Contractile Properties of Glycerol-Extracted Muscle Bundles from the Chronically Failing Canine Heart

By Ellis S. Benson, M.D., Ben E. Hallaway, B.S. and Charles E. Turbak, M.D.

Chronic congestive heart failure was induced in dogs by the surgical production of tricuspid insufficiency and pulmonary stenosis. Glycerol-extracted trabecular bundles from the right and left ventricles of these dogs developed significantly less tension than did similar preparations from the hearts of normal dogs. The maximum working capacity of the bundles from failing hearts was lower than that of bundles from normal hearts, but the rate of hydrolysis of adenosinetriphosphate was the same as that of normal heart preparations. Since glycerol-extracted muscle bundles retain the basic contractile properties of fresh, surviving whole muscle but are free from membrane, neurohormonal, ionic and pH effects and are isolated from energy-supplying systems of muscle, defective contractility and decreased working capacity of muscle bundles from failing hearts may be appropriately ascribed to physiochemical changes in the contractile protein, actomyosin. Such alterations are undoubtedly of structural significance, and are related to the conformation changes in actomyosin which characterize the contractile cycle of muscle.

ACTOMYOSIN extracted from the myocardium of dogs in chronic heart failure exhibited a quantitatively altered viscosity response to the addition of adenosinetriphosphate (ATP) from that of myocardial actomyosin of normal dogs. This finding suggests an altered state of the contractile protein of the myocardium in chronic failure. In an attempt to evaluate this possibility further, a study was made of the contractile properties and the adenosinetriphosphatase activity of glycerol-extracted trabecular muscle bundles from the hearts of normal dogs and dogs with chronic heart failure.

The glycerol-extracted muscle preparation was first used by Szent-Györgyi, who demonstrated that this preparation, while not excitable by electric stimulation, developed tension in the presence of ATP and that the tension developed was of the same approximate magnitude as that developed by fresh muscle fibers in contraction.

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MATERIALS AND METHODS

Eighteen adult mongrel dogs which appeared to be in good health were included in this study. Nine of these animals served as controls. Nine dogs had pulmonary stenosis and tricuspid insufficiency produced surgically by methods described by Baronofsky and co-workers. These dogs showed the following signs of congestive heart failure: (a) peripheral edema and ascites (ascitic fluid ranged from 20 to 450 ml./Kg. body weight); (b) mean right atrial pressures ranging from 10 to 20 mm. Hg; (c) end-diastolic right ventricular pressures ranging from 10 to 15 mm. Hg; (d) engorged and dilated superior and inferior venae cavae; (e) enlarged livers with histologic evidence of acute and chronic passive congestion; (f) dilated right atria and right ventricles and increased heart weight: body weight ratios. The heart weight: body weight ratios in the experimental animals was 8.9±1.2 Gm./Kg. body weight and in the control animals 7.3±1.1. The experimental animals were sacrificed between 5 and 24 months after production of the valvular lesions.

Preparation of the experimental material was based essentially on the method described by Szent-Györgyi. Control and experimental dogs were killed in approximately alternate order by anesthetizing them with pentobarbital and quickly excising the beating heart. The heart was immediately opened and the chambers washed free of blood with cold tap water. Strips of trabecular muscle were excised from the lateral walls of the right and left ventricles and immediately placed in an ice-cold solution of 50 per cent glycerol in water. The strips were then quickly tied on glass rods in an extended position without stretching.
CONTRACTILE PROPERTIES OF THE FAILING HEART

They were stored in 50 per cent glycerol at 0 C. for 72 hours then transferred to fresh 50 per cent glycerol and stored at -20 C. for at least 2 weeks before use. (No change in the contractile properties of the fiber bundles was noted in preparations stored at -20 C. for at least 6 months.)

Before use, the extracted bundles were placed in 15 per cent glycerol in water at 4 C. for 1 hour. The endocardium was carefully dissected free and beneath it lay linear fiber tracts which were gently separated into small bundles 0.1 to 0.2 mm. in diameter and 8 to 20 mm. in length (fig. 1). This was done by grasping fiber bundles at one end using fine jewelers forceps and gently pulling them away from adjacent bundles. The ends of the fiber bundle tested were attached to thin aluminum plates with de Khotinsky cement and placed in the muscle chamber of an electronic isometric myograph, modified from that described by Ramsey (fig. 2). The myograph was calibrated before each use with milligram weights. The muscle chamber contained a bathing solution of 0.05 M KCl, 0.005 M MgCl₂, and 0.0008 M CaCl₂ buffered at pH 8.2 with tris(hydroxymethyl)aminomethane. The ionic strength of this solution was 0.115 M. Temperature of the muscle bath was maintained at 25±0.2 C.

Slackness of the mounted fiber bundle was taken up by adjustment of its length until tension was first indicated by the transducer circuit. This length was measured and is defined as the equilibrium length. ATP, disodium salt, pH 7, was then added to give a bath concentration of 0.0046 M. Tension developed immediately and became maximum in 1 to 3 min. When measurements were complete, the bundle was cut free at its terminal attachments and placed in 1 ml. of 0.1 N NaOH. In 24 hours, protein concentration was determined by measurement of ultraviolet light absorption at 280 μ. in a Beckman model DU spectrophotometer.

Samples of ventricular myocardium of a normal dog were divided into two portions, on one of which the protein concentration per unit of mus-
icle volume was estimated by micro-Kjeldahl analysis and the other portion was extracted and analyzed in the same manner as the fiber bundles on which tension measurements were made. From these measurements and measurement of equilibrium length the cross-sectional area of the fresh fiber was computed (assuming uniform water content of all fibers tested).

In assessing the effect on tension of changes in length of the fiber bundles the following procedure was carried out. After stable tension was obtained in the presence of ATP, the length of the bundle was changed and the tension allowed to restabilize. In this way, measurements of tension developed by fiber bundles below and beyond equilibrium length were obtained for each of the ventricles included in the study. In each case, tension developed by stretching uncontracted bundles was also measured.

Isotonic shortening was assessed in the following manner: fiber bundles were placed in a Petri dish containing the bathing solution described above and their length estimated to the nearest 0.1 mm. under a dissecting microscope (X 10). ATP was then added to give a bath concentration of 0.0046 M. Length of the bundle was measured at 1 min. intervals until maximum shortening had taken place (usually in 4 to 6 min. after the addition of ATP). Temperature was maintained at 25 ± 0.2°C.

Studies of adenosinetriphosphatase activity were carried out on glycerol-extracted trabecular bundles prepared in the same manner as those used in the measurements of contractility. Finely minced 0.4 to 0.5 Gm. samples of extracted muscle were homogenized for 3 min. in 6 ml. of KCl borate buffer (0.025 M KCl and 0.039 M Na borate, pH 7.1) using a tissue grinder of the Potter-Elvehjem type with Teflon pestle. Three milliliters of this homogenate were used for the preparation of myofibril fractions according to the method of Perry and Grey, following each stage of fractionation microscopically. The particulate fraction of the remaining 3 ml. was sedimented at 15,000 g, washed once in KCl borate buffer, and resuspended in this buffer to give a final tissue dilution of 1:80.

Adenosinetriphosphatase activity of the whole particulate and myofibrillar fractions was assessed as the amount of inorganic phosphorus liberated during incubation for 5 min. at 25°C. One milliliter of suspension containing 0.60 to 0.90 or 0.08 to 0.20 mg. protein/ml of particulate fraction or myofibrillar fraction respectively was mixed with 1 ml. of pH 8.2 buffer (of same composition as used in bath solution in determinations of tension). The reaction was initiated by the addition of sufficient ATP to give a concentration in the reaction mixture of 0.005 M. The particulate frac-

- **FIG. 3.** Top. Isometric tension developed by fiber bundles from right ventricles on the addition of ATP, expressed as a function of P/L, where P is protein content of bundles in mg. and L is equilibrium length in mm.: ○ normal myocardial fiber bundles; • failing myocardial fiber bundles.

- **FIG. 4.** Bottom. Isometric tension developed by fiber bundles from left ventricles on the addition of ATP, expressed as a function of P/L, where P is protein content of bundles in mg. and L is equilibrium length in mm.: ○ normal myocardial fiber bundles; • failing myocardial fiber bundles.

Results of measurement of isometric ten-
TABLE 1.—Isometric Contractile Tension* of Trabecular Fiber Bundles at Equilibrium Length

<table>
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<th>L</th>
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N 3.31 3.56 2.00 2.35
σ 0.53 0.31 0.21 0.21
p 0.001 0.001

* In Gm. mm.⁻¹ cross-sectional area fresh fiber, in presence of 0.0046 M ATP.

Tension in the presence of ATP are illustrated in figures 3 and 4, which demonstrate measurements made on trabecular fiber bundles from the right and left ventricles respectively. Tension in milligrams is plotted against the ratio of the protein content of each fiber bundle to its equilibrium length. Regression lines for values obtained on control dogs and on experimental dogs were calculated by the method of least squares. The intercept on the y axis was not found to significantly differ from zero in any case, thus it was assumed that the regression lines passed through the origin and they were so drawn. The slopes of the regression lines for fiber bundles from the right ventricles of control dogs and experimental dogs were 104.7 and 63.9 respectively and, for left ventricles, 115.6 and 73.3. Test of significance of the differences in slopes of control dogs and experimental dogs gave a p value of less than 0.001.

Mean values of isometric tension were obtained for fiber bundles tested in each of the 18 dogs (table 1). In two of the experimental dogs, preparations were obtained from the right ventricle only. Over-all precision of determinations on bundles from one ventricle was 19 per cent expressed as a coefficient of variation. Fiber bundles from right and left ventricles of dogs in chronic heart failure developed consistently lower tension than did bundles from right and left ventricles of control dogs.

When unloaded fiber bundles were allowed to contract isotonically on addition of ATP, they shortened to a length ranging from 30 to 40 per cent of the initial length. No difference was noted in the extent of shortening of bundles from the hearts of control dogs and of experimental dogs. The extent of shortening of bundles from control right ventricles averaged 32.8 per cent of initial length; left ventricle averaged 33.2 per cent. Corresponding values for experimental dogs were: right ventricles 33.7 per cent, left ventricles 31.1 per cent.

Measurements of contractile tension at lengths below and beyond equilibrium length were obtained for each of the ventricles included in the study. In each case, tension developed by stretching uncontracted fibers was also measured. Bundles from failing ventricles developed consistently less tension at equivalent lengths than did bundles from the corresponding ventricles of control dogs. This is illustrated in figure 5 which shows a typical set of experimental measurements. On the other hand, resistance of uncontracted fiber bundles to stretch was approximately...
the same in the control and experimental groups.

Because of the intimate relationship between contractile tension and rate of hydrolysis of ATP, it was of interest to study the adenosinetriphosphatase activity of glycerol-extracted fibers from the same trabeculae on which measurements of isometric tension were made, under the same conditions of pH, temperature and ionic concentration. The rate of liberation of inorganic phosphorus from ATP was measured on particulate fractions of the fibers and on the isolated myofibrillar fractions. The results are summarized in table 2 which shows that in the ventricles studied adenosinetriphosphatase activity was essentially the same in the two groups.

**DISCUSSION**

Szent-Györgyi demonstrated that the maximal contractile tension produced by glycerol-extracted rabbit psoas muscle fibers was essentially equivalent to that of fresh intact muscle. Other close parallelisms in the contractile properties of the glycerol-extracted muscle model and living muscle have been observed repeatedly. We conclude, therefore, that muscle bundles prepared in the manner we have used retain the basic contractile properties of living muscle.

The calculated thickness of the bundles used in this study was greater than the limit of permissible thickness of 0.03 mm. defined by Weber and Portzehl for striated muscle bundles exposed to bath concentration of ATP in the proximity of $5 \times 10^{-3}$ M. In the analysis presented in figures 3 and 4, no deviation was noted in the linear relationship between tension and a function of the cross-sectional area, even for higher values of the latter. Cardiac muscle bundles apparently have a higher limit of permissible thickness than striated muscle bundles and this may be due to: (1) the looser structure of cardiac muscle bundles and a resultant more rapid rate of intrabundle diffusion of ATP; (2) the rate of hydrolysis of ATP by cardiac myosin is lower than that of myosin of striated muscle; (3) the calculated path of diffusion based on a circular cross-sectional area may be falsely high, since microscopic examination of representative bundles revealed them to be ribbon-shaped, as noted by Bowen and Kerwin.

Our observation that the extent of isotonic shortening was the same in both experimental and control bundles may be explained by the fact that this measurement is not a sensitive one nor as critical as the measurement of tension. We did not attempt to estimate rate of shortening, since this measurement is made ambiguous by variations in bundle diameter and their effect on the steady state diffusion constant of ATP.

The increased rate of hydrolysis of ATP by myofibrils as compared with the particulate fraction is explained on the basis of increased purification of the protein enzyme in the

<table>
<thead>
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<th>Failing</th>
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<tbody>
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</table>

* mM phosphorus liberated min.⁻¹ mg.⁻¹ protein.
myofibrillar fraction. The uniform rate of hydrolysis by myofibrils from failing ventricles and from normal ventricles is in accord with previous findings on extracted actomyosin.1

Szent-Györgyi2 estimated the maximum working capacity of psoas muscle fibers from the area enclosed by the length tension curve of the muscle and the length axis. This relationship was applied to the data illustrated in figure 5. Work was calculated for each fiber length at which tension was measured. The results of these calculations are illustrated in figure 6 and suggest that fiber bundles from failing hearts can do less work than fiber bundles from normal hearts under equivalent conditions of length, temperature, pH and ATP concentration.

It is realized that this derivation of the work performed by the fibers may be of questionable validity from a physical standpoint since all measurements of tension were made under isometric conditions; nevertheless, maximum tension at equilibrium length has been considered the best estimate of maximum working capacity of surviving muscle.12,19

The relationship between these properties of isolated, extracted fibers and the performance of the living, intact heart may be a tenuous one, but some considerations concerning this relationship are interesting and may be significant. Though these extracted bundles retain the basic contractile properties of living muscle, they differ from the living muscle with respect to the influence of a number of extrafibrillar factors. For example, these preparations lack a functioning membrane and therefore are not influenced by membrane effects. They are probably also free of neurohormonal influences and are isolated from the energy-supplying systems of whole muscle. Ionic and pH effects are experimentally controlled. It seems appropriate, therefore, to ascribe the differences noted in contractile tension and working capacity between fiber bundles from normal and failing hearts to alterations in the contractile protein of the latter. Such changes are undoubtedly of a structural nature, related to the conformity of actomyosin units or to their state of orientation in the fiber network. In certain nonphysiologic conditions, such as the “delta state” of muscle14 and actomyosin threads,8 the longitudinal orientation of actomyosin is disturbed and the mechanical properties of the fibers altered, i.e., they produce less tension under isometric conditions than do normal fibers. The contractile protein of the failing myocardium may be in a similar state of altered conformation or orientation, one poorly favored energetically, without impairment of its ability to hydrolyze ATP. Possibly, such a deformation of actomyosin is produced in the chronically failing overdistended heart by stretch and increased resting tension. An explanation such as this has been offered for the decrease in contractile tension sometimes seen in stretched muscle.15

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BOOKS RECEIVED

Books received by CIRCULATION RESEARCH are hereby acknowledged. Those of special interest to investigators in basic aspects of the circulation will be reviewed as space permits.


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