

# Microvascular Spasm in the Cardiomyopathic Syrian Hamster: A Preventable Cause of Focal Myocardial Necrosis

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**SUMMARY** The cardiomyopathic Syrian hamster develops focal myocardial necrosis beginning at 1 month of age, which leads to eventual ventricular failure within 1 year. The pathogenesis of this myocytolytic necrosis is unknown. Based on the nature of the cell necrosis, cytochemical evidence of vascular alterations, and the sensitivity of the hamsters to catecholamines and other vasoactive substances, we believe that the cardiomyopathy may be mediated by abnormalities of the microcirculation. Nonetheless, until the present study, no significant changes have been observed in these vessels. To elucidate the pathogenesis of this disease, we perfused living cardiomyopathic hamsters with silicone rubber solutions, which revealed numerous areas of microvascular constriction, diffuse vessel narrowing and luminal irregularity. Fixed structural lesions in these vessels could not be demonstrated. Pretreatment of young hamsters with verapamil during the period when they normally develop myocardial necrosis prevented myocytolytic lesions and abolished microvascular hyperreactivity. We believe that focal, transient spasm of small blood vessels, probably secondary to vasoactive substances, may cause myocytolytic necrosis (a form of reperfusion injury) in this model. This may also be a multifactorial disease with myocellular as well as vascular abnormalities leading to myocardial degeneration. The similarity of this disease to human and experimental cardiomyopathy suggests that microvascular spasm may be a common denominator of many different cardiomyopathic syndromes.

THE GENETICALLY INBRED Syrian hamster develops cardiomyopathy and muscular dystrophy in a reproducible and predictable fashion. Beginning at about 30 days of age, and extending over the next 4 months, the hamsters form multiple focal areas of myocytolytic necrosis in the heart and skeletal muscle, which heal (in the heart) with fibrosis and calcium deposition.<sup>1-4</sup> The subsequent course, depending on the strain of hamster, is generally cellular hypertrophy, progressive congestive heart failure, and death, usually within 1 year.<sup>5-7</sup> The morphology of the focal myocytolytic lesions in the heart is similar to myocytolytic, noncoronogenic necrosis in a variety of human cardiac diseases.<sup>8-12</sup>

The pathogenesis of the focal myocardial necrosis is unknown. Although several abnormalities have been identified, research studies have focused primarily on abnormal cellular calcium metabolism.<sup>13</sup> Wrogemann et al.<sup>14, 15</sup> suggested that there is increased calcium flux across the sarcolemma leading to cellular hypercontraction, mitochondrial calcification, and cell death. These changes may be mediated by endogenous catecholamines, explaining the sensitivity of the hamsters to these agents.<sup>16</sup> Jasmin and Bajusz<sup>17</sup> suggested that sarcolemmal permeability to calcium, presumably caused by endogenous catecholamines, may be prevented by treatment of these hamsters with calcium channel inhibitors, but not by  $\beta$ -adrenergic blockers.

The genetic defect producing either abnormal sensi-

tivity to catecholamines or membrane alterations leading to increased sarcolemmal permeability to calcium may be generalized, and affect every muscle cell. However, if the defects reside in all striated muscle cells, then it is paradoxical that the characteristic lesion of this disease should be focal necrosis of cells in discrete groups. The focal nature of the disease suggests either that the basic cellular defect is heterogeneous (which is improbable, since this is a genetic condition likely causing a diffuse abnormality in an enzyme or structural component), or that the primary locus of disease is at a level affecting the survivability of small groups of cells, i.e., the microcirculation. Most routine studies of this model, including our own,<sup>4, 18</sup> have noted only minimal morphologic changes in small intramyocardial vessels. However, we recently adapted improved methods for observing the cardiac microcirculation to the study of hamster cardiomyopathy by perfusing the beating heart in vivo with silicone rubber. This has revealed the results of dynamic alterations in small myocardial vessels, which may mediate focal cell necrosis.

In this report we examine the histologic, cytochemical, and perfusion findings that suggest a focal nature of hamster cardiomyopathy and its possible vascular origin. The prevention of myocytolytic-type necrosis with the calcium-channel blocking agent verapamil is further correlated with effects of this coronary vasodilator on spasm-like lesions in the microcirculation.

## Materials and Methods

We studied hamsters of the BIO 53.58 strain obtained from Telaco laboratory. Both male and female hamsters were evaluated, predominantly at 30, 50 and  $150 \pm 14$  days of age. A few hamsters were also studied at 90 and 210 days of age. Each cardiomyopathic hamster was compared with an age- and sex-

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matched noncardiomyopathic control shipped in the same batch. Hamsters were gently handled, and were fed standard chow as desired. Fifty-one cardiomyopathic hamsters and 51 controls were studied.

### Histology

All hamsters underwent routine histologic studies. After light ether anesthesia, beating hearts (except those perfused with silicone rubber, detailed below) were removed above the atria, blotted dry, weighed and sliced transversely from apex to base. Slices no more than 2–3 mm thick were immediately fixed in 3.7% phosphate buffered formaldehyde for 24–48 hours and processed routinely for light microscopy. Portions of latissimus dorsi and supraspinatus skeletal muscles, tongue, liver and lungs were sectioned also and embedded in paraffin. Tissues were stained with hematoxylin-eosin, Masson's trichrome and von Kossa's method for calcium salts. Selected tissues were reacted with van Gieson's stain for elastica and alizarin red for calcium. Each section was examined for the presence of degeneration or necrosis, cellular infiltrate, scarring and calcification. Specific attention was paid to small intramyocardial blood vessels. Skeletal muscle and tongue were evaluated for similar lesions and for regenerative activity. Lungs and liver were examined for evidence of congestive heart failure. The specific location of cardiac lesions was noted, particularly endocardial vs epicardial, and left vs right ventricles.

For ultrastructural studies<sup>4</sup> and for histologic evaluation of 1- $\mu$  Epon-embedded sections described below, small portions of myocardium were removed from the mid-left ventricular wall, and diced into 1-mm cubes under fixative. Tissues were fixed in 2.5% glutaraldehyde with 0.1 M cacodylate buffer, pH 7.4, for 4–6 hours, and were then rinsed overnight in buffer. Then, tissues were postfixed for 1 hour in osmium tetroxide, dehydrated in increasing concentrations of alcohol and propylene oxide, and embedded in Epon 812. One-micron sections were prepared with glass knives and were stained with alkaline toluidine blue. Sections in which small arterioles or capillaries were especially well seen were selected for serial analysis and reconstruction of the vessels along their longitudinal axis; approximately 100 sections were examined from each pellet chosen. These sections were ideally suited for evaluating fixed structural lesions in the microvasculature.

### Cytochemistry

Thin transverse rings of myocardium, including left and right ventricles, were fixed in cold formol calcium for 12–24 hours. Ten-micron sections were prepared on a freezing microtome. Lillie's modification of Gomori's  $\beta$  glycerophosphate method was used to study endothelial alkaline phosphatase reaction product.<sup>19</sup> For acid phosphatase activity, sections were processed with Barka and Anderson's method.<sup>20</sup> Lysosomal activity was evaluated in myocardial cells, interstitial histiocytes and vascular smooth muscle.

### Perfusion Studies

White silicone rubber (Microfil; Canton Bio-Medical Products), an opaque liquid that perfuses the arterial and venous circulation, hardens *in situ* and maintains its shape within the vascular tree, was used in these studies. Both cardiomyopathic and control hamsters were lightly anesthetized with ether. A sternum-splitting incision was used to expose the beating heart. Five milliliters of heparinized saline (5000 units/250 ml) were injected into the left ventricular apex before Microfil perfusion. Initial studies were performed with hand injection of 5–8 ml Microfil through the left ventricular apex (12 hamsters in groups of four at 30, 50, and 150 days with controls). The hearts continued to beat for approximately 1 minute during the injection. Adequacy of microvascular perfusion was judged by the white blush that developed in the myocardium. With left ventricular injection, the subepicardial and midwall vessels filled well, but subendocardial vessels were not consistently perfused. Because the majority of the necrotic lesions were in the outer two-thirds of the ventricular wall (see Results), the vascular filling pattern was considered adequate for the purposes of this study. However, in order to evaluate the transmural microcirculation, later studies were performed with retrograde aortic perfusion, using animals at 30, 90 and 150 days (two cardiomyopathic hamsters and two controls), and at 30 days alone (15 cardiomyopathic and controls). The descending thoracic aorta was freed and isolated over a fine hemostat. A 26-gauge needle was inserted and 5 ml of Microfil was injected slowly. As before, the hearts continued to beat for 1 minute.

After perfusion, the hearts were rapidly excised and placed on ice for 5 minutes to "cure" the Microfil. The hearts were sliced 2–3 mm thick from apex to base. One slice was fixed in 3.7% buffered formaldehyde for routine histologic study. Three hamsters from each age group treated with verapamil had heart slices fixed in formol calcium for alkaline phosphatase reactions. Portions of skeletal muscle and tongue, when well perfused with Microfil, were also taken for routine histologic and microvascular examination. The one or two slices that remained were processed for clearing by the method of Schaper.<sup>21</sup> Briefly, tissues were fixed in 3.7% formaldehyde for 14 days, bleached in hydrogen peroxide, washed, dehydrated in alcohol and benzol, and cleared in methyl salicylate. Tissues became semitranslucent and amber with loss of all cellular detail; however, the Microfil-perfused vessels were easily observed. For viewing under the microscope, cleared slices were immersed in methyl salicylate in a shallow Petri dish, and were visualized with transillumination and epi-illumination. With transillumination, the opaque Microfil absorbed light and appeared black; with epi-illumination the color and three-dimensional features of the vessels could be appreciated. By focusing through the depth of the tissue, tilting the specimen, or altering the epi-illumination source, individual vessels could be examined in detail.

Vascular lesions were compared with vessels in age- and sex-matched controls, using our experience with Microfil-perfused human, canine and rat circulations. Normal vessels taper gently down to the capillary level, with numerous branches forming loops and arcades.<sup>22</sup> Because partially filled vessels and vessel branches viewed on end may mimic true lesions, we only considered as abnormal vessels with focal narrowing, aneurysmal dilation or tortuosity in which the area in question occurred away from a branch point with good Microfil perfusion proximally and distally. Subtle variations or slight irregularities were not evaluated as abnormal.

To determine if microvascular lesions (i.e., pronounced constriction) occurred in anatomically normal vessels, we took selected cleared slices of tissue perfused with Microfil, rehydrated them progressively, embedded them in paraffin, and stained them with hematoxylin-eosin. Although the histologic detail was not ideal, it was sufficient to examine vascular anatomy. We also took Microfil-perfused uncleared tissue, embedded it directly in paraffin, and sectioned it at 50  $\mu$ . These sections, when stained with hematoxylin-eosin, enabled us to study vessels in detail and to determine the anatomy of the perfused vessel along with the features of the surrounding tissue.

### Verapamil

To evaluate the effects of verapamil on the extent of tissue necrosis and alterations in the microvasculature, a therapeutic trial was performed. We determined drug effects at the earliest stages of heart disease by injecting verapamil (1 mg/day in two divided doses for 2 weeks, subcutaneously) into 10 30-day-old female cardiomyopathic hamsters. These hamsters normally do not develop cardiac lesions until after 30 days of age, although skeletal muscle necrosis has usually commenced by this time. The treated hamsters were compared with seven cardiomyopathic hamsters and seven noncardiomyopathic controls not given verapamil, and eight noncardiomyopathic controls treated with verapamil. Two hamsters each at 30, 90 and 150 days of age, with matched controls, were treated with verapamil and studied for endothelial alkaline phosphatase activity. At the end of the treatment period, all hamsters were perfused with Microfil and studied by the methods described above, for histologic changes and microvascular detail. During the 2-week verapamil treatment, two 30-day-old cardiomyopathic hamsters died, and thus were eliminated from the study.

## Results

### Histopathology

At 30–35 days of age, almost no necrotic lesions were observed in the myocardium of cardiomyopathic hamsters. In contrast, the skeletal muscles and tongue had active cellular necrosis, with groups of necrotic muscle cells infiltrated by a prominent mononuclear inflammatory response. Foci of cellular calcification were noted. Myocardium studied later revealed increasingly more extensive alterations. Discrete foci of

cells were undergoing active contraction band and myocytolytic necrosis, with the presence of granular calcium deposits within the cellular outlines (figs. 1–3). The myocytolytic foci developed as either relatively acellular zones of cytoplasmic dissolution with residual perisarcolemmal stroma, or more cellular areas with marked inflammatory reaction. The inflammatory response was generally mononuclear; however, rare foci of necrosis elicited a polymorphonuclear infiltrate.

The active cellular necrosis involved small groups of myocytes, usually 10–50 cells, sharply delimited from the surrounding tissue, which appeared normal. Small muscular arteries, arterioles and capillaries had no specific alterations. The necrotic foci progressively healed at later stages of the disease, so that at 150–210 days of age, most lesions were composed of collagen and calcium deposits. After 150 days of age, only rare active necrosis was present in these hearts. The necrosis was localized transmurally and in both ventricles, but there appeared to be a predilection for the midventricular and subepicardial zones, and for the left ventricle compared with the right. Judging by the absence of chronic congestion in the lungs and livers and the lack of difference in control-matched heart weight/body weight ratios, none of the cardiomyopathic hamsters in our studies was in congestive heart failure.

### Cytochemistry

Endothelial alkaline phosphatase activity was diffusely present in control hamsters at all ages, producing an outline of the capillary wall. In the cardiomyopathic hamsters, discrete zones of absent alkaline phosphatase activity were observed in areas of normal muscle, even in 30-day-old hamsters without myocardial necrosis. No histologically apparent abnormalities of the microvasculature could be appreciated in these non-reactive zones. Regions of absent alkaline phosphatase activity were generally of the same size or slightly larger than the necrotic foci and myocardial scars.

Lysosomal acid phosphatase activity was present in myocardial cells, inflammatory infiltrates, and muscular blood vessel walls. Myocellular activity showed no difference between control myocardium and the non-necrotic myocardium of the cardiomyopathic animals. Interstitial inflammatory cell activity was prominent in the diseased hamsters because of the greater numbers of these cells in necrotic foci. Small arteries and arterioles had increased smooth muscle acid phosphatase reaction product in the cardiomyopathic hearts compared with controls; however, we could not discern anatomic abnormalities in these vessels.

### Microfil Perfusion Studies

Smoothly tapering and branching Microfil-perfused vessels from a 44-day-old control hamster are shown in figure 4. Control hamsters had rare areas of vascular narrowing, but never demonstrated the marked constrictions and irregularities seen in the cardiomyopathic hamsters.

Beginning at 30 days of age, before the development

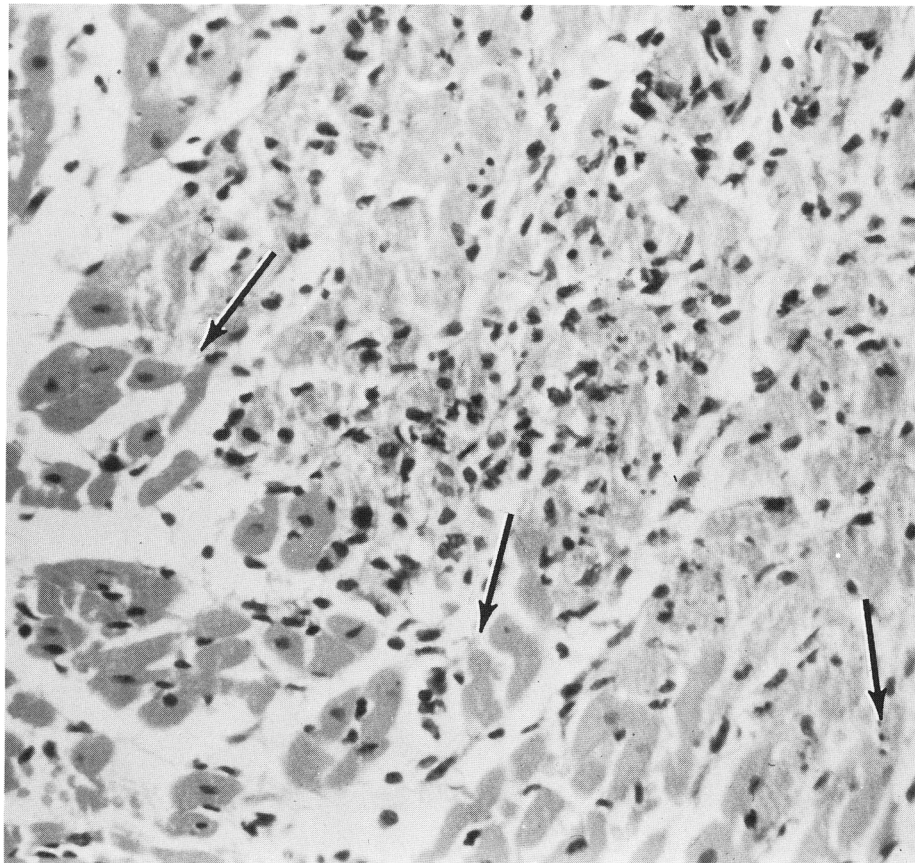


FIGURE 1. Area of left ventricle from a 50-day-old cardiomyopathic hamster with a small zone of myocardium undergoing active myocytolytic necrosis, with a mixed mononuclear and polymorphonuclear inflammatory cell response. The necrotic tissue is sharply demarcated from the surrounding unaffected myocardial cells (arrows). Hematoxylin-eosin stain; magnification  $\times 125$ .

of myocytolytic necrosis in the cardiomyopathic hamsters, arteriolar-sized vessels had numerous areas of pronounced constriction (figs. 5–7). These narrowed zones were generally in short segments of the vessel, but occasionally extended for considerable lengths. Areas of pre- and poststenotic dilatation were often associated with these constricted zones, giving the appearance of microaneurysm formation (fig. 8). The frequency of these constricted vessels appeared to increase with age; hamsters studied at 44 days, 50–60 days and 90 days of age with active cellular necrosis seemingly had more numerous lesions than those at 30 days without necrosis. Vascular lesions were seen in 150-day-old hamsters, but there were fewer than in hamsters in the more active necrotizing stage. Constrictions generally occurred peripheral to areas of active or healed necrosis in normal myocardium, so that distortion of the vessel wall by fibrotic scarring could not explain the presence of these lesions. Microfil perfusion of tongue and skeletal muscle was not adequate to make definitive statements regarding the state of the microvasculature. However, in myopathic hamsters in which perfusion was good, similar areas of constriction were noted (fig. 9).

Rehydration of Microfil-perfused myocardium with subsequent paraffin embedding allowed us to visualize

the vascular anatomy in the constricted zones. Vessels sectioned longitudinally were observed with localized areas of narrowing, and the vascular walls in these regions were not thickened by endothelium, smooth muscle proliferation, or collagen deposition (fig. 10). A sectioned longitudinal vessel in Epon-embedded myocardium from a 150-day-old cardiomyopathic hamster revealed several areas of occlusion or narrowing, which could provide the anatomic counterpart for the appearance of constriction with Microfil (fig. 11).

#### Verapamil Therapy

Pretreatment of 30-day-old hamsters for 14 days into the stage of active cellular necrosis prevented myocardial necrosis. Only one small focus of calcified scar was present in the left ventricular apex of one of eight surviving hamsters in the treated group. Treatment for 2 weeks beginning at 90 or 150 days did not alter the development of scars or necrosis, as these lesions were presumably already present. By the same token, tongue and skeletal muscle lesions were not prevented in 30-day-old hamsters with 2 weeks of verapamil therapy. In all age groups studied, even in those in which myocardial necrosis was prevented, verapamil treatment did not alter the focal absence of alkaline phosphatase reaction product in the capillary endo-



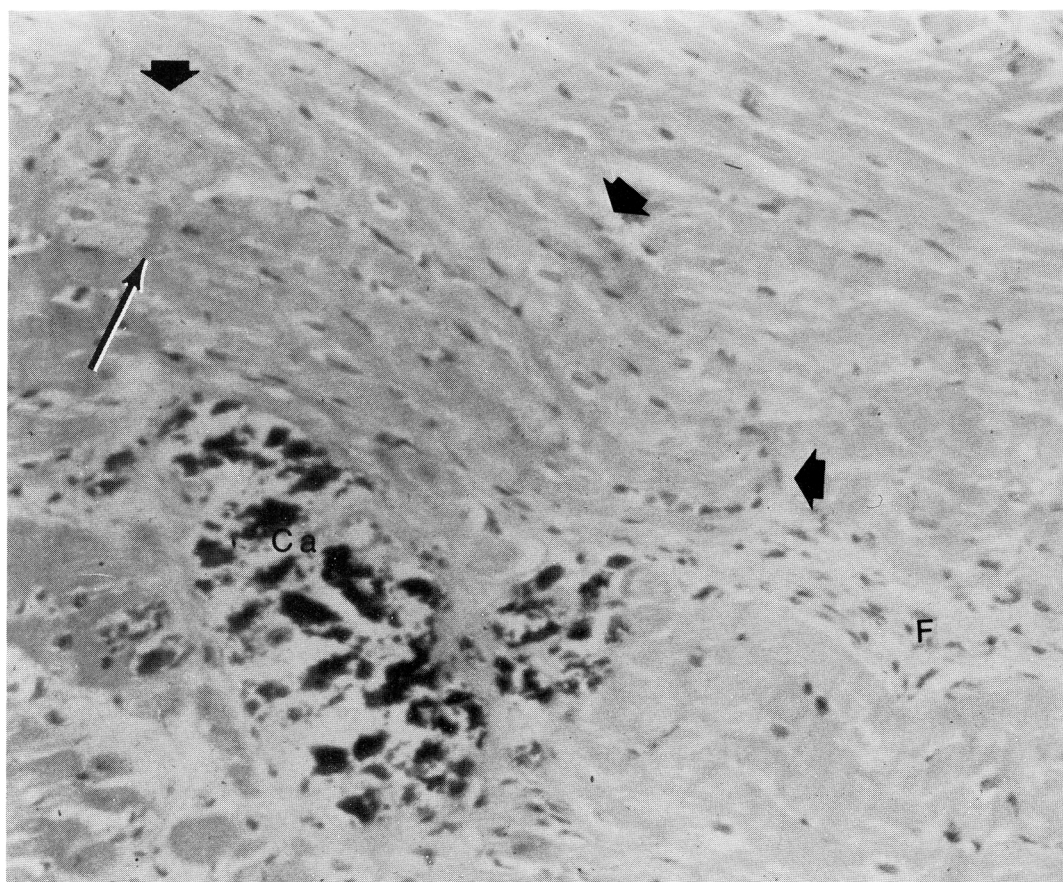


FIGURE 2. (above) Both acute and old necrosis in a 150-day-old cardiomyopathic hamster. The healed zones include a scar with clumped calcium granules (Ca) and fibrosis (F). The tissue undergoing active myocytolytic necrosis (thick arrows) has prominent contraction bands (thin arrow). Note the absence of an inflammatory cell response. Hematoxylin-eosin stain; magnification  $\times 125$ .





FIGURE 4. A Microfil-perfused 44-day-old control reveals tapering arterioles and capillaries in this transilluminated cleared specimen of the left ventricle. The vascular casts have smooth, regular contours, with no areas of constriction or dilatation. Magnification  $\times 125$ .

thelium. Control hamsters were not affected by verapamil.

Besides preventing myocardial necrosis, verapamil also had positive effects on the myocardial microvasculature. With rare exception, the Microfil-perfused vessels from treated hamsters had smoothly tapering contours with no regions of constriction or diffuse narrowing (fig. 12). Occasional focal lesions were observed; however, they were no more frequent than in the control hamsters. Constrictions also were prevented in focally scarred 90- and 150-day-old hamsters when they were compared with the untreated cardiomyopathic controls. Verapamil treatment of normal hamsters at all ages had no effects on the microcirculation.

### Discussion

This study provides the first direct evidence of the presence of a preventable microcirculatory lesion in the cardiomyopathic Syrian hamster, which may be important in the pathogenesis of cellular necrosis and the devel-

opment of congestive heart failure. Although Jasmin and co-workers<sup>17, 23</sup> speculated that the microvasculature may play a role in this disease, neither routine histologic evaluation nor electron microscopy revealed significant structural abnormalities of the vessels. We believe that the generally negative observations reflect the absence of fixed anatomic lesions in the microcirculation and the limitations of standard histopathologic techniques. With Microfil perfusion *in vivo*, however, we have studied long segments of these vessels in three dimensions, allowing us to observe what we consider to be the results of dynamic vascular hyperreactivity, which may produce transient, focal microcirculatory obstruction.

Several lines of indirect evidence support the concept that the microcirculation may be involved in cardiomyopathy. The focal nature of hamster cardiomyopathy has not been explained, with discrete areas of necrosis or scar surrounded by relatively normal myocardium. Most studies purporting to demonstrate generalized myocardial abnormalities have usually

FIGURE 3. A 210-day-old cardiomyopathic hamster with multiple discrete, focal, calcified scars in the left ventricular wall. No active necrosis is present at this stage. Most of the scarring is in the midventricular wall. LV = left ventricular cavity; RV = right ventricular cavity. Hematoxylin-eosin stain; magnification  $\times 50$ .



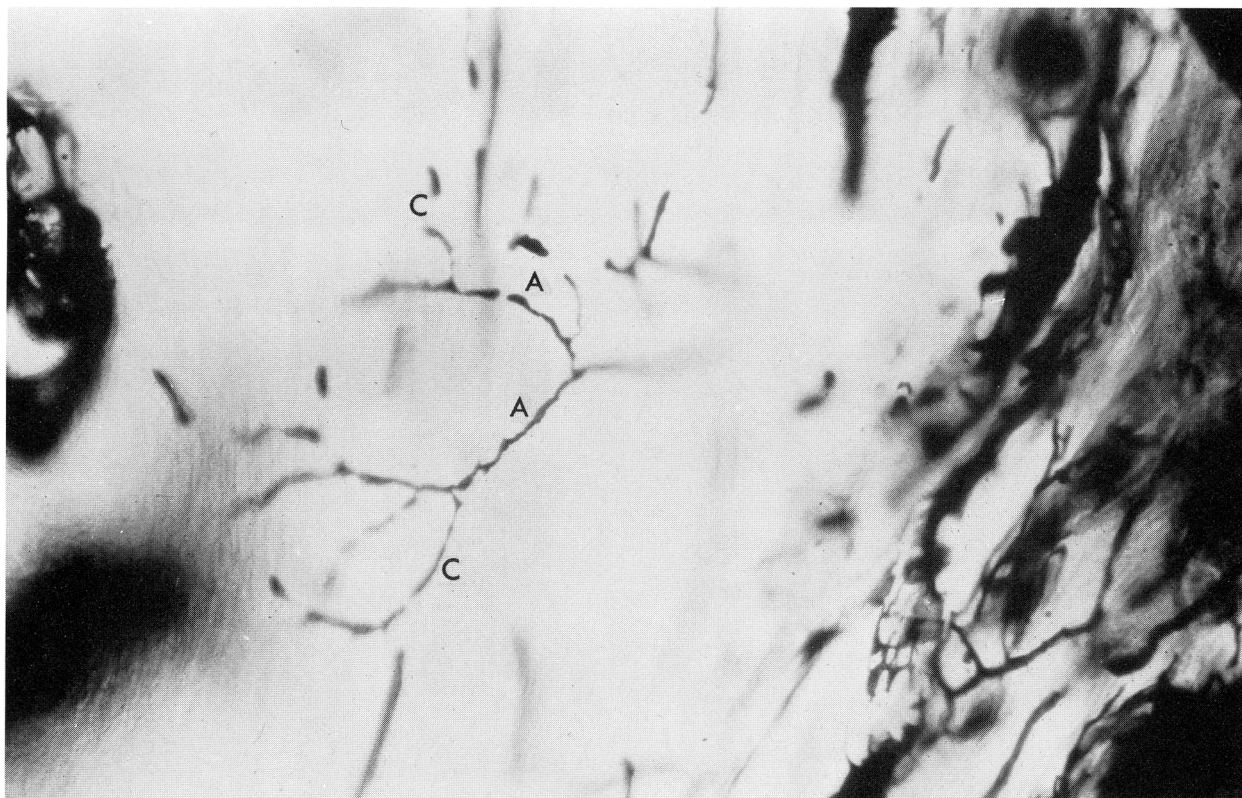
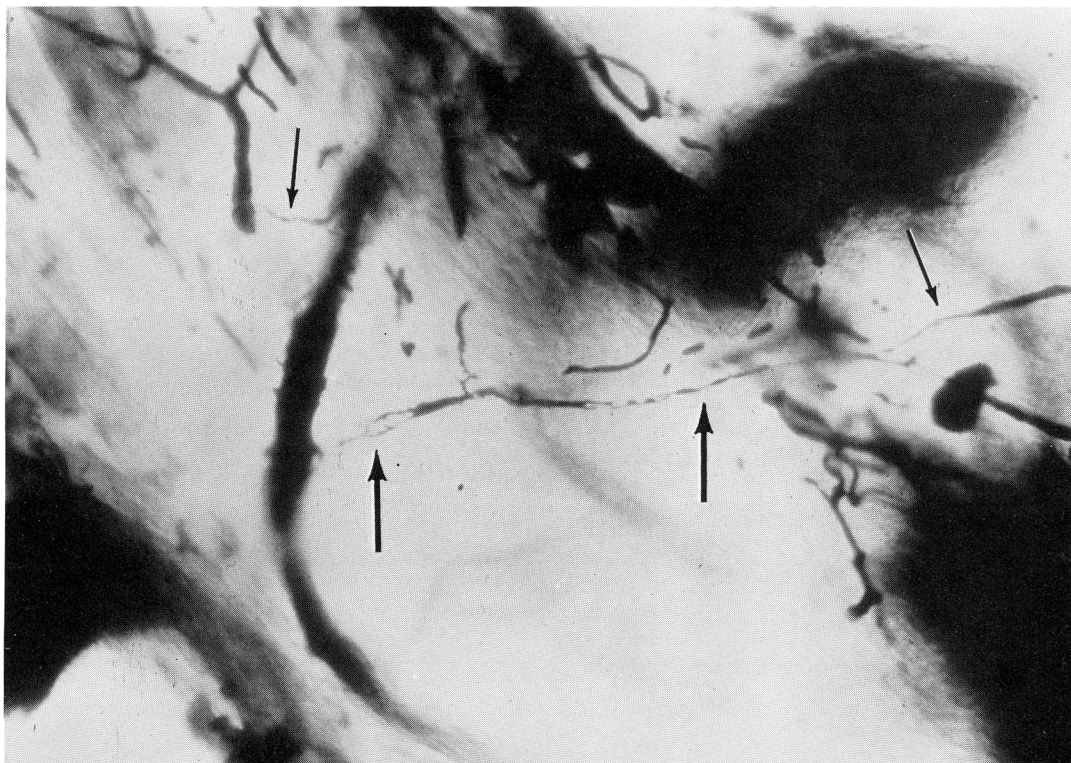


FIGURE 5. (above) A Microfil-perfused 30-day-old cardiomyopathic hamster heart before the development of overt necrosis. There is marked irregularity and focal narrowing of several connected arteriolar- (A) and capillary- (C) sized vessels, giving them a serrated rather than a smooth contour. Because of the pathologic alterations, we could not identify the vessels precisely by their luminal diameters. Transilluminated, cleared tissue; magnification  $\times 125$ .



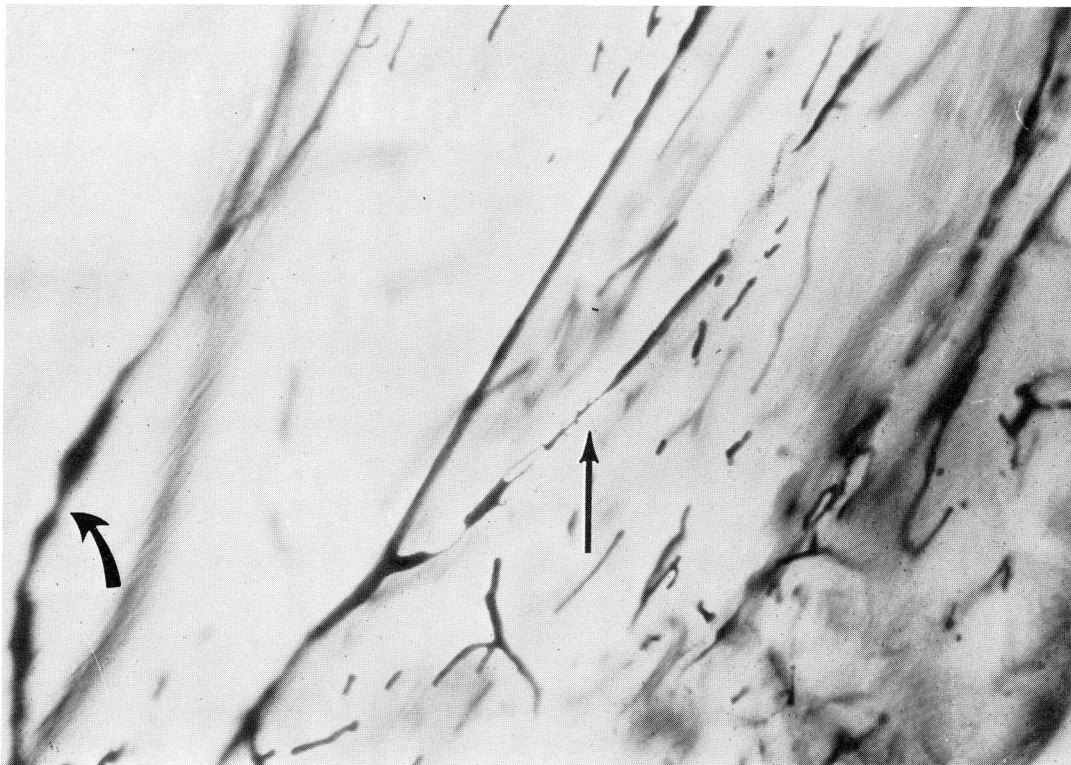


FIGURE 7. This 150-day-old cardiomyopathic hamster has a focally constricted small vessel (straight arrow), in which the lumen is reconstituted toward normal in several places along its length. The only way in which such a contour could be produced is secondary to an anatomic stricture of the vessel wall or as a result of spasm. Inadequate Microfil perfusion would not allow for reconstitution of the lumen. Note the slightly larger, out-of-focus vessel (curved arrow) with several constrictions. Transilluminated, cleared tissue; magnification  $\times 125$ .

used animals in the later stages of disease, when observed alterations may be due to compensatory myocardial hypertrophy or congestive heart failure.<sup>24, 25</sup> In contrast, histologic, ultrastructural, and cytochemical studies generally have shown insignificant or absent abnormalities in the nonnecrotic tissue at earlier periods.<sup>1, 4, 26</sup> This would not be expected if there was a generalized hereditary cellular defect (for example, the deficiency of alpha-1,4-glucosidase in Pompe's disease, which leads to diffuse glycogen accumulation and vacuolization of myocardial cells<sup>27</sup>).

If there is no consistent defect in the preserved myocardial cells, then localized tissue ischemia could cause focal necrosis. This concept is supported by our recent studies of normal myocardial arteriolar and capillary anatomy in dogs<sup>22</sup> and humans (unpublished observations, 1980), in which we showed that the microcirculation is organized as an end-capillary network, with no anastomoses between two groups of capillaries derived from two separate larger coronary vessels. Thus, obstruction of an appropriately sized

small vessel may cause necrosis of a volume of tissue supplied by that vessel, minus some preservation of peripheral myocardium kept viable by substrate diffusion from the normally perfused area.

Further support for microcirculatory involvement in this disease is provided by cytochemical studies. Jasmin and Bajusz reported focal absence of endothelial alkaline phosphatase activity in the heart, and suggested that vascular alterations may be implicated. They based their conclusions on the observation that known vasoactive substances, such as serotonin, have adverse effects on these animals.<sup>17</sup> We confirmed the focal absence of alkaline phosphatase activity at all stages of disease, and we noted that the zones of absent activity are generally in the same size range as the zones of cellular necrosis. We cannot explain the focal loss of enzyme reaction. Moreover, prevention of myocardial necrosis with verapamil did not alter the alkaline phosphatase staining pattern in the cardiomyopathic hamsters. Curiously, although catecholamine hypersensitivity has been implicated in this disease,<sup>16</sup>

FIGURE 6. This 30-day-old cardiomyopathic hamster has many small vessels in the same field with diffuse constriction. The arteriole to the left of center gives rise to a narrowed vessel, which maintains its irregular contour for many microns. Between the two large arrows, the narrowed vessel dilates slightly and then re-constricts. In the upper portion of the field, the thin arrows mark several other small vessels with diffuse constriction. Transilluminated, cleared tissue; magnification  $\times 125$ .

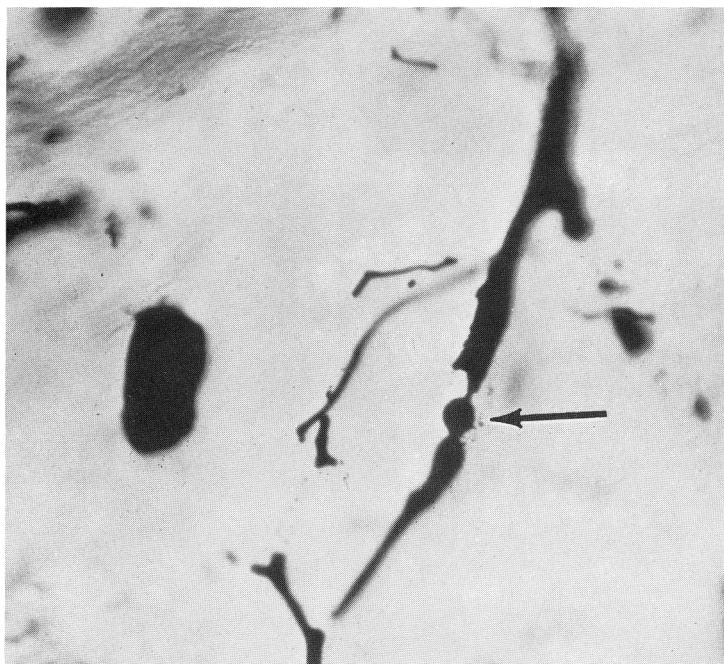


FIGURE 8. Two short zones of constriction in this arteriolar-sized vessel from a 50-day-old cardiomyopathic hamster have given rise to an expansion of the lumen resembling a microaneurysm (arrow), with dilatation of the vessel in the pre- and poststenotic segments. Transilluminated, cleared tissue; magnification  $\times 125$ .

catecholamines have been shown to increase alkaline phosphatase activity in the right atrium of the rat heart.<sup>28</sup> Finally, we noted increased acid phosphatase activity in muscular vessels of the cardiomyopathic hearts. We are uncertain of its significance, but it suggests that the vessels of cardiomyopathic hamsters differ from those of control hamsters.

Not only the focal discrete pattern of necrosis, but

also the type of necrosis seen in this model, may be consistent with a vascular pathogenesis. Contraction band and myocytolytic necrosis is thought to result from catecholamine effects on the sarcolemma, inducing overload of cellular calcium, hypercontraction of myofilaments due to calcium-dependent myofibrillar ATPase, and deposition of electron-dense calcium apatite granules within mitochondria.<sup>8, 13, 29-31</sup> Direct

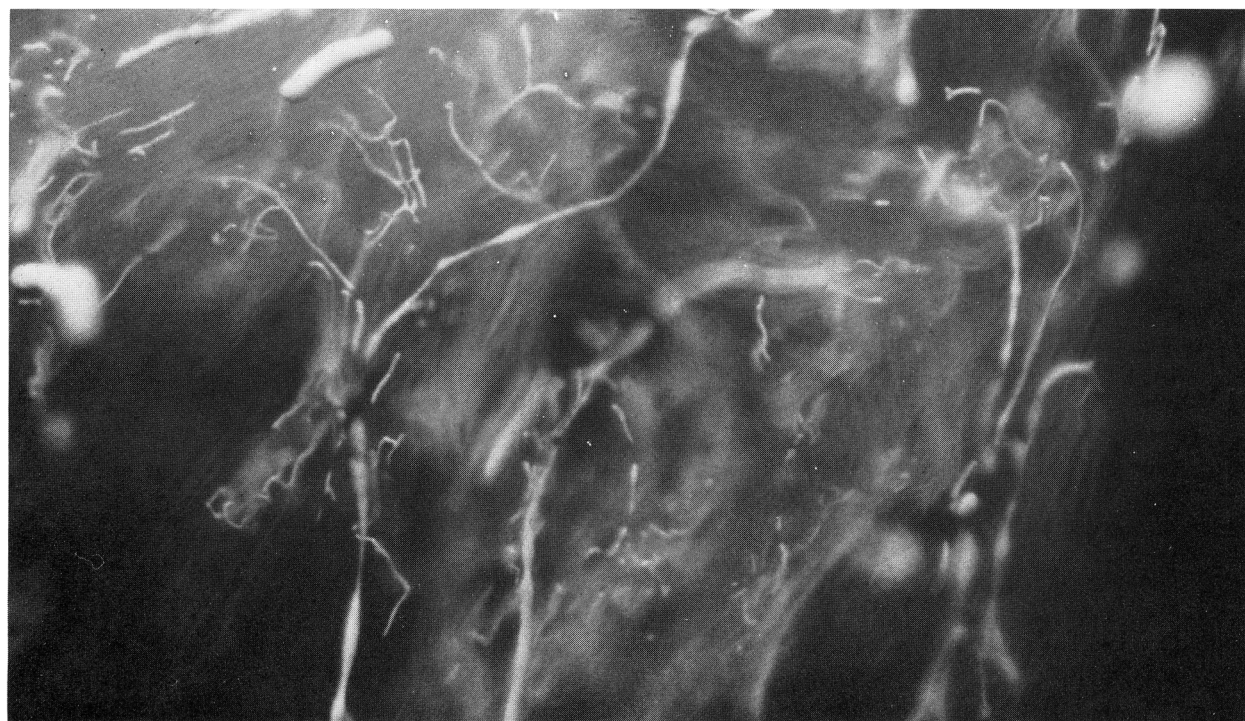


FIGURE 9. Numerous focal microvascular constrictions in a well-perfused tongue from a 44-day-old cardiomyopathic hamster. At this stage of disease, many active and healing areas of necrosis are present. Epi-illuminated, cleared tissue; magnification  $\times 125$ .



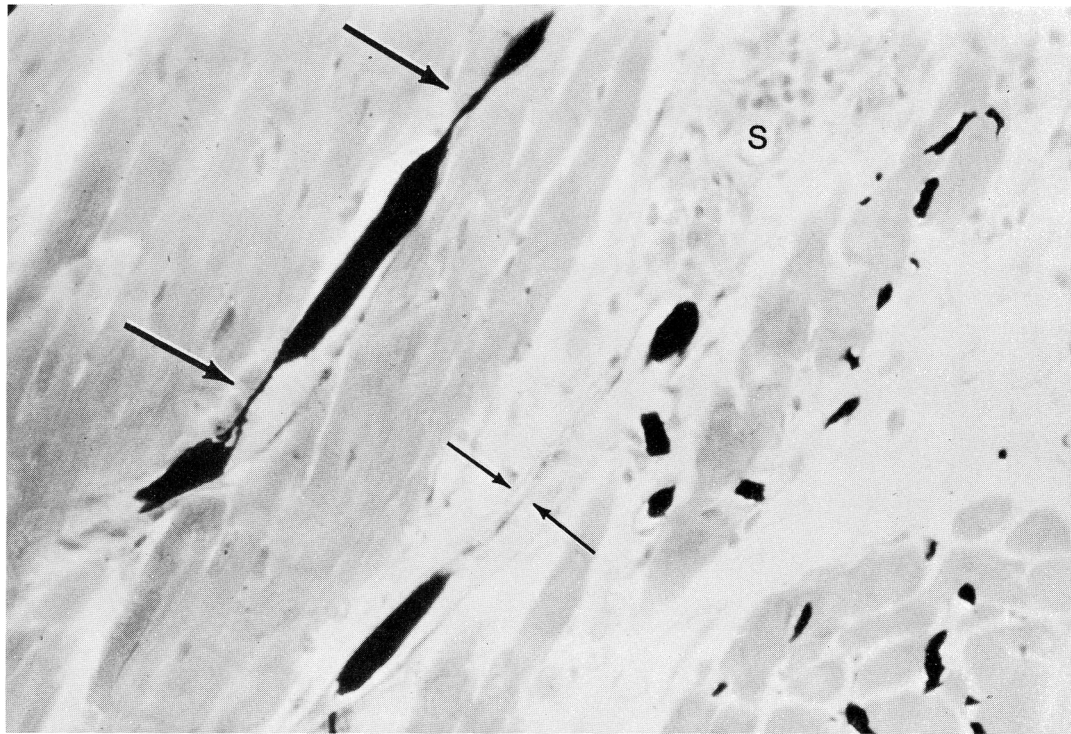


FIGURE 10. Rehydrated, paraffin-embedded, previously cleared tissue from a 150-day-old cardiomyopathic hamster. This area was selected because the cleared specimen revealed several vessels with narrowing interpreted to be spasm. The longer vessel, which is coursing through normal myocardium, has two zones of constriction (large arrows) not related to any pathologic change in the vessel wall. The shorter vessel, which is peripheral to an area of scarring (S), has a long, constricted center (thin arrows) into which no Microfil has perfused. The presence of Microfil on either side of the constriction, however, suggests that perfusion did occur at some time. Hematoxylin-eosin stain; magnification  $\times 125$ .

myocellular involvement in this form of necrosis is supported by its development in experimental treatment with the coronary vasodilating catecholamine isoproterenol,<sup>29-31</sup> as well as its occurrence with potassium depletion.<sup>32</sup> The relationship between catecholamines, calcium and necrosis is what prompted several investigators to use calcium-channel inhibitors in hamsters.<sup>13, 17, 33, 34</sup> Despite the concept that catecholamine-induced necrosis does not occur as a result of tissue ischemia, a role for the microcirculation cannot be ruled out. In fact, an identical form of necrosis can be produced in either clinical situations<sup>35</sup> or experimental models<sup>36</sup> in which coronary reperfusion follows transient coronary obstruction. Thus, catecholamines (or other vasoactive substances) may be etiologically significant in hamster cardiomyopathy, by affecting the coronary circulation directly, in addition to any alterations they may induce in individual myocardial cells to produce focal myocardial necrosis.

This latter concept leads us to believe that the lesions identified with Microfil perfusion are significant in the pathogenesis of this disease. We do not claim that the focal microvascular constrictions in any one area are the mediators of a specific region of necrosis; we cannot make such 1:1 correlations. We do think that the lesions are general markers of vascular hyperactivity in these hamsters, stimulated at the time of

sacrifice by the trauma of surgery or the perfusion of Microfil itself. That this is not an induced epiphenomenon of all microcirculations is apparent from its absence in the controls and its prevention with appropriate drug therapy in the cardiomyopathic hamsters. Because these constrictions are present in anatomically nondeformed vessels, and because they often are associated with both pre- and poststenotic dilatation of the same vessel segment, we cautiously conclude that they represent spasm identified by an *in vivo* perfusion of the coronary circulation. Transient obstruction of a small vessel and its capillaries for a period sufficient to produce ischemia may, upon relaxation of the constriction and reperfusion of the tissue, lead to typical focal myocytolytic necrosis. Verapamil may be beneficial in this model because of its coronary vasodilatory effects.<sup>37</sup>

Although the vascular constrictions revealed with Microfil perfusion resemble angiographically demonstrated large vessel spasm, one could argue that they represent unusual artifacts. We believe, however, that the only way in which a constriction of a silicone rubber luminal cast can be present between a lumen of either normal or dilated caliber is to have a fixed anatomic obstruction or a dynamic vessel closure. We did not identify structural vascular abnormalities in our routine histologic, rehydration or Epon-embedded

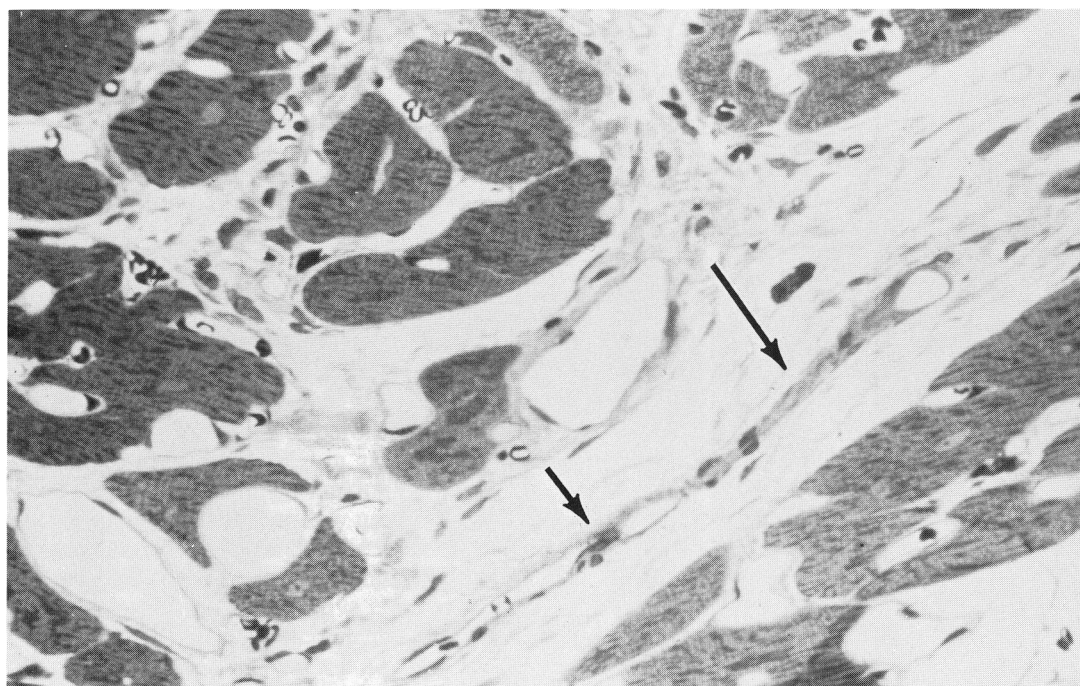


FIGURE 11. This long, thin-walled vessel, peripheral to a region of interstitial scarring, from a 150-day-old cardiomyopathic hamster shows that active vascular constriction may account for the Microfil contours seen in the previous figures. The vessel is not anatomically abnormal, but the lumen is diffusely (long arrow) and focally (short arrow) obliterated where endothelial nuclei from opposite walls abut. Serial sections demonstrated that the abnormalities were not the result of tangential sectioning. Epon-embedded, 1- $\mu$  section, alkaline toluidine blue stain; magnification  $\times 320$ .

serial section studies, nor have there been published reports of such lesions. If these lesions did exist, they would not be expected to disappear with verapamil treatment of the cardiomyopathic hamsters. These dynamic vascular obstructions have not been observed by other investigators of this model, probably because they could not examine these vessels in three dimensions. Clearly, a two-dimensional cross section of a constricted vessel would have to pass through the zone of constriction to be seen; and even then, unless serial sections were prepared, it would not be obvious whether the vessel dilated on either side of the narrowing. Only with a perfusion cast technique can this type of lesion be observed with confidence.

Although vascular hyperreactivity may provide a rational explanation for the pathologic alterations in the Syrian hamster, we do not believe that these microcirculatory lesions are limited to this model. We demonstrated very similar abnormalities in the hypertensive-diabetic rat, an experimental model of a human disease.<sup>38, 39</sup> The rats develop focal, discrete areas of fibrosis in the myocardium similar to those seen in the hamster. Perfusion of these rats with Microfil *in vivo* revealed constrictions, tortuosities and true microaneurysms. We do not know if these lesions can be prevented with calcium-blocker therapy. That such constricted lesions may also be demonstrable with postmortem perfusions can be adduced from our Microfil studies of human diabetic hearts.<sup>40</sup> In this investigation, we observed typical microaneurysms similar

to those in diabetic retinas, but in addition, we also saw focal vascular constrictions identical to those in the present study.<sup>40</sup>

Confirmation of the concept that the microvasculature may undergo closure in pathologic states was presented by Bohlen.<sup>41</sup> He showed that spontaneously hypertensive rats had a statistically significant closure of third-order arterioles compared with controls, with absent capillary perfusion for a distance of 100–200  $\mu$  around the arteriole. We also demonstrated constricted lesions with Microfil in renal hypertensive rats, used as part of a control group in our hypertensive-diabetic studies.<sup>38</sup>

If microvascular hyperreactivity is present in the Syrian hamster and the hypertensive-diabetic rat, two unrelated animal models typified by focal cell necrosis and subsequent fibrosis, could such circulatory lesions be a feature of congestive cardiomyopathy in general? Many, if not most, congestive cardiomyopathies in humans have focal myocardial fibrosis as the most significant pathologic feature.<sup>42, 43</sup> Several, such as those associated with hypertension-diabetes,<sup>44</sup> pheochromocytoma,<sup>45</sup> systemic lupus erythematosus<sup>46</sup> and progressive systemic sclerosis,<sup>47</sup> have associated myocytolytic necrosis causing patchy fibrosis. Since adult myocardial cells do not proliferate, microvascular spasm with focal myocytolytic necrosis will result in fewer ventricular myocytes. This will increase the load on the remaining myocytes leading to their compensatory hypertrophy. In late congestive cardiomyopathy, one

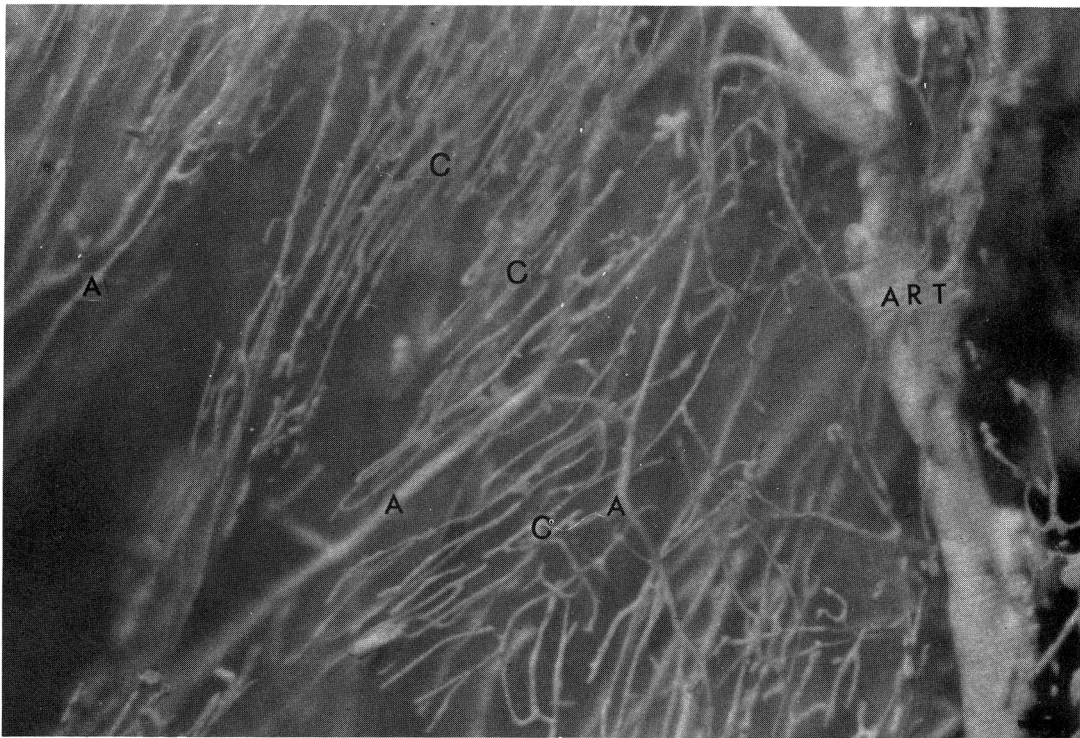


FIGURE 12. A Microfil-perfused specimen from a 44-day-old cardiomyopathic hamster pretreated for 2 weeks with verapamil. At this age in nontreated hamsters, active myocytolytic necrosis and microvascular spasm is normally evident. Note the normal smooth contours of the arterioles (A) and capillaries (C), which appear no different from vessels of control hamsters. The large artery (ART) has an irregular contour without true constriction, secondary to several branches extending above and below the sectioning plane. In addition to the restitution of the vascular lumens, verapamil treatment prevented myocardial necrosis. Epi-illuminated, cleared tissue; magnification  $\times 125$ .

would thus expect to find areas of focal necrosis and fibrosis alternating with hypertrophied myocardium; this is often the case. Ultimately, severe hypertrophy itself can lead to a failure of myocardial contractility,<sup>48, 49</sup> and these changes may well characterize the end stage of the process. What began as widespread focal necrosis secondary to microvascular spasm may terminate as hypertrophic myopathy with ventricular failure. Vascular perfusion studies will have to be performed postmortem or in experimental models to test this hypothesis. The appeal of this concept, however, based on the lessons derived from Syrian hamster cardiomyopathy, is that microcirculatory lesions may be amenable to appropriate drug therapy to ameliorate or prevent the disease.

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