AN ELECTRON MICROSCOPIC STUDY OF CARDIAC NECROSIS PRODUCED BY 9 α-FLUOROCORTISOL AND SODIUM PHOSPHATE

A. N. D'Agostino, M.D.

From the Division of Neurology and Department of Pathology, University of Utah College of Medicine, Salt Lake City, Utah

Focal cardiac necrosis has been observed repeatedly in rats after the administration of steroids and sodium phosphate.[1-3] Suggestions offered to explain the pathogenesis of such lesions include the sensitization and conditioning effects of steroids and electrolytes,[1] potassium deficiency[2] and depletion of intracellular magnesium or potassium.[3]

This study will report the ultrastructural changes present in the rat myocardium after the combined administration of 9 α-fluorocortisol and sodium phosphate.

Method

Adult female albino rats of the Sprague-Dawley strain, weighing approximately 100 gm., were used. Eleven rats were given daily subcutaneous injections of a microcrystalline suspension of 100 μg. of 9 α-fluorocortisol acetate (generous quantities were supplied by the Upjohn Company, Kalamazoo, Michigan) in 0.2 ml. of water. In addition 2 ml. of a 1.0 molar solution of monobasic sodium phosphate (NaH_2SO_4) were given twice daily by gavage. Four additional animals given distilled water subcutaneously and tap water by gavage served as controls. All animals had free access to Purina Lab Chow and tap water. Two rats in the steroid-phosphate group died and tissue was not available for electron microscopy.

The animals in the steroid-phosphate group were killed 4, 5, 10, 11, 12, 13, 14 and 18 days after the beginning of the experiment. One animal was killed on each of the specified days; on day 18, two animals were killed. Tissue from the myocardium of the left and right ventricles was fixed in cold (4° C.) phosphate-buffered 1 per cent osmium tetroxide (Millonig) for 1 hour, dehydrated in alcohol, cleared in toluene and embedded in Epon 812. Sections were cut with glass knives in a Porter-Blum microtome, stained with lead citrate[7] and examined with an RCA EMU 3-G electron microscope.

Tissue from the heart, lung, kidney, liver, spleen, skeletal muscle and adrenal gland of the steroid-phosphate group and controls was fixed in 10 per cent formalin and embedded in paraffin. All sections were stained with hematoxylin and eosin. Sections from the heart and kidney were also stained by von Kossa's method for phosphates and carbonates and Gomori's method for iron.

Observations

The rats in the steroid-phosphate group developed anorexia and diarrhea and lost weight a few days after the initiation of the experi-

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ment. After 5 to 6 days the diarrhea subsided. None of the animals developed skin lesions or convulsions. At the time of death the rats given steroid-phosphate weighed approximately 30 to 40 gm. less than the control animals. At necropsy all animals in the steroid-phosphate group had moderate to extensive calcification in the kidneys. The kidneys were yellow-tan in color and of approximately twice normal size. Focal white areas were observed in the myocardium of the left and right ventricles in 4 animals. Two animals had focal hemorrhagic lesions in the lungs. The liver, spleen and adrenal glands were unremarkable. No gross lesions were observed in the control group.

With the light microscope, examination of hematoxylin and eosin stained sections of the myocardium in rats in the steroid-phosphate group revealed focal alterations manifested by intense eosinophilia of the muscle fibers and moderate histiocytic and granulocytic cellular response. The smallest lesions seen in the myocardium of both the left and right ventricles often involved as few as 5 to 10 muscle fibers. Sections of the myocardium stained by von Kossa's method and Gomori's method failed to reveal phosphates and carbonates or iron. The renal tubules showed calcification with scattered necrosis and a focal granulocytic cellular response. Glomeruli appeared normal. No abnormalities were detected in the liver, spleen, skeletal muscle or adrenal glands. The lungs in 2 animals showed focal areas of intra-alveolar hemorrhage with a numerical increase in histiocytes within the alveolar walls. No microscopic abnormalities were detected in the animals of the control group.

With the electron microscope, examination of sections of the myocardium from animals in the control group revealed no abnormalities (Fig. 1). However, in animals receiving steroid and phosphate there were numerous pathologic changes. One of the earliest and most striking alterations was observed in the mitochondria. In animals killed on the fourth, fifth, tenth, twelfth and 14th days mitochondria contained numerous electron-dense inclusions. Some contained an electron-dense core, but in others the central portion of the inclusion was electron-lucent (Figs. 2 and 3). The inclusions ranged in size from 1,000 to 3,000 Å. The particles comprising the inclusions were deposited primarily in the matrix of mitochondria but also in parallel fashion along cristae. The inclusions were also present in unstained sections (Fig. 4). Microcrystalline deposits were not identified with certainty.[4] The relationship of these deposits to pre-existing mitochondrial matrix granules could not be determined. In the myocardium of rats killed on the fourth and the fifth days, many muscle fibers containing such intramitochondrial inclusions showed moderate dilatation of the sarcoplasmic reticu-

lum but myofibrillar components were only slightly disorganized. Glycogen granules were decreased in number (Fig. 5).

In muscle fibers undergoing necrosis myofibrillar components were replaced by structureless masses, in which mitochondria containing numerous electron-dense inclusions were recognized (Figs. 6 to 8). Macrophages with lipid inclusions and phagocytosed inclusion-bearing mitochondria in cytoplasmic compartments were present within the sarcolemma (Figs. 9 and 10). Strands of fibrin were scattered about within the necrotic muscle fiber (Fig. 11).

An alteration seen only in the animal killed on the 14th day was a peculiar modification of the myofibrils adjacent to intercalated discs. In contrast to the normal disc, masses of densely packed, haphazardly arranged fibrils were seen on both sides of the intercalated disc. The desmosomal attachments of the intercalated disc remained intact.

In animals of the steroid-phosphate group killed on the 18th day of the experiment no definite lesions were present. However, myofibrils were more widely separated and interfibrillar components such as glycogen appeared more prominent.

Discussion

The light microscopic observations noted in this experiment are similar to those reported by Selye[1] and Lehr and Krukowski.[3] Selye stated that the pathogenesis of the cardiac lesions was linked to the interaction of steroids and electrolytes, the former conditioning the myocardium for the pathogenic effects of electrolyte imbalance. He also observed that administration of K+ prevented the development of necrosis. In similar studies Nickerson, Karr and Dresel,[2] impressed by the cathartic effect of orally administered sodium phosphate, found that the incidence and severity of cardiac necrosis correlated well with the degree of myocardial potassium depletion, and concluded that potassium deficiency and its sequelae were responsible for the myocardial lesions. Lehr and Krukowski,[3] in an extensive study of steroid-phosphate induced cardiac necrosis, found that parathyroidectomy hastened the appearance of cardiac necrosis.

The intramitochondrial electron-dense inclusions observed in this investigation have been noted in other experimental conditions. Morphologically identical inclusions have been seen in the myocardium of rats after poisoning with Plasmocid (8-aminoquinoline).[5] Caulfield and Schrag[4] have noted similar inclusions in the renal tubules of rats after the administration of calcium gluconate and parathyroid extract and suggested that the inclusions represented deposits of calcium carbonate. In studies of liver mitochondria of rats loaded with Ca++ and inorganic

phosphate, electron-dense granules have been described.[6] Reynolds[7] described similar inclusions in the mitochondria of rat livers after CCl_4 poisoning and suggested that they were a form of apatite, the phosphate being derived from the degradation of mitochondrial organophosphates. Studies by Peachey[8] indicated that in addition to calcium, strontium and barium may accumulate within mitochondria and appear as electron dense granules.

In vitro studies of mitochondria isolated from various organs confirm the ability of mitochondria to bind ions such as inorganic phosphate and magnesium,[9] calcium[10] and potassium.[11] In each case the ionic binding was respiration-dependent and coupled to electron transfer.

The relationship between the electron-dense mitochondrial inclusions and the development of cardiac necrosis in rats given 9 α-fluorocortisol and sodium phosphate is not clear. From the evidence cited[4,6,7,8] it appears likely that the inclusions reported in this study are associated with the binding of cations and represent calcium deposits. Since myocardial potassium is known to be decreased in steroid-phosphate-induced cardiac necrosis,[2,12] it would seem possible that the intramitochondrial calcium deposition in this study is associated with a co-existing potassium deficiency. However, electron microscopic studies of the myocardium in dietary potassium-deficient rats have failed to reveal the presence of similar mitochondrial inclusions.[13]

The deposition of calcium within mitochondria may be the primary factor concerned in the production of steroid-phosphate cardiac necrosis. It has been postulated that the intramitochondrial accumulation of calcium and phosphate during electron transfer may replace the oxidative phosphorylation of ADP.[10,14] Since in this study the presence of intramitochondrial inclusions seemed to precede overt cardiac necrosis, the latter change may be secondary to an interference with ATP synthesis. It is possible but highly speculative that the presence of such intramitochondrial inclusions may be a morphologic indication of the uncoupling of oxidative phosphorylation. However, since in rats with extensive renal calcification the continued administration of sodium phosphate and steroids will induce profound electrolyte disturbances, the presence of intramitochondrial calcium may be secondary to other cation deficiencies such as potassium and magnesium and bear no direct relation to the development of cardiac necrosis.

Summary

Cardiac necrosis produced by the combined administration of 9 α-fluorocortisol and monobasic sodium phosphate was investigated with the electron microscope. Electron-dense particulate inclusions contain-

ing either an electron-dense or lucent core were observed in mitochondria of degenerating muscle fibers. Macrophages present in such fibers contained similar inclusion-bearing mitochondria within cytoplasmic compartments. The intramitochondrial inclusions are presumed to represent deposits of calcium.

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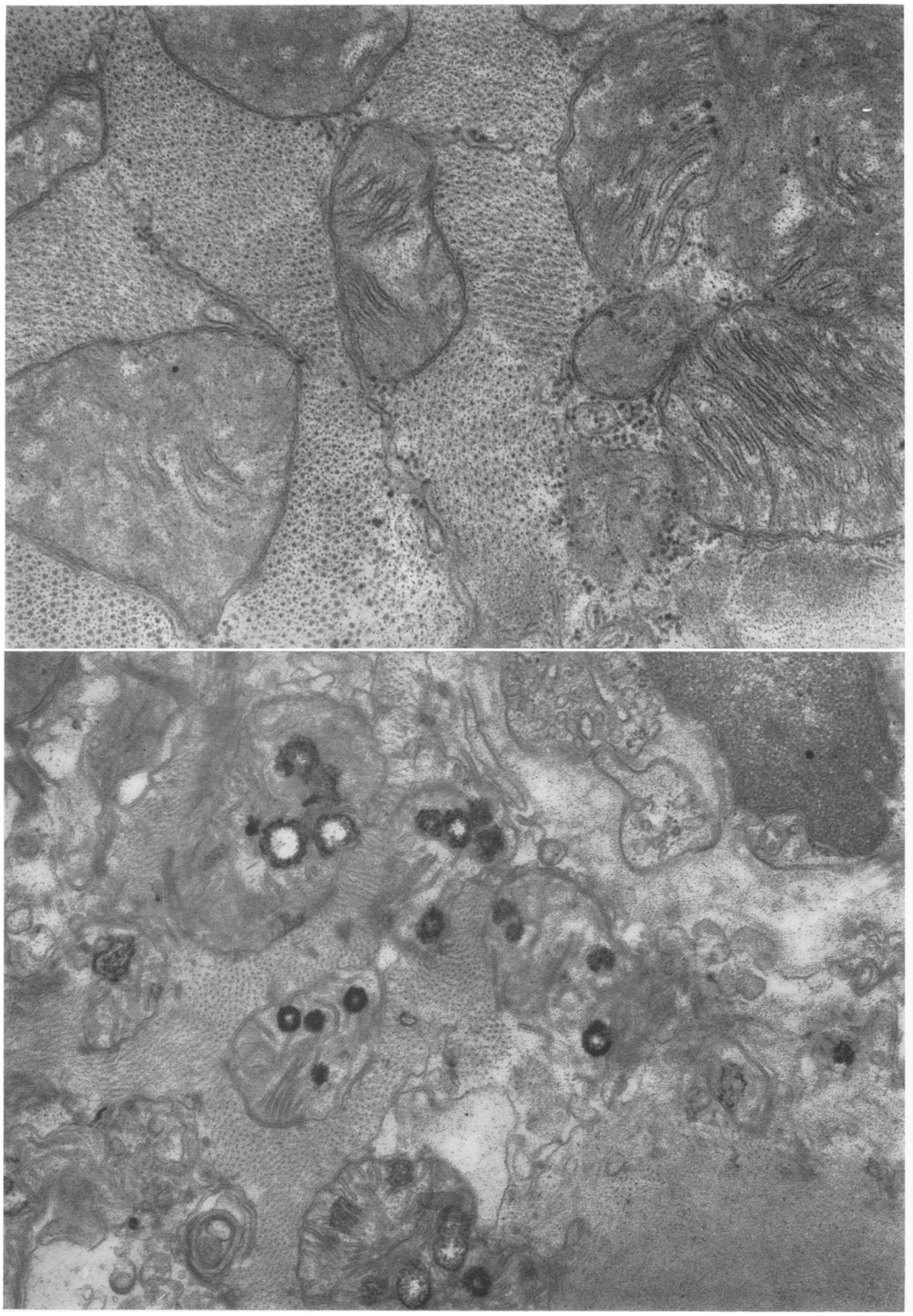
[*Illustrations follow*]

Legends for Figures

Fig. 1. Cross section, myocardium, control animal killed on the sixth day. The myofilaments and mitochondria appear normal. Lead citrate. × 37,000.

Fig. 2. Oblique section, left ventricle, rat in the steroid-phosphate group killed on the fifth day. Mitochondria contain numerous electron-dense inclusions, many with an electron-lucent center. The myofilaments in the center are essentially normal, but those at the lower right appear to have lost their fine structural detail. At the upper right is a portion of an endothelial cell. Lead citrate. × 32,000.



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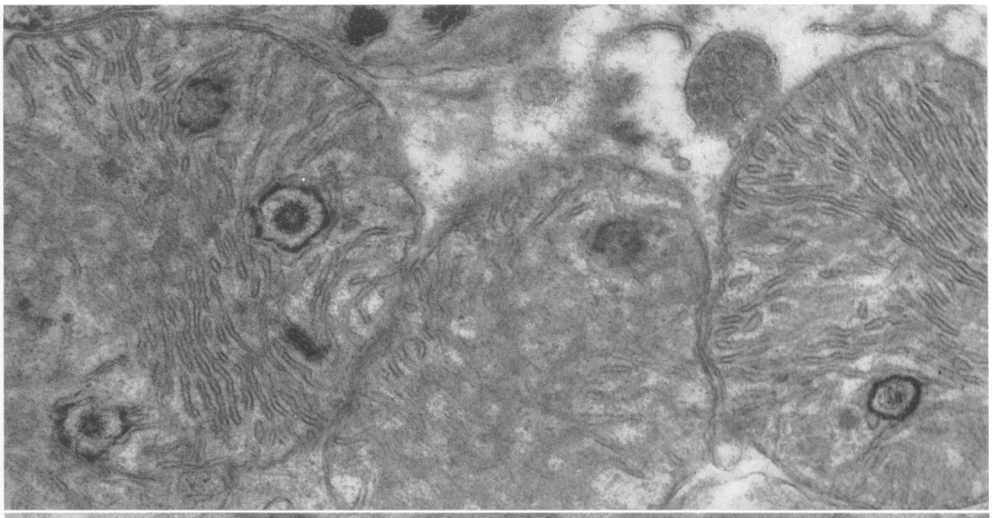

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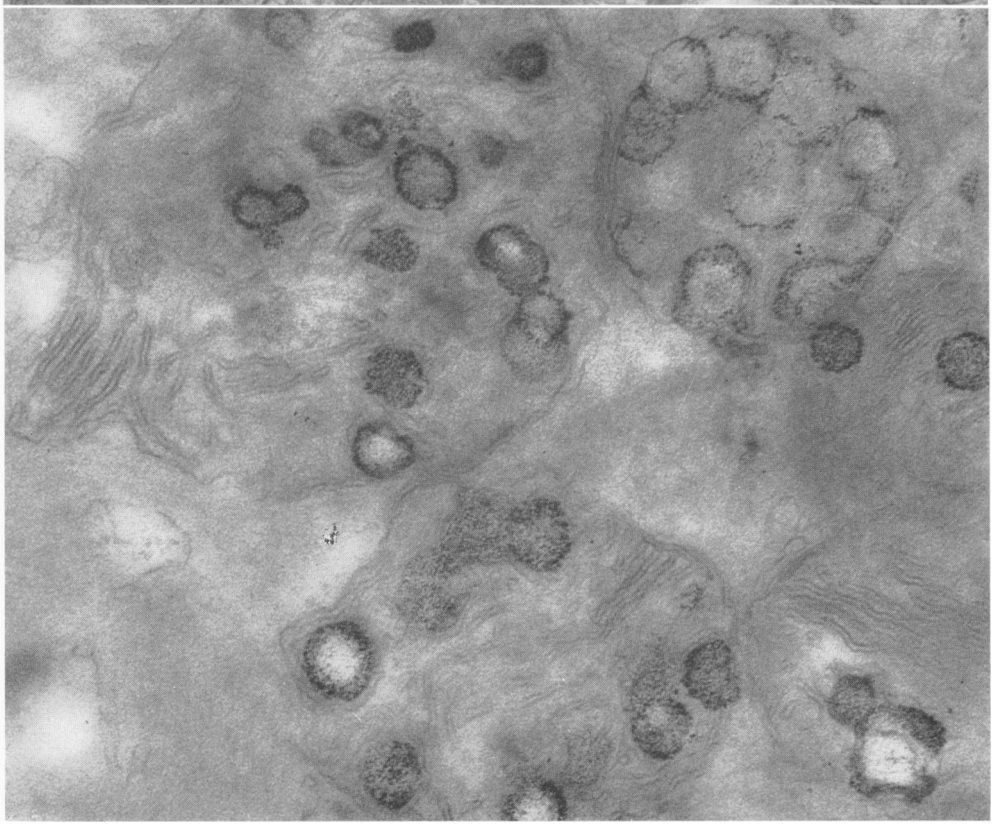
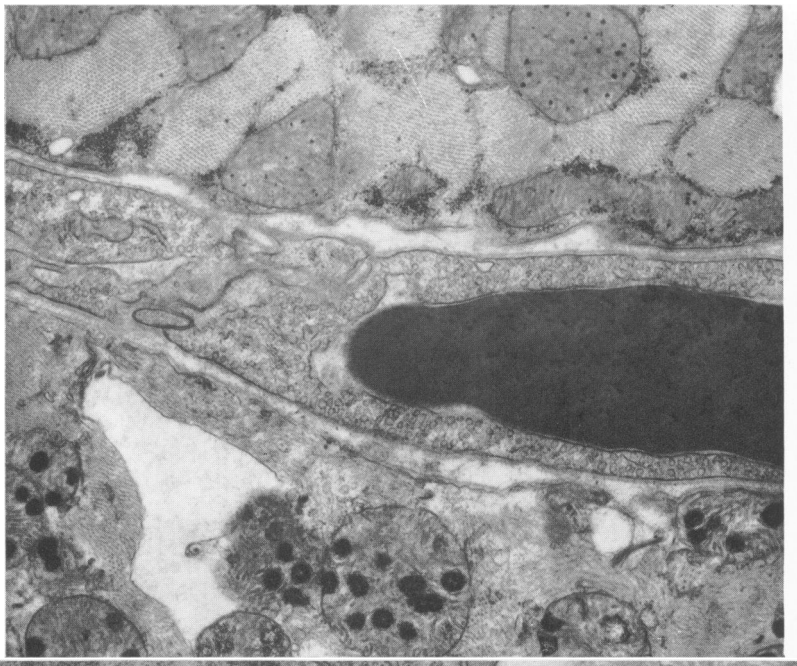


FIG. 3. Mitochondria within the myocardium of an animal in the steroid-phosphate group killed on the 14th day. The core present within the inclusions is electron-dense. The inclusions are approximately 3,000 Å in diameter. Lead citrate. × 32,000.

FIG. 4. Mitochondria within the myocardium of an animal in the steroid-phosphate group killed on the fifth day. In the mitochondrion at the upper right, electron-dense particles are seen deposited on cristae. Unstained. × 37,000.



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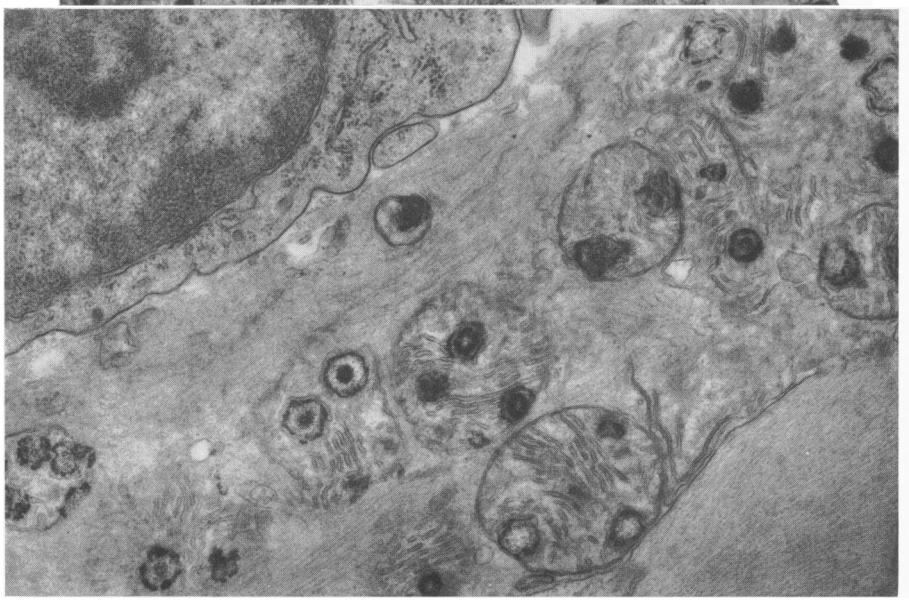

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FIG. 5. Cross section, myocardium, rat in the steroid-phosphate group killed on the fifth day. In the center is a capillary. Fiber at top contains glycogen granules and perhaps an increase in number of mitochondrial matrix granules. The degenerating fiber at the bottom shows mitochondria with numerous inclusions. The myofilaments are disorganized. The dilated sac at the lower left is probably a component of the sarcoplasmic reticulum. Lead citrate. × 13,000.

FIG. 6. Rat in the steroid-phosphate group killed on the 14th day. At the upper left is a portion of a macrophage. In addition to the presence of inclusion-bearing mitochondria, the myofilaments in the center are disorganized while those at the lower right appear as a mass of compact fibrils. Lead citrate. × 18,000.

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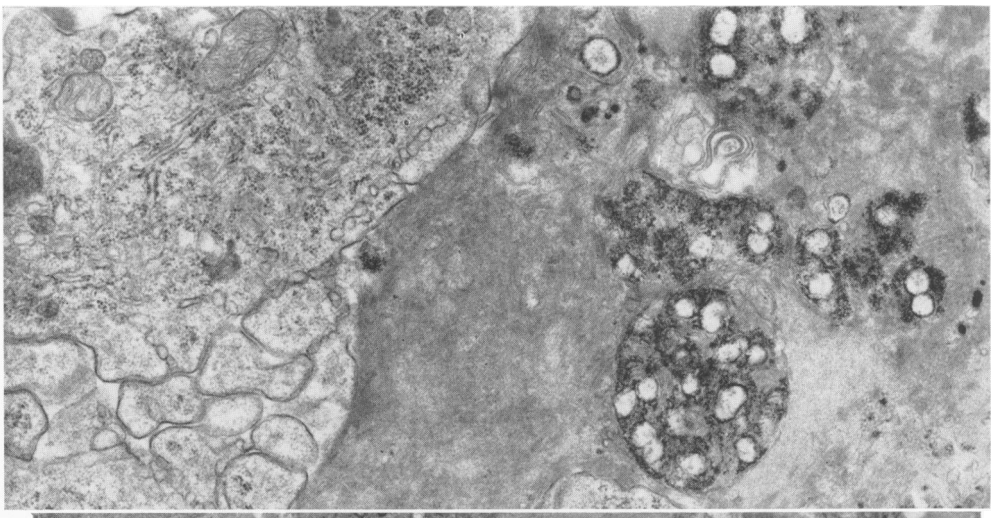

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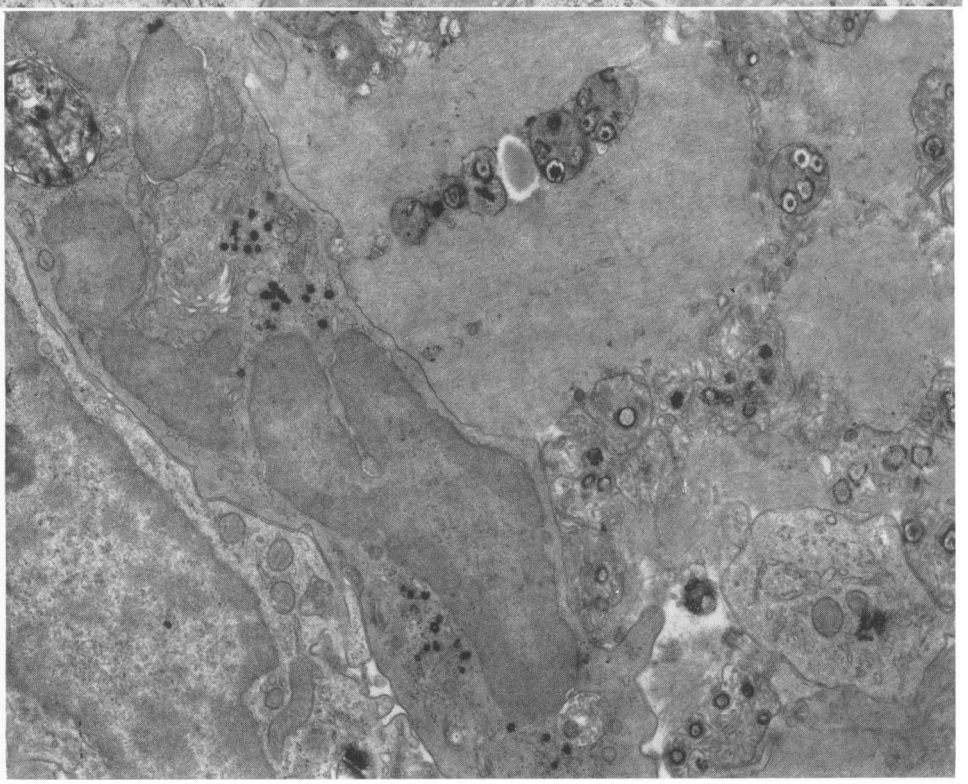


FIG. 7. Rat in the steroid-phosphate group killed on the fourth day. Within the sarcolemma are portions of macrophages surrounding an irregular mass of degenerating fibers. The spherical bodies containing electron-dense inclusions are presumably derived from mitochondria. Lead citrate. × 19,000.

FIG. 8. Rat in the steroid-phosphate group killed on the 14th day. Inclusion-bearing mitochondria are present in a mass of degenerating myofibrils. Myofilaments are still present but completely disorganized. A macrophage with perinuclear electron-dense inclusions is present at the left. Lead citrate. × 12,000.

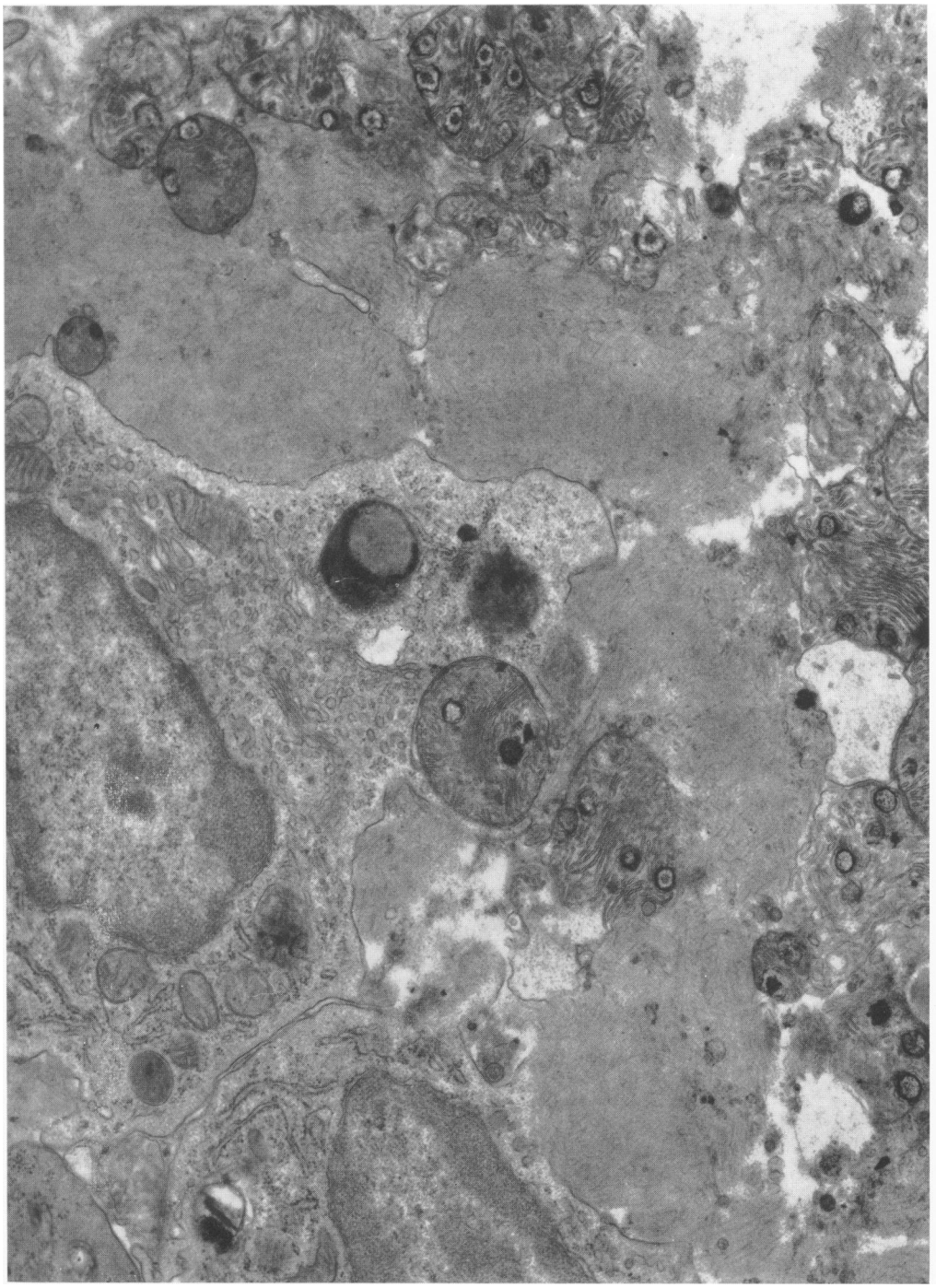
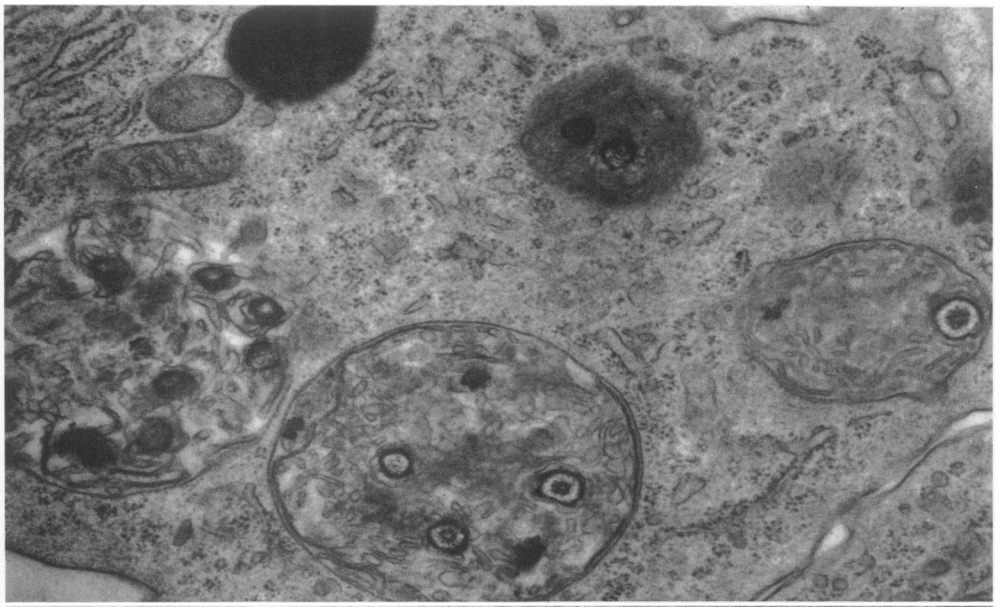


FIG. 9. Rat in the steroid-phosphate group killed on the 14th day. Normal myofibrils are replaced by a homogeneous mass of filaments. Inclusion-bearing mitochondria are evident. At the left is a macrophage which appears to be engulfing an inclusion-bearing mitochondrion (center). Lead citrate. × 16,000.



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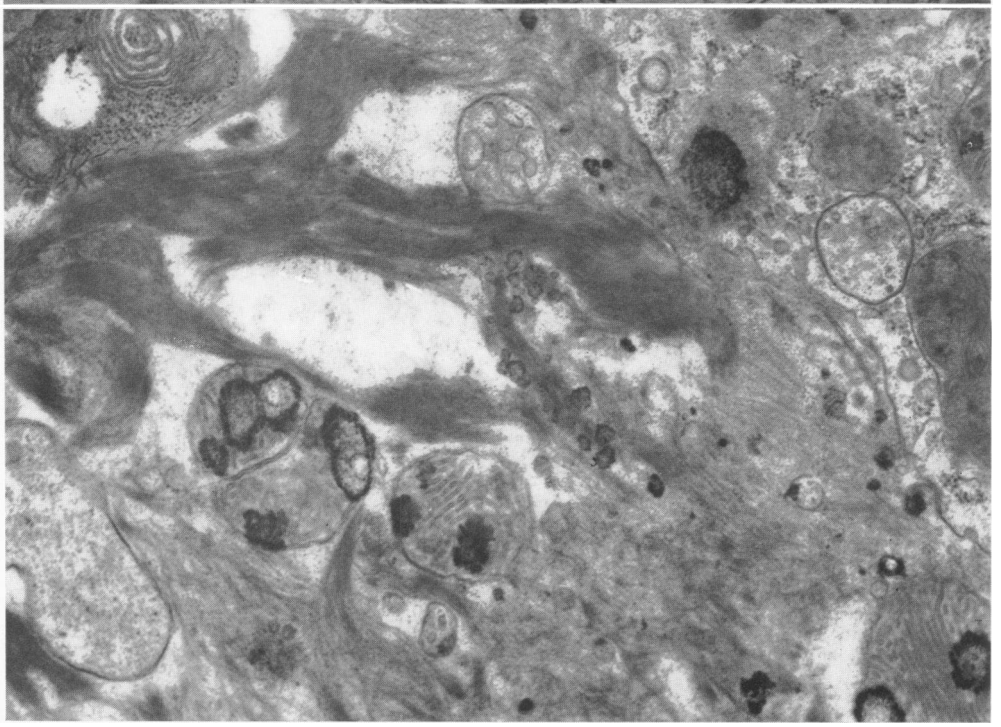

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FIG. 10. Macrophage within the myocardium of an animal in the steroid-phosphate group killed on the 14th day. Phagocytosed mitochondria with electron-dense inclusions are present within cytoplasmic compartments. Lead citrate. × 24,000.

FIG. 11. Rat in the steroid-phosphate group killed on the fifth day. Normal myofibrils are not recognized. The fibrils in the center, displaying a regular periodicity, presumably represent fibrin. Inclusion-bearing mitochondria are present. Lead citrate. × 22,000.