Myofibrillar adenosine triphosphatase activity in congestive heart failure

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ALPERT, NORMAN R., AND MICHAEL S. GORDON. Myofibrillar adenosine triphosphatase activity in congestive heart failure. Am. J. Physiol. 202(5): 940-946. 1962.—Myofibrillar adenosine triphosphatase (ATPase) activity from seven failing and nine control hearts was studied. The failing hearts came from patients with a clinical and pathological diagnosis of congestive heart failure following benign essential hypertension. Normal hearts were obtained following traumatic death in otherwise normal subjects with no evidence of pathology. As the MgCl₂ concentration was increased, the myofibrillar ATPase from the failing hearts exhibited a significantly lower rise in activity than that from the normal hearts. Increasing the substrate concentration to 5 mM adenosine triphosphate (ATP) increased the myofibrillar ATPase activity to an optimum of .95 μM/mg/15 min in the normal group and .69 μM/mg/15 min in the failing group. Further increases in substrate concentration decreased the ATPase activity in both groups. It was concluded that myofibrils from a heart in congestive failure following benign essential hypertension hydrolyze ATP at a slower rate than those from normal hearts. Since myofibrillar ATPase activity and tension development go hand in hand, the decrease in ATPase activity in the failing heart may account for its inability to meet the work load imposed upon it. Thus these experiments offer evidence that a lesion in congestive heart failure may reside in the contractile protein itself.

CONGESTIVE HEART FAILURE sometimes follows chronic benign essential hypertension. Before the heart fails, the patient performs his daily work load and may even exercise without subjective discomfort. At some point in the natural history of the disease, often without any observable exacerbation of the hypertension, the heart goes into failure. This occurs when the heart is no longer able to pump a quantity of blood adequate for the needs of the organism.

The inadequacy of power output in this type of failing cardiac muscle has been attributed to a decrease in the ability of the contractile protein, actomyosin, to utilize available phosphate bond energy (1–3). The above inference stems from the following indirect evidence.

1) Adenosine triphosphate (ATP) and creatine phosphate (CrP) levels are a function of the rate of ATP production and ATP utilization. If the contractile protein were unable to hydrolyze ATP at a normal rate the ATP and CrP levels would be normal or high. In the failing heart the ATP level is normal, while the CrP concentration is high, thus suggesting the possibility of inadequate utilization (4–6).

2) As the heart goes into failure, the oxygen consumption falls. This fall parallels the work output (7). If the availability of adenosine diphosphate (ADP) and inorganic phosphate (Pi) control the oxygen consumption (8), then the decrease in oxygen consumption of the failing heart suggests the possibility of inadequate ATP hydrolysis by the contractile protein.

3) Physical and physiological alterations in the contractile protein have been reported in heart failure. Olson et al. noted that myosin from the failing heart had a much higher molecular weight than normal myosin (9). In addition actomyosin bands from failing heart muscle shorten less than those from normal hearts (10), while the tension development of glycerinated fibers from failing hearts is depressed (11).

This data suggests the site of the lesion in congestive heart failure may be in the contractile protein itself.

The ability of actomyosin to do work is directly

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2 There is an extensive literature indicating that myocardial O₂ consumption does not fall in heart failure. In measurements made on human subjects, the myocardial O₂ consumption of normal and of failing hearts was not significantly different, while the external work performed was lower in the failing group (Circulation 2: 513, 1950). The crucial point is the diastolic volume of the heart. The hearts of the group in failure were probably dilated. When the heart is dilated it works at a mechanical disadvantage. In order to develop a given intraventricular pressure P, the tension in the myocardial fibers follows the relationship P = T (1/R₁ + 1/R₂) where R₁ and R₂ are the radii of horizontal and longitudinal curvature. Thus in this situation it is erroneous to make a correlation between external work and myocardial O₂ consumption. In experiments where an isolated cat heart preparation, kept at constant diastolic volume, was studied, it was found that the external work fell while the O₂ consumption remained constant (Circulation Research 1: 398, 1953). This is further evidence that the lesion in heart failure is probably not one of energy production.
Hypertension is in the myofibril itself. The Mg++ activation curve is additional evidence that the lesion in human heart failure following prolonged human hearts. At the optimal substrate concentration with the increased work load, and failure would follow. ATPase activity was determined in normal and failing output of the heart. If this is the case for the hypertension can be extrapolated to the intact heart, then an alteration in actomyosin which resulted in a decreased alteration in actomyosin which resulted in a decreased ability to utilize ATP could readily decrease the power rate of hydrolysis was always associated with a decrease in ATPase activity of 10, 20, and 30 % resulted in a decrease in tension of 12, 25, and 38 %. If the experiments on the glycerinated preparation can be extrapolated to the intact heart, then an alteration in actomyosin which resulted in a decreased ability to utilize ATP could readily decrease the power output of the heart. If this is the case for the hypertensive patient, the heart might then be unable to keep up with the increased work load, and failure would follow.

In the experiments reported below, myofibrillar ATPase activity was determined in normal and failing human hearts. At the optimal substrate concentration the myofibrils from the failing heart had an ATPase activity which was 35 % lower than the normal ATPase activity. This difference and a difference in the slope of the Mg++ activation curve is additional evidence that the lesion in human heart failure following prolonged hypertension is in the myofibril itself.

**METHODS**

**Choice of specimen.** Twenty grams of left ventricular muscle was obtained from each of 16 postmortem human hearts. These included seven specimens from patients in heart failure and nine from normal subjects. The tissue was obtained from 2.5 to 11.5 hr after death.

Tissues in the failure group met the following clinical and pathological criteria: 1) history of traumatic death an otherwise normal subject; 2) no pathologic evidence of heart disease, with heart weight less than 375 g.

Myofibrillar preparation. Immediately after obtaining the tissue, approximately 3 g of the total sample was used to prepare the myofibrils. The remaining tissue was kept at room temperature, wrapped in sponges and moistened with physiologic saline solution. Myofibrils were prepared from the tissue kept at room temperature at successive 3-4 hr intervals. No preparation was begun later than 13 hr post mortem. In this manner, 13 of the 16 hearts were run at two or three different postmortem intervals in order to assess the effect of time after death on ATPase activity. As a result, a total of 16 experiments were performed on the failing group and 18 on the control group.

Myofibrils were isolated according to the methods of Perry (13). The isolated myofibrils were suspended in a volume of KCl-tris solution equal to 30 times the weight of the original tissue samples (about 90 ml). The concentration of KCl and tris in this final diluent was such that the proper molarity of all constituents would be obtained.

This final myofibrillar suspension was used to measure retinopathy, cerebral symptoms, Cheyne-Stokes respiration, elevated venous pressure, and increased circulation time, 3) radiologic or electrocardiographic evidence of left ventricular hypertrophy and/or dilatation; 4) pathologic diagnosis of hypertensive heart disease with evidence of hypertrophy and dilatation and heart weight greater than 450 g (minimal arteriosclerotic changes did not exclude a subject from this category).

In one experimental heart, an additional clinical and pathologic diagnosis was made of advanced pulmonary silicosis with cor pulmonale and right ventricular hypertrophy and dilatation.

Normal tissue in all cases met the following clinical and pathological criteria: 1) history of traumatic death in an otherwise normal subject, 2) no pathologic evidence of heart disease, with heart weight less than 375 g.

**TABLE 1. Clinical and pathological history of normal male subjects**

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Age</th>
<th>Sex</th>
<th>Hours Post-mortem*</th>
<th>Clinical History and Pathology; Heart Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>39</td>
<td>M</td>
<td>9</td>
<td>Gunshot to heart; normal heart; 315 g</td>
</tr>
<tr>
<td>2a</td>
<td>40</td>
<td>M</td>
<td>4.5</td>
<td>Traumatic cerebral injuries; normal heart; 395 g</td>
</tr>
<tr>
<td>2b</td>
<td>65</td>
<td>M</td>
<td>8</td>
<td>Stab wound to left chest; normal heart; 350 g</td>
</tr>
<tr>
<td>3a</td>
<td>27</td>
<td>M</td>
<td>5</td>
<td>Gunshot to head; normal heart; 340 g</td>
</tr>
<tr>
<td>3b</td>
<td>27</td>
<td>M</td>
<td>8</td>
<td>Stab wound to chest; normal heart; 375 g</td>
</tr>
<tr>
<td>4c</td>
<td>9</td>
<td>M</td>
<td>13</td>
<td>Fell 13 stories; normal heart; 355 g</td>
</tr>
<tr>
<td>5a</td>
<td>46</td>
<td>M</td>
<td>9.5</td>
<td>Auto wreck, multiple injuries; normal heart; 325 g</td>
</tr>
<tr>
<td>5b</td>
<td>46</td>
<td>M</td>
<td>6.5</td>
<td>Stab wound to chest; normal heart; 330 g</td>
</tr>
<tr>
<td>6a</td>
<td>24</td>
<td>M</td>
<td>5</td>
<td>Gunshot to the head; normal heart; 330 g</td>
</tr>
<tr>
<td>6b</td>
<td>36</td>
<td>M</td>
<td>7</td>
<td>Acute alcoholic intoxication; normal heart; 305 g</td>
</tr>
<tr>
<td>7a</td>
<td>34</td>
<td>M</td>
<td>8</td>
<td>Stab wound to left chest; normal heart; 350 g</td>
</tr>
<tr>
<td>7b</td>
<td>25</td>
<td>M</td>
<td>9</td>
<td>Stab wound to chest; normal heart; 375 g</td>
</tr>
<tr>
<td>8a</td>
<td>25</td>
<td>M</td>
<td>12</td>
<td>Auto wreck, multiple injuries; normal heart; 325 g</td>
</tr>
<tr>
<td>8b</td>
<td>25</td>
<td>M</td>
<td>6.5</td>
<td>Stab wound to chest; normal heart; 375 g</td>
</tr>
</tbody>
</table>

* Various times listed represent time after death at which heart tissue was assayed.

TABLE 2. Clinical and pathological history of subjects in congestive heart failure

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Age</th>
<th>Sex</th>
<th>Hours Post-mortem*</th>
<th>Clinical History and Pathology; Heart Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>85</td>
<td>F</td>
<td>4</td>
<td>Failure; HHD with minimal ASHD; 490 g</td>
</tr>
<tr>
<td>1b</td>
<td>85</td>
<td>F</td>
<td>10.5</td>
<td>Failure; HHD with minimal ASHD; 490 g</td>
</tr>
<tr>
<td>1c</td>
<td>12</td>
<td>F</td>
<td>9</td>
<td>Failure; HHD and moderate ASHD; 500 g</td>
</tr>
<tr>
<td>2a</td>
<td>65</td>
<td>M</td>
<td>7</td>
<td>Failure; HHD and cor pulmonale secondary to silicosis; 450 g (lung wt. 3700 g)</td>
</tr>
<tr>
<td>2b</td>
<td>65</td>
<td>M</td>
<td>11</td>
<td>Failure; HHD and cor pulmonale secondary to silicosis; 450 g (lung wt. 3700 g)</td>
</tr>
<tr>
<td>3a</td>
<td>53</td>
<td>M</td>
<td>9</td>
<td>Failure; HHD and moderate ASHD; 500 g</td>
</tr>
<tr>
<td>3b</td>
<td>53</td>
<td>M</td>
<td>12</td>
<td>Failure; HHD and moderate ASHD; 500 g</td>
</tr>
<tr>
<td>4a</td>
<td>50</td>
<td>M</td>
<td>6.5</td>
<td>Failure, HHD; 550 g</td>
</tr>
<tr>
<td>4b</td>
<td>50</td>
<td>M</td>
<td>10.5</td>
<td>Failure, HHD; 550 g</td>
</tr>
<tr>
<td>5a</td>
<td>71</td>
<td>M</td>
<td>9.25</td>
<td>Failure; HHD and moderate ASHD; 600 g</td>
</tr>
<tr>
<td>5b</td>
<td>71</td>
<td>M</td>
<td>12.25</td>
<td>Failure; HHD and moderate ASHD; 600 g</td>
</tr>
<tr>
<td>6a</td>
<td>72</td>
<td>F</td>
<td>11.5</td>
<td>Failure; HHD; 575 g</td>
</tr>
<tr>
<td>7a</td>
<td>44</td>
<td>F</td>
<td>7.5</td>
<td>Failure, HHD; 470 g</td>
</tr>
<tr>
<td>7b</td>
<td>44</td>
<td>F</td>
<td>10.5</td>
<td>Failure, HHD; 470 g</td>
</tr>
</tbody>
</table>

* Various times listed represent time after death at which heart tissue was assayed. HHD = hypertensive heart disease; ASHD = arteriosclerotic heart disease.
of the myofibrillar suspension was added to each of eight test tubes, and one milliliter of the different ATP-MgCl₂ solutions was added with a tris and KCl concentration of 0.29 - 0.55 mM. The protein ranged between 0.37 - 0.94 mg/ml.

The final millimolar concentrations of the eight different ATP-MgCl₂ solutions were:

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Concentration, mm</th>
<th>Concentration, mm</th>
<th>Concentration, mm</th>
<th>Concentration, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.38</td>
<td>0.69</td>
<td>1.00</td>
<td>1.31</td>
</tr>
<tr>
<td>2a</td>
<td>0.48</td>
<td>0.66</td>
<td>0.90</td>
<td>1.02</td>
</tr>
<tr>
<td>3a</td>
<td>0.40</td>
<td>0.55</td>
<td>0.67</td>
<td>0.81</td>
</tr>
<tr>
<td>4a</td>
<td>0.29</td>
<td>0.31</td>
<td>0.75</td>
<td>1.04</td>
</tr>
<tr>
<td>5a</td>
<td>0.31</td>
<td>0.29</td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>6a</td>
<td>0.27</td>
<td>0.28</td>
<td>0.59</td>
<td>0.84</td>
</tr>
<tr>
<td>7a</td>
<td>0.24</td>
<td>0.26</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>8a</td>
<td>0.22</td>
<td>0.26</td>
<td>0.66</td>
<td>1.14</td>
</tr>
<tr>
<td>9a</td>
<td>0.20</td>
<td>0.27</td>
<td>0.66</td>
<td>1.09</td>
</tr>
</tbody>
</table>

* ATPase activity is expressed as µg Pi liberated/mg myofibrillar protein/15 min. Final concentration of ATP is 5 mM.

ATPase activity. Suitable aliquots were taken for the protein determination. These were checked microscopically for the appearance and adequacy of separation of the myofibrils.

Experimental design and statistical methods. Two milliliters of the myofibrillar suspension was added to each of eight test tubes, and 2 ml of deionized distilled water was added to a similar number of control tubes. The water used throughout the experiment was distilled water which had passed through a mixed-bed ion-exchange column (Amberlite MB-1). Eight solutions of various concentrations of ATP and MgCl₂ in tris buffer at pH 7.1 were then prepared. The myofibrils, water, and ATP-MgCl₂ solutions were then placed in a 37.1°C shaking water bath and allowed to equilibrate for 5 min. One milliliter of the different ATP-MgCl₂ solutions was then added to the test tubes containing the myofibrils and the water. Protein ranged between 1.5 and 3.0 mg with a tris and KCl concentration of 10 and 100 mM. The final millimolar concentrations of the eight different ATP-MgCl₂ solutions were:

<table>
<thead>
<tr>
<th>Solution</th>
<th>ATP</th>
<th>MgCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

3 ATP was obtained as the crystalline disodium salt from Sigma Chemical Co., St. Louis, Mo. In one case (normal heart no. 9), the ATP used was obtained as the crystalline disodium salt from Nutritional Biochemicals Corp., Cleveland, Ohio.

In three hearts (one failing and two normal), solutions 6, 7, and 8 were not used, i.e., there was no measure of the effect of varying ATP concentration.

The reaction was started by adding the ATP-MgCl₂ solution to the myofibrils and stopped 15 min later by the addition of 12 cc of 10% trichloroacetic acid (TCA), final concentration 8%. Those test tubes containing the precipitated myofibrils were then centrifuged for 20 min at 2,000 g. An aliquot of the supernatant fluid was removed from these and the water controls for the determination of inorganic phosphate. The entire procedure discussed under experimental design was done in duplicate.

ATPase activity, Pi liberated per milligram protein per 15 min, was calculated as follows: ATPase activity = (A - B)/C where A = Pi in the media containing myofibrils and ATP following 15 min incubation; B = Pi in the media containing ATP with no myofibrils; C = protein in milligrams. Inorganic phosphate was not liberated by the myofibrils when ATP was absent.

The experiment was designed to permit the following types of statistical analyses (14): 1) correlation between ATPase activity and time after death; 2) correlation between ATPase activity and MgCl₂ concentration; 3) correlation between ATPase activity and ATP concentration; 4) correlation between ATPase activity and time after death; and 5) comparison of ATPase activity of normal and failing groups at each ATP and MgCl₂ concentration.

Chemical methods. Phosphate was determined by the method of Fiske and Subbarow (15).

Protein was determined by the Weichselbaum-Kirk biuret method as modified by Beisenherz (16), and

**TABLE 4.** Effect of altering ATP concentration on myofibrillar ATPase activity of normal hearts

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Adenosine Triphosphate Concentration, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>

* ATPase activity is expressed as µg Pi liberated/mg myofibrillar protein/15 min. Concentration of MgCl₂ is 1 mM. When myofibrils are incubated with ATP no Pi is liberated.
kept at increasing the substrate concentration while MgCl₂ was concentration. Further increase in substrate concentration decreased ATPase activity (Tables 3 and 4).

Correlation between ATPase activity and MgCl₂ concentration. A significant correlation was found between ATPase activity and MgCl₂ concentration at the same ATP concentration. In both normal and failing groups, increasing the MgCl₂ concentration resulted in an increase in activity. This was tested by linear regression analysis. The effect of increasing MgCl₂ concentration was found to be of different magnitude in normal and failing groups. The failures did not respond as much as the normals. This was reflected in the significant difference in slopes of the normal and failing groups in the regression analysis (Fig. 1, Table 7).

Correlation between ATPase activity and ATP concentration. A significant correlation was found between ATPase activity and ATP concentration at the same MgCl₂ concentration. In both normal and failing groups, increasing the ATP concentration up to 5 mM resulted in an increase in ATPase activity. Further increase in ATP concentration resulted in a progressive decrease in ATPase activity (Fig. 2).

Comparison of ATPase activity of normal and failing groups. Normal and failing groups were compared at each different ATP-MgCl₂ concentration by means of an unpaired t test. A highly significant difference was found at a 5 mM ATP and 1 mM MgCl₂ concentration. ATPase activity was also highest at this ATP-MgCl₂ concentration in both normal and failing groups. Statistically significant differences in activity were found in four of the seven different ATP-MgCl₂ concentrations (Tables 7 and 8).

Correlation between ATPase activity and age of patient. There is no correlation between ATPase activity and age of the patient. This was tested at each ATP-MgCl₂ concentration. Normal and failing groups were tested.

### TABLE 5. Effect of altering MgCl₂ concentration on myofibrillar ATPase activity of failing hearts*

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Magnesium Chloride Concentration, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>1a</td>
<td>0.21</td>
</tr>
<tr>
<td>1b</td>
<td>0.31</td>
</tr>
<tr>
<td>1c</td>
<td>0.28</td>
</tr>
<tr>
<td>2a</td>
<td>0.20</td>
</tr>
<tr>
<td>2b</td>
<td>0.15</td>
</tr>
<tr>
<td>2c</td>
<td>0.27</td>
</tr>
<tr>
<td>2d</td>
<td>0.16</td>
</tr>
<tr>
<td>2e</td>
<td>0.35</td>
</tr>
<tr>
<td>2f</td>
<td>0.34</td>
</tr>
<tr>
<td>2g</td>
<td>0.26</td>
</tr>
<tr>
<td>2h</td>
<td>0.26</td>
</tr>
<tr>
<td>2i</td>
<td>0.21</td>
</tr>
<tr>
<td>2j</td>
<td>0.23</td>
</tr>
<tr>
<td>2k</td>
<td>0.30</td>
</tr>
<tr>
<td>2l</td>
<td>0.26</td>
</tr>
<tr>
<td>2m</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* ATPase activity is expressed as μM Pi liberated/mg myofibrillar protein/15 min. Final concentration of ATP is 5 mM.

### TABLE 6. Effect of altering ATP concentration on myofibrillar ATPase activity of failing hearts*

<table>
<thead>
<tr>
<th>Spec. No.</th>
<th>Adenosine Triphosphate Concentration, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3a</td>
<td>0.49</td>
</tr>
<tr>
<td>3b</td>
<td>0.51</td>
</tr>
<tr>
<td>3c</td>
<td>0.48</td>
</tr>
<tr>
<td>3d</td>
<td>0.61</td>
</tr>
<tr>
<td>3e</td>
<td>0.84</td>
</tr>
<tr>
<td>3f</td>
<td>0.71</td>
</tr>
<tr>
<td>3g</td>
<td>0.73</td>
</tr>
<tr>
<td>3h</td>
<td>0.64</td>
</tr>
<tr>
<td>3i</td>
<td>0.50</td>
</tr>
<tr>
<td>3j</td>
<td>0.62</td>
</tr>
<tr>
<td>3k</td>
<td>0.52</td>
</tr>
<tr>
<td>3l</td>
<td>0.62</td>
</tr>
<tr>
<td>3m</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* ATPase activity is expressed as μM Pi liberated/mg myofibrillar protein/15 min. Concentration of MgCl₂ is 1 mM. When myofibrils are incubated without ATP no Pi is liberated.
FIG. 1. Correlation between MgCl₂ concentration and ATPase activity. All reaction vessels contained tris buffer 10 mM, pH 7.1; ATP 5 mM, KCl 100 mM and approximately 2 mg protein. Reaction was started by adding ATP and MgCl₂ to the protein mixture and was run at 37.1°C.

FIG. 2. Correlation between ATP concentration and ATPase activity. All reaction vessels contained tris buffer 10 mM, pH 7.1; MgCl₂ 1 mM; KCl 100 mM; and approximately 2 mg protein. Reaction was started by adding ATP and MgCl₂ to the protein mixture and was run at 37.1°C.

TABLE 7. Correlation between MgCl₂ and ATPase activity

<table>
<thead>
<tr>
<th>MgCl₂ mM</th>
<th>Mean ATPase Activity*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Failure</td>
</tr>
<tr>
<td>0.00</td>
<td>34.6</td>
<td>34.5</td>
</tr>
<tr>
<td>0.05</td>
<td>44.0</td>
<td>34.5</td>
</tr>
<tr>
<td>0.50</td>
<td>74.5</td>
<td>55.5</td>
</tr>
<tr>
<td>1.00</td>
<td>99.5</td>
<td>69.5</td>
</tr>
</tbody>
</table>

Probability of positive correlation between MgCl₂ concentration and ATPase activity

Probability that slope of two regression lines are <.02 different

* ATPase activity expressed as μmol Pi/mg protein X 15 min.
† Difference in ATPase activity between normal and failing hearts was evaluated by means of an unpaired t test.
‡ The correlation between MgCl₂ concentration and ATPase activity was evaluated by means of linear regression analysis.

DISCUSSION

Myofibrils from hearts in congestive failure exhibit a lower ATPase activity than those from normal hearts. This raises the question as to the quantitative relationship between myofibrillar ATPase activity measured several hours post mortem, and ante-mortem activity of the same preparation. Since it is very difficult to procure 0 time samples it is almost impossible to answer this question using human tissue. Therefore, two canine hearts were studied at 0 and 4 hr post mortem. No change in myofibrillar ATPase activity was observed over the 4-hr period. Arnold and co-workers (17) reported no change in myofibrillar ATPase activity of normal canine hearts from 0 to 6 hr post mortem, and only a 5% change after 24 hr.

In our study, no correlation between the postmortem interval was found from 2.5 to 13 hr after death for the normal hearts, and between 4 and 12 hr after death for the failing hearts.

Thus, on the basis of both the human and dog experiments, in the case of the normal heart, it may be inferred that the activity measured up to 13 hr post mortem is the same or very close to the same as ante-mortem activity. Unless the rate of autolysis in the failing heart is significantly greater than in the normal heart, the activity measured up to 13 hr post mortem is the same or very close to the same as ante-mortem activity. Therefore, postmortem activity is a valid measure of in vivo activity in this group as well.

The activating effect of Mg ions on the myofibrillar actomyosin system of rabbit skeletal and heart muscle (13, 18) as well as on normal human myofibrillar ATPase activity (17) has been described. Our demonstration of a positive correlation between MgCl₂ concentration and ATPase activity in normal hearts confirms these earlier observations. In addition, the same positive correlation was found in the failing heart. However, the degree of response is significantly different in the two groups. The slopes of the regression lines in Fig. 1 and the data of Table 7 are significantly different. Increasing MgCl₂ concentration did not activate the failing group as much as the normal group. The finding suggests that there may be some alteration in the myofibrillar protein from failing death, postmortem activity is a valid measure of in vivo activity in this group as well.

Normal subjects ranged in age from 24 to 46 years. Failures ranged from 44 to 85 years. As these are different populations, no correlation may be made between activity and age in both groups together. Figure 3 demonstrates the effect of age on activity from 24 to 85 years inclusive, irrespective of normal or failing history. While such a correlation is not permissible, the need for data on young heart failures and old normal hearts is apparent.
TABLE 8. Effect of ATP concentration on ATPase activity of myofibrils from normal and failing hearts

<table>
<thead>
<tr>
<th>ATP, mM</th>
<th>Mean ATPase Activity*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Failure</td>
</tr>
<tr>
<td>1</td>
<td>0.77 (.078)</td>
<td>0.59 (.089)</td>
</tr>
<tr>
<td>5</td>
<td>0.99 (.055)</td>
<td>0.69 (.045)</td>
</tr>
<tr>
<td>10</td>
<td>0.67 (.083)</td>
<td>0.43 (.033)</td>
</tr>
<tr>
<td>20</td>
<td>0.15 (.071)</td>
<td>0.38 (.073)</td>
</tr>
</tbody>
</table>

* ATPase activity is expressed as µM Pi liberated/mg myofibrillar protein/15 min. Concentration of MgCl₂ was 1 mM. Figures in parentheses are standard deviations of the mean. † Difference in ATPase activity between normal and failing hearts was evaluated by means of an unpaired t test.

ATPase activity was found to increase with a rise in substrate concentration up to 5 mM ATP. Concentrations of ATP higher than 5 mM depressed the activity. Substrate inhibition in myofibrillar preparations has been described by Perry (18). He noted that ATP inhibited the reaction when it exceeded the concentration of MgCl₂. In our preparation, inhibition did not occur until ATP levels greater than five times the MgCl₂ concentration were reached. However, Perry used 5 mM MgCl₂ as the activator while 1 mM MgCl₂ was used in these experiments. The inhibitory effect of increasing substrate concentration was similar in both normal and failing groups (Fig. 2).

ATPase activity was significantly lower in the failing group in four of the seven different ATP-MgCl₂ concentrations (Tables 7 and 8).

Our hypothesis was that the decrease in contractility of heart muscle in chronic congestive failure resulted from a diminution in myofibrillar ATPase activity. This was based on: 1) the demonstration of a decrease in contractility of actomyosin bands (10) and glycérinated fibers (11) from failing heart muscle; and 2) the constant association between the rate of ATP hydrolysis and the extent of shortening on tension development in muscle models (12, 19). The demonstration of a decrease in ATPase activity in failing hearts supports the hypothesis.

The mechanism underlying such a change in activity would seem likely, on the basis of this study, that one of the biochemical lesions in congestive heart failure following hypertension is located in the contractile mechanism itself. The apparent stimulus for this change is the increased pressure work which the heart must carry out in the disease.

A word of caution with respect to the difference in ATPase activity between normal and failing hearts must be injected at this point. It has been shown that age of the patient has no correlation with activity in the normals (ages 24-46) or the failures (ages 44-85). This was tested in each group at all seven different ATP-MgCl₂ concentrations. The data do not permit an assessment of the effect of age on normal or failing ATPase activity from ages 24 to 85.

The difference demonstrated then, is between "young" normals and "old" failures (Fig. 3). The absolute lack of correlation with age within each group at all ATP-MgCl₂ concentrations the data do not permit an assessment of the effect of age on normal or failing ATPase activity from ages 24 to 85.

The difference demonstrated then, is between "young" normals and "old" failures (Fig. 3). The absolute lack of correlation with age within each group at all ATP-MgCl₂ concentrations strongly supports the view that this is a difference due to a clinical history of congestive heart failure. Studies are now being performed to measure the myofibrillar ATPase activity of young failures and old normals. In addition a comparison must be made between hypertrophied hearts which are in failure and those which have compensated adequately.

We express our appreciation to the Departments of Pathology of Cook County and of Illinois Research Hospitals, and to the Cook County Coroner's pathologist, for making heart tissue available to us.

REFERENCES