Reduced Unloaded Sarcomere Shortening Velocity and a Shift to a Slower Myosin Isoform in Acute Murine Coxsackievirus Myocarditis

Burt B. Hamrell, Sally A. Huber, Kevin O. Leslie

Abstract We developed a mouse myocardial preparation to study cellular dysfunction in acute coxsackievirus myocarditis. Thin right ventricular papillary muscles from normal mice (n=8) were compared with muscles from mice 7 days after coxsackievirus infection (n=7). Sarcomere shortening was studied with laser diffraction (HeNe, λ=623.8 nm). A servomotor was used to shorten a muscle until slack early in isometric contraction. Unloaded sarcomere shortening velocity (V_s) was measured at the start of zero force at slack length. V_s was independent of the extent of slack release and was the same as that estimated with an isotonic force--sarcomere shortening velocity relation. Resting muscle stiffness was calculated from shortening perturbations in resting muscles. The histology of some papillary muscles (normal, n=4; infected, n=3) was studied. There was no ventricular hypertrophy. Resting sarcomere length (SL) in infected preparations (2.11±0.07 μm) (mean±1 SD) was the same as in normal preparations (2.11±0.08 μm). In isometric switches in normal and infected muscles, total peak force (4.31±1.07 and 3.77±1.86 g/mm², respectively) resting force (0.81±0.37 and 0.81±0.35 g/mm², respectively), and time to peak force (129.5±20.3 and 125.2±13.0 milliseconds, respectively) were not significantly different. V_s was 4.14±0.84 μm/s in normal muscles at an SL of 2.08±0.09 μm and 1.70±0.33 μm/s in infected muscles at an SL of 2.06±0.08 μm. Resting stiffness was the same for normal and infected muscles. There was inflammation but no fibrosis or necrosis. Thus, V_s was depressed early in acute viral myocarditis without hypertrophy, myocyte necrosis, fibrosis, or altered resting stiffness. Pyrophosphate gel electrophoresis showed a shift from predominantly fast to slow myosin isoforms. Apparently, there is remodeling of the contractile apparatus early in acute coxsackievirus myocarditis that is caused either by the direct effects of the virus or the immune response. (Circ Res. 1994;75:462-472.)

Key Words • sarcomeres • myocarditis • unloaded sarcomere shortening velocity • slack-release method • myosin

The clinical manifestations of acute viral myocarditis vary from that of a benign disease to a fulminant life-threatening illness, which occurs particularly in children. Ventricular enlargement may be present, and left ventricular regional wall motion abnormalities have been demonstrated with echocardiography or radionuclide imaging. Ventricular dysfunction can be severe enough to result in congestive heart failure. There is, to the best of our knowledge, no demonstrated cellular basis for ventricular dysfunction in the early stages of the disease, when muscle cell death and fibrosis are minimal or not evident. In humans and in experimental studies of myocarditis, myocardial injury usually is defined in terms of proliferation of virus in the myocardium, antibody-antigen interactions, inflammatory cell infiltration, and myocardial connective tissue proliferation. These tissue changes may not be predictive of the extent of dysfunction in viral myocarditis. This is especially true if muscle cell death is not a prominent feature of the pathology.

Recently, it was observed experimentally that ventricular function is compromised at an early stage of one type of viral myocarditis. The combined left and right ventricular ejection fraction in acute murine viral encephalomyocarditis is substantially depressed by 10 days after infection. Pigs with encephalomyocarditis exhibit left ventricular dilatation, increased right ventricular filling pressure, and reduced left ventricular fractional shortening. Ventricular dysfunction in both animal models may be linked to the marked histopathologic changes. There are substantial hypertrophy, connective tissue proliferation, and myocyte necrosis in the pig and marked inflammatory cell infiltration and myocyte necrosis in mice. It is not clear in this type of animal model how intracellular myocyte dysfunction per se contributes to ventricular dysfunction.

Since the myocardial cellular dysfunction in acute viral myocarditis is not defined, we set out to develop an isolated myocardial mechanical preparation in a mouse model of coxsackievirus myocarditis. In this model, myocardial architecture is well preserved, and muscle cell death and fibrosis are not prominent features of the pathology. Our goal was to study at the sarcomere level the discrete mechanical properties of normal heart muscle and heart muscle exhibiting myocarditis in an animal model in which immunologic and pathological mechanisms also can be assessed. We chose a mouse model because of our previous extensive immunologic and pathological studies of murine myocarditis and because of the availability of a large library of immunologic reagents for mice.
The results presented here indicate that intrinsic myocardial contractile function is abnormal in acute viral myocarditis. Unloaded sarcomere shortening velocity, measured in a novel way, was depressed below normal levels. This depression occurred in the presence of intact myocardial cells with normal alignment and striations but with evident myocardial inflammation and occasional subtle focal myocyte structural changes. Connective tissue accretion was not evident, and resting muscle compliance was normal. There was no ventricular hypertrophy; therefore, abnormal ventricular loading with increased wall stress was not a likely factor here.

Our findings were consistent with a less than normal maximum rate of crossbridge cycling in acute murine viral myocarditis that was due to either the direct effects of the virus or the resulting immune response. We found a shift to a slower type myosin isoform in coxsackievirus-infected myocardium, which was consistent with the idea that the maximum rate of crossbridge cycling was reduced below normal. Apparently, early in coxsackievirus myocarditis, when inflammation was evident but there was no ventricular hypertrophy, there was a change induced in intrinsic sarcomere function based on remodeling of the contractile apparatus.

Materials and Methods

Mouse Model

We obtained BALB/c Cum mice (H-2d) originally from Cumberland Farms, Clinton, Tenn. A breeding colony of these animals was maintained in the Central Animal Facility of the University of Vermont College of Medicine. Adult male mice 6 to 8 weeks of age were used for the present experiments.

Our original stock of CVB3 virus was obtained from Dr J.F. Woodruff. The virus stock is maintained by passage through HeLa cells. We derived a plaque-purified variant of the stock virus and designated it H3. Characterization of H3 virus has been described elsewhere. All virus stocks are free of mycoplasma. We infected the mice in the present study with an intraperitoneal injection of 104 plaque-forming units of virus suspended in 0.5 mL phosphate-buffered saline. All animals were humanely killed 7 days after infection. We studied eight normal mice and seven mice with myocarditis.

Muscle Preparation

On the day of an experiment, a mouse was given an intraperitoneal injection of pentobarbitonal sodium (100 mg/kg). When the mouse was unresponsive to deep pain, a midline incision exposed the sternum. The ribs were cut on either side of the sternum, and the sternum was retracted to expose the heart. The heart was cut free of its attachments, but the aortic arch remained attached. The aorta was intubated with a blunt 24-gauge needle, through which the coronary circulation was perfused for 3 minutes. The perfusate consisted of (mmol/L) NaCl 95.5, KCl 3.0, CaCl2 5.0, MgSO4 1.2, KH2PO4 2.0, NaHCO3 35, and glucose 11.0, to which was added 1000 U catalase and 500 U superoxide dismutase (Sigma Chemical Co). The perfusate was bubbled with a mixture of 95% O2/5% CO2 and was maintained at 27°C.

The heart was then transferred to a dissection bath and pinned to a silicon disk (Sylgard, Dow Chemical Corp). The bath was superfused with a cardioplegic solution that was identical to the coronary perfusate, except CaCl2 was 0.901 mmol/L, KCl was 20 mmol/L, and no enzymes were added. The free wall of the right ventricle was cut free at its juncture with the interventricular septum. A thin septal wall papillary muscle suitable for study was present in 60% to 70% of the hearts. Such a muscle was free of any connections except at its ends. A Teflon clip was tied with 16-0 monofilament suture onto septal wall tissue at the base of the papillary muscle. Another clip was tied onto valve tissue attached to the muscle’s chordae tendineae. The muscle preparation with clips attached was cut free from the right ventricle and transferred to a study chamber.

Experimental Setup

The study chamber was a narrow rectangular hole machined into a rectangular plastic block. A depression was machined into the block adjacent to one end of the rectangular chamber, and a capacitance force transducer was secured in the depression. A thin wall separated the study chamber from the force transducer. A length of thin-wall stainless-steel hypodermic tubing (outer diameter, 0.2 mm) was glued to the movable plate of the force transducer. The tubing passed through a narrow slit in the thin plastic wall, and the end of the tubing was bent into the shape of a hook. Each Teflon clip tied to a muscle had a hole drilled in it, and one of the clips was threaded onto the force transducer hook.

A servo-controlled galvanometer (General Scanning model G120DT) was clamped onto the plastic block. A lever arm was attached to the galvanometer axle, and a stainless-steel tubing hook was attached to the other end of the lever arm. The galvanometer was positioned so that the lever hook was aligned with the force transducer hook. The other Teflon clip was threaded onto the lever hook.

The muscle was now suspended from the force transducer to the servomotor in the chamber and immersed in a solution composed of (mmol/L) NaCl 95.5, KCl 3.0, CaCl2 5.0, MgSO4 1.2, KH2PO4 2.0, NaHCO3 35, and glucose 11.0. The solution was maintained at 27°C and bubbled with a mixture of 95% O2/5% CO2. The chamber was superfused with the solution recirculating at 40 mL/min. The floor of the study chamber consisted of a 150-μm-thick cover glass held in place with a light grease seal. There were channels machined into the plastic block alongside the study chamber and the force transducer depression. Coolant circulated through these channels to maintain study chamber solution at 25°C. The same coolant circulated through the jacket of the superfusate reservoir and of a heat exchanger, through which the superfusate flowed just before entering the study chamber. A pair of narrow rectangular platinum electrodes was bonded to the walls of the chamber. The electrodes were on either side of and parallel with the suspended muscle preparation. The resting length of the muscle preparation was set with the position control of the servomotor control system. The servomotor control system consisted of a General Scanning Control Amplifier (model CX660) plus circuitry designed and built with the assistance of the University’s Instrumentation and Model Facility.

A voltage square wave of 7-millisecond duration was applied to the platinum electrodes once every 5 seconds. The voltage was set 15% above threshold, and polarity was changed before each stimulus. The entire plastic block was affixed to a stage micrometer, and the stage micrometer was now clamped onto the stage of a Zeiss microscope. The study chamber was positioned so that the muscle could be illuminated with incandescent light through the coverslip bottom and imaged with a Zeiss water immersion objective (x40; numerical aperture [NA], 0.75). During an initial hour of equilibration, the muscle preparation was held isometric and periodically stretched slightly until peak active force was maximal or near maximal and resting sarcomere length was 2.1 μm.

Sarcomere Length Measurements

Resting sarcomere length was determined with videomicroscopy. A Dage 70 video camera (Dage-MTI, Inc) with a Newvicon tube was attached to one port of the Zeiss microscope. A ×10 focusing eyepiece (Zeiss) with a graticule was used to image the muscle in the focal plane of the video.
camera. The image was optimized with a Dage image control system and displayed on a Panasonic 12-in black and white monitor. Resting sarcomere length was determined by counting 25 to 30 striations along the length of the linear graticule scale. The graticule scale was calibrated by replacing the muscle preparation on the microscope stage with a stage micrometer.

Our sarcomere length change measurements are based on the diffraction of monochromatic light by heart muscle; previous descriptions of the measurements include data on the sensitivity of our system.5-12 As in earlier studies, we used a HeNe laser (\( \lambda = 632.8 \) nm, 5 mW, model 3235H-PC, Hughes Aircraft Co) to illuminate the muscle with monochromatic light. A front-surface mirror directed the laser beam along the optical axis of the microscope in place of incandescent light. An objective (x40; NA, 0.65; Zeiss) was used as a condenser for incandescent light and to focus the laser onto the muscle.

The diffracted light exiting from the muscle was collected with the x40 water immersion objective described in the previous section (x40; NA, 0.75; Zeiss). The image at the rear focal plane of the microscope was projected with a focusing eyepiece telescope (Leitz). A positive cylindrical lens focused the image of a first order (1°) into the meridional plane at the surface of one of two light-sensing devices. One was a photodiode array (Reticon RL512 C/17), which images the spatial density of a diffraction pattern.9 The other was a position-sensing diode (United Detector Technology, PIN-LSC/30D).9 Movement of a 1° along the position-sensing diode surface produced a current proportional to 1° movement but independent of its intensity. We measured movement of a 1° with this diode and referred the measurement to resting sarcomere length to compute sarcomere length change. We took care to eliminate influence of the skirt of the zeroth order (0°) on measured 1° movement in a way we have previously described.11,12

We use the spatial density to assess the quality of a diffraction pattern. It is essential that the spatial density of a 1° remain discrete as it moves away from the 0°. We have published criteria for adequate separation of a 1° from the 0° band.10 It is also important that sarcomere length change be uniform along the length of a preparation during muscle contraction. In this current set of experiments, a 1° did remain discrete during contraction, and 1° movement was uniform along the length of a mouse papillary muscle (Fig 1).

Cardiac muscle cells interconnect in a branching array. There is a variable and probably random distribution of the amount of tilt of the myofilament lattice relative to an incident laser beam. Also, the myofibrils within each muscle cell may be tilted.12 Tilt of the myofilament lattice relative to the laser beam will result in Bragg reflection.13 Bragg reflection results in asymmetry of the spatial density of diffraction maxima.13 We evaluated this potentially confounding phenomenon in our preparations by measuring the movement of both 1° maxima during a contraction (Fig 2). The measurement of sarcomere shortening relative to force and time during an isometric twitch was not significantly influenced by Bragg reflection in our preparations.

**Active and Passive Force**

An isometric twitch was recorded at the end of the equilibration period at the start of an experiment. The muscle was then subjected to a ramp decrease in resting muscle length just before a twitch. The shorter resting length was briefly held constant to record the lower resting force. A series of these ramps was done to record a range of resting muscle lengths and forces. The muscle was considered to have reached resting slack length when an increase in the amount of ramp shortening did not further decrease resting force. Resting muscle elasticity was analyzed by relating resting muscle lengths to resting force levels.

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**Unloaded Sarcomere Shortening Velocity**

Unloaded sarcomere shortening velocity was measured by rapidly reducing muscle length to slack length early in an isometric twitch (Fig 3). We determined when a muscle first became slack by gradually increasing the amount of muscle length reduction. When the force level at the conclusion of an imposed muscle length decrease was unchanged from a previous lesser muscle length decrease, the muscle was slack. A slack release was associated with a brief period of steady output from the force transducer (Fig 3). In subsequent contractions, muscle length was reduced a small amount more than was needed for a muscle to become just slack. Duplicate measurements of unloaded sarcomere shortening velocity were made with each of three or four extents of slack release.

The linear regression of sarcomere length decrease versus time at the onset of zero force at slack length was computed with the method of least squares. Ten to 20 digitized values for decreasing sarcomere length during the initial 3 to 7 milliseconds of zero force (Fig 3) were used for the calculations. The sarcomere length at which unloaded sarcomere shortening velocity was measured was the average of the digitized values used in the linear regression computation.

Unloaded sarcomere shortening velocity also was estimated from the force–sarcomere shortening velocity relation. Iso- tonic contractions were obtained at the same time in the twitch as slack-length releases. The slope of a ramp change in muscle length early in an isometric twitch was adjusted to hold force...
sarcomere shortening was measured (Fig 4) and, with isotonic force, was used to plot the force–sarcomere shortening velocity relation (Fig 5).

**Gel Electrophoresis**

Pyrophosphate gel electrophoresis was modified from that of Hoh et al. The major blood vessels and atria were removed from the ventricles. Four normal and four infected ventricles were weighed and either then prepared for electrophoresis or stored at −70°C for later analysis. Frozen ventricles being prepared for electrophoresis were allowed to thaw on a chilled watch glass. The ventricles and all glassware were kept constantly chilled. The chilled ventricles were minced with a fine scissors and scalpel. The minced tissue was washed into a glass homogenizer with chilled extraction solution, at 16 μL to 1 mg tissue. The extraction solution consisted of (mmol/L) Na₃P₄O₁₀, 100, EGTA 5, β-mercaptoethanol 5, and dichlorovos 2.6. The tissue was gently homogenized by hand, and the homogenate was then centrifuged at 1900g for 20 minutes at 4°C. The supernatant was mixed with an equal volume of ice-cold 100% glycerol and stored at −70°C.

Electrophoresis was carried out in an electrophoresis chamber (model GE-24, Pharmacia Biotech Inc), which circulates buffer between the upper and lower chambers. The chamber buffer consisted of 20 mmol/L Na₃P₄O₁₀, 3.5 mmol/L cysteine, and 10% glycerol at pH 8.8. We used gels of 4.4% T and 2.9% C. Each gel was loaded with 4 μL of supernatant, and the electrophoresis was performed at 14 V/cm for 16.5 hours at 2°C. The gels were then stained overnight in a solution consisting of 0.025% Coomassie brilliant blue R-250, 25%
isopropranol, and 10% acetic acid. This was followed by destaining in 10% acetic acid.

**Histology**

Representative normal papillary muscles (n=4) and papillary muscles exhibiting myocarditis (n=3) were fixed and prepared for detailed histological study. At the end of a mechanical experiment, a muscle was maintained at initial resting muscle length as the chamber superperfusate was replaced with Karnovsky’s fixative, which consisted of 2.5% glutaraldehyde and 1% formaldehyde in 0.1 mol/L Millonig’s phosphate buffer, pH 7.2. Muscle length was maintained constant over the 1-hour period of fixation. Sarcomere length was determined at the end of the hour. The muscle was then removed from the chamber and placed in Millonig’s phosphate buffer and refrigerated until processed for histological study the next day.

Each papillary muscle was cut into ~1-mm³ pieces. The pieces were washed three times, 10 minutes for each wash, in Millonig’s buffer. Next they were fixed in 1% osmium tetroxide in 0.15 mol/L Millonig’s buffer for 45 minutes at 4°C, followed by dehydration in graded ethanol before embedment in Spurr’s resin. Thin sections were cut with a diamond knife and mounted on glass microscope slides. Sections on slides were stained with toluidine blue. They were examined using a light microscope, and representative images were photographed.

**Data Collection and Analysis**

Experimental data were digitized during collection with EASIEST LX software (Asyst Technologies, Keithly Instruments). The data were digitized with an analog-to-digital card (DT2801, Data Translation) in a personal computer. Isometric twitch data were collected with a sampling rate of 300 Hz. Twitches with slack releases or isovelocity ramps were sampled at 3000 and 2000 Hz, respectively. The data were saved in files suitable to be imported into a spreadsheet program (QUATTROPRO, Borland Corp) for detailed analyses. Graphics were prepared with plotting software (SIGMAPLOT, Jandel Scientific).

We used Student’s unpaired t test to compare normal body weights and ventricular weights, isometric twitch data, and levels of unloaded shortening velocity with values from coxackievirus-infected mice and their hearts. ANOVA and Student’s t test for linear regression were used to compare linear regressions derived from the resting muscle length and force data. A value of P<.05 indicates significance for values obtained from the same experimental group.

**Results**

**Animal Model**

The normal mice (n=8) weighed 22.6±3.9 g, and the mice with myocarditis (n=7) weighed 19.5±2.4 g (mean±1 SD unless otherwise stated). The weight of the right ventricular free wall was 19.7±3.3 mg in the normal mice compared with 17.0±3.0 mg in the mice with myocarditis. Right ventricular free wall weight was 21.8±1.5% of total ventricular weight in the normal tissue and 22.8±1.7% in infected tissue. When right ventricular free wall weight in milligrams was divided by body weight in grams, it was 0.854±0.079 in the normal hearts and 0.910±0.084 in hearts from mice with myocarditis. In each of the above comparisons, there was no significant difference (P>.05).

**Muscle Preparations**

The undamaged length of the papillary muscles was 0.92±0.24 mm for normal mice and 1.09±0.40 mm for mice with myocarditis. The cross-sectional area was 0.039±0.009 mm² in normal muscles and 0.043±0.014 mm² in the muscles from mice with myocarditis. Cross-sectional area was elliptical in both types of muscles. The greater diameter of the muscles was 0.31±0.05 mm for normal mice and 0.31±0.08 mm for mice with myocarditis. The lesser diameters were 0.16±0.03 and 0.18±0.04 mm, respectively. There were no significant differences when the data from normal mice were compared with data from mice with myocarditis.

**Unloaded Sarcomere Shortening Velocity**

The sarcomere length at which unloaded sarcomere shortening velocity was measured with slack releases in
the normal muscles (2.08±0.09 μm) was not significantly different from that in the muscles from mice with myocarditis (2.06±0.08 μm). Unloaded sarcomere shortening velocity was 4.14±0.84 μm/s in the normal muscles and 1.70±0.33 μm/s in muscles from mice with myocarditis (P<0.01).

Unloaded sarcomere shortening velocity also was estimated by fitting force–sarcomere shortening velocity data with a rectangular hyperbola (Fig 5). Unloaded sarcomere shortening velocity estimated from the force–sarcomere shortening velocity data in a representative normal muscle was 5.75 μm/s, and it was 5.6±0.79 μm/s measured with a set of three slack releases, with duplicates for each extent of slack release. The hyperbolic fit of the force-velocity data was done as we have previously reported.17

The unloaded velocity of sarcomere shortening should be independent of the amount of slack release.18 In the representative examples in Fig 6, muscle length was rapidly reduced until the muscles were just slack. Muscle length was rapidly reduced to lengths shorter

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Fig 6. Graph showing relation of unloaded sarcomere shortening velocity with relative slack length in a normal muscle (○) and in a muscle from a mouse with myocarditis (□). Relative slack length was the shortened muscle length divided by the length at which the muscle first became slack. In the muscle from mice with myocarditis, sarcomere shortening velocity after a release to just slack length (relative slack length of 1.00) is presented along with velocity measurements from slack releases to muscle lengths 1.0% (relative slack length of 0.990), 2% (0.980), and 2.5% (0.975) less than just slack. In the normal muscle, the values less than slack length are 0.8% and 1.9%. There is no significant regression of unloaded sarcomere shortening velocity on relative slack length in either preparation.

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than just slack in subsequent twitches. In both muscles shown in Fig 6 and in the other muscles studied here (Fig 3, for instance), unloaded sarcomere shortening velocity was independent of the extent of slack length release (Fig 6). Extents of slack release larger than in Fig 6 were not used because they resulted in lateral shift of a muscle and spurious effects on the diffraction pattern.

Resting Muscle Stiffness

The tangent slope of resting muscle force relative to resting muscle length at each force level is a measure of resting muscle stiffness.19 Tangent slopes could differ for muscles from mice with myocarditis even if resting muscle force level at a comparable resting sarcomere length was the same as the level in normal muscles (Table). Of particular importance is the fact that resting muscle stiffness may influence sarcomere shortening.11,12 Stiffness was measured for that reason.

The force level at each of the resting muscle lengths reached with ramp shortening was divided by unperturbed initial resting force to calculate stress. Strain was calculated by subtracting muscle length change during each ramp from the muscle length change to reach zero force. Each resulting value was divided by muscle length at zero force to calculate strain. The relation of stress/strain with stress was linear in individual muscles (Fig 7), as has been reported by others.19 The average of the slopes and constants of linear regression was, respectively, 10.29 and 3.25 for normal muscles and 9.85 and 3.35 for muscles from mice with myocarditis (Fig 8). There was much overlap of data and no significant difference among the two types of muscles (Fig 8).

Isometric Data

Resting sarcomere length was set at a comparable length in the two groups of preparations (Table). Resting force at the above resting sarcomere length was the same as normal in myocarditis (Table). Sarcomere shortening and lengthening occurred during isometric contraction (Fig 9) because of the presence of series-compliant elements in the damaged muscle tissue cut free from the ventricular wall. Peak total force and
sarcoplasma length at the peak of the twitch were the same as normal values in muscles from mice with myocarditis (Table). Likewise, the time from the onset of isometric force to peak force was the same in the two types of preparations (Table). It is clear from the above that the isometric twitch in the muscles from the hearts of mice with myocarditis was normal.

Protein Electrophoresis

The densest band had the greater electrophoretic mobility in normal ventricular myocardium (Fig 10). This electrophoretic pattern with pyrophosphate gel electrophoresis was consistent with predominant V1 isomyosin consisting of α-myosin heavy chains in the normal myocardium. The electrophoretic mobility pattern was reversed in ventricular myocardium from coxsackievirus-infected mice. The densest band had the lesser electrophoretic mobility (Fig 10) and was consistent with an isomyosin consisting of β-myosin heavy chains in myocarditis. The predominant isomyosin species shifted from V1 in normal muscle to V3 in muscle from mice with myocarditis.

Histology

In the normal papillary muscles and papillary muscles from mice with myocarditis, the muscle fibers were approximately parallel and cross striations were evident (Fig 11). The most prominent feature of the muscles from mice with myocarditis was the presence of mononuclear inflammatory cells, particularly surrounding blood vessels (Fig 11B and 11D). In some sections, there was the occasional myocyte with subtle changes, such as a questionably swollen nucleus, that may indicate early myocyte deterioration. Of importance for the present experiments was that the preparations from mice with myocarditis had generally normal-appearing myocytes and that the presence of mononuclear inflammatory cells (Fig 11B and 11D) was the major histopathologic finding.

Discussion

Our present findings indicated that there was a profound change in sarcoplasmic function in acute coxsackievirus myocarditis despite minimal observable pathological changes (Fig 11). Also, there was no ventricular hypertrophy. Apparently, acute viral myocarditis was associated with an alteration in myocardial function not due to muscle cell death, myocardial fibrosis, or hypertrophy. The
reduction in unloaded sarcomere shortening velocity reported in the present study (Figs 5 and 6) can occur on the basis of a shift to a slower myosin isoform.\textsuperscript{20} The results in this animal model demonstrated that early in the natural history of viral myocarditis, there were fundamental changes in sarcomere function. It may be that in acute viral myocarditis in humans, changes in the contractile apparatus play a role in early morbidity and mortality and in the subsequent development of end-stage cardiomyopathy.\textsuperscript{21}

Unloaded sarcomere shortening velocity is a reflection of the intrinsic rate of crossbridge cycling.\textsuperscript{2,20} The speed of sarcomere shortening in the absence of force development is limited by the speed with which a crossbridge can change its angle of attachment to an active site and then fully detach.\textsuperscript{22} The immediate energy source for this mechanical sequence is thought to be the hydrolysis of ATP by myosin.\textsuperscript{23} In skeletal\textsuperscript{24} and cardiac\textsuperscript{2,20} muscle, the ATPase activity of myosin is correlated with unloaded shortening velocity.

Adult rat cardiac muscle is known to have three isoforms of myosin, V\textsubscript{1}, V\textsubscript{2}, and V\textsubscript{3}, as demonstrated with gel electrophoresis.\textsuperscript{15,25} Rabbit cardiac muscle also can manifest the three isoforms, although the V\textsubscript{2} isoform appears to be less prominent in the rabbit than in the rat.\textsuperscript{26,27} Adult mouse myocardium consists almost exclusively of the V\textsubscript{1} isoform when analyzed with polyacrylamide gel electrophoresis.\textsuperscript{25} We noted a predominance of the V\textsubscript{1} isoform in normal mouse myocardium with a definite but faint band with slower mobility representing the V\textsubscript{2} isoform when analyzed with pyrophosphate gel electrophoresis (Fig 10). Myosin ATPase activity increases as a function of the relative amount of V\textsubscript{1} isoform present in the myocardium.\textsuperscript{26} In rat and rabbit myocardium, unloaded shortening velocity is increased relative to the extent to which the V\textsubscript{1} myosin predominates among the three isoforms.\textsuperscript{26,27} The shift from predominantly V\textsubscript{1} isoform in normal myocardium to predominantly V\textsubscript{1} isoform in myocardium from mice with myocarditis was consistent with the reduction in unloaded sarcomere shortening velocity below normal in preparations from infected mice.

Strains of mice that develop myocarditis after infection with coxsackievirus also develop serum autoantibodies against myosin.\textsuperscript{28} Specific portions of the \(\alpha\)-myosin heavy chain are immunogenic and can induce murine myocarditis as defined by histopathologic criteria.\textsuperscript{29} Studies in animals\textsuperscript{30} and humans\textsuperscript{31} suggest that heart tissue autoantibodies can cause injury to myocytes. Autoimmune injury may be a mechanism for altered myosin in acute myocarditis. Similar phenomena occur in skeletal muscle inflammatory disorders. There

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**Fig 11.** Longitudinal sections of a representative normal and a coxsackie virus-infected papillary muscle. A and B, Photomicrographs show a normal and an infected papillary muscle, respectively, at \(\times 50\) original magnification. Note the distinct cross striations and parallel array of the myocytes. C and D, Photomicrographs show the normal and the infected papillary muscle, respectively, at \(\times 100\) original magnification. Again, the orderly parallel arrangement of muscle fibers with clearly visible cross striations is evident in both. In the myocarditic papillary muscle sections in panels B and D, a blood vessel is surrounded by mononuclear cells (arrows). Connective tissue in the myocarditic muscle sections in panels B and D is not more prominent than in the normal sections in panels A and C (toluidine blue stain).
is an inflammatory disorder of canine masticatory muscles associated with circulating autoantibodies specific for myosin heavy chain and a light chain of a specific muscle fiber type.\textsuperscript{32} In this masticatory muscle disease, there is substantial inflammation and necrosis.\textsuperscript{32}

There can be internal loads during shortening, even with a muscle seemingly unloaded.\textsuperscript{33} For instance, some hindering crossbridges that do not promptly detach during sarcomere shortening could produce an internal load that reduces measured \( V_o \). Under these circumstances, an increase in myofilament lattice calcium would increase the number of normally cycling crossbridges relative to the number of hindering crossbridges and increase measured \( V_o \). Unloaded sarcomere shortening velocity of heart muscle does increase with an increase in calcium concentration up to 1.2 mmol/L Ca\textsuperscript{2+}, but \( V_o \) is constant at higher calcium concentrations.\textsuperscript{34} At sarcomere lengths \(<1.85 \mu m\), deactivation or geometrical constraints can result in reduced measured \( V_o \), but at 2.5 mmol/L Ca\textsuperscript{2+}, \( V_o \) is independent of sarcomere length from 1.85 to 2.3 \( \mu m \).\textsuperscript{35} The balance of resting elasticity and restoring forces in isolated, quiescent, but functional myocytes results in an average resting sarcomere length of 1.93 \( \mu m \).\textsuperscript{35} Here, we measured \( V_o \) at a calcium concentration and sarcomere length at which hindering crossbridges and restoring forces were not a likely problem. In our experiments, calcium concentration was 5.0 mmol/L, and average sarcomere length was 2.06 and 2.08 \( \mu m \) in normal muscles and muscles from mice with myocarditis, respectively.

Another type of internal load can originate external to the sarcomeres. For example, myocardium that has hypertrophied because of pressure overload has increased resting stiffness when compared with normal myocardium.\textsuperscript{56} A shift of load from shortening parallel elastic elements, which support resting force, onto shortening sarcomeres can slow and reduce sarcomere shortening differently in hypertrophied myocardium than in normal heart muscle.\textsuperscript{11,12} In the preparations from the mice with acute coxsackievirus myocarditis, there was no hypertrophy, resting force (Table) and the resting stress/strain relation (Figs 7 and 8) were the same as in normal preparations, and there was no evidence of increased connective tissue (Fig 10). From the above, we concluded that internal loads did not likely influence the present results.

Slack-length releases have been used before by others to measure unloaded shortening velocity.\textsuperscript{38} Our use of slack releases differs from these earlier experiments in that we measured unloaded sarcomere shortening velocity directly. Other investigators have noted that there is a linear relation of the extent of slack release with the amount of time for a muscle to take up the slack. The slope of the relation is unloaded shortening velocity.\textsuperscript{18} The linear relation indicates that unloaded shortening velocity is independent of the extent of slack release.\textsuperscript{18} Likewise, in our experiments, unloaded sarcomere shortening velocity was independent of the extent of slack release (Fig 6).

The absolute level of normal unloaded sarcomere shortening velocity that we measured in mice was less than reported in rat right ventricular trabeculae\textsuperscript{37} but similar to lightly loaded shortening velocity in cat ventricular myocytes.\textsuperscript{38} It is difficult to compare absolute levels with experiments done in another species and under conditions not identical with ours. There was internal consistency in the measurement of unloaded sarcomere shortening velocity in our experiments. The level of unloaded sarcomere shortening velocity measured with the slack-release technique was the same as that estimated from the force-velocity relation in the same muscle (Fig 5).

ATP metabolism in heart muscle cells may be altered in certain types of myocarditis.\textsuperscript{39-41} In toxic-allergic myocarditis in rabbits, there is a decreased rate and amount of ATP hydrolysis enthalpy in myofibrillar preparations.\textsuperscript{41} A decreased rate of ATP hydrolysis is consistent with slower cycling of crossbridges and reduced unloaded sarcomere shortening velocity,\textsuperscript{20} but toxic-allergic myocarditis in rabbits is an animal model quite different from ours. In guinea pigs immunized with beef heart mitochondrial ATP/ADP carrier, antibodies bind to mitochondrial structures.\textsuperscript{30} The ratio of ATP to ADP concentrations in mitochondria increases and decreases in the cytosole.\textsuperscript{39} Cytosolic ATP concentrations did not significantly fall in the guinea pig left ventricles, and submaximal work in the ventricles remained normal, albeit with a trend toward greater lactate production.\textsuperscript{39} Again, this animal model is quite different from our murine model. Coxsackievirus B3 infection in mice may increase membrane energy usage and reduce cytosolic ATP levels as a result.\textsuperscript{40} Unloaded sarcomere shortening velocity will decrease with enough of a decrease in myofilament lattice ATP concentration,\textsuperscript{23} but we are not aware of evidence that cytosolic ATP levels decrease to such an extent in acute viral murine myocarditis.

The isometric twitches of papillary muscles from mice with myocarditis were indistinguishable from normal muscles (Table). The level of isometric force per unit cross-sectional area at a particular sarcomere length reflects the number of sarcomeres acting in parallel during contraction. The fact that peak force levels were not significantly affected by myocarditis in these mice was consistent with the absence of muscle cell deterioration or muscle cell death and replacement with connective tissue (Fig 11).

In pressure-overload hypertrophy, there is a consistent increase in the duration of the first portion of isometric contraction, from onset to peak.\textsuperscript{42,43} Time to peak isometric force is a parameter that is a composite reflection of, among other things, the time course of movement of Ca\textsuperscript{2+} from locations in the cytosole into the interstices of the myofilament lattice and activation of the interaction of myosin with actin. In this regard, the prolonged time to peak force in pressure-overload hypertrophy occurs with and is likely related to changes in sarcolemmal, sarcoplasmic reticulum, and mitochondrial function.\textsuperscript{43} In the present experiments, the time from isometric twitch onset to peak force was the same as normal in myocarditis (Table). Apparently, there was no overall change in activation in acute viral myocarditis. To the best of our knowledge, there are no reports of measurements of myocyte internal membrane or sarcolemmal function in myocarditis. Arrhythmias in acute viral myocarditis may be due to involvement of the conduction system and focal myocyte necrosis. There is not yet, to our knowledge, evidence of discrete electro-
physiological changes in intact myocytes due to viral myocarditis.

In summary, we interpret our results to mean that there is a substantial depression in unloaded sarcomere shortening velocity early in the natural history of acute murine coxsackievirus myocarditis. Unloaded sarcomere shortening was depressed in the absence of ventricular hypertrophy, muscle cell structural changes, or increased connective tissue. This is, as far as we have been able to determine, the first such demonstration of depressed intrinsic myocardial function and of contractile apparatus remodeling in myocarditis.

It is important to note that these studies were made possible by developing a new mechanical preparation in a well-established murine model of viral myocarditis. We also developed a novel way of measuring unloaded sarcomere shortening velocity. With this model, we will be able to explore further the mechanisms of an apparent change in the contractile apparatus in this disease. An important area of interest for us is whether the virus in the myocardium directly alters myocytes or whether an associated T-cell–mediated immune reaction alters myocyte function.

Acknowledgments

This study was supported in part by the American Heart Association (AHA); the AHA, Vermont Affiliate, Inc; basic research support grants from the College of Medicine; and National Institutes of Health grants HL–31260, HL–28833, and PHS P01-28001. The authors appreciate the excellent technical assistance of Susan Porter and Elaine Mohrbach. We wish to thank Dr Nancy Drucker for her thoughtful input and encouragement at all stages of this work. Dr Miles Hacker gave generously of his time to advise us in the use of the free oxygen radical scavenging system in the aortic perfusate, and we are most grateful to him. The skills of the University's Instrumentation and Model Facility staff contributed significantly to the success of these experiments. The authors wish to thank the referees for their constructive suggestions and for their supportive comments.

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Reduced unloaded sarcomere shortening velocity and a shift to a slower myosin isoform in acute murine coxsackievirus myocarditis.

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Circ Res. 1994;75:462-472
doi: 10.1161/01.RES.75.3.462

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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