A COMPARATIVE STUDY OF THE FINE STRUCTURE OF NORMAL AND ISCHEMIC DOG MYOCARDIUM WITH SPECIAL REFERENCE TO EARLY CHANGES FOLLOWING TEMPORARY OCCLUSION OF A CORONARY ARTERY

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Occlusion of the circumflex coronary artery in the dog produces a large infarct of the posterior papillary muscle in the left ventricle.[1] This lesion is well suited for electron microscopic studies of myocardial ischemia because of its uniform necrosis and its predictable site in an easily identifiable and oriented region of the myocardium. Moreover, non-ischemic control material is readily available, as the blood supply to a large part of the left ventricle is unaffected by occlusion of this artery.[1]

Although the size and distribution of the lesion is about the same following either temporary occlusion for periods of 40 to 60 minutes, or permanent ligation,[2] some of the early changes affecting irreversibly injured myocardial cells differ considerably according to whether the circulation is, or is not, restored. Histologically, characteristic contraction bands are distributed at random throughout the area of infarction following temporary occlusion, whereas in myocardium rendered permanently ischemic for a similar time, they are seen only at the periphery of the infarct.[2,3] The restoration of blood flow also greatly accelerates tissue-electrolyte alterations associated with myocardial necrosis.[4]

We have investigated the early fine structural changes which occur when blood flow is restored to a myocardium rendered ischemic for 40 minutes, and have compared these appearances both with normal myocardium and with the different changes seen after similar periods of permanent ischemia.[5,6] The present communication reports these observations, and also draws attention to the transverse tubular system in the normal dog myocardium.

MATERIAL AND METHODS

Eight healthy adult mongrel dogs which had been housed in air-conditioned quarters maintained at 68° F, with free access to Borden's Dog Chow and water, were divided into 2 groups. In the first group of 4 animals, the circumflex coronary artery

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was occluded for 40 minutes, after which blood flow was restored for 20 minutes in 3 cases, and 200 minutes in the fourth instance. In the second group, which served as controls, the circumflex coronary artery was permanently occluded for 40 minutes in 2 dogs, and for 60 minutes in 1; the remaining dog underwent a sham operation being anesthetized for 60 minutes with identical heart exposure and isolation of the circumflex coronary artery.

The operative procedure has been reported in detail previously.[2] Briefly, each dog was anesthetized with 25 to 30 mgm of sodium pentobarbital (Nembutal®) per kg body weight, administered intravenously. Respiration was maintained through a cuffed endotracheal tube by means of a Harvard respirator pump (Model 1063). The right femoral artery was cannulated, and continuous blood pressure recordings were made on a Grass direct-writing polygraph (Model 5P1) using a Statham (Model P23) strain gauge. Continuous electrocardiographs from standard limb leads were also recorded on the polygraph. Approximately 30 minutes before occluding the coronary artery, a total of 50 mgm per kg of procaine amide (Pronestyl®) was injected intramuscularly in 2 divided doses.

The chest was opened through an incision in the left fifth intercostal space, and the parietal pericardium was reflected widely. The circumflex branch of the left coronary artery was isolated and in all cases apart from the sham-operated control, was occluded close (9 to 17 mm) to its origin by means of a Goldblatt clamp. Cyanosis developed in the myocardium supplied by this artery within 20 seconds of occlusion, and the electrocardiogram showed elevation of ST segments in leads II, III and aVF. The distribution of cyanosis was noted, the rib retractor was relaxed, and the operative area was covered with sterile towels.

In the first group, the clamp was released after 40 minutes; in 2 cases, this precipitated a period of ventricular tachycardia which was controlled within 3 minutes by small additional amounts of procaine amide administered intravenously. Following 20 minutes of restored flow in 3 animals, and after 200 minutes in the fourth, the hearts were excised. In the second group, the hearts were excised after coronary occlusion for 40 minutes in 2 dogs, 60 minutes in 1, and in the case of the sham-operated control, 60 minutes after isolating the artery.

In each instance, the excised heart was cut open, and a segment including the posterior papillary muscle was removed immediately. Except for the sham-operated control, all cases showed the expected pale area of infarction involving the posterior papillary muscle, and a variable amount of the subendocardial region in the adjacent left ventricular wall.[1] From the center of the papillary muscle, 2 adjacent sagittal slices were cut, each about 0.2 cm thick. For conventional light microscopy, 1 slice was fixed in 10 per cent aqueous formalin, buffered with phosphate to pH 7.0. After paraffin embedding, sections were cut and stained with hematoxylin and eosin.

For light microscopy of thin sections, and for electron microscopy, tissue from the pale area of the other slice was cut under cold phosphate-buffered osmic acid[7] into 1 mm cubes which were fixed at 4° C for 60 minutes. In each dog, tissue from the anterior superior area of the interventricular septum served as normal control, and was prepared in the same manner. In addition, some control myocardium was cut under, and fixed for 4 hours at 4° C in 5 per cent glutaraldehyde, buffered to pH 7.4 with 0.2 M sodium cacodylate.[8] These small cubes were washed overnight at 4° C in cacodylate buffer containing 7.5 per cent sucrose,[8] and were then post-osmicated for 60 minutes at 4° C. All the blocks were dehydrated at room temperature through graded alcohols and embedded in Epon 812, in the manner of Luft.[9] Sections were cut on a Porter-Blum MT-1 ultramicrotome using glass knives. Sections 0.5 μ thick from each block were mounted on glass microscope slides, stained with toluidine blue,[10] and examined with a light microscope to ascertain general appearances and orientation of the block. Ultra-thin sections were mounted on plain or carbon-coated[11] "Athene" or "Effa" copper grids, stained with lead[12] or left unstained, and examined with an RCA (Model EMU-3c) electron microscope.

Results

Non-ischemic Control Myocardium. The fine structure of the myocardium from the interventricular septa in all 8 animals, and from the posterior papillary muscle in the sham-operated control, conformed in general to previous descriptions of normal dog myocardium.[13–16] As is usual in well oxygenated normal tissue fixed in osmic acid, the myofibers were moderately contracted, and both sarcolemmal and nuclear membranes frequently showed prominent indentations (Fig. 5).

Throughout the myocardial cells, characteristic sarcoplasmic organelles referred to as intermediary vesicles in cardiac and skeletal muscle,[17] or as elements of the transversal system in skeletal muscle,[18] were noted at or near the level of many Z bands, usually between adjacent mitochondria (Figs. 5 and 6). From their differing shapes according to the plane of section, these structures appeared to be tubules running transversely between myofibrils at the Z band level. Their walls were morphologically identical with the sarcolemma, and were therefore quite distinct from the membranes of the sarcoplasmic reticulum. Continuity between the two systems was not observed, although in cross-section, transverse tubules frequently were seen to be partially enwrapped by one or two elements of sarcoplasmic reticulum, constituting diads or triads as described by Porter and Palade[17] (Fig. 6).

In fortuitous longitudinal sections of myocardial fibers, sarcolemma was seen to penetrate across the width of up to 4 myofibrils at the level of successive Z bands (Fig. 9). These apparently valid connections between transverse tubules and the sarcolemma are to be distinguished from a similar but misleading appearance illustrated in Figure 12, in which a resemblance to longitudinally sectioned tubules is seen at the level of some Z bands. Such "pseudo-tubules" occur when the plane of an approximately longitudinal section has passed coronally, close to the periphery of a myocardial cell across successive indentations of sarcolemma.

A variable number of small dense granules which almost certainly represented glycogen[19–21] were noted in perinuclear, perimitochondrial and subsarcolemmal sites. In addition, similar granules were aligned in rows between some myofilaments in longitudinal sections (Figs. 5, 6 and 9). Although such an appearance could have resulted from sections having passed along the periphery of the myofibril rather than from the presence of glycogen between myofilaments, some cross sections revealed identical granules deeply within the myofibril between myofilaments (Fig. 7), thereby confirming a similar recent observation.[6]

Intramitochondrial granules which are commonly observed in other

normal parenchymatous tissues,[22] were not seen in normal dog myocardium after osmium fixation.[15] Very small granules were noted, however, in some of the mitochondria of control tissue after glutaraldehyde fixation (Fig. 9).

Permanently Ischemic Myocardium. The morphologic changes induced in the posterior papillary muscle by occlusion of the circumflex coronary artery for 60 minutes were identical with those seen under the same circumstances in a recent study,[6] and the appearances were similar but somewhat less pronounced after 40 minutes of ischemia. Thus, by light microscopy, the overall architecture was seen to be preserved (Figs. 1 and 2); a few darkly staining contraction bands were present at the periphery of the lesion, although the remainder of the infarct was homogeneous. Myocardial nuclei were rather pale but retained their usual position within the cells, and the intercalated discs were unremarkable.

Electron microscopy confirmed the preservation of major cellular and intracellular relationships in this permanently ischemic tissue, in which the sarcolemmal membranes and intercalated discs remained intact. Following 40 minutes of ischemia, the myofibers were relaxed and glycogen particles were reduced in number (Fig. 10). After 60 minutes of ischemia, there was extreme relaxation of myofibers and a virtual absence of glycogen (Figs. 11 and 12). Sarcolemmal scalloping was prominent over many of these markedly relaxed fibers, showing that the degree of scalloping may not always reflect the state of contraction of the cell.[16] Myocardial nuclei invariably showed margination of chromatin material (Fig. 10). In many mitochondria, the cristae were reduced to a few irregular fragments, and the matrices were abnormally clear, whilst in some, the mitochondrial limiting membranes were disrupted (Figs. 10 to 12). Dense, apparently homogeneous granules measuring up to 80 mμ in diameter were noted in many mitochondria after 60 minutes of ischemia. Capillary integrity was preserved apart from some endothelial cell nuclei which showed abnormal chromatin margination.

Temporarily Ischemic Myocardium. The posterior papillary muscles in the 4 dogs in the temporarily ischemic group all showed similar abnormalities. Histologically, the lesion in each case was precisely the same as those of a larger series reported in detail recently.[3] The infarct was clearly demarcated from normal tissue and showed extensive disruption of architecture (Figs. 3 and 4). There was considerable separation of myofibers, and dark-staining contraction bands were seen distributed at random throughout the infarct. These bands occurred anywhere along the myofiber without reference to intercalated discs, and usually extended across the whole myofiber.

Fine structural abnormalities in these lesions included several features

which were quite distinct from those seen after similar periods of permanent ischemia. The orderly arrangement of myofibrils and mitochondria within the fiber was lacking (Figs. 8, and 13 to 15). Each myofiber included areas where the Z bands were abnormally thickened and in which over-stretched myofibrils had been torn apart in the H-band region, together with other regions in which the filaments had become extraordinarily shortened to form characteristic large contraction bands made up of several successive sarcomeres (Figs. 8, and 14 to 16). It should be noted that Caulfield and Klionsky[5] used the expression "contraction bands" to refer to thickened Z bands which they observed in rabbit myocardium rendered ischemic for up to 20 minutes and in some non-ischemic control tissue, whereas in the present study we have reserved the term for these large, dense bands consisting of a condensation of several sarcomeres.

In a few instances involving relatively less dense contraction bands, orderly filamentous arrays were present (Fig. 17), but for the most part, such a pattern was not seen in these abnormally contracted areas. The lack of filamentous pattern was not due simply to problems of section orientation,[23] for a large number of sections in different planes were examined, but appeared to result rather from a lack of the usual parallel organization of myofilaments.

Large collections of mitochondria, quite distinct from the normal perinuclear aggregations, were seen with random distribution within the disrupted fibers (Figs. 8, 13, 14 and 16). Some mitochondria had been pushed out of the myofibers, and the few mitochondria remaining in the contraction bands were very much smaller and somewhat denser than usual, as if they had been squashed in the contraction process (Fig. 16).

Very dense, large granules were noted in most of the mitochondria of these extensively disrupted cells. They appeared identical in stained and unstained sections (Figs. 18 and 19), and measured up to 200 mμ in diameter. Thin sections of mitochondria included from 1 to 10 of these granules which were made up of dense particles about 60 Å in diameter, apparently arranged in the form of thick-walled spheres. In many instances, the whole granular structure was partially or completely surrounded in the plane of section by the paired membranes of a crista.

In addition to these striking myofibrillar and mitochondrial abnormalities, other changes induced by restoring blood flow after ischemia for 40 minutes included disruption of sarcolemmal membranes and displacement of myocardial cell nuclei along myofibers. In tissue to which blood flow had been restored for 20 minutes, extensive margination of chromatin material was noted, as in the permanently ischemic group. After 200 minutes of restored flow, there appeared to be less chromatin material in

the nuclei and margination was less prominent (Fig. 15). Glycogen was virtually absent, except for a few particles which appeared to have become trapped in the contraction bands (Fig. 17), and the sarcoplasmic reticulum and transverse tubular system were completely disorganized. The capillaries, including endothelial nuclei, were unremarkable.

The structure of intercalated discs was well preserved, despite such extensive myofiber damage. The intercellular space appeared normal in width, varying for the most part from approximately 95 Å in some regions of the disc to 170 Å in others. However, in both normal and temporarily or permanently ischemic myocardium, occasional expansions of the intercellular space were noted, in which there were round structures about 40 mμ in diameter (Figs. 20 and 22). In temporarily ischemic tissue, a thin dense line bisecting the intercellular space could be discerned in those segments of the disc where dense material at the termination of myofibrils was prominent (Figs. 20 and 21). Such an intermediate line was not seen in the intercalated discs of normal or permanently ischemic myocardium.

Discussion

The present results have shown that early fine structural changes occurring in myocardial cells irreversibly injured by ischemia were markedly different according to whether blood flow was, or was not, restored. In addition the observations on control tissue drew attention to the structure of the transverse tubular system and its connections with sarcolemma in normal dog myocardium.

Permanent ischemia for 40 or 60 minutes produced fine structural changes which included marked relaxation of myofibers, loss of glycogen, margination of nuclear chromatin and mitochondrial abnormalities. The sarcolemmal membranes remained intact, and overall intracellular relationships were preserved. Similar alterations have been reported in rat, rabbit and dog heart under conditions of permanent anoxia,[24] hypoxia[25,26] or ischemia.[6,27,28]

Much more severe morphologic damage occurred after blood flow was restored to such ischemic myocardium, and was in accord with the more extensive and rapidly developing abnormalities of tissue-electrolytes which occur in these circumstances.[4] The most striking morphologic changes were the development of contraction bands throughout the infarct together with marked disruption of sarcolemmal membranes and intracellular organelles, and the appearance of remarkable dense granules in the mitochondria. Margination of chromatin again was prominent in the myocardial nuclei, but in contradistinction to permanently ischemic tissue, was not seen in endothelial nuclei.

It appeared that contraction bands were produced in infarcts by the contractile force of surrounding viable fibers acting on irreversibly injured myocardial cells. Cell death was a prerequisite for their development, because when blood flow was restored to myocardium rendered ischemic for 20 minutes or less, which are periods of ischemia known to cause only reversible cell changes,[2,6] such bands did not occur.[29] Their presence throughout infarcts after temporary ischemia, but only at the periphery of permanently ischemic lesions, presumably was due to restored blood flow in the former case allowing surrounding cells rendered ischemic but not killed by the coronary occlusion to again contract.

The changes induced by permanent or temporary ischemia were different from the zonal lesions caused by hemorrhagic shock, which appeared to be restricted to the ends of myocytes on each side of intercalated discs.[16] Although such zonal lesions were not as densely compact as the contraction bands we have described, they too were thought to be due to mechanical forces acting on the myocardium.[30]

Intramitochondrial granules are seldom seen in normal heart muscle,[22,31] and were not observed in well oxygenated, osmium-fixed, control myocardium during this or a previous experiment.[15] They have been reported in apparently normal rat myocardium [13,22,32] and can be seen in some of the excellent micrographs of Stenger and Spiro in their articles on the structure of mammalian cardiac muscle.[21,33] Weiss has suggested that such mitochondrial dense granules are more prevalent in cells across which significant quantities of water and cations flow.[31] In the light of such an hypothesis it is of interest that in the present study relatively small intramitochondrial granules were seen after a period of permanent ischemia which was long enough to alter cell permeability,[6] whilst the more striking and ubiquitous examples were found only in severely deranged cells whose sarcolemmal membranes were disrupted, and around which blood flow had been restored for 20 minutes or longer. Possibly the mitochondria of such markedly disrupted cells may be likened to isolated mitochondria, which are known to be able to accumulate calcium, magnesium and manganese.[34]

Intramitochondrial granules of similar appearance to those seen after temporary ischemia have been reported in dog myocardial mitochondria following hypoxic cardiac arrest,[26] in the mitochondria of fast-acting fish muscle,[35] and in the cells of toad urinary bladder.[36] In the latter case, they were considered to represent accumulations of calcium, strontium or barium ions present in the medium bathing the cells. Dense bodies which may be of a similar nature were seen in some mitochondria of rabbit myocardium rendered ischemic for 35 or more minutes,[5] and Porter [37] noted the development of prominent granules in the mitochondria

of cells from day-old rat heart tissue explants in the presence of calcium and other divalent ions at 38°C. With these observations in mind, and because of the known deposition of calcium in myocardial infarcts in dogs,[3] it appears likely that the granules which we have observed were in fact some form of calcium salts. Currently we are investigating this thesis.

Structural preservation of intercalated discs in myocardial cells rendered ischemic and subjected to considerable contractile force further emphasizes the remarkable tensile strength of these intercellular junctions.[38] Similarly it has been observed that intercalated discs were unaffected by the cellular disruption associated with hemorrhagic shock [16] and ischemia.[5] The thin osmiophilic line present in the center of the intercellular layer in temporarily ischemic myocardium possibly may reflect changes induced in the lipid components of the two plasma membranes which are assumed to be in mutual contact at this site.[38] The segments of intercalated discs in which this intermediate line was observed are very similar in structure to the *maculae adherens* or desmosomes described as part of the junctional complexes in various epithelia by Farquhar and Palade.[39] The nature of the expansions of the intercellular layer of intercalated discs in normal and ischemic myocardium is unknown.

Observations on normal myocardium in the present study show that the transverse tubular system was different from, and not continuous with the sarcoplasmic reticulum. This conclusion which is at variance with some opinions [15,40] but in agreement with several other observations,[14,18,41,42] is based on the different membrane structure in the two systems, and the absence of any connections between them. The present report also appears to confirm the existence of continuities between the sarcolemma and elements of the transverse tubular system. Such connections which may have profound importance relative to impulse conduction and coordination throughout the myocardial cell,[18,41–43] have long been suspected, and recently have been demonstrated in dog, sheep, rabbit and human myocardium.[40,42,43] Analogous findings have been reported in insect fibrillar flight muscle [44] and in copepod striated muscle,[45] in both of which continuity has been shown conclusively between sarcolemma and deeply penetrating tubules. With regard to mammalian cardiac muscle, however, it should be emphasized that it is difficult to distinguish true connections between transverse tubules and the sarcolemma from "pseudo-tubules" which can occur when the section passes coronally through the indentations formed at the level of Z bands by sarcolemmal scalloping. The problem would be more clearly resolved with a three dimensional model constructed from serial thin sections of a myocardial cell.

Summary

Characteristic early fine structural changes were produced in the posterior papillary muscle of the dog by temporarily occluding the circumflex coronary artery for 40 minutes. These abnormalities, which were similar whether blood flow had been restored for 20 or 200 minutes following the ischemic period, included the development of many widespread contraction bands, together with disruption of the sarcolemma, translocation and disorganization of mitochondria, and the appearance of large dense intramitochondrial granules which were probably collections of calcium. Despite such profound cellular disruption, the structure of intercalated discs was normal except for the appearance in some segments of a thin line bisecting the intercellular space.

These fine structural changes were different from and much more extensive than those produced by equivalent periods of permanent ischemia, in which contraction bands were seen only at the periphery of the infarct, and sarcolemmal membranes of the uniformly relaxed fibers appeared intact.

Observations on control tissue appear to confirm the existence of connections between elements of the transverse tubular system and the sarcolemma in normal dog myocardium, and draw attention to the distinction of this system from the sarcoplasmic reticulum.

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[*Illustrations follow*]

Legends for Figures

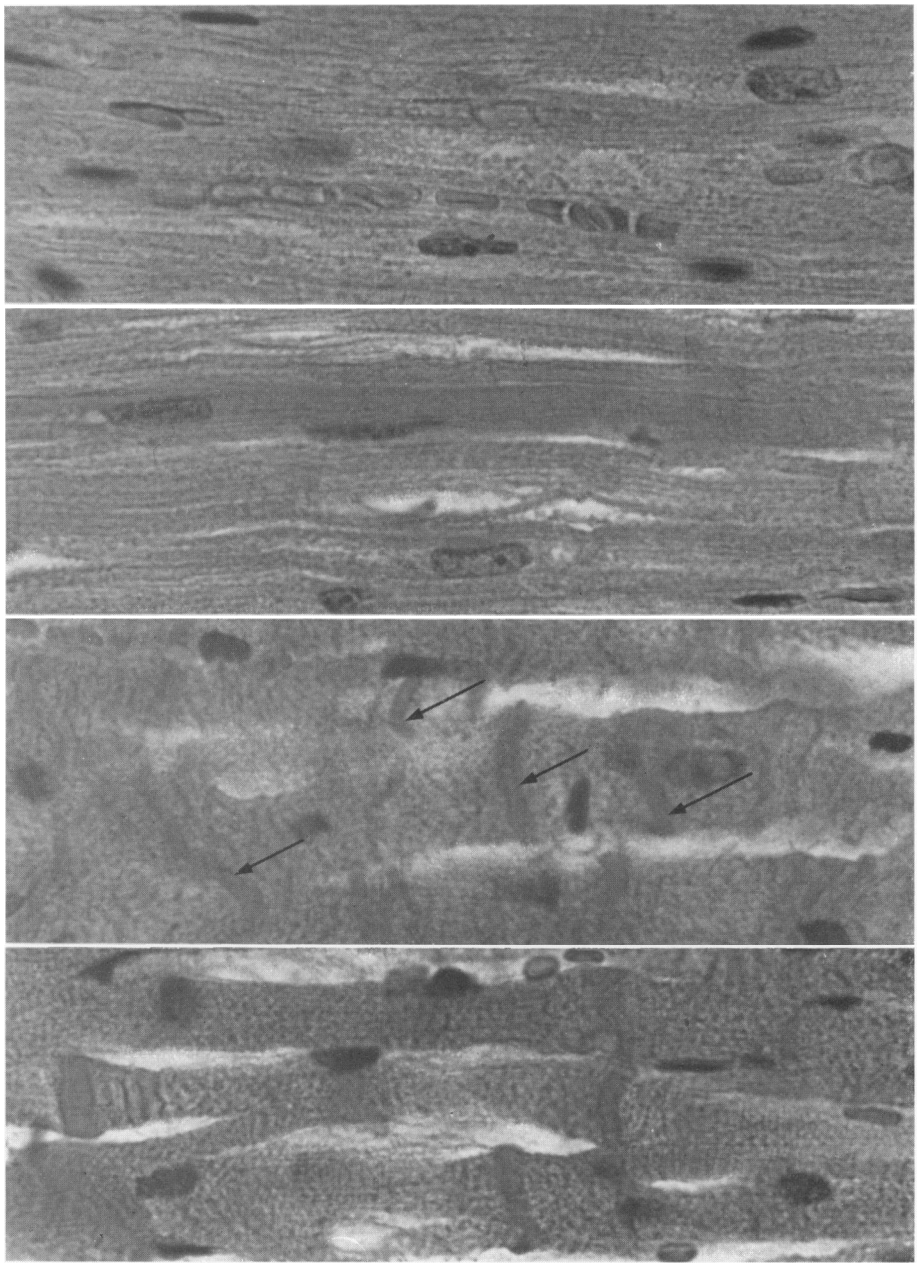
Figures 1 to 4 were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Infarct produced by occlusion of the circumflex coronary artery for 40 minutes. No contraction bands are seen. × 1,200.

Fig. 2. Infarct after 60 minutes of ischemia. One fiber is strongly eosinophilic and there is some separation of myocardial cells. No contraction bands are seen. × 1,200.

Fig. 3. Typical appearance after restoration of blood flow for 20 minutes to an area rendered ischemic for 40 minutes. Contraction bands (arrows) are prominent, occurring at random within the myofibers which are considerably separated. × 1,100.

Fig. 4. Following 200 minutes of restored flow after 40 minutes of ischemia, the appearances are similar to those in Fig. 3, with prominent contraction bands and separation of myofibers. × 1,100.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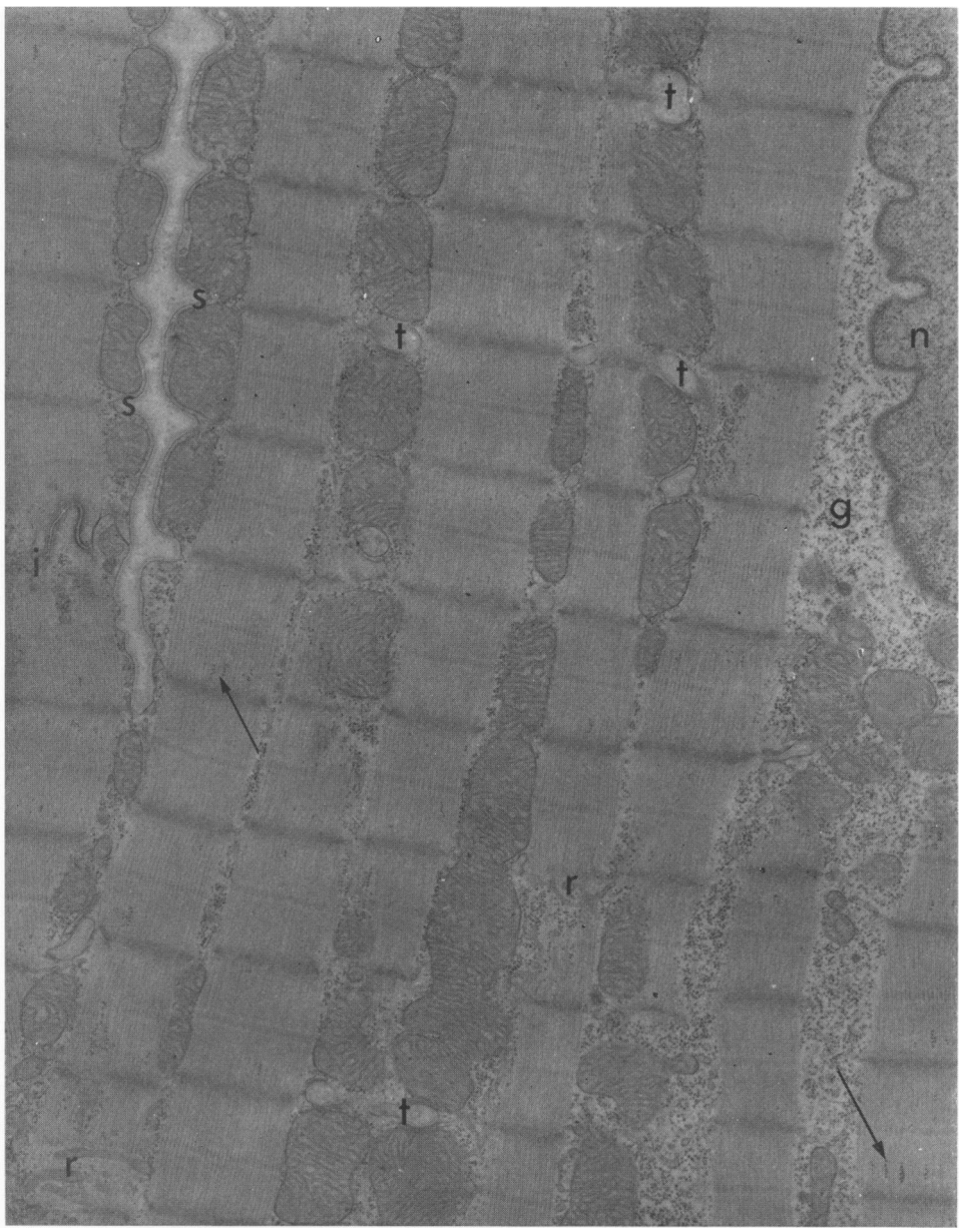

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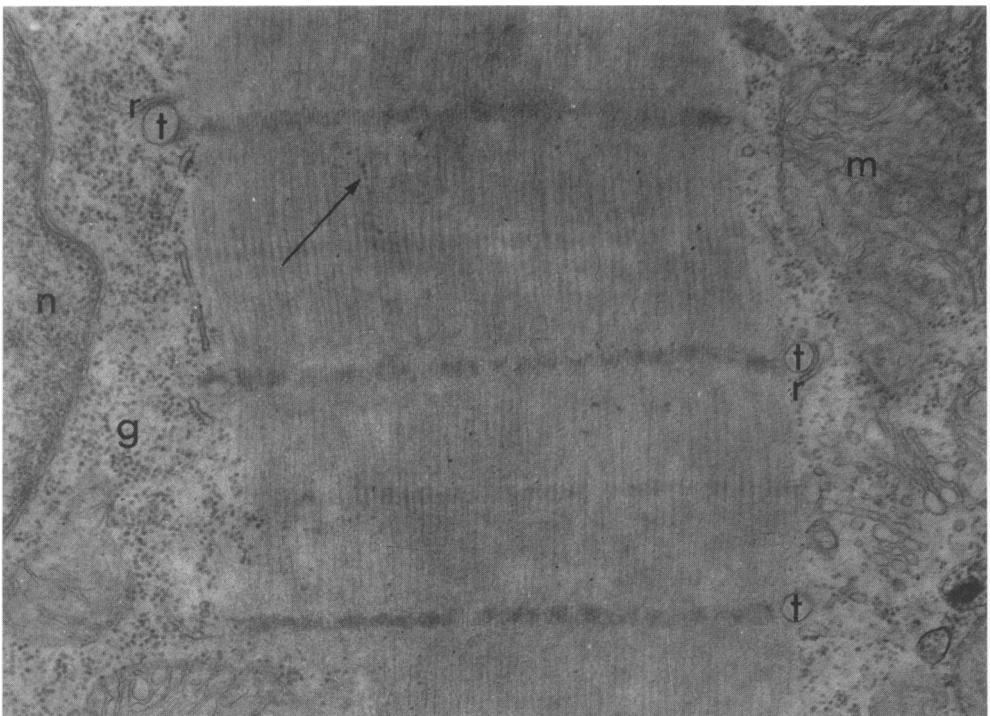
Figures 5 to 8, and 10 to 22 are electron micrographs of osmium-fixed material. Figure 9 is of glutaraldehyde-fixed tissue which was post-osmicated. In all cases except Figure 19, the sections were stained with lead.

FIG. 5. Control myocardium. Myofibrils are moderately contracted; nuclear and sarcolemmal membranes (s) are scalloped. Transverse tubules (t), with walls identical to the sarcolemma, are seen at or near the level of many Z bands. Glycogen particles appear to be arranged longitudinally in relation to myofilaments at some sites (arrows). Nucleus, n; intercalated disc, i; sarcoplasmic reticulum, r; glycogen, g. × 15,000.



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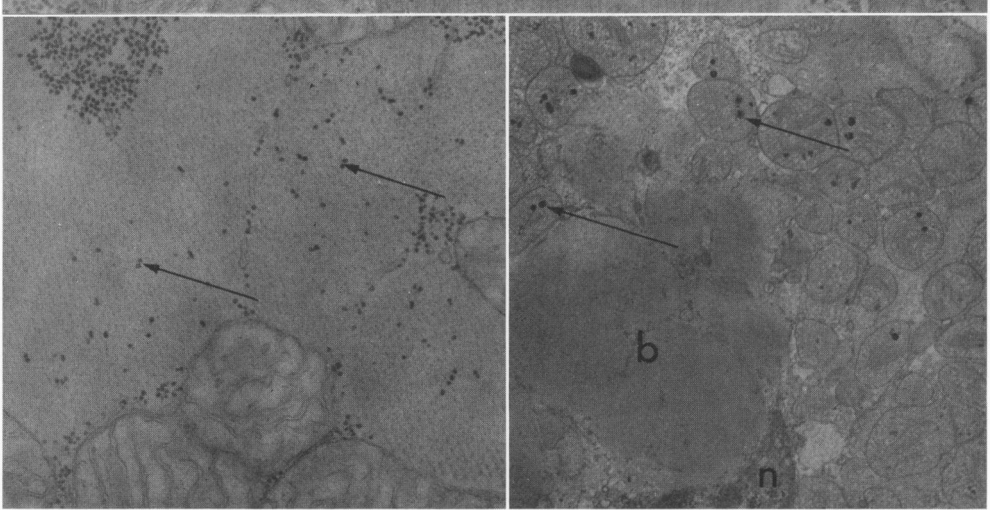

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FIG. 6. Control myocardium. The intimate relation of transverse tubules (t) with elements of the sarcoplasmic reticulum (r) is well shown. Glycogen particles are apparently aligned longitudinally (arrows). Nucleus, n; mitochondrion, m; glycogen, g. × 29,000.

FIG. 7. Control myocardium. In cross section glycogen particles (arrows) are seen to lie within the myofibrils, between some myofilaments. × 25,000.

FIG. 8. Temporarily ischemic myocardium. A cross-section of tissue after 20 minutes of restored blood flow. There is complete disorganization of the usual intracellular relationships. Arrows denote prominent intramitochondrial granules. Nucleus, n; contraction band, b. × 7,000.

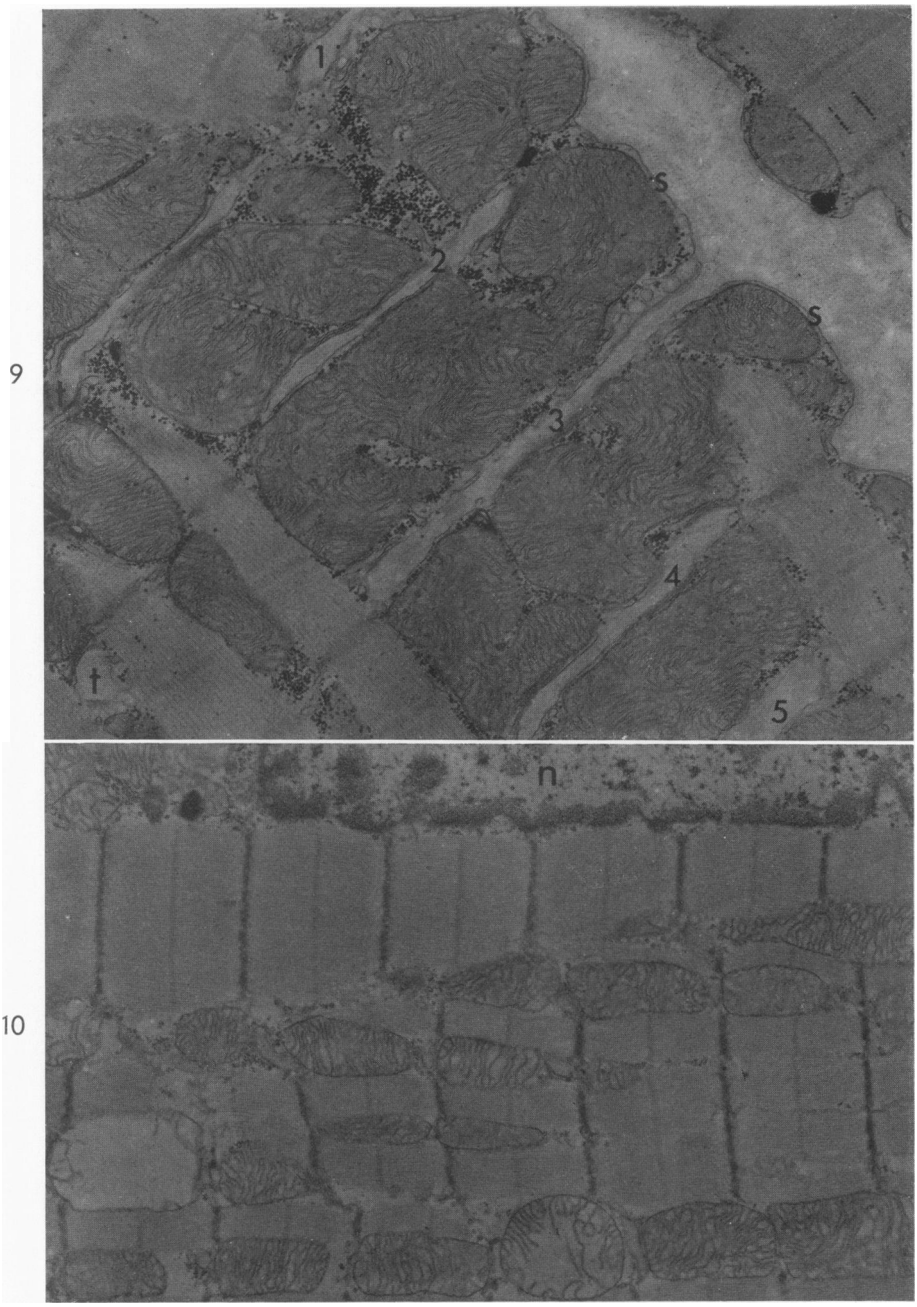

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FIG. 9. Control myocardium. Continuity between sarcolemma (s) and transverse tubules (t) is apparently maintained. Consideration of the plane of this section makes it unlikely that the deep invagination (3) of sarcolemma is due simply to sarcolemmal indentation; if this were the case, the appearances at 1, 2, 4 and 5, in which cellular continuity is present across the invaginations, would necessitate a peculiarly irregular depth to such sarcolemmal indentations. × 19,000.

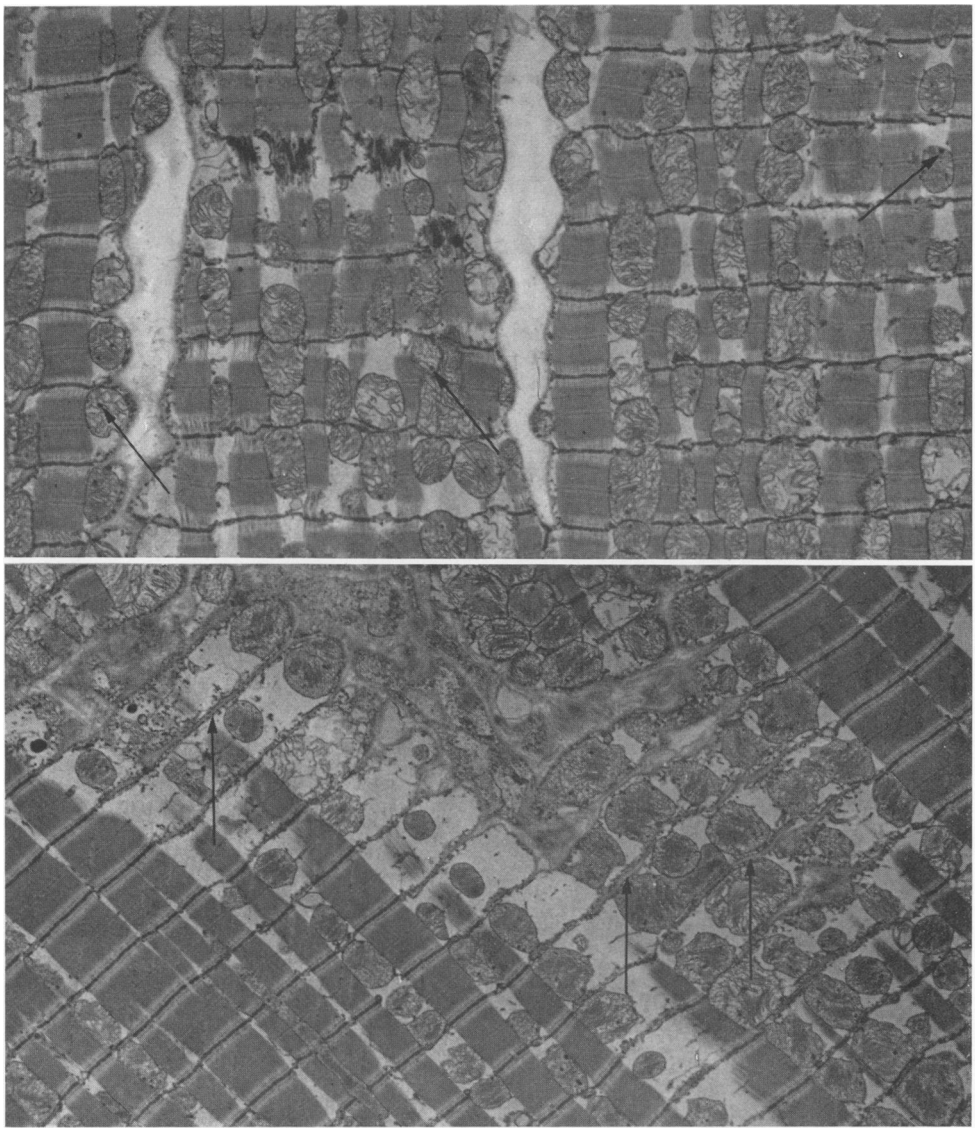

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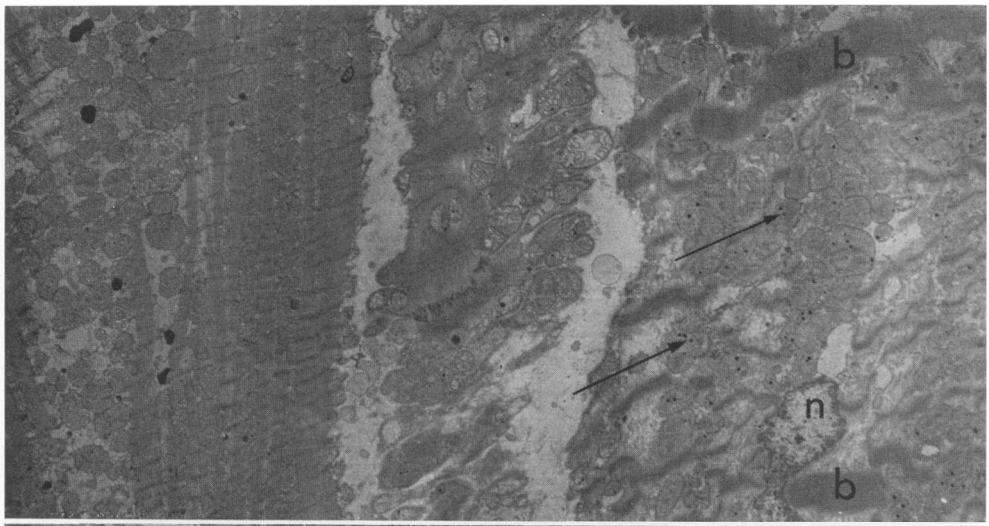
FIG. 10. Permanently ischemic myocardium; typical appearance after 40 minutes of ischemia. The fibrils are moderately relaxed, there is margination of chromatin in the nucleus (n), and some of the mitochondria show considerable disruption of cristae. × 12,000.

FIGS. 11 and 12. Permanently ischemic myocardium, after coronary occlusion for 60 minutes. The fibrils are strikingly relaxed, the mitochondria show disruption of cristae and loss of dense matrices; interfibrillar spaces are prominent. Intercalated discs retain their integrity. There is considerable sarcolemmal scalloping despite the relaxation of myofibers. Glycogen is virtually absent. Arrows in Fig. 11 denote dense intramitochondrial granules. In Fig. 12, the oblique section has produced "pseudo-tubules" (arrows) by passing across sarcolemmal indentations. Fig. 11, × 5,000; Fig. 12, × 4,500.

13



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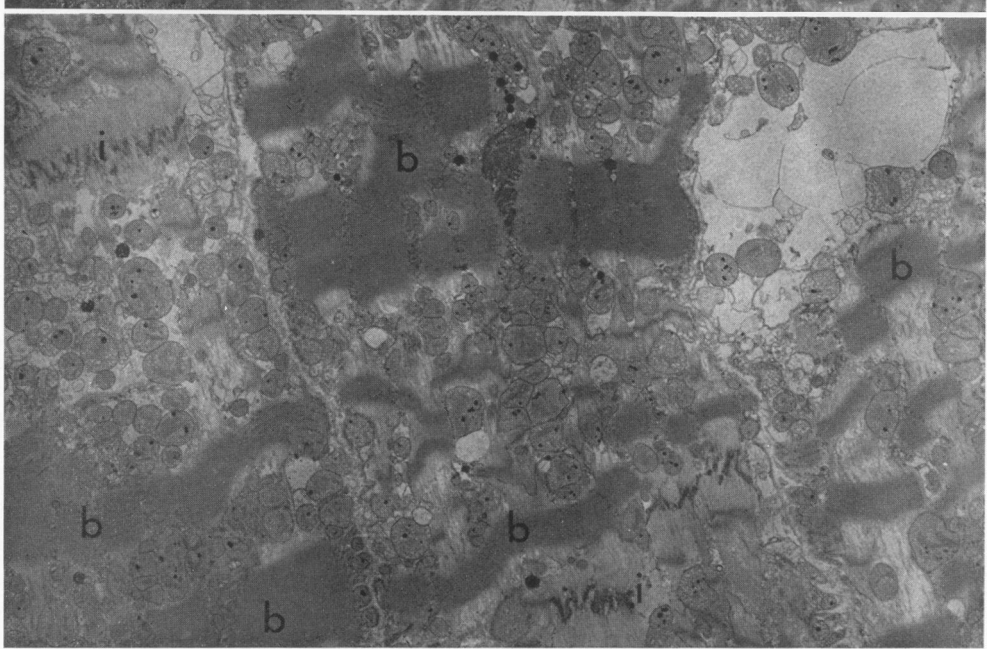


FIGS. 13 and 14, and 16 to 21 are of myocardium rendered ischemic for 40 minutes to which blood flow was restored for 20 minutes. In the case of Fig. 15, blood flow was restored for 200 minutes after the same period of ischemia.

FIG. 13. A fortuitous section near the edge of an infarct, in which a relatively normal myofiber is seen on the left, together with two typically altered fibers in the center and on the right. The gross distortion and variability in sarcomere length present after 20 minutes of restored blood flow are emphasized. Dense intramitochondrial granules (arrows) appear in the typically altered cells, but not in the more normal one. The nucleus (n) shows chromatin margination and is markedly abnormal in shape. Contraction bands, b. × 3,000.

FIG. 14. Typical appearance of the lesion after 20 minutes of restored flow. Contraction bands (b) are prominent and the myofilaments of several sarcomeres have been torn apart at the H-band level. Sarcolemma is disrupted; some mitochondria have been extruded from the cells. Intercalated discs (i) are intact. There are large granules in most of the mitochondria. × 4,000.

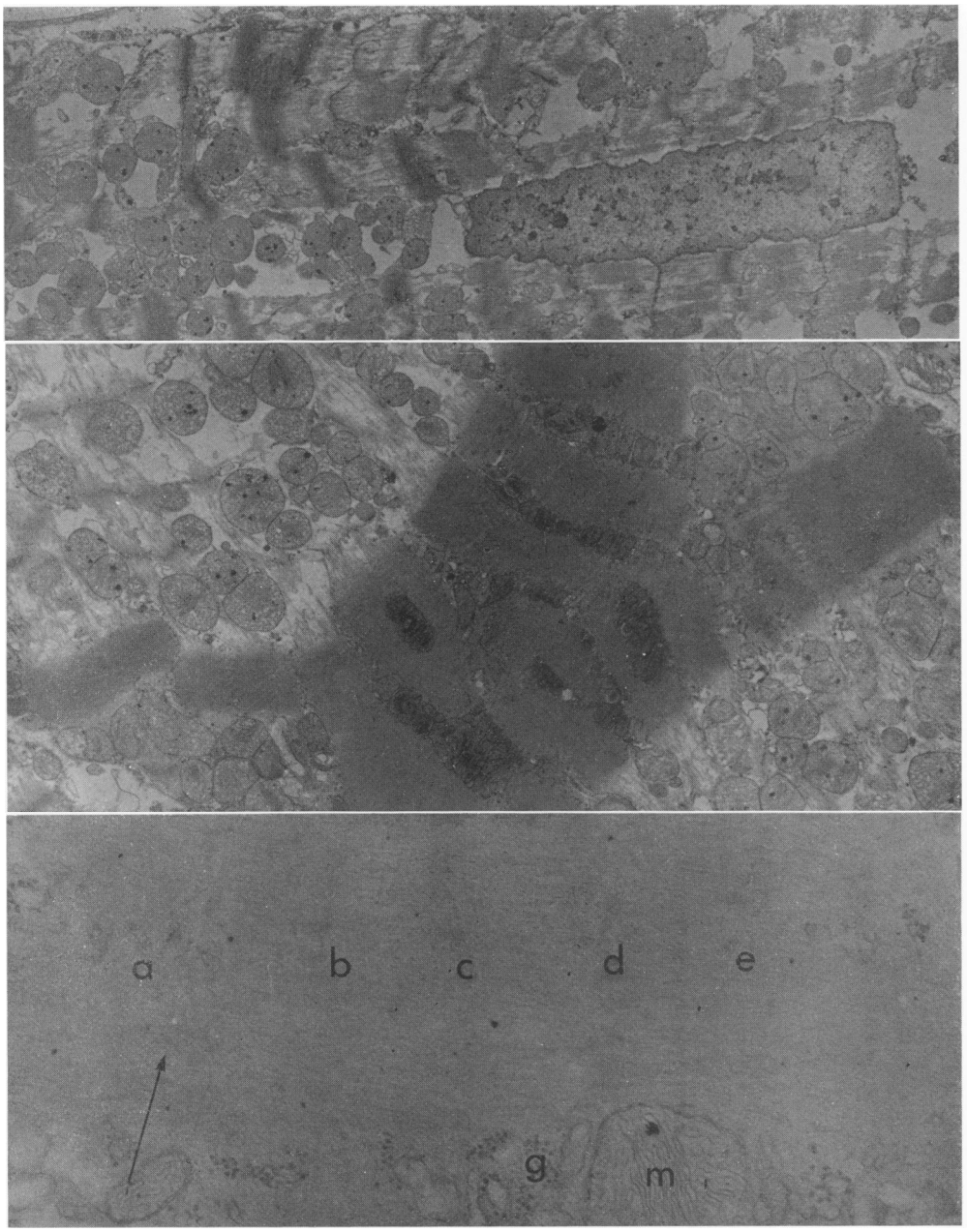
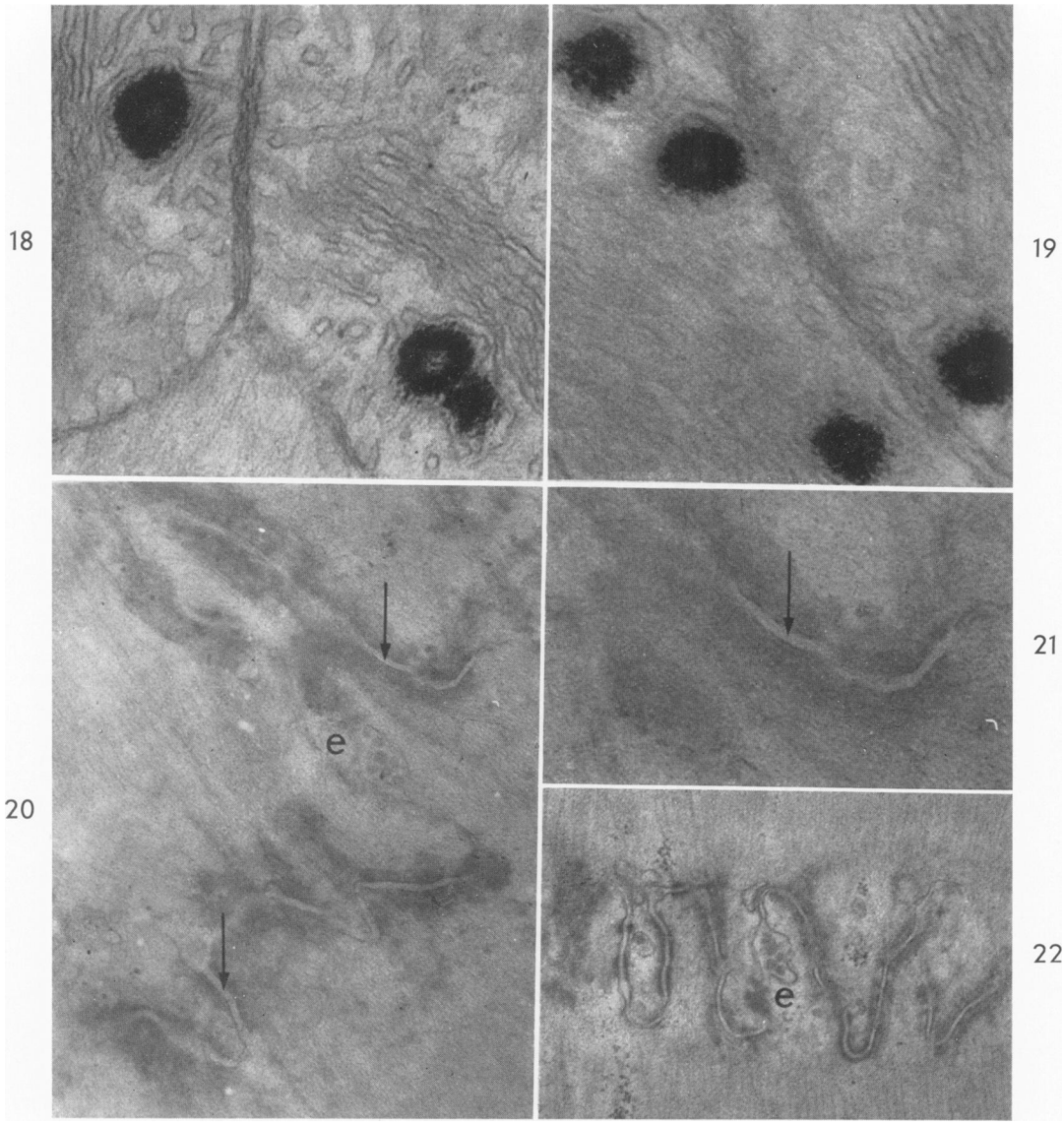


FIG. 15. Part of a myofiber to which blood flow was restored for 200 minutes. The appearances are similar to those after 20 minutes. × 4,000.

FIG. 16. A dense contraction band in which some mitochondria have become "trapped". Granules are prominent in many surrounding mitochondria. × 5,500.

FIG. 17. Part of a less dense contraction band, in which longitudinal myofilaments can be seen. The cross-striations (a, b, c, d, e) probably represent successive Z bands. That they are not due to chatter or other cutting artifact is emphasized by an unusually well placed knife mark (arrow) which runs diagonally. Glycogen, g; mitochondria, m. × 43,000.



FIGS. 18 and 19. Intramitochondrial granules, in lead-stained (Fig. 18) and unstained (Fig. 19) sections. Each granule appears to consist of a hollow sphere made up of many dense bodies approximately 60 Å in diameter. Most of the granules are partially enveloped by cristae. Fig. 18, × 65,000; Fig. 19, × 75,000.

FIGS. 20 and 21. Part of an intercalated disc from temporarily ischemic myocardium. General structure is preserved, including an expansion (e) of the intercellular space in which there are dense structures. A thin osmiophilic line (arrows) bisects the intercellular space in some regions of the intercalated disc. The pattern of such segments is similar to that of desmosomes. Fig. 20, × 45,000; Fig. 21, × 105,000.

FIG. 22. Part of an intercalated disc from normal myocardium. Expansions (e) of the intercellular space appear as in ischemic tissue (Fig. 21). × 34.000.