Disorder in Excitation-Contraction Coupling of Cardiac Muscle from Cats with Experimentally Produced Right Ventricular Hypertrophy

By Raimund L. Kaufmann, Hermann Homburger, and Heike Wirth

ABSTRACT

The contractile and electrical activity of papillary muscles from hypertrophied right ventricles of cats with artificial stenosis of the pulmonary artery was investigated. Contractility was considerably decreased along the entire force-velocity relationship, whereas no measurable alterations could be detected in the electrical activities as recorded by intracellular microelectrodes. By supramaximal Ca²⁺ activation, the contractility of both the hypertrophied and the normal control preparations was increased to about the same final value. These findings are consistent with the concept that a disorder in the mechanism of excitation-contraction coupling underlies the depressed contractile state of hypertrophied cardiac muscle. In addition, it could be shown that the increase in volume of each cellular unit is clearly related to the decrease in contractility. This can tentatively be explained by the following assumptions. If the amount of Ca²⁺ entering the cell per unit area is not changed in hypertrophy, then in a cell of increased diameter, the amount of Ca²⁺ distributed per unit cell volume will be diminished. Since the excitation-contraction coupling of the heart is very sensitive to Ca²⁺, this Ca²⁺ deficit should be reflected in a depression of contractility.

KEY WORDS contractile system calcium cell volume force-velocity relation cell surface

In the past, most investigations concerning the functional state of the chronically hypertrophied heart muscle were done in the beating heart in situ. In this way the measurable variables of the heart's mechanical activity could be related to the performance of the whole heart as a pump but not to the contractile state of each functional unit, that is, of the single myofibril within the cell. Recent investigation of the mechanics of isolated papillary muscles obtained from cats with experimentally produced right ventricular hypertrophy (1) showed clearly for the first time that there was a considerable decrease in the isometric tension developed as well as in the maximum velocity of shortening.

The finding of diminished contractility in the cellular unit of the chronically hypertrophied myocardium raises the question of what kind of altered basic function might be responsible for the decrease in contractile performance. There are at least four points at which the disturbance could theoretically be attributed: (1) the level of the transmembrane electrical potentials, (2) the process of excitation-contraction coupling, (3) the contractile machinery itself and (4) the energy-producing chemical system.

According to a concept which Fleckenstein and co-workers (2-6) outlined from a study of the metabolic aspects of acute experimental heart failure, contractile heart failure might be related mainly to a disturbance in the excitation-contraction coupling or to disorders in the energy supply system. In the first, which is characterized by a high muscular
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content of energy-rich phosphates, the depressed contractility can be restored by improving the excitation-contraction coupling, as by adding Ca\(^{2+}\). In the second, in which the stores of the energy-rich phosphates are exhausted because of anoxia or metabolic poisons, Ca\(^{2+}\) is not effective in restoring the diminished contractility. According to these criteria, one may assume that, in chronically hypertrophied heart muscle (where the content of energy-rich phosphates is at least not considerably diminished), the depression of the contractility might be due to a disorder in excitation-contraction coupling rather than to an exhaustion of the energy supply system (3, 4). Until now, however, direct experimental proof for this idea has not been available.

Methods

Production of Right Ventricular Hypertrophy.—Male and female adult cats weighing 1.5 to 2.5 kg were anesthetized with sodium pentothal (10 to 15 mg/kg, iv or ip). For muscular relaxation, succinylcholine (0.5 to 1.0 mg/kg iv) was given, and respiration was supported by intermittent endotracheal positive pressure. The left side of the thorax was opened in the fifth intercostal space. A flexible Teflon tube (outer diameter 0.6 mm) was wound around the root of the pulmonary artery, and a closed loop was formed by pulling the ends of the Teflon tube through a close-fitting Teflon sleeve. With further pulling, the loop was constricted to an inner diameter of about 2.5 to 3.5 mm, narrowing the pulmonary artery to 10 to 20% of its original cross-sectional area.

Material and preparation.—One to 80 days after the operation, the cats were killed under light ether anesthesia. The hearts were then rapidly excised and transferred into an oxygenated Tyrode solution. The right ventricle was opened and papillary muscles were removed and immediately transferred to the muscle chamber, which was perfused with a well-oxygenated Tyrode solution at 31°C. The muscles were stimulated with supramaximal stimuli at 24/min. The weight of the right ventricle (without the septum) and of the left ventricle (with septum) was determined after the atria were cut away.

Before some of the animals were killed, right ventricular pressure curves were recorded in control animals as well as in those operated on. This was done by putting a flexible catheter into the right ventricle via the right jugular vein and using a Statham pressure transducer to record onto a Hellige Multiscriptor.

Force-Velocity Measurements.—The main characteristics of the special device for force-velocity measurements in small heart muscle preparations are shown in Figure 1. Briefly, the lower end of the papillary muscle was fixed to the elongated pin of a force transducer tube (RCA 5734) by suction. The tendinous end was attached to the lever of an isotonic displacement transducer. This transducer was made from an old-fashioned coil-type galvanometer, in which the reset spring was detached and the lever arm was replaced by a device to hold the tendinous end. By the use of a controlled d-c current flow through the coil, a force of up to 10 g could be generated at the tip of the lever, and this force contributed the muscle load. Adjustable screws allowed fixation of the initial muscle length for isotonic or isometric measurements. Displacement of the isotonic lever arm was detected by a photoelectric system, the electrical output of which was linear over a range of about 3.5 mm displacement of the lever tip. The maximal rise time for linear recording was about 1500 cm/sec, and the equivalent mass of the whole movable system was about 350 mg. The active overall length of the muscle and its diameter were determined by a micrometer in the eyepiece of an observation microscope.

Action Potential Recording.—Intracellular potential recordings were done in the usual way. Microelectrodes (tip diameter less than 1 μ) were freely suspended on the end of a thin silver wire attached to the lever of a micromanipulator. In this way they could easily follow the displac-
ment of the contracting muscle. The resistance of
the microelectrodes used were in the range of 10
to 30 megohms.

Recording System.—All data were simulta-
neously displayed on a four-channel storage
oscilloscope (Tektronix 564). The shortening
velocity was measured by electrical differentiation
of the output of the isotonic length transducer. A
cathode follower was used for impedance trans-
formation of the microelectrodes.

Light Microscopy.—For histological purposes,
parts of the right ventricular wall and small
papillary muscles were initially fixed for 24 hours
in a buffered 10% formaldehyde solution and then
transferred into a solution of 4% formaldehyde.
The papillary muscles were pinned down on a
small piece of Teflon at the length they assumed
in the muscle chamber at a load of 0.3 g/mm².
The preparations were dehydrated in alcohol and
embedded in paraffin. Sections of 4 to 6 µm
were prepared in the longitudinal and in the transverse
axis of the fibers and were stained according to
Goldner's method.

Solutions.—The composition of the Tyrode
solution used in these experiments was (mM):
NaCl 136.9; KCl 2.68; NaHCO₃ 11.9; CaCl₂,
2.5; NaH₂PO₄ 0.42; glucose 5.6. The Ca-rich
solution in which 25 g sucrose/liter replaced 25%
of the normal sodium content contained 11.0 m
Ca²⁺.

Results
The pulmonary artery was constricted in
113 cats. Fifty animals died during the
operation or within the first 24 hours, and no
studies were made. Twelve animals died 1 to 3
days after the operation; their ventricular
weights and the dry weight-wet weight ratio
were determined but no other experimental
analysis was carried out. The remaining 51
animals were considered as the experimental
group, and the results were compared to those
of a control group consisting of 25 cats with
no operation and 10 with sham operations.

Analysis of the muscle mechanics of the
animals with sham operations gave results
identical to those from cats with no operation.

The papillary muscles chosen for force-
velocity measurements were in a limited range
of 5 to 8 mm in length and 0.8 to 1.4 mm in
diameter, though it was recently shown that
the force developed per unit area is consider-
ably greater in small preparations than in
larger ones. It was not always possible to find
papillary muscles of suitable size, particularly
in each hypertrophied heart because the cross-
sections of the hypertrophied muscles (aver-
age 1.29 ± 0.19 mm² [SE]) were larger than
the control preparations (0.91 ± 0.11 mm²).

For comparative analysis of muscular mechan-
nics, muscles could thus be obtained from only
23 of the experimental group and 24 of the
control group. In all, 30 papillary muscles
excised from 23 hearts with right ventricular
hypertrophy were investigated and compared
with the results of 24 muscles excised from 24
hearts of the control animals. Mean data of
muscle length, diameter, and cross section of
the muscles selected for force-velocity analysis
are given in Table 1; there were no significant
differences between the size of the papillary
muscles chosen from the control and the
experimental hearts.

In the upper part of Figure 2, the degree of
right ventricular hypertrophy, as defined by
the ratio of right ventricle to body weight, is
plotted against the duration of the postopera-
tive interval. It is clearly shown that shortly
after the operation there is a considerable
increase in right ventricular weight. This
figure also contains the values from the
animals that died within the first three days

<p>| Muscle Diameter, Length, and Cross-Section Area of Papillary Muscles of Control and Hypertrophied Hearts |
|---------------------------------------------------------------|---------------------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>Length (mm)</th>
<th>Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Hypertrophy</td>
<td>Control</td>
</tr>
<tr>
<td>1.07 ± 0.10 (n = 24)</td>
<td>1.22 ± 0.14 (n = 25)</td>
<td>6.2 ± 0.3 (n = 26)</td>
</tr>
<tr>
<td>P &gt; 0.3</td>
<td>P &gt; 0.2</td>
<td>P &gt; 0.3</td>
</tr>
</tbody>
</table>

Values are means ± 1 SE.

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ventricular weight seemed to be due to tissue edema rather than to a true muscular hypertrophy (see wet weight/dry weight, Fig. 2).

In four experimental and in three control animals, right ventricular pressure curves were recorded. In the four experimental cats the systolic peak pressure was considerably elevated (Table 2). The end-diastolic pressure was significantly increased only in cat 6.

**Myocardial Mechanics**

The preparations were stimulated at a frequency of 24/min. Initial muscle length (l₁) was adjusted by a small preload of 0.3 g/mm². At this length the muscle was suitably stretched. This initial muscle length was kept constant during the whole force-velocity analysis. By step increases in the afterloads, the force-velocity relation was determined by plotting the maximal velocity of shortening (in terms of resting muscle length (l₀) per second) against the corresponding load (g/mm²). Vₚ₅₀ in the sense defined by A. V. Hill could be estimated only by extrapolating the force-velocity curve to zero load. To define the left end of the measured force-velocity relation, i.e., the velocity of shortening at the smallest load (0.3 g/mm²), the term V₀.₃ was introduced.

Figure 3 contains all the force-velocity data from both the experimental and the control groups. Only papillary muscles of animals with a ratio of right ventricle to body weight of more than 1.2 are represented in this figure. We find, in accordance with the results of others (1), that the contractility of the hypertrophied preparations is significantly depressed by about 50% along the entire force-velocity curve. The values of both variables, V₀.₃ and P₀ are markedly below normal, averaging 0.9 l₀/sec and 2.2 g/mm², respectively, as compared with 1.4 l₀/sec and 3.9 g/mm² in control muscles.

Intracellular action potentials recorded from six papillary muscles of the experimental group were compared to those of six control muscles. No measurable disturbance of the electric activity was detected in the hypertrophied preparations (Table 3).

These results make it less likely that the depressed contractility of the hypertrophied heart muscle is due to some alteration of the biochemical events. To test the possibility that

<table>
<thead>
<tr>
<th>RV/body wt (g/kg)</th>
<th>Peak syst. press. (mm Hg)</th>
<th>End-diast. press. (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>0.84</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>0.72</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>1.05</td>
<td>62</td>
<td>5</td>
</tr>
<tr>
<td>1.17</td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>1.62</td>
<td>83</td>
<td>15</td>
</tr>
<tr>
<td>1.76</td>
<td>81</td>
<td>6</td>
</tr>
</tbody>
</table>

*Comparison of Peak Systolic and End-Diastolic Pressures in Four Experimental and Three Control Cats*
the contractile insufficiency might be the result of a weakness of the contractile proteins, force-velocity measurements were carried out in a Ca-rich medium containing 11 mM Ca and only 75% of the normal sodium content. In this solution, the contractile system of isolated cardiac preparations is activated to the extent that no further increase of tension development or shortening velocity could be brought about by the usual positive inotropic interventions (7, 8). If the depression in contractility of hypertrophied muscles arises from a weakness of the contractile proteins, then a muscle working at the saturation point of Niedergerke's (9, 10) Ca-tension curve should develop considerably less mechanical activity with respect to the control preparations.

However, as can be seen from Figure 4, the force-velocity curve of the hypertrophied muscles after half an hour in the Ca-rich solution is only slightly below that of the control preparations. For example, the hypertrophied preparations contracting against a load of 1 g shortened with an average velocity of 1.34 cm/sec as compared with 1.59 cm/sec in the control group (a difference of 16%). In normal Tyrode's solution, the corresponding values were 0.42 cm/sec in the experimental and 0.98 cm/sec in the control groups, a difference of 54%.

The modest but still significant difference between the maximal contractile performance of the hypertrophied and the control preparations, particularly along the lower portion of the force-velocity curves can be mostly attributed to an increase in connective tissue in the total mass of hypertrophied muscle. Under our conditions, the percent of noncontractile tissue was augmented from a 5.5% cross-sectional area in control muscles to 15% in hypertrophied preparations. If the force-velocity data were corrected for a 10% reduction in active cross-sectional area in hypertrophied preparations, the contractility

<p>| TABLE 3 |
|---------------------|---------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Muscle type</th>
<th>Resting potential (mV)</th>
<th>Upstroke velocity (cm/sec)</th>
<th>Recruitment (mV)</th>
<th>Active potential after repolarisation of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10% (mV)</td>
</tr>
<tr>
<td>Normal Muscles</td>
<td>83 = 1.3</td>
<td>165 ± 5.5</td>
<td>22 ± 0.7</td>
<td>94 ± 6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertrophied Muscles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>21</td>
<td>83</td>
<td>295</td>
<td>295</td>
</tr>
<tr>
<td>82</td>
<td>150</td>
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<td>82</td>
<td>275</td>
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<td>86</td>
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<td>98</td>
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<td>320</td>
</tr>
<tr>
<td>84</td>
<td>175</td>
<td>96</td>
<td>290</td>
<td>316</td>
</tr>
<tr>
<td>80</td>
<td>18</td>
<td>93</td>
<td>275</td>
<td>310</td>
</tr>
<tr>
<td>Mean</td>
<td>82</td>
<td>159</td>
<td>21</td>
<td>92</td>
</tr>
</tbody>
</table>

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Excitation-contraction coupling

Force-velocity relations of 15 hypertrophied papillary muscles (broken lines) and 18 control preparations (solid lines). Measurements were first made in normal Tyrode’s solution (Ca\(^{2+} = 2.5 \text{ mM}\)) and again after equilibration for 30 min in a Ca\(^{2+}\)-rich, Na-reduced medium (Ca\(^{2+} = 11 \text{ mM}, \ Na = 75\% \text{ of normal content}).

The velocity of contraction of these muscles was still lower, but no longer significantly different from the contractile state of the control muscles (Fig. 5).

These results do not fit into the assumption of an alteration within the contractile proteins but are more indicative of a change in excitation-contraction coupling.

Histo logical results

Length and diameter of myocardial fibers were determined by light microscopy in control and hypertrophied preparations. Figure 6 compares the data of 36 hypertrophied papillary muscles with the corresponding averages of the control hearts (hatched bands). It can be seen from this figure that the fiber length (as defined by the distances between the intercalated discs) increased very rapidly after constriction of the pulmonary artery and reached a final value of about 60\(\mu\) 30 days after operation. The mean fiber length of the control muscles was 36 ± 1.1\(\mu\) SE. Also, the diameter of the myocardial fibers increased significantly but rather slowly after operation. Distribution of the measured variables is given in Figure 7. After 38 postoperative days, the fiber diameter ranged between 16.4\(\mu\) and 26.5\(\mu\) as compared with a mean of 8.1 ± 0.4\(\mu\) in control preparations. From the data of Figure 6, the surface area and the volume of the myocardial cells were calculated and plotted against the postoperative interval; the results are given in Figure 8. In hypertrophied cardiac cells, the surface-to-volume ratio is considerably changed in favor of the cellular volume. In normal control preparations, the average surface-to-volume ratio of myocardial fibers was 0.63 × 10\(^8\) (\(\mu^3\)) to 1.26 × 10\(^8\) (\(\mu^3\)), a relation of 1\(\mu^2\) to 2\(\mu^2\). Forty days after pulmonary artery constriction, the surface-to-volume ratio was 4 × 10\(^8\) (\(\mu^2\)) to 20 × 10\(^8\) (\(\mu^2\)), a relation of 1\(\mu^2\) to 5\(\mu^2\). We believe that these changes might be an important factor leading to the depression of contractility.

The increase of cellular volume from 1.26 × 10\(^8\) (\(\mu^3\)) to about 2 × 10\(^8\) (\(\mu^3\)) means that in the hypertrophied heart muscle each cellular unit has increased its mass about 15 times. Such extreme values were found only in the papillary muscle preparation (Fig. 9). In other parts of the right ventricular wall, both the myocardial fiber length and diameter seemed only moderately increased. Until the present, however, histological data from different parts of the hypertrophied right ventricle have not been systematically collected.

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Constitutive muscular mass, wall thickness, ventricular radius, and fiber arrangement. In analyzing systolic peak pressure, $dP/dt$, stroke volume, and other hemodynamic variables we cannot be sure about the contractile state of the single cellular unit. However, of the multiple causes of heart failure, one rather simple mechanism may be found in some acute conditions. For instance, acute ischemia, hypoxia, or metabolic poisons inhibit or restrict the supply of chemical energy in each or some of the myocardial cells and contractile insufficiency results. In contrast to such acute forms of heart failure, little is known about the mechanism underlying chronic cardiac hypertrophy and subsequent failure.

The experimental and theoretical approaches to the problem of myocardial hypertrophy and congestive heart failure have been countless during the last four decades. Abundant but mostly controversial results have been obtained. "Therefore," as Katz (11)

Preliminary results of a subsequent ultra-microscopic study comparing hypertrophied and control muscles under our experimental conditions (initial muscle length adjusted always by 0.3 g/mm preload) showed no differences of sarcomere length. The average of 72 measurements in six preparations was $2.12 \pm 0.05 \mu$ (se) for controls and $2.1 \pm 0.05 \mu$ for hypertrophied muscles.

**Discussion**

Following the conventional usage we can define heart failure as the inability of the heart to pump sufficient amounts of blood. From the viewpoint of clinical cardiology this is an acceptable and practicable definition but provides a rather "imprecise definition of the true functional state of the myocardium" (11). The performance of the whole heart as a pump depends mainly on factors such as muscular mass, wall thickness, ventricular radius, and fiber arrangement. In analyzing systolic peak pressure, $dP/dt$, stroke volume, and other hemodynamic variables we cannot be sure about the contractile state of the single cellular unit. However, of the multiple causes of heart failure, one rather simple mechanism may be found in some acute conditions. For instance, acute ischemia, hypoxia, or metabolic poisons inhibit or restrict the supply of chemical energy in each or some of the myocardial cells and contractile insufficiency results. In contrast to such acute forms of heart failure, little is known about the mechanism underlying chronic cardiac hypertrophy and subsequent failure.

The experimental and theoretical approaches to the problem of myocardial hypertrophy and congestive heart failure have been countless during the last four decades. Abundant but mostly controversial results have been obtained. "Therefore," as Katz (11)
The calculated cellular surface area (•) and volume (○) of hypertrophied papillary muscles plotted against the postoperative interval. The solid horizontal line represents the average cellular volume of normal control preparations; \( V_{\text{cell}} = 1.26 \times 10^4 \, \mu m^3 \). The broken line indicates the cellular surface area of normal control preparations; \( S_{\text{cell}} = 0.38 \times 10^4 \, \mu m^2 \). Left ordinate = cellular volume \( \times 10^4 \, \mu m^3 \), right ordinate = cellular surface \( \times 10^4 \, \mu m^2 \).

Pointed out, "it is not possible to define an unequivocal mechanism to account for a decrease in the contractile force of the hypertrophied myocardium" found in the present and some recent investigations (1, 12, 13). We believe that it is beyond the aim of the present paper to reconsider extensively the different concepts applied to the problem of cardiac hypertrophy in the past. Only the main features of the most discussed ideas will be outlined again before a new working hypothesis is proposed on the basis of the present experimental findings.

Among the ideas which have heretofore strongly attracted the attention of many cardiologists, is a hypothesis, first proposed by Eppinger (14), that is based on the concept of a cellular hypoxia, which was thought to be the result of a disproportion between the vascularization and the increasing muscular mass or was attributed to an impairment of the oxygen diffusion within the hypertrophied cellular unit. Many results (mainly biochemical) failed to prove the existence of a hypoxic situation in the chronically hypertrophied heart (15-17). However, some other findings (mainly morphological) strengthen the idea of altered cardiac energetics due to hyper-...
trophy. For example, Wollenberger et al. (18) and more recently Poche et al. (19) and Novi (20) determined the relation between mitochondria and myofibrils in heart cells after hypertrophy caused by training or hypertension. They showed that during experimental hypertrophy induced by an increase in peripheral resistance there was a significant reduction in the ratio of mitochondria to myofibrils. However, in a later stage of the chronically hypertrophied myocardium, the relation appears to return to normal (13, 18, 21).

A lot of experimental work has also been done to find the crucial factor in the contractile proteins themselves. Direct measurements of the actomyosin content of the hypertrophied or failing heart mostly failed to show a significant decrease in the amount of extractable contractile proteins (12, 22, 23). On the ultrastructural level, abnormalities in the lattice or structures of contractile filaments could not be demonstrated (24-26). Finally, it appears that at least no gross physicochemical alterations exist in the contractile proteins from hypertrophied or failing hearts (27-31).

Our own results also indicate that the ability of myofibrils to generate tension or shortening is unchanged as long as sufficient calcium is provided for the activation of the contractile system. However, one has to take into account that, in the hypertrophied cardiac muscle, the affinity of troponin for calcium may be reduced. In such a case, the contractility of both hypertrophied and control preparations would be the same in a medium in which troponin is saturated with Ca$^{2+}$ anyway, but would be different at a normal extracellular Ca$^{2+}$ concentration (as in our results).

Also the energy supply system seems not to be critically affected. If the energy supply were the limiting factor of the mechanical output, then the contractile insufficiency should become more evident in a fully calcium-activated preparation. In our results this is not the case. This agrees with measurements of the content of energy-rich phosphates in chronically hypertrophied hearts, in which, under various experimental and pathophysiological conditions, no significant reduction of the energy stores could be found (32-35). The utilization of high-energy phosphates in experimental heart failure was reduced, but only in relation to the reduction in contractile element work (36). It was concluded from this experiment that a normal efficiency of energy conversion is preserved in hypertrophied or failing hearts. Therefore, as Fleckstein suggested, the possibility arises that the main disturbance underlying the depressed contractility of hypertrophied myocardium is located within the chain of cellular events which precede and control the transformation of chemical energy to mechanical work, i.e., the mechanism of excitation-contraction coupling (2, 4, 5).

Before discussing this idea, we should deal with some other possibilities not yet mentioned which might be responsible for the decreased contractility found in the hypertrophied muscles. The observed shift of the force-velocity curve may tentatively be explained by a shorter sarcomere length. However, sarcomere lengths in the hypertrophied heart are normal (12, 20, 37, 38). Also, in our experiments no differences in sarcomere spacing could be detected (2.12\mu m and 2.10\mu m). Another factor to be considered is the cardiac force-frequency relation which might exhibit altered characteristics after hypertrophy. The frequency response of isolated papillary muscles from hypertrophied cat hearts was investigated by Spann et al. (1). From their results it appears that the main features of the force-frequency relation are unchanged in hypertrophied muscles but develop on a generally depressed level of contractility.

Despite the already intricate situation, we wish to outline now a new working hypothesis explaining many experimental findings besides those shown in the present paper.

Excitation-contraction coupling in the heart critically depends on the amount of Ca$^{2+}$ available to the cell from the extracellular space. However, according to recent findings, the Ca$^{2+}$ entering the mammalian cardiac cell during excitation is probably not directly involved in the activation of the contractile
proteins but seems first to exchange with an internal compartment, probably the calcium-storing system of the sarcoplasmic reticulum (39-41). From these compartments of the sarcoplasmic reticulum which closely surround each myofibril, Ca\(^{2+}\) that initiates the contractile process is released by a mechanism as yet unknown.

In contrast to skeletal muscle, the total volume of the myocardial sarcoplasmic reticulum seems to be rather modest (42). Thus any changes of Ca\(^{2+}\) passing across the plasma membrane should subsequently be reflected—with a few seconds delay—in the amount of calcium available from the stores of the sarcoplasmic reticulum. This calcium, which is available from the sarcoplasmic reticulum at a given moment, finally determines the active state of the heart.

Keeping this model in mind, we may ask how and where in the hypertrophied heart cell the calcium-mediated excitation-contraction coupling might become defective. First, from the electrophysiological measurements, there is no evidence that in hypertrophy the ionic conductivity of the plasma membrane is altered in a way which could explain the depressed contractility. Further, Poche et al. (19) have shown that during hypertrophy the volume of the sarcoplasmic reticulum increases in proportion to the total cell volume. That means that a disproportion between the masses of myofibrils and sarcoplasmic reticulum is unlikely to be the cause of the suggested disturbance in excitation-contraction coupling. It seems more likely that the first step in our model—the calcium supply from the extracellular space into the cell—is impaired. This can be accepted if one takes into account the following arguments. If, in hypertrophy, the amount of Ca\(^{2+}\) entering the myocardial cell across the membrane per unit area is not markedly changed, then, in a cell of increased diameter, the amount of calcium distributed per unit volume will be diminished. This is based on the simple mathematical fact that, in a cylinder of a given length but of increasing radius, the volume increases by the square of the radius, whereas the surface is only proportional to the radius. Therefore, in a hypertrophied heart cell, whose diameter has markedly increased, the surface-to-volume ratio is decreased. Our histological data show that this ratio falls from 1.2 in control muscles to 1.5 in hypertrophied fibers (Fig. 8). Consequently, the amount of Ca distributed per unit cell volume should be about 40% of the calcium supply in cells of control preparations. Since the contractile performance in the heart is roughly proportional to the calcium supply (9, 10), a reduction of contractility to the same degree is expected under our experimental conditions.

However, one may argue that the effect might be exactly compensated by changes in calcium efflux which would also be expected to result from a decrease of the surface-to-volume ratio. Therefore, to make the proposed mechanism work, one must assume that the calcium efflux "grows" in proportion to the surface area. Such a disproportion between calcium influx and efflux in growing cardiac cells is, of course, as yet purely speculative. However, one may postulate that the way calcium leaves the cell is different from its way of entry. For instance, the pumping mechanism could be located in the transverse tubular system, which, in fact, seems to grow in proportion to the volume (43).

Finally, one may ask whether the in vitro preparation has already inadequate excitation-contraction coupling compared with the in vivo myocardium and whether hypertrophied muscles, although showing not much of an insufficiency in vivo, are likely to develop a larger degree of coupling insufficiency than normal muscles when they are beating in a tissue bath. This question arises from comparative studies of the mechanical performance in the excited and in the in situ papillary muscle (44, 45). The experiments indicate that the strength of contraction developed by papillary muscles in contact with their normal blood supply cannot be increased drastically by paired pulse stimulation or a higher stimulation rate. On the other hand, excised papillary muscles developed much less tension in
Tyrode's solution but could be made to contract about as strongly (5 g/mm²) in vivo as in vitro by increasing [Ca], or decreasing [Na]. At the present stage, our results do not exclude the possibility that the contractile weakness of hypertrophied muscles might be found only in vitro. We believe, however, that such an explanation is rather unlikely, for there is no evidence of a coupling insufficiency, at least in our control preparations, when compared with the in vivo myocardium. The only small difference between the tension developed in Kavaler's preparation (in vivo myocardium) and our in vitro control muscles (5 g/mm² as compared to 4 g/mm²) has to be seen in the light of the cardiac force-frequency relation. At a stimulation rate of 24/min the isometric force developed is of course lower than at a higher rate of 60 to 80/min. When force-frequency measurements were made in our in vitro preparations, a still higher performance (6 to 8 g/mm²) than that in Kavaler's preparation was obtained at the same stimulation rate.

References


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Circ Res. 1971;28:346-357
doi: 10.1161/01.RES.28.3.346

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

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