An Electron Microscopic Study of Myocardial Ischemia in the Rat

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Myocardial infarcts were produced in rats by ligating the anterior descending branch of the left coronary artery. After one hour, changes in the ultrastructure of myofibers in the infarcts were readily demonstrated by electron microscopy. These changes became progressively severe with time, and consisted primarily of swelling of mitochondria and sarcoplasmic reticulum followed by increased lipid droplets and myolysis. Autolysis of myocardium was also studied and the changes found to be similar to those of myocardial infarcts, but more uniform and slightly delayed in development. Perhaps the early ultrastructural changes are related to hyperosmolarity of mitochondria and sarcoplasmic reticulum, especially after cell death. It is hoped that definition of ischemic ultrastructural change will be useful in defining electron microscopic lesions in other forms of myocardial damage.

The rapid development of technic in electron microscopy and the description of normal histologic ultrastructure have made possible the use of this method in many fields that are mainly dependent on light microscopy. The application of this technic to pathologic study may permit correlation of metabolic disturbance with ultrastructural change. Muir, Hodge, Price et al., and Moore have described the normal electron microscopic structure of cardiac muscle. The fine structure of skeletal muscle has been studied by Bennett, Porter and Palade, Edwards et al., and by Spiro. Barnett and Palade have reported the ultrastructural changes occurring with autolysis of rat myocardium. Moore, Ruska and Copenhaver described the degenerative process in leg muscles distal to a tourniquet.

In the investigation described here the anterior descending coronary artery was ligated and early morphologic changes in the ischemic rat myocardium were studied. The definition of ischemic changes may eventually lead to a better understanding of conditions that limit the reversibility of cardiac arrest or the induction of subtler lesions of a chronic nature.

Methods

Healthy, adult male rats of the Sprague-Dawley strain were used. The results to be described were of experimentation on 12 suitable rats. Others were unsuitable because of early death or improper ligation of the coronary artery. Ether anesthesia was used; the animals were maintained during open thoracic surgery with oxygen-ether mixture under positive pressure. The anterior descending branch of the left coronary artery was ligated 2 to 4 mm. from its origin with 0000 silk suture according to the method of Johns and Olson. Respiration was maintained with positive-pressure oxygen until the animals regained consciousness. The rats were killed at 0, 1, 2, 3, 4, 5, 24 and 48 hour postoperative intervals. From each rat a 3 by 3 mm. piece of the ventricular wall was removed 2 to 3 mm. distal to the ligature, and a similar block of tissue was taken from the posterior portion of the left ventricular myocardium as a control. To determine the effect of autolysis and to compare it with the early changes in myocardial infarction, two animals were killed immediately after operation and their bodies kept at a temperature of 37 C. Blocks of tissues were removed from the left ventricle immediately, at 20 min. and thereafter at hourly intervals for 5 hours.

All tissues were placed in Eulitt's osmium tetroxide fixative within 5 min. of removal, dehydrated in graded concentrations of ethanol and embedded in methacrylate. Thin sections were cut on a Porter-Blum microtome and examined
Figs. 1, 2 and 3. See legend on opposite page.
in an RCA-EMU-3B electron microscope at 1,000 to 7,000 diameters. Thicker sections of each block were examined by phase microscopy for general orientation. Paraffin sections of formalin-fixed tissue were stained with phosphotungstic acid hematoxylin and hematoxylin eosin and studied by light microscopy.

RESULTS

General Observations

The ventricles of hearts, of rats killed after ligation of a coronary artery, were consistently flabby and slightly dilated. Gross changes in infarcted areas were essentially the same as those described by Johns and Olson. No definite gross changes could be detected in infarcts of less than 5 hours' duration. The 5 hour infarcts were pale, fairly well demarcated areas. Borders of 24 and 48 hour infarcts were sharply defined by narrow zones of hyperemia with pale-grey central areas. These changes clearly defined the location of infarcts which were about 5 mm. in diameter and always 1 to 2 mm. distal to the ligature.

Infarcts of 5 hours or less duration could not be differentiated from normal myocardium by light microscopy. Obvious necrosis of muscle and a peripheral zone of inflammatory infiltrate were apparent in the 24 and 48 hour infarcts. In both the latter, fibers were eosinophilic and structureless, with loss of cross striations, identical to the appearance of early myocardial infarcts in human autopsy material.

Electron Microscopic Observations

Normal Myocardium. The ultrastructure of myocardium of the rat was similar to that described by Muir,2 Palade,18 and Bennett and Porter17 in various laboratory animals (fig. 1). (For letters of identification used in figures see table at end of discussion. We were unable to demonstrate lesions attributable to the anesthesia or surgical procedures. Myocardial fine structure was alike in the unanesthetized unoperated control animals, in those killed immediately after operation and in the muscle removed from the posterior portion of the left ventricular wall in the rats subjected to ligation of the left coronary artery. Banding phenomena were seen and interpreted as representing various stages of contraction6,18 (figs. 1 and 2). The double membrane forming the sarcolemmal sheath16 was 'scallop ed' by regular indentations at insertions of Z bands where they end after crossing the myofibers.3,5,20 Within the myofibers the individual myofibrils were separated by rows of mitochondria possessing abundant cristae. The latter were formed of double membranes and appeared to fill so nearly the mitochondria that only occasional areas of granular matrix lay between them.21-23 Sarcoplasmic reticulum6-8,16 was widely distributed within

![Fig. 1 Top](image-url) Normal rat myocardium from uninvolved area of left ventricle (same rat as figure 4). Muscle is contracted and demonstrates only Z, A and H bands, I bands not being visible. Rows of mitochondria parallel myofibrils in the long axis of the muscle, forming a complete cylinder around them. Sarcoplasmic reticulum is most conspicuous in areas adjacent to mitochondria or between rows of myofilaments. One lipid droplet is present. Mitochondria are uniformly dense and filled with cristae. Note variation in apparent mitochondrial size. X 23,400.

![Fig. 2 Middle](image-url) 1 hour autolysis. In relaxed state I bands can be seen. Interfibrillar space is widened and vesicular sarcoplasmic reticulum easily apparent. Myofibrils not remarkably swollen but rounded, and small areas of dense clumping are present within random mitochondria. Only 2 lipid droplets present (lower right). The sarcoplasm can be seen at lower right with insertions of the Z bands. X 20,700.

![Fig. 3 Bottom](image-url) 1 hour infarction. Swollen mitochondria have dense, clumped cristae and clear, prominent, mitochondrial matrix. Clumping and sites of density irregularly located in the mitochondria. The change is not uniform. Interfibril distance is increased and sarcoplasmic reticulum swollen. Z-bands indistinct but not uniformly affected. Myofilaments sharply defined. X 17,100.
Figs. 4, 5 and 6. See legend on opposite page.
the cell; because of its tubular structure it was usually seen as small vesicles, most prominent when adjacent to mitochondria and in the space between the sarcolemma and the outermost myofibril of the cell. Intercalated discs, the lines of end-to-end attachment between longitudinally arranged muscle cells, were heavy, tortuous double membranes which zig-zagged across the fibers in the same plane as Z bands and perpendicular to the long axis of the fiber. Occasional lipid bodies were seen in the sarcoplasm (fig. 1).

Myocardial Infarction. Definite alterations in intracellular structure were demonstrated by electron microscopy in tissue that had been removed from the anterior wall of the left ventricle even 1 hour after ligation of the coronary artery (fig. 3). Enlargement of the vesicles made the saroplasmin reticulum prominent. The sarcoplasm also appeared swollen, especially beneath the sarcolemma, with a decrease in density of granular material and an increase in separation of the fibrils. Some mitochondria were relatively unaffected but many were moderately enlarged with increased matrix between the coarse, clumped cristae. The alterations of mitochondria were by far the most prominent changes.

After 2 hours of ischemia the swelling of sarcoplasm and vesiculation of sarcoplasmin reticulum had progressed (fig. 4). Lipid bodies were slightly increased in number and were usually found adjacent to mitochondria and sarcoplasmin reticulum. Mitochondria had become prominent and almost uniformly enlarged, with a marked increase in the ratio of matrix to cristae. The latter were clumped and their double membranes were coarsened. Separation of myofibril was increased by the enlargement of mitochondria and sarcoplasmin reticulum. In other areas separation was seen without mitochondrial swelling. Sarcolemma was separated from the Z bands at many points. Coarsely granular, dense material, either clumped chromatin or lipid, was present in small amounts in nuclei of the muscle cells. The dark, osmophilic substance composing the borders of intercalated discs had become granular and less sharply defined.

The changes described above were more marked in the tissues infarcted for 3 hours. By 4 hours the sarcoplasmin reticulum was remarkably swollen and some vesicles had ruptured (fig. 5). Coarse (lipid) bodies were contained in nuclei of muscle cells. Mitochondrial enlargement was great in this period (fig. 6). Myofilaments were coarsened and no longer sharply outlined or individually distinct; muscle bands were similarly affected, so that Z bands appeared granular and their borders irregular and indistinct.

At 5 hours, Z bands and myofilaments had become even less distinct in many areas. Sarcoplasmin reticulum was further distended. Internal structure of many mitochondria was completely lost and many were collapsed, presumably after their rupture. Lipid bodies had increased in size and number at this stage.

Twenty-four hours after ligation of the coronary artery the myocardium showed a variety of changes, depending on the relation

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**Fig. 4 Top.** 2 hour infarct. Mitochondrial swelling has progressed. Several empty mitochondria are seen. Swollen sarcoplasmin reticulum easily seen. An intercalated disc (right) is granular in appearance and its membranes are indistinct. Unusual double contour of lower portion of disc is due to plane of tissue section. × 12,400.

**Fig. 5 Middle.** 4 hour infarct. Marked vesiculation of sarcoplasmin reticulum (K) but only moderate swelling of mitochondria present. Interfibril space increased. Zig-zag discontinuous intercalated disc and several lipid droplets are seen. Vesicular changes of the Z bands at lower right are marked. Myofilaments sharply defined and H, A, Z and T bands demonstrable. × 25,900.

**Fig. 6 Bottom.** 4 hour infarct. Mitochondria enlarged. Cristae in several areas swollen, producing clubbed appearance (arrow). Myofilaments and Z bands homogeneous and poorly defined in contrast to figure 5. × 21,400.
Fig. 7 Top left. 48 hour infarct. Intracellular structures predominantly shrunken mitochondria, large lipid droplets and amorphous strands of fibrillar material. × 13,200.

Fig. 8 Bottom left. 48 hour infarct. Degenerate cells contain scattered lipid and fragments of myofibrils. Sarcolemma and shrunken mitochondria identifiable. Nucleus fairly well preserved. × 5,000.

Fig. 9 Top right. 24 hour infarct. In histologic preparation for light microscopy, characteristic infiltrate of inflammatory cells is seen. Muscle fibers widely separated by edematous stroma and cross striations lost. Phosphotungstic acid hematoxylin. × 600.

Fig. 10 Bottom right. Light microscopic micrograph of margin of 48 hour infarct. Inflammatory infiltrate marked. Muscle cells homogeneous and degenerated. Hematoxylin-eosin. × 600.
of the section to the area of ischemia. At the border of the infarct the gradations of degenerative change in the 1 to 5 hour infarcts were seen, but in the central portion of the infarct only clumps of swollen mitochondria and cytoplasmic vacuoles were found, scattered among homogeneous, necrotic muscle fibrils which were in disarray. Intercalated discs were relatively spared. Many lipid droplets were present. In capillaries, small lipid bodies were present in the cytoplasm of swollen endothelial cells. We did not identify inflammatory cells in the interstitial tissue, probably because most of our sections were from the central area of infarction.

In the 48 hour infarct, macrophages and neutrophilic leucocytes were numerous. Phagocytosis of cellular fragments had occurred, although precise identification of these fragments was often difficult. Many muscle fibers were represented by almost empty wrinkled sace containing only a few shriveled mitochondria and fatty debris (figs. 7, 8, 9, 10).

**Autolysis.** Electron microscopic changes during the 5 hour period of autolysis were similar to those of myocardial infarcts, and therefore only the dissimilarities will be described. Most obvious of these was the uniformity of changes during autolysis. Mitochondrial swelling progressed more slowly in autolysis than in infarction (fig. 11). The vesicular transformation of mitochondrial cristae after two hours of autolysis was more prominent than that in myocardial infarcts (fig. 12).

**DISCUSSION**

**Significance of Early Alterations.** Even the earliest of the ischemic changes we have described are probably not reversible. Blumgart, Gilligan and Shlesinger found that permanent myocardial damage followed coronary arterial occlusion in experimental animals unless the duration of occlusion was less than 5 to 20 min. Gollan and Nelson later reported that reversible cardiac arrest, induced by potassium citrate, could be maintained for 1 hour, but only with hypothermia. The latter authors demonstrated that periods of ischemia of less than 1 hour were "injurious" to the myocardium of the beating heart, which indicates that it is more susceptible to ischemia than is the unbeating heart. Our observation, that autolytic changes in the unbeating heart progress less rapidly than ischemic changes in the beating heart, offers a morphologic parallel to the experiment of Gollen and Nelson. This parallel suggests that the early mitochondrial alterations reflect the conditions determining irreversibility of myocardial damage, but no morphologic criteria of irreversibility have yet been established by electron microscopy.

**Myocardial Infarction.** Distinct and consistent morphologic changes in experimental myocardial infarcts can be seen by electron microscopy as early as 1 hour following ligation of a coronary artery. Of all the cellular organelles, mitochondria are affected earliest and most noticeably; they are enlarged, apparently from an increase in the amount of fluid in the matrix. Swelling of sarcoplasm and sarcoplasmic reticulum occurs approximately 1 hour later than the alteration of mitochondria. Lipid bodies are increased in number after 2 hours of ischemia, appearing free in the cytoplasm of the muscle cells and usually adjacent to sarcoplasmic reticulum (figs. 4 and 5). We were unable to demonstrate that there was a relationship of lipid bodies to recognized intracellular structures. Their origin is unknown. Membranes of the sarcoplasmic reticulum, mitochondrial walls and cristae are not often found broken until ischemia has been present for 4 or 5 hours. At this time myofilaments have become indistinct (fig. 6), causing an almost complete loss of normal banding of the muscle fibers.

The early pathologic alterations were somewhat variable, even among similar organelles in the same muscle cell. This variation was greater within areas of the same infarct, probably indicating different degrees of ischemia even in adjacent areas, especially near the borders of the infarct. However, examination of adequate samples of each infarct revealed consistent patterns of change in the ultrastructure of the myocardial fibers. An increasing degree of damage to cell structure
Figs. 11 and 12. See legend on opposite page.
could be demonstrated by comparing the progression of changes with increasing duration of ischemia. Uninfarcted tissue from the posterior left ventricular wall consistently failed to reveal abnormalities, which indicates that only local ischemia, produced by arterial ligation, was a significant factor in the development of lesions.

The changes in fine structure in early myocardial infarction do not suggest new methods or ready criteria for the diagnosis of recent myocardial infarcts in man. Autolytic structural alterations associated with the usual delay in performing autopsies (fig. 11), and the unknown effects of circulatory failure present in the period preceding death, would make diagnostic interpretation of electronmicrographs difficult or, more likely, impossible.

**Autolysis.** The early autolytic changes in myocardial fibers are similar to those in early myocardial infarction but have certain differences. The autolytic process in the first 5 hours is distinguished from myocardial infarction of the same duration by (1) a 1 to 2 hour later appearance of marked mitochondrial swelling (fig. 11), (2) a greater uniformity of cellular alteration, especially noticeable among mitochondria of the same cell and (3) more prominent vesicular changes in mitochondrial cristae (fig. 12). These differences suggest that accumulation of metabolites, edema and possibly mechanical trauma of the beating heart cause the progression of changes to be more rapid in myocardial infarction than in autolysis.

**Pathogenesis of the Alteration in Ultrastructure.** The changes in structure of the myocardium in early infarction and in autolysis are indicative of an inhibition of fluid by mitochondria, by sarcoplasmic reticulum and, to a lesser degree, by the entire cell. Using phase microscopy, Watanabe and Williams\(^2\) have shown that isolated mitochondria increase in size when suspended in hypotonic solution, and that the enlargement is followed by a rupture of the outer membrane, extrusion of their contents and collapse. Our observations indicate that the mitochondria and sarcoplasmic reticulum act as osmometers during ischemia and autolysis, their swelling demonstrating their hyperosmolarity in relation to the interstitial fluid.\(^2\) Luft and Hechter\(^2\) have demonstrated a similar alteration of mitochondria in adrenal cortical cells during early autolysis and have postulated that this is reversible by perfusion with oxygenated blood.

The pathologic changes we have described are not necessarily pathognomonic of ischemia, and similar lesions have been described in other tissues following damage of a different sort. Okada and Peachey\(^2\) observed swelling and vacuolization of mitochondria of rat liver following gamma irradiation. The mitochondria of adrenal cortical cells undergo a somewhat similar change after hypophysectomy.\(^2\) Moore, Ruska and Copenhaver\(^1\) observed multiple lipid droplets, swollen mitochondria and sarcoplasmic reticulum, degenerative 1-band zones and hyalinized myofilaments in leg muscle of rats after 2 hours of ischemia produced by tourniquet. These changes were not uniform throughout the cell and did not vary remarkably during a 16 hour period following removal of the tourniquet. Their study offers an interesting combination of autolytic and ischemic effects and serves to emphasize the dynamic nature of ischemia. The similarity of response of mitochondria to various forms of injury suggests the presence of a common factor, probably anoxia.

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*Fig. 11 Top.* 4 hour autolysis. Mitochondria swollen, their cristae vesiculated. Several mitochondrial "ghosts." The sarcoplasmic reticulum and spaces between fibrils enlarged. \(\times 8,700\).

*Fig. 12 Bottom.* 2 hour autolysis. Prominent, swollen sarcoplasmic reticulum adjacent to Z bands (upper left). Mitochondria moderately enlarged and vesicular. Cristae swollen, producing clubbed appearance not unlike that seen in 4 hour infarct (fig. 6). \(\times 27,800\).
importance of mitochondria in cellular oxidation and energy production is well established.24-26,31,32 Disruption of this system is especially significant in heart muscle because of the high-energy demands placed on this organ.

Study of the intracellular enzyme activity following cell injury10,11,20,32 suggests that many of these processes continue during autolysis and ischemia, but decreased utilization of nutrients has been demonstrated early in experimental myocardial infarction.33 These alterations of function may be reflected anatomically by swollen, disrupted mitochondria. If the sarcoplasmic reticulum is important in electrical conduction, the electrocardiographic changes of infarction may parallel swelling of the sarcoplasmic reticulum.

SUMMARY

Definite changes can be demonstrated by electron microscopy in the early stages of acute myocardial infarction, before lesions are apparent by light microscopy. The earliest and most prominent alterations are swelling of mitochondria and sarcoplasmic reticulum, probably reflecting relative hyperosmolality of these structures.

The autolytic process is distinguished from myocardial infarction by greater uniformity of damage and less rapid development of structural changes, but otherwise the two processes appear similar in the first five hours. An increase in the number of lipid droplets and size of tubules forming the sarcoplasmic reticulum is noticeable in both autolysis and infarction. The changes observed in ischemia may be useful in evaluating electron microscopic lesions of other forms of myocardial damage.

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SUMARIO IN INTERLINGUA

Per medio de microscopia electronic, definite alterationes pote esser demonstrate durante le studios precoce de acute infarcimento myocardial, ante que le presentia del lesion es apparente sub le microscopio a lumine. Le prime e plus prominentes alterationes es phenomenon de tumescentia in le mitochondrios e le reticulo sarcoplasmic, reflectente probablemente un relative hyperosmolalitate de iste structuras.

Le processo autolytic es distingute ab infarcimento myocardial per un plus grande uniformitate del injuria e un plus lente disvelloppamento del alterationes structural, sed alteremente le duo processos pare esser simile durante le prime cinque horas. Un augmento del numero de guttetas lipidic e del dimensiones del tubulos que forma le reticulo sarcoplasmic es a notar in autolysis e etiam in infarcimento. Le alterationes observate in ischemia va possibilmente esser de adjuta in le evaluation, per microscopia electronic, de lesiones in altere forums de injuria myocardial.

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