MYOCARDIAL ISCHEMIA AND EARLY INFARCTION: AN ELECTRON MICROSCOPIC STUDY*

JAMES CAULFIELD, M.D., and BERNARD KLIONSKY, M.D.
From the Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, Kans.

This investigation is part of a study of the early morphologic alterations in myocardial ischemia and infarction produced by ligation of the coronary artery of the rabbit. Tissue obtained from the experimental animals was investigated by various histochemical techniques and by electron microscopy. Ventricular myocardium was selected because the cells here are relatively uniform in structure and function. It was hoped that by varying the period of time between arterial ligation and fixation of the tissue, the sequence of cellular events could be elucidated. This report deals primarily with the electron microscopic features.

Experimental ligation of the coronary arteries has been performed in a wide variety of animals. Although chemical determinations have revealed losses of glycogen and increases of lactic acid within a few minutes after coronary occlusion,[1-3] the effects of ischemia were not usually noted histologically before 4 to 6 hours had elapsed.[4,5] Chemical measurements have revealed marked loss of glycogen in anoxic hearts within 4 minutes.[6] Progressive chemical and histologic alterations of infarction did not follow temporary occlusions of less than 20 to 30 minutes.[7] The earliest histochemical change recorded is the loss of glycogen in from 30 to 60 minutes.[7,8] In view of the evidence indicating loss of glycogen within minutes and the apparent induction of necrobiosis within 30 minutes, it was felt that structural alterations not observable by the light microscope might be demonstrated with the electron microscope.

MATERIAL AND METHODS

Male and female albino rabbits weighing 4 to 5 pounds were anesthetized with intravenous sodium pentobarbital (50 to 75 mg.). Following tracheotomy and intubation, a left lateral thoracotomy was performed, using positive pressure respiration. After opening the

* This investigation was supported by grants H-2922 and HTS-5130 from the National Heart Institute and C-3308 and CRT-5006 from the National Cancer Institute, United States Public Health Service.

Presented before the International Academy of Pathology in Cleveland, Ohio, April, 1958.

Received for publication, September 15, 1958.

pericardium, a small curved-eye needle was passed deep to a branch of the left coronary artery, which was occluded by ligation with a cotton thread. Within one minute after successful arterial occlusion, the affected myocardium blanched and subsequently became cyanotic. The lung was then re-expanded, and an airtight closure of the chest in multiple layers was performed.

After a chosen interval, the chest was reopened through the previous incision, and the entire heart was removed and transected below the ligature. This second procedure was carried out with positive pressure respiration and additional anesthesia as required. Eighty rabbits were operated upon and investigated by histochemical methods. Electron microscopic studies were carried out in 37 of the experimental hearts and in 6 control hearts. In the experimental animals the intervals were from 5 minutes to 5 hours after coronary ligation. Animals were excluded from the study if a vein was inadvertently included in the ligature or if the characteristic blanching and cyanosis did not occur.

A slice of the ischemic region was removed, cut into cubes measuring 1 to 2 mm. on a side, fixed in buffered osmium tetroxide, dehydrated in alcohols, and embedded in methacrylate.[9-11] Numerous muscle blocks from non-ischemic portions of damaged hearts were processed in similar fashion. Thin sections of the methacrylate blocks were cut with a Porter-Blum microtome and mounted on grids with carbon films.[11-14] All photographs were taken with an RCA EMU 3B at initial magnifications of 3,000 to 12,000. Adjacent blocks of heart tissue were processed for histochemical studies. These will be reported subsequently.

Results

Normal Hearts

The structure of normal cardiac and skeletal muscle has been well described.[15-21] Although there has been disagreement concerning the surface structure,[22,23] the anatomic features relevant to the present investigation are depicted in Figures 1 to 3. In electron micrographs the surface membrane was clearly demonstrated to be a complex membrane system consisting of two dense areas separated by a lighter zone[15,17] (Figs. 1 to 3). The membrane complex is referred to as the sarcolemma which is adherent to the myofibrils at the "Z" band. As can be seen in Figure 2, this indented and was continuous with the endoplasmic reticulum, a structure which is well developed at the "Z" bands.[16,17] Small vesicles in the sarcoplasm were manifest near the membrane. In sections stained with the periodic acid-Schiff (PAS) stain, the sarcolemma was magenta and continuous with the intercalary discs. The PAS-positive material was not removed by diastase. The

general distribution and continuation with intercalary discs indicated that the sarcolemma (Figs. 1 and 3) was identical with the PAS-positive membrane observed with the light microscope. Specimens prepared by freeze drying or freeze substitution and stained with PAS demonstrated the polar localization of magenta-staining, perinuclear material which was removed by diastase. Figure 3, an electron micrograph from a region with abundant glycogen, demonstrates many granules 150 to 250 Å in diameter which were not sharply defined and were not associated with the endoplasmic reticulum as in the case of ribonucleic acid (RNA) granules.[24] The former have been tentatively identified as glycogen.[20,21] Although glycogen, as demonstrated by PAS staining and diastase, appeared to be located in other regions of the sarcoplasm, in electron micrographs it was clearly seen only around the nucleus.

The bundles of myofilaments, which occasionally branched, were frequently surrounded by mitochondria and elements of the endoplasmic reticulum (Fig. 1). Usually a mitochondrion was found in the fork of a branch. Cross sections of myofibrils showed close packing of filaments with hexagonal configuration (Fig. 8).[19]

In sections of fixed normal heart, contraction bands were generally in the region of the "Z" band (Fig. 1). These were the result of placing pieces of muscle directly in fixative with no attempt to stretch or maintain fiber length. In most longitudinal sections, a dense "H" zone appeared halfway between two "Z" bands. "I" bands were not visible in any of the normal, contracted preparations; however, "A" bands were prominent. Myofilaments coursed through a given cell and terminated at intercalary discs.[20,21]

Mitochondria were found throughout the heart muscle fiber. They were located about the nucleus at either pole, often in the same regions where glycogen was demonstrable. They were also found in long chains between bundles of myofilaments (Figs. 1 and 3). The structure of these bodies was similar regardless of their location within the myocardium.[25] The mitochondrial membranes were the sites of the greatest concentrations of the oxidative electron transfer systems.[26,27] The endoplasmic reticulum of striated muscle consists of tubules, vesicles and cisternas.[16-18] These are organized about the myofibrils in units that repeat with the sarcomeres. The endoplasmic reticulum of cardiac muscle was similar to that of skeletal muscle (Figs. 1 to 3). Its association with the sarcolemma is shown by the arrow in Figure 2. Points of continuity between the endoplasmic reticulum and the external portion of the nuclear envelope, described in other cell types, have been seen in heart muscle also.[28]

The nucleus was bounded by a double membrane (Fig. 3). Nucleoplasm was evenly distributed, without dense aggregates. The nuclear material itself was comprised of a random array of filamentous structures and denser granules. Nucleolar substance frequently appeared as an aggregate of closely packed granules.

Myocardial Infarction

Morphologic alterations occurred quickly in ischemic muscle. Five minutes after ligation of a coronary artery, perinuclear PAS staining was markedly decreased in intensity. This was reflected in electron micrographs by the absence of perinuclear glycogen particles of the order of 150 to 250 Å (Fig. 5). At no time after the onset of ischemia were these particles evident. There was a sharp division between fibrils having a normal glycogen content and those in which it was absent. The regions previously occupied by glycogen were now pale, and the endoplasmic reticulum was more prominent. There was no position shift of the mitochondria, endoplasmic reticulum, nucleus, or myofibrils in the regions depleted of glycogen. The fine fibrillar background was undisturbed by the loss of glycogen.

Light microscopy clearly demonstrated the sarcolemma in regions rendered ischemic for 12 hours. Electron micrographs of areas ischemic for 5 hours revealed rupture of sarcolemma but no disturbance of the spatial relationships of the dense and pale regions (Fig. 12). The external membrane was still in continuity with collagen fibrils. The middle pale layer was not swollen, nor was there any evidence of fibrillar material. The dimension of the inner membrane was still 80 Å; however, there was a decrease in number of the small vesicles formerly seen touching it and adjacent to it. This decrease was gradual and was first apparent about 1½ hours after arterial ligation. There was evidence of slight separation of adjacent cells in preparations ischemic for 10 to 15 minutes. In view of cellular shrinkage during fixation and dehydration and the minimal increase in intercellular space, this early change could represent artifact.

A definite accentuation of the intercalary discs was evident in the specimens ischemic for one hour or more (Fig. 10). Adjacent to the disc, an intracellular pale zone appeared which contained a few randomly placed fine filamentous structures. The vacuole formation associated with decrease in density of the entire muscle cell accentuated the darker intercalary disc. The distance between two cells at an intercalary disc did not change appreciably during 5 hours.

Within 5 minutes of arterial ligation, intracellular spaces appeared between myofilaments in the perinuclear region and around the

mitochondria (Fig. 4). Later these spaces increased in size and number. For one-half hour these spaces contained a loose array of very fine filamentous structures; subsequently they appeared empty. At half an hour the bundles of myofilaments showed longitudinal cracking and separation. The separation of myofilaments was random, since the size of the resulting bundles was variable. After 4 to 5 hours, transverse tearing of myofilaments was evident.

Comparison of tissues anoxic for 20 and 35 minutes revealed a marked change in the mitochondria. At 20 minutes they were almost normal, whereas at 35 minutes they had begun to swell. The cristae appeared more distinct because of decrease in the density of the matrix and separation. Throughout this period of swelling, the cristae maintained a rough semblance of their previous order in that they all projected perpendicularly from the surface toward the center. Because of swelling they did not approach the opposite side as closely. After 4 hours of ischemia the cristae of many mitochondria had disappeared while the cristae of others had lost spatial organization (Fig. 11). In a number of instances the limiting membrane was altered, and the overall appearance was similar to that of ruptured mitochondria.[26] The size of the ruptured mitochondria was decreased in relation to the swollen ones observed at 2 to 3 hours. In the specimens procured after 5 hours or longer, collapsed mitochondria were frequent but swollen ones still remained.

During the periods of cellular swelling the endoplasmic reticulum underwent similar modification. The first definite evidence of alteration of the tubules was seen in the specimens at 35 minutes in that simple enlargement occurred. They contained no electron-dense material. At 4 and 5 hours the swelling was more pronounced. Again because of the separation of myofibrils and surrounding loss of density, in the later preparations the system was more clearly seen. Similar alterations, i.e., swelling and enlargement of the vesicles and tubules, also could be seen in the Golgi apparatus near nuclei.

A unique feature of muscle tissue is the presence of various bands under different conditions of stretch. In our investigation two states of contraction were clearly visible. In most of the tissue ischemic for periods up to 20 minutes, the muscle showed contraction bands at the "Z" region, and darkening of the sarcomere midway between the contraction bands was often apparent. In muscle ischemic for 30 minutes or more, contraction bands were absent, but "Z," "I," "A," "H," and "M" bands were evident in most longitudinally sectioned fibers.

The alterations induced by ligation of rabbit coronary artery have

been well described.[29] Two features were very clearly demonstrated by electron microscopy: preservation of the subendocardial tissue, and extremely sharp limits to the lesion. Repeated examinations at the margins of lesions indicated that the transition zone between normal tissue and that maximally damaged measured about 0.1 mm. Within the area of ischemia the extent of damage was variable, so that adjacent fibers showed different degrees of swelling, vacuolization, nuclear alteration and the other features described above. Sections from muscle anoxic for 22 hours frequently exhibited cells undergoing dissolution and immediately adjacent cells still apparently capable of contraction.

Nuclear ischemic alterations were detectable within 5 minutes (Fig. 5). Early, the nucleoplasm showed clumping, and adjacent areas were the seat of rarefaction. Aggregation of nucleoplasm at the nuclear envelope was accentuated with prolongation of ischemia.

The degree of damage manifest in the muscle from one heart after 20 minutes of ischemia was comparable to that seen in the 3 and 4 hour preparations (Fig. 8). It was noted at the time the heart was removed from the carcass that it was fibrillating. The same reaction pattern was observed in two other hearts fibrillating at the time of removal, and it was assumed that the marked damage was attributable to fibrillation.

In order to determine whether the alterations were a result of curtailed blood flow, small fragments of normal heart tissue were excised and placed in .01 M Krebs-Ringer phosphate buffer with sufficient sucrose added to provide an osmolarity in the neighborhood of .34 (assuming 100 per cent ionization of the electrolytes). The tissue was incubated for 3 hours at 37° C. As shown in Figure 14, there was a deviation from normal, but the overall change was slight and not comparable to that seen 3 hours after coronary ligation.

The myocardium contains a number of other tissues than muscle. The fibrous tissue exhibited no change in the abundant material evaluated during periods up to 5 hours after ligation. The vascular endothelial cells developed cytoplasmic and nuclear alterations, though more slowly, than those noted in muscle.

Not uncommonly, red cells seemed to lose hemoglobin (Fig. 12). Since such a small portion of any cell was present in an electron micrograph, the overall volume could not be determined; however, the red cell margins were not crenated although the cells appeared to be swollen. In a number of instances red cells exhibiting leaching lay adjacent to red cells showing no loss of hemoglobin. Apparently, 2 to 3 hours after ligation there was an extensive diapedesis of both

red cells and neutrophils. At no time could a break in capillary continuity be detected, nor could a cell be seen in the process of leaving a vessel.

Discussion

A reasonable prelude to any discussion of observations should include consideration of possible sources of error. In these experiments many such sources existed, and some are not well understood. Procurement of specimens required two thoracotomy procedures with positive pressure respiration and on occasion two administrations of sodium pentobarbital anesthesia. It has been shown that 5 to 10 minutes of breathing 10 per cent oxygen is sufficient stimulus to reduce the cardiac glycogen by 80 per cent or more.[6] The general structure and the glycogen content were similar in both the control myocardiums and in the non-ischemic regions of hearts 3 hours after coronary ligation. It would seem reasonable, therefore, to discount the effects of the surgical and related trauma.

Alterations due to fixation are difficult to evaluate in electron microscopy since the appraisals of artifacts of this nature have been gathered from preparation techniques for conventional microscopy. Anoxia may alter tissue response to osmium tetroxide and other components of the fixative. This might be a constant artifact, or it might easily fluctuate in kind as well as degree under anoxic conditions. To our knowledge there is no satisfactory method of evaluating this point.

It has been demonstrated by Ring[29] that the extent of infarction induced by experimental coronary occlusion cannot be determined until approximately 5 days after ligation. The degree of collateral circulation in normal heart muscle has not been established.[30] The existence of a collateral circulation could result in inconstant damage to muscle fibers thus introducing another variable factor.

Our electron microscopic investigations have shown that very shortly after the blood supply to the myocardium was interrupted, clear spaces appeared in various portions of the muscle. It was apparent that these represented an acquired alteration since they were not present in the normal state and they occupied positions in the cell previously held by normal components. Early, the spaces had a fibrillar background similar to that in the normal cell matrix but less dense. Within 30 minutes the matrix became sufficiently diluted to become invisible.

The easiest explanation of this phenomenon is to assume an influx of fluid from the extracellular space. Currently there are two major concepts concerning intracellular osmolarity. If one accepts the idea

of an intracellular osmolarity considerably higher than that of the extracellular fluid (heart muscle, isotonic with .3 M sodium chloride) a rapid influx of fluid is easily explained.[31] This, of course, assumes either a ready permeability of the sarcolemma or an early and reversible loss of selective permeability in the absence of recognizable morphologic alteration and the continued utilization of energy by other components of the cell. Conway,[32] on the other hand, has expressed the belief that the intracellular and extracellular compartments are iso-osmolar, and that the differences observed by others were due to the breakdown of phosphocreatine and other energy-yielding compounds to form more particles, thus increasing the intracellular osmolarity. In the first instance, energy is utilized to maintain an osmotic gradient; in the second, reconstitution of metabolic products is necessary. This breakdown and subsequent freezing point depression take place rapidly at o°C. The observations made here do not aid in resolving the problem since the muscle in a low state of activity (excised and incubated control) showed little alteration, and the muscle in a state of increased activity (fibrillation) showed maximal alteration. In the first case little breakdown took place, or sufficient energy was available to maintain the concentration gradient. In the case of fibrillation, increased activity necessitated maximal utilization of energy sources and rapid production of many molecules. Thus insufficient energy was present to maintain concentration gradients resulting from excessive utilization by other cellular components.

The continuation of the plasma membrane with the endoplasmic reticulum in the region of the "Z" bands (Fig. 2) provides a mechanism by which the difference in potential between the sarcoplasm and intercellular space may be rapidly conducted laterally to all portions of a given cell. This is in agreement with evidence put forth that local contraction within a single cell may be initiated only in the region of the "I" band, and that the contraction is propagated for a short distance into the cell.[33]

The sarcolemma was maintained in a relatively normal morphologic state during the first 3 to 4 hours of swelling. At about the time that the mitochondria ruptured, the sarcolemma also was disrupted. Examination of intact portions of the sarcolemma in 4 hour preparations showed normal structure, and the disruption was thought to be due to simple cellular swelling. With bursting, the pressure on the sarcolemma was eliminated and cytoplasmic components freed. The more soluble elements appeared in plasma while those less soluble were probably destroyed *in situ*. Blood enzyme values in these rabbits were elevated, presumably because of the large amount of skeletal

muscle traumatized during the surgical procedures. The PAS reaction of the sarcolemma remained unchanged throughout the period of investigation.

Cardiac glycogen is claimed to be present in acid soluble and acid insoluble forms. Bloom demonstrated a 93 per cent reduction of acid soluble and a 75 per cent reduction of acid insoluble glycogen following anoxia for 4 minutes.[6] For this reason it was felt that the glycogen granules, which disappeared promptly in the ischemic myocardium, probably represented acid soluble or unbound glycogen. So far we have not found any particles corresponding to the glycogen accumulations at the "A," "I," and "Z" bands described by Studnitz[34] and Dempsey.[35]

Marked clumping of nucleoplasm occurred before very extensive cytoplasmic changes had taken place. The alteration consisted primarily of aggregation of nuclear substance and some tendency toward accumulation near the nuclear envelope. Associated with the clumping was a loss of density in many regions. Clumping of a similar nature had been encountered in cells fixed in acid solutions or those of high sodium chloride concentration (.9 per cent), in virus-infected cells and in cells which were unfixed for a period sufficient to permit a reduction of the pH of a .01 molar phosphate buffer to a point below 7. There are undoubtedly many other circumstances that can bring about nuclear clumping. Since this feature repeatedly occurs with little cytoplasmic alteration one may assume that excessive penetration of extracellular sodium ions or loss of intracellular potassium ions is not of paramount importance. In view of the rapid change in glycogen content, a shift in pH or an alteration of cytoplasmic osmolarity might affect the nucleus. Muscle cells are not well suited to solve this problem since they have a large component of highly stable cytoplasmic substance. For instance, mild pH or ionic shifts would not be expected to alter the myofilaments.

The mitochondrial alterations were those which might be expected in an "osmometer" placed in hypotonic solution. The earliest evidences of mitochondrial swelling appeared in 20 to 30 minutes and progressed for 3½ to 4 fours, at which time disruption and collapse began. Not all mitochondria proceeded through the complete cycle. Since swelling occurred so early and was accompanied by minimal cytoplasmic changes, one is faced with the same problem encountered in attempting to explain cellular swelling. However, in this instance there are a number of additional features. The mitochondrial swelling does not commence until the ischemia has existed for 20 or more minutes; from this point on it parallels cellular swelling. Moreover, swelling

of the mitochondria was proportional to the cytoplasmic alteration in the fibrillating heart. Thus, the mitochondrial alterations began at about the time the myofibrils lost their ability to contract, or at the time when there was no further energy available for contraction. It appears, therefore, that mitochondrial form is maintained in relation to evidences of available energy. It would seem that the source of energy for maintenance of mitochondrial integrity is related to that necessary for muscular contraction.

A striking feature was the existence of contraction bands at the "Z" region in all specimens of ischemic muscle at all periods up to 20 minutes after ligation; after 30 minutes the contraction bands were absent and did not return. The band was attributed to the irritative effects of the fixative on viable muscle. Its absence and the appearance of a distinct "I" band were thought to result either from an inability of fibrils to contract, or a loss of the energy necessary for contraction.

The spatial relationship of myofilaments to each other became more clearly manifest in lesions of longer duration. This probably resulted from loss of substance as well as density with stretching of muscle. Groups of myofilaments exhibited splitting, but the aggregates that remained showed no appreciable alteration of the hexagonal packing observed on cross section.

Ring[29] had shown that the extent of necrotic tissue 5 days after coronary ligation did not necessarily coincide with the initial distribution of blanching and cyanosis observed after coronary ligation and suggested that a minimum of 5 days was necessary to determine the extent of injury. In each instance, however, he noted a very sharp line of demarcation between damaged and normal muscle. In the rabbit hearts we examined, a similar line of demarcation was apparent; that the sharpness was related to the distribution of the blood supply was self evident. The exactness of demarcation could best be related to a functional end-artery status. The intermixture of intact and necrotic fibers at 5 days and the persistence of intact muscle in the fibrous scar at 6 weeks suggests the existence or the development of sufficient circulation to maintain their viability. This was brought out in electron micrographs of muscle fibers lying within the ischemic area in which variable degrees of damage were evident. Barely sufficient circulation may be augmented by varying degrees of muscular activity. Both light and electron microscopy demonstrated the preservation of a subendocardial zone. This has been a common observation and has been attributed to diffusion of nutrients from the cardiac chamber or the flow of blood through thebesian veins.

Endothelial cells have been considered to be sensitive to oxygen deficit; indeed, they exhibit alterations similar to those observed in muscle although the alterations develop at a slower rate. The energy expenditure of endothelium is less than that of muscle, a factor which may well explain the discrepancy in rate of degeneration.

Specimens of heart incubated for 3 hours at 37° in .01 M phosphate buffer, pH 7.4, showed only slight alteration in contrast to the lesions encountered in ischemic myocardium. The difference was probably related to the failure of contraction in the excised fragment; the ischemic muscle continued to contract and use energy for varying periods of time. The presence or absence of glucose in the incubating medium had little influence on the structure of the myocardium from the controls, indicating that exogenous sources of energy were not essential for periods as long as 3 hours.

SUMMARY

The premise that chemical alterations in experimentally induced ischemia of the myocardium are associated with morphologic alterations was substantiated. As early as 5 minutes after coronary arterial ligation in rabbits there was disappearance of glycogen from ischemic myocardium; simultaneously, structural changes became manifest. This was characterized by clumping of nucleoplasm and cytoplasmic distortion. Ischemia continuing for 30 minutes was sufficient to result in loss of contractile capacity and cell death.

The actual disintegration of muscle fibers proceeded at a varied rate, seemingly dependent in part upon the activity of the muscle during the period of ischemia.

Electron microscopy clearly demonstrated continuity of the sarcolemmal membrane of heart muscle and its endoplasmic reticulum. This was thought to provide a morphologic basis for the conduction of surface potentials throughout the cardiac muscle fiber.

REFERENCES

1. Himwich, H. E.; Goldfarb, W., and Nahum, L. H. Changes of the carbohydrate metabolism of the heart following coronary occlusion. *Am. J. Physiol.*, 1934, 109, 403–408.
2. Tennant, R.; Grayzel, D. M.; Sutherland, F. A., and Stringer, S. W. Studies on experimental coronary occlusion. Chemical and anatomical changes in the myocardium after coronary ligation. *Am. Heart J.*, 1936, 12, 168–173.
3. Bronson, L. H. Anatomical and chemical changes in the myocardium following short term coronary artery occlusion in dogs. *Yale J. Biol. & Med.*, 1937–1938, 10, 405–410.
4. Miller, J. L., and Matthews, S. A. Effect on the heart of experimental obstruction of the left coronary artery. *Arch. Int. Med.*, 1909, 3, 476–484.

5. Karsner, H. T., and Dwyer, J. E., Jr. Studies in infarction. IV. Experimental bland infarction of the myocardium, myocardial regeneration and cicatrization. *J. Med. Res.*, 1916, **34**, 21–39.
6. Bloom, W. L. Glycogenolysis in the anoxic heart. *Am. J. Physiol.*, 1956, **186**, 518–520.
7. Lowry, O. H.; Gilligan, D. R., and Hastings, A. B. Histochemical changes in the myocardium of dogs following experimental temporary coronary arterial occlusion. *Am. J. Physiol.*, 1942, **136**, 474-485.
8. Kent, S. P., and Diseker, M. Early myocardial ischemia: study of histochemical changes in dogs. *Lab. Invest.*, 1955, **4**, 398–405.
9. Yokoyama, H. O.; Jennings, R. B.; Clabaugh, G. F., and Wartman, W. B. Histochemical studies of early experimental myocardial infarction. Periodic acid-Schiff method. *A. M. A. Arch. Path.*, 1955, **59**, 347–354.
10. Palade, G. E. A study of fixation for electron microscopy. *J. Exper. Med.*, 1952, **95**, 285–298.
11. Caulfield, J. B. Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 827–830.
12. Porter, K. R., and Blum, J. A study in microtomy for electron microscopy. *Anat. Rec.*, 1953, **117**, 685–710.
13. Latta, H., and Hartmann, J. F. Use of a glass edge in thin sectioning for electron microscopy. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 436–439.
14. Watson, M. L. The use of carbon films to support tissue sections for electron microscopy. *J. Biophys. & Biochem. Cytol.*, 1955, **1**, 183–184.
15. Robertson, J. D. Some features of the ultrastructure of reptilian skeletal muscle. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, 369–380.
16. Bennett, H. S., and Porter, K. R. An electron microscope study of sectioned breast muscle of the domestic fowl. *Am. J. Anat.*, 1953, **93**, 61–105.
17. Porter, K. R. The sarcoplasmic reticulum in muscle cells of *Amblystoma* larvae. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, No. 4 Suppl., 163–170.
18. Porter, K. R., and Palade, G. E. Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 269–300.
19. Huxley, H. E. The double array of filaments in cross-striated muscle. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 631–648.
20. Fawcett, D. W., and Selby, C. C. Observations on the fine structure of the turtle atrium. *J. Biophys. & Biochem. Cytol.*, 1958, **4**, 63–72.
21. Muir, A. R. An electron microscope study of the embryology of the intercalated disc in the heart of the rabbit. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 193–202.
22. Gutmann, E., and Young, J. Z. The re-innervation of muscle after various periods of atrophy. *J. Anat.*, 1944, **78**, 15–43.
23. Perry, S. V. Relation between chemical and contractile function and structure of the skeletal muscle cell. *Physiol. Rev.*, 1956, **36**, 1–76.
24. Palade, G. E., and Siekevitz, P. Liver microsomes; an integrated morphological and biochemical study. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, 171–200.
25. Palade, G. E. An electron microscope study of the mitochondrial structure. *J. Histochem.*, 1953, **1**, 188–211.
26. Watson, M. L., and Siekevitz, P. Cytochemical studies of mitochondria. I. The separation and identification of a membrane fraction from isolated mitochondria. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, 639–652.

27. Barrnett, R. J., and Palade, G. E. Histochemical demonstration of the sites of activity of dehydrogenase systems with the electron microscope. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 577–588.

28. Palade, G. E. The endoplasmic reticulum. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, No. 4 Suppl., 85–98.

29. Ring, P. A. Myocardial regeneration in experimental ischaemic lesions of the heart. *J. Path. & Bact.*, 1950, **62**, 21–27.

30. Bing, R. J.; Castellanos, A.; Grado, E.; Lupton, C., and Siegel, A. Experimental myocardial infarction: circulatory, biochemical and pathologic changes. *Am. J. M. Sc.*, 1956, **232**, 533–554.

31. Opie, E. L. Osmotic activity in relation to the movement of water under normal and pathological conditions. *Harvey Lect.* (Series L, 1954–1955). Academic Press, Inc., New York, 1956, 292–315.

32. Conway, E. J. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.*, 1957, **37**, 84–132.

33. Huxley, A. F., and Taylor, R. E. Function of Krause's membrane. (Letter to the editor.) *Nature, London*, 1955, **176**, 1068.

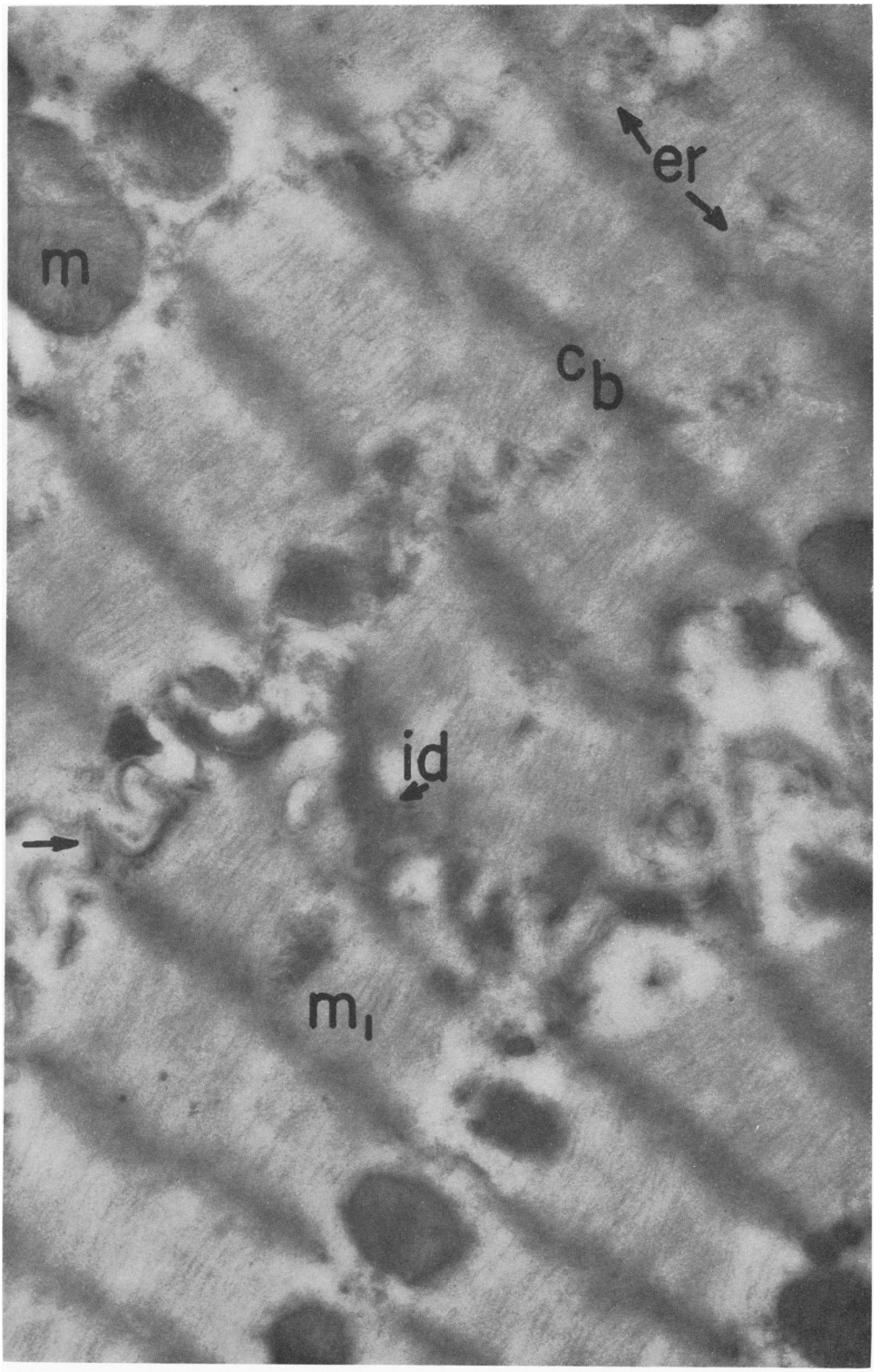
34. Studnitz, G. Versuche zur Deutung der Querstreifung des Muskels; der Glykogenspiegel der A- und J-Schichten während der Ruhe. Kontraktion und Erholung. *Ztschr. f. Zellforsch. u. mikr. Anat.*, 1935, **23**, 270–279.

35. Dempsey, E. W.; Wislocki, G. B., and Singer, M. Some observations on the chemical cytology of striated muscle. *Anat. Rec.*, 1946, **96**, 221–247.

[*Illustrations follow*]

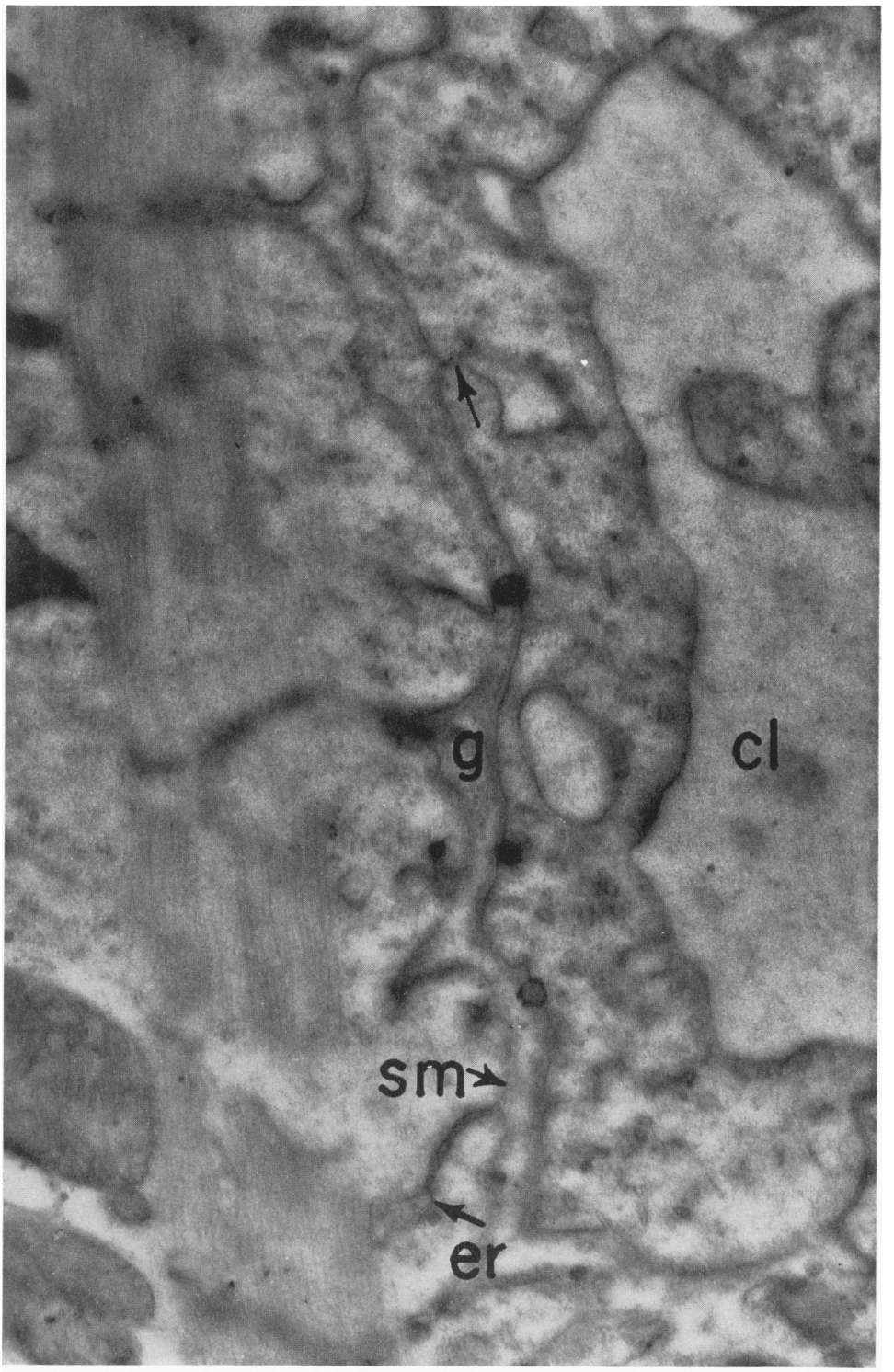
LEGENDS FOR FIGURES

FIG. 1. Normal rabbit myocardium. A prominent intercalary disc (id) is continuous with the cell membrane (arrow). This relationship holds at the right margin as well. Elements of the endoplasmic reticulum (er) are particularly evident in the region of contraction bands (cb). Mitochondria (m) have a linear arrangement between bundles of myofilaments. A tangential section of a mitochondrion is present at m_1. There are two lipid bodies visible adjacent to the mitochondrion in the lower right corner. × 50,000.



1

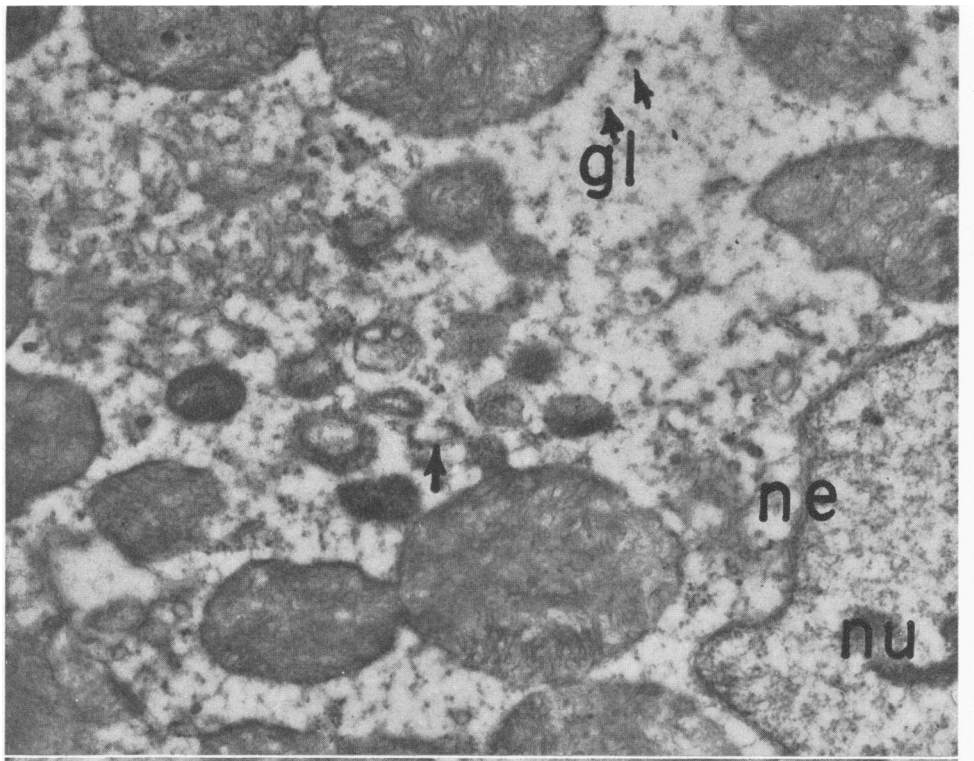
FIG. 2. Normal myocardium. The sarcolemma (sm) is indented in the regions of contraction bands. Arrows in the lower portion of the photograph indicate the indentation of the sarcolemma to be continuous with the endoplasmic reticulum (er). Between a capillary and the muscle fiber there are numerous unidentifiable granules (g), some of which may represent cross sectioned collagen. Arrows in the upper portion designate an invagination of the capillary membrane forming a large intracytoplasmic vesicle. The granular precipitate within the capillary lumen (cl) is frequently seen and probably represents precipitated plasma protein. × 40,000.



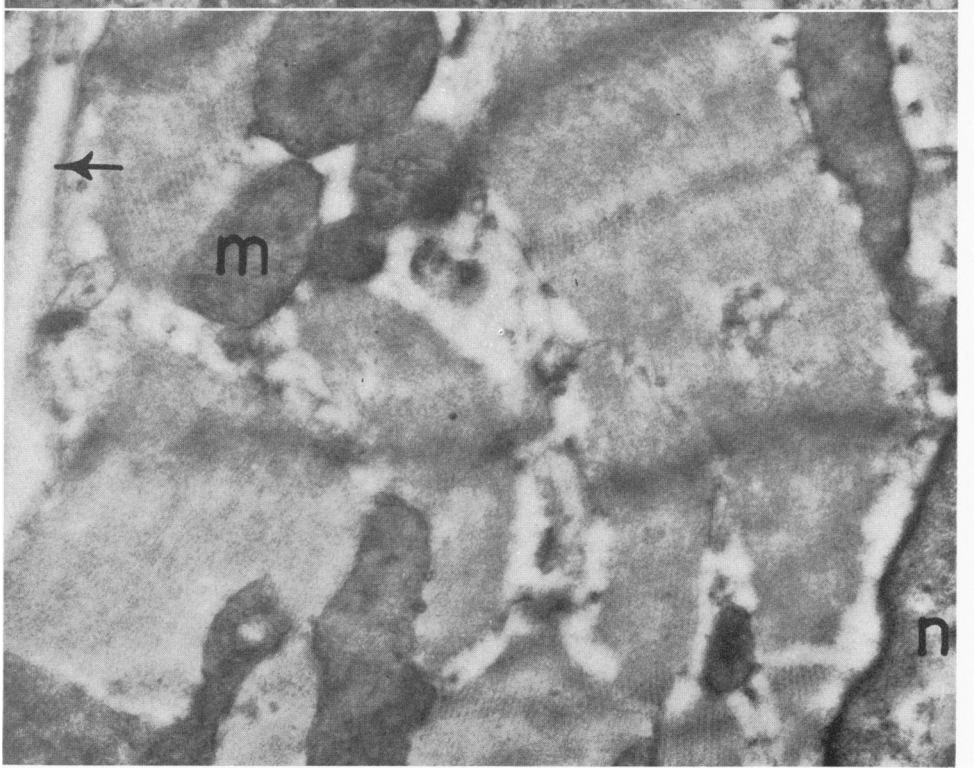
2

FIG. 3. Normal myocardium. Glycogen granules (gl) are clearly visible in the perinuclear region. A segment of endoplasmic reticulum with ribonucleic acid granules is indicated by the arrows. The smooth vesicular structures are present in random arrangement in perinuclear location. The nuclear envelope (ne) encloses a mass of evenly distributed granular and fibrillar substance. The nucleolus (nu) is much more dense than the nucleoplasm. × 23,000.

FIG. 4. Myocardial ischemia, 5 minutes. Clear spaces are evident around unaltered mitochondria and between the myofibrils. The space between two cells (arrow) is not increased appreciably. A small segment of nucleus may be seen (n). × 23,000.

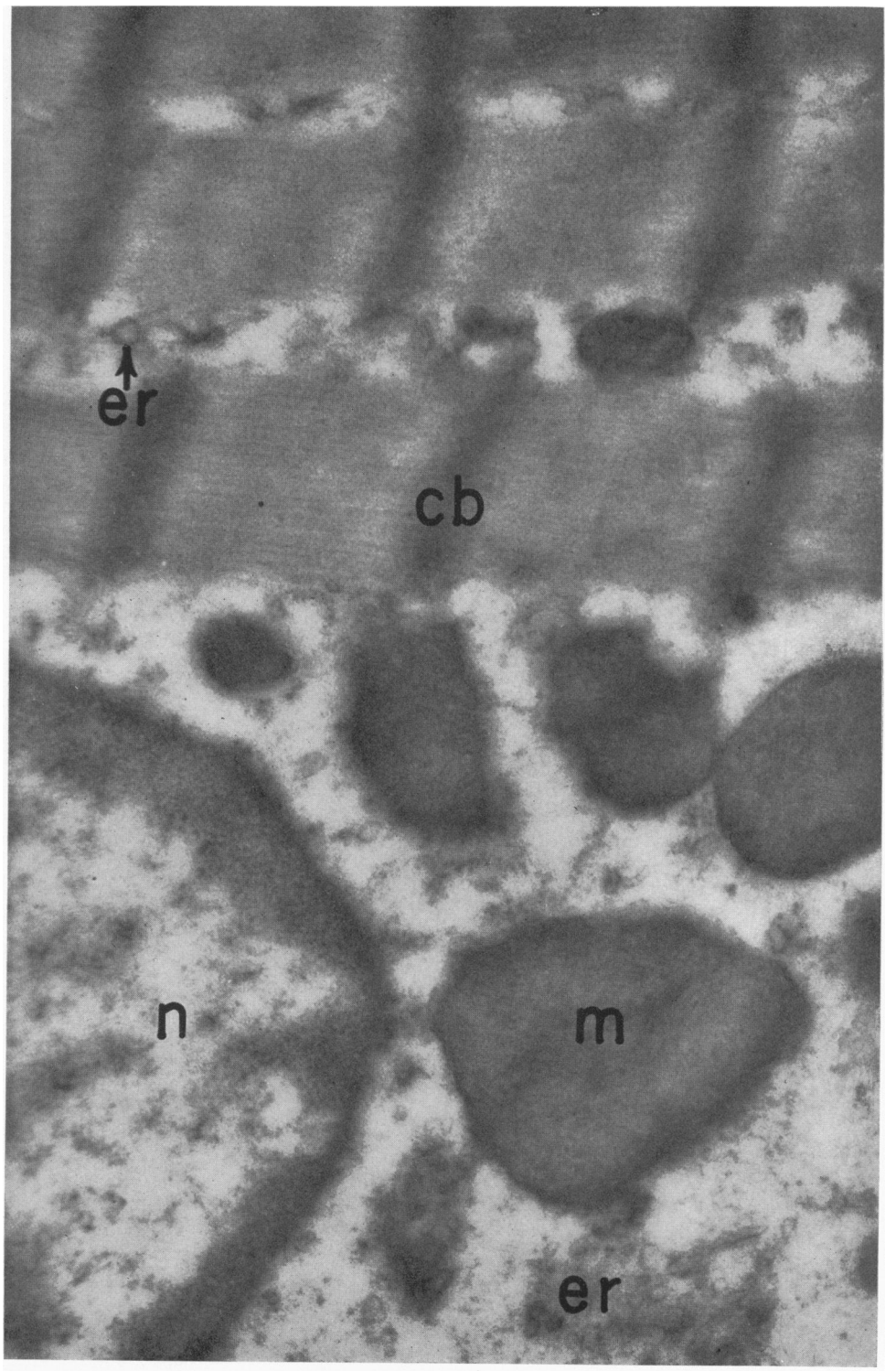


3



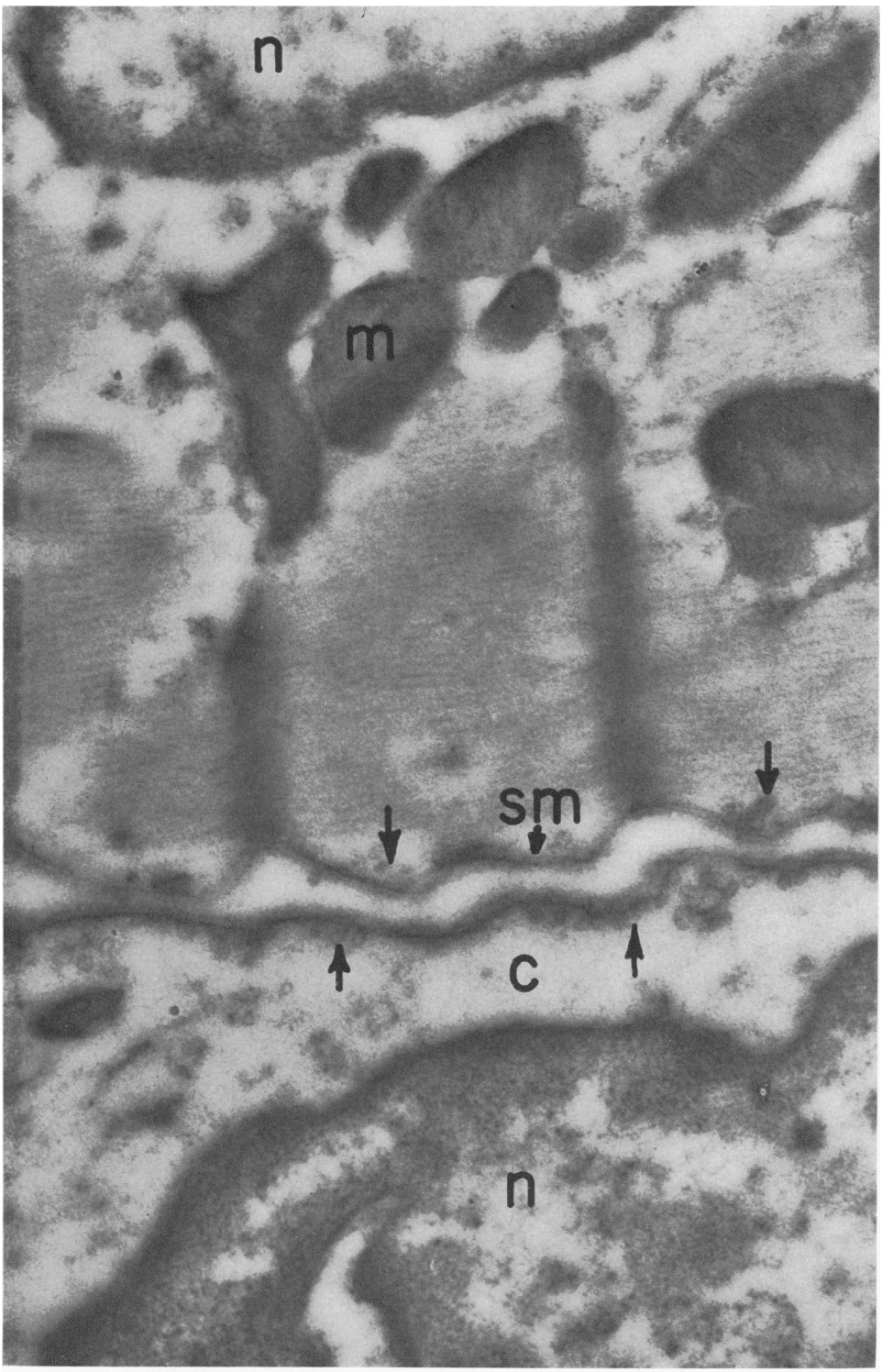
4

FIG. 5. Myocardial ischemia, 5 minutes. Perinuclear glycogen has disappeared. The nucleoplasm (n) shows clumping and margination. Endoplasmic reticulum (er) is normal at this stage and contraction bands are visible. × 50,000.



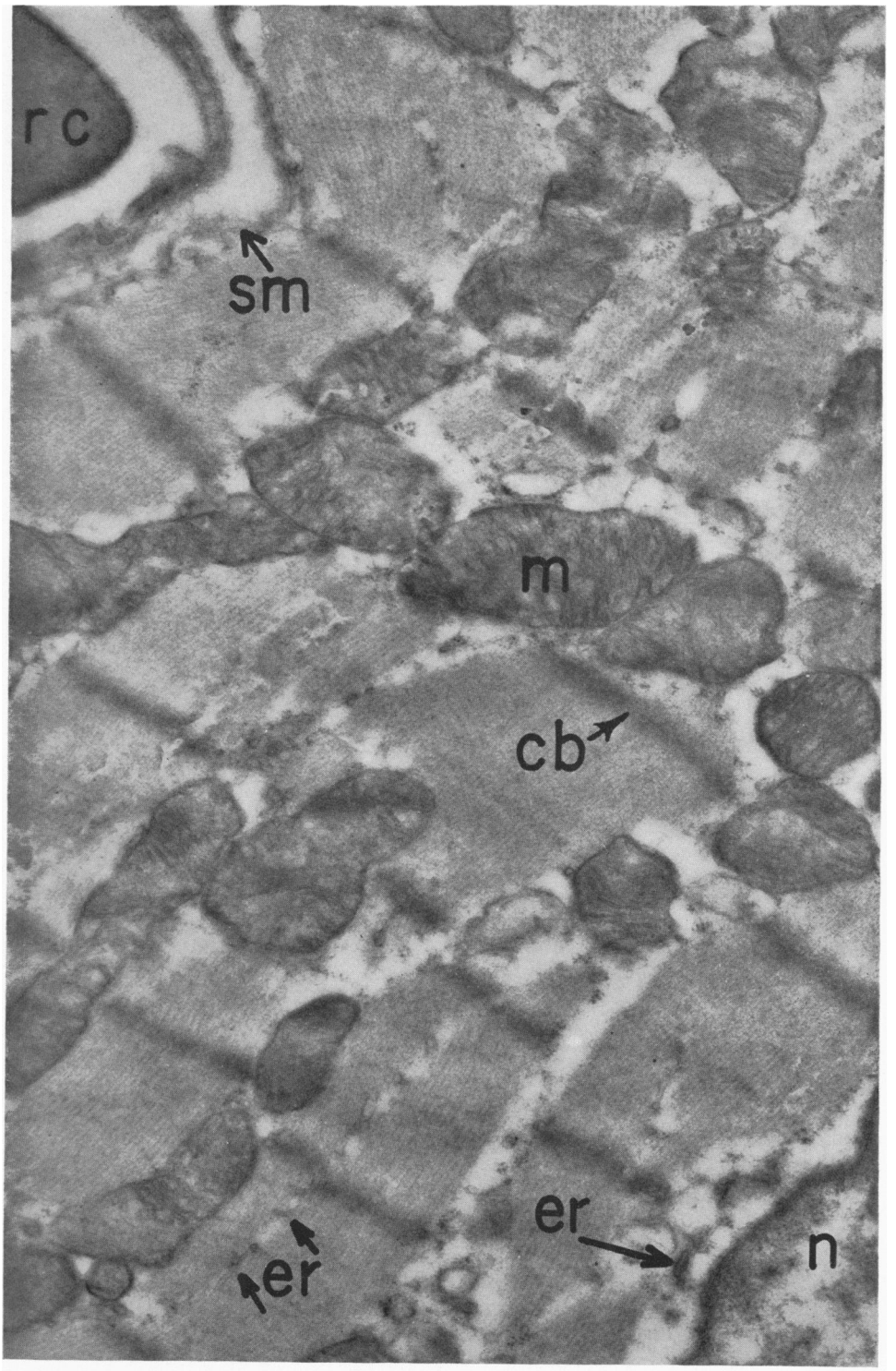
5

FIG. 6. Myocardial ischemia, 10 minutes. Segments of muscle and endothelium (c) are visible. Numerous small vesicles appear near the cytoplasmic membrane (arrows) of each cell type. There is some clumping of the endothelial nucleoplasm (n). The attachment of the sarcolemma at the "Z" band region is shown. Alterations of the muscle nucleus are similar to those seen in endothelium, but are of a more advanced nature. × 40,000.



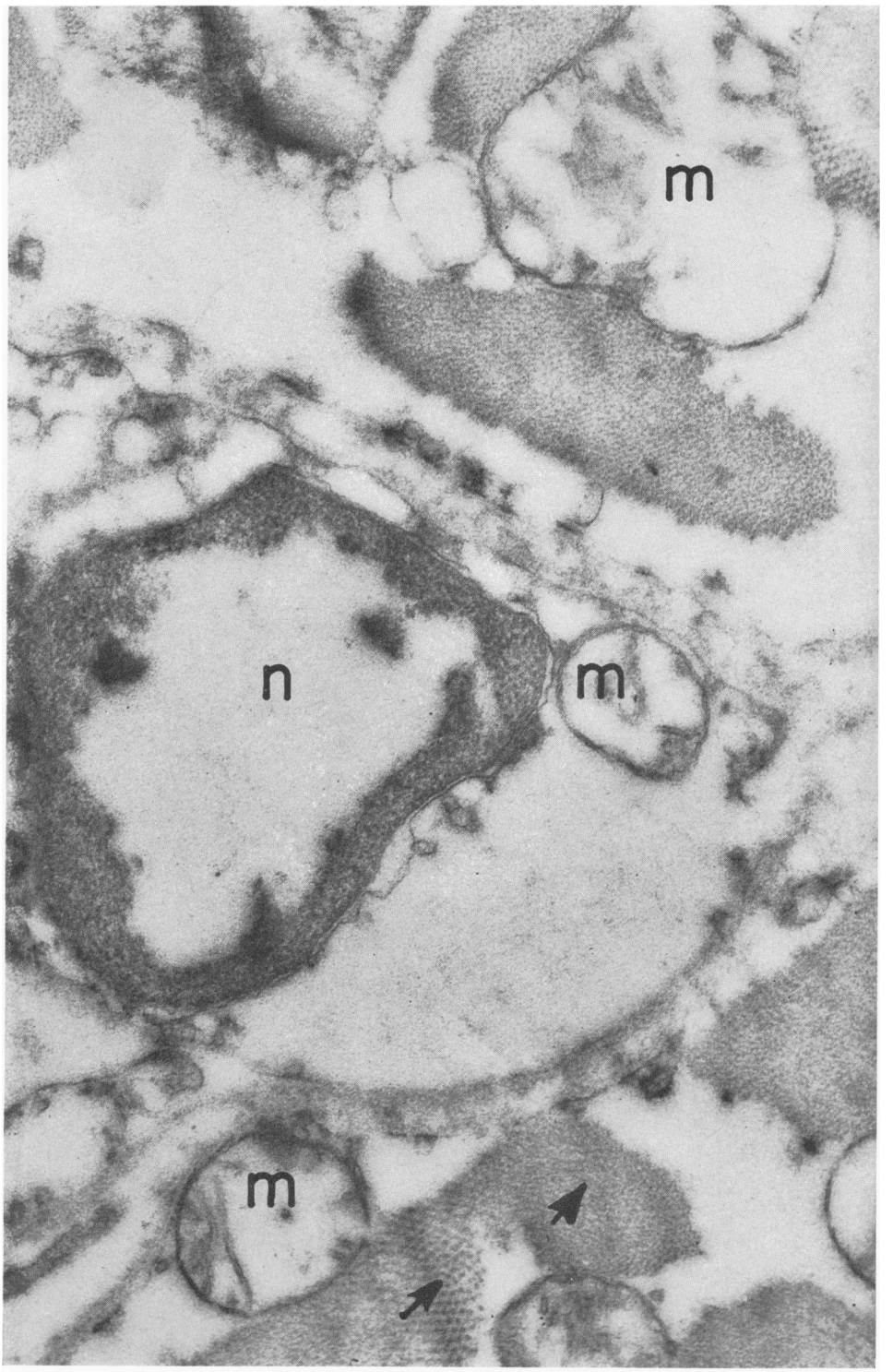
6

FIG. 7. Myocardial ischemia, 20 minutes. There is little more than progression of the intracellular swelling seen in figures 4 to 6. Mitochondria retain normal appearance. A portion of an erythrocyte may be seen (rc). × 23,000.



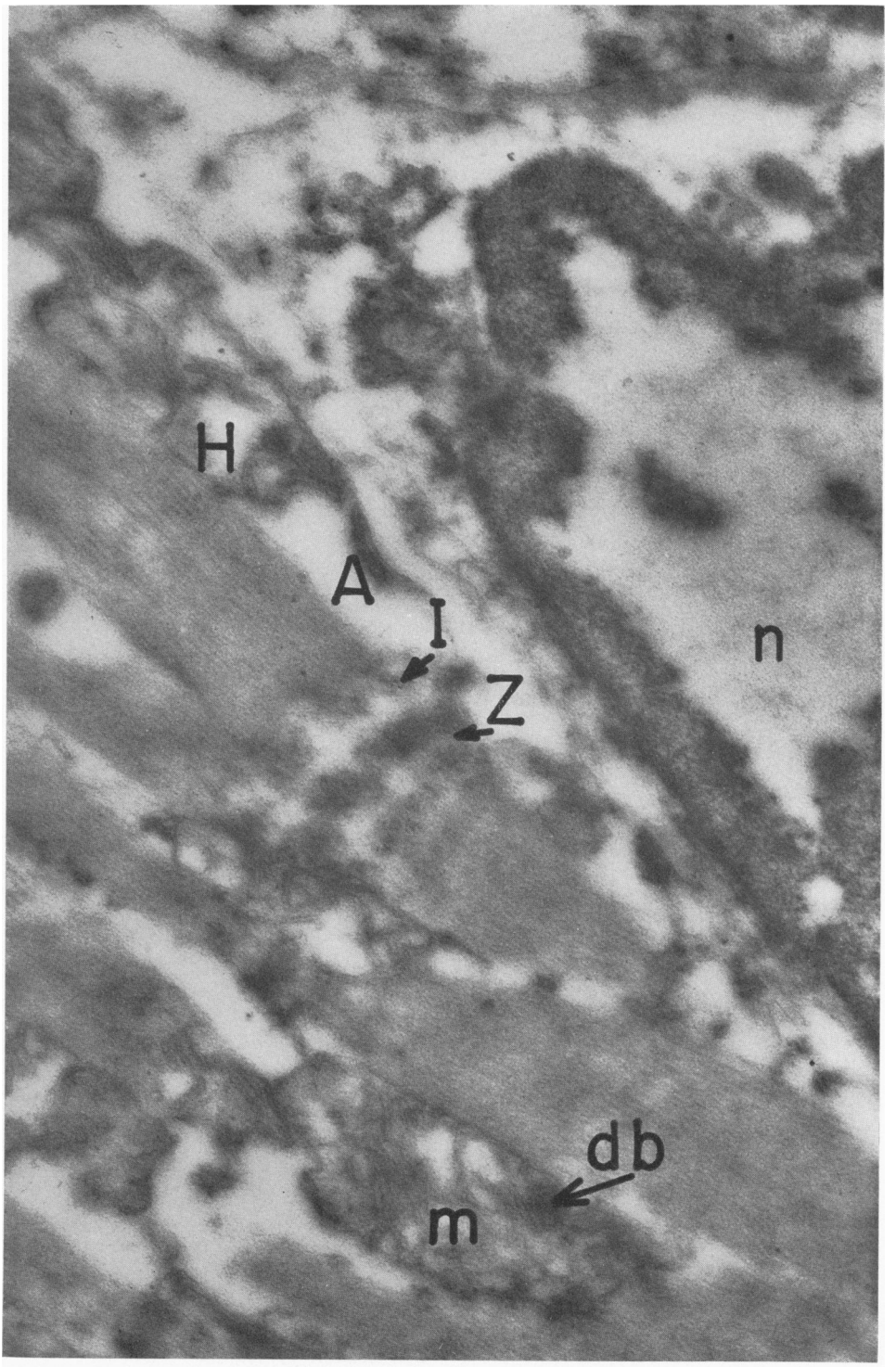
7

FIG. 8. Myocardial ischemia, 20 minutes; heart fibrillating at the time of removal. The large endothelial nucleus (n) appears in the center. The myocardial nuclei are comparable in appearance. Much intracellular edema results in separation of myofilaments. The usual fibrillar cytoplasmic matrix is not visible. Mitochondria are markedly swollen and are of decreased density. Tangential sections of two myofilaments are indicated by arrows. × 50,000.



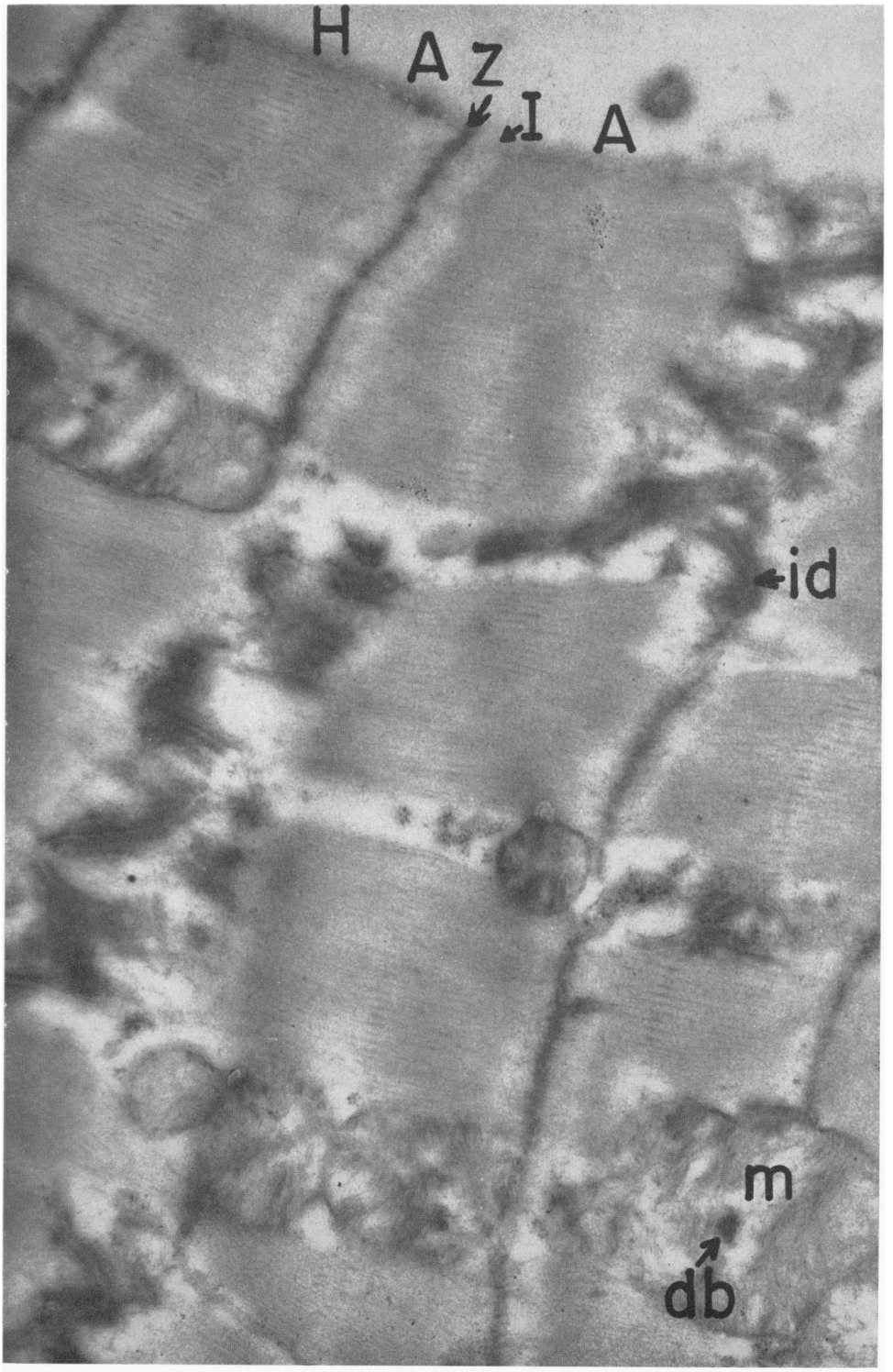
8

FIG. 9. Myocardial ischemia, 35 minutes. Nuclear and cytoplasmic alterations are in more advanced state. Myofibrils lack contraction bands, and an "I" band is present. Mitochondria are swollen, dark bodies (db) appear within them and the spatial arrangement of their cristae is disturbed. × 50,000.



9

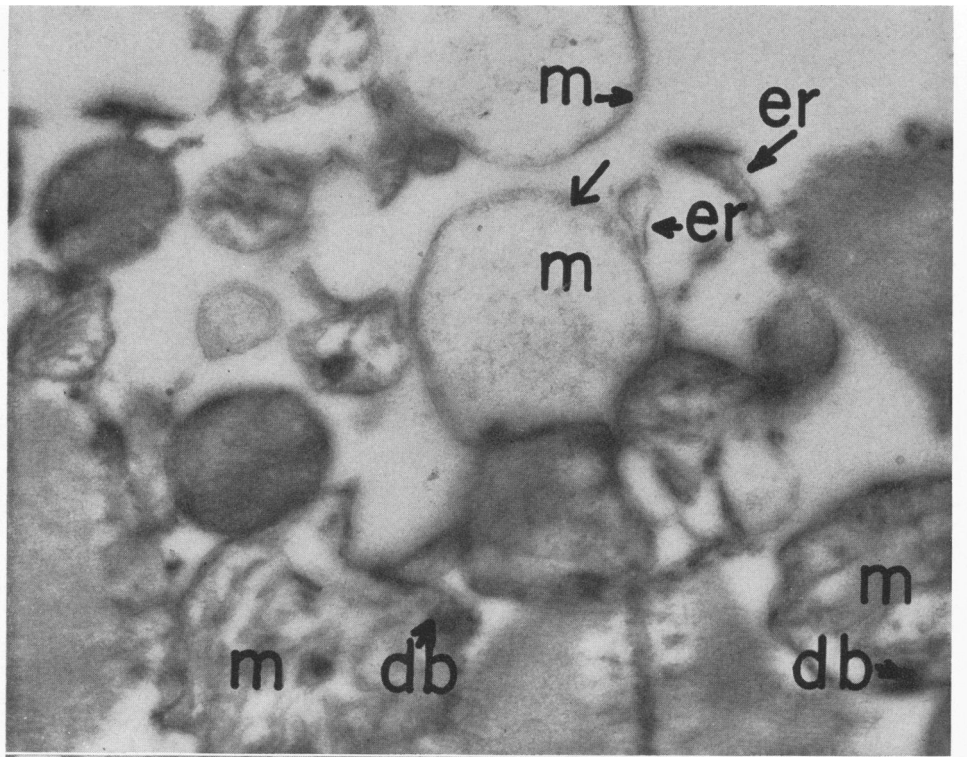
FIG. 10. Myocardial ischemia, one hour. The intercalary disc (id) shows no evidence of separation but clear areas are evident adjacent to it. These are in continuity with the spaces between myofibrils and apparently represent a similar process. Mitochondria resemble those seen in Figure 9. × 28,000.



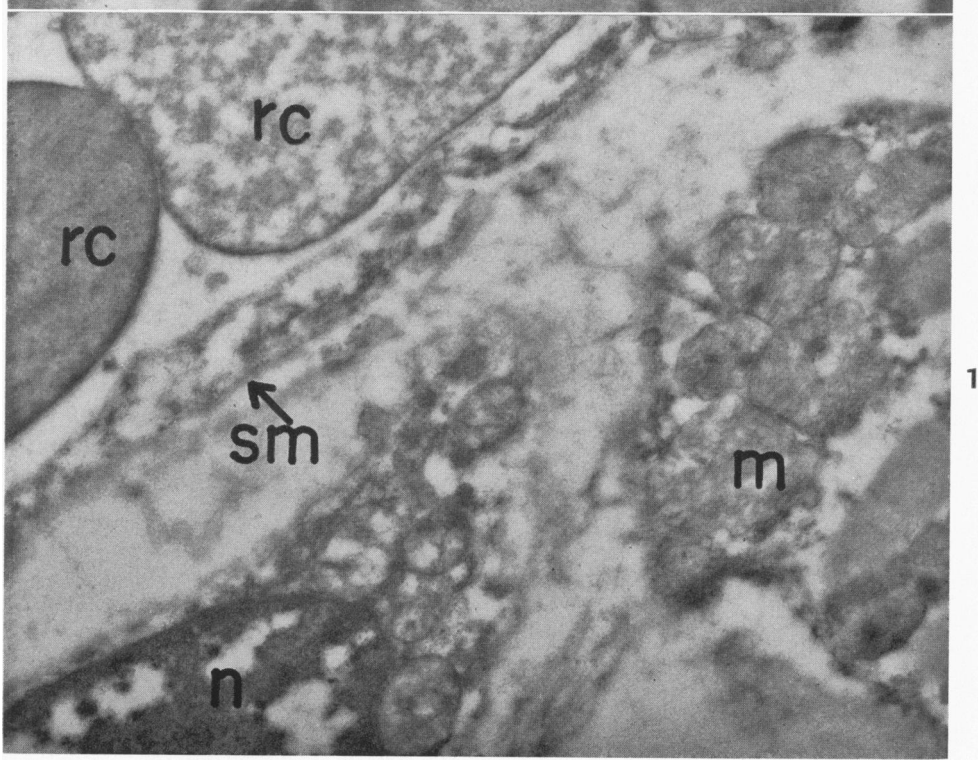
10

FIG. 11. Myocardial ischemia, 4 hours. There is disruption of mitochondria (m) and loss of their content. Swollen endoplasmic reticulum (er) is common at this stage. × 35,000.

FIG. 12. Myocardial ischemia, 5 hours. Two red cells (rc) show very different degrees of hemoglobin loss. There is continued progression of the degenerative changes in muscle. Loss of continuity of the sarcolemmal membrane is now visible although the fibers still maintain their relations and organelles are not extruded. × 16,800.



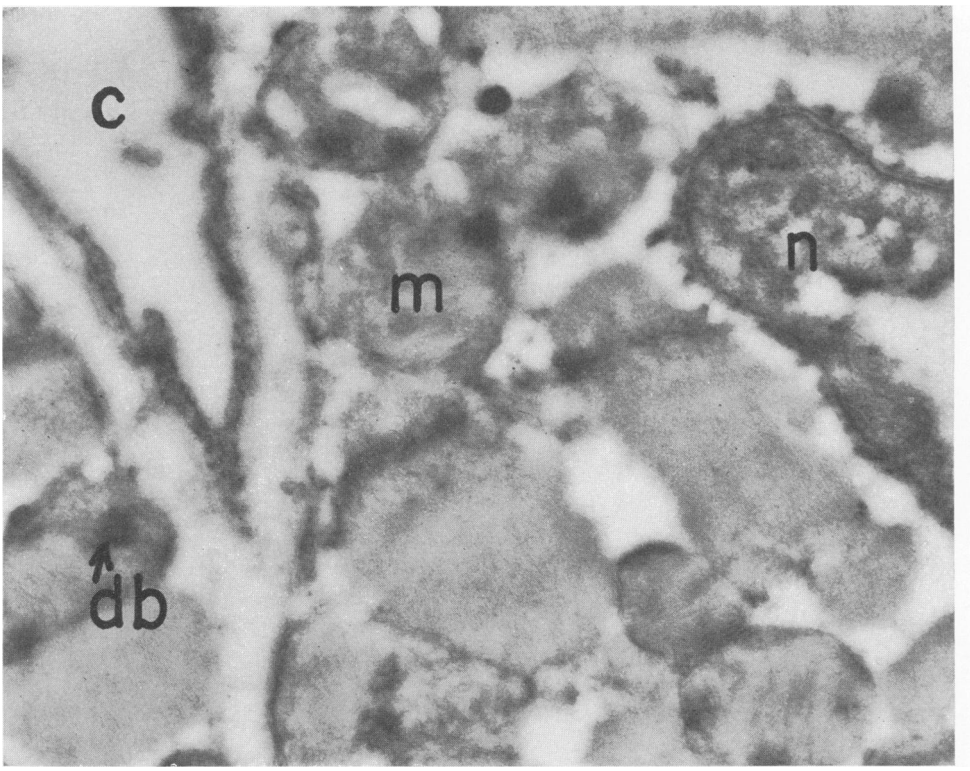
11



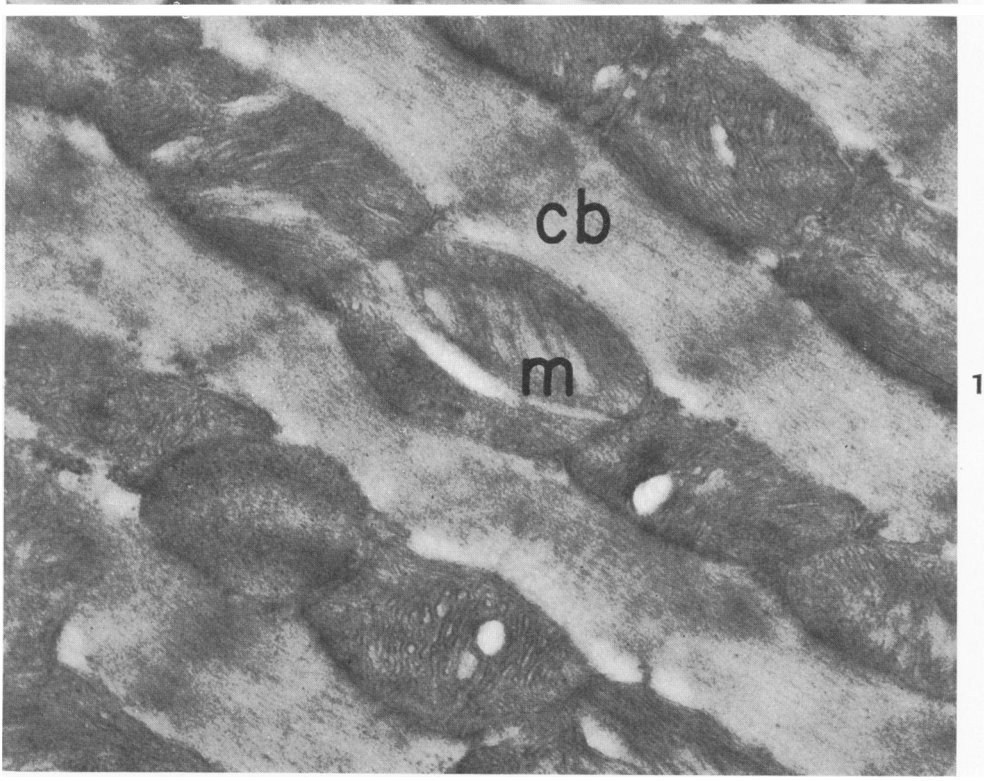
12

FIG. 13. Myocardial ischemia, 22 hours. Large numbers of dense bodies (db) are visible both inside and outside of mitochondria. Despite progression of alterations no new features are noteworthy. × 23,000.

FIG. 14. Normal heart incubated at 37°C. for 3 hours in .01 M Krebs-Ringer phosphate buffer. There is only slight edema of the muscle fiber and minor alterations of the mitochondria. Contraction bands (cb) are maintained. The endoplasmic reticulum appears normal. The nucleus, not illustrated, shows some margination of nucleoplasm. × 50,000.



13



14