The American Journal of PATHOLOGY

July 1978 • Volume 92, Number 1

Influence of Methylprednisolone on Ultrastructural and Cytochemical Changes During Myocardial Ischemia

Selective Effects on Various Cell Inclusions and Organelles Including Lysosomes

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Occlusion of the circumflex branch of the coronary artery of rabbit hearts for 45 minutes elicits structural and cytochemical changes in myocytes similar to those observed in ischemic dog myocardium, which are indicative of irreversible cell injury. When methylprednisolone is administered prior to occluding the artery, myocytes are transiently protected and many of the electron microscopic signs of irreversible damage are delayed for 15 minutes or more. During this period, the steroid preferentially protects mitochondria, lysosomes, and sarcolemma from the ischemic changes that normally develop. However, some other events, including depletion of glycogen and margination of nuclear chromatin, are only minimally influenced by the therapy, if at all. In all hearts, treated and untreated, the development of severe cell damage, whenever it occurs, is closely associated with cell swelling, mitochondrial dilation with concomitant appearance of amorphous osmiophilic densities, and abnormalities in and, ultimately, disappearance of lysosomes, suggesting that damage to cell membranes is a central event in the progression of reversible injury to irreversible infarction and that protection of membrane integrity should be a reasonable aim in efforts to ameliorate or delay ischemic injury. (Am J Pathol 92:1–22, 1978)

RECENT OBSERVATIONS on ischemic rabbit myocardium disclose that structural evidence suggestive of irreversible myocytic injury is recognizable by 45 minutes after occlusion of the circumflex branch of the

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Supported by Grants HL 17669 and HL 70125 from the National Heart and Lung Institute and by Grant-in-Aid 77-6570 from the American Heart Association.

Accepted for publication March 14, 1978.

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0002-9440/78/0710-0001$01.00

coronary artery.[1,2] Margination of nuclear chromatin, depletion of intracellular glycogen stores, and the formation of osmiophilic densities within dilated mitochondria occur in ischemic rabbit hearts in the same sequence and at approximately the same time as in ischemic dog heart, in which such changes are closely correlated with the onset of functional irreversibility.[3-5] It has been suggested that changes in lysosomes also accompany early myocytic injury and may contribute to the production of the cellular damage that accompanies ischemia. (For a critical review of this topic, see Reference 6.) We have reported the release of cathepsin D from myocytic lysosomes within 30 minutes of coronary occlusion,[1,7] and we and others have described a subcellular redistribution of lysosomal acid phosphatase and aryl sulfatase activities within 1 to 2 hours.[2,8,9] It remains unclear, however, whether such changes are of causal importance in provoking cell injury or, instead, merely reflect loss of enzyme from lysosomes as a passive result of severe myocytic damage or death.

Although the central event that transforms a reversibly injured myocyte into an irretrievably damaged one is unknown, much evidence indicates that changes in cellular and organellar membrane function are early and crucial factors.[1,5,9,10] If alterations in sarcolemmal permeability, mitochondrial integrity, and lysosomal latency transpire as the result of membrane defects, agents like corticosteroids, which are reputed to stabilize cell membranes,[11] might be expected to protect myocytes from the damaging influences of ischemia. Several studies have yielded results that support the concept that steroids protect the ischemic or hypoxic myocardium in experimental animals [12-18] and that this protection is associated with lysosomal stabilization.[9,14,19,20] On the other hand, some studies have failed to reveal any protective effect of steroids on lysosomes in ischemic or hypoxic hearts.[21-23] As a rule, observations on the effects of steroids have been conducted 1 or more hours after ischemia, when it is difficult to ascertain the sequence of subcelluar changes before irreversible necrosis has supervened; thus, the early influences of steroid action on reversibly injured myocytes have not been described. Accordingly, the present study was undertaken to evaluate the effects of a pharmacologic dose of methylprednisolone on the sequential ultrastructural and cytochemical changes in ischemic rabbit myocardium during the first 15 to 120 minutes following coronary occlusion.

Materials and Methods

Experimental Protocol

New Zealand white male rabbits, aged 4 to 6 months (body weight, 2.5 to 3.0 kg), were fed Wayne rabbit chow *ad libitum*. The rabbits were anesthetized with sodium pentobar-

bital, maintained on artificial ventilation during open-chest surgery, and prepared for circumflex artery ligation as described previously.[1] Thirty minutes prior to coronary occlusion, half the rabbits were given methylprednisolone (Solu-Medrol, Upjohn, Inc., Kalamazoo, Mich.), 50 mg/kg, intravenously.

At 0, 15, 30, 45, 60, 90, or 120 minutes after ligation of the circumflex branch of the left coronary artery, hearts were excised from matched control and steroid-treated rabbits, and ischemic tissue (identified by cyanosis) was removed from the center of the area supplied by the ligated artery. Samples of distant, presumably nonischemic, left ventricle that appeared normal were removed simultaneously, as were portions of the right ventricle. Left ventricular tissue in the area of circumflex artery perfusion was also obtained under identical conditions from sham-operated control rabbits (both control and methylprednisolone-treated) whose coronary arteries had not been ligated. At each interval, 6 to 10 ischemic and steroid-ischemic hearts were studied in conjunction with a similar series of sham-operated control hearts. From the center of each section, the subendocardium was dissected free in the fixative and diced into small pieces; then 8 to 10 samples were selected at random, dehydrated, embedded, and sectioned for light and electron microscopy and cytochemistry. All experiments were carried out in a double blind fashion so as not to prejudice the observer.

Electron Microscopy

Small samples of ischemic or nonischemic myocardium were preserved for 60 to 90 minutes at 4 C in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.1 M sucrose, as suggested by Brunk and Ericsson.[24] Following fixation, the tissue was embedded in 7% agar and 20- to 40-μ nonfrozen sections were prepared in a Smith-Farquhar TC-2 tissue chopper. Sections were washed overnight in 0.1 M cacodylate buffer (pH 7.4) plus 7% sucrose and incubated the next morning for acid phosphatase, aryl sulfatase, or thiolacetic acid esterase. After incubation, the sections were rinsed in 0.05 M acetate–veronal buffer (pH 5.5) containing 5% sucrose for 1 hour and then postfixed for 1 hour in 1% OsO_4 in 0.1 M cacodylate buffer. Some sections were then stained *en bloc* in 0.5% uranyl acetate for 1 hour to enhance membrane staining. Then all sections were dehydrated in a graded series of ethanols and propylene oxide and were embedded in Epon. Thin sections were cut with a diamond knife on a Porter–Blum MT-2B ultramicrotome and either stained with uranyl acetate and lead citrate or viewed unstained in a Philips 200 electron microscope.

Cytochemistry

Acid phosphatase activity was detected employing two incubation media. First, we utilized a modification of the Barka and Anderson technique[25] in which sections are incubated in the presence of 10 mM *p*-nitrophenyl phosphate or 2.5 mM β-glycerophosphate for 1 to 2 hours at pH 5.0 and 37 C. Controls were run in the absence of substrate or in the presence of 10 mM sodium fluoride. Dimethylsulfoxide (DMSO) was also included in this medium at a concentration of 10%. The second acid phosphatase medium was that described by Hoffstein et al[8] in their modification of the method of Brunk and Ericsson,[24] in which β-glycerophosphate is employed as the substrate and DMSO is used to aid substrate penetration into the tissue sections. Tissue sections were incubated for 1 to 2 hours at 37 C with continuous agitation. Controls were incubated with 10 mM sodium fluoride or without substrate.

Aryl sulfatase activity was, likewise, assessed with two cytochemical media. In the first, aryl sulfatase was localized by using a modification of Goldfischer's medium[26] containing 6.25 mM *p*-nitrocatechol sulfate, 10% DMSO, 7% sucrose, and 1.6% lead nitrate in 0.05 M acetate buffer, pH 5.5. The second medium was Hoffstein's modification of the aryl sulfatase reaction mixture of Hopsu-Havu et al,[27] which utilizes either lead or barium salts

as capturing ions. Incubations were conducted for 1 to 2 hours at 37 C, and controls were incubated identically except without substrate.

Thiolacetic acid esterase (lysosomal esterase) reaction product was localized with Bell and Barrnett's medium,[28] consisting of 50 mM thiolacetic acid and 10 mM lead nitrate in 0.1 M cacodylate buffer (pH 5.5) with 10% DMSO and 7% sucrose. Sections were preincubated for 30 minutes in the medium with 10 μM ethyl-*p*-nitrophenyl phosphate and then rinsed and incubated in the cytochemical medium for 1 to 2 hours at 25 C. Controls were run in the absence of substrate or in the presence of 100 mM sodium fluoride.

Thiamine pyrophosphatase or *inosine diphosphatase* activity was assessed by the technique of Novikoff and Goldfischer,[29] which employs thiamine pyrophosphate or inosine diphosphate as substrates. Incubations were conducted at 37 C for 30 to 90 minutes, with renewal of media every 30 minutes. In control incubations, the substrate was omitted from the media.

Results

Nonischemic and Sham-Operated Myocardium

Nonischemic myocytes from sham-operated controls and from sites distant from the ischemic area of ligated hearts displayed well-ordered sarcomeres exhibiting a relaxed configuration with prominent A and I bands (Figure 1). In paranuclear regions of these cells, large densely stained mitochondria, abundant β-glycogen, randomly scattered lysosomes, and a small Golgi complex could be recognized. Myocytic nuclei were elliptic and generally revealed finely dispersed chromatin with a centrally located nucleolus.

A variety of lysosomal types [30] could be readily identified in the paranuclear area of most myocytes (Figure 1, insets a and b). These included dense bodies; autophagic vacuoles; multivesicular bodies; and large, polymorphic residual bodies which contained variable amounts of lipid and membranous residues (Figure 1, insets a and b). Many of these lysosomal profiles were observed in close approximation to the Golgi complex (Figure 1, inset a). However, only a few of these secondary lysosomes divulged reaction product, whereas elements of the Golgi complex consistently displayed activity (Figure 1, insets a and b). Of those few lysosomal granules which did exhibit acid phosphatase and aryl sulfatase activity, dense bodies and autophagic vacuoles were most reactive. Little or no activity could be demonstrated in residual bodies (Figure 1, inset a), and this class of lysosomes failed to display any reaction product even after sections were frozen and thawed. Likewise, elements of the sarcoplasmic reticulum which have been reported to be rich sources of lysosomal acid phosphatase or aryl sulfatase in dog heart [8] exhibited little evidence of hydrolase activity (Figure 1, inset a).

No differences could be discerned between nonischemic cells from

hearts that had received methylprednisolone and those that had not. No significant structural changes were encountered in the nonischemic and right ventricular myocardium during the 2-hour period of circumflex artery occlusion.

15-Minute Ischemic Myocardium

A brief ischemic episode induced only minor alterations in the morphology of untreated heart myocytes (Figure 2). The changes included some condensation of chromatin along the inner aspect of the nuclear envelope (Figure 2), but the majority of the chromatin was still evenly distributed throughout the nucleoplasm. Mitochondrial dilation and loss of matrix staining was minimal; however, the majority of the mitochondrial matrix granules disappeared (Figures 2 and 3). Another feature of reversibly injured myocytes was a congregation of lysosomes in the paranuclear regions of most cells (Figures 2 and 3). Many of the dense bodies and autophagic vacuoles exhibited acid phosphatase reaction product (Figure 3), whereas residual bodies failed to stain. Saccules of the Golgi complex, likewise, stained intensely (Figure 3). Some of the myocytes had moderately contracted sarcomeres disclosing readily identifiable A bands but poorly resolvable I bands (Figure 2).

Prior administration of a pharmacologic dose of methylprednisolone to rabbits subsequently made ischemic for 15 minutes inhibited some but not all of the morphologic and cytochemical changes previously noted. Margination of nuclear chromatin occurred and sarcomeres were somewhat contracted, but swelling of mitochondria and disappearance of their matrix granules did not develop (Figure 4). Fewer lysosomes (Figure 4) were concentrated in the paranuclear areas of most steroid-treated cells than were encountered in the untreated hearts. Nevertheless, those lysosomes that were encountered revealed identical staining patterns, ie, dense bodies generally reacted positively (Figure 4) and residual bodies displayed little activity.

30-Minute Ischemic Myocardium

Prolonging ischemia to one-half hour initiated obvious signs of myocytic injury in hearts that had not received methylprednisolone. A distinctive band of marginated chromatin was evident in the nuclei of most cells, and, additionally, small clumps of heterochromatin were visible in the nucleoplasm (Figure 5). Although mitochrondria were only moderately swollen, a significant decrease in matrix density could be noted after 30 minutes. Osmiophilic densities, which are indicative of irreversible cell damage in the dog myocardium,[4] could not yet be discerned between the

cristae of these dilated mitochondria. Many secondary lysosomes displaying hydrolase reaction products were present in the paranuclear regions of the injured myocytes. As in nonischemic cells, few of the residual bodies disclosed acid phosphatase activity (Figure 5, inset). In addition, significant portions of the intracellular glycogen stores had been utilized by the damaged myocytes by this time (Figure 5).

In contrast, a 30-minute ischemic insult in corticosteroid-pretreated rabbit hearts provoked only minor changes in myocyte morphology, and there were minimal alterations in the distribution of lysosomal enzyme reaction products. Nuclei displayed evenly dispersed chromatin (Figures 6 and 8) and mitochrondria remained structurally unaltered (Figures 6 through 8). Lysosomes of various types were observed in paranuclear sites (Figures 6 through 8), and reaction products for all three lysosomal enzymes were visible to a variable degree in saccules of the Golgi complex and elements of GERL (Golgi–encoplasmic-reticulum–lysosome complex). Small coated vesicles (primary lysosomes), dense bodies and autophagic vacuoles, disclosed considerable acid hydrolase activity (Figures 6 through 8). On the other hand, glycogen distribution appeared similar to that observed in unprotected ischemic myocytes.

45-Minute Ischemic Myocardium

In untreated hearts, mitochondrial swelling and the margination of nuclear chromatin were markedly enhanced after 45 minutes of ischemia (Figure 9). Along with dilation of the mitochrondria, there was fragmentation of cristae and a decrease in matrix staining; osmiophilic densities were observed occasionally, but the outer mitochrondrial membranes appeared intact in these damaged organelles. Lysosomal staining was still evident in the paranuclear areas, with acid phosphatase reaction product confined to components of the vacuolar apparatus; no reaction products could be visualized in the cytosol (Figure 9).

The only morphologic changes that were encountered in 45-minute methylprednisolone-treated, ischemic hearts were a distinct margination of nuclear chromatin and a severe depletion of intracellular glycogen (Figure 10 and inset). Mitochrondria and lysosomal structures still appeared unaltered, and the localization of acid hydrolase reaction products was identical to that seen in nonischemic myocytes.

60-Minute Ischemic Myocardium

Almost all subendocardial myocytes in the center of the area supplied by the ligated circumflex artery had developed obvious signs of irrevers-

ible damage by 1 hour of ischemia in animals that had not received methylprednisolone. Nuclear chromatin was aggregated along the inner aspect of the nuclear envelope, leaving an empty appearance to the nucleoplasm (Figure 11). The mitochondrial matrix was rarified and osmiophilic densities could often be observed within the matrix (Figure 11, inset; Figure 12). Mitochrondrial cristae frequently appeared fragmented. Deposits of β-glycogen in the paranuclear regions and among myofibrils were much reduced over controls but were not completely absent. Most lysosomal profiles were observed in the paranuclear regions, but at this juncture, deposits of acid phosphatase (Figure 11) and aryl sulfatase (Figure 12, inset b) reaction product could be visualized over the myofibrillar elements as well as within lysosomal organelles. Even after *en bloc* staining with uranyl acetate, lead phosphate precipitates could be localized primarily over the A bands (Figure 11, inset). Thiolacetic acid esterase activity, on the other hand, could be detected in dilated elements of the Golgi complex but rarely over myofibrils (Figure 12, inset a).

Observations on corticosteroid-protected ischemic hearts after 1 hour revealed a different morphology. Myocytic nuclei displayed marginated chromatin, but its distribution was comparable to that following only 45 minutes of ischema in untreated animals. (Compare Figure 13 with Figure 9.) Mitochondria were slightly swollen but retained much of their dense matrix and some of their matrix granules. Osmiophilic densities were never observed in steroid-treated myocytes at this time. Lysosomal reaction products, likewise, were distributed quite differently from those in untreated hearts. Lead sulfide (Figure 13) and phosphate (Figure 13, inset) deposits were restricted to saccules of Golgi complex and portions of the GERL but were also found in some autophagic vacuoles and dense bodies (Figure 13). Reaction products could not be visualized over myofibrils or other regions of the sarcoplasm: they were confined within organelles. The major similarity between steroid-protected and untreated myocytes at 1 hour was the sparse distribution of glycogen in both hearts.

90- and 120-Minute Ischemic Myocardium

By 90 minutes of ischemia, myocytes in steroid-treated animals had become as severely damaged as in untreated animals. The cells appeared irreversibly damaged, with most manifesting severe swelling and almost complete depletion of intracellular glycogen stores. Mitochondria were dilated and revealed amorphous matrix densities and fragmented cristae (Figure 14), and recognizable lysosomal images were rare (Figure 14). Prolongation of the ischemic period to 120 minutes further intensified the damage inflicted on both steroid and nonsteroid ischemic hearts; more-

over, no morphologic or cytochemical differences could be discerned between these groups.

Discussion

The present observations illustrate that a single pharmacologic dose of methylprednisolone briefly protects rabbit cardiac myocytes from the ultrastructural damage normally incurred during acute, severe myocardial ischemia. Administration of the corticosteriod prior to ligating the circumflex coronary artery postpones irreversible cell injury as judged by electron microscopic criteria described in dog hearts [4,5] for at least 15 minutes in the center of the area of severe ischemia. While the mode of action of the steroid is unresolved, ultrastructural studies disclose that membrane-bound organelles appear to be the preferential target of the drug's protective effects. Thus, after 1 hour of ischemia, unprotected myocytes reveal severely damaged mitochondria, significant cell swelling, and a breakdown of lysosomes with a concomitant appearance of their reaction products in the cytosol, compared with the steroid-treated ischemic cells (Figures 11 through 13). Conversely, events that are less directly related to membrane damage, such as glycogen utilization and the margination of nuclear chromatin, progress with little or no protection from glucocorticoid therapy. However, the protection of myocytic membranes is only transient, and, by 90 minutes in this experimental model, methylprednisolone-treated ischemic myocytes display features compatible with irreversible injury (Figure 14). These morphologic and cytochemical observations concur with our biochemical and immunocytochemical studies which demonstrate that significant biochemical and anatomic redistribution of cathepsin D, likewise, is delayed approximately 15 to 30 minutes in steroid-treated ischemic hearts.[31]

Several studies have suggested that pharmacologic doses of glucocorticoids reduce the myocardial damage which normally ensues during experimental ischemia or hypoxia,[9,12,14,15,17,18] possibly due to their ability to "stabilize" a variety of subcellular membranes.[11] Other studies, however, have failed to disclose any differences between steroid-treated and untreated hearts [21-23] with regard to nonspecific protective effects or specific stabilization of lysosomes. Our results suggest a possible explanation for the apparent discrepancies; steroids appear to delay damage transiently but are unable to prevent necrosis indefinitely in areas of severe ischemia. A similar delay in biochemical signs of tissue damage in hypoxic perfused hearts has been reported by others.[16,32] Thus, since the time at which evidence of protective effects has been sought in a given experimental preparation has usually been limited to a single point (and not necessarily

the same point in different laboratories), it seems likely that some investigators have evaluated the heart during the temporary period of protection, whereas others have studied the tissue at a relatively later time. It should also be noted that the duration of the period of protection might well vary with the degree of ischemia and that apparent protection might be longer than observed in our model if areas of moderate or minimal ischemia are evaluated.

Little information has been available on the disposition of steroids in the heart until recently. Okuda and associates [33] reported on the subcellular distribution of radiolabeled methylprednisolone and dexamethasone in ischemic, adjacent "non-ischemic," and uninvolved regions of feline hearts. These investigators concluded that lysosomal "stabilizing activity" is apparently proportional to the amount of steroid incorporated into a membrane fraction which is enriched in lysosomal enzyme activity. Moreover, extracted membrane preparations reveal little degradation of either steroid, indicating that the native molecule rather than a metabolite is responsible for the stabilization. Assays of 5′-nucleotidase activity, a sarcolemmal marker enzyme,[34] likewise disclosed that both steroids partially prevent the precipitous decline in enzymatic activity in ischemic zones of the heart, illustrating that these drugs probably act on membranes in general rather than a specific class of biomembranes, like lysosomal membranes.[35] On the basis of previous studies,[14] it also seems unlikely that any of the protective effects of methylprednisolone administered *in vivo* are mediated by the vehicle used rather than the steroid itself.

Thus, available evidence is compatible with the postulate that the ability of steroids to reduce or delay ischemic necrosis may be related to their ability to prevent membrane damage, at least temporarily, and thereby to reduce the potentially detrimental effects of labilization of degradative enzymes. It should be emphasized, however, that neither our current observations nor those of others are able yet to establish definitively that membrane damage is the critical factor that leads to the "point of no return" in ischemic necrosis [36] or that the apparent beneficial effects of steroids on the process of necrosis are a function of their membrane actions. It remains possible that the two events (the development of membrane damage and lysosomal enzyme release and the establishment of irreversible necrosis), although related temporally, are not related causally. Finally, it should be noted that, in addition to whatever beneficial effects steroids may have in delaying membrane damage and cell death, they may prevent institution of repair processes as well.[37-39] Since there is also some evidence that the lysosomal alterations that accompany

ischemic injury may be more importantly related to the initiation of subcellular repair processes [40] than the process of injury *per se*, it remains possible that the ultimate effect of inhibiting normal lysosomal responses could reduce the ability of injured myocardium to recover. Therefore, extrapolation of these results to the clinical setting, where evidence for a beneficial effect of steroids in patients with myocardial infarction is less than clear-cut,[41,42] should be made only with caution.

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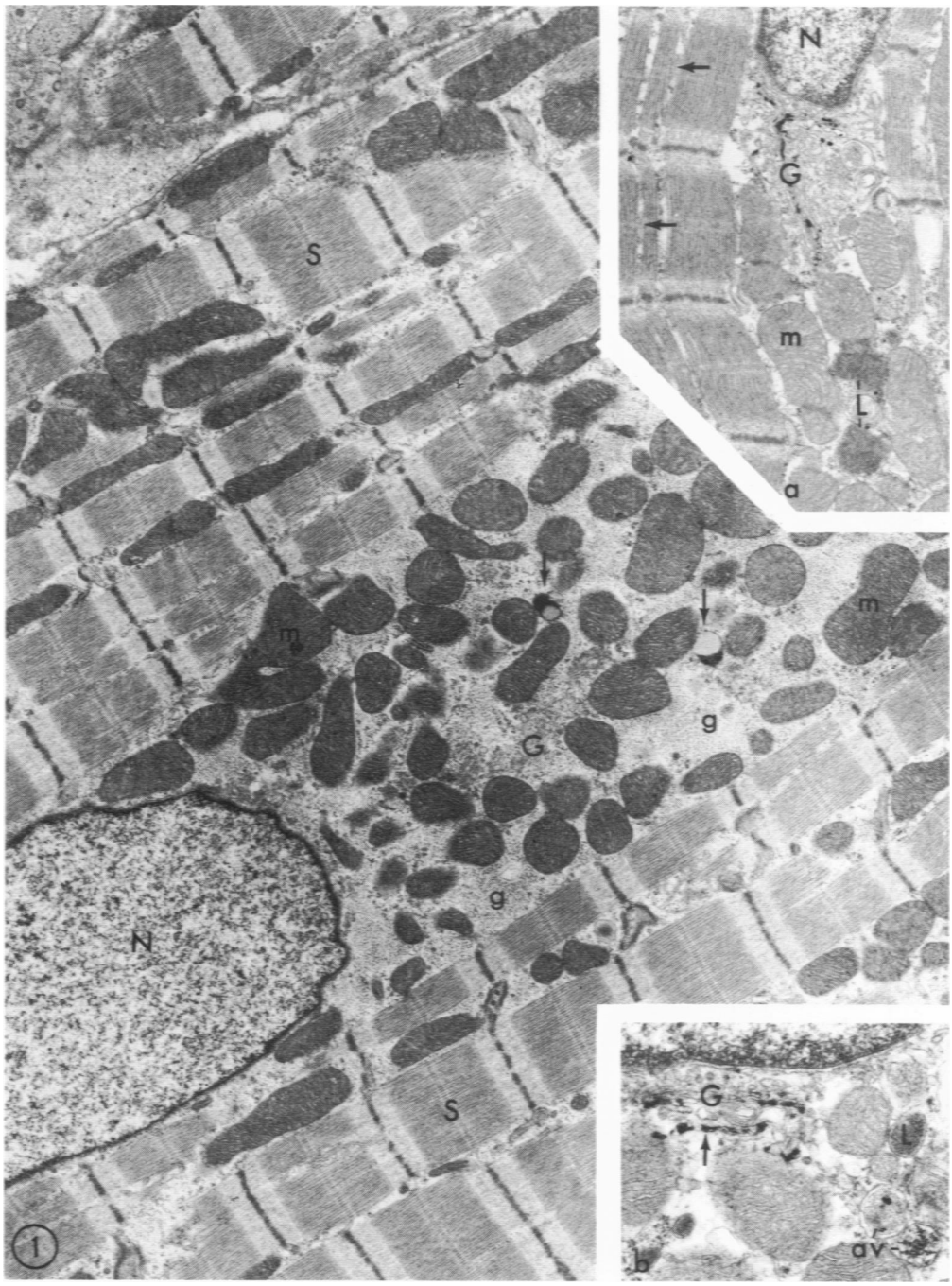
Acknowledgments

The authors express their thanks to Mrs. Marlene Decker, Ms. Marina Kennedy, and Mr. John Lloyd for their invaluable technical assistance. Methylprednisolone (Solu-Medrol) was kindly provided by Upjohn, Inc.

Figure 1—Morphology of sham-operated control subendocardial myocytes. Glycogen (*g*) is distributed in the paranuclear regions of uninjured myocytes. Secondary lysosomes (*arrows*), densely stained mitochondria (*m*), and a small Golgi complex (*G*) are also observed. Nuclei (*N*) disclose a uniform distribution of chromatin, and sarcomeres (*S*) exhibit a relaxed configuration. **Insets a and b**—Location of acid phosphatase and aryl sulfatase reaction product after 1 and 2 hours of open-chest sham surgery. Lead phosphate and barium sulfide precipitates are readily apparent in saccules of the Golgi complex (*G*) and elements of GERL (*arrow*, inset b), but most secondary lysosomes (*L*) react negatively, as do elements of the sarcoplasmic reticulum (*arrows*, inset a). Some reaction product is evident in autophagic vacuoles (*av*, inset b). Such myocytes retain a normal complement of glycogen (*g*), and no changes in mitochondrial structure are apparent. The nuclei (*N*) usually reveal some heterochromatin, with the majority, however, being evenly dispersed. (× 10,000; **inset a**, × 10,000; **inset b**, × 20,000)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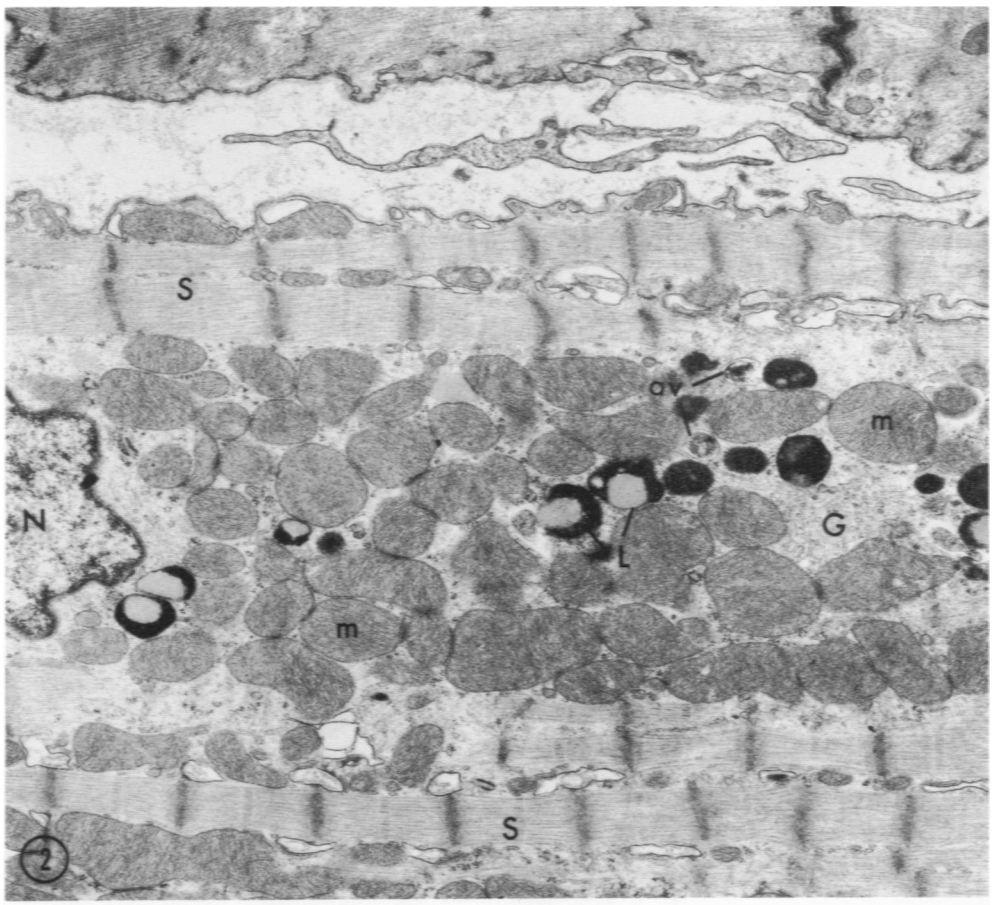
Figures 2 through 4—Alterations in myocyte structure and lysosomal staining following 15 minutes of myocardial ischemia in untreated (Figures 2 and 3) and steroid-treated (Figure 4) animals. The most obvious change in untreated hearts is an aggregation of secondary lysosomes in the paranuclear region of affected myocytes (Figure 2). Of these, autophagic vacuoles (*av*) and dense bodies (*d*) display considerable acid phosphatase reaction product (Figure 3), whereas residual bodies (*L*) do not. Some mitochrondrial (*m*) dilation can be discerned, matrix granules are rarely encountered, and minimal glycogen depletion is apparent; little margination of nuclear (*N*) chromatin is observed (Figures 2 and 3). Methylprednisolone-treated myocytes (Figure 4) reveal normal mitochrondria (*m*) with an electron-dense matrix and granules. Lysosomal dense bodies (*d*) and autophagic vacuoles (*av*) exhibit aryl sulfatase reaction product as does the Golgi complex (*G*), but lysosomal number and size in the paranuclear regions are reduced compared with untreated ischemic hearts. Nuclear (*N*) chromatin condensation and glycogen distribution after steroids are similar to that in untreated ischemia myocytes. *S*=sarcomere. (**2,** × 8000; **3,** × 14,000; **4,** × 14,000)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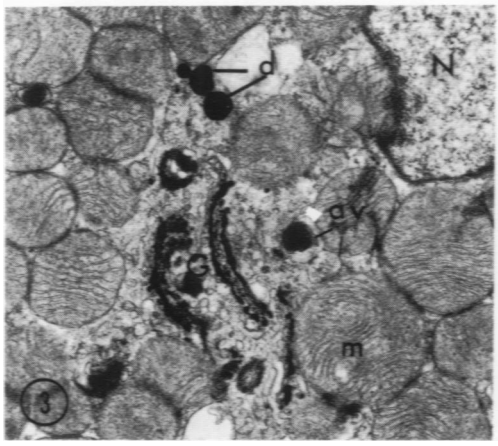
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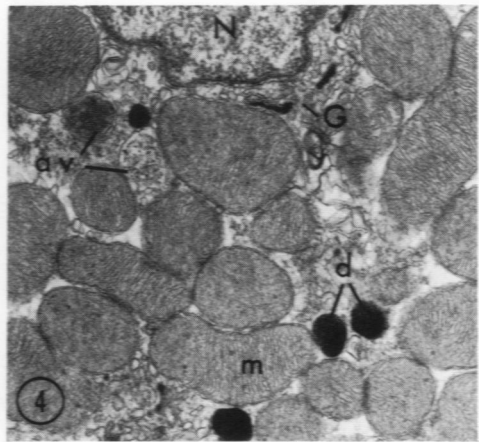
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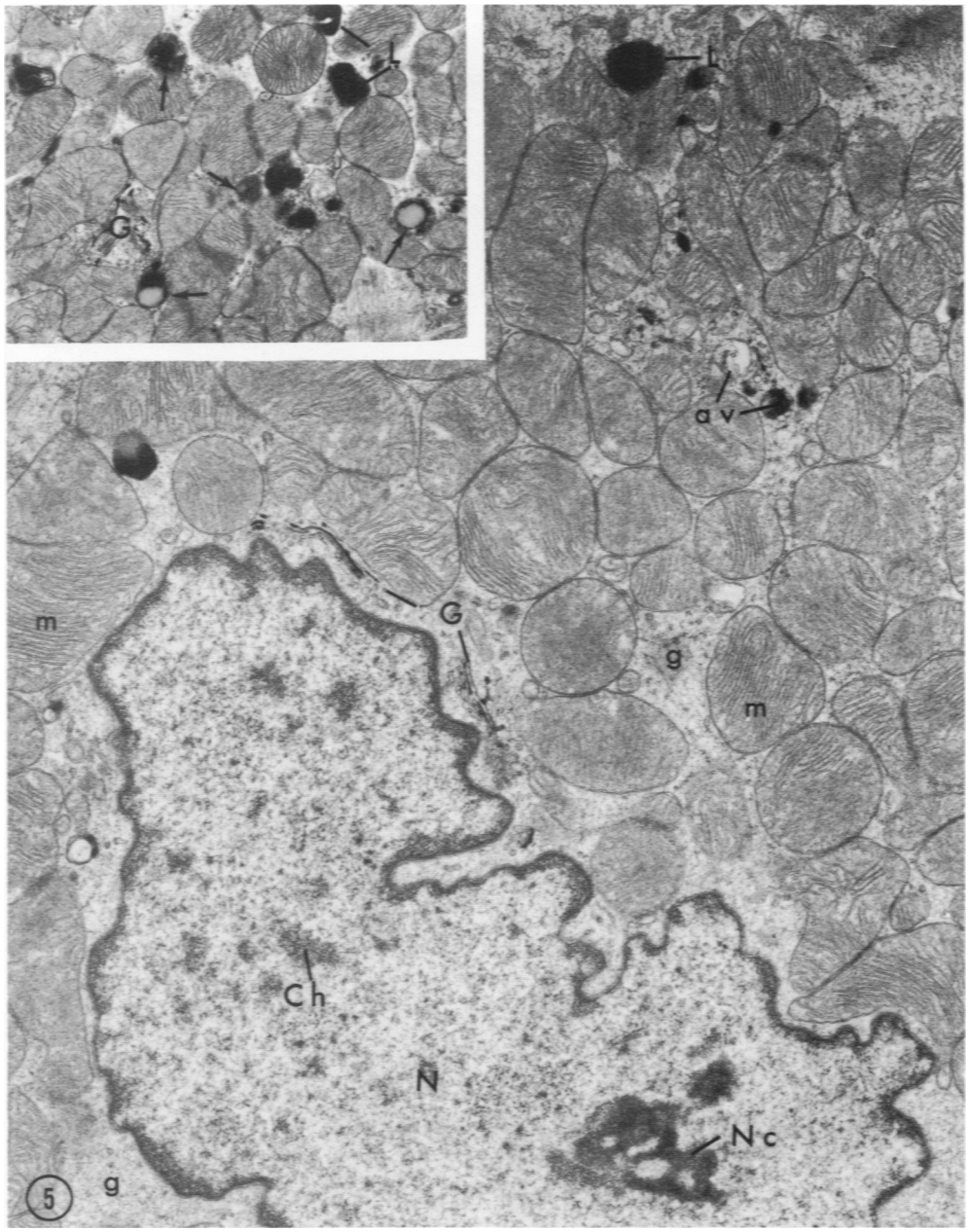
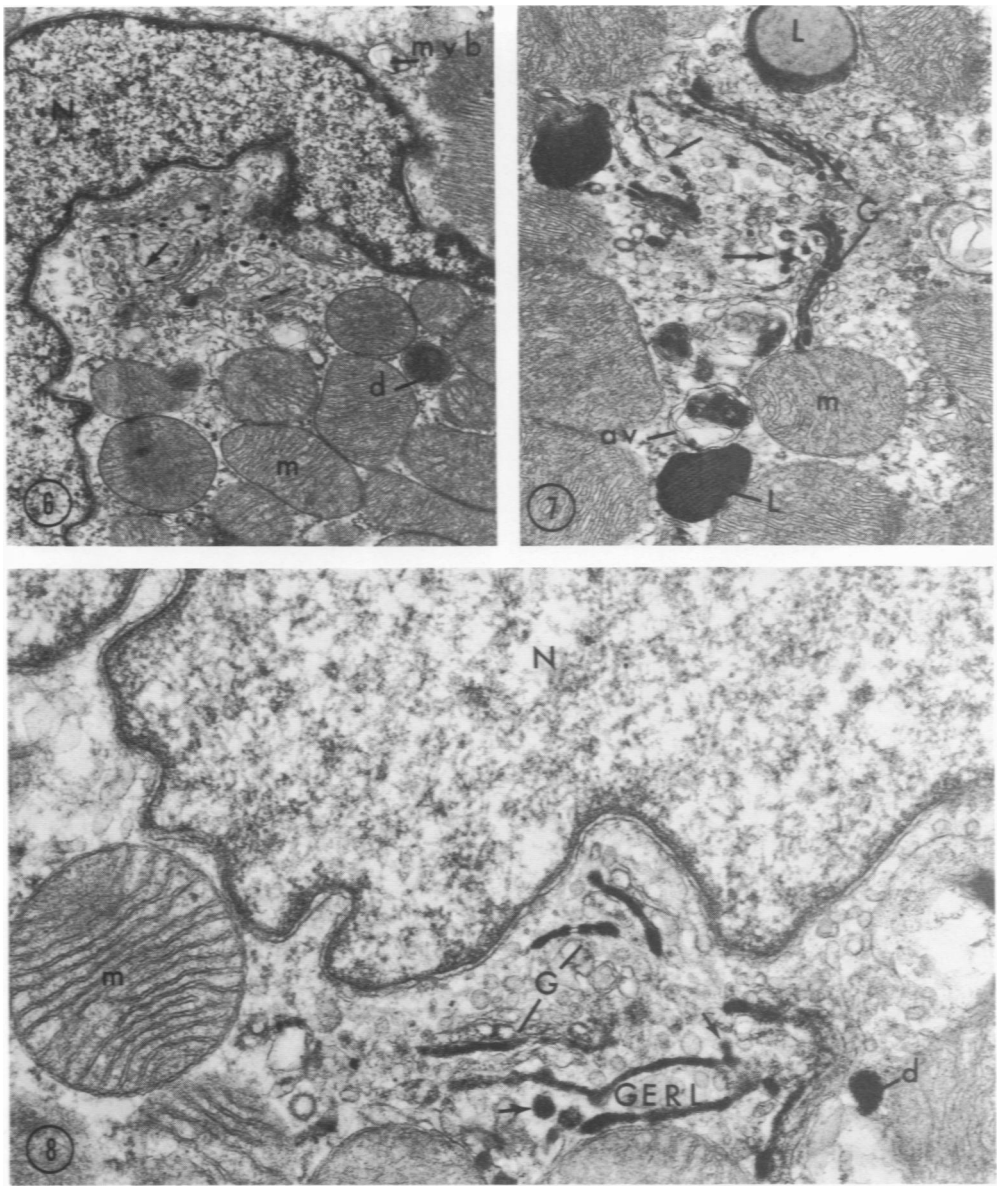
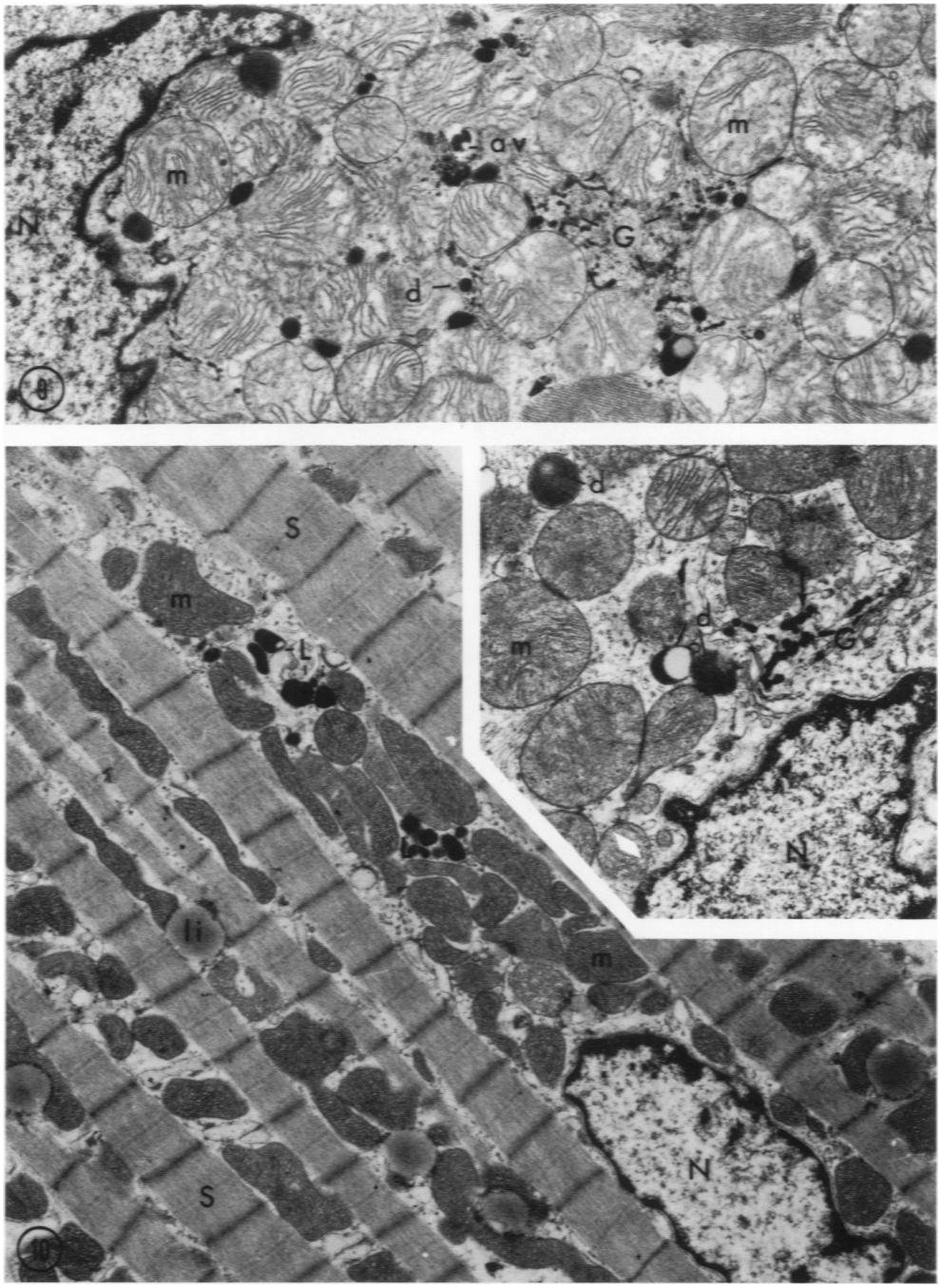


Figure 5—Major changes in untreated myocyte structure after 30 minutes of ischemia. Moderate mitochondrial (*m*) swelling and loss of matrix can be noted at this time. Glycogen (*g*) depletion is moderate and condensation of chromatin (*Ch*) is evident along the inner nuclear envelope. Lysosomes still stain heterogeneously, with some residual bodies (*arrows*) revealing little acid phosphatase activity while the Golgi complex (*G*), dense bodies (*L*), and autophagic vacuoles (*av*) exhibit considerable reaction product (**inset**). *N*=nucleus, *Nc*=nucleolus. (× 10,000; **inset,** × 5000)



Figures 6 through 8—Distribution of thiolacetic acid esterase (Figure 6), acid phosphatase (Figure 7), and aryl sulfatase (Figure 8) reaction products in steroid-treated 30-minute ischemic hearts. In methylprednisolone-treated hearts, mitochondria (*m*) appear normal, but margination of chromatin (*N*) and moderate glycogen depletion appear as severe as in untreated ischemia. Lysosomal reaction products are distributed in a manner identical to that observed in nonischemic controls; elements of the Golgi complex (*G*), *GERL* (*arrows*), and small coated vesicles (primary lysosomes) display some reaction products, but dense bodies (*d*) and autophagic vacuoles (*av*) exhibit the bulk of the activity. *L* = secondary lysosomes, *mvb* = multivesicular body. (**6,** × 12,500; **7,** × 20,000; **8,** × 35,000)



Figures 9 and 10—Myocytes subjected to a 45-minute period of ischemia. In untreated hearts (Figure 9), severe mitochondrial swelling (*m*), glycogen depletion, and condensation of nuclear (*N*) chromatin are signs of "near" irreversible cell injury. Acid phosphatase reaction products in the Golgi complex (*G*) and some secondary lysosomes such as dense bodies (*d*) and autophagic vacuoles (*av*) appear unchanged from 30-minute ischemic myocytes (Figure 9). Significant chromatin condensation and glycogen loss are also apparent in methylprednisolone-protected hearts (Figure 10). However, neither mitochondrial (*m*) structure nor lysosomal staining appears abnormal in ischemic hearts given steroid. The Golgi complex (*G*) and GERL (*arrows*) are the predominant sites of acid phosphatase reaction products (Figure 10, **inset**). Dense bodies (*L*) also frequently exhibit aryl sulfatase reaction product (Figure 10). Occasional lipid droplets (*li*) are seen in steroid-treated and untreated ischemic cells. *S* = sarcomere. (**9**, × 11,500; **10**, × 8000; **inset**, × 15,000)

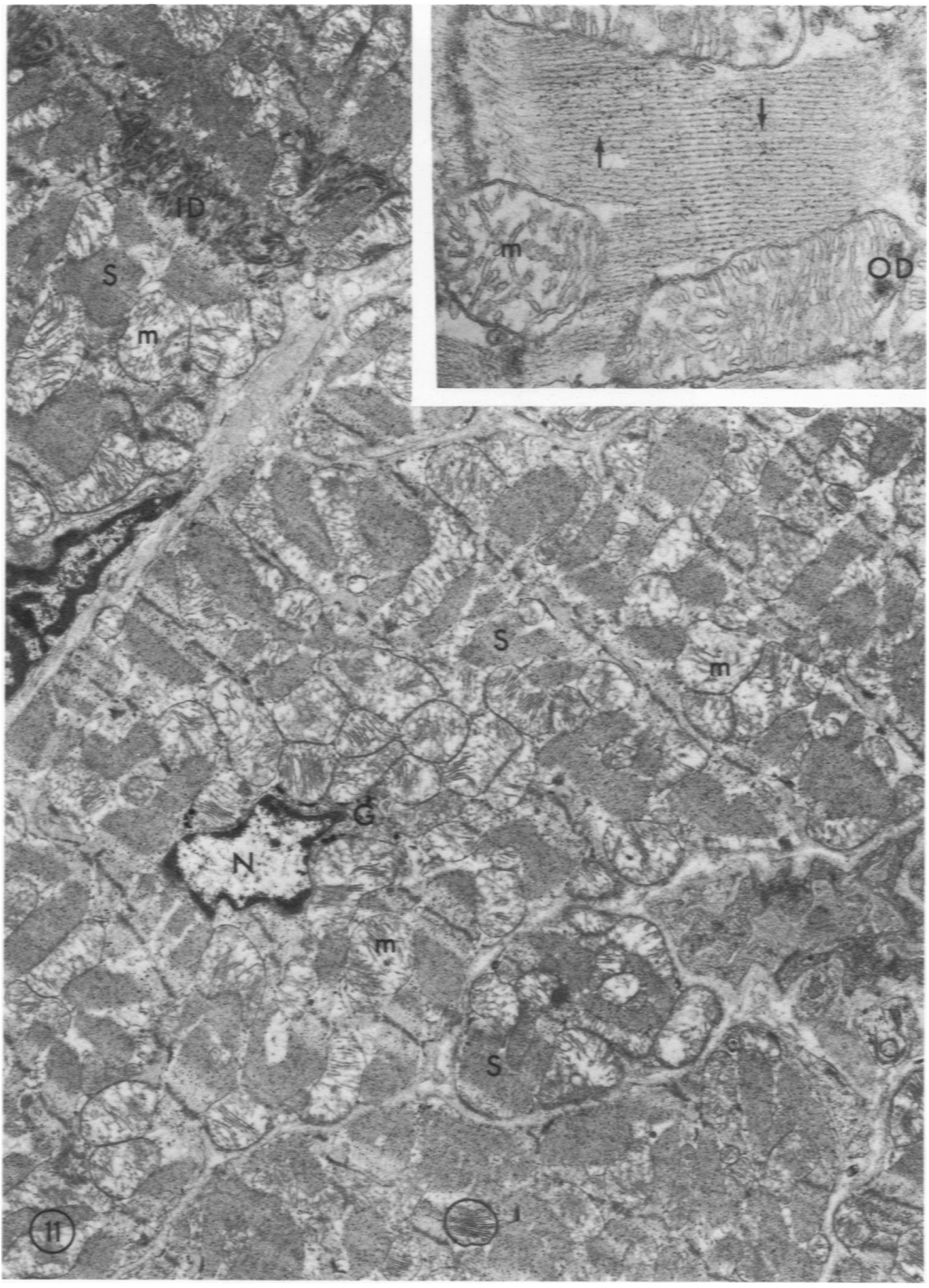


Figure 11—Localization of acid phosphatase reaction product in myocytes exposed to a 1-hr ischemic insult. Damaged cells display pyknotic nuclei (*N*), swollen mitochrondria (*m*) with occasional osmiophilic densities (*OD*), severe glycogen depletion, and fine particulate deposits of lead phosphate localized over myofibrillar elements (**inset**). Although dilated Golgi saccules (*G*) and an infrequent dense body stain positively, much of the reaction product (*arrows*) is observed over myofibrils (*S*). *ID*=intercalated disc. (× 6500; **inset**, × 25,000)

Figure 12—Distribution of thiolacetic acid esterase (Figure 12 and **inset a**) and aryl sulfatase (**inset b**) reaction products in 1-hr ischemically injured myocytes. Esterase activity can be observed in dilated elements of the Golgi complex (*G*), and lead nitrate deposits are lightly distributed over lysosomal dense bodies (*L*). There is little evidence, however, of reaction product over the relaxed myofibrils that exhibit characteristic n lines (*n*) and H bands (*H*) (**inset a**). In contrast, barium sulfide deposits are apparent over A bands (*arrows*) and, to a lesser extent, over other components of the sarcomere (*S*) (*Z to Z*, **inset b**). (× 6500; **inset a**, × 16,000; **inset b**, × 30,000)

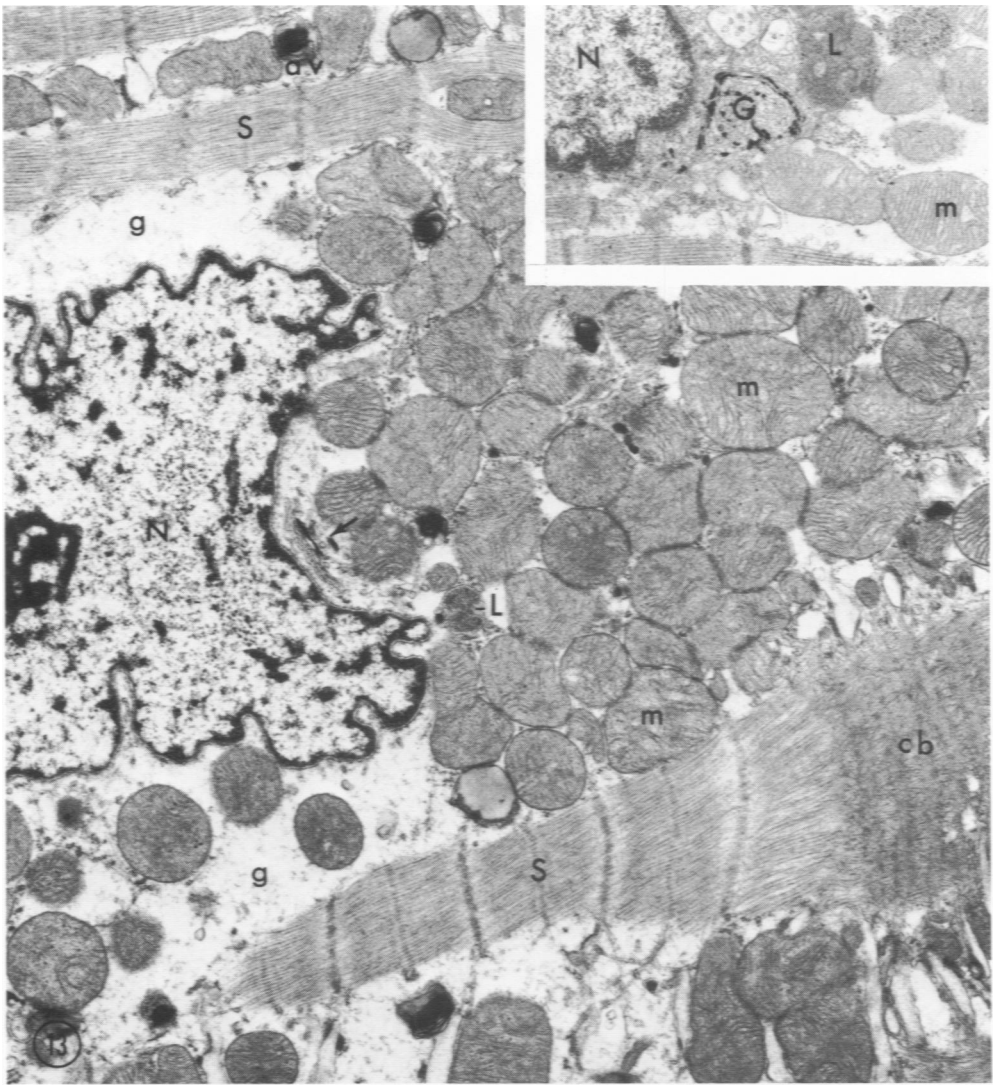


Figure 13—Fine structural features of methylprednisolone-pretreated myocytes after 1 hr of ischemia. Although condensation of nuclear (*N*) chromatin is apparent and glycogen stores (*g*) are nearly exhausted, other aspects of myocyte morphology seem normal. Mitochrondria (*m*) are somewhat swollen, but their matrix is present as are their matrix granules. Myofibrils (*S*) are generally relaxed, but n lines are not obvious; occasional contraction bands (*cb*) are evident. Aryl sufatase reaction product can be observed in the GERL (*arrow*) and in autophagic vacuoles (*av*). Acid phosphatase activity is predominantly displayed in both the Golgi complex (*G*) and GERL. Dense bodies (*L*) generally reveal little acid hydrolase activity, and no evidence has been uncovered of any lysosomal reaction products distributed over myofibrils (*S*). (× 12,000; **inset,** × 12,000)

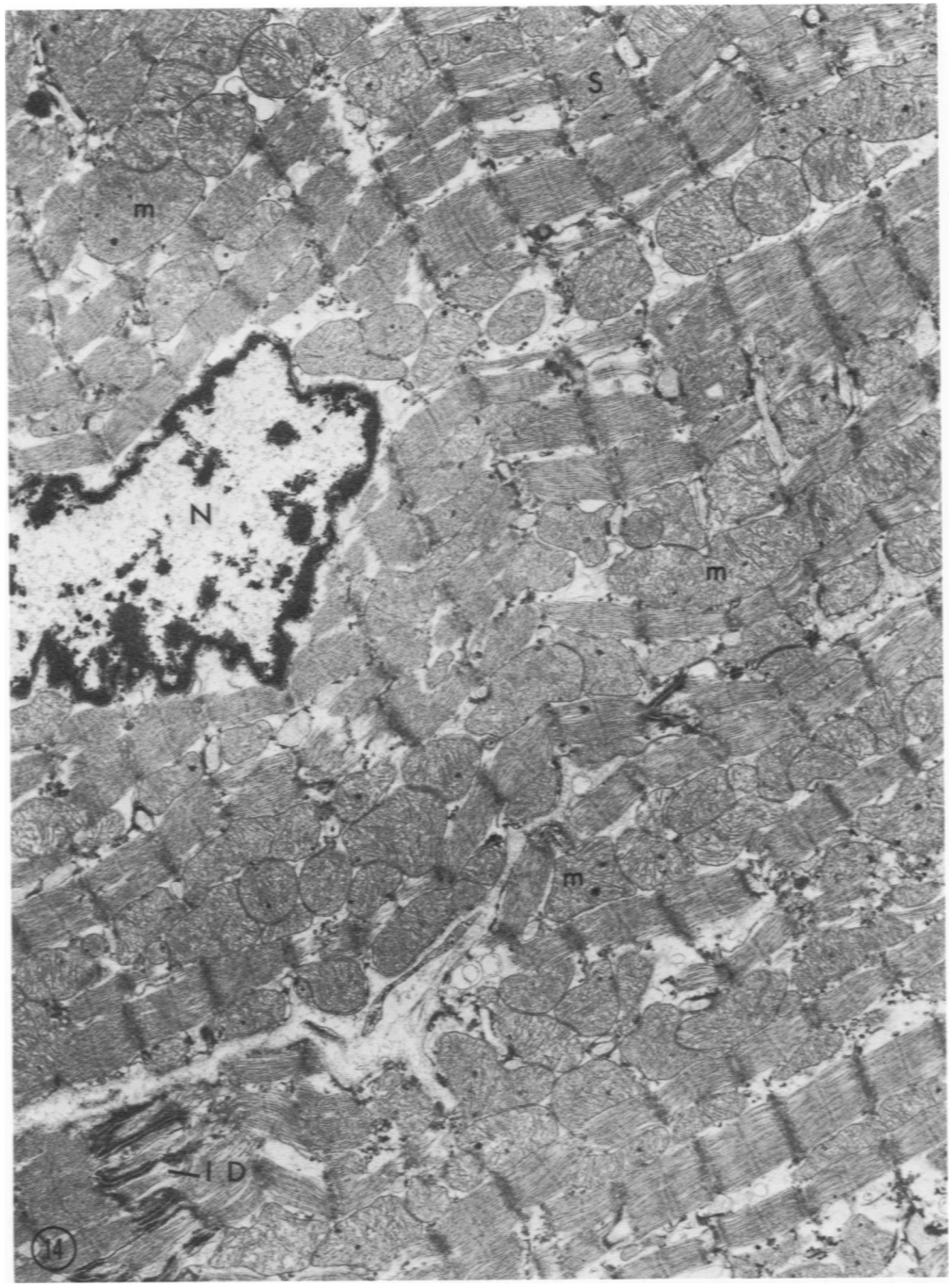


Figure 14—Structural damage incurred by corticosteroid-treated myocytes after 90 minutes of ischemia. Massive chromatin (*N*) condensation, numerous osmiophilic densities within mitochrondria (*m*), a complete loss of ground substance, depletion of glycogen stores, and a disappearance of lysosomal profiles attest to the irreversible injury inflicted on these cells. *ID* = intercalated disc, *S* = sarcomere. (× 8000)