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# *Transmural Progression of Morphologic Changes During Ischemic Contracture and Reperfusion in the Normal and Hypertrophied Rat Heart*

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**The purpose of this study was to compare the functional and morphologic changes that occur during ischemic contracture and reperfusion in the normal and hypertrophied heart. Hearts from Sprague–Dawley, spontaneously hypertensive (SHR), and normotensive Wistar–Kyoto rats were evaluated using a modified Langendorff perfusion apparatus. After obtaining control data, hearts were potassium-arrested, made ischemic, and studied at various time points. Regional coronary flow was assessed with the use of radiolabeled microspheres or Microfil dye infusion, and morphologic changes were evaluated by means of light and electron microscopy. Sarcomere length changes and qualitative morphologic changes during global ischemia demonstrate a transmural progression of ischemic damage starting at the endocardium and extending, with time, epicardially. The progression of ischemic changes in hypertrophied hearts of SHRs was similar to that of normal hearts; however, hypertrophied hearts developed ischemic contracture sooner than normal hearts. In addition, the development of contraction band change after ischemic contracture occurred only when hearts were reperfused and was related to the development of no-reflow. (Am J Pathol 1987, 129:152–167)**

THE DEVELOPMENT of ischemic contracture or "stone heart" has been observed after cardiac arrest in patients undergoing cardiac surgery.[1-3] This phenomenon was first noted in patients with left ventricular hypertrophy,[2] which suggests that the hypertrophied myocardium may have an enhanced susceptibility to ischemic injury. The mechanisms responsible for the development of ischemic contracture are not well understood and mechanisms responsible for the apparent increased sensitivity of hypertrophied hearts to ischemia are also not known. Many experimental studies have evaluated the functional and structural changes that occur in the normal heart subjected to ischemia[1,4-8]; however, few studies have evaluated the response of the hypertrophied heart to ischemia. Peyton et al[9] and Attarian et al,[10] using hypertrophied rat and dog hearts, noted that ischemic contracture occurred sooner in hypertrophied hearts compared to normal hearts. These investigators suggested that hypertrophied hearts were more susceptible to ischemic damage because of decreased endomyocardial blood flow, decreased adenosine triphosphate (ATP) levels, and depressed mitochondrial function.

Ischemia causes alterations in the coronary vasculature, as well as changes in the myocardium. Reperfusion after ischemic contracture is not homogeneous, due to areas of subendomyocardial no-reflow. The mechanism(s) responsible for the development of no-reflow are not well understood. Various investigators have studied this no-reflow phenomenon and have suggested that intramyocardial pressure, edema, increased coronary resistance, or coronary vascular damage may be responsible for the development of areas of no-reflow.[11-14] Recently, Humphrey et al,[15] using an isolated rat heart preparation with an incompressible left ventricular (LV) balloon, found that if the left ventricle remained distended during ischemic contracture, and if the balloon was deflated during

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reperfusion, reflow was not impaired. This supports the theory that increased intramyocardial pressure, which occurs during ischemic contracture, may compress the coronary vessels and thereby produce areas of no-reflow. Reflow in the hypertrophied heart subjected to ischemic contracture and reperfusion has not been studied.

The purpose of this study was to compare the functional and morphologic changes that occur during ischemic contracture and reperfusion in normal and hypertrophied hearts. In established cardiac hypertrophy, morphologic changes characteristic of ischemia occur more rapidly than in normal hearts, thus substantiating the hypothesis that the hypertrophied myocardium is more susceptible to ischemic damage.

## Materials and Methods

### Experimental Preparation

Sprague–Dawley (SD), spontaneously hypertensive (SHR), and normotensive Wistar–Kyoto (WKY) rats were obtained from the Charles River Breeding Laboratories, Inc. (Kingston, NY) at 8–10 weeks of age. Animals were maintained and handled in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 85-23). The animals were housed in temperature-controlled rooms on a 12-hour light/dark cycle and were fed rat diet and water *ad libitum*. At 8 months of age the SHR and WKY rats were randomly assigned to one of four groups: 1) untreated SHRs (n = 7); 2) hydralazine-treated SHRs (SHR + HYD, n = 6); 3) untreated WKYs (WKY, n = 5); and 4) hydralazine-treated WKYs (WKY + HYD, n = 6). Hydralazine-treated groups received drinking water containing hydralazine (120 mg/l) which was made up fresh each week, and drinking water bottles were changed twice weekly. Systemic arterial blood pressure and heart rate were obtained weekly by means of the indirect tail-cuff method without anesthesia.[16] The median value for peak systolic pressure of at least five pressure determinations from each recording session was used, and the heart rate was determined from these systemic pressure tracings. All animals had four pressure determinations before cohorts were begun on hydralazine treatment. Hydralazine-treated and control SHR and WKY animals were maintained for 12 weeks. Studies were conducted when SD rats were 14 weeks of age and SHR and WKY rats were 12 months of age. Animals were given 300 IU heparin intraperitoneally and 30 minutes later were anesthetized with sodium pentobarbital (5 mg/100 g). The hearts were quickly extirpated and immersed in ice-cold buffer, the aorta was cannulated and antegrade perfusion of the coronary arteries begun with oxygenated buffer by retrograde perfusion of the aortic stump.

### Isolated Perfused Heart Apparatus

The isolated isovolumic perfused rat heart model was used in these experiments.[6] All hearts were perfused at a constant pressure of 100 mm Hg with Krebs–Henseleit buffer containing Na (143 mM), K (5.9 mM), Mg (1.2 mM), Ca (2.5 mM), Cl (127.7 mM), $HCO_3$ (25 mM), $SO_4$ (1.2 mM), $H_2PO_4$ (1.2 mM), glucose (11 mM). The buffer was gassed with 95% $O_2$ and 5% $CO_2$ to produce a pH of 7.4. High potassium buffer, used to arrest the hearts, had a potassium concentration of 30 mM, with sodium being lowered correspondingly to maintain normal osmolality. A fluid-filled latex balloon, used to monitor intraventricular pressure, was inserted into the left ventricle via the left atrium. Latex balloons of known volume were made daily in our laboratory and had a volume approximately twice that of the left ventricular chamber, such that changes in balloon volume used to assess left ventricular function did not record balloon wall tension. Left ventricular pressure and dP/dt were recorded through a 15-cm length of ridged tubing connecting the intraventricular balloon to a Statham P23 ID pressure transducer. A Hewlett-Packard 7758B recorder with a high frequency thermal writer was utilized and dP/dt obtained from an HP derivative computer (HP 8814A). A needle thermistor probe (Webster Laboratories) was inserted into the right ventricle to monitor temperature of the heart, which was immersed in nonaerated buffer surrounded by a water jacketed warming chamber and kept at 37 C.

### Experimental Protocol

Hearts from 250–280-g SD rats were perfused with oxygenated buffer, a drain was placed in each left ventricular apex, and the intraventricular balloon was inserted. The volume of the left ventricular balloon was increased until the heart produced 60 mm Hg pressure while maintaining less than 4 mm Hg diastolic pressure. A right atrial pacing wire was installed, and the hearts were paced at 300 bpm. After a 15-minute equilibration period a pressure volume curve was generated by inflating the intraventricular balloon in four 0.01 ml increments and recording the developed and diastolic pressure at each volume increment. Any hearts with abnormal pressure volume responses were rejected. The balloon was then deflated back to the original volume. For some experimental protocols, control regional coronary flow was quantitated with

the use of 15-$\mu$ radioactive tracer microspheres after the 15-minute equilibration period.

After completion of the initial baseline studies (approximately 15 minutes of perfusion with Krebs–Henseleit buffer), hearts were arrested by perfusion with oxygenated high potassium buffer (30 mM $K^+$). The LV diastolic pressure was slightly above zero, due to hydrostatic pressure within the coronary vascular bed. After 5 minutes of high-potassium perfusion, control nonischemic hearts (Figure 1, group N) either were perfusion-fixed with 2% phosphate-buffered glutaraldehyde for light and electron microscopy or were infused with latex rubber (Microfil) to delineate coronary competence. Infusions were performed with a controlled constant pressure perfusion system (100 mm Hg) attached to a side port of the aortic cannula.

In experimental groups, the coronary flow was stopped for production of global ischemia. With cessation of coronary perfusion LV pressure dropped to zero. LV pressure was monitored throughout the ischemic interval, and the onset of ischemic contracture was noted. During the development of ischemic contracture, hearts in the contracture group (Figure 1, Group C) were either perfusion-fixed with 2% phosphate-buffered glutaraldehyde or were infused with Microfil. Additional hearts were allowed to progress to complete ischemic contracture and 5 minutes after the onset of contracture were either perfusion-fixed or infused with Microfil (Figure 1, Group PC). At this time point (5 minutes after onset of ischemic contracture), some hearts were reperfused with normokalemic buffer and after 45 seconds of reflow were injected with microspheres or Microfil. Another group of postcontracture hearts were reperfused with oxygenated high-potassium buffer for 5 minutes, after which the hearts were either perfusion-fixed or infused with Microfil. The remaining postcontracture hearts were reperfused with oxygenated normokalemic buffer (Figure 1, Group R) for 30 minutes, and at the conclusion of the 30-minute reperfusion period, a final microsphere injection was made. The hearts were then perfusion-fixed with 2% glutaraldehyde or were infused with Microfil.

### Ischemic Contracture in the Hypertrophied Heart

Hearts from the untreated hypertensive and hydralazine-treated (normotensive) SHRs and control WKY rats were evaluated when the animals were 12 months of age by means of a perfusion protocol similar to that described above for SD rats. One-year-old SHRs and WKY rats were studied because this is a well-characterized model of cardiac hypertrophy without congestive heart failure. The hydralazine treatment in SHRs lowers blood pressure but does not cause regression of cardiac hypertrophy. This produces a model of cardiac hypertrophy in a normotensive animal which allows us to evaluate the separate contributions of cardiac hypertrophy and hypertension on the response of the heart to ischemia. All hearts were given injections of radioactive microspheres during the control perfusion period for determination of normal regional coronary flow distribution. The hearts from all groups were potassium-arrested by perfusing with oxygenated high potassium buffer for 5 minutes prior to the cessation of coronary flow. The time required to develop ischemic contracture after cessation of coronary flow was determined from the LV pressure tracing. After ischemic contracture and 30 minutes of reperfusion with normokalemic buffer, a second set of microspheres was injected. Pre- and postcontracture regional coronary flows and endomyocardial to epimyocardial flow ratios were determined in each heart.

### Morphologic Studies

Figure 1 is a representative LV pressure tracing of a typical experiment. SD rat hearts used for measurement of sarcomere lengths were studied after normal perfusion (N), during the development of ischemic contracture (C), after complete ischemic contracture (PC), and after 30 minutes of reperfusion, which was started 5 minutes after the onset of contracture.

Eight transmural tissue samples (approximately 3×8×1 mm in size) from the left and right ventricle of each perfusion-fixed heart were embedded in Spurr epoxy resin. Serial 1-$\mu$ sections from each block were affixed to a glass slide and stained with toluidine blue for light-microscopic examination. From these sections, representative areas with longitudinally aligned myofibers from the subendomyocardium, midwall, and the subepimyocardium were identified, the tissue blocks trimmed, and ultrathin sections cut for examination by transmission electron microscopy. Six grids from each heart (two endomyocardial, two midmyocardial, and two epimyocardial) were examined, and four prints from each grid were processed. Sarcomere lengths were measured from these prints using a Graf/pen sonic digitizing system interfaced with a Hewlett-Packard 9825A computer.

### Coronary Flow Assessment

Qualitative assessment of coronary flow distribution was evaluated with aortic infusions of latex (Microfil). A constant pressure (100 mm Hg) system was

used to infuse the Microfil into the aortic cannula in order to assure reproducible controlled injections. The hearts were then immersion-fixed in 10% buffered formalin and cut into six equal transverse slices. These slices were photographed, and each slice was weighed. Microfil delineated the areas of flow within the myocardium. By using a Graf/pen sonic digitizing system and knowing the weight of each tissue section, we were able to trace photographs of heart slices and quantitate the percent of myocardium that was not reperfused. Transmural left ventricular tissue sections were embedded in plastic, cut at 1 $\mu$, and examined by light microscopy. In these sections the coronary arteries retained the Microfil injectate, and the morphologic characteristics of the myocardium as well as the vasculature were assessed. Other sections from these Microfil-infused hearts were cleared in alcohol-methyl salicylate and examined with a dissecting microscope using trans- and epiillumination. With these specimens we were able to characterize the vascular morphology, especially at the flow/no-reflow border zone.

Quantitation of coronary flow was performed with 15-$\mu$ radioactive tracer microspheres ($^{46}$Sc, $^{51}$Cr, $^{141}$Ce, $^{85}$Sr).[17] Microspheres were diluted such that a 0.2-ml injectant given to each heart contained approximately 80,000–100,000 microspheres. The small volume of injectant and the number of microspheres did not affect heart function or coronary flow. Microspheres were sonicated for 3 minutes and then mixed with a vortex mixer for 1 minute prior to injection into the aortic cannula. During the injection, coronary flow was measured as a reference flow by timed collections of coronary effluent. The number of microspheres in the effluent was assessed for determination of the number of microspheres that did not remain in the tissue (less than 5%) and for testing aortic valve competence. At the completion of the experiments, the total counts for each isotope were determined for each heart. The total counts per heart and the reference flow rate during each microsphere injection were used for calculation of regional coronary flow. Hearts were sliced into six equal transverse sections, and each slice was divided into endomyocardial, midmyocardial, and epimyocardial regions. These regional samples were counted in an LKB CompuGamma counter (Model 1282-002) and were then desiccated. Coronary flow calculations were done using dry tissue weights which were then converted to milliliters per minute per gram wet weight, with the assumption that the tissue contained 80% water. Coronary flow in the different regions was expressed as absolute flow or as a ratio of endomyocardial flow/epimyocardial flow.

### Data Analysis

All data are expressed as mean ± the standard error of the mean (SEM) except where otherwise noted. Statistical evaluation among groups was done by one-way analysis of variance, and when $F$ values were significant at a 95% confidence limit, group means were evaluated by means of the Tukey-Kramer multiple range test. In the tables and figures, letters have been used to denote statistical relationships between groups. Groups sharing the same letter designations are not statistically different; those with dissimilar letter designations are significantly different.

## Results

### Ultrastructural Morphology

Figure 1 is a representative LV pressure tracing of a typical experiment. Initially, the LV balloon volume was adjusted such that each heart produced 60 mm Hg developed pressure. After the equilibration period, the perfusate was switched to oxygenated high-potassium buffer (Figure 1, potassium arrest). The LV pressure after potassium arrest was not zero, but ranged from 2 to 6 mm Hg, because of hydrostatic pressure in the coronary vasculature produced by the perfusate (100 mm Hg perfusion pressure). After 5 minutes all flow to the heart was stopped (Figure 1, "Ischemia"), and LV pressure dropped to zero. After a given period of ischemia, the LV pressure gradually began to increase as the heart developed ischemic contracture. Maximum contracture was attained in 3–4 minutes, after which the LV pressure gradually declined, indicating slight relaxation of the contracted heart. Five minutes after the onset of contracture the hearts were reperfused (Figure 1, reperfuse). The heart demonstrated in Figure 1 was reperfused with oxygenated normokalemic buffer. Immediately after reperfusion the hearts began to contract, and the end diastolic pressure decreased to levels below the LV pressure seen after contracture. Approximately 1 minute after reperfusion, end diastolic pressure began to increase and LV developed pressure decreased. Thus, initially after reperfusion the hearts relax and LV function returns; however, within 1–2 minutes of reperfusion LV function is markedly depressed. Often, as is demonstrated in Figure 1, the LV end diastolic pressure after ischemic contracture and reperfusion was higher than the LV pressure at peak contracture, suggesting severe myocardial damage and loss of compliance. After 30 minutes of reperfusion, LV developed pressure was improved, but end diastolic pressure was still elevated (Figure 1, R).

Nonischemic isolated perfused SD hearts perfu-

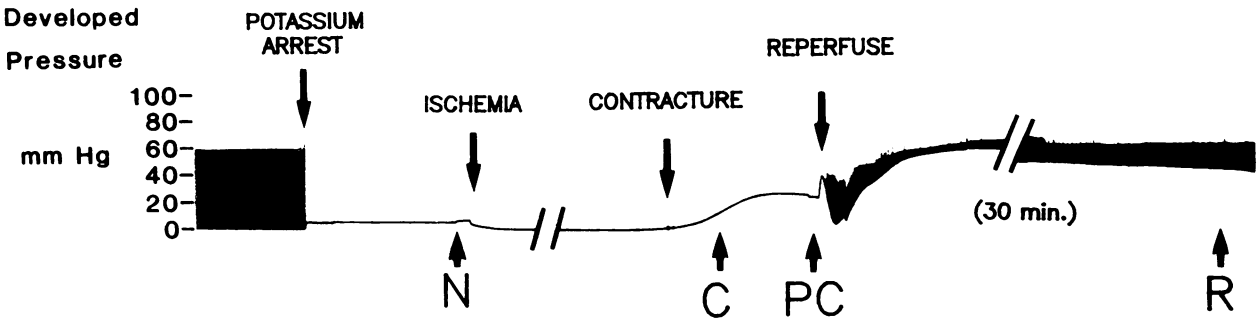


**Figure 1**—LV pressure tracing of a typical experiment. After potassium arrest, control nonischemic hearts (*N*) were studied. Experimental hearts were made ischemic and were studied during ischemic contracture (*C*) or 5 minutes after peak contracture (*PC*). Remaining hearts were perfused and studied after 30 minutes of reperfusion (*R*).

sion-fixed after 15 minutes of buffer perfusion and 5 minutes of potassium arrest (Table 1, Group N) had uniform sarcomere lengths across the wall, and tissue morphology was normal (Table 1 and Figure 2). Subendomyocardial sarcomere lengths of hearts perfusion-fixed during the onset of ischemic contracture (Table 1, Group C) were significantly shorter than control and were shortened, compared with the midwall and subepimyocardial regions of the same hearts (Table 1). Subendomyocardial tissue had few glycogen granules, the mitochondria were mildly swollen, with loss of matrix granules, and the myocyte nuclei demonstrated central chromatolysis with peripheral clumping of chromatin (Figure 3). There was, however, no evidence of irreversible ischemic injury. The midwall and subepimyocardium had similar qualitative morphologic changes indicative of ischemic injury, though of much lesser severity. The sarcomere lengths of the midmyocardium tended to be shorter than in control hearts or in the epimyocardium of the same heart, but these values did not reach statistical significance.

Five minutes after the onset of ischemic contracture (Table 1, Group PC) the intraluminal left ventricular pressure was slightly decreased, compared with that at the peak of contracture, but the pressure was still significantly elevated, compared with precontracture LV pressure. This suggests that after peak contracture there is slight relaxation of the myocardium. Subendomyocardial sarcomeres demonstrate this relaxation and were significantly longer than in nonischemic hearts (Table 1). In the subendomyocardium mitochondrial swelling was evident, the sarcotubular system was dilated, and there was increased intracellular space indicative of intracellular edema (Figure 4). Significant shortening of sarcomeres was observed in the midmyocardium (Figure 5). Mitochondrial and nuclear changes consistent with ischemia were also evident. Qualitative morphologic changes consistent with ischemia were also noted in the subepimyocardial region, but sarcomere lengths were no different from control (Table 1). Despite the marked increase in intraluminal left ventricular pressure and the severe shortening of sarcomeres in the midwall region, no contraction bands were observed.

Table 1—Regional Sarcomere Lengths

| Group | LV endo | LV mid | LV epi |
|---|---|---|---|
| Normal perfusion (N, n = 4) | 1.80 ± 0.013 | 1.86 ± 0.011 | 1.84 ± 0.017 |
| Contracture (C, n = 6) | 1.39* ± 0.019 | 1.77 ± 0.044 | 1.89 ± 0.016 |
| Postcontracture (PC, n = 5) | 2.01* ± 0.019 | 1.32* ± 0.028 | 1.88 ± 0.013 |
| 30-minute reperfusion (R, n = 5) | 1.25* ± 0.036 | Contraction bands | 1.69 ± 0.025 |

Sarcomere lengths in microns, mean ± SEM.
*$P < 0.05$, compared with the normal perfusion group.

Significant morphologic changes occurred with reperfusion after ischemic contracture. Sarcomere lengths in the subendomyocardium were shortened after reperfusion, compared with control (Table 1). Electron micrographs taken from this region demonstrated marked heterogeneity of morphologic changes. In many regions, myocytes were stretched with expanded I bands and dense accumulations in the I bands suggestive of N bands (Figure 6). In other sections within the subendomyocardium, near the midwall region, the sarcomeres were markedly shortened (Figure 7). This heterogeneity of sarcomere lengths often occurred in adjacent cells and accounted for the increased standard error in measurements of sarcomere length (Table 1, "LV endo," "R"). The midwall region was characterized by marked contraction band change (Figure 8). In this region, myocytes contained dense accumulations of myofibrillar material and large areas of disrupted mitochondria with no myofilaments (Figure 9). Mi-

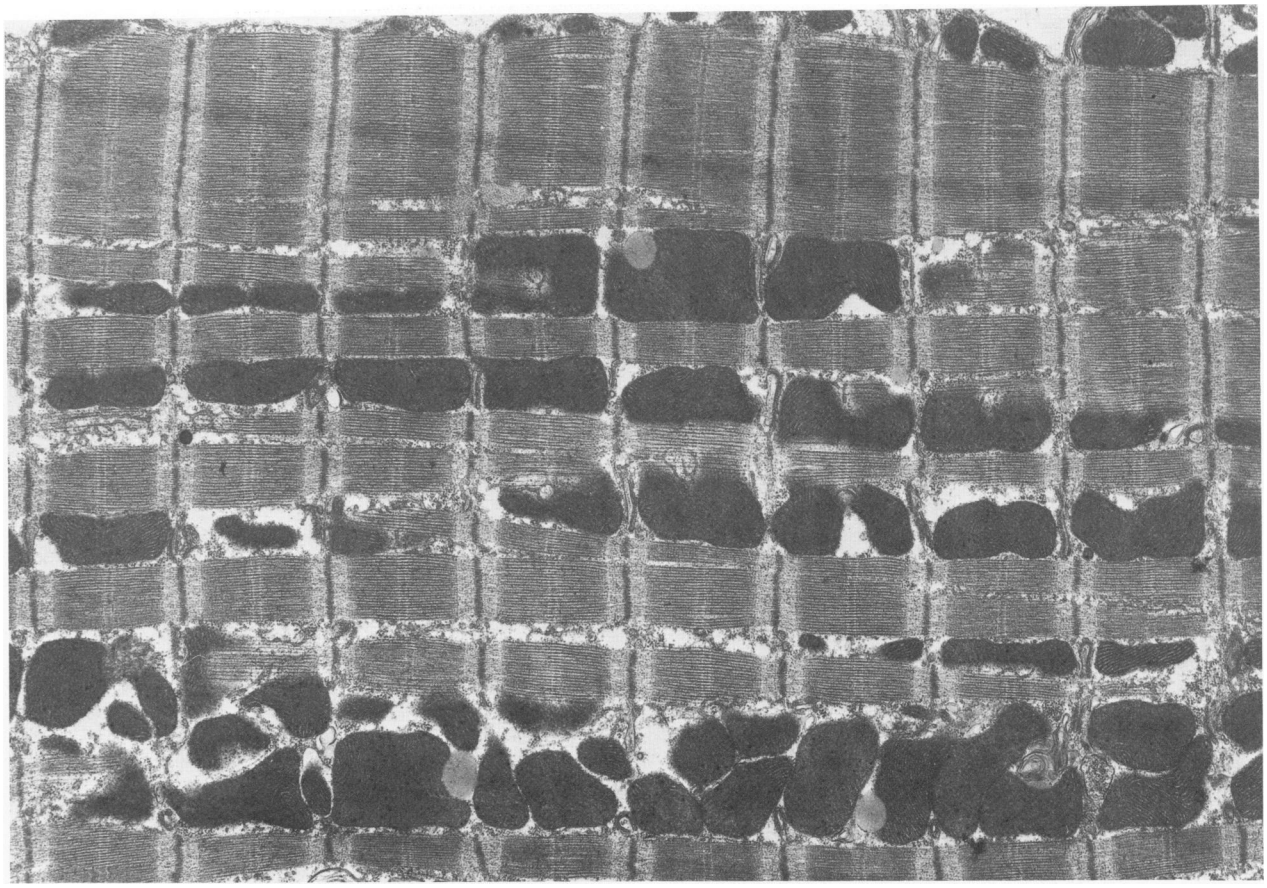

**Figure 2**—Electron micrograph of control nonischemic tissue from heart in Group N. Normal appearance of myofibrils, mitochondria with normal matrix granules, and abundant glycogen granules. (×10,540)

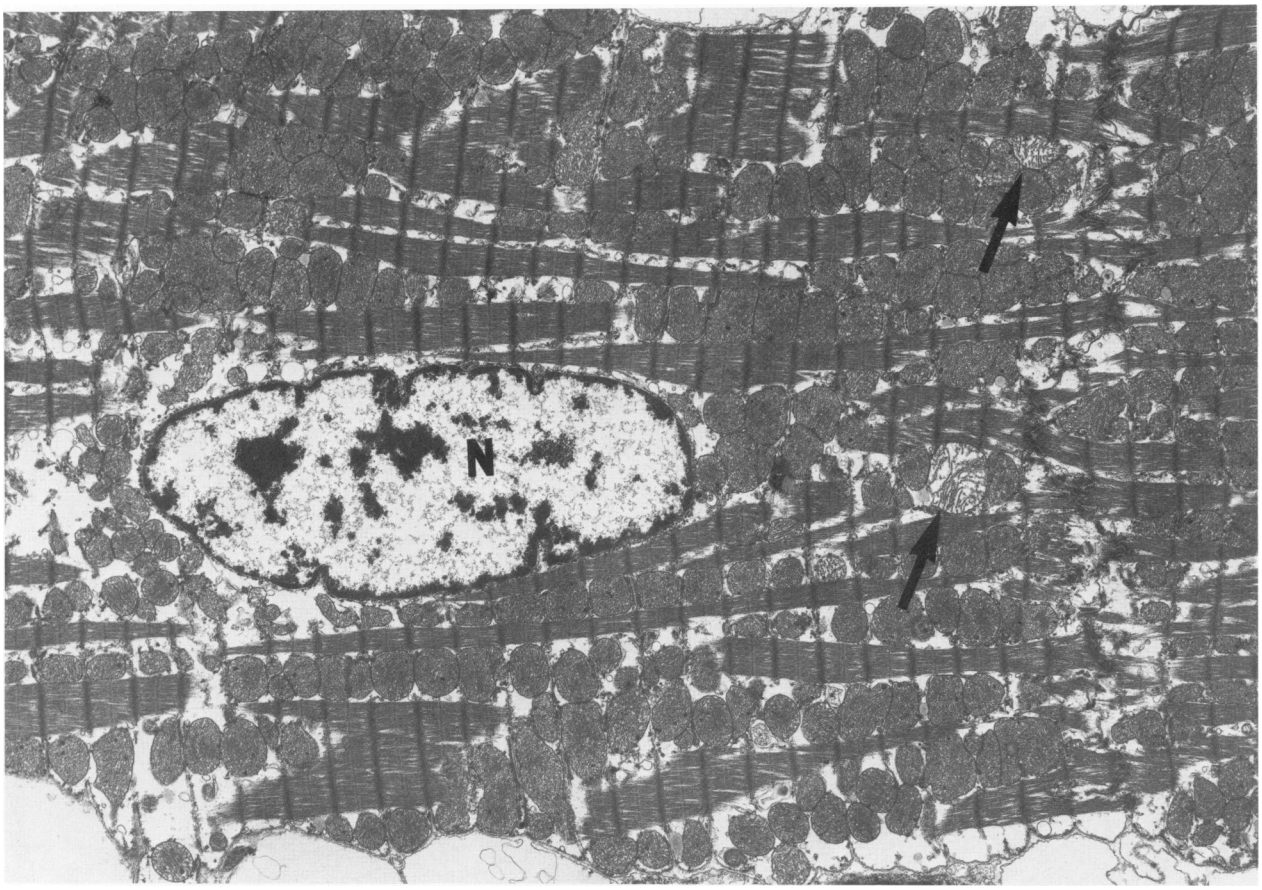


**Figure 3**—Electron micrograph of subendomyocardium of heart fixed during ischemic contracture (*C*). Note contracted sarcomeres with loss of I bands, some swollen mitochondria (*arrows*), central clearing with margination of chromatin within the nucleus. (×4560)

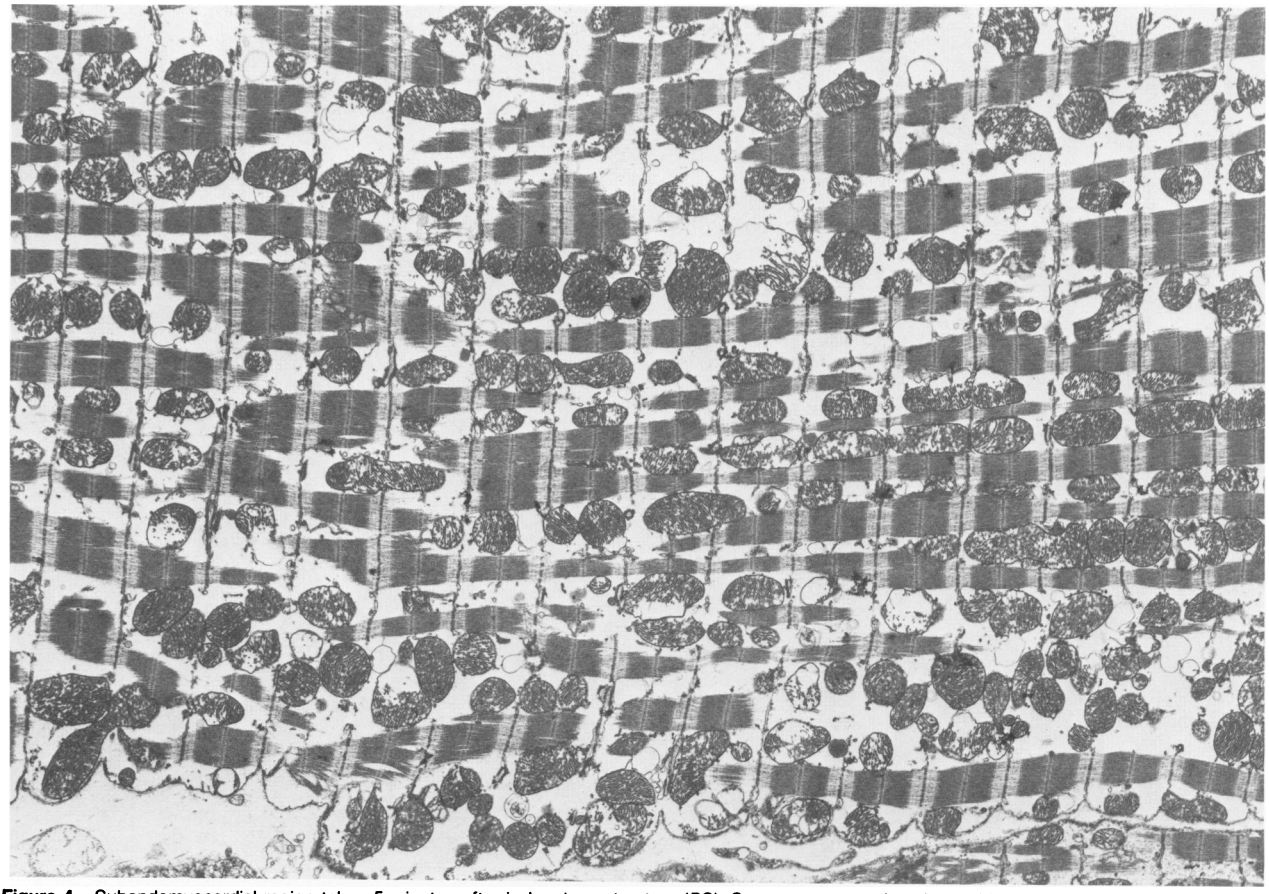

**Figure 4**—Subendomyocardial region taken 5 minutes after ischemic contracture (*PC*). Sarcomeres are relaxed as evidenced by the prominent I bands, mitochondria are markedly swollen, and there is evidence of intracellular edema. (×5480)

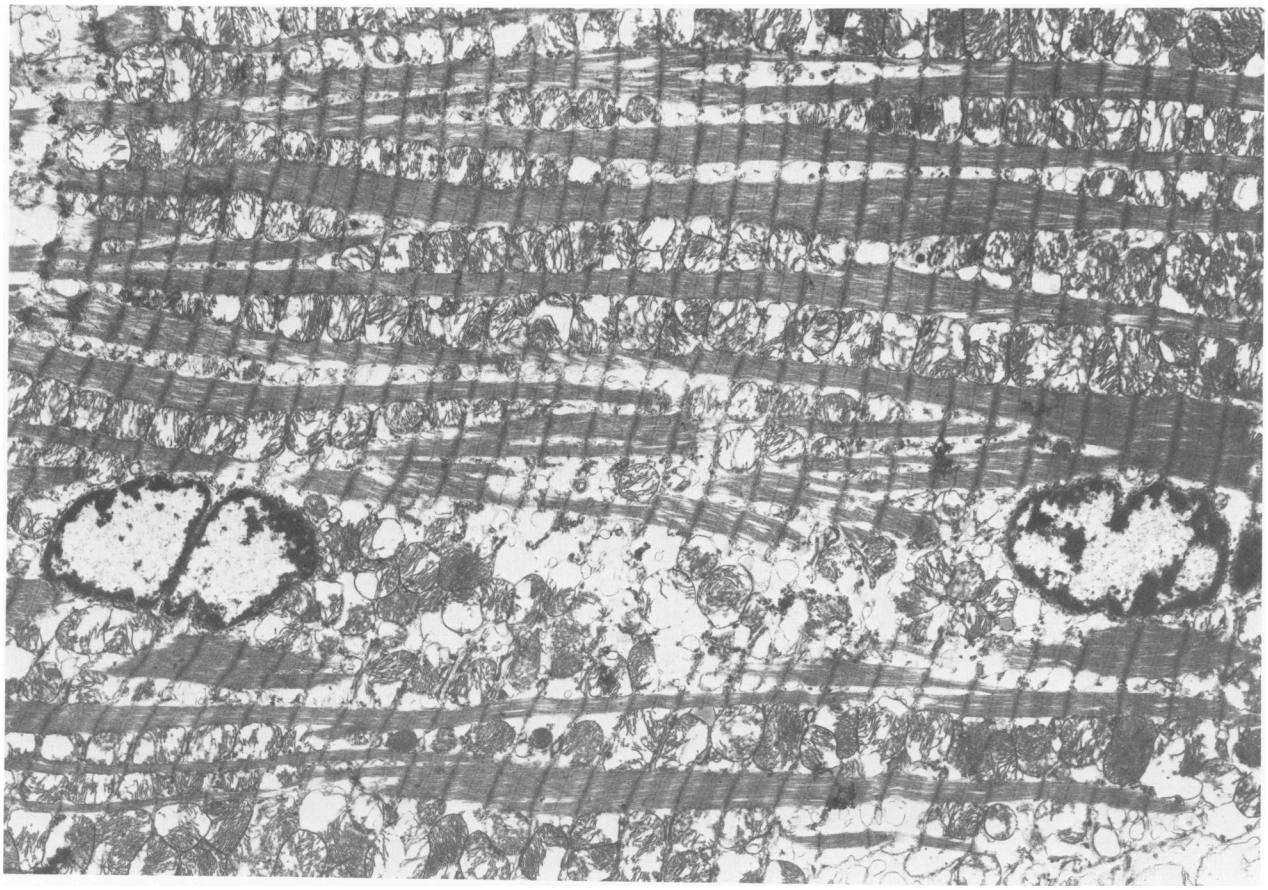

**Figure 5**—Midmyocardial section taken 5 minutes after ischemic contracture (*PC*). In this region there is significant shortening of sarcomeres, swelling of mitochondria, and margination of nuclear chromatin, consistent with ischemic injury. (×4260)

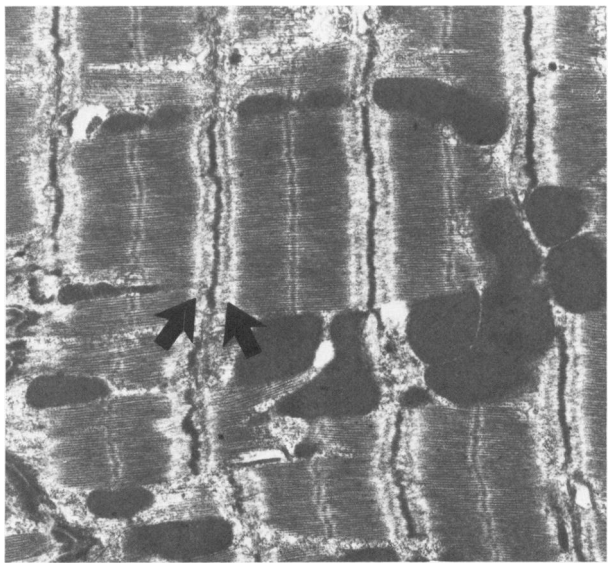

**Figure 6**—Electron micrograph of myocyte from subendomyocardium after ischemic contracture and 30 minutes of reperfusion (*R*). Sarcomeres are stretched, with evidence of N line formation (*arrows*) in some I bands. (×10,460)

tochondrial, sarcotubular, and sarcolemmal membranes were also disrupted throughout this region. Endothelial cells in this region had significant cytoplasmic blebbing (Figure 10). Occasional myocytes in the subepimyocardium exhibited mild dilation of the sarcoplasmic reticulum and evidence of intracellular edema, but were otherwise normal in appearance. Thus, after ischemic contracture and 30 minutes of reperfusion the subepimyocardium has very little evidence of ischemic damage. The midwall region contains a zone of contraction band change (approximately 20–30 cells wide) that comprises the flow/no-reflow border. The subendomyocardium is not reperfused, the cells are swollen and demonstrate a heterogeneous pattern of cell injury, and no contraction bands are present within this region.

## Regional Coronary Flow

The distribution of coronary flow was assessed quantitatively by means of radioactive tracer micro-

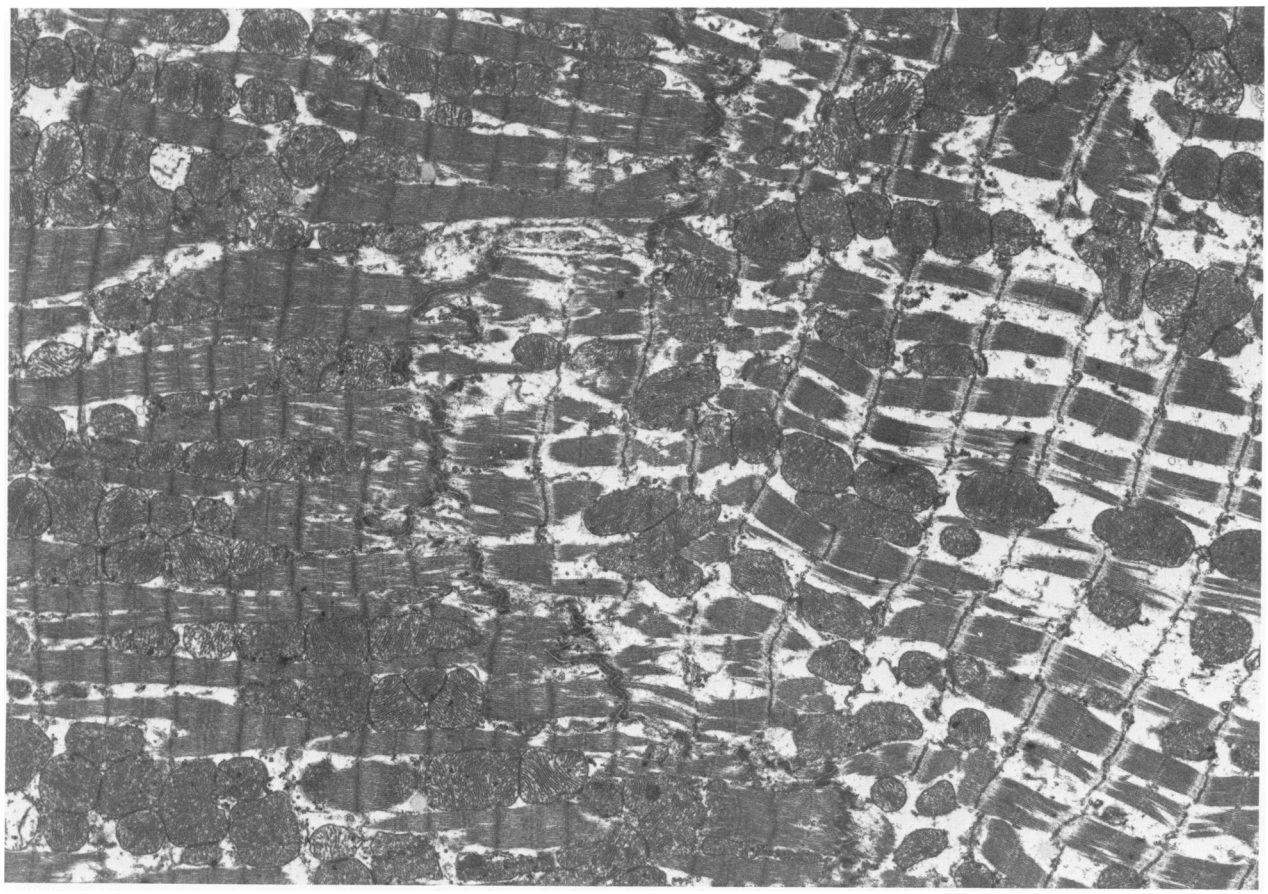

**Figure 7**—Midwall region from Group R. Adjacent myocytes demonstrate marked heterogeneity of structure. Sarcomeres are contracted in some cells (*left*), while in adjacent cells sarcomeres are stretched (*right*). (×6240)

**Figure 8**—Light-microscopic view of the LV midwall region after ischemic contracture and reperfusion. Note severe contraction band formation, disruption of cellular architecture, and intra- and extracellular edema. (×425)

spheres, and the regional flow distribution was characterized by vascular injection of Microfil. Regional myocardial blood flow in the nonischemic isolated perfused rat heart was equally distributed throughout the heart with a slight, but statistically insignificant, increase in subendomyocardial flow relative to the subepimyocardium (Table 2).

Microfil injection of the nonischemic perfused heart resulted in transmural vascular filling (Figure 11). In contrast, reperfusion with normokalemic buffer after ischemic contracture resulted in a well-defined subendomyocardial no-reflow zone (Figure 12). Examination of heart slices cleared in methyl salicylate demonstrated vascular filling in the epimyocardium with a sharp line of demarcation at the flow/no-reflow border. At this interface, capillaries and small vessels did not fill; however, several large-caliber vessels did penetrate further into the no-reflow zone than the smaller vessels (Figure 13). Microscopically, there was a well-defined area of contraction band change at the flow/no-reflow border (Figure 14). This area of contraction band change did not extend

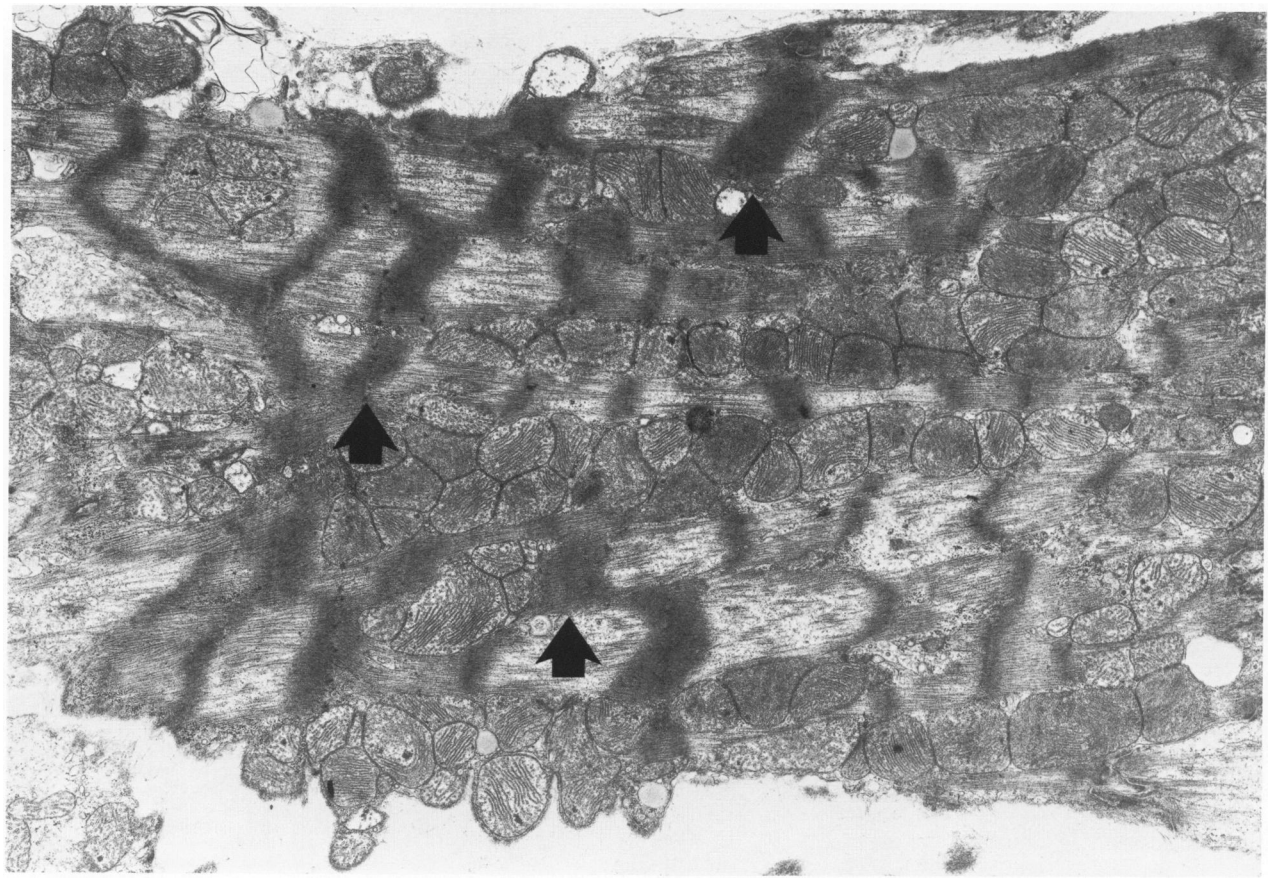

**Figure 9**—Contraction band change in myocyte from midwall region of Group R. Hypercontraction (condensation) of myofibrillar material (*arrows*) with marked disruption of cellular architecture. (×10,260)

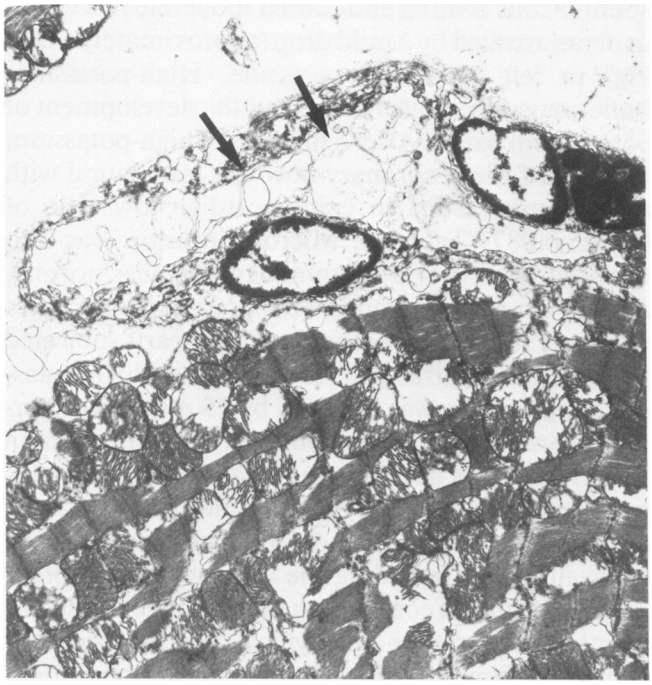

**Figure 10**—Myocytes and blood vessels in the midwall region of Group R demonstrate severe ischemic damage. Endothelial cells exhibit severe cytoplasmic blebbing within the vessel lumen (*arrows*). (×5250)

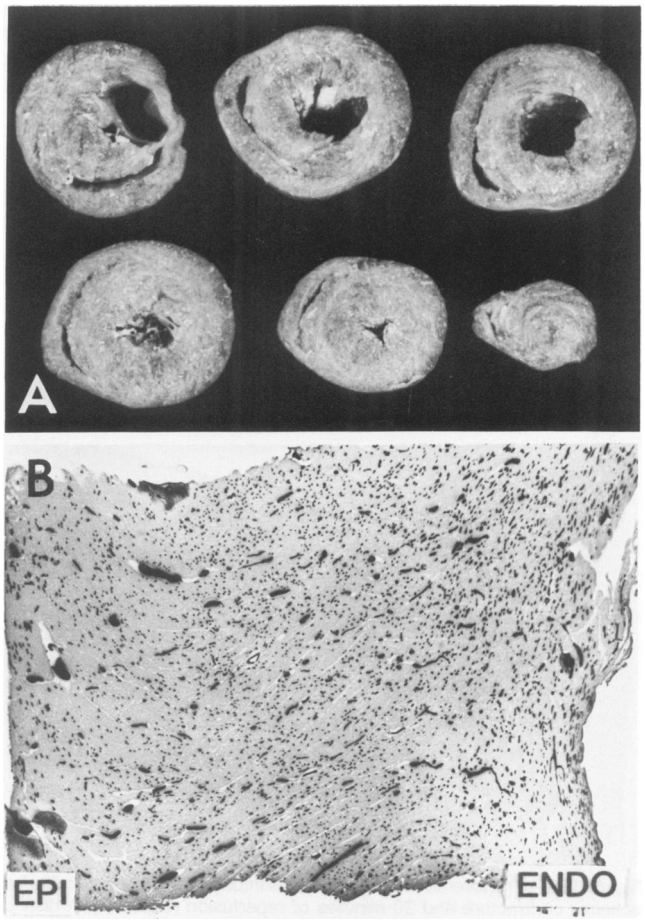


**Figure 11A**—Transverse sections of control nonischemic SD rat heart infused with white Microfil (Group N). Note transmural distribution of Microfil. **B**—Low-power photomicrograph of a plastic-embedded 1-$\mu$ transmural section of heart from **A**. Note transmural patency of the vasculature. *Epi*, epimyocardium; *endo*, endomyocardium. (×15)

into the nonreperfused subendomyocardial region. Regional blood flow assessed with microspheres also demonstrated a marked decrease in subendomyocardial flow (Table 2). After ischemic contracture and 30 minutes of reperfusion the coronary flow to the right ventricle and the LV epimyocardium was mildly decreased, compared with control flows. Coronary flow to the LV subendomyocardium was markedly decreased, but was not zero. Evaluation of Microfil-infused hearts suggests that subendomyocardial flow is zero, because no dye is visible grossly or microscopically in the subendomyocardial region (Figure 12). However, regional coronary flow assessed with microspheres demonstrates low coronary flow in the subendomyocardium. In fact, this low flow in the subendomyocardium calculated from microsphere data may be due to small focal areas of reflow into the subendocardial region. As can be seen in Figure 12A and in Figure 13, the anterior papillary muscle contains Microfil dye, even though the remainder of the subendocardium is devoid of dye. This small area of reperfusion into the anterior papillary muscle was present in all hearts that were examined. The cause of this small area of reflow is not known, but we suspect that a large-caliber vessel must supply this region and must remain partially patent even after ischemic contracture and reperfusion. Because, for microsphere evaluation of blood flow, this small region of reperfused myocardium is pooled with the remaining subendomyocardial tissue, which is not reperfused; the result is a low mean total subendomyocardial flow.

In order to evaluate the temporal changes in flow after ischemic contracture with reperfusion, we injected microspheres 45 seconds after the initiation of reflow. This time point was chosen because, as noted

Table 2—Regional Coronary Flow

| Group | RV | LV endo | LV epi | Endo/Epi |
|---|---|---|---|---|
| Normal perfusion (N, n = 6) | 16.02 ± 0.682 | 18.03 ± 1.356 | 16.77 ± 0.935 | 1.08 ± 0.062 |
| 45-second reperfusion (n = 5) | 14.23 ± 0.996 | 15.16 ± 1.044 | 16.27 ± 1.685 | 0.93 ± 0.074 |
| High $K^+$ reperfusion (n = 7) | 16.65 ± 1.340 | 14.87 ± 1.961 | 15.46 ± 1.657 | 0.96 ± 0.087 |
| 30 min reperfusion (R, n = 6) | 13.77 ± 0.946 | 4.66* ± 0.327 | 12.73 ± 1.328 | 0.37* ± 0.046 |

Flows expressed as milliliters per minute per gram wet weight.
*$P < 0.001$, compared with the normal perfusion group.

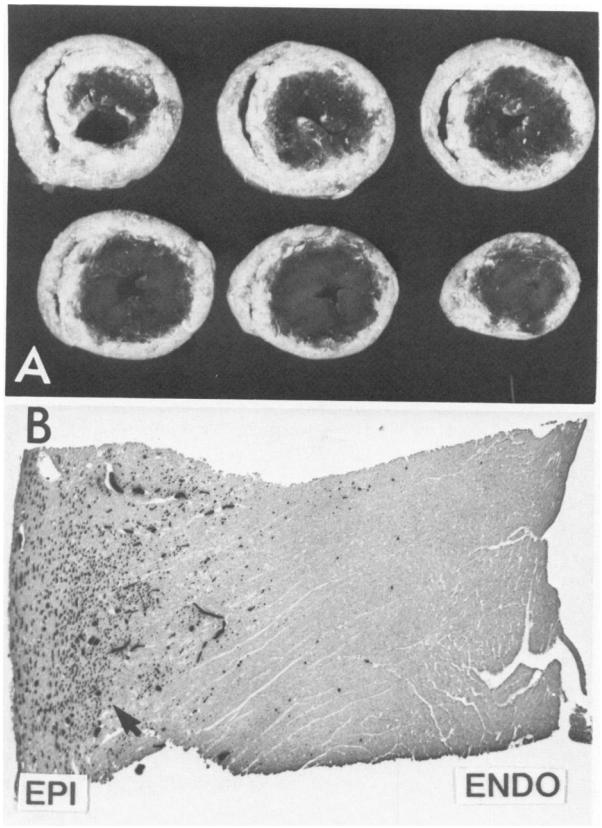


**Figure 12A**—Transverse sections of hearts infused with white Microfil after ischemic contracture and 30 minutes of reperfusion (Group R). A distinct region of subendocardial no-reflow is delineated by Microfil injection. **B**—Photomicrograph of a plastic-embedded 1-$\mu$ transmural section from the heart in **A**. Microfil fills coronary vasculature in the epicardial region (*arrow*), but the subendomyocardium is devoid of dye. (×20)

earlier, during the first minute of reperfusion the contracted heart relaxes and beats spontaneously, which suggests that initially cardiac function is not severely compromised. At this early time point (after 45 seconds of reperfusion) coronary flow was transmural, as evidenced by an endomyocardial/epimyocardial flow ratio of $0.93 \pm 0.074$ (Table 2). Microfil injection at this time point also demonstrated transmural flow. Microscopically, the myocytes in the subendomyocardium and midmyocardium were somewhat swollen; however, only rare contraction bands were seen adjacent to blood vessels in the midwall. Therefore, during early reperfusion, the hearts relaxed, myocytes were not irreversibly injured, contraction band change was only rarely seen, and reperfusion was transmural.

To determine whether reflow alone was able to cause contraction band formation, we perfused hearts subjected to ischemic contracture with oxygenated high-potassium buffer to eliminate electromechanical activity. The high-potassium buffer prevented spontaneous beating and caused moderate relaxation as demonstrated by a mild drop (approximately 5 mm Hg) in left ventricular pressure. High-potassium buffer reperfusion also prevented the development of contraction bands. After 5 minutes of high-potassium buffer perfusion, coronary flow was transmural with an endomyocardial to epimyocardial flow ratio of $0.96 \pm 0.087$ (Table 2). Microfil infusion was also transmural, myocyte morphology was near normal, and no contraction bands were present in myocytes from any region. In another group of hearts subjected to ischemic contracture, 5 minutes of high-potassium reperfusion was followed by 25 minutes of normokalemic buffer perfusion. Microfil injection in these hearts was not transmural, and there was a distinct area of subendomyocardial no-reflow. A large area of contraction band change was present in the midwall region including the flow/no-reflow border area. This zone of contraction band change was significantly broader than the typical contraction band zone at the flow/no-reflow border in hearts reperfused with normokalemic buffer (Group R). High-potassium buffer reperfusion after ischemic contracture prevented contraction band formation and allowed transmural perfusion. However, when the perfusate was switched back to normokalemic buffer, contraction band change and subendomyocardial no-reflow did develop.

The ischemia induced changes in sarcomere length, and morphologic features of ischemic injury progressed from the endomyocardium to the epicardium with increasing ischemic time. In addition, with increasing ischemic time the amount of myocardium

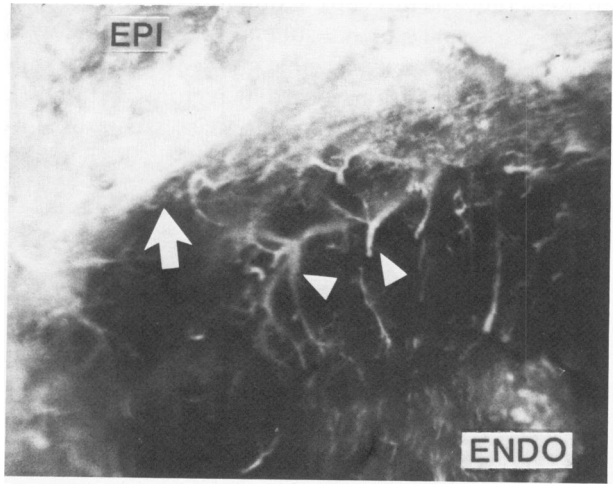


**Figure 13**—Transmural slice of heart from Figure 12A cleared in alcohol-methyl salicylate and viewed with trans- and epiillumination. In the epicardial region (*EPI*) all vessels, including capillaries, are filled. At the flow/no-reflow border (*arrow*) only large caliber vessels are filled (*arrowheads*). These Microfil-filled vessels penetrate into the no-reflow zone but eventually lose their patency as they enter the endocardial region (*ENDO*).

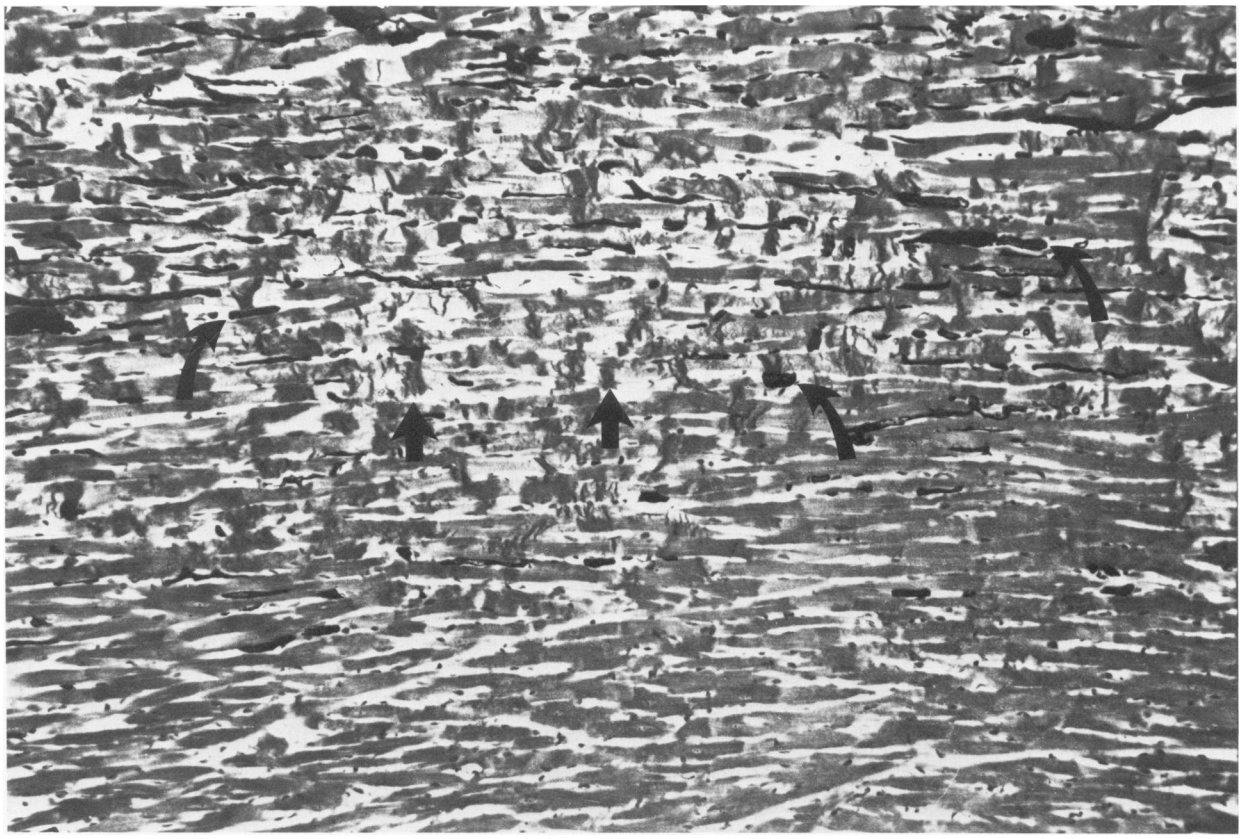

**Figure 14**—Photomicrograph of a plastic-embedded tissue section of a Microfil infused heart from Group R. The vasculature is filled with Microfil (*curved arrows*) in the epicardial region (*top*), but little vascular filling is present beyond the dense band of contraction band change (*arrows*) present at the flow/no-reflow border. (×125)

that was not reperfused (no-reflow zone), increased. Reperfusion initiated at the peak of ischemic contracture resulted in a no-reflow zone that encompassed 25.6% ± 1.8% of the left ventricle. When reperfusion was initiated 15 minutes after contracture the subendomyocardial no-reflow zone was increased in size, involving 36.3% ± 2.6% ($P < 0.05$) of the left ventricle.

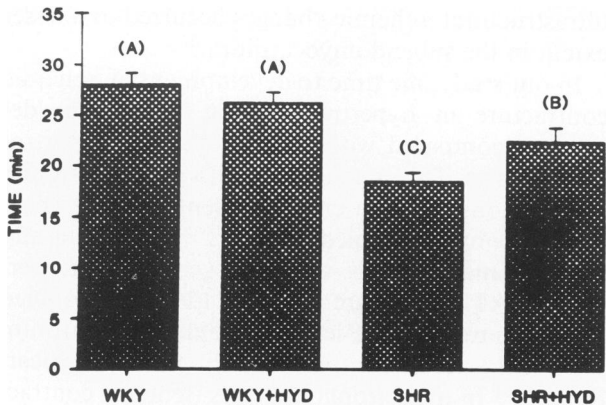


**Figure 15**—Time to ischemic contracture. Length of ischemic time (in minutes) before the onset of LV pressure rise due to contracture. Letters in parentheses designate multiple range test grouping, $P < 0.05$.

## Ischemic Contracture in the Hypertrophied Myocardium

The time to development of ischemic contracture was evaluated in 1-year-old spontaneously hypertensive rats and in normotensive WKY rats. The time till onset of contracture was significantly shorter in SHRs, compared with WKY (Figure 15). Compared with untreated SHRs, the time to ischemic contracture was significantly longer in SHRs treated with hydralazine for 12 weeks, but it was not as long as for WKY. In SHR + HYD rats, blood pressure was normalized (128 ± 9.4 mm Hg versus 198 ± 14.2 mm Hg for untreated SHRs; WKY = 116 ± 7.2, WKY + HYD = 109 ± 8.4). However, the heart weight to body weight ratio was not significantly different from untreated SHRs (SHR + HYD 4.18 ± 0.086 versus SHR 4.31 ± .095), but was greater than that for WKY (3.18 ± 0.092) and WKY + HYD (3.23 ± 0.106) rats. Thus, SHR + HYD rats were normotensive for 12 weeks prior to study, but cardiac hypertrophy did not regress. In all groups there was an area of subendomyocardial no-reflow after ischemic contracture and reperfusion. The microscopic char-

acteristics of the hypertrophied hearts were similar to those of the nonhypertrophied WKY and SD hearts.

## Discussion

The significant findings in this study are that with global ischemia in the potassium-arrested rat heart, there appears to be a transmural progression of ischemic damage starting in the endomyocardium and extending, with time, epicardially. This transmural progression occurs in the absence of differences in regional coronary flow and wall tension in the endomyocardium, compared with the epimyocardium. Hypertrophied hearts appear to be more sensitive to global ischemia than normal hearts, again in the absence of any difference in functional activity between hypertrophied and control hearts. In addition, the development of contraction band change occurs only after reperfusion and appears to be related to the development of no-reflow.

The increased sensitivity of the endomyocardium to ischemic damage has been well known for many years. Studies of acute myocardial infarction in dogs have shown that there is a wavefront of ischemic necrosis that begins in the endomyocardium and progresses toward the epimyocardium.[18] It has been suggested that this transmural progression of ischemic change may be caused by decreased coronary flow and/or increased wall tension in the endomyocardium, compared with the epimyocardium.[18-20] In a recent study, Lowe et al suggested that the transmural progression of ischemic damage may be due to inherent differences between the endomyocardium and the epimyocardium, irrespective of coronary flow or wall tension.[20] In their study, slabs of left ventricle were excised and incubated *in vitro* at 37 C. With this preparation, coronary flow and wall tension were both zero; however, ischemic contracture and decreases in ATP levels occurred first in the endomyocardium. Other studies have also demonstrated heterogeneity of metabolic activity between various regions of the heart, including mitochondrial respiratory activity, glycolytic enzyme activity, and metabolite levels.[21-25] Recent studies have also demonstrated increased levels of V3 myosin isoenzyme in the endomyocardium versus the epimyocardium of the rabbit, again suggesting underlying metabolic differences across the wall of the heart [26]

In our studies using the isolated perfused rat heart exposed to global ischemia, we were able to control wall tension and collateral coronary flow during ischemia. All hearts were potassium-arrested for 5 minutes prior to cessation of coronary flow. Because the hearts were potassium-arrested, they were not performing any work and wall tension was zero. Thus, differences in functional activity prior to the onset of global ischemia were prevented. With this model, any differences in response to ischemia would be expected to be due to inherent differences in metabolic activity between different regions of the heart. This is especially important in comparisons between normal and hypertrophied hearts, because functional activity and high-energy phosphate stores have been shown to be different in hypertrophied hearts as compared with normal hearts performing work.[9] Global ischemia was achieved by occlusion of the aortic cannula, resulting in complete cessation of coronary flow. Regional differences in collateral flow were not present in this model. Despite these various factors, which made the ischemic conditions identical in the endomyocardium and the epimyocardium, we noted significant transmural differences in the severity and time course of ischemic change. Our studies show that the endomyocardium develops ischemic contracture first, followed by the midwall. After peak contracture the subendomyocardial cells relaxed slightly. This corresponds to the slight decrease in LV pressure noted after peak contracture (Figure 1). The mechanisms responsible for this relaxation are unknown; however, the sheer stresses placed on the subendomyocardial myocytes during the transmural progression of contracture may play a role. Our findings of a transmural progression of ischemic injury in the isolated perfused rat heart model are consistent with studies by Lowe et al using isolated dog myocardium.[20] In addition, Edoute et al[27] have also shown a transmural progression of ischemic injury and ATP decline in the isolated working rat heart. In their studies morphologic evidence of ischemic injury occurred first in the subendomyocardium, progressed to the midwall, and with time affected the epimyocardium. With reperfusion, the subepimyocardium showed almost complete morphologic recovery; regression of ultrastructural ischemic changes occurred to a lesser extent in the subendomyocardium.[27]

In our study, the time to development of ischemic contracture in hypertrophied rat hearts was decreased, compared with age-matched nonhypertrophied hearts. This is consistent with previous studies that demonstrated an enhanced sensitivity to ischemia in the hypertrophied heart.[10,28-30] In patients and experimental animals with left ventricular hypertrophy, ATP levels are lower in the hypertrophied myocardium; the ATP levels in the endomyocardium were decreased, compared with the epimyocardium.[30,31] In hypertrophied hearts ischemic contracture also developed sooner. These findings suggest that the decrease in ATP may occur sooner in the

hypertrophied myocardium, compared with the normal heart, resulting in a decreased time to development of ischemic contracture. To test this, Peyton et al[9,32] arrested isolated perfused rat hearts with oxygenated high-potassium buffer before the initiation of global ischemia. This preischemic arrest caused the ATP levels in the hypertrophied hearts to increase, up to the level of nonhypertrophied control hearts. Even when initial ATP levels were identical, the hypertrophied hearts still developed ischemic contracture sooner than nonhypertrophied hearts. These findings suggest differences in metabolic rates and an increased susceptibility to ischemia in hypertrophied hearts.[9,31,32] Recent studies by Neeley et al[33] suggest that ATP levels may not be the only factor responsible for ischemic damage to the myocardium. In their studies using isolated perfused rat hearts, the extent of ischemic damage did not correlate with ATP levels, and recovery of function was inversely related to tissue lactate content. These authors suggest that accumulation of byproducts of anaerobic glycolytic metabolism (lactate, hydrogen ion, NADH) rather than loss of tissue adenine nucleotides may be important in the pathogenesis of myocardial ischemic damage.[33]

In our studies, ATP levels were not measured. However, all hearts were potassium-arrested for 5 minutes before the onset of ischemia. As demonstrated by Peyton et al,[9] this preischemic arrest should have caused the ATP in the hypertrophied hearts to increase up to control levels. Under these conditions, the hypertrophied hearts in our study still developed ischemic contracture earlier than nonhypertrophied hearts. This suggests that there are inherent metabolic differences between normal and hypertrophied hearts which make the hypertrophied heart more susceptible to ischemic injury.

Of interest is the difference in time to ischemic contracture between the hydralazine-treated SHR and the untreated SHRs. In SHR + HYD rats the blood pressure was reduced to normotensive levels for 12 weeks; however, cardiac hypertrophy did not regress. In these animals, despite cardiac hypertrophy equal to that of the untreated SHRs, the time to ischemic contracture was increased to near the time to ischemic contracture of nonhypertrophied hearts. This suggests that relief of the stress imposed on the myocardium by hypertension may alter the way the hypertrophied heart responds to ischemia. Several investigators have shown that pressure overload hypertrophy causes modifications in the myosin isoenzyme patterns.[34–36] One-year-old SHRs have increased levels of V3 myosin isozyme, as do other models of pressure overload hypertrophy.[37,38] The V3 myosin isozyme is associated with a slower than normal rate of contraction and a decreased rate of ATP hydrolysis. Scheuer et al[39] have shown that the changes in myosin isozyme pattern in hypertrophied hearts can be modified by exercise and other factors. The SHRs used in this current study would be expected to have increased levels of V3 myosin isoenzyme, as has been previously reported for SHRs.[37,38] In a recent report by Nagano et al,[40] SHRs treated with hydralazine experienced a shift in myosin isozyme pattern with a decrease in V3 isozyme and an increase in V1. This pattern more closely resembles the isozyme pattern in hearts of normotensive WKY rats. These shifts in myosin isozyme patterns, as well as the expected modifications in other metabolic pathways in the hydralazine-treated normotensive SHRs, may be responsible for the partial normalization in time to ischemic contracture seen in SHR + HYD rats.

Because our hearts were potassium-arrested prior to ischemia, the rates of myosin ATPase activity would not be important during ischemic arrest. However, because there are obvious differences in ATPase activity, differences in other metabolic pathways may also be seen in hypertrophied hearts. These differences may be responsible for the increased susceptibility of the hypertrophied heart to ischemic insult. Additional factors, other than myosin isoenzyme pattern shifts, may be responsible for the improvement in response to ischemia in SHR + HYD rats. Although not addressed in this study, decreased wall tension due to decreased afterload may have caused modifications in other metabolic pathways that could cause an improvement in response to ischemia.

### No-Reflow Phenomenon

Reperfusion after ischemic contracture results in the development of areas of no-reflow in normal and hypertrophied hearts. This no-reflow phenomenon has been observed in infarcted myocardial tissue *in vivo* and in some cases has been attributed to thrombotic occlusion of vessels.[41] However, no-reflow also occurs in buffer-perfused hearts in which thrombotic occlusion of vessels cannot occur. In the isolated rat heart, no-reflow has been attributed to increased intramyocardial pressure or edema compressing the coronary vasculature, increased coronary resistance, or damage to the coronary vasculature.[11–14] Humphrey et al[12] and Gavin et al[42] have suggested that intramyocardial pressure compressing the coronary vessels may be the most important factor causing no-reflow. In their studies, hearts were subjected to ischemic contracture with reperfusion and were then infused with methacrylate resin, which was allowed to harden, forming casts of the coronary vessels. Exami-

nation of these casts revealed that only the larger vessels penetrated part of the way into the no-reflow zone, and that the depth of penetration was proportional to the caliber of the vessel.[12,42] Larger vessels with thicker walls and increased intraluminal pressure were able to penetrate deeper into the no-reflow zone. This suggests that external compressive forces may be responsible for the constriction of the smaller coronary vessels.[42] Further studies, in which the reperfusion pressure was increased, demonstrated that areas of no-reflow could be decreased or prevented if the vascular reperfusion pressure was increased in order to offset the compressive forces.[43]

In our studies, discrete areas of no-reflow were evident after ischemic contracture and reperfusion. Use of radioactive microspheres allowed us to quantitate regional coronary flow, and stereologic evaluation of Microfil-treated heart slices allowed us to quantitate the areas of no-reflow. Microscopic evaluation of Microfil-infused heart tissue taken at the flow/no-reflow border demonstrated extensive contraction band change in the midwall region, with no contraction bands in the endomyocardium or the epimyocardium. It is possible that the zone of contraction bands may cause the increased intramyocardial pressure that compresses the coronary vessels, resulting in no-reflow. We have seen that shortly after reperfusion, coronary flow is transmural (Table 2, 45-second reperfusion) and contraction band change is not a significant finding. This corresponds with the LV pressure tracing (Figure 1), which demonstrates good recovery of contractility and decreasing end diastolic pressure during the first few minutes of reperfusion. With time, however, contraction bands do form, and a discrete area of subendomyocardial no-reflow develops. If indeed contraction band formation is associated with the development of areas of no-reflow, then prevention of contraction band formation should prevent the no-reflow phenomenon. In our studies in which ischemic contracture was followed by reperfusion with oxygenated high-potassium buffer, contraction bands did not form and coronary flow was transmural (Table 2, high-$K^+$ reperfusion). This lends further support to the theory that the formation of contraction bands may be responsible for the development of areas of no-reflow. However, we cannot rule out other causes of no reflow. Microscopic examination of tissue from the flow/no-reflow border zone in all groups, including potassium-reperfused hearts, demonstrated endothelial cell damage, including cytoplasmic blebbing. It is possible that the milieu created during ischemia, which predisposes to contraction band necrosis, may also cause vascular damage that could cause spasm and vascular constriction upon reperfusion. However, the discrete zone of contraction band change at the flow/no-reflow border in all hearts examined suggests an association between contraction band formation and the development of areas of no-reflow after ischemic contracture.

In summary, these studies demonstrate that in hearts subjected to total global ischemia, morphologic changes indicative of ischemic injury first appear in the subendomyocardium and progress, with time, epicardially. With reperfusion, a zone of contraction band change develops at the flow/no-reflow border and appears to impede reperfusion. In addition, there is an enhanced sensitivity to ischemia in the hypertrophied heart. The morphologic characteristics of ischemic injury in the hypertrophied heart are similar to those seen in the normal heart, although the time course is accelerated.

## References

1. Hearse DJ, Garlick PB, Humphrey SM: Ischemic contracture of the myocardium: mechanisms and prevention. Am J Cardiol 1977, 39:986–993
2. Cooley DA, Reul GJ, Wukasch DA: Ischemic contracture of the heart: "Stone heart." Am J Cardiol 1972, 29:575–577
3. Hutchins GM, Silverman KJ: Pathology of the stone heart syndrome: Massive myocardial contraction band necrosis and widely patent coronary arteries. Am J Pathol 1979, 95:745–752
4. Alanen K, Nevalainen TJ, Lipasti J: Ischaemic contracture and myocardial perfusion in isolated rat heart. Virchows Arch [Pathol Anat] 1980, 385:143–149
5. Ganote CE, Humphrey SM: Effects of anoxic or oxygenated reperfusion in globally ischemic, isovolumic, perfused rat hearts. Am J Pathol 1985, 120:129–145
6. Digerness SB, Tracy WG, Andrews NF, Bowdoin B, Kirklin JW: Reversal of myocardial ischemic contracture and the relationship to functional recovery and tissue calcium. Circulation 1983, 68(Suppl II):II33–II40
7. de Leiris J, Harding DP, Pestre S: The isolated perfused rat heart: A model for studying myocardial hypoxia or ischemia. Basic Res Cardiol 1984, 79:313–321
8. Humphrey SM, Thomson RW, Gavin JB: The influence of the no-reflow phenomenon of reperfusion and reoxygenation damage and enzyme release from ischaemic isolated rat hearts. J Mol Cell Cardiol 1984, 16:915–930
9. Peyton RB, Trigt PV, Pellom GL, Jones RN, Sink JD, Wechsler AS: Improved tolerance to ischemia in hypertrophied myocardium by preischemic enhancement of adenosine triphosphate. J Thorac Cardiovasc Surg 1982, 84:11–15
10. Attarian DE, Jones RN, Currie WD, Hill RC, Sink JD, Olsen CO, Chitwood JR WR, Wechsler AS: Characteristics of chronic left ventricular hypertrophy induced by subcoronary valvular aortic stenosis: II. Response to ischemia. J Thorac Cardiovasc Surg 1981, 81:389–395
11. Apstein CS, Mueller M, Hood WB Jr: Ventricular contracture and compliance changes with global ischemia and reperfusion, and their effect on coronary resistance in the rat. Circ Res 1977, 41:206–217
12. Humphrey SH, Gavin JB, Herdson PB: The relation-

ship of ischemic contracture to vascular reperfusion in the isolated rat heart. J Mol Cell Cardiol 1980, 12:1397–1406
13. Jennings RB, Kloner RA, Ganote CE, Hawkins HK, Reimer KA: Changes in capillary fine structure and function in acute myocardial ischemic injury, Microcirculation of the Heart. Vol 5, Changes in Capillary Permeability. Edited by H Tillmanns, W Kubler, H Zebe. Berlin, Springer-Verlag, 1982, pp 87–97
14. Lee BY, Wilson GJ, Domenech RJ, MacGregor DC: Relative roles of edema versus contracture in the myocardial post ischemic "no-reflow" phenomenon. J Surg Res 1980, 29:50–61
15. Humphrey SM, Thomson RW, Gavin JB: The effect of an isovolumic left ventricle on the coronary vascular competence during reflow after global ischemia in the rat heart. Circ Res 1981, 49:784–791
16. Pfeffer JM, Pfeffer M, Frohlich ED: Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. J Lab Clin Med 1971, 78:957–962
17. Bishop SP: Radioactive tracer microspheres: Practical considerations to determine regional organ blood flow in experimental animals. Med Electronics 1981, 69:66–73
18. Reimer KA, Jennings RB: The wavefront phenomenon of myocardial ischemic cell death. Lab Invest 1979, 40:633–644
19. Lundsgaard-Hansen P, Meyer C, Riedwyl H: Transmural gradients of glycolytic enzyme activities in left ventricular myocardium: I. The normal state. Pflugers Archiv fr die gesamte Physiologie des Menschen und der Tiere 1967, 297:89–106
20. Lowe JE, Cummings RG, Adams DH, Hull-Ryde EA: Evidence that ischemic cell death begins in the subendocardium independent of variations in collateral flow or wall tension. Circulation 1983, 68(1):190–202
21. Camici P, Ursini F, Galiazzo F, Bellitto L, Pelosi G, Marzilli M, L'Abbate A, Barsacchi R: Different respiratory activities of mitochondria isolated from the subendocardium and subepicardium of the canine heart. Basic Res Cardiol 1984, 79:454–490
22. Dunn RB, Griggs SM Jr: Transmural gradients in ventricular tissue metabolites produced by stopping coronary blood flow in the dog. Circ Res 1975, 37:438–445
23. Griggs DM Jr, Tchokoev VV, Chen CC: Transmural differences in ventricular tissue substrate levels due to coronary constriction. Am J Physiol 1972, 222:705–709
24. Jedeikin LA: Regional distribution of glycogen and phosphorylase in the ventricles of the heart. Circ Res 1964, 14:202–211
25. Takala TES, Ruskoaho HJ, Hassinen IE: Transmural distribution of cardiac glucose uptake in rat during physical exercise. Am J Physiol 1983, 244(Heart Circ Physiol 13):H131–H137
26. Eisenberg BR, Edwards JA, Zak R: Transmural distribution of isomyosin in rabbit ventricle during maturation examined by immunofluorescence and staining for calcium-activated adenosine triphosphatase. Circ Res 1985, 56:548–555
27. Edoute Y, van der Merwe E, Sanan D, Kotze JCN, Steinmann C, Lochner A: Normothermic ischemic cardiac arrest in the isolated working rat heart. Circ Res 1983, 53:663–678
28. Lorell BH, Wexler LF, Momomura S, Weinberg E, Apstein CS: The influence of pressure overload left ventricular hypertrophy on diastolic properties during hypoxia in isovolumically contracting rat hearts. Circ Res 1986, 58:653–663
29. Menasche P, Grousset C, Apstein CS, Marotte F, Mouas C, Piwnica A: Increased injury of hypertrophied myocardium with ischemic arrest: Preservation with hypothermia and cardioplegia. Am Heart J 1985, 110:1204–1209
30. Peyton RB, Jones RN, Sabina RL, Swain JL, Trigt PV, Spray TL, Holmes EW, Wechsler AS: Transmural high-energy phosphate gradients in patients with ventricular hypertrophy. Surgical Forum 1981, 32:268–270
31. Sink JD, Pellom GL, Currie WD, Hill RC, Olsen CO, Jones RN, Wechsler AS: Response of hypertrophied myocardium to ischemia: Correlation with biochemical and physiological parameters. J Thorac Cardiovasc Surg 1981, 81:865–872
32. Peyton RB, Pellom GL, Currie WD, Wechsler AS: Preischemic ATP enhancement to improve tolerance to ischemia to hypertrophied myocardium. Surg Forum 1980, 31:315–317
33. Neely JR, Grotyohann LW: Role of glycolytic products in damage to ischemic myocardium: Dissociation of ATP levels and recovery of function of reperfused ischemic hearts. Circ Res 1984, 55:816–824
34. Bugaisky L, Siegel E, Whalen RG: Myosin isozyme changes in the heart following constriction of the ascending aorta of a 25-day old rat. FEBS Lett 1983, 161:230–234
35. Alpert NR, Mulieri LA: The inhomogeneity and appropriateness of the myocardial response to stress. Hypertension 1984, 6(Suppl III):III50–III57
36. Rupp H: The adaptive changes in the isoenzyme pattern of myosin from hypertrophied rat myocardium as a result of pressure overload and physical training. Basic Res Cardiol 1981, 76:79–88
37. Rupp H, Jacob R: Response of blood pressure and cardiac myosin polymorphism to swimming training in the spontaneously hypertensive rat. Can J Physiol Pharmacol 1982, 60:1098–1103
38. Rupp H: Association of ventricular myosin heavy chains in functional states which lead to isoenzyme populations encompassing the whole range of possible distribution. Basic Res Cardiol 1985, 80:608–616
39. Scheuer J, Malhotra A, Hirsch C, Capasso J, Schaible T: Physiologic cardiac hypertrophy corrects contractile protein abnormalities associated with pathologic hypertrophy in rats. J Clin Invest 1982, 70:1300–1305
40. Nagano M, Takeda N, Kanemura M, Noma K, Ohkubo T: Influences of a long term treatment with antihypertensive drugs on left ventricular myosin isoenzyme pattern in spontaneously hypertensive rats. J Mol Cell Cardiol 1986, 18(Suppl 3):50
41. Gavin JB, Nevalainen TJ, Seelye RN, Webster V, Thomson RW: An association between the onset of rigor and loss of vascular competence in early myocardial infarcts. Pathology 1978, 10:219–225
42. Gavin JB, Thomson RW, Humphrey SM, Herdson PB: Changes in vascular morphology associated with the no-reflow phenomenon in ischemic myocardium. Virchows Arch [Path Anat] 1983, 399:325–332
43. Humphrey SM, Gavin JB: The effect of coronary pressure on contracture and vascular perfusion in the hypoxic isolated rat heart. Basic Res Cardiol 1984, 79:350–362

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