Properties of Myocardium in Cardiomegaly

By Arthur F. Grimm, D.D.S., Ph.D., Ryo Kubota, B.S., and William V. Whitehorn, M.D.

The ventricular myocardium responds to a chronic increased work load by an increase in the myocardial mass. The functional significance of this adaptation has been an area of interest particularly because of the common clinical finding of an increased cardiac mass in cardiac failure. Since an increased myocardial mass is predominantly the result of the enlargement rather than the multiplication of the myocardial cells,^ some have suggested that this response may be detrimental since it must increase diffusion distances for metabolites. However, functional studies, such as those of Beznák, Dieckhoff, and Hasenfeld and Romberg have shown an increased cardiac work capacity with an increase in myocardial mass. Few studies have examined the basic properties of the enlarged myocardium. A preliminary report by the authors later supported by the findings of Kerr, Winterberger, and Giambattista suggested that the enlarged myocardium might even be capable of producing greater than normal tension per unit weight of muscle.

The present studies are an attempt to re-examine the question of the functional value of the enlarged heart with emphasis on the basic properties of the cardiac muscle itself. These studies attempted a quantitation and crude characterization of the contractile protein, actomyosin; the determination of the electrical and mechanical properties of surviving papillary muscles; and some histologic studies of their contractile systems.

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Methods

Three separate though complementary investigations were carried out on Sprague-Dawley albino male rats. Ventricular enlargement was produced either by chronic severe exercise on a treadmill or by subdiaphragmatic aortic constriction with a thick nylon ligature, a method similar to that of Beznák. The degree of constriction was predetermined by placing a rod 1.1 mm in diameter next to the aorta of 150 g rats, tying the ligature about the rod and the aorta until the aorta was completely occluded, and then withdrawing the rod.

The ventricular weights were compared with values predicted from a regression equation relating heart size and body weight in Sprague-Dawley rats. As seen in figure 1, this equation is based on randomly selected individual values from normal animals arranged so that there were 20 in each 100 g division of body weight between 100 and 500 g. Beyond a body weight of 100 g the body-ventricular weight relationship is rather linear and permits the derivation of the following equation (similar to one derived by Beznák):

\[ \text{Ventricular weight in g} = (197.2 + (1.869 \times 10^{-3} \times \text{Body wt in g}) \]

In these studies the ventricular weights of the animals were predicted by this formula and compared to the experimentally determined values. The differences were then expressed as a percentage of the predicted values.

Details of the procedures were as follows:

EXPERIMENT 1

Twelve control and 19 exercised litter mates were used. Exercising began when the animals weighed about 90 g and continued five days a week for about eight weeks. The rate and duration of running were progressively increased to a maximum of 65 feet per minute for four hours each day by the third week and maintained at this level for five weeks.

At the termination of the experiment the animals were killed and the hearts were removed and the ventricles freed of auxiliary tissue. The properties of one left ventricular papillary muscle were determined by using the method of Ullrick and Whitehorn. A small piece of the ventricles was dried to constant weight to determine the water content. The remainder was used for the isolation of the contractile protein, actomyosin, using with only minor modifications, the method of Benson, Hallaway, and Frier. The total...
ventricular protein concentration and the actomyosin concentrations were determined by the micro-Kjeldahl method. The actomyosin was characterized in Ostwald viscosimeters at 25°C by the viscosity response to ATP using the "ATP sensitivity" of Portzehl, Schramm, and Weber.  

\[
\text{"ATP sensitivity"} = \frac{Z_q - Z_{q,\text{ATP}}}{Z_{q,\text{ATP}}} \times 100.
\]

where \(Z_q\), the viscosity number = \(2.3 \log q_{\text{rel}}\)  

\(C\) (protein concentration).

where \(\log q_{\text{rel}}\) = the log of the relative viscosity; the ratio of the outflow time of the protein solution to that of the solvent.

The subscript ATP refers to the values obtained after the addition of ATP.

EXPERIMENT 2

Nine sham-operated control animals and 10 with aortic constriction were studied 3–6 weeks postoperatively. This study was limited to those techniques already described for the quantitation and characterization of the contractile proteins.

EXPERIMENT 3

Four groups of animals were employed in this study:

Group A: Weight-control; 12 sham operated animals. Group B: Exercised; 8 sham operated animals. Group C: Constricted; 9 animals with the aortic constriction. Group D: Constricted-exercised; 11 animals with aortic constriction, and exercised identically with B. Since the influence of exercise alone had already been investigated in experiment 1 and since this degree of exercise results in a marked weight loss, this study employed a younger non-exercising, non-constricted, sham-operated group, A, as a body weight control rather than the more usual age control. The other groups were litter mates. The operative procedures were carried out when the animals weighed about 150 g. The exercised animals, groups B,

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of animals</th>
<th>Terminal body wt, g</th>
<th>Terminal ventricular wt, mg</th>
<th>Per cent deviation from prediction</th>
<th>Ventricle per body wt, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>351 ± 19</td>
<td>820 ± 60</td>
<td>−3.9 ± 5.4</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Exercised</td>
<td>19</td>
<td>282 ± 30</td>
<td>847 ± 70</td>
<td>+18.6 ± 6.3*</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>291 ± 37</td>
<td>796 ± 81</td>
<td>−1.9 ± 7.1</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Constricted</td>
<td>10</td>
<td>288 ± 37</td>
<td>1049 ± 172</td>
<td>+42.6 ± 20.5*</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>254 ± 29</td>
<td>621 ± 58</td>
<td>−7.4 ± 6.0</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Weight control</td>
<td>8</td>
<td>279 ± 33</td>
<td>776 ± 40</td>
<td>+8.5 ± 8.3</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Exercised</td>
<td>9</td>
<td>358 ± 15</td>
<td>1083 ± 109</td>
<td>+25.0 ± 9.8</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Constricted-exercised</td>
<td>11</td>
<td>299 ± 39</td>
<td>997 ± 133</td>
<td>+31.5 ± 10.2</td>
<td>0.33 ± 0.03</td>
</tr>
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*"* "** vs. control < 0.001

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Mild I), ran for a period of three weeks after a 2–3 week post-operative convalescent period at a rate of 30 feet per minute for four hours, five days a week.

In addition to the experimental procedures described for experiment 1, the second papillary muscle of each left ventricle was examined with the light microscope. The muscles were tied in situ to an applicator stick, freed from the ventricle, and fixed with acrolein. Standard histologic procedures were then followed. The length of 10 adjacent sarcomeres was measured with an ocular micrometer. Forty of these measurements were made for each muscle examined and the means of the mean sarcomere lengths determined.

Results
The effects of the experimental procedures on body and ventricular weights are indicated in table 1. As expected, exercise resulted in significant reductions of body weight. No significant changes in body weight were associated with aortic constriction. Exercise produced a moderate though significant cardiomegaly. The degree of cardiomegaly was greater with chronic aortic constriction.

There were no significant changes in the electrical properties of surviving papillary muscles. Data are presented in figure 2 and table 2.

Length-tension relationships of muscles of experiment 1 are presented in figure 3. There were no differences in either developed or resting curves. Analysis of methods of expressing developed tension showed that, with this preparation, the best correlation exists between the developed tension and the wet weight of the muscle. Tensions were accordingly expressed in terms of unit weight. Data for experiments 1 and 3 are presented in table 3. Tension production of muscles of the exercised animals of experiment 1 did not differ from that of the controls. The litter mate groups of experiment 3 similarly showed no significant differences but the younger animals of group A showed a significantly greater tension production as compared to the other three groups. The mean

<table>
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<th>Table 2</th>
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<tbody>
<tr>
<td><strong>Refractory Periods</strong></td>
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<tr>
<td><strong>Mean Values ± sd</strong></td>
</tr>
<tr>
<td>No. of animals</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Exercised</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
</tr>
<tr>
<td>Weight control</td>
</tr>
<tr>
<td>Exercised</td>
</tr>
<tr>
<td>Constricted</td>
</tr>
<tr>
<td>Constricted-exercise</td>
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resting tensions per weight or cross-section of muscle did not differ between the groups.

Data on the chemical characteristics are presented in Table 4. There were no significant differences between groups in either the water or the total protein concentrations of the ventricles. The protein values are based on the nitrogen content of the samples as determined by the micro-Kjeldahl analysis with 6.25 as the conversion factor.

There were no significant differences in the actomyosin concentrations of experiments 1 or 3. In experiment 2, the actomyosin concentration was moderately but significantly elevated in the constricted animals when expressed as either a percentage of the wet ventricular weight or as a percentage of the total protein.

Table 5 lists the mean values of the standards used to qualitatively characterize the actomyosin. There were no significant changes in any of the parameters within experiments 1 or 2. In experiment 3, the weight-control group of younger animals had a statistically significant lower "ATP sensitivity." This was due to the significantly lower viscosity number prior to the addition of the ATP. After the ATP addition, the viscosities were the same for the 4 groups.

Estimation of the sarcomere length in histologic preparations of selected muscles of experiment 3 are listed in Table 6. No significant differences were found.

Discussion

Some comments are in order as to the degree of cardiomegaly present in these studies. Though the ventricular weight when expressed in absolute terms was only slightly increased, this increase when expressed in terms of body weight amounted to about 20%. The increase in ventricular weight with aortic constriction, both on an absolute and on a relative basis, amounted to about 30%-40%. These percentile differences are far short of those seen in some enlarged human hearts, and if such a general comparison can be made between mice and men, would fall below the "critical heart weight" of Linzbach1-2 and would lie in the range he designated as "physiologic hypertrophy."

The histologic studies gave clear evidence of hypertrophy of the papillary muscles. Moreover, in the few suitable cross-sections studied, this increase in the measured mean fiber cross-sectional area was proportional to the increase in myocardial mass if one assumes a symmetric increase in the mean cylin- drical myocardial cell. However, it is clear from Table 6 that cardiomegaly was accomplished without a change in the length of the individual sarcomere. This value of about 2.05 μ is in good agreement with that found by Hort.18

Cardiomegaly did not significantly alter the electrical properties of the papillary muscle bundle.
The general shape of the resting and developed tension curves of surviving muscle bundles were not modified by cardiomegaly. When suitable corrections were made for the size of the muscle bundles, cardiomegaly produced no significant differences in resting tensions. The ability of the isolated surviving muscle bundles to develop tension is of primary interest. Preliminary studies\(^7\) and the report of Kerr et al.\(^8\) suggested that tension production was enhanced in hypertrophied cardiac muscle. The studies reported here, however, do not support such a concept. Differences in tension production per unit weight between control and exercised muscle in experiment 1 were not significant. There were also no differences between the litter mate groups of experiment 3 though there was a marked difference in ventricular weights. These studies rather support the work of Bretschneider, Bücherl, Frank, and Husten\(^11\) who found no difference in cardiac work per gram of heart between normal dog heart lung preparations and those prepared from hearts.

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enlarged by the combination of aortic stenosis and exercise.

In experiment 3 the tension development of the experimental groups was significantly less than that of the younger weight controls. Kelly and Hoffman demonstrated a progressive fall in the developed tension per unit weight with increasing bundle weight. However, since there were no real differences in tension production in the three litter mates groups although the size of the muscle bundles varied, this interpretation does not seem to hold, at least within this relatively narrow weight range. The most obvious possibility is that this difference is somehow related to the age of the animal and that an increase in weight does not modify the ventricle's ability to produce a unit developed tension per unit weight of muscle.

The absolute magnitude of the developed tension is of interest. This amounted to approximately 200 g/cm², a value not too different from the estimates of Abbott and Monamaerts for the cat and rabbit papillary muscle and Linzbach for the human ventricle. It is also in fair agreement with the values obtained by Briggs for the glycinated trabecular muscle of dogs. As pointed out by Briggs and others, these values are well below the tensions reported for skeletal muscles.

It was hoped that the chemical isolation of the contractile proteins would make it possible to define the myocardial contractile capabilities more precisely. The absolute values for the myocardial actomyosin content are somewhat lower than other estimates (Benson, Davis et al., Meerson et al., Sobel and Cohen). These differences are partially due to the lack of correction for non-protein nitrogen. Differences in extraction techniques probably account for the remaining dissimilarities. The importance of slight technical modifications is illustrated by the differences in values of experiments 1 and 2.

Cardiomegaly results in little or no change in the ventricular actomyosin concentration. Although aortic constriction produced a slight but statistically significant elevation in experiment 2, there were no differences between the groups in any of the other experiments. Changes in the total ventricular protein concentration were similarly lacking.

The characteristics of the isolated actomyosin in experiments 1 and 2 and in the litter mate groups of experiments 3, were not altered. In experiment 3, the weight-control group of younger animals showed a significant reduction of ATP sensitivity. This was obviously due to the lower viscosity per unit protein prior to the addition of ATP, a finding that may indicate a reduced actomyosin content. Since the viscosity numbers after ATP were identical, it would seem that this may be the result of a reduced actin content in the younger muscles, a finding supported by unpublished studies in this laboratory. It may be more than coincidental that the group with the reduced "ATP sensitivity" is also the group with the greater developed tensions per unit of muscle weight.

Summary

The adult rat's ventricular myocardium is able to increase its mass markedly while maintaining its unit quality. It does so by maintaining constant the design of the sarcomeres: an increase in length is accomplished by the addition of sarcomeres in series; an increase in tension production is accomplished by the addition of more cross-sectional area of a uniform quality. This was shown by the almost constant concentration of the contractile protein, actomyosin, as well as by the histologic evidence of the constancy in the sarcomere lengths. Functional support was obtained by the finding of an identity

**TABLE 6**

<table>
<thead>
<tr>
<th>Sarcomere Lengths</th>
<th>Mean Values ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of animals</strong></td>
<td><strong>Mean sarcomere length (in micra)</strong></td>
</tr>
<tr>
<td>Weight control</td>
<td>6 2.05 ± 0.13</td>
</tr>
<tr>
<td>Exercised</td>
<td>4 2.08 ± 0.06</td>
</tr>
<tr>
<td>Constricted</td>
<td>4 2.07 ± 0.03</td>
</tr>
<tr>
<td>Constricted-exercised</td>
<td>4 2.06 ± 0.13</td>
</tr>
</tbody>
</table>
in the parameters of the length-tension curves; the curves differed only in the absolute magnitude of the tensions, a difference that completely disappeared when suitable corrections were made for the size of the muscle. The electrical parameters also indicated a lack of change in the quality of the excitatory membrane phenomena. However, some data were presented that suggest that the myocardium may show altered properties dependent on the age of the animal. No evidence was found in support of the concept of detrimental consequences at least with this degree of cardiomegaly. It is rather concluded that this degree of cardiomegaly is accomplished without change in the basic architecture, properties, or concentration of the contractile mechanism.

References
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