Studies of Actomyosin from Cardiac Muscle of Dogs with Experimental Congestive Heart Failure

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With the surgical assistance of Alfred Casper

Comparative studies were made on cardiac actomyosin from normal dogs and from dogs with experimental heart failure. Actomyosin was characterized by ultracentrifugal sedimentation velocity, viscosity and ATP-ase measurements. The data on actomyosin from normal cardiac muscle showed a striking similarity to the findings reported by others for skeletal muscle actomyosin. The only difference found between cardiac actomyosin from the normal and experimental material was an abnormal component (S20w = 5.0-6.7) in the sedimentation pattern for actomyosin from 4 of 11 dogs with cardiac failure. It seems likely that the changes in actomyosin which resulted in the abnormal sedimentation pattern were produced during extraction or preparation of the actomyosin and that they do not reflect an altered state of actomyosin in the functioning heart. The explanation for the occurrence of this slow sedimentation component solely in the experimental material is not clear.

The nature of the biochemical changes in the failing heart is one of the important unsolved problems in cardiovascular physiology and disease. Normal myocardial function is dependent upon a series of biochemical and biophysical changes which terminate in cardiac contraction. Myocardial failure may result as a consequence of interruption of these sequential changes at some critical stage. At an organismal level, a decrease in stroke work per unit of end diastolic volume occurs and the well-known syndrome of congestive heart failure ensues.

Many aspects of the biochemical changes leading to contraction of cardiac muscle have been investigated. Bing,1 Goodale2 and Olson3 have studied the uptake of substrate and of oxygen by the failing heart but they found no evidence of a defect. Also, studies of the high energy phosphate compounds (adenosine triphosphate and phosphocreatine) in the myocardium of failing heart-lung preparations by Wollenberger4 and in hearts of dogs with chronic low output failure by Olson and associates5 have failed to demonstrate a depletion of these sources of energy. Instead, available evidence has favored the view that a biochemical defect is present in the assimilation of phosphate bond energy by the contractile proteins, or in actomyosin itself.

Attention has been directed, therefore, at the contractile proteins in the failing myocardium. Benson and co-workers5-7 reported that actomyosin is partially broken down into actin and uncombined myosin. Olson and associates8 have suggested that myosin from the failing heart has a molecular weight of 500,000 to 750,000 which is approximately 2 to 3 times the value they found for normal cardiac myosin. Both groups of workers made no distinction between contractile proteins obtained from the right and the left ventricles of dogs with right-sided lesions of tricuspid insufficiency and pulmonic stenosis. Kako and Bing9 reported that the contractility of actomyosin bands from failing heart muscle of humans was reduced, but they failed to mention whether the actomyosin came from the right or the left ventricle, and some of their patients had hypertensive heart disease.

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The present observations were undertaken to characterize cardiac actomyosin from normal dogs and from dogs with experimental heart failure by ultracentrifugal sedimentation velocity, viscosity and adenosine triphosphatase (ATP-ase) measurements. Physiologic studies were made throughout the clinical course of heart failure to define as completely as possible the physiologic state associated with the pathogenesis of cardiac failure and to determine changes secondary to failure which might conceivably alter the cardiac muscle proteins. The principal part of the physiologic data is presented elsewhere. 10

METHODS

Cardiac muscle was obtained from 15 normal dogs, 7 dogs with cardiac failure produced by controlled progressive pulmonic stenosis, 11 5 dogs with chronic congestive failure secondary to tricuspid insufficiency and pulmonic stenosis, 12 and 3 dogs with chronic ascites produced by thoracic inferior vena cava constriction. 13 On the day of sacrifice, physiologic studies were made to define (1) the degree of sodium retention, (2) the quantity of ascites present, (3) mean right atrial pressure (RAP), and (4) left ventricular end diastolic pressure. The methods are described elsewhere. 10

To obtain the cardiac muscle, the animals were anesthetized lightly with intravenous sodium pentobarbital. The chest was opened quickly, the heart excised and washed with cold water, and placed in a container with chipped ice. All samples were taken from the right and left ventricular walls. The muscle was cut into small pieces of approximately 0.1 Gm. from which 1.0 Gm. (0.95 to 1.05) samples were weighed to within 0.1 mg. Immediately after weighing, the muscle samples were placed in a cold room (0 to 5 C). All muscle referred to hereafter as fresh was homogenized and extraction begun immediately; the remaining samples were frozen at — 20 C.

Homogenization and extraction of cardiac muscle for actomyosin were performed at 0 to 5 C. The extraction procedure was the same as that employed by Benson. 5-7 One gram samples were homogenized in 7 ml. of Weber's solution (0.6 M KCl, 0.04 M NaHCO₃, and 0.01 M Na₂CO₃; pH = 9.3 at 10 C.) in Potter tissue grinders (40 ml.) by hand until all visible strands of muscle and connective tissue disappeared. This procedure was adopted after failure to obtain complete homogenization with several different types of motor driven grinders. Seven milliliters of 0.6 M KCl and 2 mg. of adenosine triphosphate (ATP) were added to the homogenate and extraction carried out for 20 to 24 hours.

Following extraction, 15 ml. of 0.6 M KCl were added to the homogenate which was then centrifuged at 2000 g. for 10 min. in a Lourdes model AT centrifuge. After decentering, the supernatant was centrifuged for an additional 15 min. at 2000 g. The resultant preparation will be called the soluble extract. For preparation of actomyosin, 5 volumes of glass distilled water were added to the soluble extract. The solution was adjusted to pH 6.8 with 0.5 N acetic acid; actomyosin appeared as a flocculent precipitate. Actomyosin was separated by centrifugation (5000 g. for 10 min.). washed with glass distilled water, and dissolved in 0.6 M KCl.

To determine the yield of actomyosin, 7 ml. samples of soluble extract were subjected to the procedure described. Two 1 Gm. samples from each ventricle were analyzed on fresh tissue; repeat analyses were made on frozen tissue. Total nitrogen was determined on the precipitated actomyosin by micro-Kjeldahl analysis and the result multiplied by 6 to obtain the amount of actomyosin. Nonprotein nitrogen was determined on the trichloroacetic acid filtrate of the soluble extract. The total protein content of heart muscle was obtained by determining the total nitrogen in 1 Gm. samples, subtracting the nonprotein nitrogen and multiplying the result by 6.

The sedimentation velocity of cardiac actomyosin was studied with a model E Spinco analytic ultracentrifuge. One preparation of actomyosin was made from fresh cardiac muscle from each dog and all subsequent preparations from each animal were obtained from frozen tissue. Centrifugation was carried out on the same day the protein preparation was complete. Actomyosin was dissolved in 0.6 M KCl and a preliminary centrifugation at 20,000 g. for 20 min. was made in a Lourdes model AT centrifuge. The sedimentation velocity was determined on 2 samples simultaneously by use of a wedge cell and a plain cell. Kel F centerpieces were used to prevent denaturation of the protein. The routine procedure was to centrifuge an actomyosin preparation in the wedge cell and the same actomyosin preparation, ATP and MgCl₂ in the plain cell. The final concentrations of ATP and MgCl₂ were 5 X 10⁻³ M and 10⁻³ M, respectively. An equivalent volume of 0.6 M KCl was added to the actomyosin preparation in the wedge cell so that the protein concentration in the 2 cells was identical. By addition of ATP, actomyosin was converted to myosin and the sedimentation velocity of both proteins was measured

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simultaneously. Ultracentrifugation was performed at 59,780 RPM or 250,000 g. at an average temperature of 11.4 C. Values of 0.74 and 0.728 were used for the partial specific volumes of actomyosin and myosin, respectively. In addition to these observations, the effects of dialysis of actomyosin against 0.1 M Na₂CO₃ for 24 hours at 0 to 5 C. were studied by ultracentrifugation.

Viscosity was measured with size I and II Ubbelohde viscosimeters with outflow times for 0.6 M KCl of 16.8 and 100.0 sec, respectively. Two viscosimeters with different flow times were used to measure viscosity at 2 widely different mean velocity gradients* (75 to 150 for size II and 1,200 to 1,500 for size I) because molecules such as actomyosin with a high axial ratio show size dependence. The use of 2 viscosimeters with different mean velocity gradients does not provide a completely satisfactory solution to the problem of shear rate dependence but only comparative data and not absolute values are of primary importance in this study.

All measurements of viscosity were made on actomyosin immediately after preparation from fresh right ventricular muscle or skeletal muscle (the latter measurements were made to obtain comparative data). The protein was dissolved in 0.6 M KCl. Viscosity measurements with the size I viscosimeter were made at 0 to 0.5 C. by placing the viscosimeter in an ice bath and constantly stirring the ice. Studies with the size II viscosimeter were made in a constant temperature bath at 22.5 ± 0.2 C. A phosphate buffer system adjusted to pH of 7.6 was used. The relative viscosity (η_rel) was determined as the ratio of outflow time for the solution of actomyosin to that of 0.6 M KCl. “ATP sensitivity” was determined on actomyosin solutions of approximately 1 mg/ml. This test is a means of characterizing the actin content of actomyosin. It is made by measuring the relative viscosity of the actomyosin solution before and after addition of ATP. One tenth milliliter of 0.200 M ATP was added to 11.1 ml of protein solution. The “ATP sensitivity” is expressed in per cent by the formula

\[ \frac{Z_n - Z_{n,ATP}}{Z_{n,ATP}} \times 100 \]

where \( Z_n \) is the viscosity number and \( Z_{n,ATP} \) is the viscosity number after addition of ATP. The viscosity number \( Z_n \) is obtained from the expression

\[ Z_n = \frac{2.303 \log \eta_{rel}}{C} \]

where \( C \) is the concentration in grams per L. The specific viscosity, \( \eta_{sp} \) is equal to \( \eta_{rel} - 1 \).

ATP-ase activity of actomyosin from the right ventricle was measured under conditions designed to yield maximal or near maximal activity. The reaction mixture contained 0.2 to 0.3 mg/ml. of actomyosin in 0.05 M KCl, either 0.1 M or 0.01 M CaCl₂, 0.002 M ATP and 0.02 M Tris buffer. The pH of the reaction mixture was approximately 7.8. All reactions were carried out in a constant temperature bath at 22.5 ± 0.2 C. After incubation for 0, 2, 4, 6, 8, or 10 min., the reaction was stopped by addition of 1 ml of 20 per cent trichloroacetic acid. The filtrate was analyzed by the Fiske-SubbaRow method for inorganic phosphorus.

RESULTS

Physiologic State of Dogs at the Time of Sacrifice

All 7 animals with progressive pulmonic stenosis showed evidence of right heart failure including a marked elevation in mean RAP, hepatomegaly, a very low rate of Na excretion and ascites (table 1). Left ventricular end-diastolic pressure was measured in 2 of the dogs and found to be normal. Elsewhere measurements of filling pressure in the left ventricle showed normal values for 7 other dogs with heart failure secondary to progressive pulmonic stenosis. Additional data on cardiovascular and renal hemodynamic function in this type of experimental heart failure have been reported previously.

The findings from studies of the dogs with chronic congestive failure secondary to tricuspid insufficiency and pulmonic stenosis are presented in table 1. There was evidence of pure right-sided heart failure. Other data are presented elsewhere.

Actomyosin Content of Ventricular Heart Muscle. To exclude the influence of increased water content of the tissue from animals with heart failure, the data are expressed as the per cent of actomyosin per gram of total protein. The yields of actomyosin were (1) 28.7 ± 3.1* for normal right ventricle

*Standard deviation.
TABLE 1.—Physiologic Data at Sacrifice

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<thead>
<tr>
<th>Dog</th>
<th>Time after last operation (Days)</th>
<th>Duration of ascites (Days)</th>
<th>Volume of ascites (L)</th>
<th>Renal Na excretion* (mEq./day)</th>
<th>Mean right atrial pressure† (mm. water)</th>
<th>LVEDP† (mm. water)</th>
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Cardiac failure secondary to pulmonic stenosis

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<th>Volume of ascites (L)</th>
<th>Renal Na excretion* (mEq./day)</th>
<th>Mean right atrial pressure† (mm. water)</th>
<th>LVEDP† (mm. water)</th>
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Cardiac failure produced by tricuspid insufficiency and pulmonic stenosis

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<th>Mean right atrial pressure† (mm. water)</th>
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Thoracic inferior vena cava constriction

*Na intake, 60 mEq/day.
†These values represent inferior vena caval pressure rather than mean atrial pressure.
‡Pressures were measured immediately before sacrifice. C, control; E, experimental; LVEDP, left ventricular end-diastolic pressure.
§A + indicates presence of ascites; volume not measured but several hundred milliliters present.

and 27.9 ± 4.0 for normal left ventricle (N = 15), (2) 29.7 ± 3.5 for right ventricle and 28.6 ± 3.0 for left ventricle from dogs with cardiac failure produced by progressive pulmonic stenosis (N = 7), and (3) 25.0 for right ventricle and 29.0 for left ventricle of dogs with congestive failure secondary to tricuspid insufficiency and pulmonic stenosis (N = 3). The actomyosin yield was essentially the same for the right and the left ventricles and no difference was observed in the actomyosin yield from normal and failing hearts. Also, no difference was found in the actomyosin yield from fresh and frozen tissue. In addition to the measurements on cardiac actomyosin, the nitrogen content of the soluble extract per unit of total muscle nitrogen was studied and found to be unaltered in the failing heart.

Sedimentation Velocity Studies of Actomyosin. Comparative data among animals were obtained for actomyosin prepared from the right ventricle only unless otherwise stated. The typical sedimentation patterns for actomyosin from normal and failing heart muscle were identical (fig. 1). A single sedimentation boundary was present at low concentrations (below 3.0 mg./ml.) whereas at higher concentrations there was evidence of polydispersity. These findings were obtained from 6 normal dogs, 5 dogs with failure secondary to pulmonic stenosis, 2 dogs with tricuspid insufficiency, pulmonic stenosis and heart failure and from 3 animals with chronic thoracic inferior vena cava constriction and ascites. No difference was observed in the sedimentation of actomyosin prepared from fresh and from frozen muscle. An abnormal sedimentation pattern for cardiac actomyosin from right ventricular muscle was found in 2 of the dogs with cardiac failure produced by pulmonic stenosis.
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Fig. 1. Ultracentrifugal sedimentation patterns of native actomyosin from right ventricular muscle (wedge cell and upper part of picture for all figures) and the same actomyosin with added ATP and the resultant formation of myosin (regular cell and lower picture in all but E). In E, sedimentation diagrams of 2 different concentrations (4.06 mg./ml. for wedge cell and 3.06 mg./ml. for regular cell) of actomyosin are presented. A, B, and C are photographs of actomyosin from normal heart muscle whereas D, E, and F show typical sedimentation patterns of cardiac actomyosin from dogs with congestive failure. The protein concentrations for A, B, C, D and F were 1.72, 4.85, 6.29, 2.34 and 4.14 mg./ml., respectively. Photographs were taken at 8, 16, 20, 8, 12 and 32 min., respectively, after reaching a speed of 59,780 r.p.m. (dogs 1 and 7) and in 2 of the animals (dogs 4 and 5) with congestive failure secondary to tricuspid insufficiency and pulmonic stenosis (table 1). A small portion of the sedimenting material had an $S_{20w}$ ranging from 5.0 to 6.7 (fig. 2 Left). This finding was observed in at least 2 separate cardiac actomyosin preparations from each of the 4 dogs. In dog 5 with tricuspid insufficiency and pulmonic stenosis, actomyosin from the left ventricle showed the same slow sedimenting component.

Additional sedimentation velocity studies were carried out on actomyosin preparations
which were precipitated 2 or 3 times. A slow component with an $S_{20w}$ similar to that described for actomyosin from the 4 dogs with heart failure was observed in preparations of normal cardiac actomyosin from right ventricle (fig. 2 Right). Also, a second or third precipitation of actomyosin from the right ventricle of dog 7 with cardiac failure secondary to pulmonic stenosis showed a greater proportion of the sedimenting material as a slow component than was present after one precipitation (fig. 3 Left). Measurements of the pH of the actomyosin preparations from the right ventricle showed that pH fell after a second or third precipitation. Adjustment of the pH to 5.7 in one-time precipitated material was associated with the appearance of a slow component not present in the same actomyosin at pH 6.7 (fig. 3 Right).

Upon addition of ATP to actomyosin, the sedimenting material formed a single boundary which moved at the rate of myosin (fig. 1). A very small portion (less than 5 per cent) of the material was not converted to myosin and sedimented faster than myosin. The response to ATP was the same for material from all animals including the 4 dogs with the slow sedimentation component.

Cardiac actomyosin from 2 of the normal dogs and from 1 dog with cardiac failure secondary to pulmonic stenosis was dialyzed against 0.1 M Na$_2$CO$_3$ before ultracentrifugation. Two components were observed in the sedimentation pattern. One component sedimented at a rate similar to that of myosin; the other component moved more slowly ($S_{20w} = 1.6-2.2$). No difference in the response of actomyosin to dialysis against Na$_2$CO$_3$ was observed for material from normal and failing heart muscle. This response was similar to that described by Komínz$^{16}$ for skeletal muscle myosin dialyzed against Na$_2$CO$_3$.

Quantitative data on the sedimentation of actomyosin and myosin are presented in figure 4. Each point for actomyosin and myosin represents a separate enzyme preparation with the exception of a few of the values for myosin obtained at concentrations below 1.8 mg/ml. No differences are evident for rates of the sedimentation of proteins from normal and failing hearts and from the hearts of dogs with caval constriction. Sedimentation of actomyosin showed a marked dependence on protein concentration. Extrapolation of the sedimentation coefficient to zero protein concentration yielded a value of 70 to 90. The rate of movement of material formed by addition of ATP to actomyosin was similar to that described for myosin from skeletal muscle.$^{17-19}$ The $S_{20w}$ extrapolates linearly at zero concentration to a value of 6.25 for normal material and to similar values for the experimental material.

Viscosity Measurements. No difference in the viscosity of actomyosin from normal and failing cardiac muscle was detected from measurements at 0 or at 22.5 C. with 2 viscosimeters which produced widely different mean velocity gradients (fig. 5). Also, cardiac actomyosin from dogs with thoracic inferior vena cava constriction showed a normal viscosity. The logarithmic plot of $\eta_{rel}$ against the concentration of actomyosin shows that the Arrhenius relation

$$\log \eta_{rel} = K \times \text{concentration}$$

holds for cardiac actomyosin as it does for skeletal actomyosin.$^{20}$ The slopes of the lines (fig. 5) were essentially the same with the 2 types of viscosimeters for measurements at 0 and 22.5 C. The explanation for this finding is that measurements with size I viscosimeter (high $\beta$) were made at 0 and with size II viscosimeter (low $\beta$) were made at 22.5 C. Apparently, the viscosity was sufficiently greater at low temperatures to counteract the effect of a high mean velocity gradient and vice versa. The data indicate, therefore, a dependence of viscosity on the mean velocity gradient, a finding described for rabbit skeletal muscle actomyosin.$^{21}$

The viscosity response to ATP was studied on actomyosin from 6 normal dogs and 6 animals with cardiac failure (table 2). The "ATP sensitivity," which is an index of the actin content of the actomyosin, ranged from
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103 to 159 per cent for the normal material. This range is comparable to that of 97 to 179 per cent reported by Portzehl and co-workers for skeletal muscle actomyosin. Actomyosin from 5 of the 6 dogs with heart failure showed a similar response (103 to 130 per cent). In the remaining animal the response to ATP was low, but the initial viscosity number was lower than all other values obtained for this animal. The drop in specific viscosity per unit of actomyosin concentration in response to ATP showed no difference between cardiac actomyosin from normal and failing hearts.

**ATP-ase Activity of Actomyosin.** Studies were first conducted to determine if freezing normal cardiac muscle before extraction of actomyosin altered the ATP-ase activity. No difference in the ATP-ase activity of actomyosin from fresh and from frozen muscle was found. Thereafter, observations on the ATP-ase activity of actomyosin were made on both fresh and frozen material. The rates of enzymatic hydrolysis of ATP by actomyosin from normal and failing cardiac muscle (dogs with progressive pulmonic stenosis) were not detectably different for measurements made with 0.1 M Ca++. Examination of the ATP-ase activity of cardiac actomyosin from dogs with tricuspid insufficiency and pulmonic stenosis was made with 0.01 M Ca++ which gave greater ATP-ase activity for the normal material than with 0.1 M Ca++. With 0.01 M Ca++, the rate of hydrolysis of ATP appears to be reduced for the actomyosin from failing muscle, but studies were conducted on actomyosin from 3 dogs only so that no definitive conclusions can be made.

**Discussion**

In previous studies of contractile proteins from the failing myocardium, little attention was given to either the source of the cardiac muscle (right or left ventricle) in relation to which ventricle was failing or the relationship of the findings for the contractile proteins to the other features characteristic of congestive heart failure. Benson found no difference between actomyosin extracted from right and left ventricles in dogs with pure right-sided cardiac failure and only 3 of the 6 dogs studied had ascites at the time of sacrifice. Olson and associates used the entire right and left ventricular mass as a source of muscle for extraction of myosin. The question arises as to whether the previously reported findings of abnormal contractile proteins in heart failure have significance in the pathogenesis of cardiac failure.

In this and other studies, observations were made to evaluate left ventricular function and to define the changes associated with congestive failure which might conceivably alter the myocardial proteins. No evidence for left ventricular failure was obtained from studies of dogs with cardiac decompensation secondary to progressive pulmonic stenosis or to a combination of tricuspid insufficiency and pulmonic stenosis. Left ventricular end-diastolic pressure was normal in both preparations. There was no evidence of left ventricular hypertrophy or of chronic passive congestion of the lungs in the dogs with tricuspid insufficiency and pulmonic stenosis. Previous studies of dogs with heart failure produced

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**Table 2. Viscosity Response of Actomyosin to Adenosine Triphosphate**

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<thead>
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<th>Dog</th>
<th>Concentration of actomyosin (mg/ml.)</th>
<th>ATP sensitivity (%)</th>
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Fig. 2 Top. Sedimentation patterns for cardiac actomyosin (wedge cell) and myosin (regular cell) from right ventricular muscle. Left, proteins from a dog with experimental heart failure; actomyosin was precipitated one time. The abnormal slow component ($S_{wo} = 5.91$) was present in the actomyosin preparation. Right, two-time precipitated actomyosin from normal heart muscle is in the wedge cell and a slow component is present while the same actomyosin plus ATP is in the regular cell. The protein concentrations were 4.68 and 5.13 mg./ml. for Left and Right respectively. Photographs were made at 20 min. after reaching a speed of 59,780 r.p.m. in both instances.

Fig. 3 Bottom. Effects of additional precipitation (Left) and lowering the pH (Right) on the
by progressive pulmonic stenosis have demonstrated marked depression of right ventricular function. In this series of animals with heart failure secondary to tricuspid insufficiency and pulmonic stenosis, there was right ventricular hypertrophy and the pressure in the right atrium was consistently elevated. Barger demonstrated impairment of right ventricular function in heart lung preparations of dogs with chronic failure secondary to tricuspid insufficiency and pulmonic stenosis; no measurements of left ventricular function were made since filling pressures were measured only on the right side of the heart. Because isolated right ventricular failure was present in both types of animal preparations, comparison of normal cardiac actomyosin with material from dogs in failure was made on actomyosin from the right ventricle only, except for studies of actomyosin yield.

No difference in the yield of actomyosin from normal and failing heart muscle was found. This result was obtained for cardiac actomyosin from both experimental animal preparations. The actomyosin yield per gram of total protein is slightly lower than Benson reported. This is attributable to the present higher values for the total protein content of heart muscle. In the present study, the total protein content of muscle was determined by digesting 1 gm. samples of muscle for Kjeldahl analysis rather than analyzing an aliquot of the homogenate. In our laboratory, this latter procedure yielded variable and unreliable results regardless of the completeness of homogenization.

Ultracentrifugal sedimentation velocity studies of actomyosin from normal right ventricular muscle showed essentially the same sedimentation pattern and the same concentration dependence of the sedimentation constants as reported for skeletal muscle actomyosin. No difference was detected in the plots of $S_{20w}$ against protein concentration for actomyosin from normal and failing heart muscle. The response of actomyosin to ATP as determined by the sedimentation pattern and sedimentation constants of the resultant myosin was identical for all material from normal and failing hearts. The sedimentation pattern of material obtained by dialysis of actomyosin against Na$_2$CO$_3$ was the same for normal and failing heart muscle.

Abnormal sedimentation patterns were observed in cardiac actomyosin from the right ventricle of a dog with cardiac failure. Left, actomyosin was precipitated one time (wedge cell) and three times (regular cell). The quantity of the abnormal slow component increased with additional precipitation. Right, the pH of the actomyosin was 6.70 in the wedge cell and 5.78 for the same actomyosin in the regular cell. Lowering the pH increased the amount of the slow component present. The protein concentrations were 5.17 and 4.90 mg/ml, respectively. Photographs were taken at 33 and 24 min., respectively, after reaching a speed of 59,780 r.p.m.
served, however, for cardiac actomyosin from 2 dogs with cardiac failure secondary to progressive pulmonic stenosis and from 2 dogs with heart failure produced by tricuspid insufficiency and pulmonic stenosis. A small portion of the sedimenting material moved with a rate similar to that of myosin. The material probably represents myosin which was uncombined with actin, or a degradation or polymerization product of myosin. This finding is similar to the reported consistent observation of uncombined myosin in actomyosin preparations from failing heart muscle by Benson.7

Viscosity studies were also made on actomyosin from 2 of the 4 dogs for which the abnormal sedimentation pattern was observed. The viscosity was not reduced, as would be expected if actomyosin were partially fragmented into myosin and actin. The viscosity response of actomyosin from these 2 dogs to ATP (ATP sensitivity) was similar to that for normal cardiac actomyosin and for skeletal muscle actomyosin.14 These data show that the actin content of the actomyosin was not reduced. The present findings are in contrast to those of Benson7 who reported a lower ATP sensitivity for normal cardiac actomyosin than that found here and a further reduction in the ATP sensitivity of actomyosin from failing heart muscle as compared with normal material. A low ATP sensitivity for actomyosin might result from fragmentation of actomyosin into actin and uncombined myosin or from inadequate extraction of actin from muscle. The present average value of 124 per cent for the ATP sensitivity of actomyosin from normal right ventricular muscle is considerably higher than the value of 86.3 per cent reported by Benson for normal cardiac actomyosin. The data show, therefore, that more complete extraction of actin was obtained in the present preparations than in those of Benson.

What is the significance of the slow component in sedimentation patterns of actomyosin from failing right ventricular muscle? The duration and degree of cardiac failure and the general condition of these 4 dogs did not differ from the other animals with heart failure. The finding of the abnormal sedimentation pattern for actomyosin from the left ventricle of one of the 4 dogs suggests that the slow sedimentation component is not characteristic of a failing myocardium since the left ventricle was not in failure.

Several other findings suggest that the abnormal sedimentation component may be an extraction or preparation artifact. First, the relative amount of the slow component was increased by repeated precipitation when the pH was not adjusted to pH 6.8. Secondly, a reduction in pH of the actomyosin preparation increased the quantity of the slow component. Finally, the slow component appeared in actomyosin prepared from normal cardiac muscle after the second and third precipitation in the absence of adjustment of pH to 6.8. Therefore, these data do not support the concept that actomyosin present in the functioning hearts of dogs with experimental congestive failure is partially fragmented into uncombined myosin and that such an abnormality constitutes a primary defect in the failing myocardium. The occurrence of one-time precipitated actomyosin preparations with a slow sedimentation component solely in the experimental material remains unexplained.

**SUMMARY**

Actomyosin from cardiac muscle of dogs with heart failure secondary to progressive pulmonic stenosis or to a combination of tricuspid insufficiency and pulmonic stenosis was characterized by ultracentrifugal sedimentation velocity, viscosity and ATP-ase measurements. Cardiac actomyosin from normal animals and from dogs with thoracic inferior vena cava constriction provided control material. The sedimentation patterns and sedimentation constants for actomyosin from the right ventricles of normal hearts and the hearts of dogs with thoracic caval constriction were not detectably different from the results reported by others for skeletal muscle.
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actomyosin. Seven of the dogs with cardiac failure showed normal sedimentation patterns and constants for cardiac actomyosin from right ventricular muscle. In the other 4 dogs with cardiac failure, a small portion of the sedimenting protein had an S_{20w} ranging from 5.0 to 6.7. Additional precipitation or reduction in the pH of the actomyosin increased the relative amount of this slow component. Also, repeated precipitation of actomyosin from normal dogs without adjustment of the pH to 6.8 yielded a similar slow component in the sedimentation pattern. Cardiac actomyosin from all normal and experimental animals responded to ATP by formation of myosin; the S_{20w} at zero concentration was 6.25, a value similar to that reported for skeletal muscle myosin. The viscosity of actomyosin and the viscosity response to ATP were the same for material from normal dogs and dogs with experimental failure; also, the present viscosity data on cardiac actomyosin are similar to those reported for skeletal muscle actomyosin. No difference was observed in the ATP-ase activity of normal and experimental material. It seems likely that the slow component present in the sedimentation patterns of actomyosin from the 4 dogs with heart failure reflects an extraction or preparation artifact. It is concluded that these data do not support the concept that the contractile proteins are altered in experimental heart failure.

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SUMMARIO IN INTERLINGUA

Le actomyosina cardia de canes normal e de canes con constriction del vena cave inferior in le thorace provideva le valores de controlo. Le configurationes de sedimentation e le constantes de sedimentation pro actomyosina ab le ventriculo dexter de cordes normal e ab le corde de canes con constriction del vena cave thorace non differeva detegibilemente ab le resultatos reportate per alteres pro actomyosina ab musculo skeletal. Septe del canes con disfallimento cardiac exhibiva normal configurationes e constantes de sedimentation pro actomyosina ab musculo dexter-ventricular. In le altere 4 canes con disfallimento cardiae, un micre portion del proteina sedimentate haveva un S_{20w} in le intervallo ab 5,0 a 6,7. Precipitation additional o reduction in le pH del actomyosina augmentava le quantitate relative de iste lente componente. In plus, le repetite precipitation de actomyosina ab canes normal, sin rectification del pH a 6,8, produceva un simile lente componente in le configuration sedimentatorii. Actomyosina cardia ab omne le animates normal e experimental respondeva a adenosino-triphosphato per le formation de myosina. Le S_{20w} al concentration zero esseva 6,25, un valor simile a illo reportate pro myosina de musculo skeletal. Le viscositate de actomyosina e le responsa de iste viscositate a adenosino-triphosphato esseva le mesme pro material ab canes normal como pro material ab canes con disfallimento cardiae de origine experimental. In plus, le presente datos pro le viscositate de actomyosina cardiae es simile al datos reportate pro actomyosina de musculo skeletal. Nulle differentia eseva observate in le activitate de adenosino-triphosphatase inter material normal e material experimental. Il pare probabile que le lente componente in le configuration del sedimentation de actomyosina ab le 4 canes con disfallimento cardiae reflecte un artefacto de extraction o preparation. Es concludite que le presente datos non supporta le conception que le proteinas contractil es alterate in disfallimento cardiae de origine experimental.
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Studies of Actomyosin from Cardiac Muscle of Dogs with Experimental Congestive Heart Failure

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