CARDIAC NECROSIS AND CALCIFICATION IN EXPERIMENTAL MAGNESIUM DEFICIENCY

A Light and Electron Microscopic Study

H. Alexander Heggtveit, M.D.*; Lawrence Herman, Ph.D., and Rajendra K. Mishra, M.D.†

From the Department of Pathology, State University of New York, Downstate Medical Center, Brooklyn, N.Y.

Myocardial lesions in experimental magnesium deficiency have been reported by a number of authors.[1-8] Dietary depletion of magnesium has been shown to cause focal myocardial necrosis in rats.[3-5] These changes may progress to fibrosis and calcification. Cold stress enhances the progression of the lesions and the addition of magnesium supplements to the diet inhibits their formation.[5] Preliminary observations on the ultrastructure of rat cardiac muscle following a magnesium-deficient regime and cold stress have been reported.[9] A more detailed account of these changes, based on additional experiments is presented in this paper.

Material and Methods

Fifty female Sprague-Dawley rats (Sprague-Dawley Farms, Madison, Wis.), weighing 60 to 78 gm (average 70 gm), were divided into 6 experimental and control groups as follows: group I was fed a magnesium (Mg)-deficient diet; group II received a Mg-deficient diet and cold stress was administered; in group III the depleted diet was replenished with a Mg supplement; group IV was also replenished but in addition the animals underwent cold stress; groups V and VI were fed a stock diet of Purina "chow checkers" and stress was administered to the rats in group VI.

The animals were individually housed in an air-conditioned room at 68°F. and all diets were given *ad libitum*. De-ionized, doubly-distilled drinking water was available at all times. The commercially prepared Mg-deficient diet (General Biochemicals, Chagrin Falls, Ohio) contained: casein (high protein) 25.00 per cent, choline chloride 0.25 per cent, corn oil 5.00 per cent, dextrose 58.28 per cent, gelatin 5.00 per cent, i-Inosital 0.10 per cent, DL-Methionine 0.30 per cent and salt mix 5.99 per cent. The salt mixture consisted of calcium carbonate 905.84 gm, calcium phosphate 226.44 gm, copper sulfate 0.92 gm, iron citrate 84.52 gm, manganous sulfate 12.08 gm, potassium iodide 2.40 gm, potassium dihydrogen phosphate 981.16 gm, sodium chloride 507.20 gm and zinc carbonate 0.76 gm per 100 lbs. of diet. Biotin 0.0140 gm, calcium pantothenate 1.1340 gm, folic acid 0.1132 gm, menadione 0.2268 gm, nico-

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Present address: * Department of Pathology, University of Ottawa, Faculty of Medicine, Ottawa, Ontario, Canada; † Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India.

tonic acid 3.1748 gm, pyridoxine hydrochloride 3.1748 gm, riboflavin 0.2720 gm, thiamine hydrochloride 0.2720 gm, vitamin B_{12} 0.0020 gm, alpha tocopherol 0.9200 gm, vitamin A conc. (200,000 units per gm) 9.0800 gm and vitamin D conc. (400,000 units per gm) 0.9200 gm were added as vitamin supplements per 100 lbs. of diet. The Mg content of this mixture was less than 1 mg per 100 gm diet. The Mg supplement utilized in groups III and IV consisted of Mg chloride 150 to 200 mg per 100 gm diet. The Purina chow contained Mg 200 mg per 100 gm diet. Cold stress was administered by swimming the animals in an ice-water bath at 1° C for 4 minutes. This was done twice daily on the 2 days prior to sacrifice.

Animals were killed by decapitation in batches of 10 on days 14, 21, 29 and 36. Each lot of 10 rats consisted of 3 from group I, 3 from group II and 1 apiece from groups III, IV, V and VI. The apical portions of the hearts were excised, cut into 1 mm cubes and fixed in cold buffered osmium tetroxide containing sucrose. The tissue blocks were fixed for 2 hours at 50° C, dehydrated through a series of graded alcohol-water solutions containing uranyl acetate, cleared in propylene oxide and embedded in an epoxy resin (Epon). Ultrathin sections were cut on Porter-Blum and LKB microtomes with glass and diamond knives. Lead hydroxide stained and unstained sections were examined with an RCA EMU-3C electron microscope.

The remaining portions of the hearts were fixed in 10 per cent neutral formalin and embedded in paraffin for light microscopy. Heart sections were stained routinely with hematoxylin and eosin, and in selected cases, Masson's trichrome, periodic acid-Schiff (PAS), phosphotungstic acid hematoxylin (PTAH) and von Kossa's silver nitrate techniques were employed. Complete necropsies were performed on all animals and all the major organs were processed for light microscopy.

Observations

Clinical Observations

Animals in groups I and II exhibited the classic signs of Mg deficiency[10,11] beginning on the fifth day. They developed intense erythema and edema of the ears and foot pads. Hyper-irritability was noted during the second week and some of the animals manifested tonic and clonic convulsions following which a few expired. During the third week on the Mg-deficient diet patchy alopecia with crusted skin lesions appeared. These rats exhibited marked growth depression; at the time of sacrifice the Mg-depleted animals weighed an average of 55 per cent less than those receiving stock diet rations. The spontaneous mortality rate of the rats in groups I and II throughout the experiment was 20 per cent.

Animals in groups III, IV, V and VI showed none of the characteristic signs of Mg deficiency. However, the rats in groups III and IV, receiving the Mg-supplemented diet, averaged 20 per cent less in weight than did those in groups V and VI on stock diet.

Gross Morphologic Observations

Gross cardiac lesions were found in none of the animals in group I, but were present in all of the rats in group II, sacrificed at 14 days. Lesions visible to the naked eye were seen in the hearts of 2 of the rats in group I, and in all of those in group II, killed at 21 days. One animal

in group I and two in group II showed lesions at 29 days. No gross cardiac abnormalities were discovered at 36 days in groups I or II. None of the animals in groups III, IV, V and VI exhibited changes at any of the 4 days of sacrifice.

The lesions varied from small, pale patches flecked with yellow in the heart muscle to large areas of necrosis and calcification extending through the entire ventricular wall. The subepicardial vessels were often severely congested and occasionally the cardiac chambers were markedly dilated.

Apart from vascular engorgement the only other structures exhibiting gross alterations were the skin and kidneys. Most of the rats in groups I and II had crusted skin lesions and several had minute yellowish foci of calcification in the renal parenchyma.

Light Microscopic Observations

Histologic lesions were found in the cardiac muscle of all Mg-depleted rats (groups I and II). These animals developed focal areas of myocardial necross and exudative inflammation. The lesions were scattered at random throughout the myocardium but the majority were situated in the subendocardial regions. In several animals extensive transmural zones of necrosis extended from the endocardial to the epicardial surfaces. Although many of the necrotic foci surrounded small ramifications of the coronary vessels, the distribution of the lesions was not consistently perivascular. Vascular dilatation and hyperemia were common, but no primary blood vessel damage was apparent.

The typical lesion consisted of focal destruction and loss of muscle cells with replacement by an inflammatory exudate composed primarily of mononuclear cells (Fig. 1). Neutrophils and eosinophils were also seen but were present only in small numbers. The mononuclear cells were mostly macrophages but occasionally Anitschkow myocytes and lymphocytes were noted. The muscle fibers at the periphery of the lesions were frequently fragmented and vacuolated. Often the sarcoplasmic vacuoles contained prominent clusters of minute reddish-brown granules. Other adjacent muscle cells stained intensely eosinophilic and exhibited loss of cross striations. PAS-positive material was usually present in such fibers. Muscle fibers exhibiting increased sarcoplasmic eosinophilia, granularity and vacuolization were sometimes found in areas remote from foci of overt necrosis.

In over half of the Mg-deficient rats, many of the lesions were accompanied by varying degrees of calcification (Figs. 2 and 3). Myocardial calcium deposition first appeared as discrete basophilic granules within the cytoplasm of degenerating muscle fibers. These von Kossa-

positive granules formed intracellular clumps and linear plaque-like aggregates. With the complete necrosis and phagocytosis of the non-calcified remnants of the muscle cells, these masses of calcium tended to enlarge, coalesce and assume an extracellular interstitial position. In some lesions, small quantities of calcium were found in relatively large foci of necrosis, while in other instances, rather large deposits of calcium were accompanied by minimal cellular reaction. Occasionally, the only visible alteration in isolated muscle fibers was granular intracytoplasmic calcification. Nuclear changes occurred late, following extensive sarcoplasmic damage, and consisted of karyorrhexis and karyolysis.

In addition to necrosis and calcification, many of the lesions were also characterized by proliferation of fibroblasts and the laying down of collagen. Calcification often preceded fibrosis. The degree of scarring was related to the magnitude of the necrotic areas rather than to the presence of calcium in the myocardium.

Cardiac lesions in all stages of evolution were found in animals of groups I and II killed on all 4 dates of sacrifice. Generally speaking, more advanced lesions, larger numbers of lesions, and those with the greatest degree of fibrosis and calcification were seen at 21 and 29 days. The severe lesions tended to occur earlier and attain greater magnitude in those Mg-depleted rats exposed to cold stress.

No myocardial alterations were seen at the light microscopic level in any of the animals in groups III or V. Minor histologic changes consisting of sarcoplasmic vacuolization and segmental eosinophilia of isolated muscle fibers were detected in the hearts of several animals in groups IV and VI.

Microscopic sections of skeletal muscles from the Mg-deficient rats revealed only a few small foci of necrosis and exudative inflammation in several animals. The kidneys from animals in groups I and II frequently disclosed calcium casts in the tubules, with tubulorrhexis and focal inflammatory changes in the interstitium. Foci of suppurative inflammation were often observed in the skin of these rats. No significant lesions were discovered in other organs or tissues.

Electron Microscopic Observations

The fine structure of cardiac muscle has been well-documented.[12-18] For purpose of comparison a low magnification view of normal cardiac muscle is shown (Fig. 4).

Ultrastructural changes in the heart muscle of Mg-deficient rats were most pronounced in and around the areas of necrosis described histologically. Collagen deposition was prominent in these regions as were numerous infiltrating cells. Large dense masses with blackened spherical

structures were present (Figs. 5 and 6). Some regions contained bundles of fibers and degenerating myofibrils (Fig. 7). Alterations in the various components of the myocardium will be considered individually.

Sarcosomes. Mitochondrial or sarcosomal alterations were the earliest lesions seen, and consisted of swelling and distortion of the internal fine structure. Vacuolization of the enlarged sarcosomes was a common feature. (Fig. 8). The appearance of one or more vacuoles resulted in further distortion of the cristae. Clusters of swollen and vacuolated sarcosomes accumulated in the cytoplasm of muscle cells in areas where loss of myofibrils was prominent, as well as in a paranuclear position. These changes were most pronounced in areas of maximum cellular damage but were also seen in varying degree throughout the areas of histologically normal myocardium.

A variety of other sarcosomal changes were seen. These included clumping of cristae and progressive deposition on and between cristae of a particulate, electron-dense material, presumably calcium (Figs. 9 to 11). This material eventually filled the entire sarcosome (Fig. 12). These altered sarcosomes matted together formed large osmiophilic masses of calcium (Figs. 5, 6, 13 and 14). Dense sarcosomes released through rupture of muscle cells were phagocytosed by macrophages (Fig. 15).

Myofibrils. Fragmentation and loss of myofibrils also took place early in the development of the lesions. This resulted in the formation of large spaces within the cardiac muscle fibers (Fig. 16). These spaces sometimes contained components of the sarcoplasmic reticulum, glycogen particles and sarcoplasmic "ground substance," but were more often filled with clusters of normal or altered sarcosomes. These spaces and their contents corresponded to the vacuoles and granules described histologically.

The cross-striations of the affected muscle cells were disrupted by the fragmentation of myofibrils. "Z" bands and "M" lines were broken and discontinuous. The fragmented myofibrils often formed irregular, osmiophilic plaque-like structures composed of condensed aggregates of frayed myofilaments.

Some myofilaments seemed continuous with dense aggregates of material which displayed a crossband periodicity of 200 Å. In such areas it was difficult to determine whether such banded material was degenerating myofilaments (Fig. 17) or fibrin[19] (Fig. 18).

Sarcoplasmic Reticulum. The sarcoplasmic reticulum in degenerating muscle cells appeared more prominent than in undamaged fibers. This was due, in part, to an "unmasking" of these structures as a result of loss of myofibrils. Also, dilatation of the channels and vesicles of the sarco-

plasmic reticulum accounted for a portion of this prominence. Whorls of osmiophilic membranes were present (Fig. 5). In some instances grossly disturbed sarcosomes and fragments of myofilaments were encompassed by these concentric whorls. Lipid droplets were occasionally encountered within the cisternae of the sarcoplasmic reticulum.

Sarcolemma. Rupture or disappearance of the sarcolemma occurred as the cytoplasmic alterations became more pronounced. In some cases macrophages aligned themselves alongside the periphery of disintegrating muscle cells (Fig. 17). Gradual ingestion of the sarcolemmal membrane and altered cellular organelles ensued. Elsewhere, phagocytosis took place in the interstitial space following rupture of the sarcolemma with spillage of the sarcoplasmic contents.

Muscle Cell Nuclei. Nuclear lesions were infrequently seen with the electron microscope and occurred only after extensive cytoplasmic damage. Nuclei were often observed essentially intact even though many of the sarcoplasmic components of their respective fibers had undergone degenerative change and partial phagocytosis. However, as necrosis of muscle cells progressed some nuclei exhibited coarse clumping of chromatin, loss of nucleoli, vesiculation and irregularity of the nuclear membranes. This was followed by rupture of the nuclear membranes with dispersion and complete disappearance of nuclear material.

Interstitial Tissues. The majority of cells comprising the inflammatory exudate were mononuclear and actively phagocytic. Lymphocytes, neutrophilic and eosinophilic granulocytes were occasionally encountered. The cytoplasm of the macrophages often contained ingested organelles of cardiac muscle cells. The large vacuolated mitochondrion with dense deposit within (Fig. 17) was so much larger than the other mitochondria that it probably represented an ingested sarcosome.

Loss of muscle fibers created additional interstitial spaces (Figs. 5 to 7) which were only partially filled by exudate cells and large masses of calcium. These empty gaps, as well as the exudate, were gradually replaced by proliferating fibroblasts. Bundles of collagen fibrils were laid down in the healing lesions and extended peripherally between viable myocardial cells. Muscle fibers were sometimes split and partly replaced by groups of collagen fibrils formed by rapidly growing connective tissue cells.

No definitive changes were found in the capillaries and small blood vessels of the heart. Occasionally endothelial cells appeared rather plump and swollen as a result of an apparent increase in quantity of cytoplasm. Blood cells were rarely observed passing between the capillary endothelium into the interstitial space. Mast cells were occasionally found in the interstitium in all experimental and control groups. Increased

numbers of mitotic figures seen in the lesions were attributed to proliferating interstitial cells. However, the possibility that some of these dividing cells represented dedifferentiated myocytes could not be completely ruled out.

The ultrastructural changes described above were found almost exclusively in the hearts of animals in groups I and II. Quantitative but no qualitative differences existed between the lesions in the two groups. Several rats in groups IV and VI exhibited sarcosomal swelling and vacuolization with some focal fragmentation of myofilaments but no overt cellular necrosis or calcification. One animal in group III displayed focal swelling of sarcosomes with deposition of electron-dense material on the cristae. In the remaining animals receiving diet supplement the sarcosomes were essentially normal.

Discussion

The myocardial lesions of Mg-deficiency have been described and interpreted in varied manner. In 1936, Greenberg, Anderson and Tufts[2] described "myocardial degeneration with fibrosis and polyblastic infiltration" in rats reared on diets low in magnesium. Two years later, Moore, Hallman and Scholl[6] found focal cardiac necrosis and calcification in calves fed Mg-deficient rations. Blaxter[1] noted hemorrhages in the myocardium of livestock with a hypomagnesemic disorder. In a comprehensive study of the basic histologic lesions of Mg-deficiency in the rat, Lowenhaupt, Schulman and Greenberg[4] described a widespread inflammatory lesion involving loose mesenchymal tissues. In the heart, the lesions began as focal acute perivascular inflammation and progressed through stages of necrosis, granulomatous inflammation and fibroblastic proliferation to scar formation. Mishra[5] produced similar lesions in albino rats and noted that stress caused aggravation of the lesions. Vitale, Hellerstein, Nakamura and Lown[7] observed calcification of the myocardium in puppies restricted to a Mg-deficient diet. The calcium deposition was accompanied only by mild fibrosis and a few macrophages. Ko, Fellers and Craig[3] found focal degenerative and inflammatory changes in the hearts of Mg-deficient rats. Recently, Wener and associates[8] have reported myocardial degeneration, necrosis and calcification in a study of prolonged hypomagnesemia in dogs.

The Mg-deficient rats in these experiments developed multiple myocardial necroses of varying size. Calcification occurred either early or late and began within the sarcosomes of cardiac muscle fibers, later assuming an interstitial position following complete lysis of the myocyte. (Additional studies of these regions are being carried out using: selected area electron diffraction; transmission electron diffraction; and electron

microprobe analysis.) Fibroblastic proliferation was variable and often of scant proportions. No significant vascular alterations were demonstrable, thus capillary endothelial injury or myocardial ischemia were not considered to be of prime importance in the pathogenesis of the lesions.

In a preliminary electron microscopic study from this laboratory of rat cardiac muscle following a Mg-deficient regime and cold stress,[9] alterations in the sarcosomes were considered to be fundamental to the development and progression of the lesions. The experiments reported here confirm this observation. Swelling and vacuolization of sarcosomes with derangement of their internal fine structure (Figs. 9 to 12), was an early and important change, not only in zones of maximum injury, but in areas of histologically intact myocardium.

Nakamura and colleagues[20] have demonstrated sarcosomal swelling morphologically and spectrophotometrically in mitochondria isolated from Mg-deficient rat hearts. Besides swelling, there was visible loss of sarcosomes in the Mg-deficient rats. Mishra[5] has shown a statistically significant diminution in the number of sarcosomes in homogenates of Mg-deficient cardiac muscle.

The mitochondria are the site of Mg-dependent enzyme systems involved in oxidative phosphorylation.[21-23] Uncoupling of oxidative phosphorylation is known to occur in the Mg-deficient state.[24-27] A close interdependence exists between mitochondrial structure and function.[28] It is not clear whether the effect of Mg is related to the biochemical or to the physical integrity of the mitochondrial system.[29]

Mg deficiency is accompanied by a decrease in serum calcium and an increase in soft tissue calcium.[30-35] Tufts and Greenberg[35] demonstrated a 15-fold increase in the calcium content of kidneys and a 60 per cent increase in heart muscle. Vitale and co-workers[7] along with Ko and associates[3] found normal serum calcium levels in their Mg-deficient animals. The mechanism of calcium deposition in Mg deficiency is obscure. The dense intramitochondrial particulate material seen in this study probably represents calcium. Deposition begins prior to cell death and serum levels of calcium are usually not increased. A competitive or antagonistic interrelationship between calcium and magnesium ions has been suggested.[36,37] This may be a possible explanation for calcium deposition in Mg deficiency. However, it is obvious that the biochemical aspects of Mg deficiency with respect to ion transport, calcification and oxidative phosphorylation are not yet completely understood.

The role of cold stress in the potentiation of the cardiotoxicity of the Mg-deficient diet requires explanation. Cardiac lesions have been produced by stress[38,39] and degenerative changes in the myocardium have

been observed in animals subjected to prolonged hypothermia.[40] Possible synergistic effects of excess thyroxine and adrenal corticosteroids liberated in response to cold stress must be considered in the development of the cardiac lesions.

Myocardial lesions in experimental potassium deficiency have been observed for a number of years,[41-69] and like those of Mg deficiency, have been the subject of conflicting interpretations. The cardiac lesions of Mg deficiency and potassium deficiency have striking histologic similarities including focal necrosis, calcification and fibrosis.[39,47] However, the ultrastructural alterations show conspicuous differences suggesting quite dissimilar underlying pathogenetic mechanisms.

The electron microscopic changes in the potassium-depleted heart have been studied by several investigators.[48-50] The predominance of cytoplasmic abnormalities with little or no nuclear loss has been interpreted as a degenerative process rather than a focal necrosis.[48] Although extensive myofibrillar damage takes place in both conditions, the sarcosomes appear to be fairly well-preserved in the potassium-deficient lesions[48] or only slightly swollen[50] whereas these organelles exhibit early and significant changes in Mg deficiency. A certain interrelationship exists between Mg and potassium with respect to the production and prevention of cardiac necrosis.[38,39,47] It has been postulated by one of the authors (R.K.M.) that the protection offered by $MgCl_2$ and KCl against various types of experimental cardiac necrosis depends upon an inhibition of some metabolic defect related to impairment of oxidative phosphorylation.

Electron microscopic studies of ischemic cardiac and skeletal muscle[51-54] have shown that apart from severe sarcoplasmic damage, nuclear changes are early and prominent features. This is in contrast to the cardiac lesions of experimental Mg deficiency which appear to commence as intracytoplasmic degenerative processes and exhibit nuclear injury rather late in their development. Sarcosomal alterations are basic to the evolution of the lesions which may progress to overt necrosis and calcification of isolated myocardial fibers or groups of fibers. Uncoupling of oxidative phosphorylation is probably an important factor in the pathogenesis of the mature lesions. Whether this is primarily a result of Mg deficiency, or is secondary to morphologic alterations in the sarcosomes, is still not known.

Summary

Changes in rat cardiac muscle following a magnesium-deficient regime and cold stress were studied histologically and by electron microscopy. Focal myocardial necrosis, calcification and fibrosis occurred in the

Mg-deficient animals. These changes were aggravated by cold stress and inhibited by magnesium supplements. Ultrastructural alterations included swelling and vacuolization of sarcosomes and fragmentation of myofibrils. Calcification commenced with the deposition of particulate, electron-dense material on the cristae of sarcosomes. The sarcosomal abnormalities appeared to be fundamental to the pathogenesis of the lesions. A relationship of these changes to deficient oxidative phosphorylation is possible.

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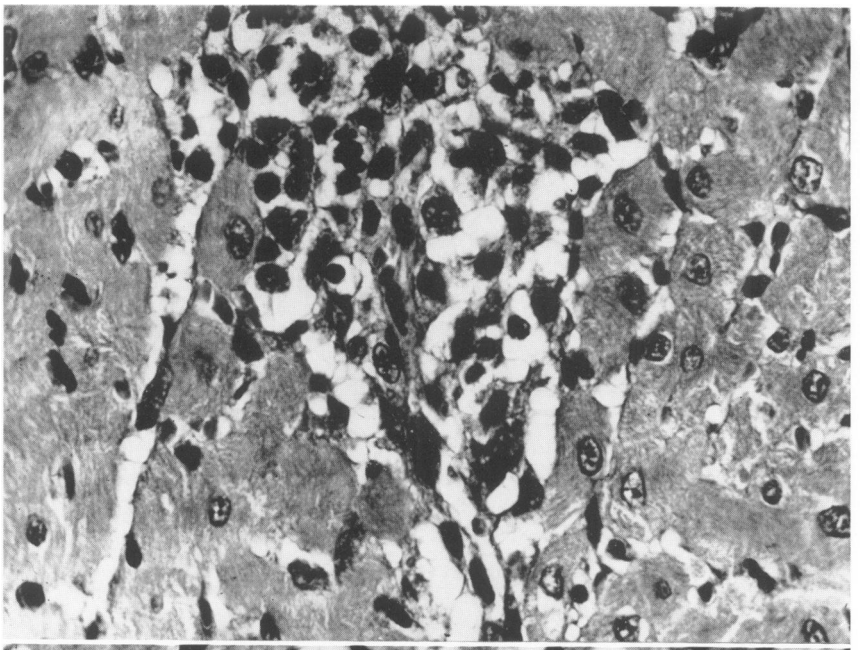
[*Illustrations follow*]

Legends for Figures

Fig. 1. Mg-deficient rat, day 21. Focal necrosis characterized by loss of muscle cells and replacement by an exudate in which macrophages predominate. Hematoxylin and eosin stain. × 500.

Fig. 2. Mg-deficient, stressed rat, day 29. Irregular dark masses represent deposits of calcium within myocardial fibers and in the interstitium. Clear areas are both intracytoplasmic vacuoles and apparently empty interstitial spaces created by loss of muscle cells. Note partially altered muscle cells with intact nuclei. Hematoxylin and eosin stain. × 500.



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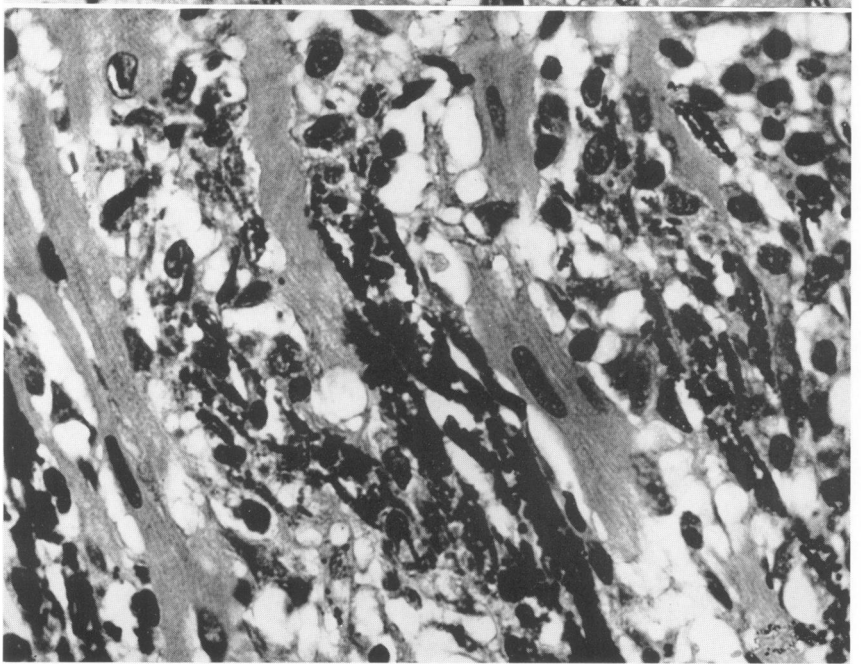

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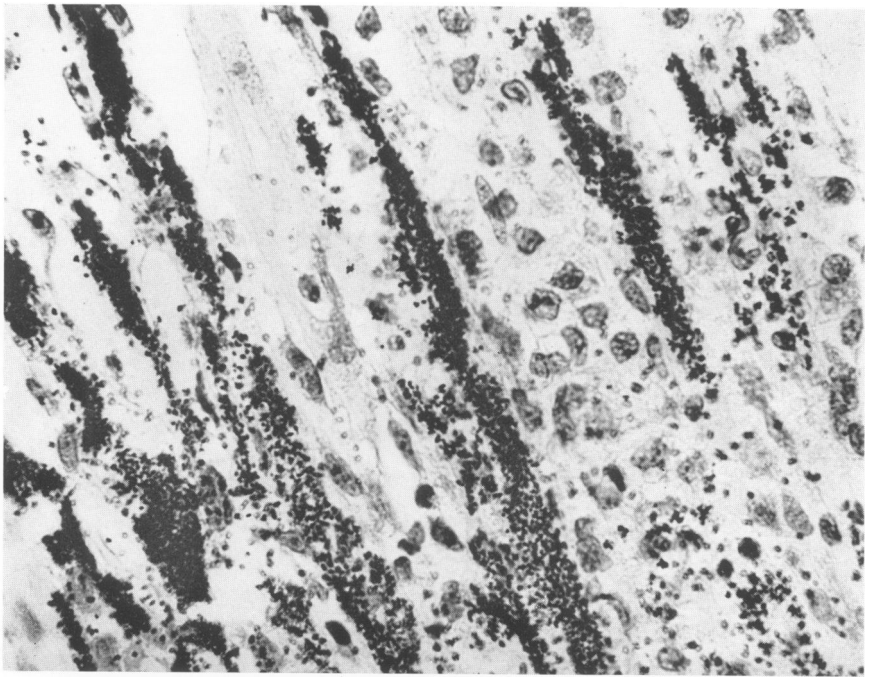
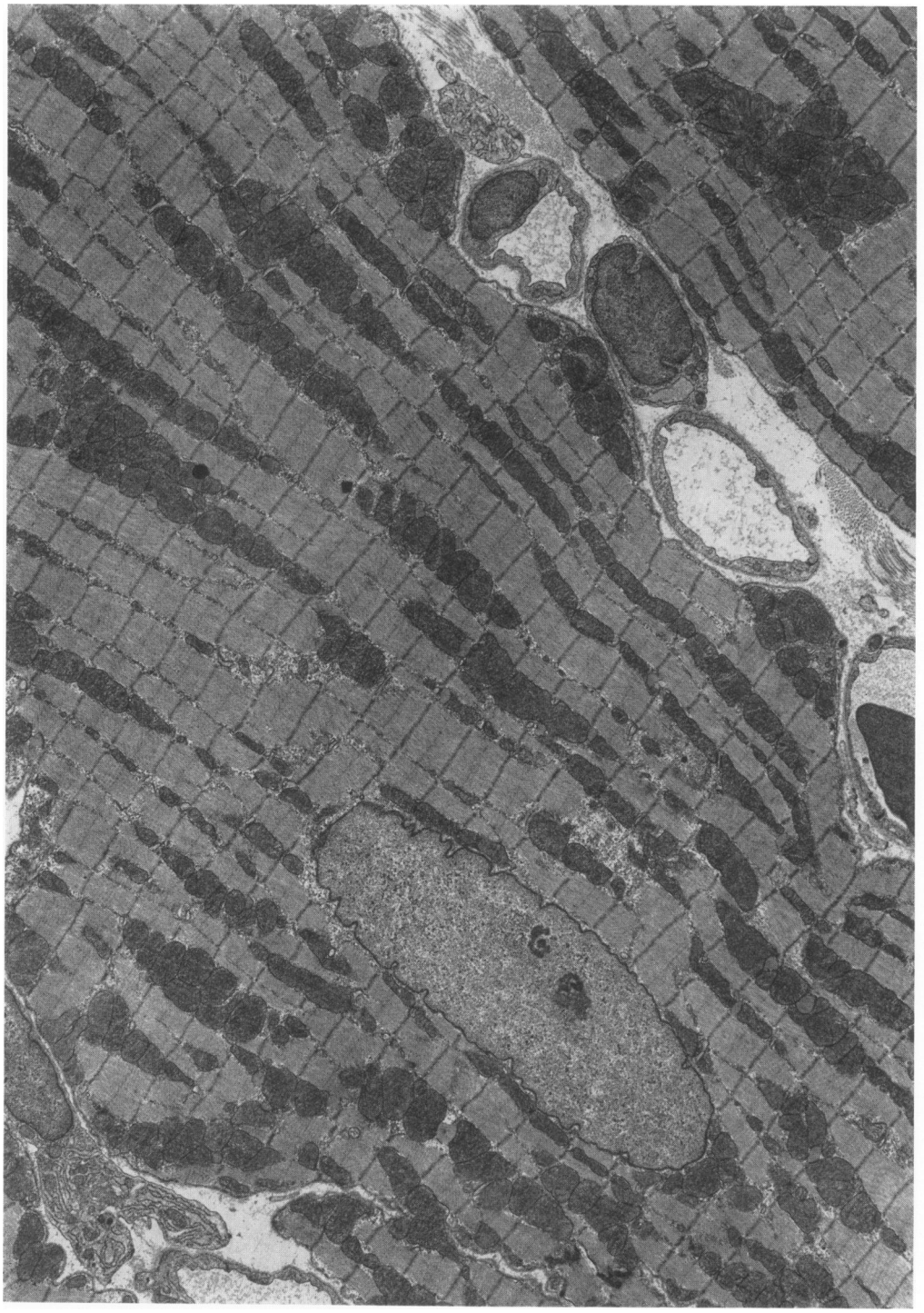


FIG. 3. Mg-deficient, stressed rat, day 29. Granular calcification appears in degenerating myocardial fibers. Von Kossa stain. × 500.

FIG. 4. Normal control, stock diet rat. × 3,800.



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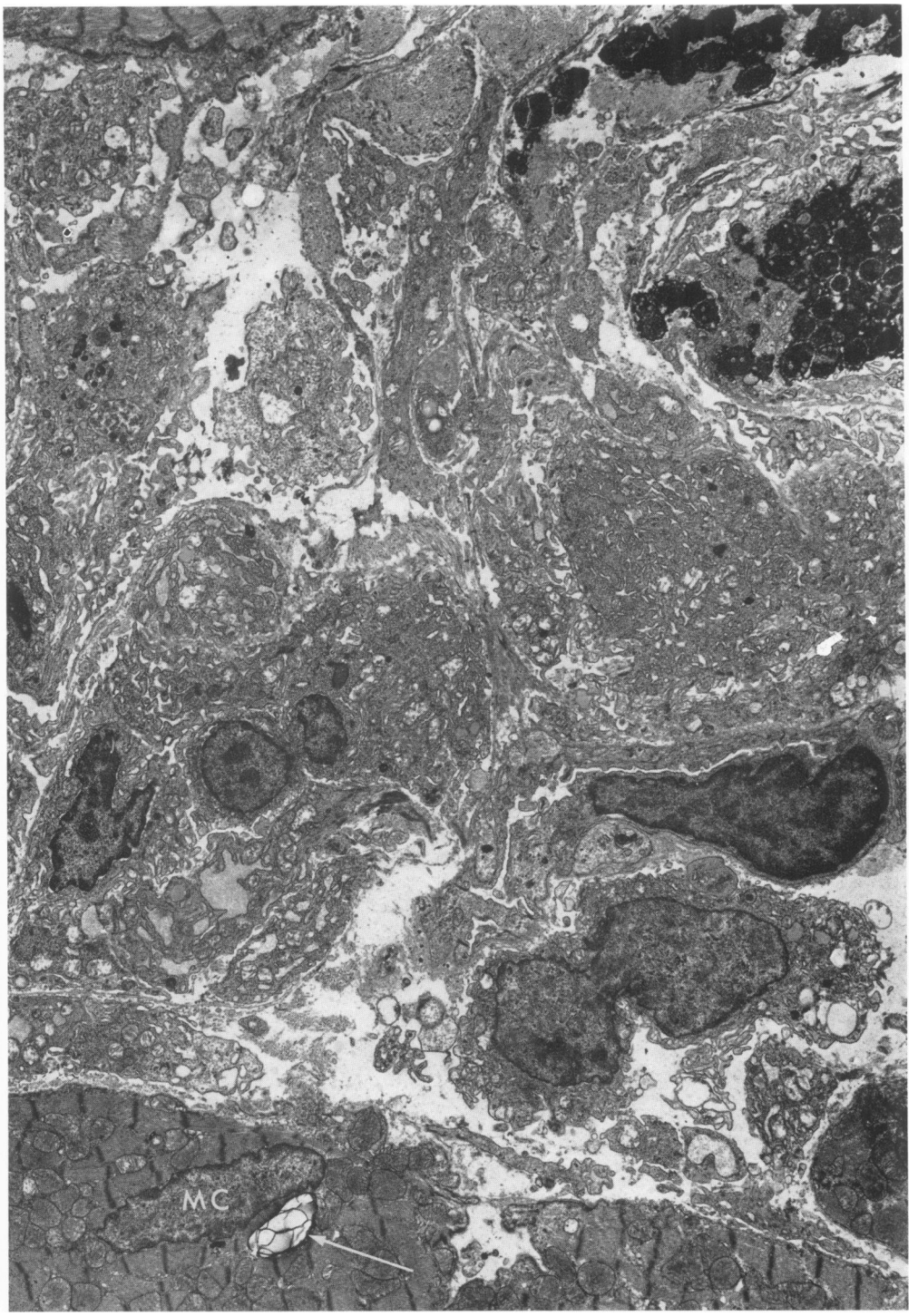

FIG. 5. Mg-deficient, stressed rat, day 29. An area filled with infiltrating cells and collagen is seen. A muscle cell (MC) persists at the bottom of the micrograph. A whorled collection of membranes (arrow) is adjacent to the nucleus. × 3,800.

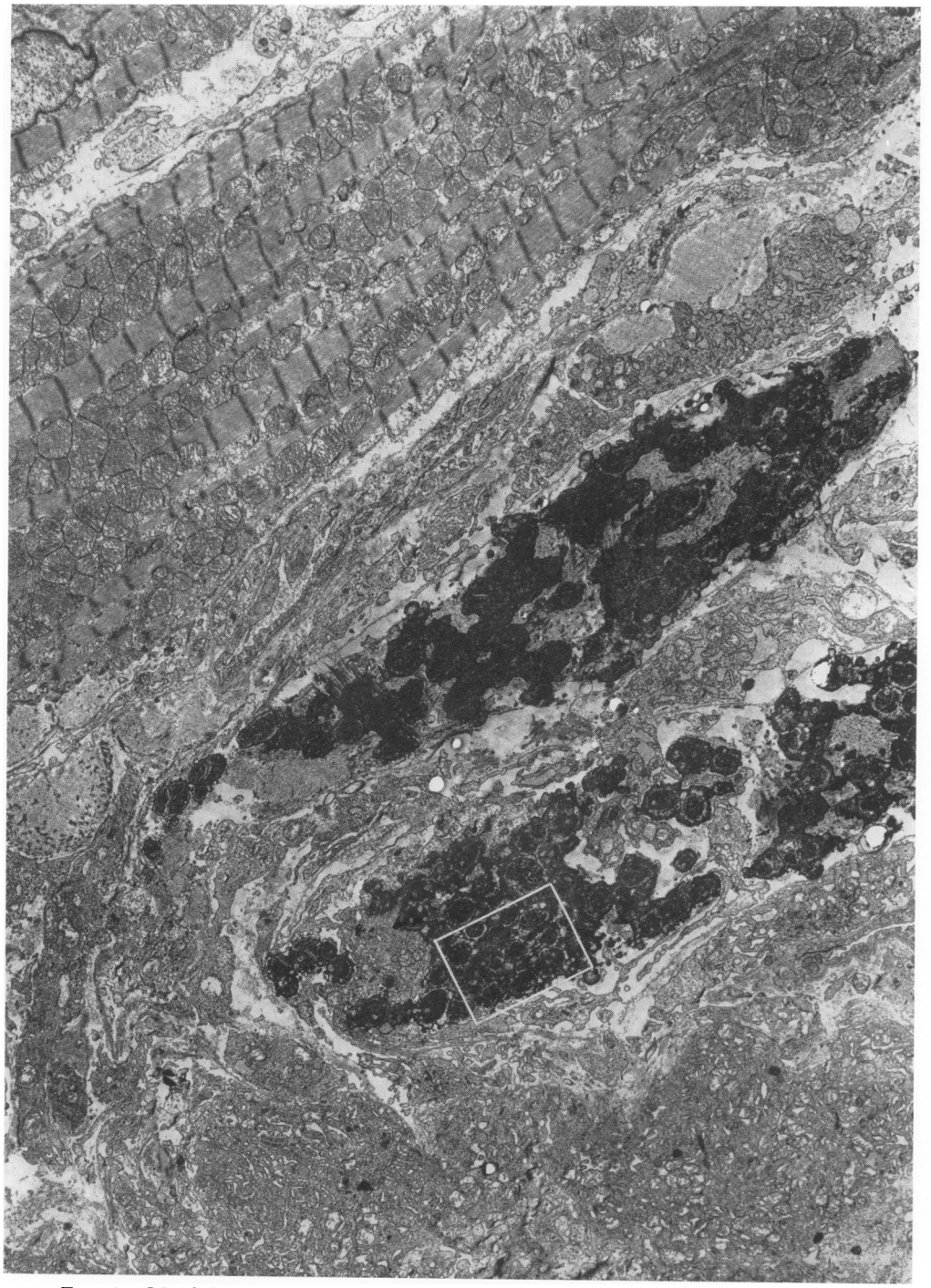

FIG. 6. Mg-deficient, stressed rat, day 29. Collections of infiltrating cells lie between muscle bundles and surround two large masses of dense material, probably representing calcium. Round dense structures, presumably sarcosomes, appear within these masses; these may represent calcified muscle cells. An area comparable to the inset may be seen at higher magnification in Figure 13. × 3,800.

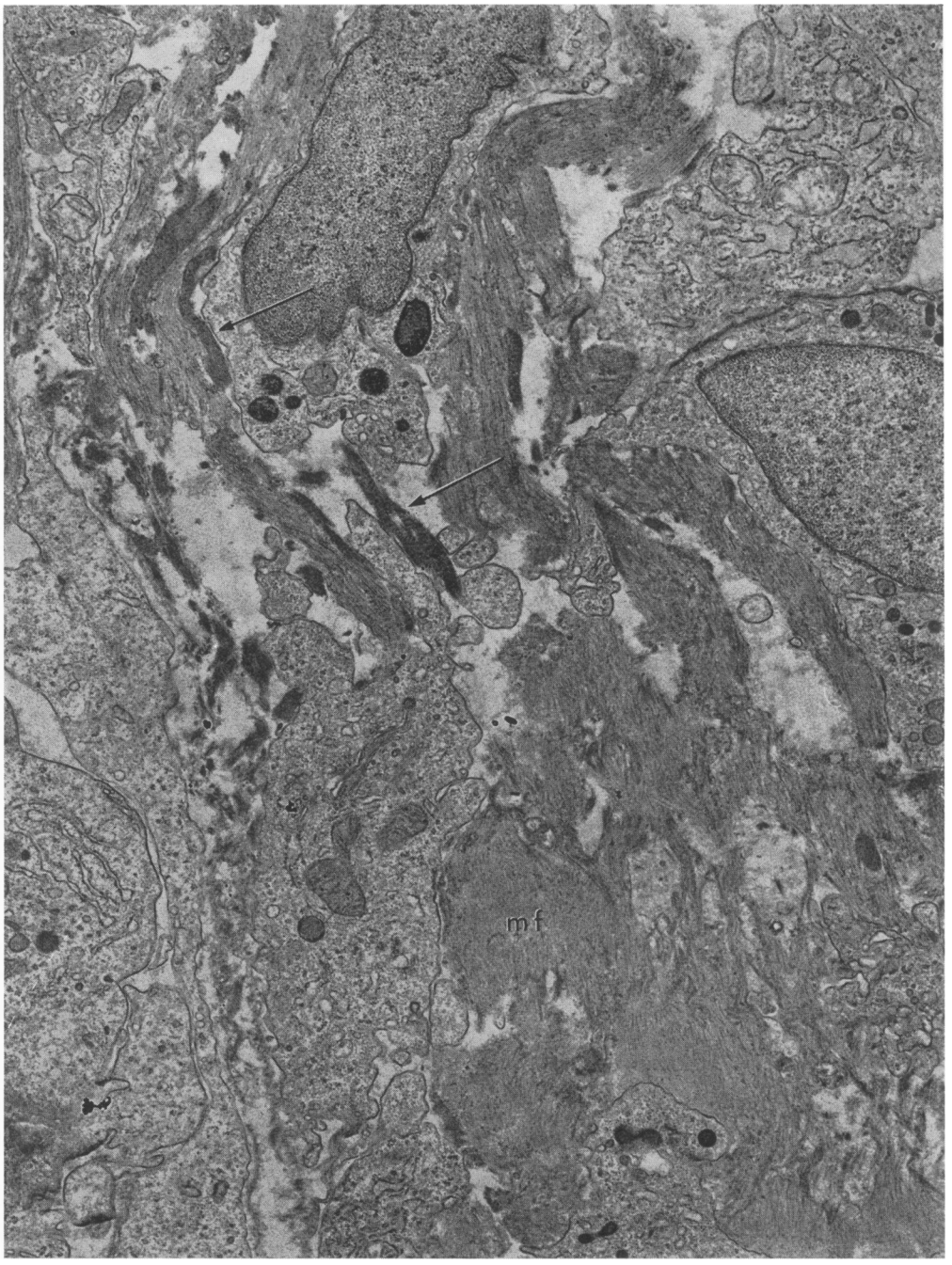

FIG. 7. Mg-deficient rat, day 21. Partially degenerated myofibrils (mf) are surrounded by macrophages. The irregular, osmiophilic plaque-like structures (arrows) in the prominent interstitial space, appear to be fragments of fibrin. × 7,300.

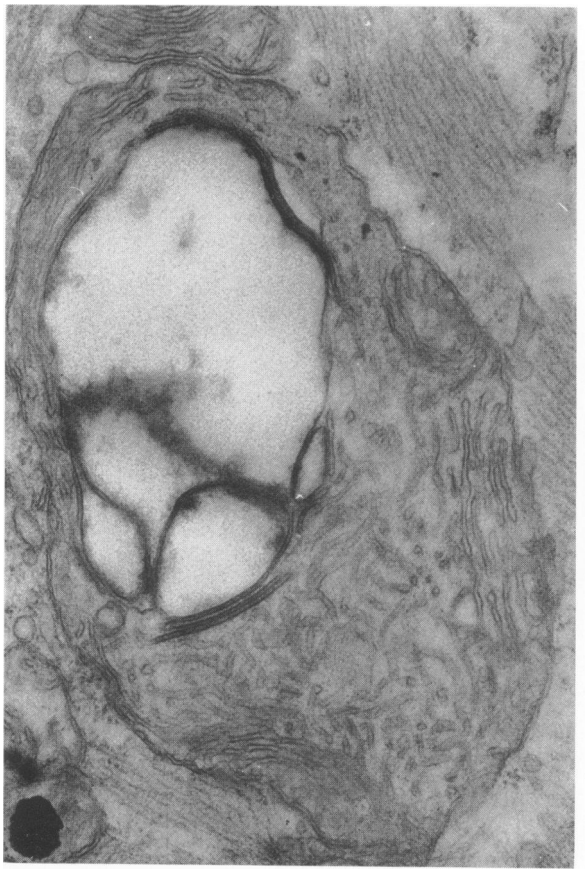

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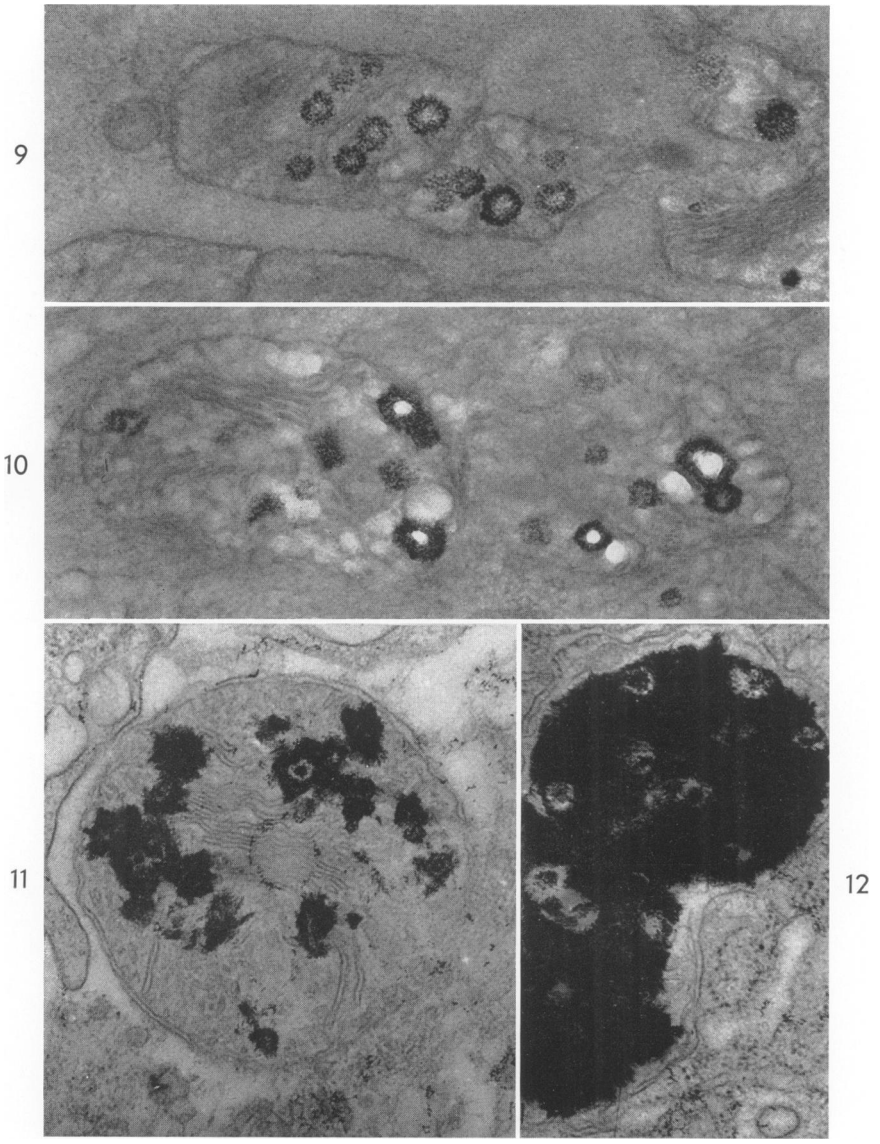
FIG. 8. Mg-deficient rat, day 14. A markedly enlarged sarcosome contains several vacuoles. The cristae are distorted, some cristae adjacent to the vacuole are highly dense and may represent the earliest deposition of calcium. × 37,500.



FIGURES 9 to 12. Progressive filling of mitochondria with electron dense granules, presumably calcium. × 32,000. FIG. 9. Mg-deficient, stressed rat, day 23. The dense granules are, at first, arranged as spheres with clear centers. FIG. 10. Mg-deficient, stressed rat, day 23. The spheres become thicker and more dense. FIG. 11. Mg-deficient, stressed rat, day 29. Electron dense granules increase in number and appear as solid dense particles. FIG. 12. Mg-deficient, stressed rat, day 29. Eventually the entire sarcosome is filled with many tightly packed particles.

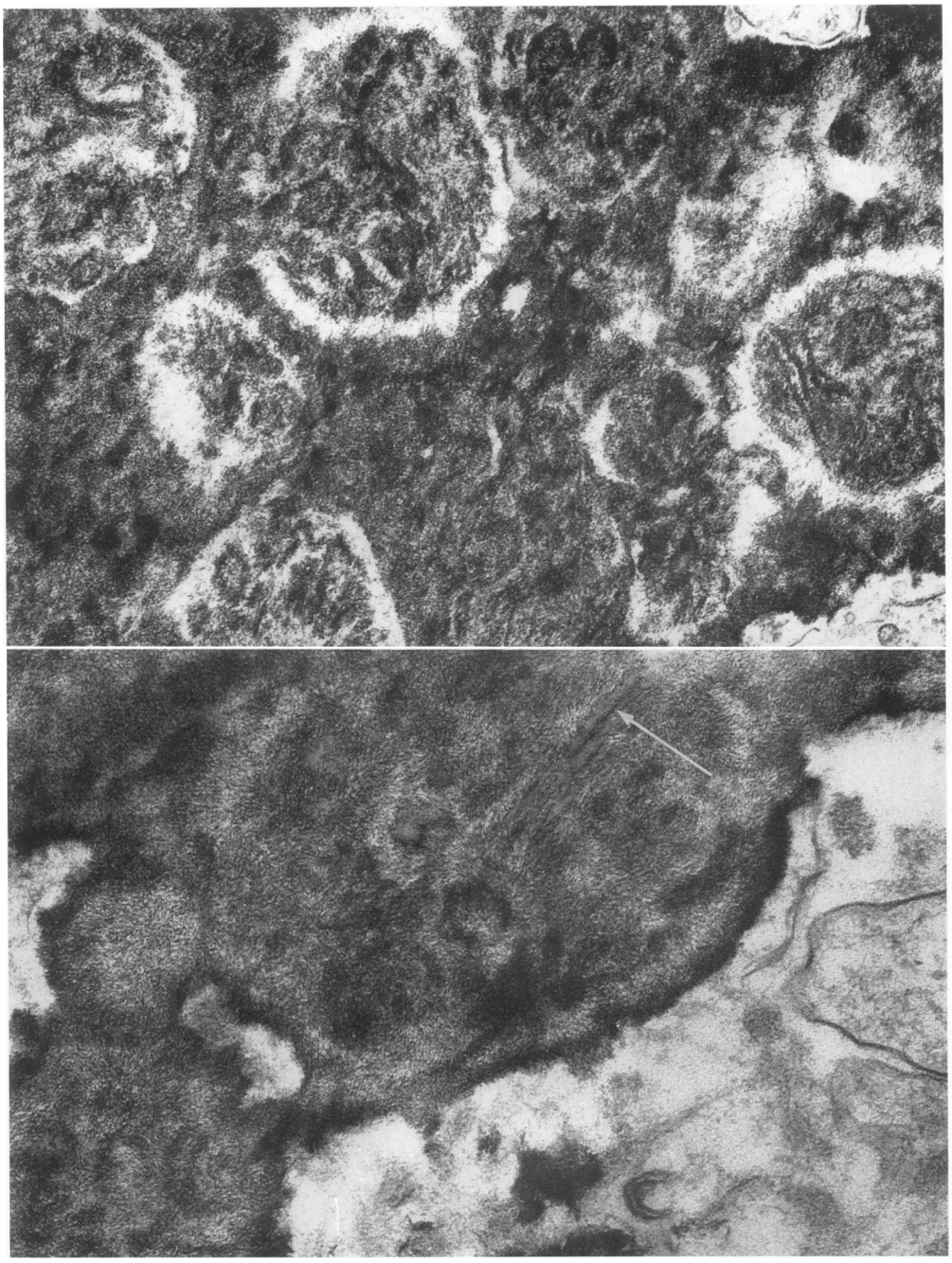


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FIG. 13. Mg-deficient, stressed rat, day 29, Details of a portion of a large dense mass similar to the inset shown in Figure 6. The round bodies representing calcified sarcosomes are encircled by clear areas. These, in turn, are embedded in a matrix of similar dense granular material. Little evidence persists to suggest that these are calcified muscle cells. × 36,000.

FIG. 14. Mg-deficient, stressed rat, day 29. Detail of structure of a calcified sarcosome. Some cristae reminiscent of the normal persist (arrow). × 65,000.

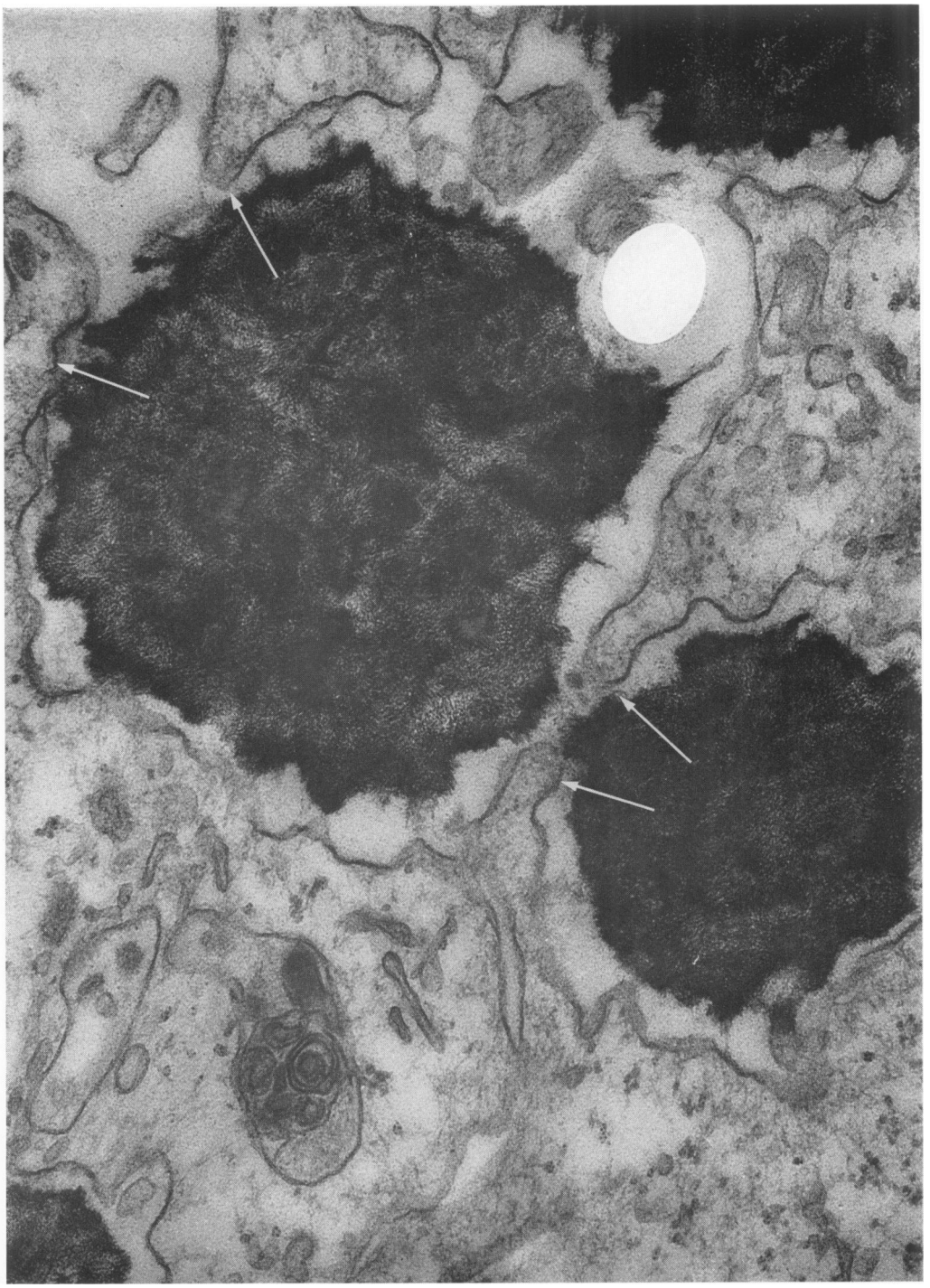

FIG. 15. Mg-deficient, stressed rat, day 29. Two calcified bodies, (presumably sarcosomes), in the interstitial space are being engulfed by the cytoplasmic extensions (arrows) of a macrophage in the process of phagocytosis. × 65,000.

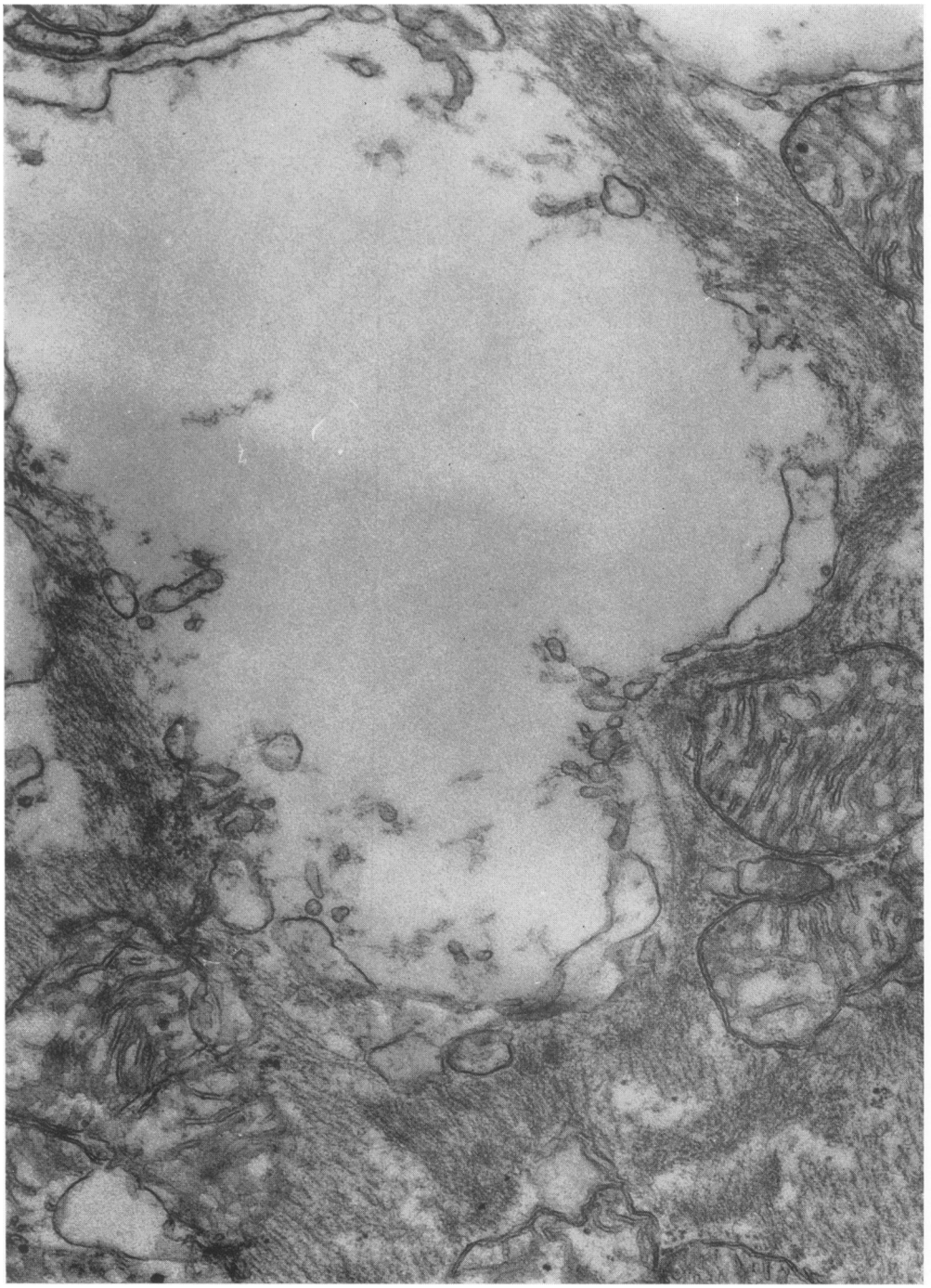

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FIG. 16. Mg-deficient, stressed rat, day 29. A large intracellular space of a muscle cell is created by the dissolution and loss of myofilaments. × 36,000.

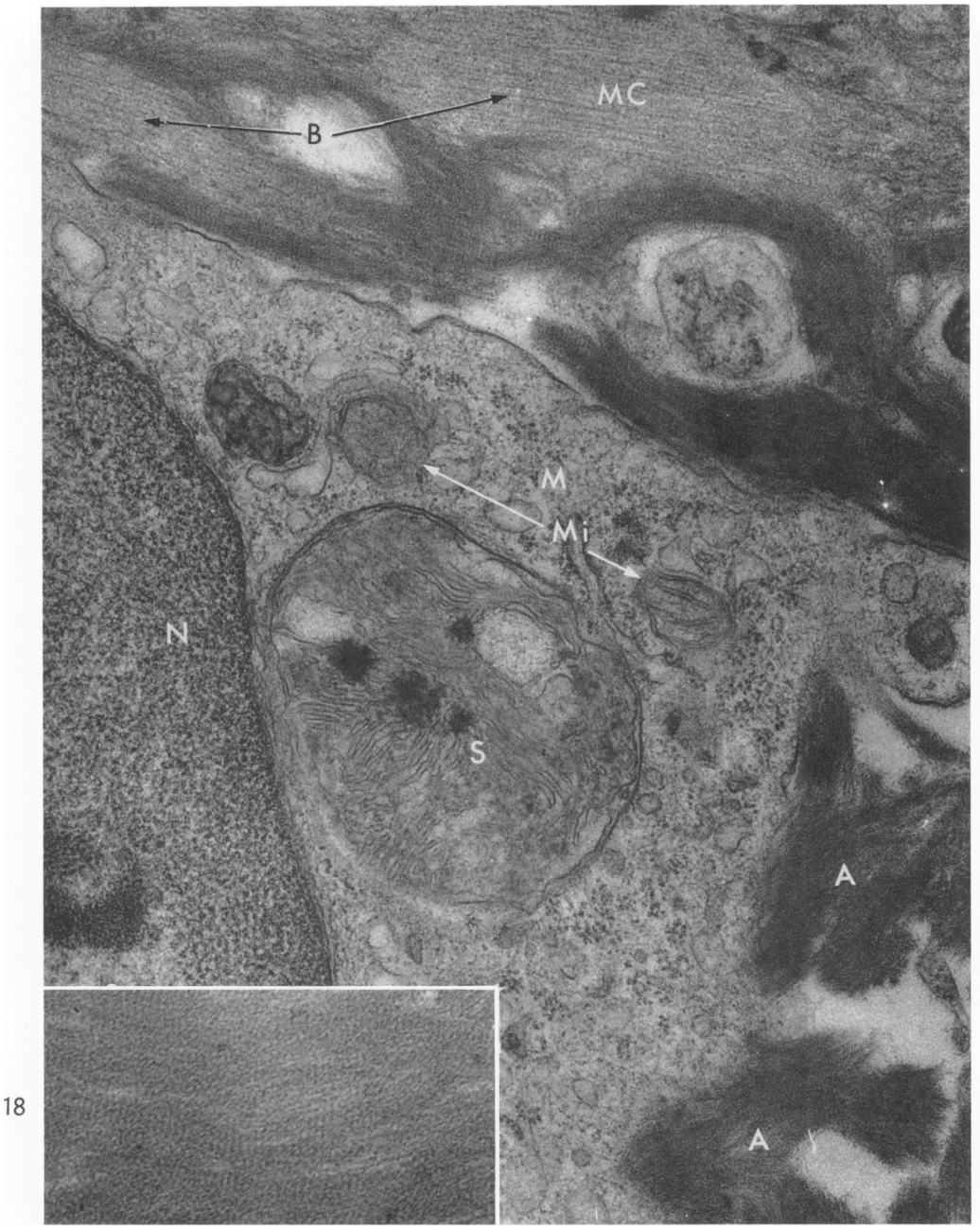

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FIG. 17. Mg-deficient rat, day 21. Parts of a macrophage (M) and necrotic muscle cell (MC) are seen. Nucleus of the macrophage, "N"; two small macrophage mitochondria, "Mi." The macrophage contains a mitochondrion (S) with dense precipitate and vacuoles. The size suggests that it represents a phagocytosed sarcosome. The macrophage appears in the process of phagocytosing osmiophilic clumps of fibrin (A). Some myofilaments are still visible as discrete parallel-arranged lines (B). × 37,500.

FIG. 18. Mg-deficient, stressed rat, day 29. A higher magnification of clumped dense material possibly represents fibrin and demonstrates a pattern of periodic cross-banding. × 56,000.