# Abnormal Myocardial Fluid Retention as an Early Manifestation of Ischemic Injury

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**Fifty-seven isolated, blood perfused, continuously weighed canine hearts have been utilized to study the development of abnormal myocardial fluid retention during early myocardial ischemic injury. Inflatable balloon catheters were positioned around the left anterior descending coronary arteries (LAD) of 54 hearts or the proximal left circumflex coronary arteries of three hearts for study of the following intervals of coronary occlusion: a) 10 minutes followed by 20 minutes of reflow, b) 40 minutes followed by either no reflow or by 20 minutes of reflow, and c) 60 minutes without reflow. After 60 minutes of fixed coronary occlusion, histologic and ultrastructural examination revealed mild swelling of many ischemic cardiac muscle cells in the absence of interstitial edema, cardiac weight gain, and obvious structural defects in cell membrane integrity. After 40 minutes of coronary occlusion and 20 minutes of reflow, significant cardiac weight gain occurred in association with characteristic alterations in the ischemic region, including widespread interstitial edema and focal vascular congestion and hemorrhage and swelling of cardiac muscle cells. Focal structural defects in cell membrane integrity were also noted. The development of abnormal myocardial fluid retention after 40 minutes of LAD occlusion occurred in association with a significant reduction in sodium-potassium-ATPase activity in the ischemic area, but with no significant alteration in either creatine phosphokinase or citrate synthase activity in the same region. Despite the abnormal myocardial fluid retention in these hearts, it was possible pharmacologically to vasodilate coronary vessels with adenosine and nitroglycerin infusion to maintain a consistently high coronary flow following release of the coronary occlusion after 40 minutes and to even exceed initial hyperemic flow values following release of the occlusion when adenosine and nitroglycerin infusion was delayed until 15 minutes after reflow. Thus, the data indicate that impaired cell volume regulation and interstitial fluid accumulation and focal structural defects in cell membrane integrity are early manifestations of ischemic injury followed by reflow, but fail to establish a major role for the abnormal fluid retention in altering coronary blood flow prior to the development of extensive myocardial necrosis. In contrast, fixed coronary occlusion for 60 minutes results in mild intracellular swelling but no significant interstitial edema and no obvious structural defects in cell membrane integrity. (Am J Pathol 87:159–188, 1977)**

THE TEMPORAL RELATIONSHIP between myocardial ischemia, alterations in myocardial sodium-potassium-ATPase activity, and the development of abnormal myocardial fluid retention has not been completely

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elucidated. Moreover, the potential role that abnormal myocardial fluid retention might play in altering coronary blood flow prior to the development of extensive myocardial necrosis has not yet been definitively established. It has been previously demonstrated in both the brain and the kidney that severe ischemia results in cell swelling and that the swollen cells encroach on the vascular space.[1-4] Such cell swelling has been considered to have the potential of reducing blood flow and acting to further perpetuate the ischemic process.[1-4] Whether the same phenomena actually occur in the heart is not yet certain. Accordingly, the present study was designed to a) explore the temporal relationship between the duration of acute myocardial ischemia and the development of abnormal myocardial fluid retention, b) determine whether fixed coronary occlusion and temporary coronary occlusion followed by reflow result in different patterns of abnormal fluid retention, c) determine whether alterations in sodium-potassium ATPase tissue activity are present in isolated, blood perfused canine hearts at the time that abnormal myocardial fluid retention develops, and d) determine whether abnormal myocardial fluid retention has a major influence on altering coronary blood flow prior to the development of significant myocardial necrosis.

## Materials and Methods

### Experimental Model

Simultaneously 2 dogs were anesthetized with Nembutal (30 mg/kg), intubated, and placed on Harvard respirators. One dog (*support animal*) had cannulae inserted in both femoral veins and one femoral artery. The cannulae were clamped, and the dog was heparinized. The other dog (*donor animal*) had its chest opened through a median sternotomy; the heart was then exposed and the pericardium dissected away. The superior and inferior vena cavae, proximal aorta, and main pulmonary artery were identified and sutures placed around them. A catheter was placed in the left subclavian artery, and the blood volume of the donor dog reduced by bleeding for approximately 3 minutes. The arterial catheter from the support dog was then quickly connected to the proximal aortic root of the donor animal, and perfusion begun as the sutures around the proximal pulmonary artery, superior and inferior vena cavae were ligated and the remaining stumps of the vessels severed. The portion of the aortic stump above the perfusing cannula was also suture-ligated and severed. The isolated heart was then removed to the cantilever beam* (designed by Mr. Bill Romans of the Bioengineering Department at The University of Texas Health Science Center at Dallas, Dallas, Texas) that had been specially constructed so that the isolated hearts could be continuously weighed; the isolated hearts were

* The weighing system (cantilever beam) for isolated hearts was designed around a 6-inch-long cantilever beam, ⅛ inch thick by ⅝ inch wide, made of stainless steel. Two strain gauges were bonded to the beam at a position 1 inch from the fixed end. These gauges formed two arms of a Wheatstone bridge with the other two arms provided by fixed resistors; electrical balance was provided by means of a variable potentiometer. Provision was made to hang the isolated hearts to the end of the beam.

When the cantilever beam is deflected downward at the end, the outer fibers of the beam are lengthened and the inner fibers are shortened; therefore, the strain gauges also change their length, which in turn changes the electrical resistance. This change of electrical resistance unbalances the bridge and can be calibrated directly as force on the end of the beam.

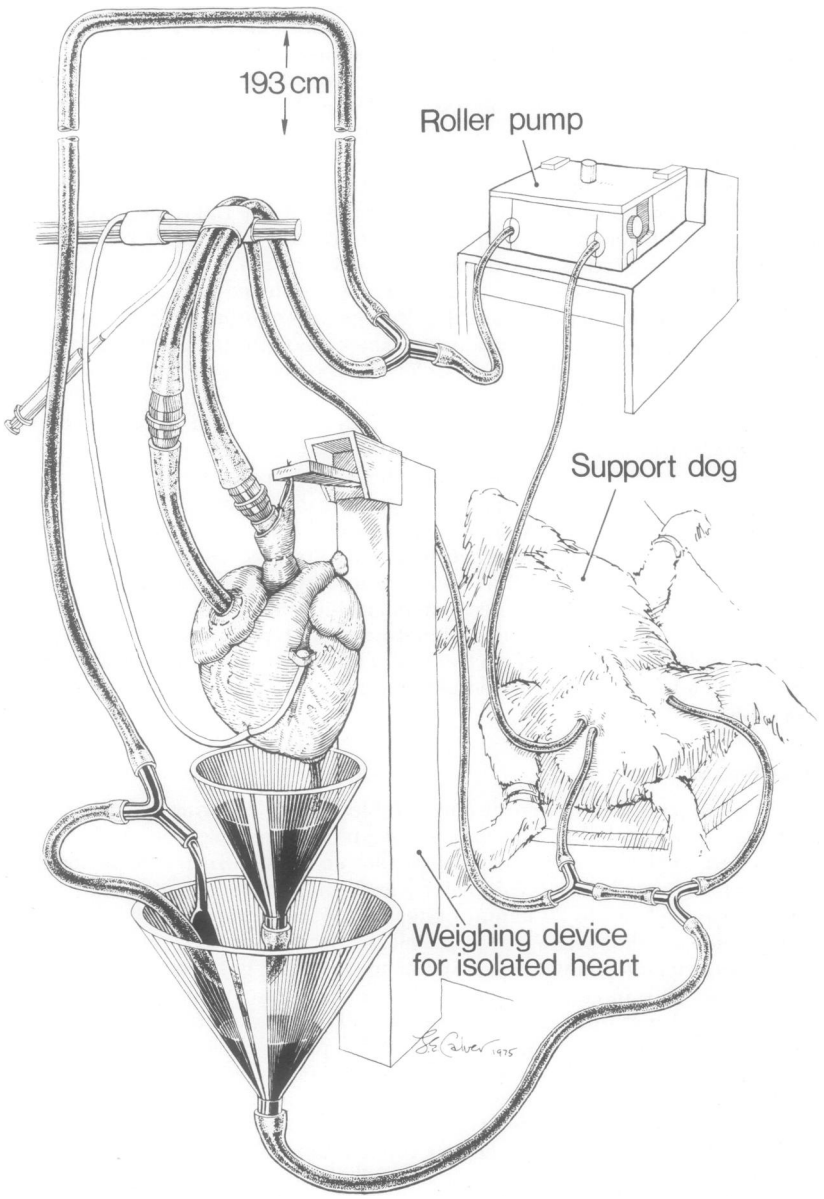
attached to the cantilever beam so that the total weight of the heart was suspended from the weighing device; the perfusion catheters were draped so that they would not interfere with suspension of the heart and would contribute in as minor a way as possible to the weight of the isolated heart (Text-figure 1). The perfusion pressure of the isolated heart was set at a constant value by using a Sarns roller pump (Model 6002) to pump to an overflow valve at a vertical height of 100 cm. Once the heart was positioned on the weighing stand, an inflatable balloon occlusive catheter was positioned around the proximal left anterior descending coronary artery (LAD) or proximal left circumflex coronary artery; when inflated this balloon device totally occluded the coronary artery. Ventricular pacing electrodes were sutured on the anterior surface of the right ventricle. A "mitral button" was placed through the mitral valve orifice and sutured in place so that it prevented mitral regurgitation. A balloon filled with enough volume to provide a left ventricular end-diastolic pressure of 4 to 5 mm Hg was fastened to the mitral button. All pressures were measured using Statham P23Db pressure transducers and recorded on an Electronics for Medicine recorder. A drainage catheter was inserted into the apical dimple of the left ventricle and allowed to freely drain any coronary blood flow that collected in the left ventricle (LV) into a funnel positioned beneath. The drainage from the funnel was returned through a catheter by gravity drainage to the femoral vein of the support dog. A large bore catheter was placed in the right ventricle to collect coronary venous drainage emptying into the right side of the heart in order to return it to the femoral vein of the support dog. The overflow from the vertical tubing (the difference between blood pumped from the support dog and that utilized by the isolated heart) was collected in the funnel positioned beneath the heart and returned by gravity drainage to the femoral vein of the support dog. Thus, when all lines were appropriately connected, the isolated heart–support dog perfusion was a closed system in which blood removed from the support dog for the isolated heart was subsequently returned to the support dog. Heart rate was kept constant by ventricular pacing. Total coronary blood flow was measured as the difference between blood pumped from the support dog and the overflow from the vertical tubing (Text-figure 1).

### Experimental Protocol

A total of 57 isolated hearts were studied (Table 1). The experimental format for the various studies was as follows. The isolated hearts were removed and attached to instruments as described above. The hearts were allowed to stabilize as determined by their recorded weights, hemodynamic parameters, and coronary blood flow. This generally occurred approximately 15 to 20 minutes after they were suspended from the cantilever beam. Since edema is known to occur in isolated perfused hearts, preliminary control studies were performed to identify when spontaneous weight gain occurred in this model. Generally, weight gain in control hearts occurred sometime following 1 hour of stable cardiac weights and performance. Consequently, all of the experiments reported herein are limited to those obtained within 1 hour of stable performance.

When recorded weight and coronary blood flow were stable, the proximal LAD was occluded by inflation of the previously positioned balloon catheter for: 10 minutes followed by 20 minutes of reflow (14 hearts), 40 minutes followed by 20 minutes of reflow (27 hearts), 40 minutes without reflow (4 hearts), or 60 minutes without reflow (9 hearts). In 3 additional hearts, 40 minutes of proximal circumflex coronary occlusion followed by 20 minutes of reflow was evaluated (Table 1).

Changes in heart weight were identified in this experimental model by setting the weight recording in the center of the recording paper with a weight scale range of 0 to 25 g. Changes in weight in these isolated hearts of as little as 0.25 g could be identified with this recording system. Since the histologic studies that were done suggested that the major morphologic changes that occurred were in the ischemic LV (see Results), increases in weight were assumed to represent LV ischemic region weight gain.




TEXT-FIGURE 1—The figure demonstrates the cantilever beam instrumented with strain elements for continuous weighing of the isolated blood-perfused canine hearts and the isolated heart and support dog experimental model. *193 cm* refers to distance from top of overflow column to floor; the overflow column was 100 cm above the arterial pressure transducer.

Table 1—Protocol Utilized in Studies of Isolated Hearts

| Experimental group | Continuous weighing | Tissue $Na^+ + K^+$-ATPase determinations | Tissue CPK and citrate synthase determinations | Nitroglycerin plus adenosine | Morphologic evaluation |
|---|---|---|---|---|---|
| 1. Controls (Includes sham-occluded isolated hearts, hearts obtained from support dogs, and unoperated control dogs) | − | + | − | − | + |
| 2. 10-minute LAD occlusion and reflow (see text for details) | + | − | − | − | + |
| 3. 40-minute LAD occlusion and reflow (see text for details)* | + | + | + | + | + |
| 4. 40-minute circumflex occlusion and reflow (see text for details) | + | − | − | + | + |
| 5. Fixed 40-minute LAD occlusion (see text for details) | + | + | − | − | + |
| 6. Fixed 60-minute LAD occlusion (see text for details) | + | − | − | − | + |

+ = determination was performed, − = determination was not performed.
* Six of these isolated hearts also had ischemic and nonischemic LV ouabain uptake measured.

In the hearts subjected to myocardial ischemia, changes in weights were recorded continuously, and developed pressure, LV dp/dt, mean arterial perfusion pressure, and total coronary blood flow were recorded at 10-minute intervals during the occlusion period. In the hearts with temporary LAD occlusion, weights, pressures, and coronary blood flow were measured 1 minute after release of the occlusion and then again after 10 minutes and 20 minutes of reflow. In the hearts with permanent LAD occlusion, the same pressures and flows were measured during the last 20 minutes of these occlusions as were measured in the reflow hearts.

In 7 of the 27 hearts with 40-minute LAD occlusions and in the 3 hearts with 40-minute circumflex coronary occlusions, nitroglycerin (0.05 mg/ml at 39 ml/min) and adenosine (1.0 mg/kg support dog body weight) were administered by Harvard infusion pump into the aortic perfusion catheter of the isolated heart beginning the infusions 35 minutes after occluding the LAD and continuing them through the release of the occlusion and for the completion of the reflow period (Table 1). In two additional hearts with 40-minute LAD occlusions, the nitroglycerin and adenosine were administered beginning after 15 minutes of reflow, and the infusions were continued for the last 5 minutes of the reflow period. In two other hearts, nitroglycerin and adenosine were given prior to LAD occlusion to identify the peak coronary flow that could be achieved and then again beginning 35 minutes after LAD occlusion and during the 20-minute reflow period.

At the end of the experiments, the hearts were examined carefully. Each of the perfusion and drainage catheters was clamped, and needle aspiration of fluid from both the left and right ventricles was performed. With the exception of hearts designated for histologic study, each ventricle was opened and directly inspected for retained fluid. The hearts that

were utilized for data analysis for all experiments, except for those to which adenosine and nitroglycerin infusions were given, were ones in which no significant fluid accumulation was found in either ventricle. As best we could determine, significant fluid collection between the mitral button and endocardium did not occur in these hearts. Hearts receiving adenosine and nitroglycerin exhibited pooling of blood in the right ventricles, and this factor was noted in the data analysis, as indicated in the Results and Discussion sections.

Since almost all of the hearts in the present experiments were utilized for either morphologic or biochemical studies, it was necessary to estimate the weight of the ischemic and nonischemic regions of the heart by using a group of 41 comparably sized dogs studied in our laboratory over the past 2 years. In these animals, ischemic LV weighed 19.0 ± 1.1 (SE) g and nonischemic region LV weighed 52 ± 6.1 g. In these animals, ischemic LV had also been identified by ST segment mapping and by identifying the portion of the LV that became blue in color when the proximal LAD was occluded. While it seems certain that the ischemic and nonischemic LV weights were not exactly the same in these two groups of dogs, they should have been quite similar.

Statistical comparisons were made for changes in heart weight, coronary blood flow, and LV performance measurements between the period just prior to LAD occlusion and end LAD occlusion, and where applicable, between 1 minute of reflow after LAD occlusion and 10 and 20 minutes of reflow. Results were compared using the paired $t$ test and were considered significant when $P < 0.05$.

Control hearts and hearts from various experimental groups were either processed for morphologic examination or were used for various biochemical assays described below (Table 1). Both routine and detailed histologic and ultrastructural examination was undertaken in these hearts. For the latter studies, the balloon occluder was removed from the coronary artery and an intracoronary catheter was inserted for perfusion fixation. Perfusion was performed for 5 to 10 minutes at a pressure of 120 mm Hg with a 340 mOsm solution of 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature. The fixative chosen conformed to established criteria for the study of cell injury.[5] Following perfusion, the hearts were divided into multiple transverse slices. Generally, uniform perfusion fixation was observed after sectioning the hearts. Large tissue blocks were obtained from the ischemic and nonischemic portions of the left ventricles, immersed in 10% phosphate-buffered formalin, and processed for routine histologic examination. In addition, small blocks were obtained from subepicardial and subendocardial myocardium for detailed light and electron microscopic examination. These blocks were given additional immersion fixation in 1% glutaraldehyde in 0.1 M phosphate buffer, washed in 0.1 M phosphate buffer with or without 5% sucrose, postfixed in 1% osmium textroxide in veronal acetate buffer (pH 7.4), dehydrated in a graded series of alcohols and propylene oxide, and embedded in Mollenhauer's Epon-Araldite mixture number one.[6] Semithin sections in the range of 1 $\mu$ were cut and stained with toluidine blue for light microscopy. Ultrathin sections also were cut and stained with uranyl acetate and lead citrate for examination in a JEOL 100C electron microscope.

In 15 isolated hearts, in two hearts obtained at the end of experiments from the support animals and in three additional hearts obtained from normal, unoperated control dogs, myocardial sodium-potassium ($Na^+ + K^+$)-ATPase activity was measured utilizing previously described methods (Table 1).[7,8] From the 15 isolated hearts, fresh tissue was obtained from subepicardial and subendocardial areas of both the nonischemic and ischemic portions of the LV. Ischemic myocardium was defined as that portion of the LV that developed bluish discoloration immediately following LAD occlusion and included anteroseptal left ventricle and generally extended laterally approximately 1 inch from the LAD. Epicardial mapping was performed to insure that the gross area of bluish discoloration also contained sites of epicardial ST segment elevation equal to or greater than 1.5 mm. In the 2 hearts obtained from the support dogs and the 3 additional hearts obtained from control animals, the same approximate regions and amounts of LV tissue

were removed; these hearts were considered as additional controls for the $(Na^+ + K^+)$-ATPase experiments to be certain that $(Na^+ + K^+)$-ATPase activity was homogeneously distributed between these two regions under normal circumstances. Both direct measurements of $(Na^+ + K^+)$-ATPase and, in 6 isolated hearts, measurements of ouabain binding were obtained (Table 1).

Partial purification and assay of $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.13) was performed by the method of Schwartz *et al.*;[7] fresh heart muscle was utilized for the assays. Inorganic phosphate ($P_i$) analysis was performed using the method of Ames and Dubin.[9] Protein concentration was determined by the method of Lowry *et al.*[10] using crystalline bovine serum albumin as a standard. The enzyme activity was expressed as micromoles of $P_i$ per milligram protein per hour. $^3$H-Ouabain binding to $(Na^+ + K^+)$-ATPase was studied *in vitro* according to the method of Schwartz *et al.*[7] The uptake of $^3$H-ouabain was expressed as picomoles ouabain per milligram enzyme protein.

In six of these hearts, creatine phosphokinase (EC 2.7.3.2) (CPK) activity, and in four hearts citrate synthase activity (EC 4.1.3.7), were also measured in the subendocardial and subepicardial muscle of both the ischemic and nonischemic portions of the left ventricle (Table 1).[11-13] These particular enzymes were chosen since one is predominantly cytoplasmic in location (CPK) and the other is a mitochondrial enzyme (citrate synthase), and their measurement might provide biochemical information regarding cell death in ischemic LV. Citrate synthase activity was measured by determining the initial velocity of CoA SH formation at 412 nm in the presence of 5',5'-dithio(2-nitrobenzoate) as described by Srere *et al.*[12] The preparation of acetyl CoA was based on the procedure of Simon and Shemin.[13] The protein was determined by the method of Lowry *et al.*[10] using crystalline bovine serum albumin as a standard.

## Results

Preliminary experiments were performed to determine whether the addition of small weight changes to these isolated hearts could be identified in this experimental model. In these experiments we found that the addition of extra weight ranging from 0.25 to 10 g to the isolated hearts was precisely recognized and recorded. As noted earlier, preliminary experiments were also performed to determine whether weight gain occurred under control circumstances just as a consequence of the passage of time. In five control hearts, 1 hour and 15 minutes of suspension from the cantilever beam did not result in significant weight gain (increase of 4 ± 5% as compared to original control weight). Longer periods of time, i.e., 2 hours, did result in significant spontaneous weight gain. Therefore, the present experiments are confined to those obtained within 1 hour of stable performance.

### General Hemodynamics

The paced heart rate for all of the isolated hearts studied was 85 ± 2 beats/min. The mean arterial perfusion pressure for all of the isolated hearts was 77 ± 1 mm Hg. Coronary blood flows for all hearts was 95 ± 8 ml/min/100 g LV before coronary occlusion, 51 ± 5 ml/min/100 g LV at the end of coronary occlusion, and 74 ± 6 ml/min/100 g LV after 20 minutes of reflow following the release of the LAD occlusion. The isolated

heart and support dog were maintained at room temperature. During the time periods utilized in these experiments there did not appear to be severe desiccation of the surface of the hearts.

Since the isolated hearts were perfused, even hearts that developed ventricular fibrillation at the time of release of the coronary occlusion could be examined for abnormal myocardial fluid retention, histologic changes, and biochemical alterations following varying periods of coronary occlusion and reflow. In these experiments each heart served as its own control for weight changes; since tissue was removed from both the ischemic and nonischemic regions, each heart also served as its own control for the morphologic and biochemical studies. In each of the various experimental groups that were studied the weight gain, morphologic and biochemical results were directionally identical irrespective of whether the heart remained in a ventricular paced rhythm or developed ventricular fibrillation at the time of release of the coronary occlusion. Therefore, all of the hearts in each experimental group have been considered together.

#### Individual Groups Studied

##### Ten-Minute Occlusion of the Proximal Left Anterior Descending Coronary Artery (LAD) and Reflow Hearts

Fourteen isolated hearts were subjected to 10-minute LAD occlusions and reflow. These hearts were studied to determine whether an extremely short period of coronary occlusion and subsequent reflow resulted in abnormal weight gain. Only one of these hearts fibrillated at the time of release of the LAD occlusion. There was no significant change in weight during the 10-minute occlusion period; in fact, there was a tendency toward weight loss during the LAD occlusion [90 ± 3% (SE), $P < 0.1$]. There was no significant gain in ischemic region weight after release of the LAD occlusion following 10 (98 ± 2%) and 20 minutes (100 ± 4.0%) of reflow as compared to the weight of the isolated hearts after 1 minute of reflow. Coronary blood flow did not change significantly during the 10-minute occlusion (97 ± 3.1%) as compared to 1 minute after occlusion. Immediately following release of the LAD occlusion, coronary blood flow increased to 192 ± 18% ($P < 0.001$). After 10 minutes of reflow, coronary blood flow had fallen to 71 ± 5%, and after 20 minutes of reflow, to 64 ± 6% as compared to the values present after 1 minute of reflow. LV dp/dt tended to decrease during the 10-minute occlusion (91 ± 5%) but returned toward normal after 20 minutes of reflow. Peak LV systolic pressure similarly tended to decrease during the 10-minute occlusion but

returned to preocclusion values at the completion of reflow. Thus, in these hearts it was not possible to document abnormal fluid retention by measuring changes in the weight of the isolated hearts with the 10-minute occlusion or during reflow after the occlusion.

#### Sixty-Minute Permanent LAD Occlusion Hearts

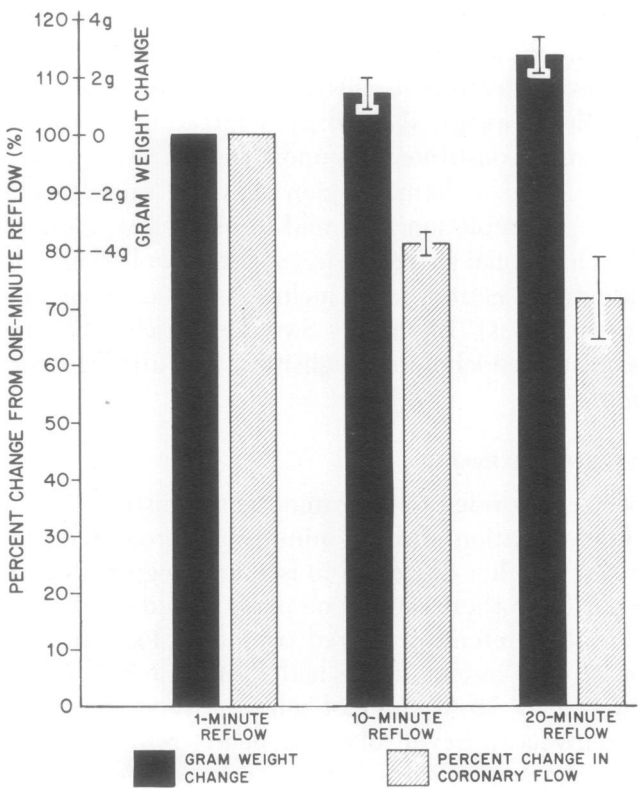
These experiments were performed to determine whether fixed coronary occlusion for 1 hour resulted in abnormal myocardial fluid retention in these isolated hearts. In nine hearts with fixed 60-minute occlusion of the proximal LAD, there was no significant weight gain, either during the initial 40-minute period of occlusion or during the last 20 minutes of the occlusion ($1 \pm 4\%$). All of these hearts remained in a ventricularly paced rhythm throughout the studies. There was a tendency for coronary blood flow to decrease during the last 20 minutes of the occlusion ($87 \pm 5\%$, $P < 0.1$) as compared to coronary flow after 40 minutes of LAD occlusion.

Histologic and ultrastructural study of nonischemic and ischemic myocardium showed that 60 minutes of fixed LAD occlusion was associated with prominent alterations of numerous cardiac muscle cells in the absence of significant interstitial edema (Figures 1 and 2). Occasional muscle cells in the ischemic region showed focal myofibrillar alterations in the form of typical zonal lesions. Other muscle cells appeared ultrastructurally normal. Many muscle cells, however, showed mild intracellular edema and swelling, and this change constituted the most frequently observed alteration of muscle cells in the ischemic region. Swollen muscle cells frequently exhibited glycogen depletion and mild to marked swelling of mitochondria (Figure 2). Occasional mitochondria of some swollen muscle cells also exhibited distinctive electron-dense inclusions (amorphous matrix densities, flocculent densities) (Figure 2). Swollen muscle cells, including those with flocculent mitochondrial densities, had ultrastructurally intact plasma membranes.

#### Forty-Minute LAD Occlusion With Reflow Hearts

These experiments were performed to determine a) the distribution of abnormal myocardial fluid retention after 40 minutes of coronary occlusion followed by reflow, b) whether decreases in ischemic region ($Na^+ + K^+$)-ATPase activity occurred at these same time periods, and c) whether the abnormal myocardial fluid retention altered coronary blood flow responses to potent vasodilators. Thirteen hearts had 40-minute ligations of the proximal LAD followed by 20 minutes of reflow without receiving either adenosine or nitroglycerin. Seven of these hearts remained in a ventricular paced rhythm throughout the occlusion and during reflow; six

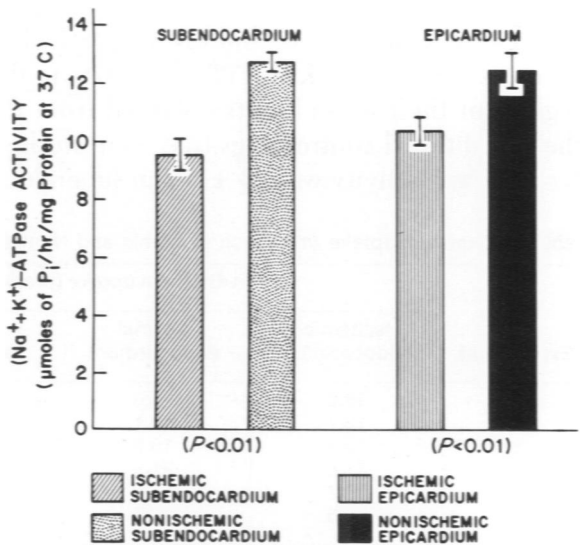
developed ventricular fibrillation following release of the coronary occlusion. In these hearts there was no significant gain in weight during the 40-minute occlusion (96 ± 7% as compared to the weight 1 minute after LAD occlusion), but with reflow significant weight gain occurred in the ischemic region of the LV after both 10 (7 ± 3%), (1.3 ± 0.6 g, $P < 0.05$), and 20 minutes (14 ± 1%), (2.6 ± 0.8 g, $P < 0.01$) of reflow as compared to weight noted after 1 minute of reflow (Text-figure 2). The percent increase in weight during reflow is calculated on the basis of an ischemic region weight of 19.0 g (see Materials and Methods). Coronary blood flow tended to decrease during the 40-minute occlusion (86 ± 7%, $P < 0.1$) compared to flow at the time of LAD occlusion; with release of the LAD occlusion, coronary blood flow increased by 235 ± 27% ($P < 0.001$) at 1 minute of reflow. Ten minutes of reflow resulted in coronary blood flow decreasing to 82 ± 4%, $P < 0.01$, and after 20 minutes of reflow, coronary blood flow had fallen to 73 ± 7%, $P < 0.01$ of the values obtained after 1 minute of reflow (Text-figure 2). The weight gain by the isolated hearts




TEXT-FIGURE 2 — Bar graphs that demonstrate the changes in coronary blood flow and ischemic LV weight that occur after 40 minutes of LAD occlusion with 10 and 20 minutes of reflow. Percent change in coronary flow and change in weight in grams are shown in the vertical axis.

with reflow at a time when coronary blood flow was decreasing demonstrates that the abnormal weight gain in the ischemic region was not simply the result of an increase in coronary blood volume as part of the hyperemic response, although from the weight measurements alone it would not be possible to exclude the theoretical possibility that a decrease in venous capacitance was a factor. LV dp/dt fell significantly during the 40-minute occlusion as compared to values noted prior to LAD occlusion ($86 \pm 5\%$, $P < 0.05$) and increased significantly with reflow (increase of $68 \pm 22\%$, $P < 0.01$).

Measurement of $(Na^+ + K^+)$-ATPase activity in the ischemic and nonischemic areas of the left ventricle in nine of these hearts demonstrated a value of $10 \pm 0.5$ in the subendocardial portion of the ischemic area as compared to $12.7 \pm 0.4$ μmoles of $P_i$/hr/mg protein at 37 C in the subendocardial portion of the nonischemic LV ($P < 0.01$) (Text-figure 3, Table 2). In the ischemic epicardium, $(Na^+ + K^+)$-ATPase activity was $10 \pm 0.4$ as compared to $13 \pm 0.6$ μmoles of $P_i$/hr/mg protein at 37 C in nonischemic epicardium ($P < 0.01$) (Table 2, Text-figure 3). Seven of these hearts remained in a ventricular paced rhythm throughout the study, and two developed ventricular fibrillation at the time of reflow. The $(Na^+ + K^+)$-ATPase activity reductions in the ischemic region were present irrespective of whether the hearts remained in a regular rhythm or developed ventricular fibrillation with reflow. In addition, in the six hearts that were tested, ouabain uptake was also decreased in the subendocardial




TEXT-FIGURE 3—The actual values for ischemic and nonischemic LV $(Na^+ + K^+)$-ATPase activity in the isolated hearts with 40 minutes of LAD occlusion and 20 minutes of reflow are shown. The bars represent mean values and the cross bars, standard errors.

Table 2—$(Na^+ + K^+)$-ATPase Specific Activity ($\mu$moles of $P_i$/hr/mg protein at 37 C)*

| No. | Ischemic endocardium | Normal endocardium | Ischemic epicardium | Normal epicardium |
|---|---|---|---|---|
| 1 | 8.8 | 13.0 | 10.0 | 13.0 |
| 2 | 8.0 | 13.3 | 10.3 | 13.8 |
| 3 | 11.9 | 13.5 | 12.6 | 14.0 |
| 4 | 6.8 | 13.6 | 9.4 | 14.5 |
| 5 | 11.1 | 13.0 | 11.7 | 13.3 |
| 6 | 10.4 | 12.8 | 11.0 | 13.0 |
| 7 | 10.1 | 10.4 | 9.5 | 9.7 |
| 8 | 9.7 | 11.6 | 9.3 | 10.4 |
| 9 | 9.0 | 13.2 | 8.6 | 10.4 |

* Represents $(Na^+ + K^+)$-ATPase activity in the precipitates after the second DOC treatment (see Materials and Methods). In these isolated hearts sodium, potassium ATPase activity was measured in the initial crude homogenate and in the first DOC-treated precipitate. The decrease in activity of the enzyme was present even in these initial measurements.

and subepicardial portions of the ischemic area as compared to that noted in the same regions in nonischemic LV (Table 3). In contrast there was no significant depression of creatine phosphokinase activity in the same area in six of these hearts (19 ± 1 IU in subendocardium of ischemic area as compared to 20 ± 2 IU in subendocardial portion of nonischemic LV and 20 ± 1 IU in subepicardial portion of ischemic versus 21 ± 0.6 in subepicardium of nonischemic LV). Neither was there a significant reduction in citrate synthase in either the subendocardial or subepicardial portions of the ischemic region as compared to nonischemic areas (0.7 ± 0.1 in subendocardium and 0.8 ± 0.1 in epicardium of ischemic area as compared to 0.7 ± 0.1 in subendocardium and 0.9 ± 0.1 in epicardium of the nonischemic area of the LV respectively). There was no significant difference in $(Na^+ + K^+)$-ATPase activity in the anterior and posterior LV regions of the control hearts obtained from the 2 support dogs and from the 3 additional control dogs that were studied; in these animals $(Na^+ + K^+)$-ATPase activity was 13 ± 0.5 in subendocardium anteriorly versus 13

Table 3—Ouabain Uptake *In Vitro* in Ischemic and Nonischemic LV (N = 6)

| Experiment | $^3$H-Ouabain uptake (pmoles/mg protein) | | | |
|---|---|---|---|---|
| | Ischemic endocardium | Normal endocardium | Ischemic epicardium | Normal epicardium |
| 1 | 18.0 | 20.0 | 15.1 | 16.7 |
| 2 | 13.7 | 16.1 | 13.4 | 15.4 |
| 3 | 13.2 | 18.9 | 12.8 | 15.1 |
| 4 | 17.6 | 21.5 | 16.0 | 18.9 |
| 5 | 16.7 | 18.1 | 15.8 | 16.7 |
| 6 | 10.5 | 15.3 | 12.7 | 16.1 |

Table 4—($Na^+$ + $K^+$)-ATPase Activity ($\mu$moles of $P_i$/hr/mg Protein at 37 C) in Isolated Canine Hearts With 40-Minute LAD Occlusion Without Reflow (N = 4)

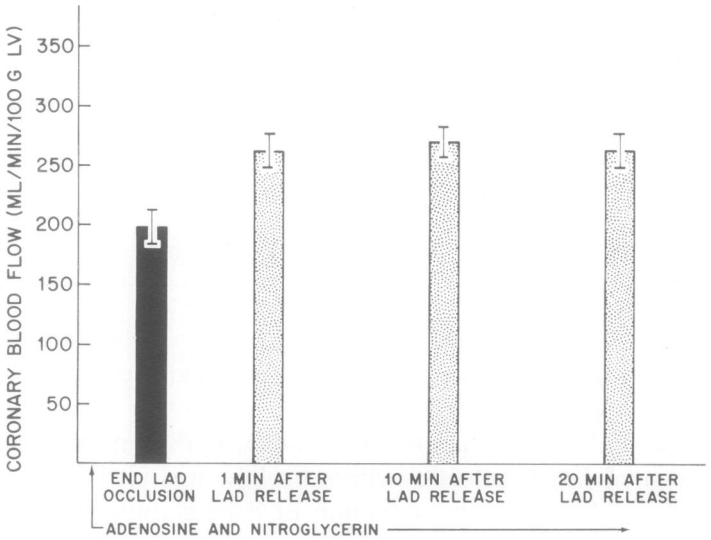
| Experiment | Normal endocardium | Ischemic endocardium | Normal epicardium | Ischemic epicardium |
|---|---|---|---|---|
| 1 | 10.2 | 9.0 | 12.2 | 10.5 |
| 2 | 12.1 | 10.3 | 13.5 | 11.7 |
| 3 | 13.7 | 11.4 | 11.9 | 10.4 |
| 4 | 13.2 | 11.4 | 13.8 | 12.2 |
| Mean | 12.3 ± 0.79 | 10.5 ± 0.57 | 12.8 ± 0.50 | 11.2 ± 0.45 |

± 0.7 in same area posteriorly and 13 ± 0.4 in epicardium anteriorly versus 12 ± 0.5 $\mu$moles $P_i$/hr/mg protein in epicardium posteriorly (see Materials and Methods).

Four additional isolated hearts were studied to determine if a fixed 40-minute proximal LAD occlusion without reflow was also associated with ($Na^+$ + $K^+$)- ATPase activity reduction in the ischemic LV. In each of these hearts ($Na^+$ + $K^+$)-ATPase activity was lower in ischemic LV than in nonischemic tissue (Table 4).

### Administration of Nitroglycerin and Adenosine to Isolated Hearts With 40-Minute LAD or Left Circumflex Occlusion and Reflow

These particular studies were performed to determine whether abnormal myocardial fluid retention developing after 40 minutes of proximal LAD occlusion followed by reflow interfered with coronary vascular responses to potent vasodilators. A total of 11 isolated hearts with proximal LAD occlusions received nitroglycerin and adenosine either immediately prior to and during reflow (9 hearts) or beginning 15 minutes after reflow was begun (2 hearts). Two of these hearts received nitroglycerin and adenosine prior to LAD occlusion and again at the end of the LAD occlusion and during the 20-minute reflow period. In the nine hearts that received nitroglycerin and adenosine with the infusions beginning 35 minutes after LAD occlusion, heart weights increased at 10 minutes of reflow by 44 ± 9% ($P < 0.005$) as compared to weights noted 1 minute after reflow. Twenty minutes of reflow was associated with a gain in weight in the isolated hearts of 218 ± 26% ($P < 0.005$). Coronary blood flow was maintained at a constant level by the nitroglycerin and adenosine infusions throughout the reflow period so that after 20 minutes of reflow coronary blood flow was 101 ± 2% of that noted after 1 minute of reflow (Text-figure 4). Thus, it was not possible to identify any major effect of abnormal myocardial fluid retention to modify a persistent hyperemic response provided by adenosine and nitroglycerin infusion during reflow after 40 minutes of proximal LAD occlusion. In the two hearts that



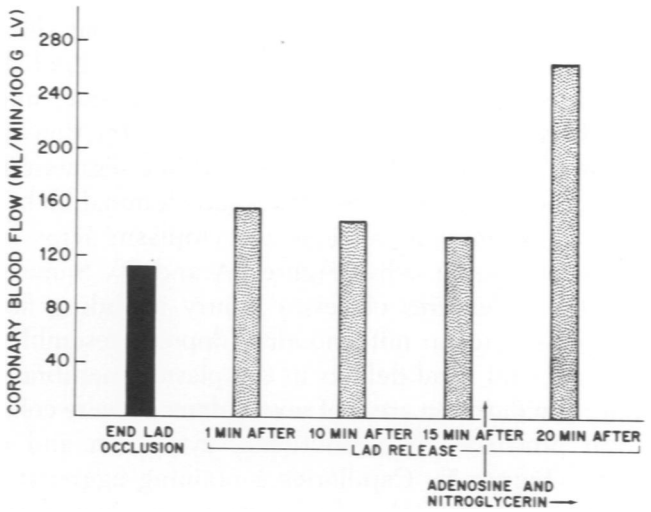

TEXT-FIGURE 4—This figure demonstrates that despite abnormal myocardial fluid retention in the isolated hearts subjected to 40 minutes of LAD occlusion and reflow, it was possible to keep coronary blood flow at the same high levels observed after 1 minute of reflow for 20 minutes of reflow when nitroglycerin and adenosine were infused.

received nitroglycerin and adenosine prior to LAD occlusion and again at the end of the occlusion and during reflow, it was possible to equal and even exceed peak coronary flows noted preocclusion during the late reflow period.

In the two isolated hearts that were given nitroglycerin and adenosine infusions beginning 15 minutes after reflow was started and continued for an additional 5 minutes, it was possible to increase coronary blood flow to levels above those present after 1 minute of reflow as well as to levels well above those after 10 minutes of reflow (Text-figure 5). This data again suggested that abnormal myocardial fluid retention, once present, did not prevent adenosine and nitroglycerin infusion from increasing coronary blood flow to levels well above those noted immediately after release of the coronary occlusion.

In three additional experiments, the influence of proximal circumflex coronary occlusion on reflow patterns was examined. Nitroglycerin and adenosine were given near the end of the 40-minute occlusion and during 20 minutes of reflow in exactly the same manner as utilized in the hearts with LAD occlusion. In these three hearts it was also possible to maintain a constant coronary blood flow throughout the 20-minute reflow period.

Histologic and ultrastructural examination revealed similar changes in hearts subjected to 40 minutes of temporary occlusion of the LAD or




TEXT-FIGURE 5—This figure shows that nitroglycerin and adenosine infusion beginning after 15 minutes of reflow following 40 minutes of LAD occlusion can increase coronary blood flow to levels even above those observed after 1 minute of reflow despite the presence of abnormal myocardial fluid retention (N = 2 hearts).

circumflex coronary arteries with or without administration of nitroglycerin and adenosine during the reflow period. Histologically, ischemic myocardium from the hearts with temporary coronary occlusion and reflow exhibited widespread interstitial edema, focal congestion of capillaries and postcapillary venules, focal hemorrhage, and multiple foci of damaged muscle cells (Figure 3). The damaged muscle cells exhibited edema and swelling with or without myofibrillar disruption and formation of dense, transverse contraction bands (Figure 3). Rare muscle cells appeared dense and hypercontracted without edema formation (Figure 3). The interstitial and intracellular alterations were more extensive in the subendocardial than the subepicardial myocardium. The hearts also showed focal endocardial hemorrhage.

Ultrastructural examination of ischemic tissure revealed a pleomorphic population of ultrastructurally intact, mildly damaged and severely injured muscle cells. Prominent interstitial edema was present in many areas with intact muscle cells as well as in many areas with damaged muscle cells. Some muscle cells showed prominent cell swelling and glycogen depletion but were otherwise structurally normal (Figure 4A). Occasional muscle cells lacked edema but exhibited focal areas of hypercontraction (zonal lesions) similar to the lesions observed after 60 minutes of fixed coronary occlusion (Figure 4B). The zonal lesions were characterized by hypercontraction of myofibrils adjacent to the intercalated discs associated with focal lysis of myofibrils and displacement of mitochondria away from the intercalated discs. Muscle cells with zonal lesions did not show other abnormalities and were not edematous. Other muscle cells

exhibited a combination of edema, vacuolar dilatation of sarcoplasmic reticulum and T tubules, accumulation of lipid droplets, mitochondrial damage with swelling or stacking of cristae, and hypercontraction of myofibrils leading to disruption and contraction band formation (Figures 5 and 6). Hypercontraction resulted in a segmental distribution of edema fluid, including formation of subsarcolemmal blebs (Figure 5) and herniation of edematous portions of cytoplasm across intercalated discs into adjacent muscle cells (Figure 6A and B). Some muscle cells exhibited additional features of severe injury including flocculent mitochondrial densities, spicular mitochondrial deposits resembling calcium-rich apatite crystals, and focal defects in the plasma membranes (Figure 6C and D). Rare capillaries in areas of severe damage were collapsed; others exhibited focal protrusions of endothelial cytoplasm and contained cytoplasmic blebs (Figure 5). Capillaries containing aggregates of erythrocytes and, rarely, platelets also were observed. Nevertheless, most capillaries throughout the ischemic area, even in areas of severe damage, were widely patent. Swelling of endothelial cells or interstitial cells or disruption of the plasma membranes of these cells were not observed.

## Discussion

Questions regarding the onset of abnormal myocardial fluid retention aften initiation of acute myocardial ischemia and the role of abnormal myocardial fluid retention in perpetuating the ischemic process generally have been considered to be of central importance.[1,14] It has been demonstrated previously in the brain and the kidney that prolonged ischemia results in intracellular swelling and that swollen cells can compress adjacent capillaries, thus providing a mechanism for perpetuation of the ischemic process.[2-4] Leaf has postulated that cell swelling occurs as a consequence of the inability of the sodium-potassium-ATPase pump to actively extrude sodium from the interior of cells during a period of insufficient blood supply and that this results in intracellular movement of sodium, chloride, and water and subsequent cell swelling.[1] Hypertonic mannitol, by virtue of its extracellular position, has been demonstrated to reduce or prevent cell swelling in both the brain and the kidney, and thus improve regional perfusion during profound ischemia in these organs.[2,4]

In the heart it has not been clear what role abnormal fluid retention might play in the perpetuation of the ischemic process prior to the development of extensive myocardial necrosis. Previous investigations have demonstrated that hypertonic mannitol reduces myocardial damage, improves total and collateral coronary blood flow, and improves ventricular function during acute (short-term) myocardial ischemia in both

anesthetized and awake intact experimental animals.[15,16] A *no-reflow* phenomenon has been demonstrated in the heart after 90 to 120 minutes of either LAD or circumflex coronary artery occlusion,[17,18] and hypertonic mannitol has been shown to be capable of modifying it,[18] but this is a time period by which extensive myocardial necrosis has begun to develop. More recently, hypertonic mannitol has also been shown to be capable of reducing myocardial infarct size when it is given prior to and continued during a relatively short period of myocardial ischemia.[19] Nevertheless, it remains uncertain whether mannitol reduces infarct size during short-term coronary occlusion by a) increasing coronary blood flow, either as a consequence of preventing cell swelling or by directly reducing coronary vascular resistance and/or b) preventing cell swelling and thus directly altering the rate of progression to cell death independent of its influence on coronary blood flow. We have performed the present experiments to determine whether there are differences in the location and types of abnormal myocardial retention with fixed coronary occlusion and temporary coronary occlusion followed by reflow. These studies also tested the influence of abnormal myocardial fluid retention in altering the ability to pharmacologically vasodilate coronary vessels with the hope that the information provided by these experiments would provide additional information regarding the mechanism of mannitol's protective effect during short-term coronary occlusion.

In the present experiments, the data demonstrate that the potential for abnormal myocardial fluid retention occurs within 40 minutes after proximal LAD or circumflex coronary occlusion and that abnormal fluid retention can be demonstrated by reflow and recognized as significant weight gain in the isolated heart. The morphologic studies demonstrate that abnormal myocardial fluid retention following temporary coronary occlusion and reflow was due to intracellular and interstitial edema and focal vascular congestion and hemorrhage. This fluid accumulation probably was mediated by functional abnormalities in vascular permeability unmasked by reperfusion. Direct evidence of such vascular permeability abnormalities has been reported in various types of muscle injury.[20,21] In contrast, following 60 minutes of permanent LAD occlusion, mild swelling of muscle cells without interstitial edema was noted; the intracellular edema appeared to represent an abnormal redistribution of fluid from the extracellular to the intracellular space since it developed in the absence of an overall cardiac weight gain.

Jennings and his associates, using an intact canine model, have previously shown that temporary occlusion of the circumflex coronary artery for 40 to 90 minutes followed by reflow results in marked cell swelling

with formation of prominent subsarcolemmal blebs.[17,22,23] The swollen muscle cells uniformly exhibited evidence of irreversible injury produced by the prior interval of severe myocardial ischemia.[17,22,23] In the present study, ischemic myocardium with abnormal myocardial fluid retention exhibited a pleomorphic population of muscle cells with variable degrees of damage, usually including edema and swelling. Many muscle cells exhibited changes of mild injury, such as glycogen depletion or formation of myofibrillar zonal lesions, which have been demonstrated to be potentially reversible.[24-26] In hearts with temporary coronary occlusion, more severe muscle cell injury was associated with marked myofibrillar hypercontraction with distortion of cellular architecture including segmentation of edematous cytoplasm into subsarcolemmal blebs and double-membrane-limited vacuoles formed by cytoplasmic herniation across intercalated discs.[17,22-24,27] Nevertheless, only a minority of the muscle cells showed changes indicative of irreversible injury, including flocculent mitochondrial densities following permanent or temporary coronary occlusion, or marked mitochondrial calcification, severe myofibrillar disruption, and breaks in the plasma membranes following temporary coronary occlusion.[17,22-24,28,29] Although evidence of explosive cell swelling as described by Jennings and his associates was observed in severely injured muscle cells after temporary coronary occlusion, cell swelling and edema was found in many cells with changes of mild as well as severe or irreversible injury. Furthermore, no significant reductions were found in CPK levels in the ischemic tissues. Thus, abnormal myocardial fluid retention was demonstrated *prior to* the development of extensive myocardial necrosis as judged by ultrastructural and biochemical criteria.

In our model, the abnormal myocardial fluid retention occurs in association with a reduction in sodium-potassium-ATPase activity in both the subendocardial and subepicardial regions of the ischemic area, thereby suggesting that a potential functional defect in cell membrane integrity occurs within 40 minutes of proximal LAD occlusion. Whether this small reduction in ($Na^+ + K^+$)-ATPase activity is the major reason for the development of cell swelling is, of course, presently uncertain. In fact, defects in plasma membrane integrity could be demonstrated in the hearts subjected to 40 minutes of coronary occlusion and reflow, but similar structural alterations in cell membrane integrity were not apparent with 60 minutes of fixed occlusion. While the average reduction in ischemic region ($Na^+ + K^+$)-ATPase activity appeared small, the significance of the abnormality would increase if future studies demonstrate that some ischemic region cells have marked reductions while others retain almost normal ($Na^+ + K^+$)-ATPase activity. Since in this present study,

intracellular edema was a widespread but not universal alteration in the ischemic region, one would like to know whether the swollen cells developing during fixed coronary occlusion are ones with reduced ($NA^+ + K^+$)-ATPase activity and whether those that do not demonstrate edema are ones with near normal levels of enzyme activity.

One might suggest that the abnormal fluid retention in the ischemic LV "diluted" ($Na^+ + K^+$)-ATPase activity, making it appear low erroneously. However, the fact that *in vitro* ouabain uptake was also reduced and that there was no similar significant reduction in levels of either CPK or citrate synthase argues strongly against that possibility. The lack of significant reduction in CPK and citrate synthase activity indicated that the reduction in ($Na^+ + K^+$)-ATPase activity was not part of a generalized reduction in ischemic region enzyme activity but occurred selectively prior to biochemical evidence of cell death. Other workers, using a different canine model of mycardial infarction, have previously presented data suggesting that the reduction in myocardial tissue ($Na^+ + K^+$)-ATPase activity becomes progressively more severe from 2 to 6 hours after myocardial ischemia and that it is associated with reduced cardiac glycoside tissue binding.[30] However, at least one previous group of investigators, using yet another experimental model of myocardial infarction, did not find a reduction in tissue ($Na^+ + K^+$)-ATPase activity until 7 days following the onset of the ischemic process.[31] Presumably, the different experimental models utilized account for the different results.

The exact amount of abnormal myocardial fluid retention that occurs after 40 to 60 minutes of LAD ligation using our model is difficult to precisely quantify. This is due to the fact that coronary blood volume itself contributes to the weight of the isolated hearts, and therefore, the weight measurements obtained during reflow are a combination of tissue weight gain by the isolated heart and a loss of weight by the heart resulting from the reduction in coronary blood flow. Abnormal weight gain was identified after 40 minutes of LAD occlusion with reflow but not following 10 minutes of LAD occlusion and reflow.

The present studies do not establish a major role for abnormal myocardial fluid retention in altering coronary blood flow following 40 minutes of proximal coronary occlusion in this model. The evidence for this statement is our ability to keep coronary blood flow at a constant level throughout a reflow period of 20 minutes with adenosine and nitroglycerin infusion after an ischemic period in which abnormal myocardial fluid retention occurred both with LAD and circumflex coronary artery occlusion. The isolated hearts that received nitroglycerin and adenosine also gained weight with reflow; however, a portion of this weight gain was due

to the increase in coronary blood flow that occurred as a result of adenosine and nitroglycerin infusion, so that the weight gain was overestimated in these hearts. In addition to the increase in weight occurring as a consequence of the increase in coronary blood flow, there was also an increase in blood that collected in the right ventricles of these hearts as the marked increase in coronary blood flow exceeded the capability of the drainage catheter to adequately remove blood, thus adding to the weight gain. So while it was still impossible to precisely quantitate the amount of abnormal fluid retention in these hearts, we found no evidence that the abnormal fluid retention significantly reduced coronary blood flow. Even when adenosine and nitroglycerin administration were delayed until 15 minutes after reflow, it was still possible to increase coronary blood flow above the values seen at one minute of reflow.

One might argue that there might be a no-reflow phenomenon in the ischemic area, and that abnormal myocardial fluid retention might have reduced flow in that area but not elsewhere, and that this might be undetectable using our model. The focal alterations noted in some capillaries in areas of severe myocardial damage following temporary coronary occlusion also could be taken as evidence for this hypothesis. These lesions included apparent compression or collapse of capillaries adjacent to swollen muscle cells with subsarcolemmal blebs as well as capillaries containing blebs or protrusions of endothelial cytoplasm. The vast majority of capillaries, however, were widely patent and devoid of such lesions; swelling of endothelial or interstitial cells was never observed. Furthermore, the physiologic significance of the rare capillary lesions observed in the present study must be seriously questioned because of the demonstration of similar changes in ischemic tissues that did not show evidence of a no-reflow phenomenon.[17,22,23,32] In addition, the rare capillary lesions were observed in association with marked contraction band formation in muscle cells, a phenomenon which appears to occur in tissue receiving significant perfusion following temporary ischemia.[17,22-24]

The present data do not exclude the possibility, once significant myocardial necrosis has developed, that cell swelling and/or interstitial edema itself might not contribute to the no-reflow phenomenon. In fact, there is some evidence to suggest this as a possibility;[17] however, once significant myocardial necrosis develops, it becomés virtually impossible to distinguish between necrosis, intrinsic vascular damage and thrombosis, and abnormal myocardial fluid retention as potential causes for the no-reflow phenomenon.

In summary: Our data demonstrate that abnormal myocardial fluid retention occurs in isolated, blood perfused canine hearts as early as

following 40 minutes of occlusion of the left anterior descending or circumflex coronary artery if reflow is provided. This abnormal myocardial fluid retention amounts to, at least, an increase in weight of 7% (1.3 g) in the ischemic LV after 10 minutes of reflow and 14% (2.6 g) after 20 minutes of reflow following 40 minutes LAD occlusion; however, both of these figures are definite underestimates of the actual amount of abnormal myocardial fluid retention, since weight loss from decreasing coronary blood volume is not taken into account. The abnormal myocardial fluid retention occurring with temporary LAD or circumflex occlusion and reflow represents intracellular and interstitial edema with focal vascular congestion and hemorrhage, and it occurs prior to the onset of extensive myocardial necrosis. These studies did not identify a major role for abnormal myocardial fluid retention *per se* in decreasing coronary blood flow prior to the development of extensive myocardial necrosis. Thus, the ability of hypertonic mannitol to reduce infarct size at this same time period[19] would appear to be the result of either its ability to directly preserve cell viability by reducing or preventing cell swelling and/or its ability to directly reduce coronary vascular resistance and increase coronary blood flow. These data also suggest that future investigation should probably focus on the metabolic and electrophysiologic consequences of abnormal myocardial fluid retention during the early phases of ischemic injury.

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[*Illustrations follow*]

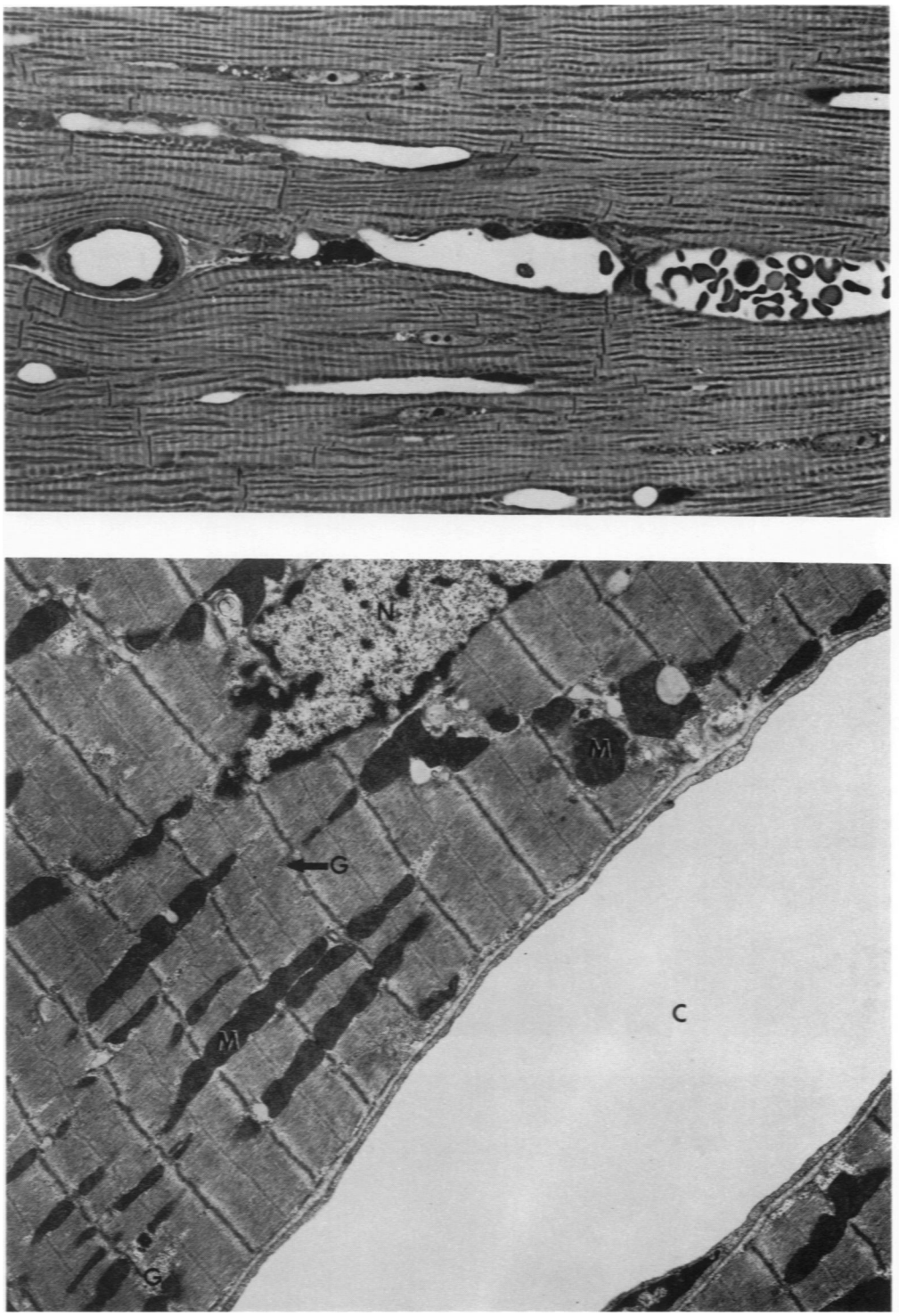


**Figure 1**—Anterior left ventricular myocardium from isolated heart suspended for 60 minutes without coronary occlusion. **A**—Myocardium shows no intracellular or interstitial edema and appears histologically normal. As a result of perfusion fixation, the capillaries are expanded and are largely devoid of erythrocytes. (Plastic section, toluidine blue stain, × 575) **B**—Myocardium also appears ultrastructurally intact. The muscle cells are fixed in a slightly relaxed state as indicated by the presence of narrow I bands. The muscle cells exhibit compact cytoplasm with no separation of organelles, and contain abundant glycogen (*G*) and normal mitochondria (*M*) and nucleus (*N*). The capillary (*C*) is expanded and has thin endothelium. The interstitial space is very narrow (× 6800)

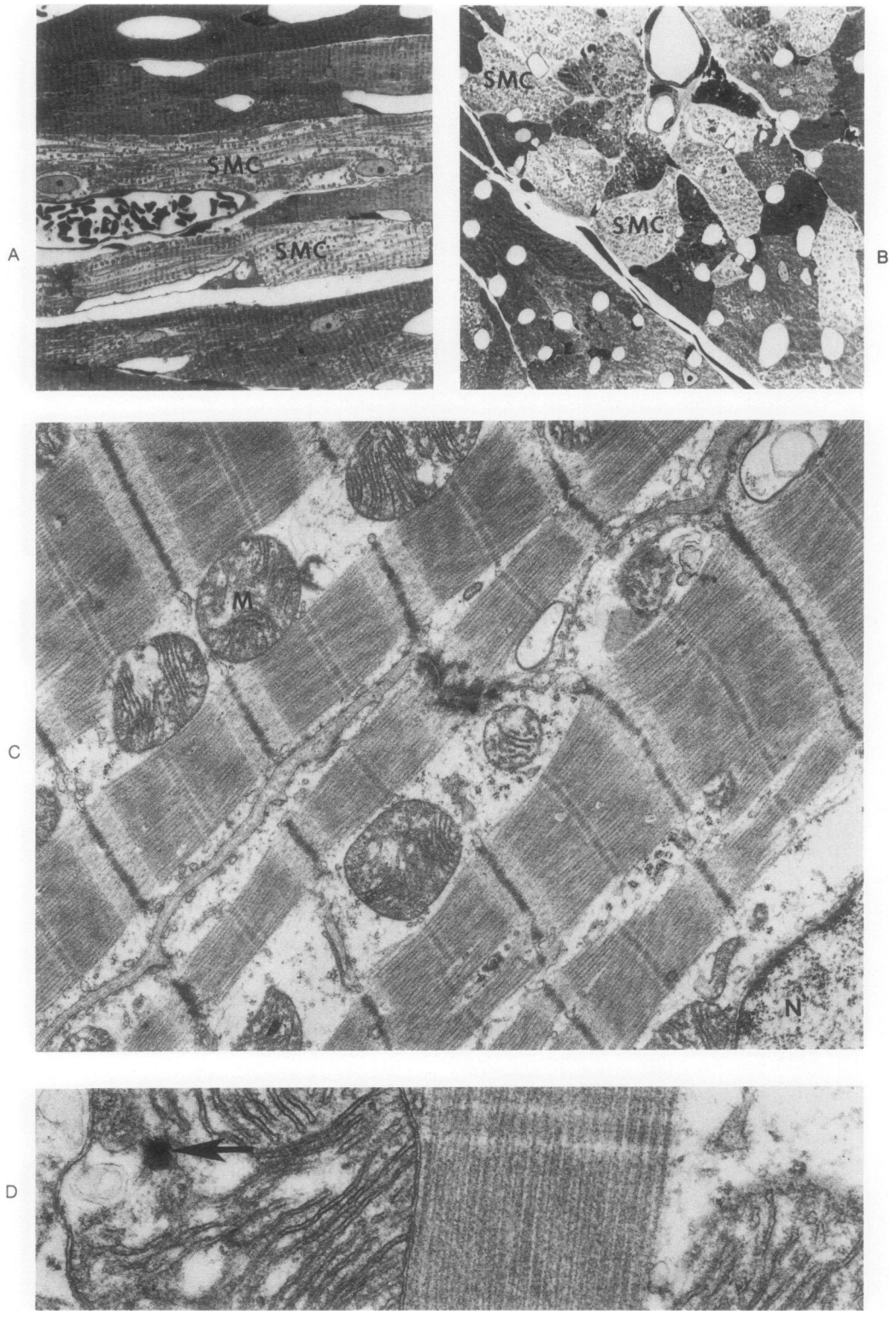


**Figure 2**—Anterior left ventricular myocardium from isolated hearts with 60-minute permanent LAD occlusion. **A**—Longitudinal histologic section demonstrates mild edema and swelling of some muscle cells (*SMC*) indicated by prominent separation of cytoplasmic organelles. There is also focal separation of cells without demonstrable interstitial edema fluid. (× 425) **B**—Numerous pale swollen muscle cells (*SMC*) with separated organelles are also present in this tangential histologic section (× 425). **C**—In this electron micrograph, the muscle cells exhibit mild swelling indicated by separation of organelles, glycogen depletion, slightly swollen mitrochondria (*M*) without inclusions, markedly relaxed myofibrils, intact plasma membranes, and a normal nucleus (*N*) (× 14,500). **D**—Another muscle cell shows similar features of damage as well as an electron-dense, flocculent mitochondrial inclusion (*arrow*) (× 48,000).

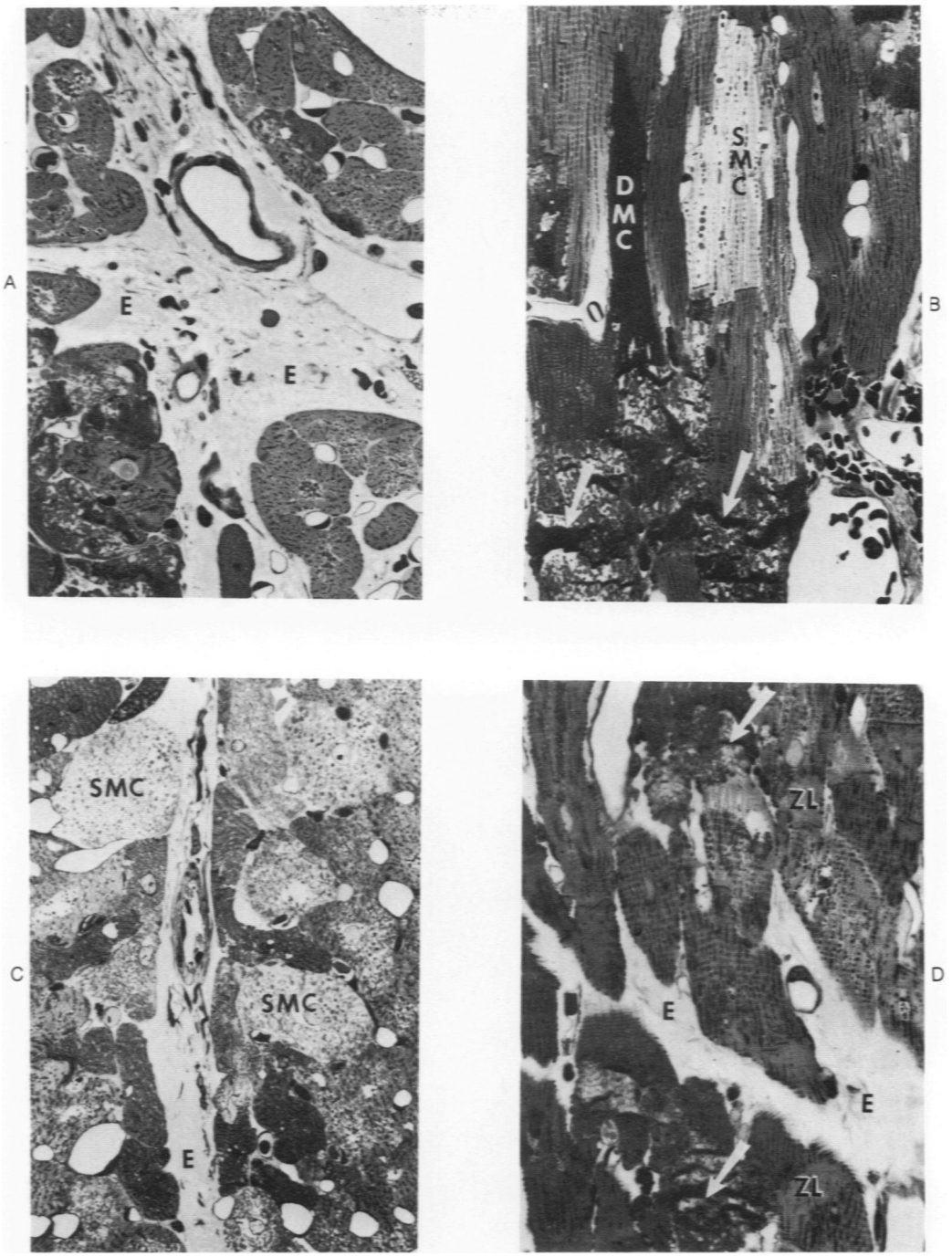


**Figure 3**—Light micrographs of left ventricular myocardium subjected to 40 minute temporary occlusion of the LAD (**A** and **B**) or circumflex (**C** and **D**) coronary arteries and 20 minutes of reflow. **A**—Interstitial space is greatly widened by pale staining edema fluid (*E*). Some muscle cells (*lower right*) exhibit cytoplasmic damage. (× 475) **B**—Pleomorphic population of muscle cells in ischemic myocardium. Some muscle cells are compact and have relaxed myofibrils. One muscle cell is pale and swollen (*SMC*); a second muscle cell is contracted and densely stained (*DMC*). Other muscle cells exhibit marked cytoplasmic disruption with formation of dense transverse bands (*arrows*) and exhibit intervening areas of pale, swollen cytoplasm with separated organelles. Extravasated erythrocytes also are present. (× 450) **C**—Several pale swollen muscle cells (SMC) are shown. The interstitial space is widened and contains edema fluid (*E*). (× 400) **D**—This area exhibits interstitial edema (*E*), muscle cells with contraction bands (*arrows*), and muscle cells with palely stained zonal lesions (*ZL*) adjacent to intercalated discs (× 400).

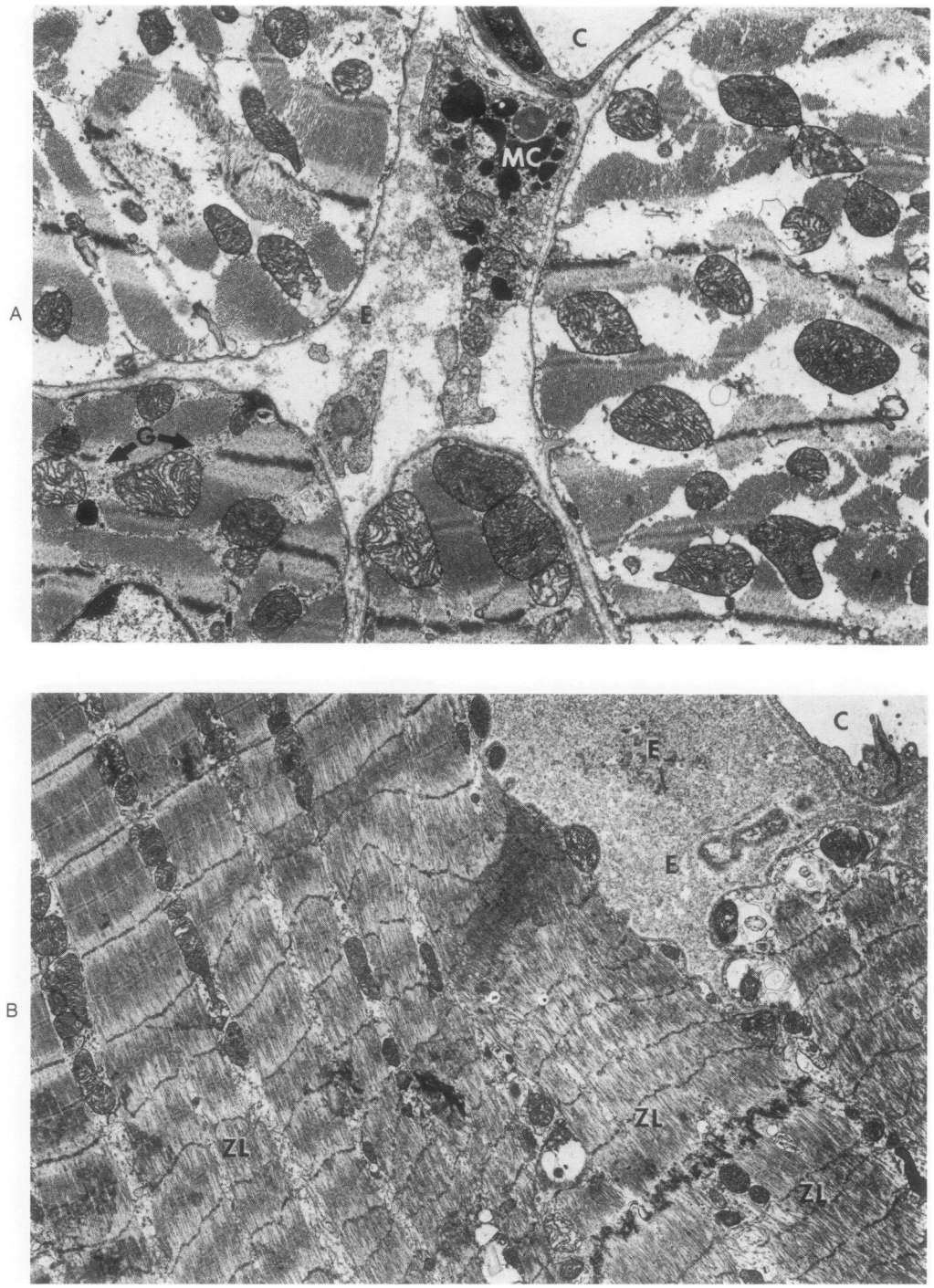


**Figure 4**—Posterior papillary muscle from isolated heart with 40-minute temporary circumflex coronary occlusion and reflow. **A**—Two muscle cells exhibit prominent swelling with marked separation of organelles, glycogen depletion and focal mitochondrial damage. Adjacent muscle cells do not exhibit swelling and contain glycogen deposits (G). The interstitial space is widened and contains precipitated edema fluid (*E*). The capillary (*C*) and adjacent mast cell (*MC*) are normal. (× 6400) **B**—Muscle cells exhibit characteristic zonal lesions (*ZL*) characterized by hypercontraction and focal lysis of myofibrils adjacent to an intercalated disc. The muscle cells are separated from an adjacent capillary (*C*) by abundant edema fluid (*E*). (× 4500)

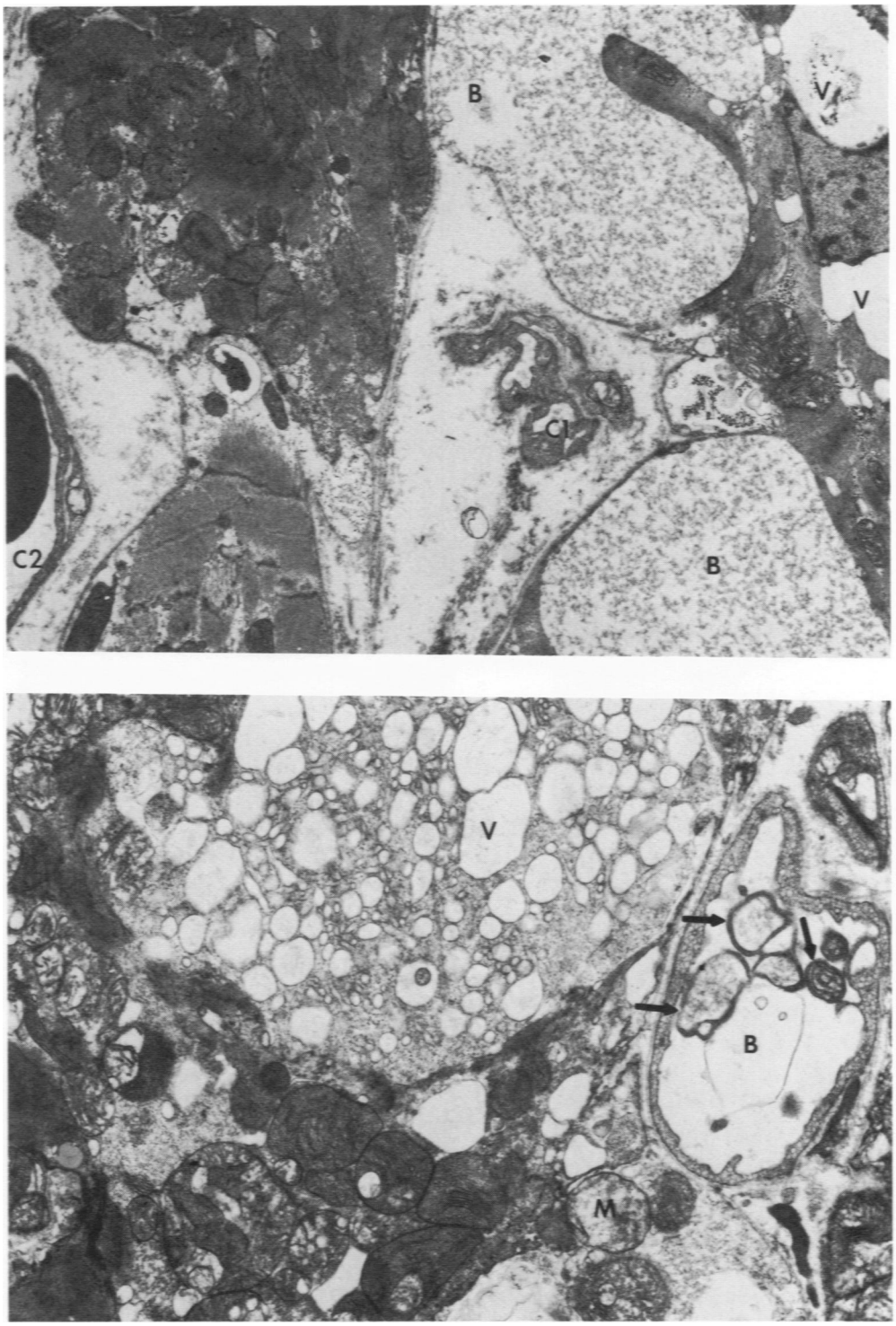


**Figure 5**—Anterior left ventricular myocardium from isolated heart with 40 minutes of temporary LAD occlusion and 20 minutes of reflow. **A**—Area of severe myocardial damage. The interstitial space is widened and contains edema fluid. Muscle cells exhibit hypercontracted myofibrils and altered mitochondria. One muscle cell has an intact nucleus but contains numerous vacuoles (*V*) and prominent blebs (*B*) filled with edema fluid. The capillary (*C1*) adjacent to this muscle cell appears to be collapsed. Another capillary (*C2*) in the area is widely patent and contains an erythrocyte. (× 6000) **B**—Another area of severe myocardial injury contains muscle cells with hypercontracted myofibrils, damaged mitochondria, and subsarcolemmal areas of edematous cytoplasm containing either mitochondria (*M*) or numerous vacuoles (*V*). The adjacent capillary exhibits numerous projections of endothelial cytoplasm (*arrows*) and a membranous bleb (*B*). (× 8300)

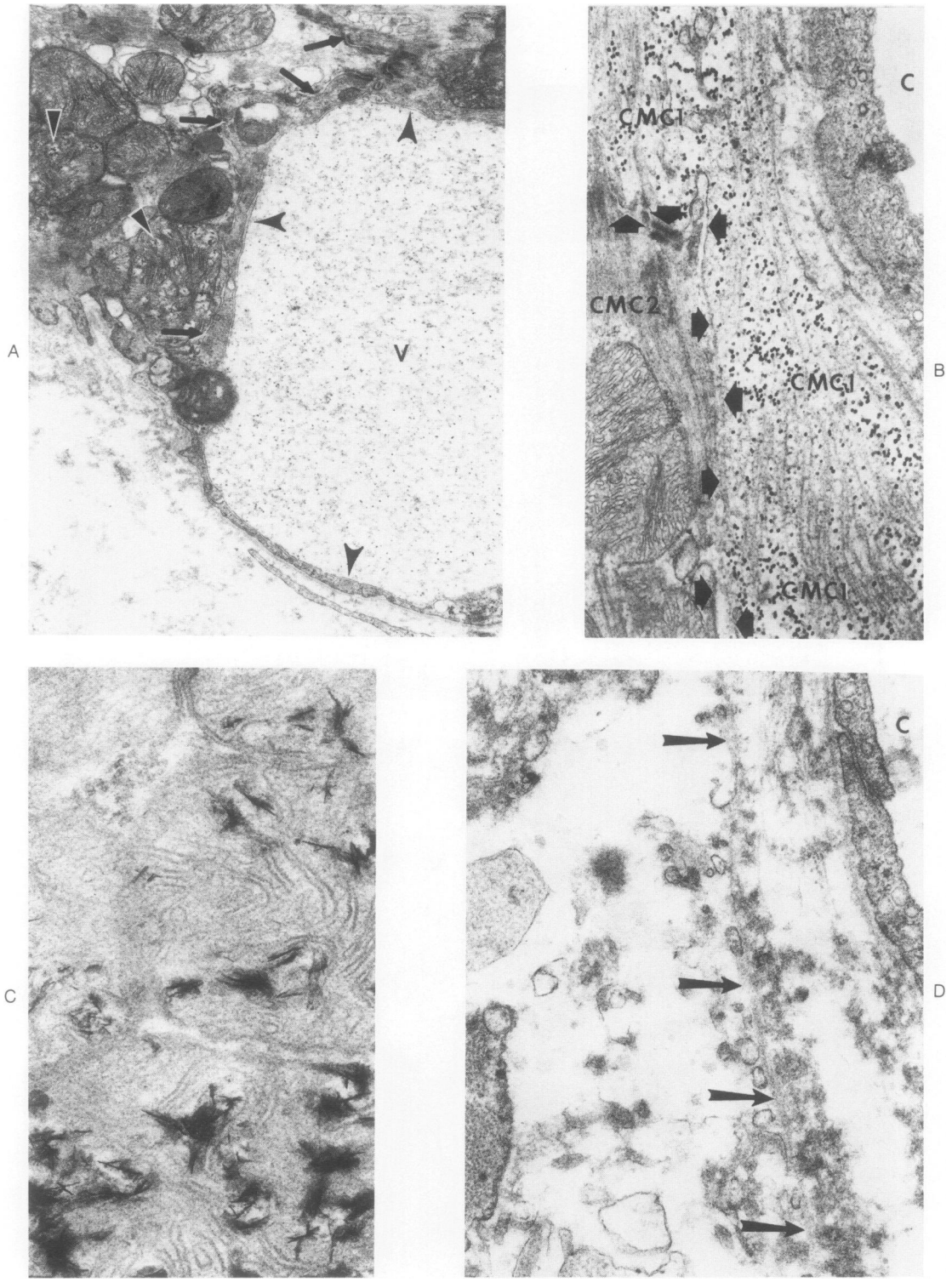


**Figure 6**—Anterior left ventricular myocardium from isolated heart with 40 minutes' temporary LAD occlusion and 20 minutes' reflow. **A**—Region of intercalated disc (*arrows*) between two severely damaged muscle cells. One muscle cell contains a large fluid-filled vacuole (*V*) lined by a double membrane (*black arrowheads*) indicating formation of the vacuole by protrusion of edematous cytoplasm of one muscle cell across the intercalated disc into the second muscle cell. Some mitochondria exhibit electron-dense inclusions of the calcium-containing type (*triangles*). Interstitial edema is evident. (× 12,000) **B**—Section showing actual herniation of cytoplasm of one muscle cell (*CMC1*) across an intercalated disc into a second muscle cell (*CMC2*). The course of the plasma membranes of the two muscle cells is indicated by the *arrowheads*. The herniated cytoplasm is slightly edematous, contains dense glycogen granules and some myofilaments. Adjacent endothelium of a capillary (*C*) exhibits pinocytotic vesicles. (× 22,600) **C**—Mitochondria from a severely damaged muscle cell contain numerous electron-dense spicular deposits with features of calcium-rich apatite crystals. (× 63,000) **D**—Portion of another severely damaged muscle cell shows numerous breaks in the plasma membrane (*arrows*) with resulting formation of membrane fragments adjacent to the basement membrane. Note that the plasma membranes of the endothelial cells of an adjacent capillary (*C*) are structurally intact. (× 30,000)