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Effect of Repetitive Brief Episodes of Ischemia on Cell Volume, Electrolytes and Ultrastructure

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The effects of repeated brief episodes of ischemia on myocardial cell volume, electrolytes and ultrastructure were studied in dogs. Seventeen animals were divided into five groups. Group 1 underwent a single 10 minute occlusion of the circumflex coronary artery, with no subsequent reperfusion. Group 2 was similarly subjected to a 10 minute coronary occlusion, but was allowed a 20 minute reperfusion period. Group 3 underwent two 10 minute occlusions separated by 20 minutes of reperfusion and Group 4 underwent four 10 minute occlusions, each separated from the next by 20 minutes of reperfusion. Group 5 was subjected to a single, uninterrupted 40 minute occlusion.

The anterior and posterior papillary muscles in each heart were sampled to compare nonischemic versus ischemic myocardium. No changes in myocardial water or electrolytes occurred during ischemia. However, reperfusion was associated with slight increases in tissue water and potassium, loss of magnesium and minimal changes in sodium or calcium ions. Electron microscopic analysis revealed signs of mild ischemic injury (absence of normal intramitochondrial granules, partial loss of glycogen and slight clumping of the nuclear chromatin) in posterior papillary muscle from Groups 1, 3 and 4. Group 2 showed complete recovery with 20 minutes of reperfusion, whereas Group 5 showed evidence of irreversible injury. There was no difference in the appearance of myocardium that had been subjected to one, two or four 10 minute occlusions. It is concluded that intermittent periods of reperfusion between brief episodes of coronary ischemia have a protective effect and prevent a cumulative deterioration of myocardial ultrastructure.

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The ultrastructural changes associated with reversible and irreversible injury in regional myocardial ischemia in the open chest anesthetized dog have been established in a series of studies from this laboratory. These have shown that the damage induced by an ischemic episode of 15 minutes or less is reversible (1–4) and that the changes in ultrastructure associated with ischemia disappear virtually completely in the myocytes after only 20 minutes or more of reperfusion (5–8). On the other hand, episodes of severe ischemia of 40 minutes or more cause irreversible injury; the damaged myocytes cannot be saved by reperfusion (2–4,8). Results of several recent studies (9–14) have indicated that some of the functional and metabolic deficits of ischemia are limited if short intervals of reperfusion are allowed between sequential episodes of ischemia. Few data exist, however, on the effect of multiple episodes of ischemia on the ultrastructure and cell volume regulation in myocytes damaged by intermittent ischemia. As part of a larger study in which we determined levels of high energy phosphates and collateral blood flow in the intermittently ischemic heart, we assessed myocardium damaged in this manner for changes in cell volume regulation and in ultrastructure with the aim of discovering whether repetitive 10 minute episodes of ischemia separated by 20 minute intervals of reflow cause cumulative damage, or whether intermittent reperfusion has a protective effect on cell volume regulation and on myocardial ultrastructure.

Methods

Experimental protocol. Seventeen healthy mongrel dogs of either sex and weighing from 15 to 25 kg were subjected to either single or repeated occlusions of the circumflex coronary artery and were studied by electron microscopy. Group 1 (n = 3) was subjected to a single 10 minute occlusion, with no subsequent reperfusion. Group 2 (n = 5) was given a single 10 minute occlusion followed by 20 minutes of reperfusion. Group 3 (n = 2) underwent two 10 minute occlusions separated by 20 minutes of reperfusion, and Group 4 (n = 2) underwent four 10 minute oc-

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clusions, with 20 minute intervals of reperfusion between each. Group 5 (n = 5) underwent a single 40 minute occlusion so that we could compare the effects of four separate 10 minute occlusions with a single occlusion of the same total duration. The hearts examined by electron microscopy were part of a larger experiment involving a total of 36 dogs in which the metabolic consequences of repetitive episodes of ischemia were assessed (15). Three dogs died of ventricular fibrillation during these experiments and were excluded. Total tissue water and electrolytes were measured in two of these dogs. The number of animals in each group is given in Table 1.

Experimental preparation. The dogs were anesthetized with 30 to 40 mg/kg of sodium pentobarbital, intubated and ventilated on a Harvard model 607 respirator at a rate of 200 ml/kg per min. The respiratory rate and the amount of supplemental oxygen were adjusted to maintain blood gases at physiologic levels. Using a Gould model 2400 recorder, lead II of the electrocardiogram was monitored and peripheral blood pressure was recorded with a Statham model DB23 pressure transducer connected to a polyethylene catheter in the right femoral artery. A left thoracotomy was performed in the fourth intercostal space, the heart was suspended in a pericardial cradle and the circumflex artery was isolated just lateral to the left atrial appendage. Left atrial pressure was recorded through a catheter inserted into the left atrial appendage, and a temperature probe was placed under the heart. After hemodynamic equilibration, the artery was occluded by snaring it with a silk suture.

Sampling of damaged and control tissue. To ensure that only severely ischemic tissue was included in the samples, we used the fluorescent dye thioflavine S to distinguish ischemic from nonischemic tissue (3). Ten to 15 seconds before excision of the heart, thioflavine S was given intravenously. In the experiments ending with a period of reperfusion (Group 2), the circumflex artery was reoccluded 15 seconds before excision of the heart and thioflavine S was injected immediately thereafter. Each heart was excised rapidly by cutting with a sharp knife just below the atrioventricular (AV) groove. About 4 seconds were required to excise the ventricles and to plunge them into 750 ml of ice cold (0°) isotonic potassium chloride where they were swirled about to accelerate heat exchange. After cooling for 1 minute, the hearts were removed from the cold potassium chloride and transmural sections obtained through the ischemic and nonischemic regions, including the posterior and anterior papillary muscles, respectively. These were frozen in freon at liquid nitrogen temperature. The remainder of the heart was then examined under ultraviolet light through a yellow filter to distinguish between nonischemic or mildly ischemic (fluorescent) and severely ischemic (nonfluorescent) tissue. Thin slices of each were fixed in glutaraldehyde for electron microscopic study or were weighed and dried overnight at 105°C, and reweighed for determination of total tissue water. Only nonfluorescent areas of the posterior papillary muscle were included in the ischemic samples and only fully fluorescent areas of the anterior papillary muscle were used as nonischemic control tissue. The nonischemic tissue of each heart was examined by electron microscopy to assess the quality and consistency of fixation and preservation.

The frozen transmural samples were freeze-dried and portions removed for metabolic and blood flow analyses as part of another study (15). Samples of the remaining tissue were analyzed for electrolytes after drying overnight at 105°C.

Electron microscopy. Thin slices of nonischemic anterior papillary and of severely ischemic or ischemic reperfused posterior papillary muscle were processed for electron microscopic examination. Multiple cubes measuring 1 mm across or less were cut under glutaraldehyde with a sharp razor blade and were transferred to 20 to 30 ml of 4% glutaraldehyde in 0.1 *M* cacodylate buffer. After 1 to 4 hours of fixation, the blocks were postosmicated in 1% osmium tetroxide in 0.1 *M* collidine buffer, dehydrated in a graded ethanol series, rinsed in propylene oxide and embedded in Epon 812. Thick sections were cut from two or more blocks per slice, stained with toluidine blue and examined by light microscopy. After the selection of longitudinally oriented, artifact-free areas, ultrathin sections were cut with a diamond knife, mounted on copper grids

Table 1. Electrolytes in Ischemic Left Ventricle After Episodes of Ischemia With or Without Reperfusion‡

Group	No.	Sodium	Potassium	Magnesium	Calcium
Control§	7	15.4 ± 0.66	37.6 ± 0.60	4.6 ± 0.08	0.39 ± 0.02
1 (10 min I)	4	15.0 ± 1.52	37.7 ± 0.44	4.5 ± 0.34	0.37 ± 0.01
2 (10 min I + 20 min R)	7	16.1* ± 0.39	44.1† ± 0.53	4.4 ± 0.11	0.40 ± 0.03
3 (10 min I × 2)	3	14.3 ± 1.34	42.2* ± 0.74	4.0 ± 0.18	0.36 ± 0.04
4 (10 min I × 4)	3	14.3† ± 0.22	42.0† ± 1.33	3.8† ± 0.19	0.36 ± 0.05
5 (40 min I)	5	15.9 ± 0.68	37.0 ± 0.68	4.4 ± 0.02	0.43 ± 0.05

Statistical significance for comparisons between control and damaged tissue from the same hearts using a two-tailed paired *t* test is indicated as *$p < 0.05$, †$p < 0.005$. ‡All electrolyte measurements are mmol/100 g dry weight. §The control groups were indistinguishable. Data from the 10 minute ischemia +20 minute reperfusion group are reported here. I = ischemia; R = reperfusion.

and stained with uranyl acetate/lead citrate. Two or more grids from each block were examined with a JEOL JEM 100B electron microscope and representative areas were photographed.

Electrolytes. Electrolytes were extracted in 5 ml of 0.75 N nitric acid according to the techniques described previously (3). Atomic absorption spectroscopy was used for analysis of sodium, potassium, magnesium and calcium ions with an IL 351 spectrometer interfaced with a Tektronix model 31 computer calculator. Standard curves were prepared for all four ions, and two standard and two reagent blanks were analyzed with every series of curves. Electrolytes were expressed as millimoles per 100 grams dry tissue.

Statistics. Tabulated results are reported as the mean ± SEM. For each group, differences between nonischemic and damaged tissue samples within the same heart have been analyzed by a two-tailed Student's paired *t* test.

Results

After the onset of ischemia, cyanosis always was observed in the posterolateral myocardium supplied by the occluded circumflex artery, and a zone of severe ischemia, identified by absence of thioflavine S, was present in every heart. Electrocardiographic evidence of ischemia also developed within 20 to 30 seconds of occlusion. Mild to marked ST segment elevation was noted in lead II and a mean decrease in systolic pressure of about 10% developed in most animals. The ischemic tissue was grossly indistinguishable from control myocardium.

Ultrastructure. A representative view of the ultrastructure of nonischemic myocardium is shown in Figure 1. In all samples, the nuclear chromatin was evenly dispersed; the sarcomeres were in register and were slightly contracted. The sarcoplasm contained little open space; granular glycogen, mitochondria and sarcoplasmic reticulum occupied most of the sarcoplasmic space between myofibrils. The mitochondria had tightly packed cristae; most mitochondrial profiles revealed normal matrix granules. Electron-dense lipofuscin granules were common in the perinuclear region; small lysosomes also were present. The sarcolemma was intact and was scalloped from Z band to Z band of the underlying myofibrils. The basal lamina of the sarcolemma was tightly applied to the underlying trilaminar unit membrane.

After 10 minutes of ischemia, the myocytes exhibited the changes of mild reversible injury (Fig. 2). The nuclear chromatin was clumped and aggregated at the periphery of the

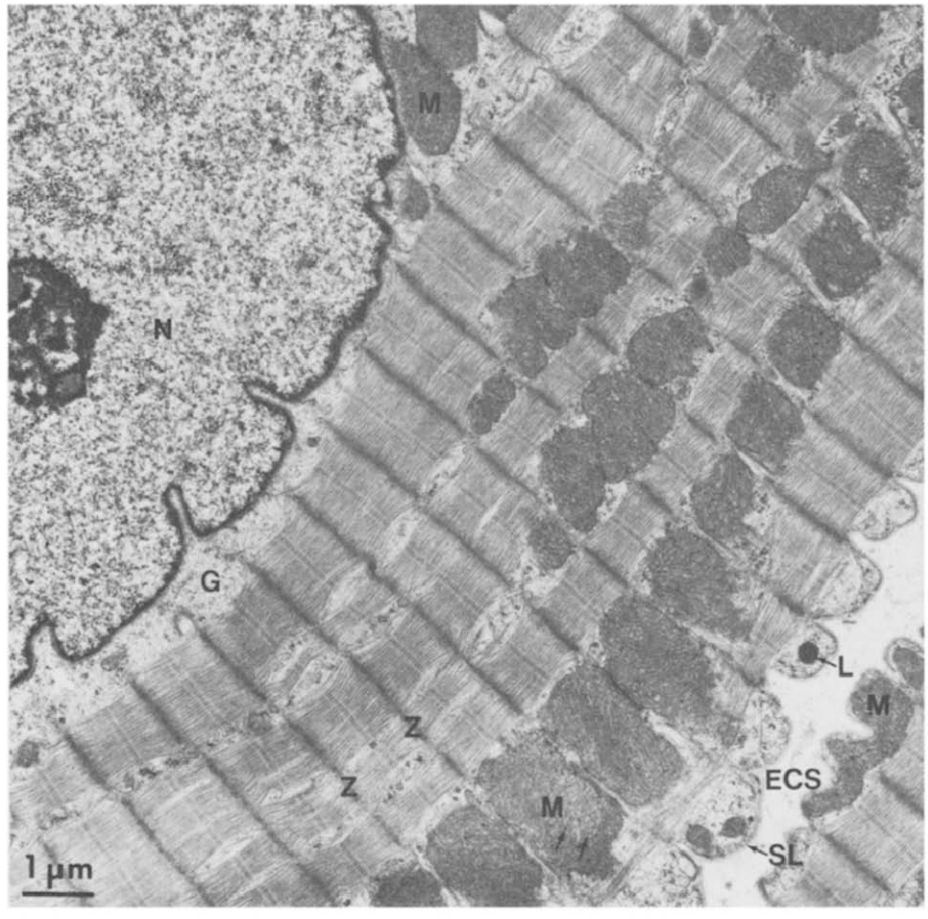

Figure 1. Control nonischemic myocardium. This medium power micrograph is representative of control myocardium from these experiments and shows parts of two myocytes. The sarcomeres of the myofibrils (see Z bands) (Z) are generally contracted and exhibit only the A band. The sarcoplasm contains palely stained glycogen particles (G) and abundant mitochondria. Most of the mitochondria (M) are interfibrillar, but they also were abundant in the perinuclear region. Tiny matrix granules are present in many mitochondrial profiles (**small arrows**). The sarcolemma (SL) is scalloped because of attachments to the underlying myofibril at each Z line. An occasional primary lysosome is present (L). The chromatin of the nucleus (N) is generally evenly distributed. ECS = extracellular space between the two myocytes. (Magnification ×13,125, reduced by 33%.)

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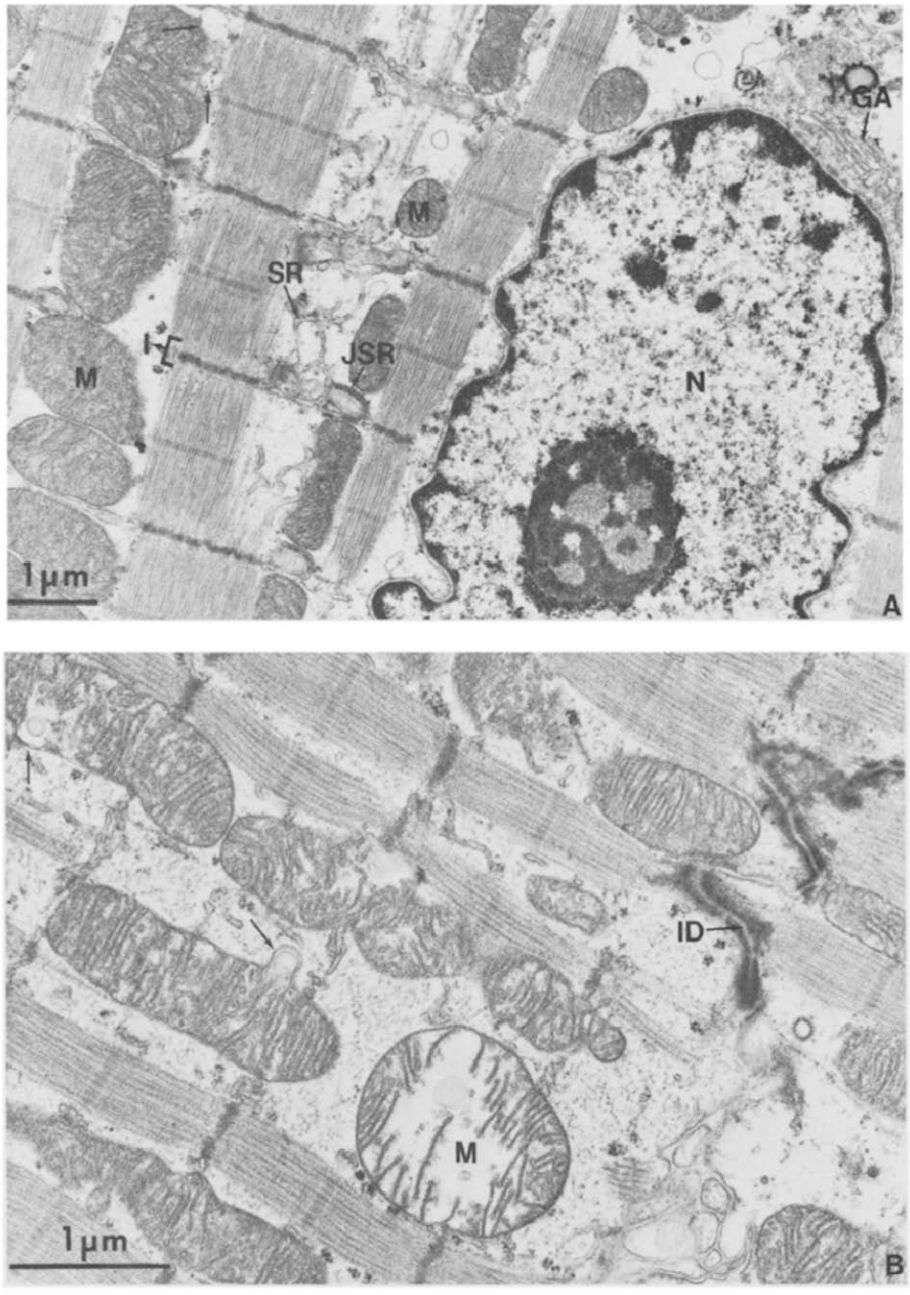

Figure 2. Ten minutes of ischemia, no reperfusion. **A**, Representative view of an ischemic myocyte after 10 minutes of permanent ischemia. Note that the chromatin of the nucleus (N) is peripherally aggregated. The sarcoplasmic space is clear and contains less glycogen than that in control myocardium. The mitochondria (M) have a density generally similar to that of control mitochondria (Fig. 1). Localized mitochondrial swelling is occasionally noted (**arrows**). The myofibrils are relaxed and show I bands (see **bracket** and I). Sarcoplasmic reticulum (SR) and junctional sarcoplasmic reticulum (JSR) are visible and are similar to those in nonischemic myocardium. The Golgi apparatus (GA) is present adjacent to the nucleus and is indistinguishable from that in nonischemic myocardium. (Magnification ×17,950, reduced by 32%.) **B**, View of the most marked mitochondrial changes after 10 minutes of ischemia. A mitochondrion (M) with a greatly enlarged matrix space is present. Most mitochondria are similar to control mitochondria. However, an occasional mitochondrion shows a localized area of swelling (**arrows**). A portion of an intact intercalated disk (ID) is present. (Magnification ×30,000, reduced by 32%.)

nucleus. The sarcoplasmic organelles were much less densely packed than observed in nonischemic myocytes; the sarcoplasm appeared relatively clear and much less glycogen was apparent. The mitochondrial matrices were slightly more electron-lucent than in control tissue and contained no matrix granules. Although most mitochondria appeared only slightly swollen, an occasional mitochondrion showed severe generalized or localized swelling (Fig. 2).

When myocytes damaged by 10 minutes of ischemia were reperfused with arterial blood for 20 minutes, the architecture returned to normal (Fig. 3). The nuclear chromatin and glycogen distribution were indistinguishable from those in the control tissues. However, rare damaged mitochondria persisted and remained as monuments to the ischemic episode. Thus, by 20 minutes of reperfusion, the time at which the second 10 minute occlusion was initiated, recovery from the initial occlusion was virtually complete.

At the end of the second 10 minute occlusion, the ultrastructure of the ischemic myocytes was virtually identical to that seen at the end of a single 10 minute episode of ischemia (Fig. 4). Mildly clumped chromatin, loss of glycogen, slight intracellular edema, loss of intramitochondrial granules and mild clearing of the mitochondrial matrix again were apparent. Rare mitochondria were swollen and exhibited fragmented cristae. Only 1 in approximately 50 mitochondria showed this abnormality. Moreover, the severely

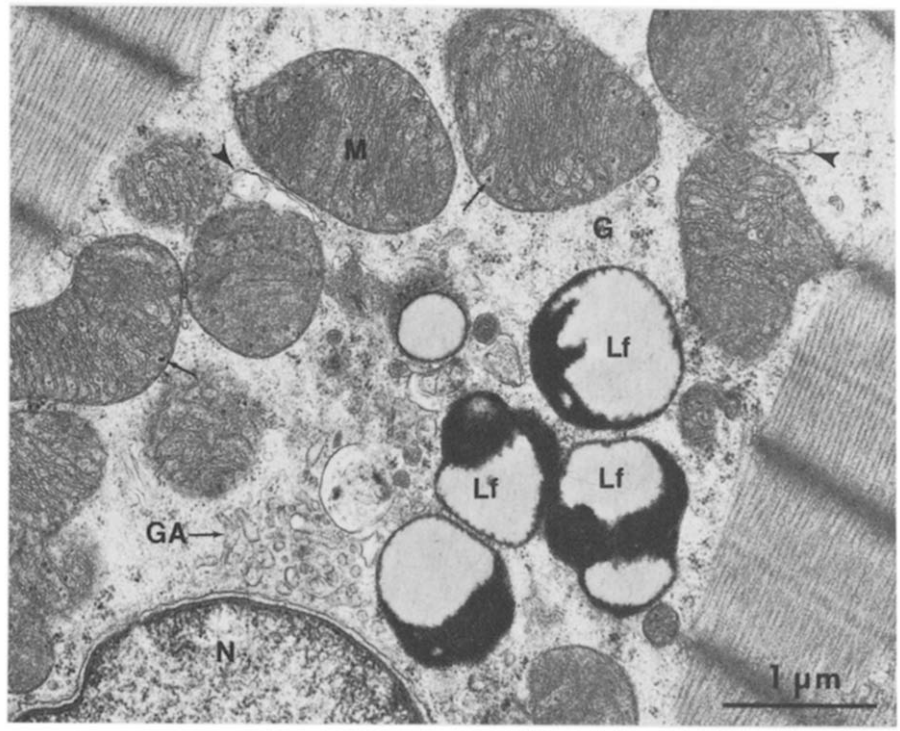

Figure 3. Ten minutes of ischemia followed by 20 minutes of reperfusion. This myocyte is representative of those seen in the damaged tissue. Note that the chromatin of the nucleus (N) is evenly distributed. Glycogen (G) is present in the sarcoplasm as are the Golgi apparatus (GA), numerous mitochondria (M) and lipofuscin granules (Lf) (**arrows**). Mitochondria are dense and have compact cristae. Note that the matrix space now contains matrix granules. Rare mitochondria show focal swelling (**arrowheads**). The myofibrils are contracted. (Magnification ×29,375, reduced by 31%.)

affected mitochondria were scattered randomly throughout the myocytes. They were not localized in any particular region.

Cardiac myocytes subjected to four separate 10 minute episodes of ischemia again showed remarkably mild ischemic changes, considering the 40 minute cumulative duration of ischemia (Fig. 5). The appearance of the ischemic myocardium at this time was very similar to the appearance of myocytes injured by one or two 10 minute occlusions. Occasional swollen mitochondria again were seen. These were more frequent than in the previously mentioned groups, but still were rare. Other organelles looked much the same as described for the one and two occlusion groups.

Careful search revealed no difference between any of these groups in the appearance of the intercalated disc or the sarcolemma. These structures were intact and unaltered in all of the samples examined, and were apparently unaffected by the episodes of ischemia.

The samples from the 40 minute single occlusion group displayed changes typical of irreversible injury (1–5) (Fig. 6). There was severe clumping and margination of the chromatin, near absence of glycogen, intracellular edema, elevation of the sarcolemma off of the underlying sarcomeres with areas of plasmalemmal disruption and stretching of the sarcomeres, with widening of the I-bands and the presence of N bands. All mitochondria were swollen and exhibited disorganized cristae and the amorphous matrix densities which are the ultrastructural hallmark of irreversible injury.

Electrolytes and water of damaged myocardium. The changes in ion content and total tissue water observed in each group are presented in Table 1 and Figure 7. There was no significant change in ion or water content after either 10 or 40 minutes of severe ischemia. These results were expected because, in the absence of significant collateral flow, no new electrolytes or water are provided to the ischemic tissue.

The effects of 20 minutes of reperfusion after 10 minutes of ischemia were quite striking. The previously ischemic tissue exhibited a slight but significant increase in sodium, a substantial increase in potassium and water and a decrease in magnesium. Calcium was unchanged. These changes persisted through two and even through four ischemic episodes. Magnesium levels appeared to decrease progressively as the cumulative period of ischemia increased, but the decrease was significant after only 40 cumulative minutes of ischemic injury.

Discussion

Reversibility of ischemic ultrastructural changes by reperfusion. The ultrastructural changes induced by 10 minutes of low flow ischemia were similar to those previously reported to be present after 15 minutes (6). These changes included clearing and enlargement of the sarcoplasmic space, a decrease in the number of glycogen particles, mitochondrial swelling with a loss of mitochondrial matrix granules and peripheral aggregation of nuclear chro-

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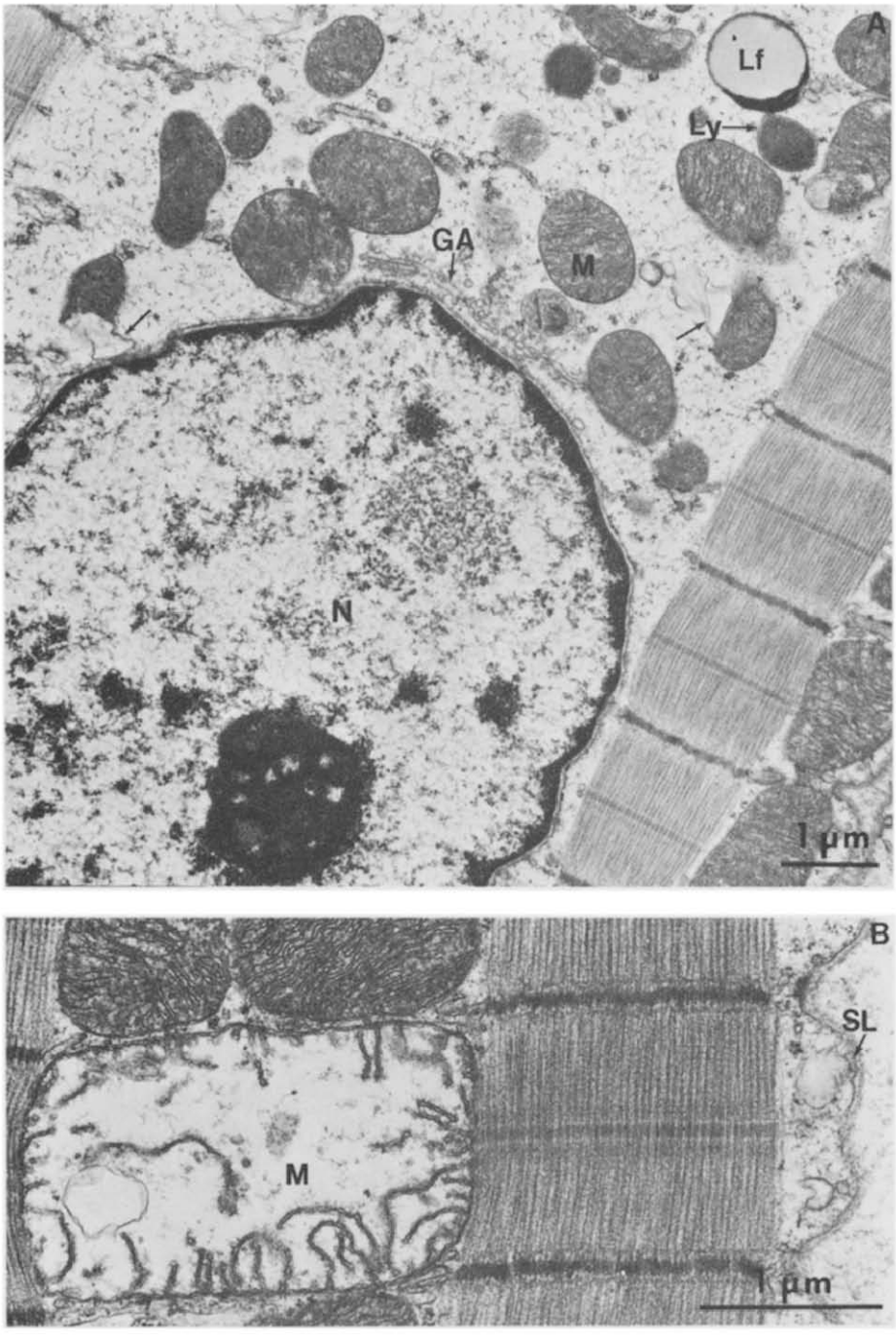


Figure 4. Two 10 minute periods of ischemia separated by a 20 minute interval of reperfusion. **A**, Representative myocyte obtained at the end of the second interval of ischemia. Its appearance is quite similar to that shown in Figure 2A except for a slight increase in a sarcoplasmic density. The chromatin of the nucleus (N) is peripherally aggregated. The mitochondria (M) have tightly packed cristae; matrix granules are absent. An occasional area of localized swelling involving a projection of the outer membrane of the mitochondria is present (see **arrows**). GA = Golgi apparatus; Ly = intact lysosome. (Magnification × 17,625, reduced by 27%.) **B**, High power view of a single mitochondrion (M) which is greatly swollen. It shows an enlarged matrix space together with disorganized cristae. Adjacent to it are two intact mitochondria which are similar to control except for the absence of matrix granules. The sarcolemma (SL) is intact. (Magnification × 32,250, reduced by 27%.)

matin. However, the sarcolemma remained intact and was scalloped over each damaged myocyte. Signs of irreversible injury such as generalized mitochondrial disorganization with the presence of amorphous matrix densities and focal sarcolemmal disruption never were detected.

Twenty minutes of arterial reperfusion restored the architecture of the affected myocytes virtually to the control condition. Similarly, rapid restoration of normal myocyte structure was previously observed 20 minutes after a 15 minute episode of ischemia (6). At the end of 40 cumulative minutes of ischemia (four 10 minute episodes of ischemia interspersed with three 20 minute episodes of reperfusion), the ultrastructural changes were identical to those found at the end of the first episode of ischemia. Thus, intermittent reperfusion protected the myocardium; the changes characteristic of irreversibility, that is, marked mitochondrial swelling and amorphous matrix densities, superstretched myofibrils and a focally disrupted sarcolemma, did not ap-

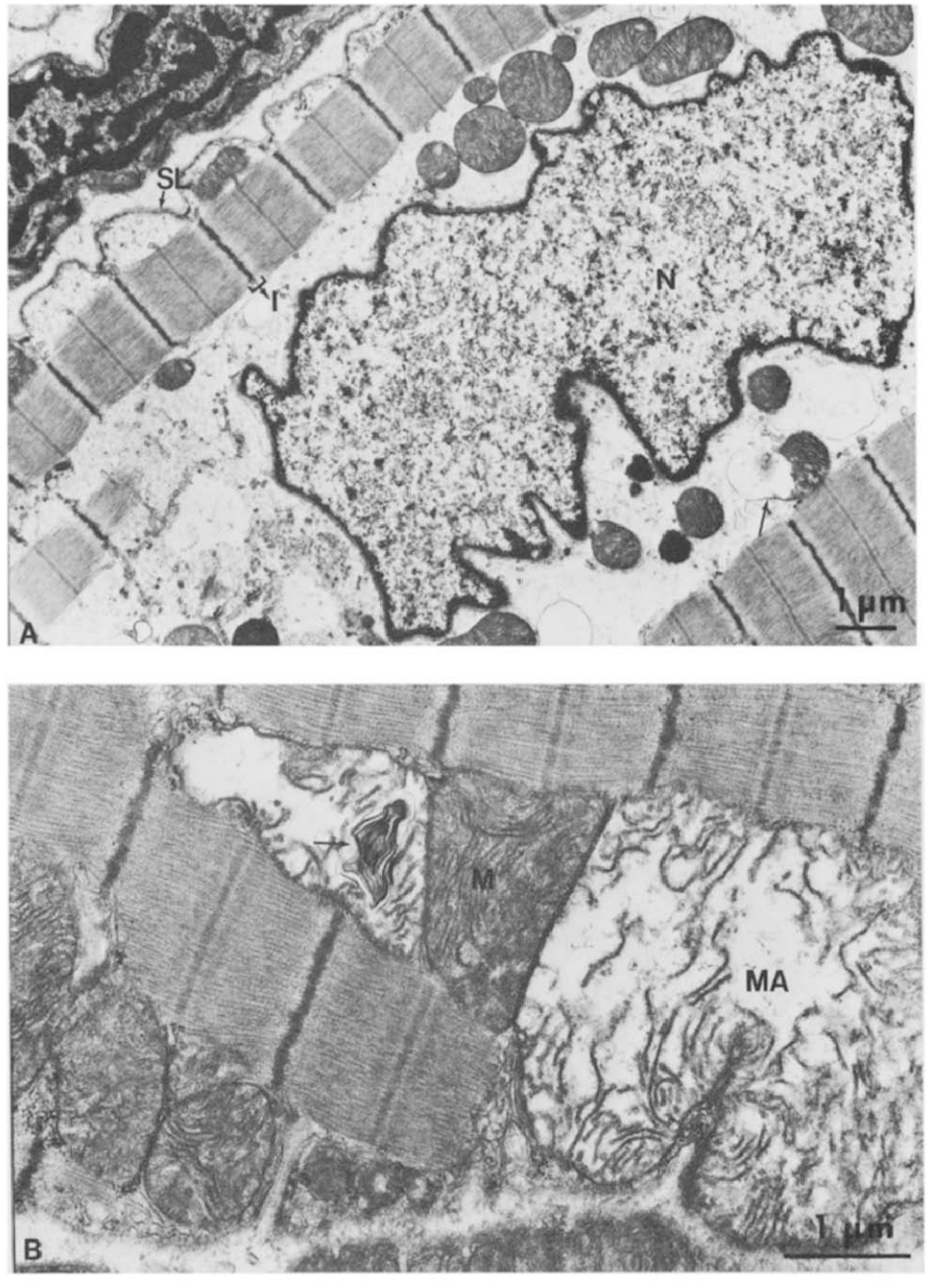


Figure 5. Four 10 minute episodes of ischemia separated by three 20 minute intervals of reperfusion. **A**, Representative low power view of a part of a myocyte obtained at the end of the fourth 10 minute episode of ischemia. The sarcolemma (SL) is intact. The myofibrils are relaxed and show prominent I bands (I [**bracket**]). The nuclear (N) chromatin is more evenly distributed than illustrated in Figures 2A and 4A, but is less evenly distributed than in control myocytes (Fig. 1). Mitochondria (M) are free of matrix granules. However, most mitochondria are dense and exhibit tightly packed cristae. An occasional mitochondrion shows focal protrusion of the outer membrane (**arrow**). (Magnification × 10,950, reduced by 30%.) **B**, Mitochondrial (M) changes at the end of 40 cumulative minutes of ischemia in more detail. Most of the mitochondria were similar to those in the control sample except for the absence of matrix granules. However, some greatly swollen mitochondria with an enlarged matrix space (MA) and myelin figures (**arrow**) were seen. About 1 of every 50 mitochondria was swollen. (Magnification × 23,000, reduced by 30%.)

pear even though a single episode of ischemia of this duration induced these and other changes characteristic of the irreversible state (Fig. 6).

The explanations for the rapid reversibility of cardiac ultrastructure following a brief transient episode of myocardial ischemia, and for the fact that cumulative ultrastructural changes do not occur after as many as four 10 minute episodes of ischemia, are currently unknown. However, it is of interest that parallel metabolic studies in our laboratory (15) and similar studies by others (10–12,14) have shown no cumulative loss of myocardial adenosine triphosphate (ATP) content during repetitive episodes of ischemia, even though repletion of ATP during intermittent reperfusion is slow. The absence of cumulative ATP depletion during reperfusion appears to be caused by a combination of three factors: first, there is a creatine phosphate "overshoot" (12,14) that helps to restore total cellular high energy phosphate reserves. Second, washout of lactate and other accumulated inhibitors of anaerobic glycolysis occurs so that the capacity for ATP production through this metabolic pathway is restored. Third, there is a lower net rate of high energy phosphate utilization by the ischemic myocytes during later episodes of ischemia compared with the first (15). The parallel preservation of high energy phosphates and cardiac ultrastructure by intermittent reperfusion is consistent with the previous observation that the ultrastructural

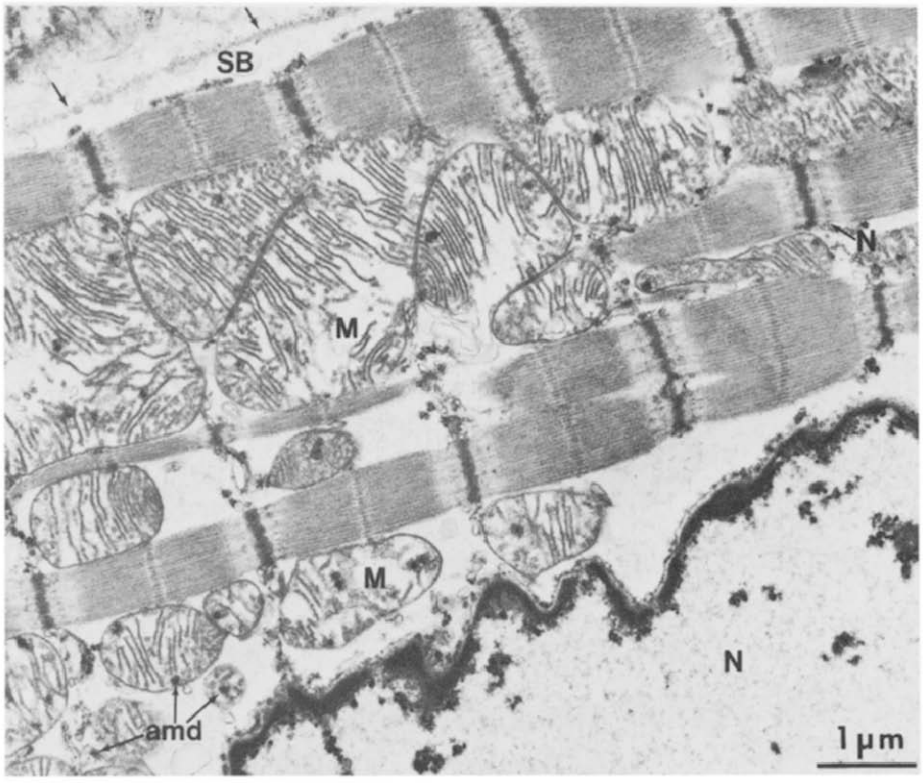
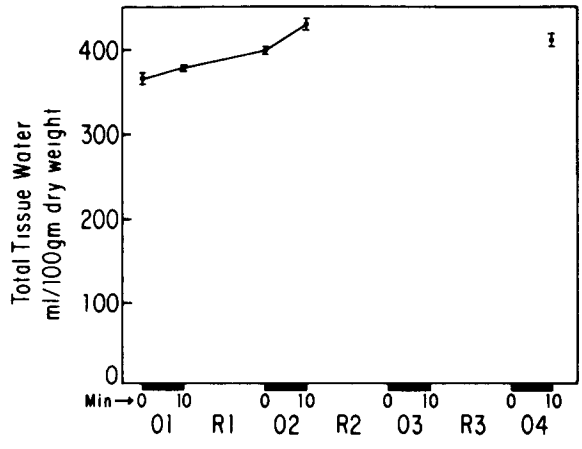


Figure 6. Forty minutes of continuous ischemia. This myocyte shows the changes shown previously to be characteristic of irreversible injury in canine ischemia. The chromatin of the nucleus (N) is markedly aggregated peripherally. The sarcoplasm contains very little glycogen and is increased in dimension. All mitochondria are enlarged and have an increased matrix space in which amorphous matrix densities (amd) are common. The myofibrils are markedly stretched and show N bands (N) in the I band regions. The sarcolemma is lifted off the underlying myofibrils to form subsarcolemmal blebs (SB). The plasmalemma of the sarcolemma (SL) overlying such blebs is focally disrupted (**arrows**). (Magnification × 18,800, reduced by 67%.)

changes associated with irreversible ischemic injury develop only after severe depletion of ATP and with the hypothesis that marked loss of ATP or high energy phosphate availability may be the underlying cause of irreversible injury (3,4).

Changes in tissue electrolytes and water content. A single 10 minute episode of severe ischemia was not associated with a significant increase in the total tissue water or a change in tissue electrolytes. The most likely explanation for these observations is that the arterial collateral flow is minimal and very little plasma influx or efflux occurs during the first few minutes of ischemia. Nevertheless, mild cell swelling probably occurs early in ischemia, as evidenced ultrastructurally by the reduced density and increased size of the sarcoplasmic space and less dense matrix space of the mitochondria. This early cell swelling may be due, in part, to the accumulation of osmotically active catabolites, such as creatine, dihydrogen phosphate, lactate, glucose-6-phosphate and so on, within the myocyte. However, cell swelling is not marked during the first 10 minutes of ischemia, probably because of the small volume of extracellular fluid available to support such swelling.

Figure 7. Total tissue water after one or more 10 minute episodes of ischemia interspersed by 20 minute episodes of reperfusion. Total tissue water was unchanged at the end of a single 10 minute episode of ischemia (O1) but increased during the subsequent reperfusion period (R1). This tissue edema persisted during additional episodes of ischemia and reperfusion (O2 to O4 and R2 and R3, respectively).



After reperfusion, the total tissue water and potassium increased markedly, but sodium increased only modestly. The increased sodium and a small part of the increase in total tissue water could be explained on the basis of increased extracellular space. The remainder of the increase in total tissue water and the increase in potassium more likely are due to persistent cell swelling. Similar changes in total tissue water and electrolytes previously have been demonstrated (6) after 15 minutes of ischemia followed by 3 or 20 minutes of reperfusion.

Paradoxically, as noted earlier, apparent intracellular edema was not obvious by electron microscopic evaluation at the end of the 20 minute reperfusion period. The explanation

for this paradox is unknown. It may be that resynthesis of glycogen obscured the mild intracellular edema.

Previous studies. The results of the present study are consistent with the findings of several other studies. Geft et al. (9) investigated the effects of repeated (up to 18 times) 5, 10 and 15 minute coronary occlusions, separated by 15 minute periods of reperfusion, on the occurrence of myocardial necrosis. Of 77 dogs, 62 had no necrosis despite cumulative ischemic periods of up to 210 minutes. Lange et al. (13) recently studied the effect of three sequential 5 or 15 minute ischemic episodes. Each ischemic period was followed by 30 minutes of reflow. Infarction was absent in all groups, and electron micrographs obtained 30 and 180 minutes after the third of three 5 minute occlusions showed essentially normal ultrastructure except for the finding of "unfolded" mitochondrial cristae. They found that regional contractile function was depressed to the same degree during the second and third reperfusion periods as during the first in both 5 and 15 minute groups. These authors concluded that two or three repeated coronary occlusions with intermittent reperfusion cause no more myocardial injury than does one.

Clinical implications. The relevance of these studies to disease in humans involves conditions where repeated episodes of ischemia may occur, as in the hearts of patients with angina pectoris. Similar changes probably occur in the hearts of patients undergoing cardiac surgery with intermittent cross clamping of the aorta. Our experiments, if applicable to patients, suggest that such repeated episodes of angina, if they are of sufficiently short duration, would not cause cumulative myocardial damage. Several investigators have examined the effects of intermittent cross clamping of the aorta during bypass surgery. Balibrea et al. (16) and Benzing et al. (17) observed myocardial protection with intermittent reperfusion although Levitsky et al. (18) did not. In the latter study, 60 minutes of continuous normothermic ischemic arrest was compared with four 15 minute intervals, each followed by 5 minutes of reperfusion in a dog model. There was no significant difference in either myocardial metabolites or contractile function between the groups; ATP content decreased at a similar rate in both groups. The explanation for these contradictory results is unknown.

Conclusion. The results of this study show that intermittent reperfusion has a protective effect on myocardial ultrastructure. We have demonstrated that two or four 10 minute periods of coronary occlusion, when separated by 20 minute periods of reperfusion, produce no more ultrastructural damage than a single 10 minute occlusion. These findings complement those of other studies that have shown a protective effect on high energy phosphate content.

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