# Ultrastructural Features of Degenerated Cardiac Muscle Cells in Patients With Cardiac Hypertrophy

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Degenerated cardiac muscle cells were present in hypertrophied ventricular muscle obtained at operation from 12 (38%) of 32 patients with asymmetric septal hypertrophy (hypertrophic cardiomyopathy) or aortic valvular disease. Degenerated cells demonstrated a wide variety of ultrastructural alterations. Mildly altered cells were normal-sized or hypertrophied and showed focal changes, including preferential loss of thick (myosin) filaments, streaming and clumping of Z band material, and proliferation of the tubules of sarcoplasmic reticulum. Moderately and severely degenerated cells were normal-sized or atrophic and showed additional changes, including extensive myofibrillar lysis and loss of T tubules. The appearance of the most severely degenerated cells usually reflected the cytoplasmic organelle (sarcoplasmic reticulum, glycogen, or mitochondria) which underwent proliferation and filled the myofibril-free areas of these cells. Moderately and severely degenerated cells were present in areas of fibrosis, had thickened basement membranes, and had lost their intercellular connections. These observations suggest that degenerated cardiac muscle cells have poor contractile function and may be responsible for impaired cardiac performance in some patients with chronic ventricular hypertrophy. (Am J Pathol 79:387–434, 1975)

CARDIAC MUSCLE evolves through three distinct stages during the time course of hypertrophy. Hypertrophy begins to develop during the first stage, in which there is an increase in energy production and protein synthesis. A stable state of cardiac hyperfunction exists in the second stage. The third stage is characterized by gradual exhaustion of the heart's ability to synthesize proteins, by failure to renew myofibrils and mitochondria, and by myofibrillar damage and cellular atrophy.[1-3]

Ultrastructural changes occurring in cardiac hypertrophy have been

studied in several animal models,[4-11] but the progression of hypertrophy has not been followed for more than 1 year in any of these studies. Studies of chronic cardiac hypertrophy in humans have been made in hypertensive [12] and rheumatic heart disease,[12-18] congestive cardiomyopathy,[19-21] asymmetric septal hypertrophy or ASH (hypertrophic cardiomyopathy),[21-24] and congenital heart diseases.[9,12,14,16-18] These studies, however, have not described the spectrum of degenerative changes that occur in cardiac muscle cells in the late stages of hypertrophy. The present communication reports various features of myocardial degeneration that we have observed during the course of ultrastructural studies on myocardium from patients with aortic valvular disease or with ASH.

## Materials and Methods

The observations in this report are based on study of tissues from two groups of patients. The first group consisted of 16 patients (12 men and 4 women, age ranging from 7 to 64 years and averaging 41 years) with aortic valvular disease. Six of these patients had predominant aortic stenosis, with peak systolic pressure gradients of 60 to 120 mm Hg between the left ventricle and the aorta; 5 patients had no aortic valvular gradient but had moderate to severe aortic regurgitation, as graded by angiography; and 5 patients had combined aortic stenosis and regurgitation, with pressure gradients of 13 to 78 mm Hg. In patients with aortic valvular disease, biopsies of the left ventricular free wall were obtained at the time of operation for aortic valve replacement (14 patients) or for aortic commissurotomy (2 patients). The second group was composed of 16 patients (9 men and 7 women, ranging in age from 10 to 67 years with an average of 42 years) with ASH. Thirteen of these patients had obstruction to left ventricular outflow, with gradients of 65 to 162 mm Hg under basal conditions; the other 3 patients had no gradient under basal conditions or with provocative maneuvers. In the patients with ASH, tissues were taken from three areas of the left ventricle, including ventricular septum (16 patients), posterior wall of the left ventricle (13 patients), and apex of the left ventricle (12 patients) at the time of left ventricular myotomy-myectomy. Details of the procedures employed for obtaining these tissues have been reported previously.[25]

All tissues were immediately fixed with cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. After washing with several changes of cold 5% sucrose in 0.1 M phosphate buffer, pH 7.2, the tissues were postfixed with 1% osmium tetroxide in Millonig's phosphate buffer, dehydrated with a graded series of ethanols and propylene oxide, and embedded in Maraglas.[26]

Semithin (0.5-μ-thick) sections were stained with alkaline toluidine blue and examined with a light microscope to select artifact-free areas suitable for ultrathin sections [21] and to measure transverse cell diameters with a calibrated micrometer eyepiece. Four to 15 tissue blocks (measuring 1 to 4 sq mm in area) were available from each biopsy for analysis. Ultrathin sections were stained with uranyl acetate, using either a saturated aqueous solution for 10 minutes at room temperature or a 2% solution in 95% ethanol for 15 to 30 minutes at 60 C,[27] and with Reynold's lead citrate.

## Results

The majority of cardiac muscle cells in the tissues examined from all

32 patients were hypertrophied and had transverse diameters measuring between 15 and 70 µ (normal, 10 to 15 µ). Hypertrophied but non-degenerated cardiac muscle cells in patients with aortic valvular disease (Figure 1) were characterized by: a) increased numbers of myofibrils, ribosomes (both free in the cytoplasm and bound to membranes of endoplasmic reticulum), mitochondria and glycogen particles; b) variability in size of mitochondria; c) enlarged Golgi complexes; d) enlarged nuclei with increased convolutions of the nuclear membranes; e) irregularly shaped and often dilated T tubules; f) markedly convoluted intercellular junctions; g) increased numbers of lipofuscin granules; h) mild, focal thickening of Z bands; i) small, focal accumulations of Z-band-like material adjacent to the sarcolemma and often contiguous with true Z bands; j) maintenance of normal cellular organization and normal relation to adjacent cells; and k) association with variable, but generally small, amounts of interstitial fibrous tissue. The ultrastructural features of hypertrophied, nondegenerated cells, as well as those of degenerated cells to be described below, are summarized in Table 1.

Some areas of ventricular septal muscle from patients with ASH contained hypertrophied, nondegenerated cells similar to those described above in patients with aortic valvular disease; other areas, however, had nondegenerated cells with a bizarre form of hypertrophy. As described elsewhere,[24,25] the latter cells showed marked irregularities of size, shape, and orientation, and often contained myofibrils coursing in different directions rather than in parallel. In the ventricular free walls, these areas of disorganization have been shown to be extensive in patients with the nonobstructive form of ASH, but minimal or absent in patients with the obstructive form of the disease.[25]

Cardiac muscle cells with features of degeneration, as defined below, were found in tissue specimens from 12 (38%) of the 32 patients studied. Such cells were present in apical left ventricular myocardium from 6 of the 16 patients with aortic valvular disease (2 of 5 with pure aortic regurgitation, 4 of 5 with combined aortic stenosis and regurgitation, and none of the 6 patients with predominant aortic stenosis) and in ventricular myocardium from 6 of the 16 patients with ASH, including ventricular septum (4 patients), ventricular septum and apex of the left ventricle (1 patient) and posterior wall of the left ventricle (1 patient). Of the 6 patients with ASH who had degenerated cardiac muscle cells, 4 had obstruction to left ventricular outflow under basal conditions; the remaining 2 patients had no obstruction under basal conditions or after provocative maneuvers. Of the 10 patients with ASH in whom

Table 1—Ultrastructural Features of Hypertrophied or Degenerated Cardiac Muscle Cells

| Morphologic feature | Hypertrophy without degeneration | Mild degeneration | Moderate degeneration | Severe degeneration |
|---|---|---|---|---|
| Cell size | ↑ | NL or ↑ | NL or mildly ↑ | ↓ |
| No. of myofibrils/cell | ↑ | NL or ↑ | ↓ | ↓↓ |
| Changes in myofilaments | 0 | Focal ↓ affecting thick > thin filaments | ↓↓ Affecting thick > thin filaments | ↓↓ Affecting thick > thin filaments |
| No. of cytoskeletal (100 Å) filaments | ↑ (Focal) | ↑ (Focal) | ↑ (Disorganized) | ↑ (Disorganized) |
| Changes in Z band material: | | | | |
| Focal thickening; marked proliferation; subsarcolemmal accumulations | + | + | Rare | 0 |
| Elongated masses in center of cell; streaming, clumping, fragmentation | 0 | + | + | + |
| Foci of proliferation of SR | 0 | Sparse | Extensive | Extensive |
| No. of T tubules | NL | NL | Variable | ↓ or absent |
| Changes in intercellular junctions | Marked convolutions | Marked convolutions | Partial dissociation | Extensive dissociation |
| Presence of intracytoplasmic junctions | Very rare | Rare | + | + |
| Changes in glycogen: | | | | |
| No. of β-particles | ↑ | ↑ | Variable | Variable |
| Presence of α-particles | + (Focal) | + (Focal) | + (Focal) | + (Focal) |
| Presence of glycogen-like basophilic degeneration material | 0 | 0 | + (Focal) | + (Focal) |
| Size of Golgi complexes | ↑ | ↑ | ↑ | 0 |
| No. of ribosomes (free or membrane-bound) | ↑ | ↑ | ↑ | ↑ |
| Changes in mitochondria: | | | | |
| Number | ↑ | ↑ | Variable | Variable |
| Size | Variable | Variable | Variable | Variable |
| Presence of myelin figures | 0 | 0 | + | + |
| Nuclear size | ↑ | ↑ | ↑ | ↑ |
| No. of lipofuscin granules | NL | NL or ↑ | Variable | Variable |
| Thickness of basement membranes | NL | ↑ | ↑↑ | ↑↑ |
| Amount of interstitial fibrous tissue | NL | ↑ | ↑↑ | ↑↑ |
| Dissociation of cells | 0 | 0 | + | + |

+ = present, 0 = absent, ↑ = increased, ↑↑ = markedly increased, ↓ = decreased, ↓↓ = markedly decreased, NL = normal, SR = sarcoplasmic reticulum.

degenerated cells were not observed, 9 had obstruction and 1 had no obstruction to left ventricular outflow.

The morphologic features interpreted as degenerative in this study were remarkably consistent in cardiac muscle cells from all 12 patients, regardless of the nature of the hemodynamic alteration or the site of the tissue sample in the left ventricle (apex, posterior wall or septum). Severely degenerated cardiac muscle cells showed greater variation in the pattern of proliferation of cytoplasmic organelles in patients with ASH than in patients with aortic valvular disease. Cardiac muscle cells with evidence of degeneration were classified as showing mild, moderate, or severe degeneration, according to the nature and extent of the morphologic changes which they exhibited. While each of these three categories of cells will be described separately, it should be emphasized that they represent three stages in a continuum of morphologic changes. Furthermore, cardiac muscle cells characteristic of all three categories usually were observed in the same area of myocardium.

### Morphology of Cardiac Muscle Cells With Mild Degeneration

Cardiac muscle cells with evidence of early degeneration were either normal in size or hypertrophied. By light microscopy, these cells could not be distinguished from hypertrophied, nondegenerated cells. Mildly degenerated cells differed from hypertrophied, nondegenerated cells only in regard to focal alterations in the ultrastructure of myofibrils and sarcoplasmic reticulum. Abnormalities of myofibrillar structure in mildly degenerated cells consisted of various alterations of Z band material and focal loss of myofilaments. The Z bands showed fragmentation, with clumping of Z band material and formation of irregular, elongated extensions of Z band material into other regions of the sarcomeres (Figures 2 and 3). These changes, which were considered to represent early stages of myofibrillar lysis, occurred in addition to the focal thickening of Z bands (Figure 2) and subsarcolemmal accumulations of Z-band-like material (Figures 4–6) which were also observed in hypertrophied, nondegenerated cells. Markedly abnormal Z bands were occasionally observed in some cells with mild or moderate degeneration. These Z bands measured up to 1.5 μ in thickness, were traversed by thin filaments and focally extended into adjacent regions of the sarcomeres (Figures 7 and 8).

The focal loss of myofilaments involved only a few sarcomeres per cell and appeared to affect the thick, myosin (120 to 160 Å in diameter) filaments to a greater extent than the thin, actin (60 to 75 Å in diameter) filaments (Figures 9–11). Therefore, this process resulted in the pres-

ence of disproportionately greater numbers of thin filaments in involved
regions of the cells (Figures 9–11). In some cells, myofibrils appeared to
have undergone focal lysis at their points of attachment to intercellular
junctions (Figure 10). The myofibrillar lysis in cells with mild de-
generation (or in cells with moderate and severe degeneration) was not
associated with contraction bands.

The alterations in sarcoplasmic reticulum of mildly degenerated cells
consisted of foci of proliferation of smooth-surfaced tubules, which
formed small meshworks in areas between myofibrils and in perinuclear
spaces (Figure 5). In contrast to normal tubules of sarcoplasmic
reticulum, these tubules did not surround myofibrils and did not form
dyads or triads with components of the transverse tubular system.

### Morphology of Cardiac Muscle Cells With Moderate Degeneration

Cardiac muscle cells showing moderate or severe degenerative
changes were usually normal sized or only mildly enlarged (transverse
diameters, 10 to 20 μ). By light microscopy, they usually were pale-
staining and appeared to have decreased numbers of myofibrils (Figures
12–15). These cells were present in areas of marked interstitial fibrosis
and often appeared to have lost their connections with adjacent cells.
By light microscopy alone it was often impossible to distinguish cells
with moderate degeneration from those with severe degeneration. Cells
with moderate degeneration showed marked alterations in the ultra-
structure of myofibrils, sarcoplasmic reticulum, T tubules, and other
organelles. These changes usually involved large areas of the cytoplasm.

The alterations of myofibrils in these cells represented further progres-
sion of the process which began in early degeneration. These changes
consisted of: a) presence of disproportionately greater amounts of thin
filaments than of thick filaments, b) decreased number of myofibrils,
c) appearance of tangled masses of thin filaments in the cytoplasm,
d) severe changes involving Z band material, and e) disorganized
arrangement of cytoskeletal filaments [28] which often were free in the
cytoplasm rather than attached to Z bands (Figures 16 and 17). These
alterations in myofibrils accounted for the pale staining of the cells.

Material which morphologically resembled that in Z bands was usually
present in cells with moderate degeneration in the following two forms:
a) large masses which were distributed throughout the cytoplasm in a
disorganized fashion, occasionally showed a square lattice type of sub-
structure, and were traversed by thin filaments that were arranged in
parallel and were separated from each other by distances of 100 Å (Fig-
ure 16), and b) elongated masses which measured up to 17 μ in length

and up to 1 μ in diameter and were interspersed with numerous, irregu-
larly arranged, thin filaments. These elongated masses were oriented
along the longitudinal axis of the cell, usually frayed into the cytoplasm
at each end, and were not attached to adjacent structures (Figures 18–
21). These masses were located either in the center of the cell (Figures
19 and 20) or near the sarcolemma (Figures 18 and 21) and often were
adjacent to normal appearing myofibrils (Figure 18). Other types of Z
band alterations, similar to those frequently found in cells with mild or
no degeneration, were less commonly observed in cells with moderate
degeneration. These changes consisted of focal widening of Z bands of
otherwise normally constituted myofibrils and focal, subsarcolemmal
accumulations of Z-band-like material.

The tubules of sarcoplasmic reticulum in cells with moderate de-
generation often showed marked proliferation and formed branching
and anastomosing networks which occupied myofibril-free areas of the
cytoplasm. These tubules were occasionally dilated (up to 2750 Å in
diameter). The surfaces of cells with moderate degeneration often were
irregular and convoluted, and exhibited shallow plasma membrane
invaginations of varying sizes (Figure 22). Basement membranes, com-
posed of a homogeneous band of finely filamentous material, often were
thickened (up to 2.5 μ). Microfibrils were usually in close contact with
the outer margins of the basement membranes. These alterations in the
cell surfaces were associated with prominent changes in the T tubule
system and intercellular junctions. True T tubules (as opposed to shal-
low surface invaginations that were not related to Z bands) were rarely
observed in these cells. Intercalated discs joining degenerated cells often
showed various degrees of dissociation (ie, separation of their apposed
membranes). The mechanisms of this dissociation have been described
in detail elsewhere.[29] Some cells contained junctional structures formed
by the apposition of two areas of the plasma membrane of the same
cell (Figures 22 and 23). Some of these structures consisted of desmo-
somes, while others were more complex and resembled parts of inter-
calated discs. These junctional structures, usually present in the periph-
eral areas of cytoplasm at the sides or ends of cells, have been termed
intracytoplasmic junctions [29] and probably result from the remodeling
of cell surfaces that occurs when degenerated cardiac muscle cells lose
their intercellular contacts. These junctions were occasionally observed
in cells with mild degeneration, although they were more characteristic
of moderately and, particularly, of severely degenerated cells. In addi-
tion to the usual β-glycogen particles (230 to 350 Å in diameter), α-
glycogen rosettes (1300 to 2400 Å in diameter) were present in some

moderately or severely degenerated cells. These changes have been described in detail elsewhere.[30] Golgi complexes and ribosomes were present in varying numbers in moderately or severely degenerated cells.

In addition to these moderately degenerated cardiac muscle cells that were characterized by extensive myofibrillar lysis, we also observed a distinct population of small cardiac muscle cells (transverse diameters usually $<10$ μ) that were dissociated from adjacent cells but which did not show myofibrillar damage (Figures 24 and 25). Some of these latter cells appeared to end abruptly in the surrounding interstitium (Figure 24). The plasma membranes at the ends of these cells had only shallow invaginations and mild irregularities of contour, and their basement membranes were markedly thickened (Figures 22 and 24). Other cells tapered gradually into the interstitium (Figure 25). The ends of some of these tapered cells had deep invaginations of the plasma membrane associated with prominent finger-like projections of the cytoplasm (Figure 25). These projections were oriented parallel to the longitudinal axis of the cell and measured up to 7.5 μ in length and 2 μ in width; they contained myofibrils, mitochondria, glycogen granules, and narrow, elongated, subsarcolemmal masses of Z-band-like material without periodic substructure. The myofibrils in the cytoplasmic projections terminated in the form of thin filaments which inserted into the subsarcolemmal masses of Z band material. Although these myofibrils were usually intact, they sometimes showed thickening of Z bands and loss of thick filaments. Intracytoplasmic junctions were present at the margins of many of the deep invaginations and often constituted sites of attachment between the sides of adjacent cytoplasmic projections.

Extremely small cardiac muscle cells (about 1 to 5 μ in diameter) were occasionally associated with larger cardiac muscle cells (Figures 26 and 27). These cells were connected by side-to-side intercellular junctions and enveloped by a common basement membrane. Some of these small cells showed no evidence of myofibrillar lysis and probably were parts of large, irregularly shaped, nondegenerated cardiac muscle cells which had been sectioned tangentially (Figure 26). Other extremely small cells had no normal appearing myofibrils (Figure 27) and were considered to be truly degenerated cells rather than fragments of larger, nondegenerated cells.

### Morphology of Cardiac Muscle Cells With Severe Degeneration

Cardiac muscle cells showing severe degeneration were usually atrophic (transverse diameter, 3 to 10 μ). By light microscopy these cells appeared palely stained with alkaline toluidine blue. They contained

very few myofibrils and were invariably separated from adjacent muscle cells by large amounts of fibrous tissue (Figures 12, 13 and 15).

Cells with advanced degeneration demonstrated marked loss of contractile elements and a spectrum of changes involving virtually every type of organelle (Figures 28–33). Cells with advanced degeneration often assumed characteristic appearances which reflected the selective proliferation or alteration of the cytoplasmic organelle which replaced the contractile elements in the cytoplasm. Therefore, three distinctive subgroups of severely degenerated cells were recognized, depending on whether the cytoplasm primarily contained: a) markedly proliferated tubules of sarcoplasmic reticulum; b) large aggregates of mitochondria, some of which were associated with electron-dense concentric lamellae (myelin figures); or c) masses of glycogen or glycogen-like material. In certain cells these proliferative processes were less selective and involved more than one of these components.

Some severely degenerated cells were characterized by marked proliferation, with or without dilatation, of tubules of sarcoplasmic reticulum (Figures 28 and 29). These tubules formed a network which often occupied virtually the entire cytoplasm. Cells showing proliferation of sacroplasmic reticulum usually had few or no normally constituted myofibrils. These cells contained (as did those with moderate degeneration) few or no thick filaments, apparently normal or increased numbers of mitochondria, and randomly arranged masses of abnormal Z band material to which thin filaments were attached. The most extreme form of this type of proliferation of sarcoplasmic reticulum was present in moderately as well as in severely degenerated cells and consisted of the formation of aggregates of hexagonally arranged tubules (500 to 1130 Å in diameter) (Figure 16). The tubules in the aggregates were continuous with tubules of sarcoplasmic reticulum and were often closely associated with accumulations of Z band material. Filaments which measured 65 Å in diameter were present in the intertubular spaces of these aggregates. These aggregates of tubules have been described in greater detail in a separate communication.[31]

In other severely degenerated cells, the myofibrils were extremely sparse and the cytoplasm was filled with large numbers of mitochondria of various sizes (Figure 30). In many of these cells the mitochondria were intact and undamaged. In other cells there were prominent accumulations of myelin figures (0.4 to 2.3 µ in diameter) associated with numerous lysosomes, glycogen particles, and fragmented membranes of damaged mitochondria (Figures 31 and 32). In the latter cells a consistent relation was found between the number of myelin figures and the

extent of mitochondrial damage, suggesting that damaged mitochondria were transformed into myelin figures. The myofibrils which remained in these cells often had a compact and homogeneous appearance with increased electron density; the Z bands and myofilaments were indistinct (Figure 31).

Other degenerated, but nonatrophic cells (transverse diameters 12 to 20 μ) were characterized by huge, confluent masses of monoparticulate glycogen (up to 35 μ in length and 9 μ in width) which occupied large, central areas of cytoplasm (Figure 33). These cells contained a few myofibrils, which did not show evidence of lysis and were located mostly at the cell periphery. Instead of glycogen, some cardiac muscle cells had large accumulations of fibrils of basophilic degeneration material[32]; other cells contained a particulate material which appeared to be intermediate in structure between normal glycogen and basophilic degeneration material. This particulate material may represent an early stage in the development of basophilic degeneration.[32]

Discrete T tubules were not observed in most severely degenerated cardiac muscle cells. Large, shallow, plasma membrane invaginations were present in some cells. Because of the plane of sectioning, some of these invaginations appeared as vacuoles (up to 2.5 μ in diameter) (Figure 30) disconnected from the cell surfaces. Plasma membrane invaginations of this type possessed basement membranes but were irregularly distributed and did not have the relation to myofibrils that normal T tubules have. These invaginations probably represent the end-stage of dilatation and disorganization of T tubules.

The nuclei in all types of severely degenerated cells were elongated and often showed marked convolutions of their membranes (Figure 30). Variable numbers of lipofuscin granules were present in central areas of the cytoplasm (Figure 30). In general, the amount of lipofuscin in degenerated cells was comparable to that in hypertrophied, nondegenerated cells. Capillaries appeared to be decreased in number in areas of fibrosis; however, normal-appearing capillaries were occasionally observed adjacent to degenerated cardiac muscle cells.

## Discussion

The observations described in this communication demonstrate that degenerated cardiac muscle cells are a common finding in severely symptomatic patients undergoing operative treatment for either aortic valvular disease or ASH. The morphologic features of these degenerated cardiac muscle cells demonstrated great variation. Mildly degenerated cells were normal in size or hypertrophied and showed: preferential loss

of thick filaments, alterations in the structure of Z bands, and focal proliferation of sarcoplasmic reticulum. The most typical findings in more severely altered cells were: a) decreased cellular size (many cells were less than 10 μ in transverse diameter), b) loss of contact between adjacent cells, c) decreased numbers of myofibrils (and continued preferential loss of thick filaments), d) abnormal masses of Z band material to which isolated thin filaments (not associated with thick filaments) were attached, e) decrease in numbers or absence of T tubules, f) disarray of cytoskeletal (100 Å in diameter) filaments, g) thickened basement membranes, and h) large accumulations of either sarcoplasmic reticulum, glycogen, mitochondria or myelin figures.

Degenerated cardiac muscle cells with the features summarized above are in some respects morphologically similar to cells of the normal atrioventricular conducting system, and to hypertrophied, nondegenerated cardiac muscle cells. An analysis of the similarities between these types of cells, with emphasis on the characteristics by which they can be distinguished from each other, is presented in the discussion that follows. Comparisons are then made of the features of degenerated cardiac muscle cells described herein and those reported in other studies. Further comparisons are made with various features of degenerated skeletal muscle cells because of certain similarities in the degenerative processes in skeletal and cardiac muscle, because of the abundance of ultrastructural data on skeletal muscle cells, and because some of these data were found to be helpful in establishing criteria for distinguishing between changes of hypertrophy and of degeneration in cardiac muscle.

### Distinctions Between Degenerated Cardiac Muscle Cells and Cells of the Normal Atrioventricular Conducting System

Specialized muscle cells in the normal atrioventricular conducting system of man [33-36] and of many animals [37-44] resemble the degenerated cardiac muscle cells described in this study in the following respects: a) paucity of myofibrils, b) decreased numbers or absence of T tubules, c) large areas of myofibril-free sarcoplasm which in some instances are filled with abundant tubules of sarcoplasmic reticulum, d) presence of large amounts of glycogen, and e) thickening of Z bands or proliferation of Z band material. Features of degenerated cardiac muscle cells that distinguish them from cells of the normal atrioventricular conducting system are: absence of the extensive intercellular connections characteristic of conducting cells, frequent loss of all connections with adjacent cells, location in areas of extensive fibrosis, and evidence of myofibrillar lysis.

### Distinctions Between Changes of Hypertrophy and Degeneration in Cardiac Muscle Cells

The static nature of ultrastructural images makes it difficult to distinguish between morphologic alterations representative of pure hypertrophy and those of degeneration. Furthermore, hypertrophy and degeneration may occur simultaneously in the same cell, which therefore may show ultrastructural changes reflecting a combination of synthetic and degenerative processes. Distinguishing ultrastructural images of degeneration from those of hypertrophy was most difficult in cardiac muscle cells undergoing early stages of either of these processes. The ultrastructural alterations most useful in making these distinctions were localized proliferation of sarcoplasmic reticulum and fragmentation of Z bands and streaming or clumping of Z band material, which we consider to be typical of cells undergoing early degeneration. We interpret these Z band alterations as indicating early myofibrillar lysis, because of their association with disruption and loss of myofilaments. Other alterations in Z bands, such as thickening or symmetric extensions of Z band material into adjacent areas of sarcomeres and accumulations of Z band material adjacent to the sarcolemma, occurred both in degenerated cells and in hypertrophied, nondegenerated cells. Because of their presence in hypertrophied hearts, these Z band changes (thickening and symmetric extensions of Z bands and subsarcolemmal accumulations of Z band material) have been thought to be indicative of sarcomerogenesis.[9,18] We believe, however, that these changes are not useful in distinguishing between hypertrophy and degeneration. This conclusion is supported by the fact that some investigators have described these types of Z band alterations in: nonhypertrophied cardiac muscle cells from normal animals,[45,46] in skeletal muscle cells from normal rats,[47,48] normal humans [49,50] and patients with psychiatric illnesses,[50] and in normal Purkinje fibers.[39,41,43,44] Other workers have found a relation between the occurrence of these Z band changes and the degree of atrial hypertrophy in patients with rheumatic heart disease,[16] or the presence of congestive heart failure in dogs with experimentally produced right ventricular hypertrophy.[9] A vast amount of evidence from ultrastructural studies of skeletal muscle supports the concept that streaming or clumping of Z bands are degenerative changes.[47,51-60] These alterations have been observed in atrophic or normal-sized skeletal muscle cells from a variety of conditions in which degeneration occurred (see Engel [47] and Santa [51] for review), including denervation,[52-57] tenotomy [58,59] or disuse.[60]

Although the precise significance of the spectrum of Z band changes in hypertrophied cardiac muscle described in this report requires further

investigation, we suggest that: a) marked accumulations of Z band material in undamaged cardiac muscle cells reflects an imbalance between the synthesis of Z band material and that of myofilaments, and in some instances may represent an abortive form of sarcomerogenesis; b) other Z band changes, including streaming and clumping, result from rearrangement of Z band material in myofibrils undergoing lysis; and c) although actual splitting of Z bands is the most convincing morphologic evidence of the formation of new sarcomeres, it is uncertain that this is a normal mechanism of sarcomerogenesis.

### Distinctions Between Degenerated Cardiac Muscle Cells in Different Pathologic Conditions

The spectrum of degenerative changes observed in this study has not been described previously in left ventricular myocardium from patients with long-standing cardiac hypertrophy. Other investigators, however, have described some degenerative alterations in atrial or ventricular muscle cells in a variety of conditions. Myofibrillar or myofilament loss is the pathologic feature common to the altered cardiac muscle cells in the following conditions: cardiomyopathies of various types,[19,20,22,23,61–63] experimentally induced pressure overloading [4,5,8] or volume overloading,[6,7] experimentally produced myocardial infarcts (at the margins of the infarcts),[64] multiple episodes of acute hypoxia,[65] hypokalemia,[66] plasmocid toxicity,[67,68] and rheumatic heart disease.[13–15,17] In certain instances, this loss of myofibrils has been observed in some cells that were isolated from adjacent cells and surrounded by fibrous tissue.[6,8,64,66] Proliferation of the sarcoplasmic reticulum [14,15] and degeneration of mitochondria associated with the formation of concentric lamellae (myelin figures)[14] have been observed in association with myofibrillar loss in atrial muscle cells from patients with rheumatic heart disease. Other studies from this laboratory have described certain cardiac degenerative changes, similar to those reported in this communication, in crista supraventricularis muscle from patients with congential heart diseases associated with obstruction to right ventricular outflow [69] and in ventricular myocardium from patients with neoplasms undergoing therapy with anthracyclines.[70,71] Ultrastructural features of degeneration also have been described in skeletal muscle from a variety of human and experimental conditions, including muscular dystrophy,[72,73] periodic paralysis,[74–76] polymyositis,[77–78] nemaline myopathy,[79,80] and systemic lupus erythematosus,[81] and following denervation,[52–57] tenotomy,[58,59] or disuse.[60]

The following three generalizations can be drawn from the present

and from previously reported studies of degeneration in cardiac and skeletal muscle cells: a) loss of myofibrils is the morphologic alteration common to the degeneration observed in all of these studies, b) loss of myofibrils proceeds along diverse morphologic pathways in different conditions, and c) the overall appearance of degenerated cells depends on the alterations which other cytoplasmic organelles undergo in association with myofibrillar loss. Evidence supporting these concepts is reviewed in detail below.

### Myofibrillar Changes

Myofibrillar lysis can occur in at least three different ways depending on the sequence in which the two different classes of myofilaments are lost. In many studies no discrepancies were noted in the relative amounts of thick and thin filaments,[4,6–8,13–15,61–63] suggesting that thick and thin filaments disappeared at the same rate. In other conditions, the relative amounts of thick and thin filaments present in cardiac [17,64–71] or skeletal [57,59,74,80,82–85] muscle cells (Tables 2 and 3, respectively) were found to differ, suggesting that they were lost at different rates. The findings in several studies show that preferential loss of thin filaments can occur under certain circumstances in cardiac [65–68] and skeletal muscle.[82,83] In contrast, other studies showed that disproportionately large numbers of thin filaments were present in various abnormal cardiac [17,64,69–71] and skeletal [57,59,74,80,84,85] muscle cells. These observations suggest the occurrence of a preferential loss of thick filaments similar to that described in this report. Under certain conditions, myofilament loss also may be part of a normal developmental process. For example, preferential loss of thick filaments, with dissolution of myofibrils, occurs in the abdominal muscles of *Pieris brassicae* as they undergo involution during the last larval period and pupation.[86] In addition, several other reports, summarized in Tables 2 and 3, have described isolated accumulations of thin filaments in cardiac [5,10,11,87–91] and skeletal [75,92–94] muscle cells. The relation of these isolated masses of thin filaments to myofibrillar lysis is unclear. Some investigators believe that the masses of thin filaments result from a degenerative process with selective lysis of thick filaments,[92,93] while others maintain that they develop from the synthesis,[5,10,11] regeneration,[87,91] or redistribution [88,89] of thin filaments. Ferrans and Buja also have found masses of isolated thin filaments in otherwise normal cardiac muscle cells of normal dogs[90]; these masses may represent instances of aberrant synthesis of thin filaments. In contrast, we consider that the accumulations of thin filaments unassociated with thick filaments in the cardiac muscle cells described in this report probably are

indicative of degeneration, since these alterations were invariably present in cells that also showed other lytic or degenerative changes.

Myofibrillar loss in degenerated cadiac muscle cells may result from any of the following three types of alterations in contractile proteins: a) inhibition of synthesis, b) acceleration of breakdown, and c) disaggregation. The relative importance of each of these mechanisms in the production of myofibrillar lysis is uncertain. Furthermore, it is unclear to what extent the disappearance of myosin and actin filaments represents actual physical loss of protein from muscle cells or simply a phenomenon of disaggregation, so that myosin and actin are still present within the cell but are not in the form of filaments. It is known that the aggregation of both myosin and actin into filaments is profoundly influenced by physical and chemical factors [95] and that myosin is not morphologically demonstrable in the form of filaments in cell types other than muscle.[96–99] It is not known, however, how the biochemical environment in degenerated cardiac muscle cells becomes altered to produce preferential lysis of one class of muscle proteins, or whether this apparent loss of filaments is a reversible process. Of particular interest in this regard is the study of Fay and Cooke [100] in which reversible disaggregation of thick and thin filaments was achieved in smooth muscle cells by incubation *in vitro* with solutions (mammalian Krebs-Ringer bicarbonate saline) in which concentrations of calcium ions were reduced below $10^{-6}$ M by the addition of chelating agents.

### Alterations in Other Organelles

The preceding discussion emphasizes that lysis of myofibrils is a complex process involving a variety of different steps. In the degenerated cardiac muscle cells described herein, the first morphologically recognizable event in this process was preferential loss of thick filaments; associated morphologic alterations in these cardiac muscle cells were varied, depending on which cytoplasmic component proliferated to fill the myofibril-free areas.

Proliferation of tubules of sarcoplasmic reticulum was a common finding in degenerated cardiac muscle cells. The amount of tubules in these cells often was far greater than that in normal cardiac muscle cells. This finding suggests that the increase in sarcoplasmic reticulum represents a phenomenon of abnormal growth and not simply a rearrangement of preexisting tubules in cells in which the myofibrils have undergone lysis. It is not known, however, how this increased volume fraction of sarcoplasmic reticulum alters certain functions, such as release and binding of calcium, in degenerated cardiac muscle cells. Although this phe-

Table 2—Occurrence of Discrepancies in Relative Amounts of Thick or Thin Filaments in Cardiac Muscle

| Author (ref) | Species | Condition | Location of tissue | Cell atrophy | Myofibrillar loss | Myofilament changes | Mitochondria | SR | T | Other findings |
|---|---|---|---|---|---|---|---|---|---|---|
| Hatt and Swynghedauw[5] | Rabbit | Constriction of abdominal aorta; CHF | LV | 0 | + | Isolated "wadded" masses of A* | ↑ | — | — | — |
| Sybers et al[87] | Dog | Glucose-insulin-potassium administration after acute coronary occlusion | LV | 0 | 0 | Isolated bundles of A‡ (located near intercalated discs) | — | — | — | |
| Onishi et al[10,11] | Dog | Constriction of ascending aorta | LV & RV | 0 | 0 | Isolated masses of A* | — | — | — | |
| Csapó et al[88] | Rat | Administration of isoproterenol | LV | 0 | + | Isolated masses of A‡ (located near intercalated discs) | NL | Dilated | — | 330 Å periodicity observed in some thin filaments |
| Ferrans and Buja[90] | Dog | NL | RV | 0 | 0 | Isolated masses of A‡ | NL | NL | NL | |
| Dušek et al[91] | Rat | Administration of deoxycorticosterone and isoproterenol | LA & RA | — | + | Areas with only A‡ | NL | — | — | |
| Hasper[65] | Mouse | Hypoxia (multiple acute episodes) | LV | + | + | A < M | ↓ | Dilated | — | |
| Maurat et al[66] | Rat | Hypokalemia | LV | + | + | A < M | Variable size | ↓ | — | Dissociation of some cells |
| Berger and Bencosme[68] | Rat | Administration of plasmocid | RA | 0 | 0 | A < M | Swollen | — | — | |
| D'Agostino[67] | Rat | Administration of plasmocid | LV | 0 | + | A < M | Swollen and damaged; dense inclusions | — | — | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Roy and Morin | Man | Mitral stenosis | LA & RA | 0 | 0 | M < A | — | — | Marked widening of Z bands |
| Buja et al[70]; Buja and Ferrans[71] | Man | Neoplasms treated with anthracycline drugs | LV | + | + | M < A | Variable numbers and size | NL | Altered nuclear chromatin§ |
| Dušek et al[64] | Rat | Myocardial infarcts (at the margins) | LV | + | + | M < A | Swollen | ↑ | Dilated Dissociation of some cells |
| Jones et al[69] | Man | Congenital heart diseases associated with right ventricular outflow tract obstruction | RV (CSV) | 0 | + | M < A | ↓ | Variable amounts | ↓ |
| Maron, Ferrans, Roberts (present study) | Man | Aortic valvular disease or ASH | LV | + | + | M < A | Variable numbers and size | ↑, Dilated | Interstitial fibrosis; dissociation of some cells |

A = actin filaments, A < M = actin (thin) filaments present in disproportionately smaller numbers than myosin (thick) filaments, ASH = asymmetric septal hypertrophy, CHF = congestive heart failure, CSV = crista supraventricularis, LA = left atrium, LV = left ventricle, M = myosin filaments, M < A = myosin (thick) filaments present in disproportionately smaller numbers than actin (thin) filaments, NL = normal, RA = right atrium, Ref = reference, RV = right ventricle, SR = sarcoplasmic reticulum, T = T tubules, + = present, 0 = absent, — = no data available, ↑ = increase in number (mitochondria) or amount (sarcoplasmic reticulum), ↓ = decrease in number (mitochondria or T tubules) or amount (sarcoplasmic reticulum).

* Interpreted by the authors as representing synthesis of new thin filaments.
† Interpreted by the authors as representing regeneration of thin filaments.
‡ Interpreted by the authors as representing redistribution of preexisting thin filaments.
§ See reference for further details.

Table 3—Occurrence of Discrepancies in Relative Amounts of Thick and Thin Filaments in Skeletal Muscle

| Author (ref) | Species | Condition | Muscle | Fiber atrophy | Myofibrillar loss | Myofilament changes | Mitochondria | SR | T | Other findings |
|---|---|---|---|---|---|---|---|---|---|---|
| Nakashima et al[92] | Man | Neuromuscular disorder* | Deltoid; vastus lateralis; peroneal | — | 0 | Isolated masses of A† | NL | Dilated | — | |
| Macdonald et al[75] | Man | Hyperkalemic periodic paralysis | Deltoid | + | + | Isolated masses of A | ↓ | Dilated | NL | Aggregates of tubules |
| Odor et al[93] | Man | Hypokalemic periodic paralysis | Vastus lateralis | — | + | Isolated masses of A† | ↓ | ↑ | — | Aggregates of tubules |
| Bergman et al[94] | Man | Myasthenia gravis; Eaton-Lambert syndrome | Gastrocnemius; quadriceps | 0 | + | Isolated masses of A | — | ↓ | ↓ | Aggregates of tubules |
| Lentz[82] | Newt | Muscle dedifferentiation during limb regeneration | Upper forelimb | 0 | + | A < M | Small size; crystalloid inclusions | — | — | |
| Price et al[83] | Rat | Administration of plasmocid | Diaphragm | 0 | 0 | A < M | NL | NL | NL | Loss of Z bands |
| Howes et al[74] | Man | Hypokalemic periodic paralysis | Anterior thigh | — | + | M < A | Irregular shapes; few cristae | — | — | Myelin figures; prominent vacuoles |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Engel[80] | Man | Nemaline myopathy | Deltoid; gastrocnemius | + | + | M < A | — | — | — | Nemaline rods; aggregates of tubules |
| Shafiq et al[57] | Man | Neurogenic muscular atrophy* | Gastrocnemius | + | + | M < A | → | → | → | |
| Zintz and Villiger[84] | Man | Progressive ocular muscular dystrophy | Sternocleidomastoid | 0 | + | M < A | Concentrically arranged cristae; paracrystalline inclusions | Dilated | — | |
| Auber-Thomay and Sriharl[86] | Pieris brassicae | Last larval period and pupation | Abdominal | 0 | + | M < A | → | → | Greatly dilated | |
| Shafiq et al[59] | Rat | Tenotomy | Soleus | + | + | M < A | → | — | — | Central core lesions; nemaline rods |
| Zacks et al[85] | Man | Interstitial hypertrophic neuritis | Anterior tibial | + | + | M < A | Swollen | Dilated | Mildly dilated | Hyaline bodies* |

A = actin filaments, A < M = actin (thin) filaments present in disproportionately smaller numbers than myosin (thick) filaments, M = myosin filaments, M < A = myosin (thick) filaments present in disproportionately smaller numbers than actin (thin) filaments, NL = normal, ref = reference, SR = sarcoplasmic reticulum, T = T tubules, + = present, 0 = absent, — = data not available, ↓ = decrease in number (mitochondria or T-tubules) or amount (sarcoplasmic reticulum), ↑ = increase in amount (sarcoplasmic reticulum).

\* See reference for further details.

† Interpreted by authors as representing an accumulation of thin filaments resulting from the breakdown of myofibrils with selective lysis of thick filaments.

nomenon has not been reported in cardiac muscle, the possibility that proliferation of sarcoplasmic reticulum in cardiac muscle cells in our patients represents a drug effect cannot be excluded. It is known that smooth endoplasmic reticulum undergoes marked proliferation in hepatocytes as a response to the administration of drugs that are metabolized in the liver.[101-105] However, proliferation of smooth reticulum appears to be a more diffuse process in hepatocytes than in degenerated cardiac muscle cells. The presence of large masses of glycogen in cardiac muscle cells suggests that an imbalance between glycogen synthesis and utilization has occurred and that increased amounts of glycogen are being stored. These accumulations of glycogen may be related to the development of the glycogen-related type of basophilic degeneration.[32]

Degenerated cardiac muscle cells also showed great variability in the number and size of mitochondria. Some severely degenerated cells that had few or no myofibrils were virtually filled with intact mitochondria. In some of these cells, the large numbers of mitochondria seemed to represent an actual proliferation. Mitochondria in many cells appeared to occupy an increased volume fraction, due to the loss of myofibrils and other cytoplasmic components. Unusually small mitochondria, similar to those previously described in hypertrophied, failing myocardium [21,106] were numerous in many degenerated cells. Some severely degenerated cells also showed a distinctive alteration characterized by the presence of concentric lamellae (myelin figures) which probably were derived from mitochondrial membranes. These cells with damaged mitochondria and myelin figures often contained hyalinized myofibrils and closely resembled the degenerated cardiac muscle cells described in patients with neoplasms undergoing therapy with anthracyclines.[70,71] The significance of the various mitochondrial alterations which occur in hypertrophied myocardium is not clearly understood at the present time.

The loss of myofibrils that occurs in degeneration of cardiac muscle is associated with survival of other organelles, including the nuclei and mitochondria. These features distinguish this type of degeneration from a true phenomenon of necrosis, either of the coagulation type or of the myofibrillar injury type (associated with contraction bands). The alterations in the degenerated cardiac muscle cells observed in this study probably represent part of the spectrum of changes described as myocytolysis by light microscopists.[107,108] Both these degenerated cardiac muscle cells and other cells described as showing myocytolysis are characterized by loss of myofibrils. Degenerated cardiac muscle cells in patients with aortic valvular disease or ASH undergo preferential loss of thick filaments and myofibrillar lysis unassociated with contraction

bands. These alterations, however, differ from those described in myo-cytolysis in patients dying after cardiac surgery. The latter changes do not involve selective lysis of thick filaments and often are preceded by the formation of contraction bands.[108] Some of the features of degeneration that we have observed in muscle cells of patients with ASH have been described in other studies of this disease.[22,23] It should be emphasized, however, that hypertrophied, bizarrely shaped and disorganized cardiac muscle cells (usually without evidence of degeneration) constitute the characteristic morphologic feature of the ventricular septum in all patients with ASH.[24,25]

The ultrastructural features of moderately or severely degenerated cardiac muscle cells suggest that they are manifestations of the end stages of cellular hypertrophy. This concept is supported by the fact that cells with advanced degeneration often have few or no normal myofibrils or T tubules and have marked alterations in the sarcoplasmic reticulum. Furthermore, some degenerated cells have lost their connections with adjacent cells and, therefore, do not have the capacity to directly transmit electrical activation to other muscle cells or to contribute effectively to myocardial contractility. Therefore, it is reasonable to conclude that these cardiac muscle cells are not capable of normal contractile function and correspond to cells in the third stage of hypertrophy as described by Meerson and co-workers.[1-3] The finding of cardiac muscle cells with similar degenerative features in crista supraventricularis muscle from patients with congential heart diseases associated with right ventricular outflow obstruction [69] and in ventricular myocardium from patients with neoplasms undergoing therapy with anthracycline drugs [70,71] suggests that these morphologic alterations represent a final common pathway of cell injury in a variety of cardiac conditions.

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*[Illustrations follow]*

## Legends for Figures

Figures 1–11 and 16–33 are electron micrographs of ultrathin sections. Figures 12–15 are light micrographs of semithin sections stained with alkaline toluidine blue. Figure 1 is from left ventricular apical myocardium of a 12-year-old boy with aortic valvular stenosis. Figure 2 is from left ventricular apical myocardium of a 67-year-old woman with obstructive ASH. Figures 3–11, 17, 19–21, 23, 28 and 29 are from left ventricular apical myocardium of a 43-year-old man with combined aortic stenosis and regurgitation. Figures 12–15, 24, 26, 27 and 31–33 are from ventricular septal myocardium of a 10-year-old boy with ASH and discrete, fibrous type of subaortic stenosis. Figure 16 is from left ventricular posterior wall of a 24-year-old man with nonobstructive ASH. Figure 18 is from left ventricular apical myocardium of a 51-year-old man with pure aortic regurgitation. Figure 22 is from ventricular septal myocardium of a 47-year-old man with obstructive ASH. Figures 25 and 30 are from left ventricular apical myocardium from a 46-year-old man with combined aortic stenosis and regurgitation.
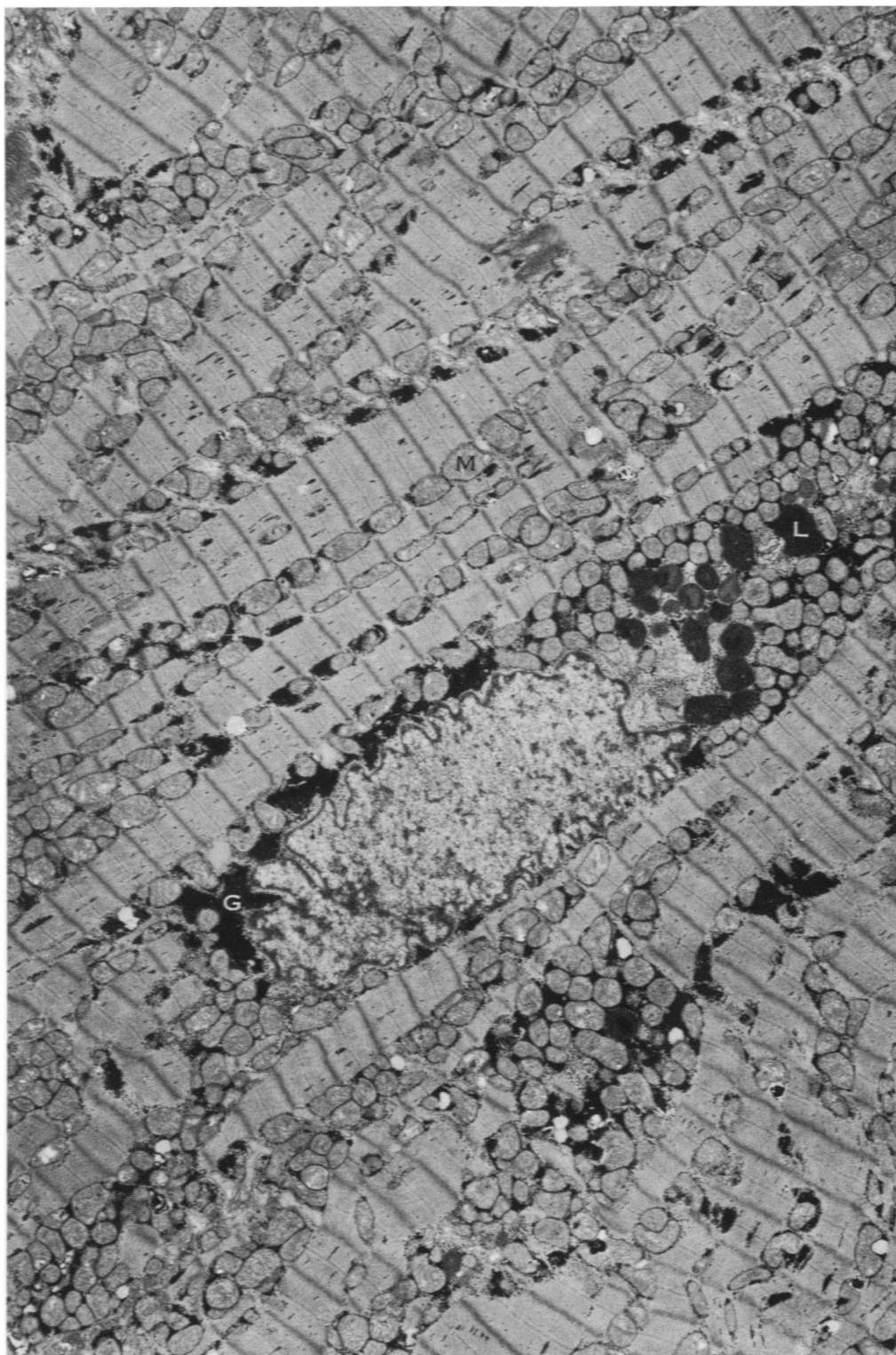
Fig 1—Hypertrophied cardiac muscle cell showing numerous intact myofibrils that are separated by mitochondria. The centrally located nucleus has convoluted membranes and is surrounded by a myofibril-free area that is filled with mitochondria (*M*), glycogen (*G*) particles, and lipofuscin granules (*L*). (× 5300)
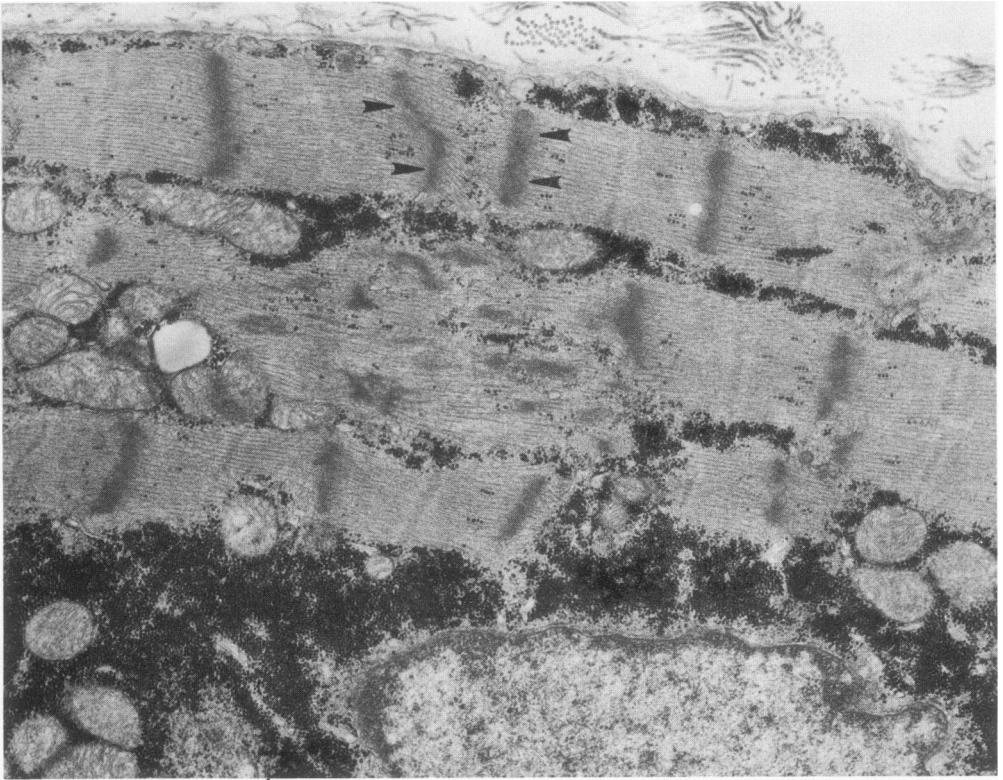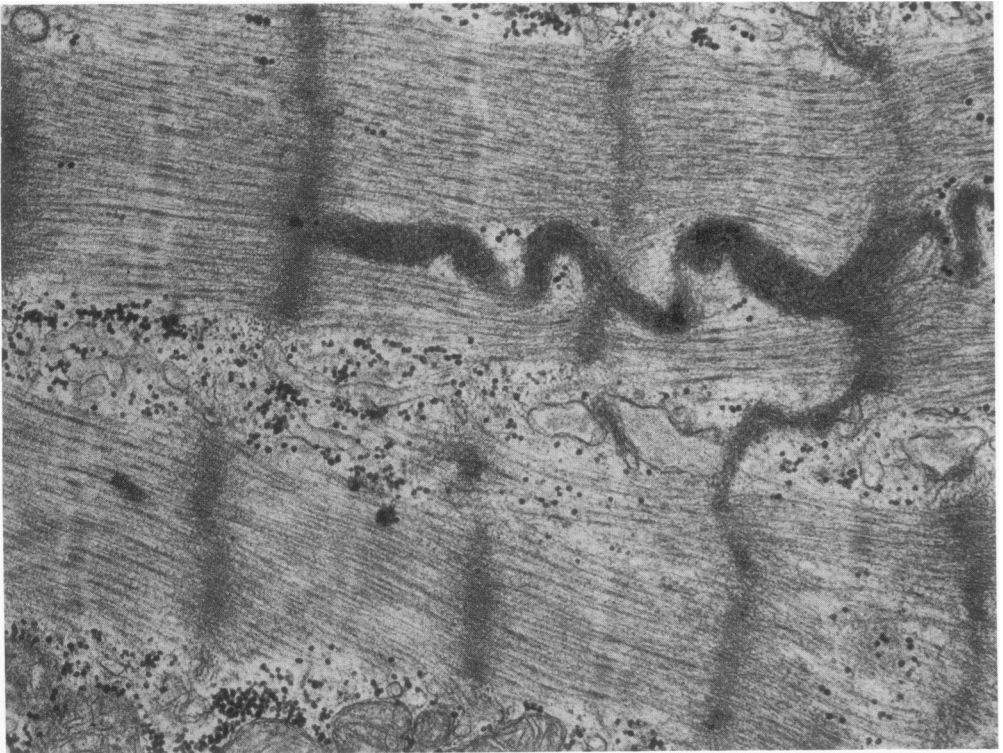
Fig 2—Part of mildly degenerated cardiac muscle cell showing streaming and fragmentation of Z bands in several sarcomeres. Other Z bands show mild thickening. An abnormally short sarcomere (*arrowheads*), which may be developing, is present just under the cell membrane. Images of this type suggest the coexistence of the processes of myofibrillar lysis and synthesis in a given cell. (× 15,000)    Fig 3—Part of a mildly degenerated cardiac muscle cell showing streaming of Z band material into adjacent areas of three sarcomeres. (× 31,700)
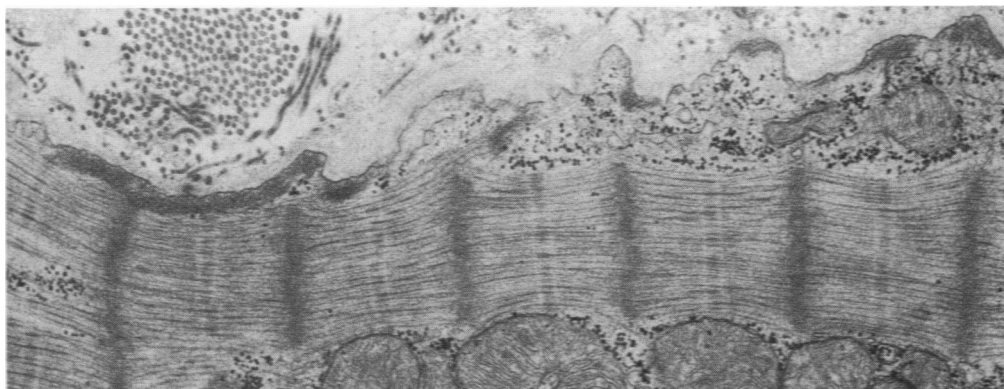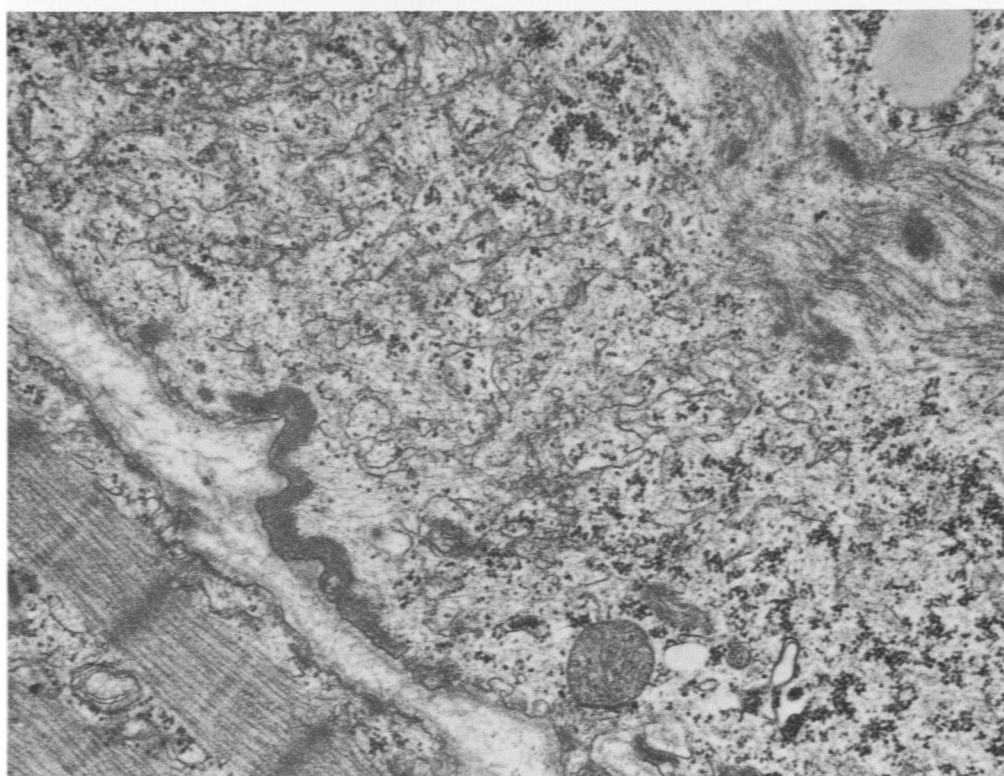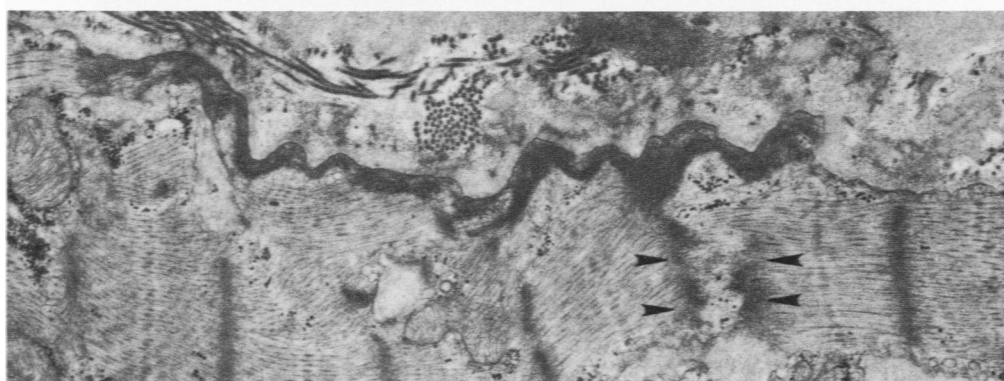
**Figs 4–6**—Three electron micrographs showing subsarcolemmal accumulations of Z-band-like material in mildly degenerated cardiac muscle cells. **4**—Elongated mass of Z-band-like material which is contiguous with an actual Z band. Several smaller accumulations of Z-band-like material are also present, and the basement membrane is markedly thickened. (× 20,900) **5**—Elongated accumulation of Z-band-like material is present in area of myofibrillar lysis and focal proliferation of tubules of sarcoplasmic reticulum. (× 26,000) **6**—An elongated accumulation of Z-band-like material is shown in a cell with a markedly thickened basement membrane. The Z-band-like material is closely associated with an actual Z band that appears to be splitting (*arrowheads*). (× 21,600)
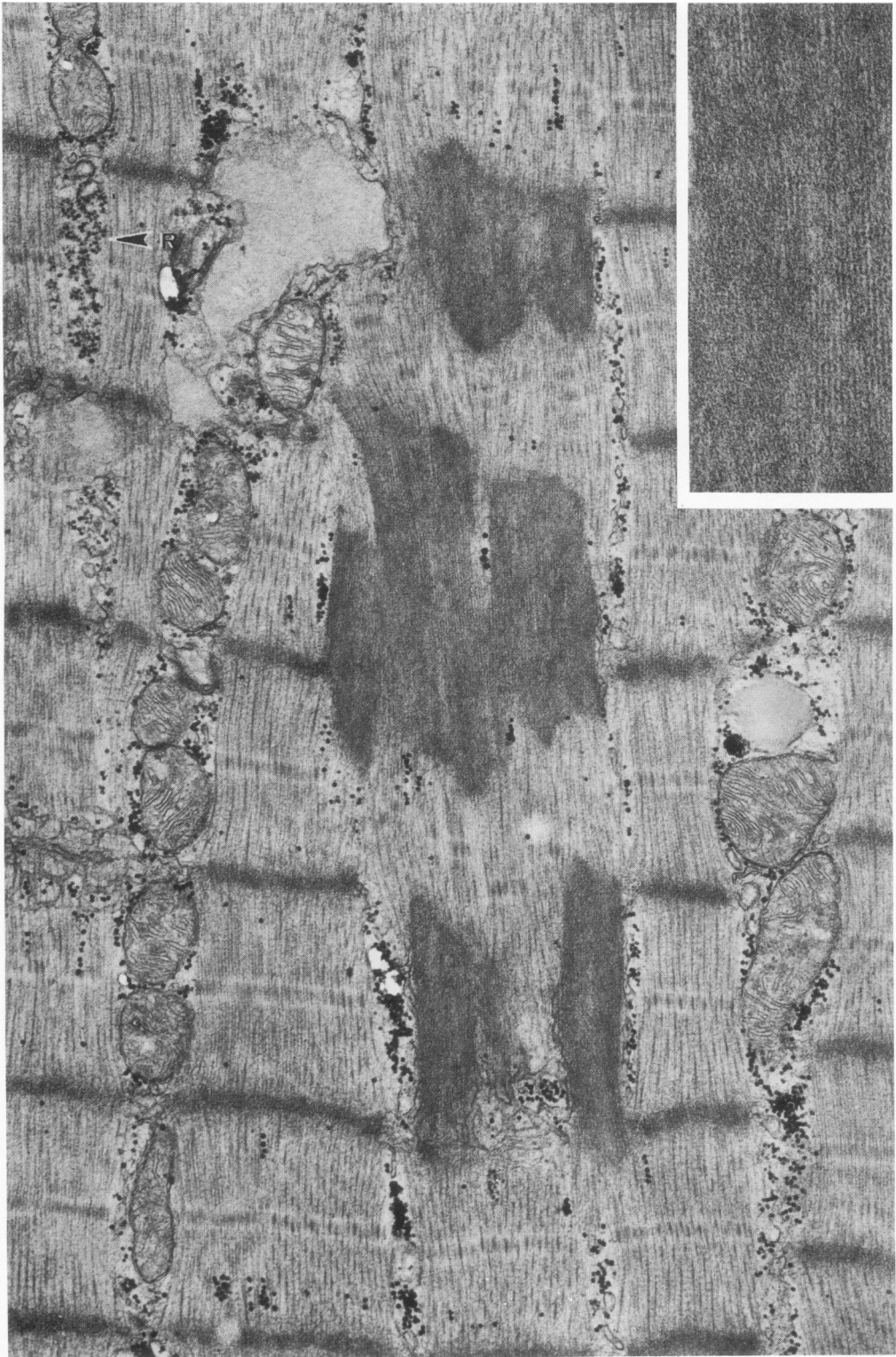
**Fig 7**—Cardiac muscle cell showing four masses of markedly proliferated Z band material. Each of these masses appears to occupy most of a sarcomere. Sarcoplasmic reticulum, T tubules, and ribosomes (R) are also shown. (× 27,300)     **Fig 8**—High magnification view of one of the masses of Z band material shown in Figure 7. Note that Z band material is traversed by thin filaments arranged in parallel. (× 60,900)
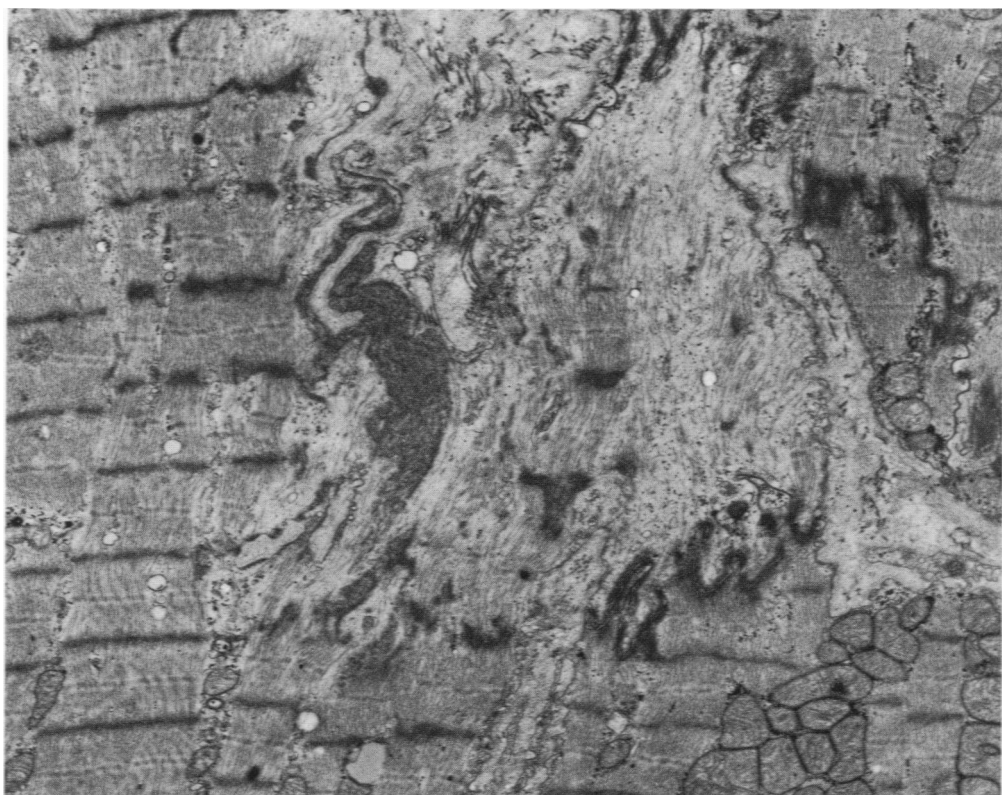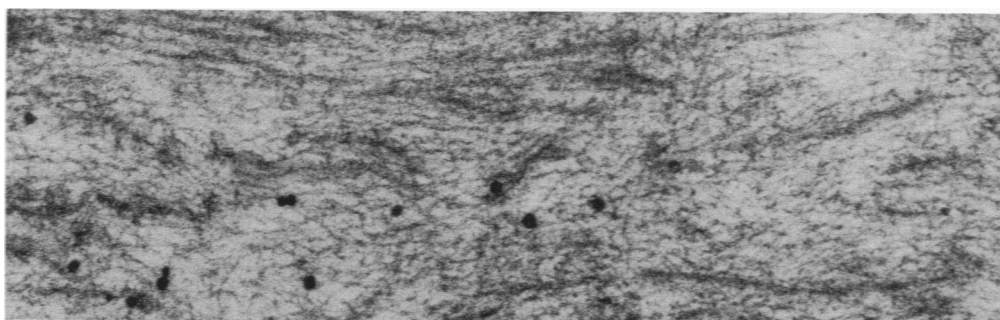
**Fig 9**—Early myofibrillar lysis in a hypertrophied cardiac muscle cell. Thick filaments are decreased in number in some sarcomeres (*arrowheads*). A subsarcolemmal mass of Z-band-like material appears continuous with an actual Z band. (× 27,800)      **Fig 10**—Parts of four cardiac muscle cells are shown. In the cell in the center the myofibrils have undergone lysis and disruption. The loss of thick filaments exceeds that of thin filaments. Several Z bands show marked thickening and a large mass of Z band material is present. (× 10,600)      **Fig 11**—High magnification view of an area of the degenerated cell shown in Figure 10. Disproportionately large numbers of thin filaments are present throughout, suggesting that preferential loss of thick filaments has occurred. (× 30,300)
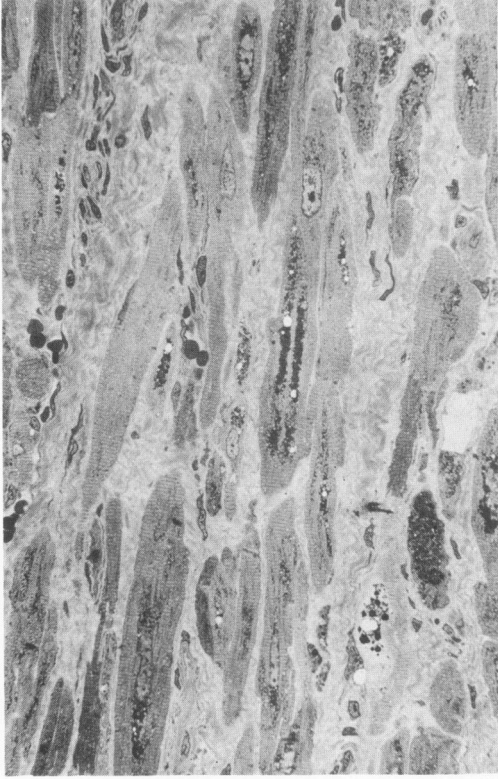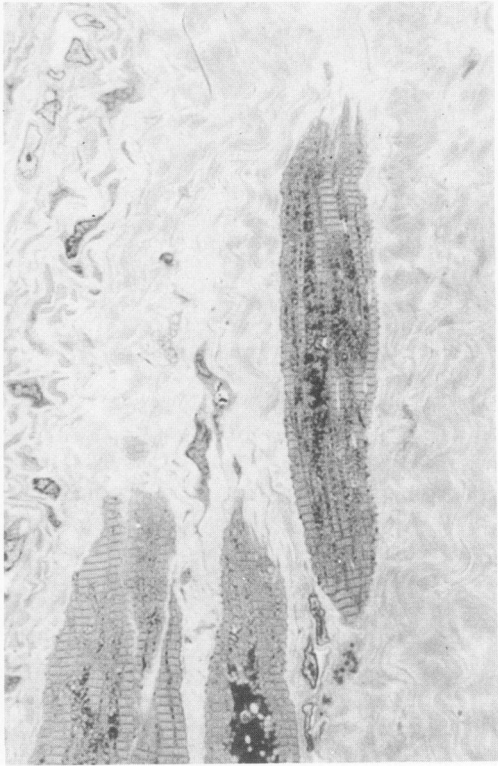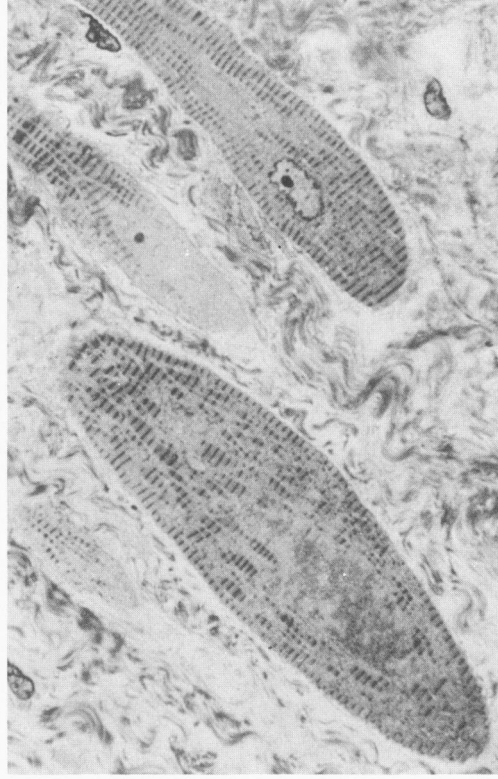
**Figs 12–15**—Light micrographs of semithin (0.5-μ-thick) sections showing spectrum of degenerative changes. **12**—Several normal-sized or small cardiac muscle cells have lost their contacts with adjacent cells and are present in area of marked interstitial fibrosis. (× 350) **13**—High magnification view of several small cardiac muscle cells separated from each other by fibrous tissue. One cell appears to have no myofibrils and is filled with myelin figures which appear as dark granules. Compare with Figure 31, which shows an electron micrograph of a similar cell from the same patient. Other cells in this area also appear to have reduced numbers of myofibrils. (× 750) **14**—Cardiac muscle cell is isolated and surrounded by large amounts of fibrous tissue. One end of this cell has finger-like cytoplasmic projections which taper abruptly into the interstitium. (× 750) **15**—Four isolated cardiac muscle cells in area of fibrosis show evidence of myofibrillar lysis. Each cell has palely staining areas of cytoplasm in which myofibrils appear to be absent. (× 750)
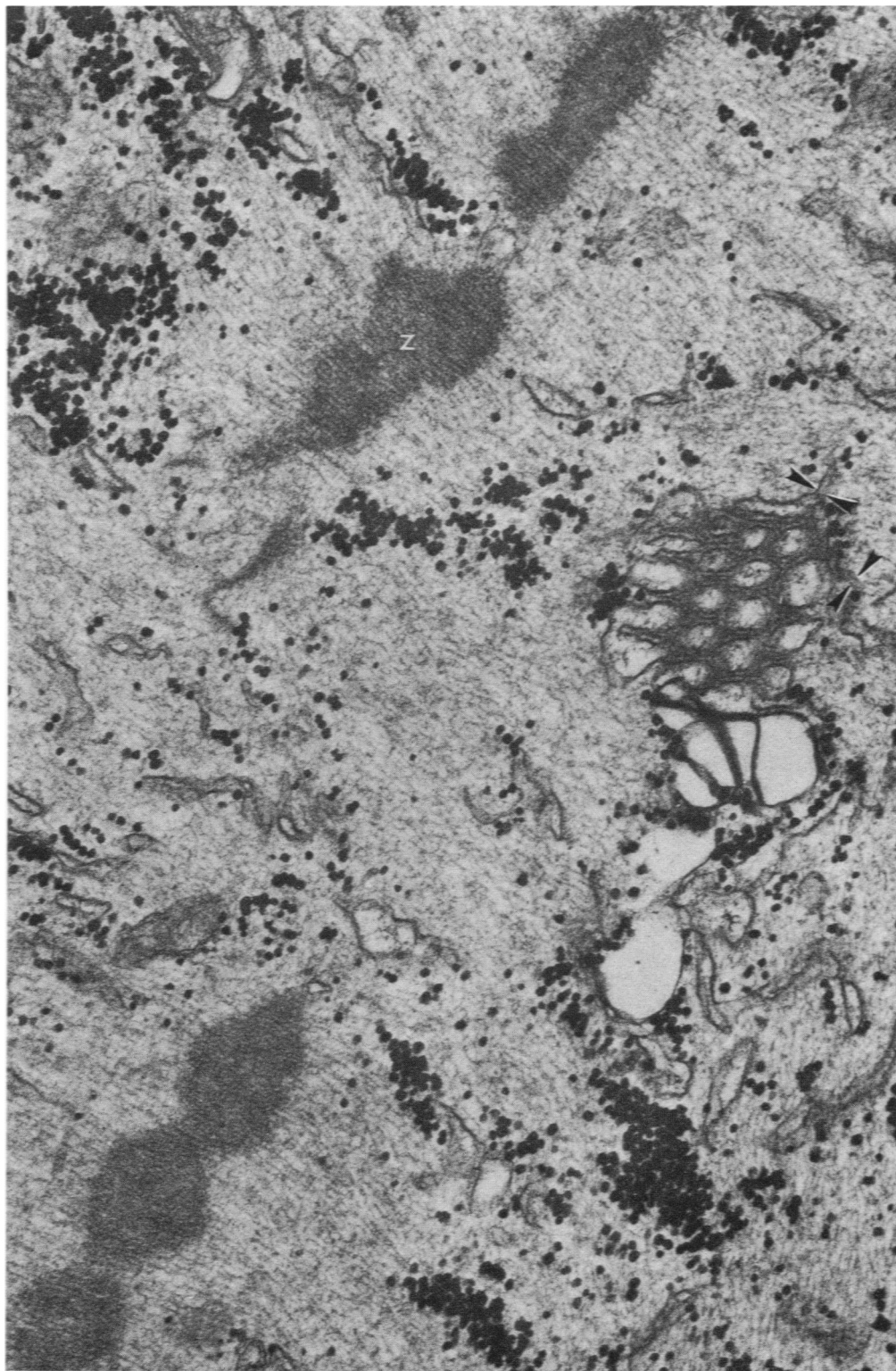
**Fig 16**—Part of a moderately degenerated cardiac muscle cell containing large numbers of thin filaments, some of which are attached to masses of Z band (Z) material. Thick filaments are not present in this area. An aggregate of tubules, in continuity with tubules of sarcoplasmic reticulum (*arrowheads*), is shown at the right. (× 52,500)
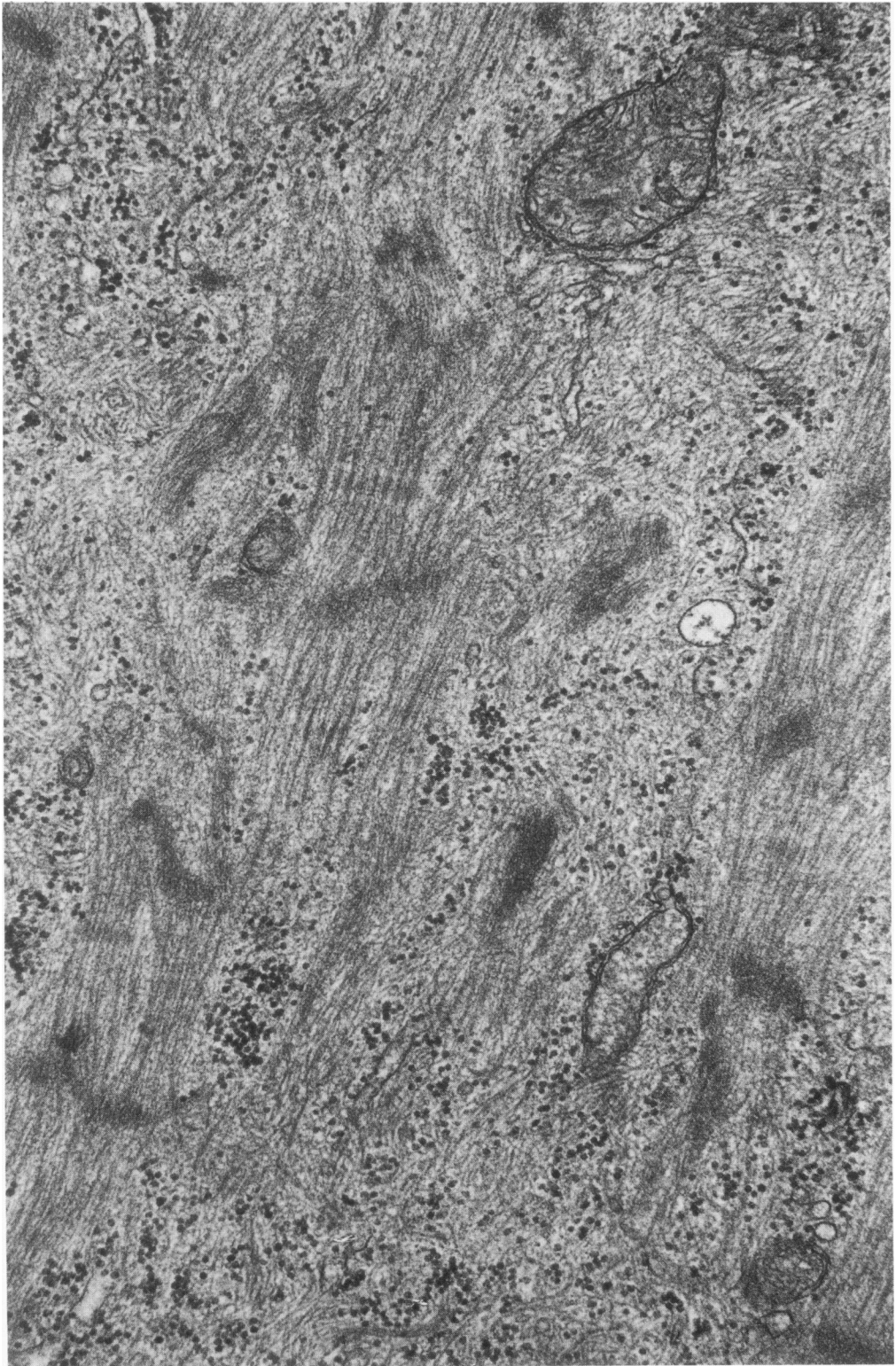
**Fig 17**—Part of a moderately degenerated cardiac muscle cell showing disrupted myofibrils, clumps of Z band material, and large numbers of tangled and disorganized cytoskeletal filaments (100 Å in diameter). (× 38,900)
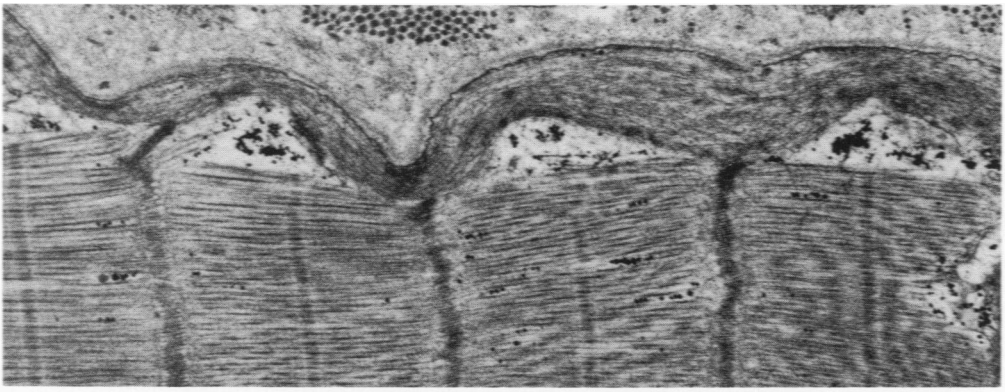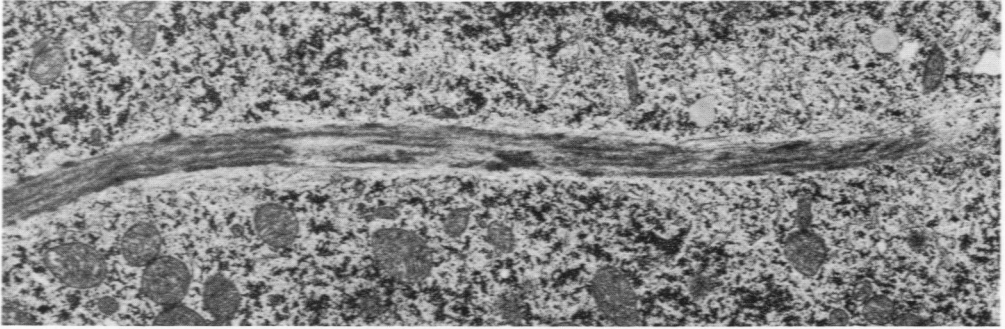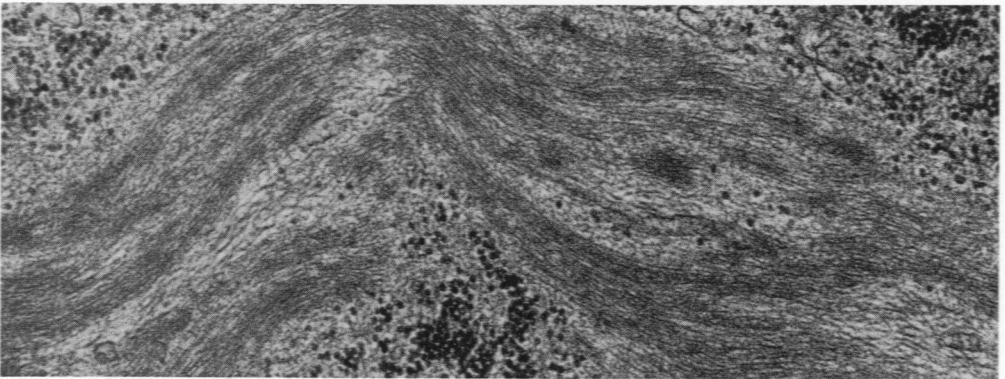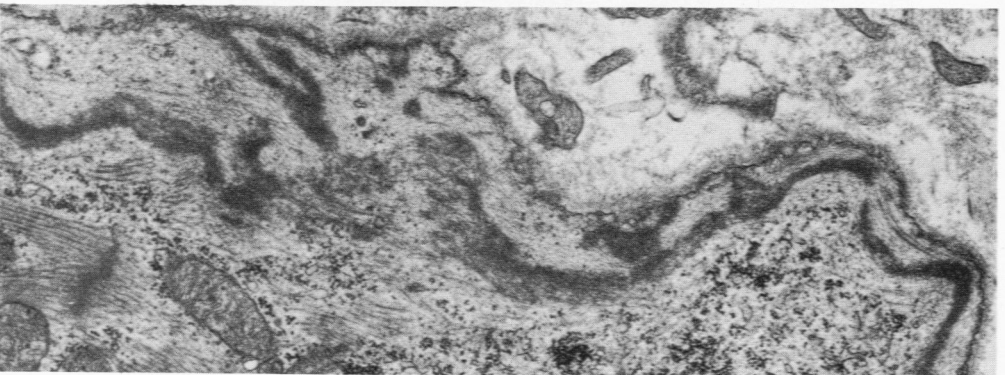
**Figs 18–21**—Four electron micrographs showing elongated accumulations of Z-band-like material associated with thin filaments, but not with thick filaments, in moderately degenerated cardiac muscle cells.        **18**—A subsarcolemmal mass of thin filaments and Z-band-like material is adjacent to a normal-appearing myofibril. (× 20,700)        **19**—A long, streaming accumulation of thin filaments and Z-band-like material tapers into the cytoplasm in an area free of myofibrils. (× 5400)        **20**—High magnification view of area similar to that in Figure 19 shows association of thin filaments with Z-band-like material. (× 40,400)        **21**—An elongated, streaming accumulation of Z-band-like material and thin filaments adjacent to the sarcolemma. (× 17,100)
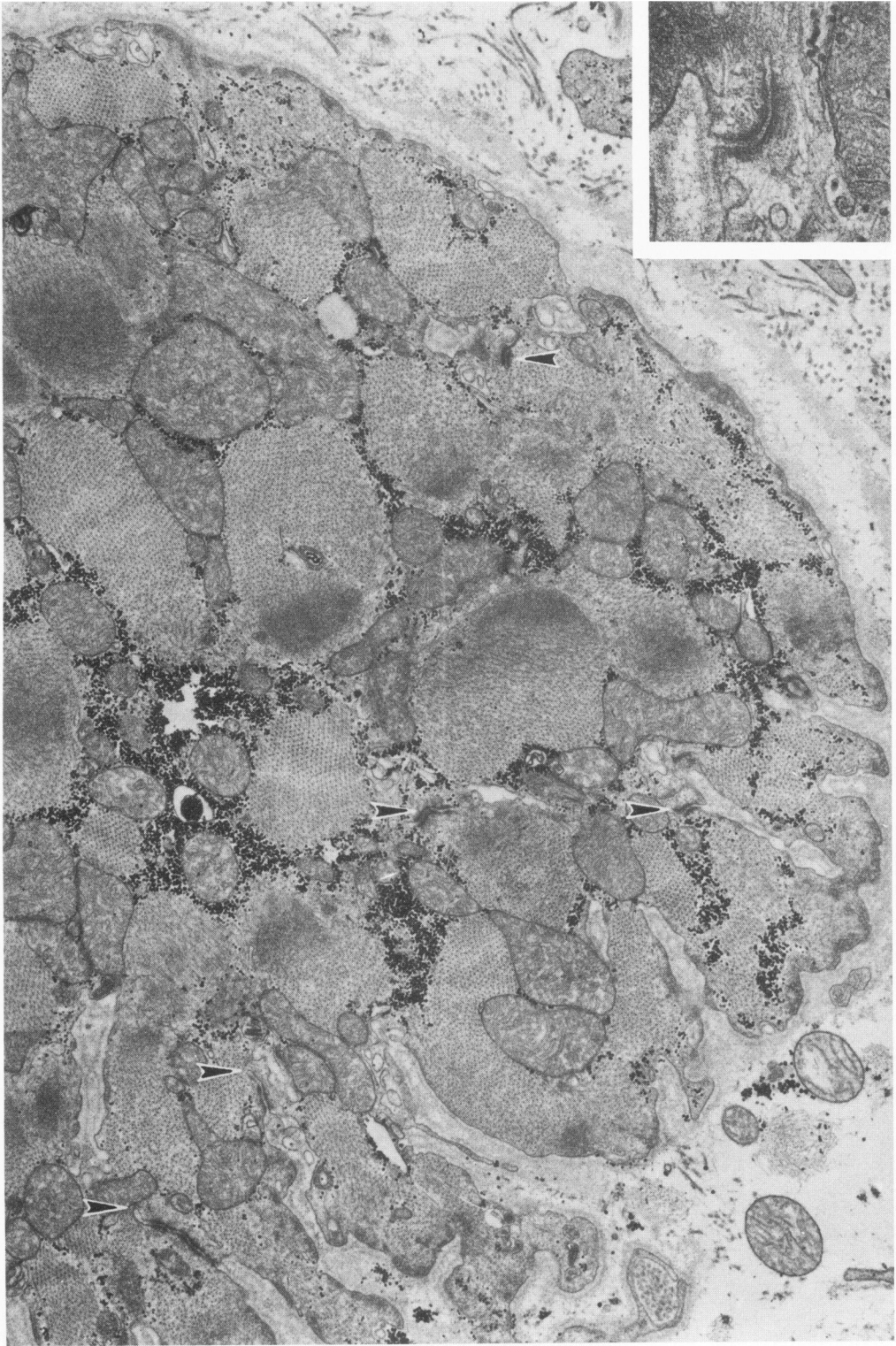
**Fig 22**—Part of cardiac muscle cell which is dissociated from adjacent cells has intact myofibrils and thickened, reduplicated basement membrane. Shallow plasma membrane invaginations are associated with several intracytoplasmic junctions (*arrowheads*). (× 20,600)      **Fig 23**—High magnification view of an intracytoplasmic junction which is in continuity with the plasma membrane. (× 48,600)
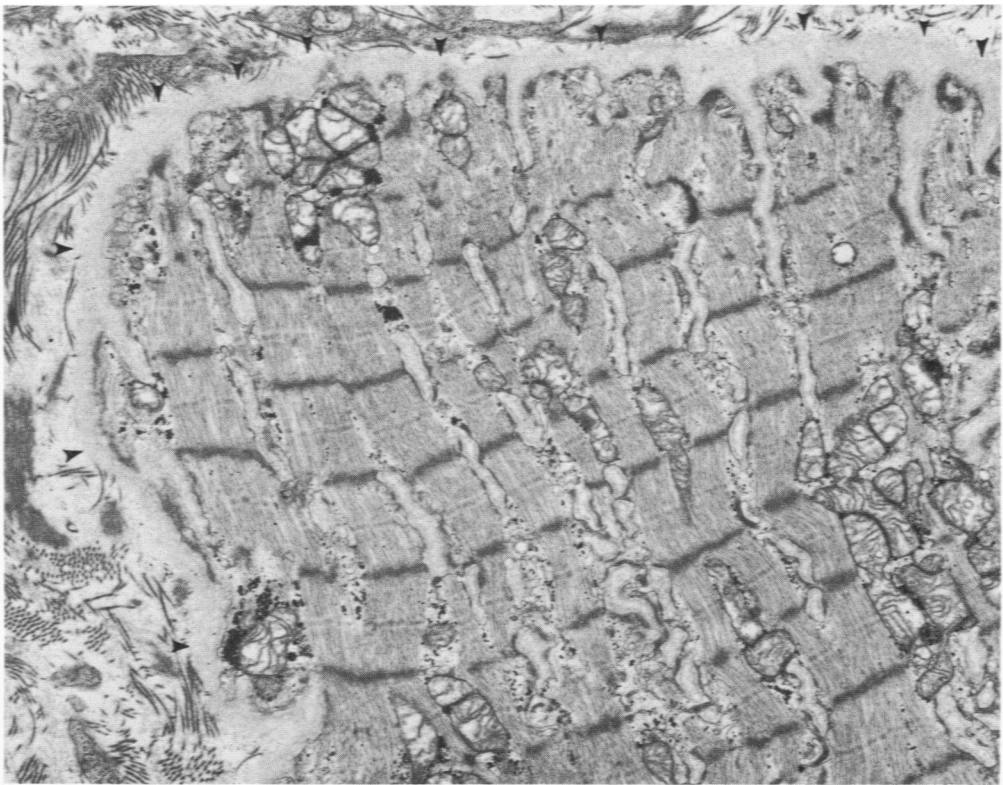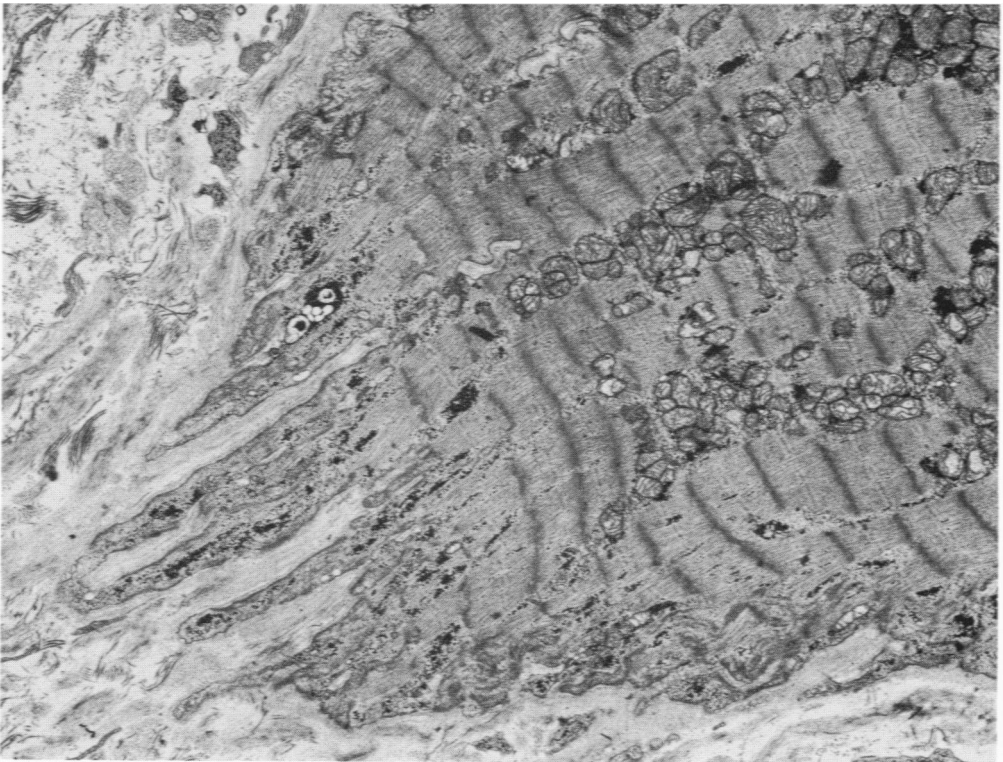
Fig 24—A dissociated cardiac muscle cell without evidence of myofibrillar lysis is sur-
rounded by fibrous tissue, ends abruptly in the interstitium, and has shallow narrow
invaginations of the plasma membrane and a markedly thickened basement membrane
(*arrowheads*). Compare with Figures 12 and 15. (× 9600)      **Fig 25**—Part of a dissociated
cardiac muscle cell which tapers gradually into the surrounding interstitium. The cell shows
deep invaginations of the plasma membrane associated with finger-like cytoplasmic
projections and a markedly thickened basement membrane. In these projections, the
myofibrils are disrupted, and accumulations of Z-band-like material are present adjacent
to the plasma membrane. Compare with Figure 14. (× 7500)

**Fig 26**—Irregularly shaped fragment of a cardiac muscle cell (darkly stained) without evidence of myofibrillar lysis is connected to a hypertrophied cardiac muscle cell (palely stained) by a side-to-side intercellular junction. Both the cell fragment and the hypertrophied cardiac muscle cell are surrounded by a markedly thickened basement membrane. ($\times$ 7700)

**Fig 27**—Part of a small (4 $\mu$ in diameter) degenerated cardiac muscle cell which has no intact myofibrils and is filled with mitochondria. Thin, elongated masses of Z-band-like material are adjacent to the sarcolemma. This cell is attached at localized points to a hypertrophied, nondegenerated cell. Both cells have markedly thickened basement membranes. ($\times$ 5500)
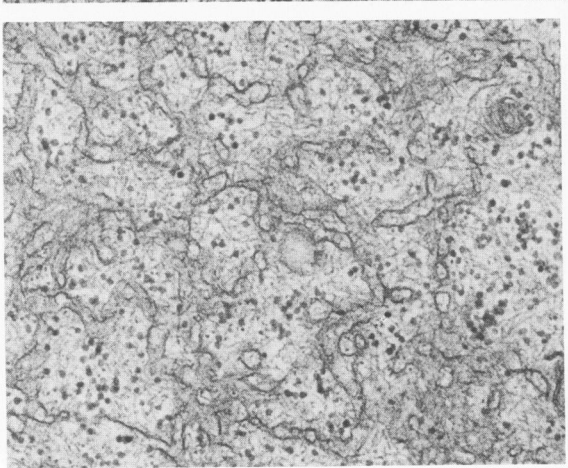
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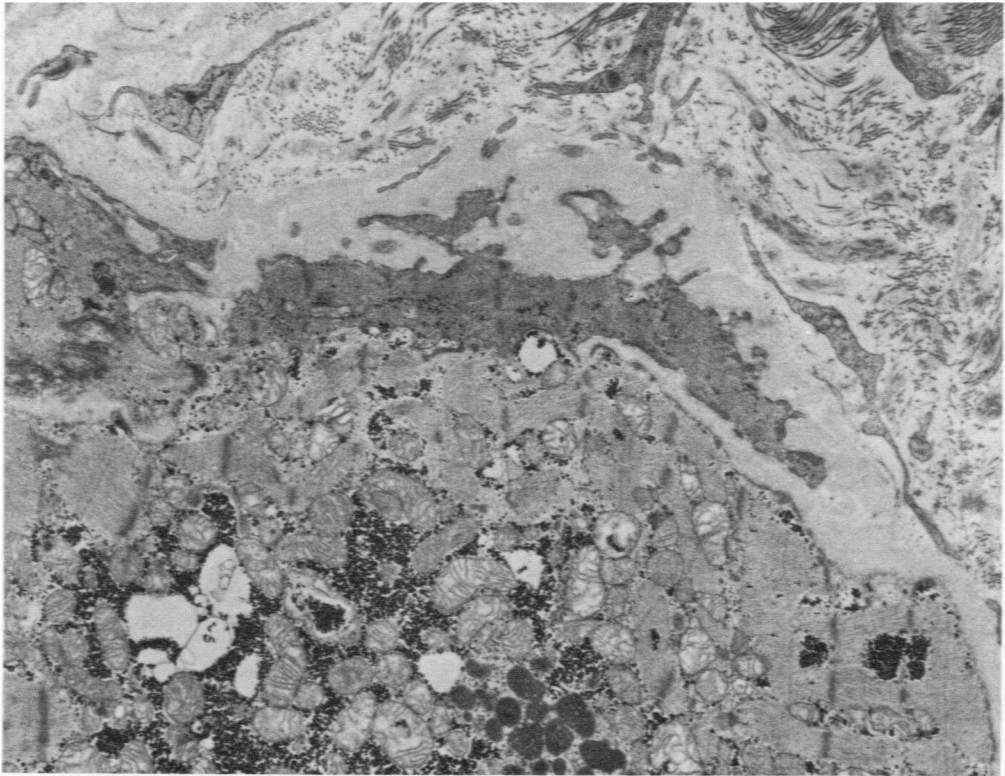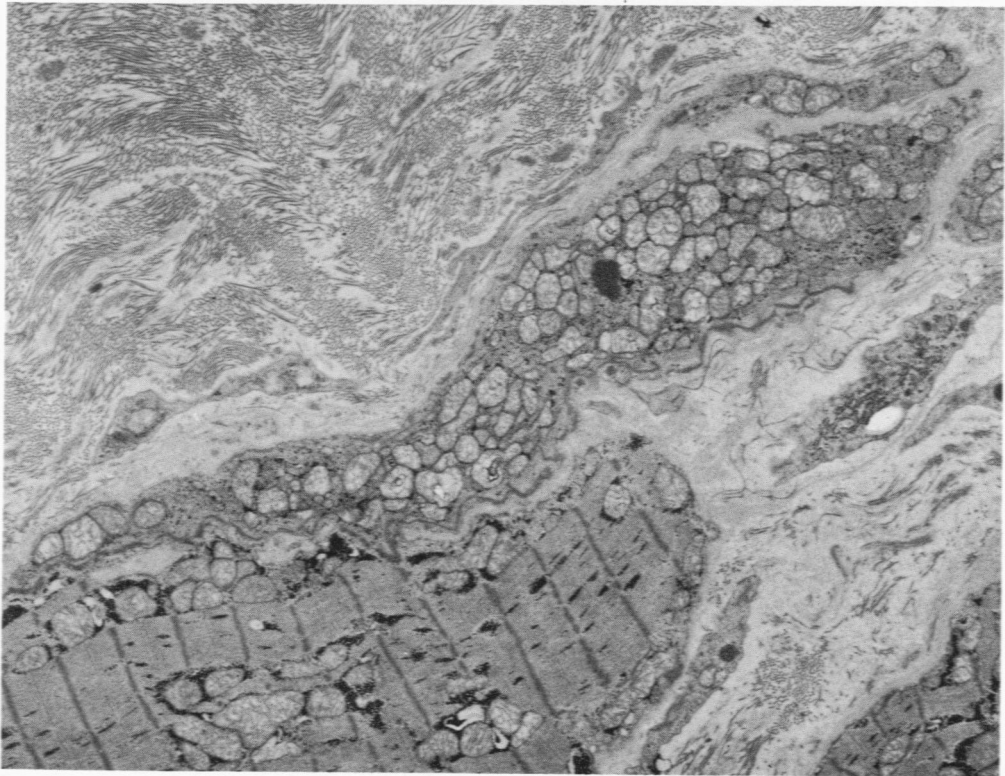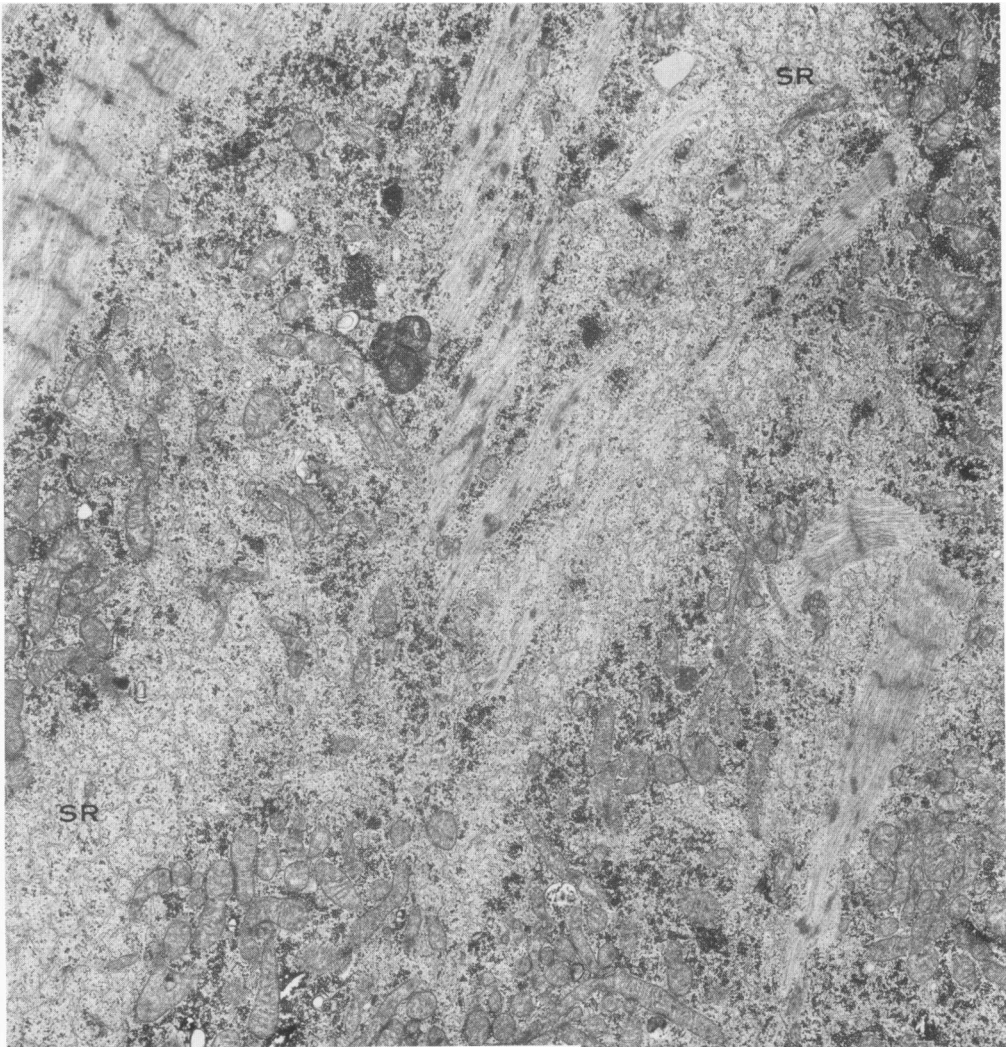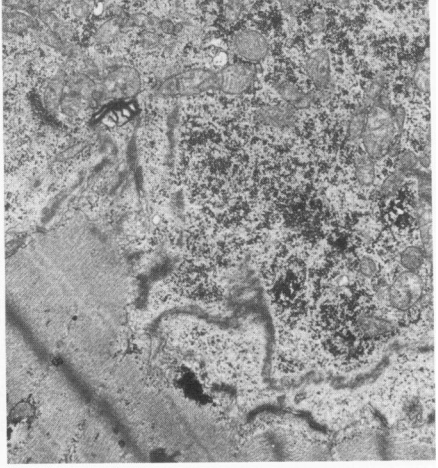
**Fig 28**—Low magnification view of cardiac muscle cell which is severely degenerated and contains sparse myofibrils (which are undergoing lysis), proliferated tubules of sarcoplasmic reticulum (*SR*) and large numbers of mitochondria. Compare with Figures 13 and 15. (× 9500)    **Fig 29**—High magnification view of markedly proliferated tubules of sarcoplasmic reticulum forming a branching and anastomosing network. (× 45,000)
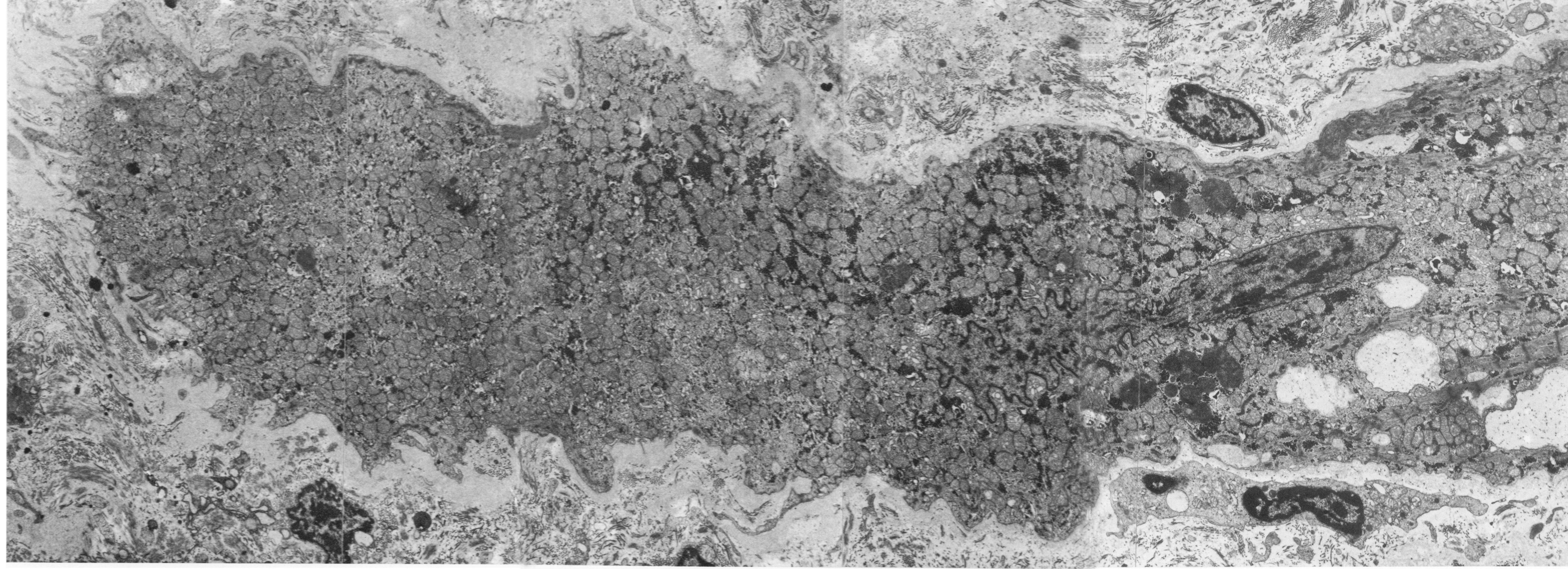
**Fig 30**—Montage of four electron micrographs shows a severely degenerated, atrophic and isolated cardiac muscle cell filled with large numbers of mitochondria, glycogen particles, lipofuscin granules, and tubules of sarcoplasmic reticulum. Large plasma membrane invaginations and part of a single myofibril are shown at the lower left. A bizarrely shaped nucleus is present. Compare with Figure 13. (× 9100)
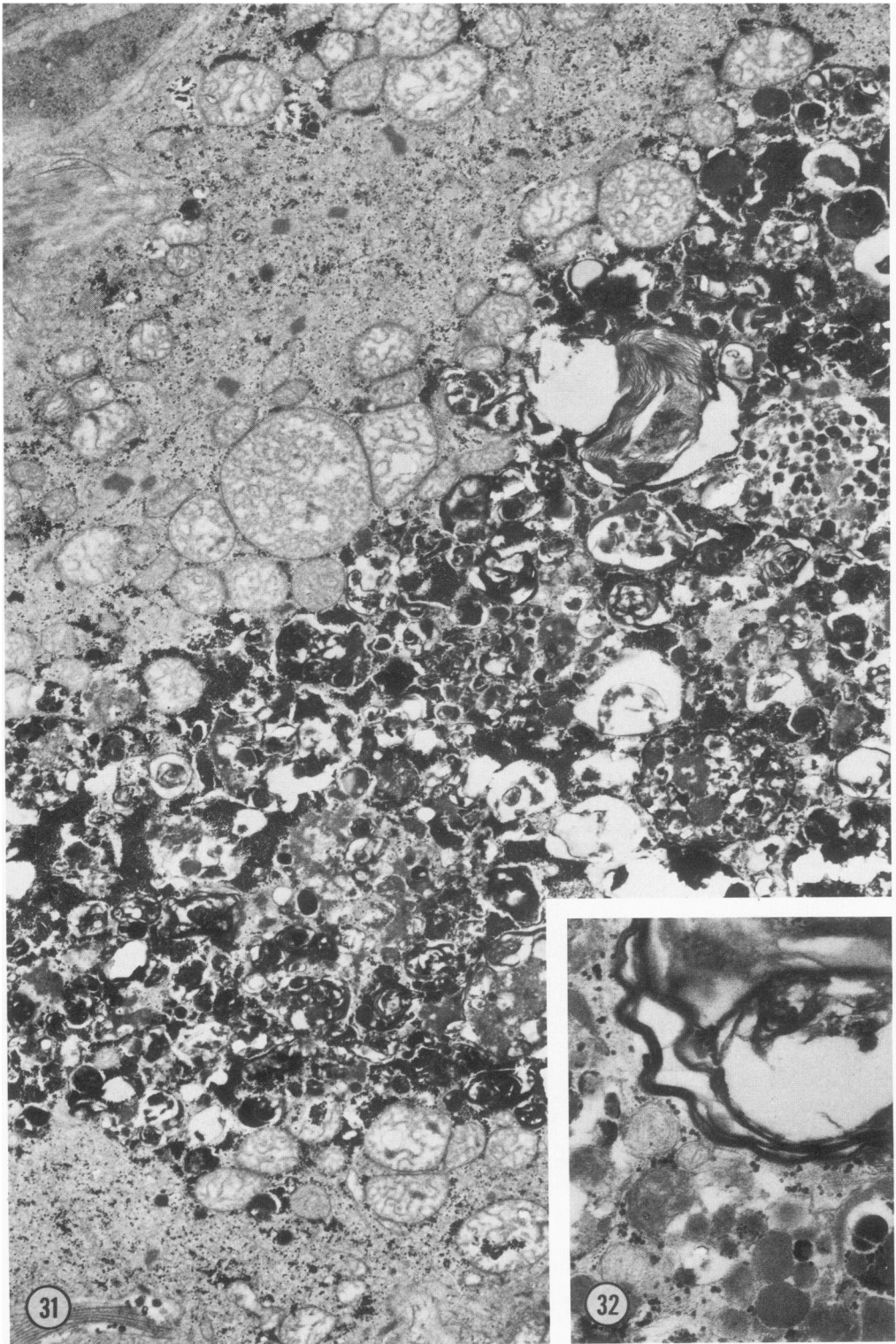
Fig 31—A degenerated, hyalinized cardiac muscle cell shows a large accumulation of myelin figures, lysosomes, glycogen particles, and fragmented membranes of damaged mitochondria. Adjacent areas are filled with granular material which has small, dense patches of electron-dense Z band material dispersed throughout. Compare with Figure 13. (× 9600)     Fig 32—High magnification view of part of a severely degenerated cardiac muscle cell similar to that shown in Figure 31. Concentric lamellae and numerous lysosomes are present. (× 34,800)
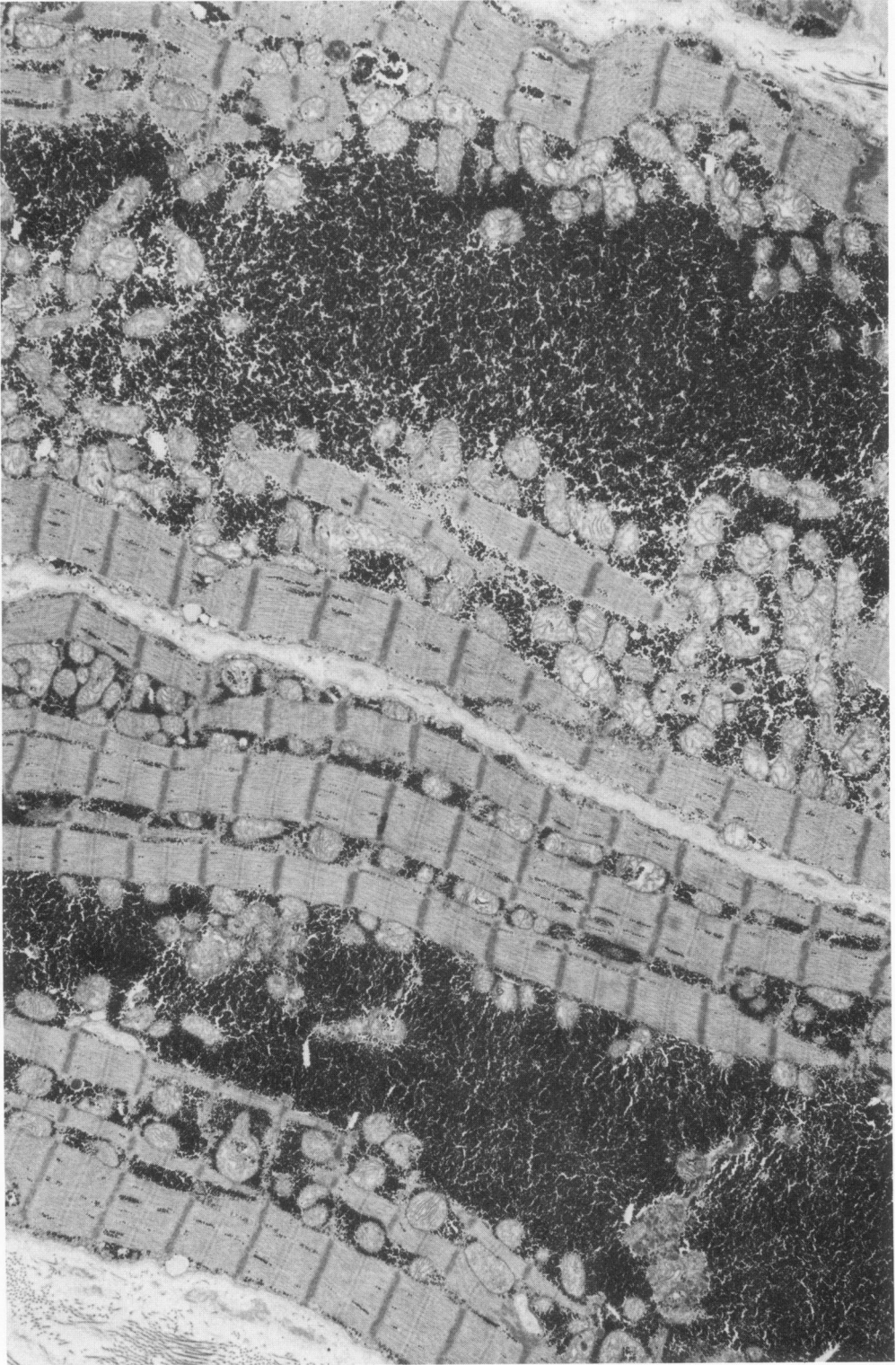
**Fig 33**—Two cardiac muscle cells with large, centrally located masses of glycogen and decreased numbers of myofibrils. (× 7600)