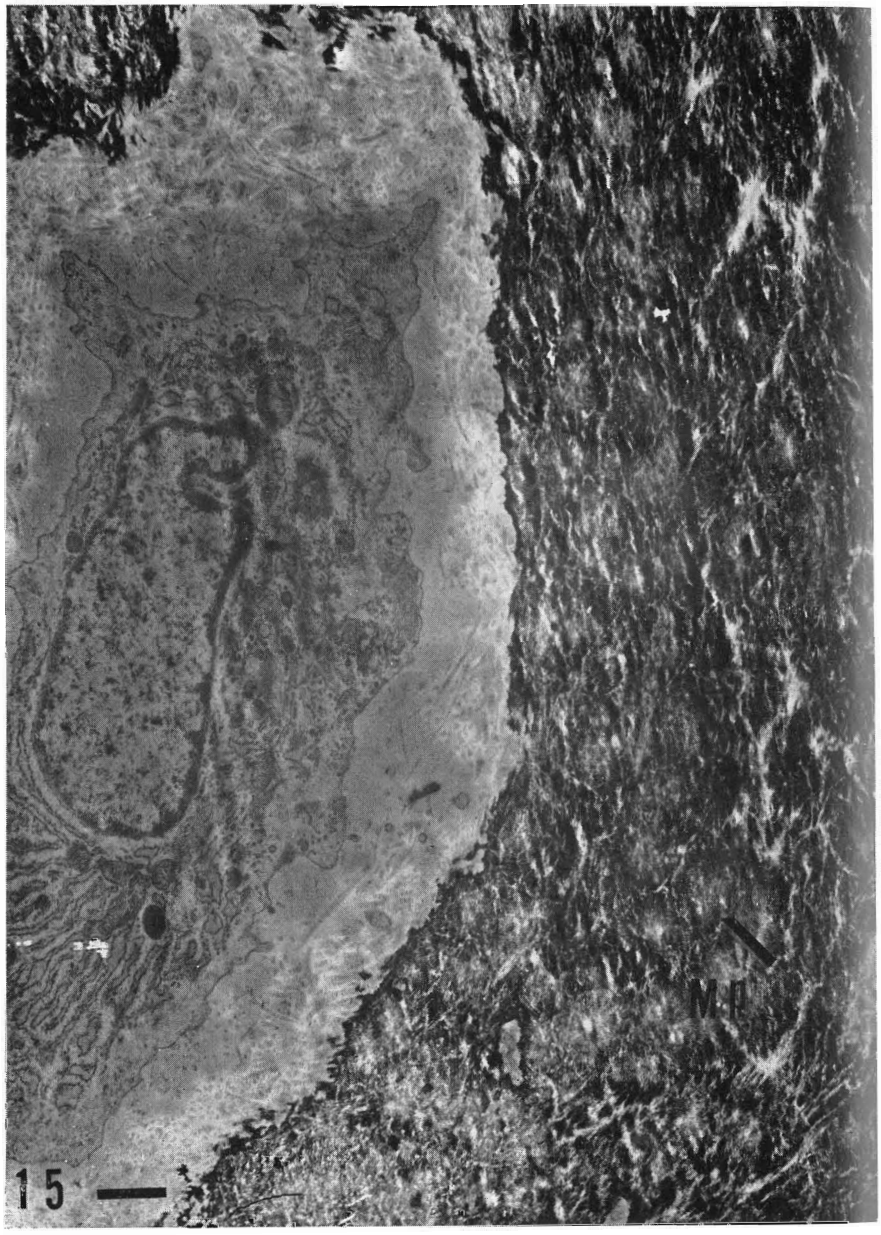

Fig. 15. Osteocyte in lathyric periosteal hyperostosis. Cytoplasmic organellae indicate rather slight involution. Osteoid is devoid of collagen fibers near osteocyte. Even calcification front. Mottled pattern of mineralized bone matrix (Mp). Central portions of the mottles show low density. Lead hydroxide.

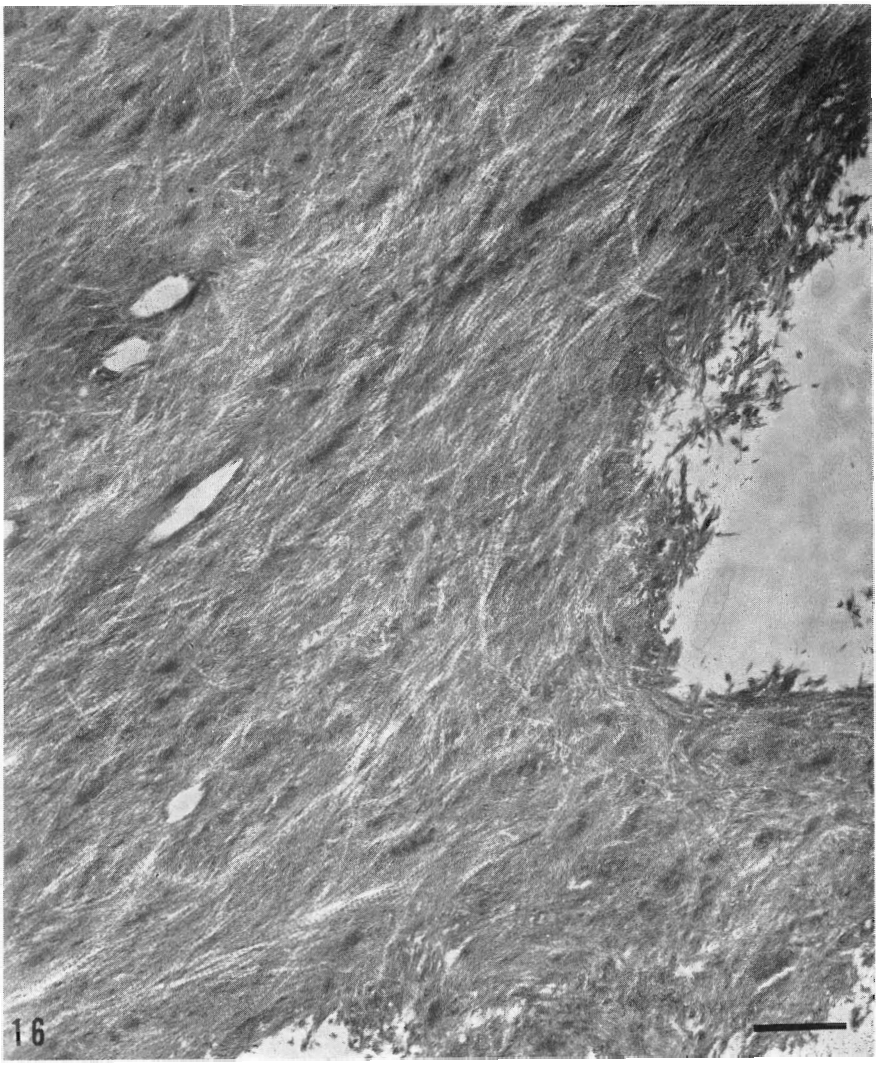

Fig. 16. Unmineralized and mineralized bone matrix of metaphyseal trabecula in normal young rat. Initial mineralization and uneven calcification front with crossing band-like projections. Occasional periodical banding of collagen fibers in mineralized bone matrix. Lead hydroxide.

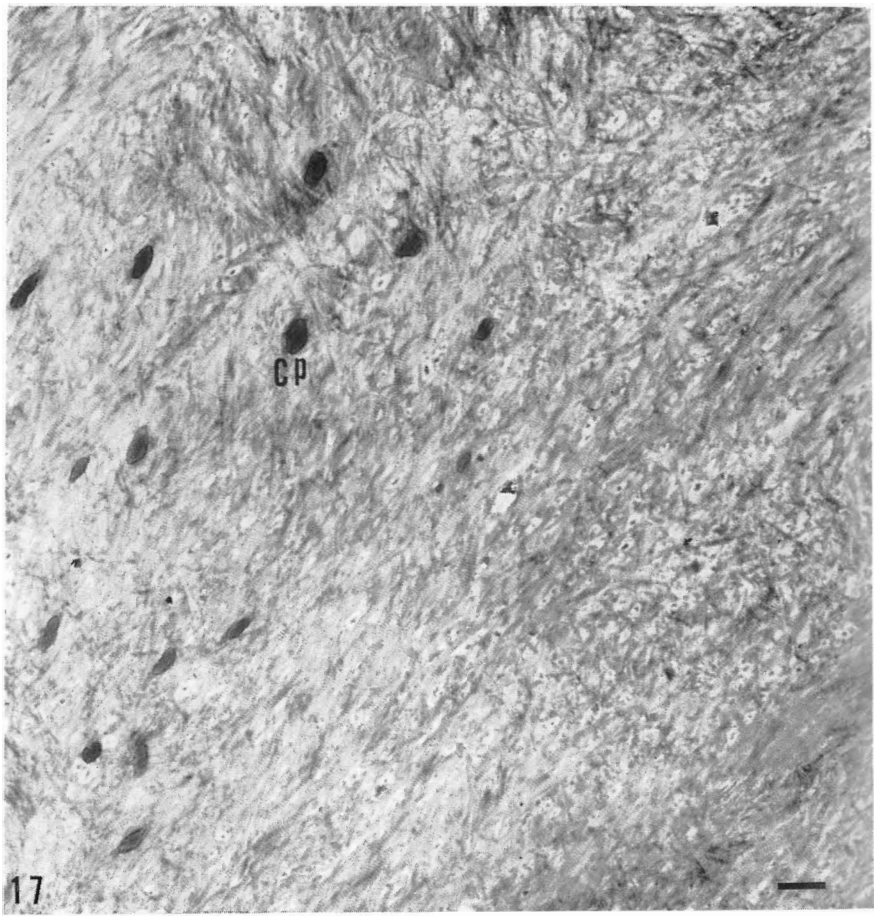

Fig. 17. Distinct periodical banding of collagen fibers in mineralized bone matrix. Scattered cytoplasmic processes in transverse section (Cp). Phosphotungstic acid.

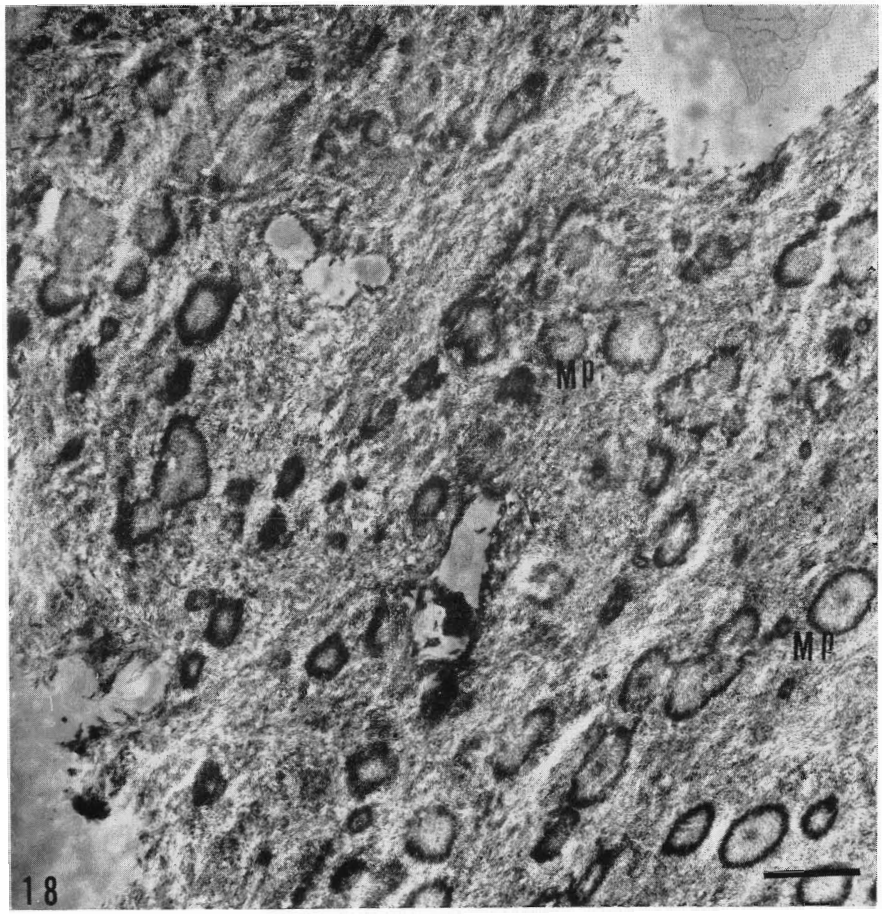

Fig. 18. Typical mottled pattern of mineralized bone matrix in lathyric periosteal hyperostosis. Periphery of each mottle (Mp) shows high mineralization, but the central portion indicates low mineralization. Lead hydroxide.

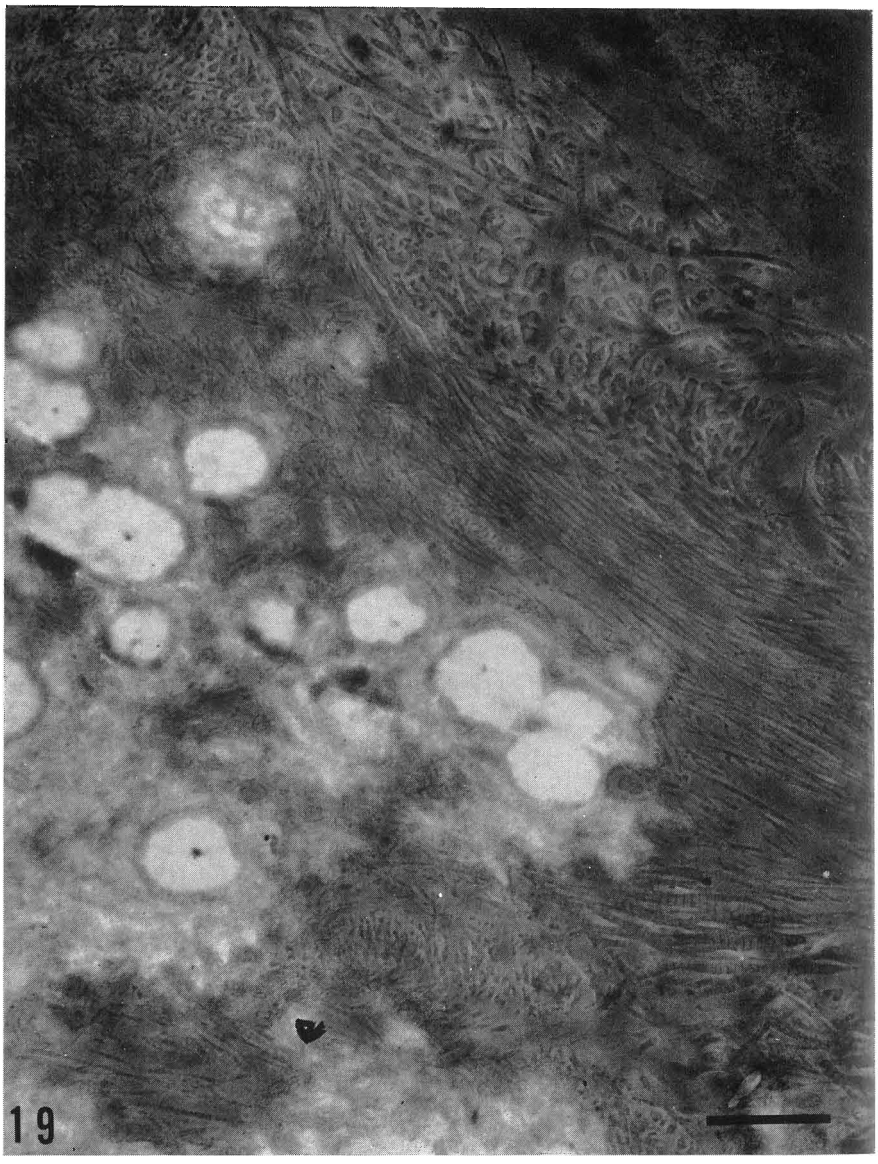

Fig. 19. Scattered mottles in mineralized bone matrix in lathyric periosteal hyperostosis. Mottles are devoid of collagen fibers. Increase in number of collagen fibers in mineralized bone matrix is not marked. Phosphotungstic acid.