Relationship between Myosin Isoenzyme Composition, Hemodynamics, and Myocardial Structure in Various Forms of Human Cardiac Hypertrophy

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SUMMARY. Hemodynamic and angiographic parameters, muscle fiber diameter, nonmuscle tissue content, and myosin light chain isoform composition were determined in the left ventricle of nine patients with primary (four with hypertrophic, five with dilated cardiomyopathy) and 27 patients with secondary hypertrophy (11 with aortic regurgitation, 16 with aortic stenosis), nine patients with coronary heart disease, and seven controls. In various forms of hypertrophy, a new atrial-like light chain 1 occurred in two-dimensional electrophoresis of total tissue homogenates amounting up to 29% of total light chain 1. Total light chain 1 content remained constant in all groups when related to tropomyosin. The mean content of this atrial light chain 1 was highest in dilated cardiomyopathy (12.1%), less in cases with pressure (6.4%) and volume overload (2.9%), but as low in hypertrophic cardiomyopathy (0.3%) as in controls (0.4%). In cases with coronary heart disease without prior infarction, it was lower (0.6%) than with infarction (1.9%). Its occurrence was not affected by digoxin administration. In ventricular myocardium, an atrial-like light chain 2 was never observed. Peptide patterns after limited proteolytic digestion of isolated myosin heavy chains from cases with pressure overload and hypertrophic cardiomyopathy were identical to those from controls. The content of the atrial-like light chain 1 was not correlated to either muscle fiber diameter or nonmuscle tissue content, both of which were increased in all hypertrophy groups. In individual cases, no firm correlation could be established between atrial-like light chain 1 content and various parameters of ventricular load and function. However, a significant correlation resulted when the mean values of atrial-like light chain 1 content of each disease group were related to the respective mean values of peak circumferential wall stress (r = 0.96). Thus, the shift of myosin light chain 1 isoforms in ventricle seems to characterize biochemically the hypertrophy process induced by mechanical stress. (Circ Res 57: 729-740, 1985)
an α,β-heterodimer, or a β,β-homodimer which correspond to the V-1, V-2, and V-3 isoforms described by Hoh and coworkers (1978). V-1 exhibits higher adenosine triphosphatase (ATPase) activity than V-3 (Hoh et al., 1978; Pope et al., 1980; Lompre et al., 1981; Litten et al., 1982). The ratio of these myosin isoforms varies according to the physiological and pathological state of the myocardium (for reviews, see Mercadier et al., 1983; Tobacman and Adelstein, 1984). The change from V-1 toward V-3 is accompanied by a decrease in ATPase activity and speed of contraction (Schwartz et al., 1981; Ebrecht et al., 1982), an improved economy of force generation (Alpert and Mulieri, 1981; 1982), and decreased oxygen consumption (Kissling et al., 1982). These changes in myosin HC composition found in animals were interpreted as an adaptation of the myocardial cell, together with compensatory hypertrophy of the muscle, to new functional requirements.

In man, however, no clear correlation has as yet been established between myosin HC isoform composition, ATPase activity, and contractility. Normal human ventricular tissue contains predominantly the V-3 species (β,β-homodimer) and only few amounts of the V-1 species (α,α-homodimer) (Mercadier et al., 1983; Gorza et al., 1984; Bouvagnet et al., 1984), and this composition does not seem to change in hypertrophy (Mercadier et al., 1983; Gorza et al., 1984). Yet, myofibrillar ATPase activity was reported to be decreased in the hypertrophied failing heart (Alpert and Gordon, 1962; Leclerq and Swinghedauw, 1976; Peters et al., 1977), a finding which could not be confirmed, however, by examining isolated myosin and its subfragments activated by pure actin (Maron et al., 1977; Schier and Adelstein, 1982). Thus, there is still a missing link between the altered contractile properties of the hypertrophied human ventricle and possible corresponding changes on the molecular level involving the contractile proteins in particular. Changes in myosin ATPase activity in hypertrophied ventricle are therefore difficult to be explained unless changes in protein subunits other than the myosin HC are occurring.

The present study focuses on changes in ventricular LC isoform composition in relation to workload and functional states in various forms of primary and secondary hypertrophy in man and, thus, may contribute to the understanding of adaptive mechanisms of the human heart.

Methods

Patient Population

Myocardial biopsy specimens of 52 patients were analyzed. There were 37 men and 15 women ranging in age from 23–72 years. All patients were evaluated clinically and by invasive methods except four of seven controls who died from traffic accidents and in whom the biopsy material was obtained within 8 hours postmortem. Based upon the clinical, hemodynamic, and angiographic findings, the patients were assigned to the following groups: primary hypertrophy due to hypertrophic (four patients) or dilated cardiomyopathy (five patients), secondary hypertrophy due to pressure (16 patients) or volume overload (11 patients), coronary heart disease (nine patients, four without and five with proven myocardial infarction 1–5 years before the investigation), and "controls," i.e., three patients with uncomplicated atrial septal defect and the four autopsy cases which had no cardiac history or any signs of hypertrophy or valvular pathology. Patients with significant combined valvular lesions were excluded from the study. None of the patients was in clinically apparent heart failure. The group with pressure overload consisted of patients with predominant aortic stenosis and the group with volume overload included patients with pure aortic insufficiency. No biopsy material was available from cases with isolated mitral valve lesions. Fourteen of 36 patients with hypertrophy were on chronic medication of 0.25 mg digoxin daily for at least 6 months before examination.

Hemodynamic and Angiographic Evaluation

After premedication with 10 mg chloridiazepoxide (Librium), given perorally 1 hour before examination, all patients underwent right- and left-heart catheterization, biplane left ventricular angangiography, and selective coronary arteriography, using standard techniques. Aside from the heart rate and the conventionally recorded left ventricular pressures, the cardiac index was estimated using the Fick principle. In the cases with volume overload due to aortic insufficiency, the regurgitant fraction was measured by the thermodilution method. All measurements were recorded on an Electronics for Medicine oscillograph VR-12. Biplane left ventricular cineangigrams performed in the right and left anterior oblique projection were recorded on 35-mm cinefilm exposed at 50 frames/sec. From the left ventricular cavity silhouette drawn in end-diastole and end-systole and corrected for image magnification, volume estimates were made according to the area-length method (Dodge et al., 1960, 1966), and the biplane ejection fraction was computed.

Moreover, left ventricular high-fidelity pressure measurements were obtained in 36 patients (16 with pressure overload, 11 with volume overload, and four with hypertrophic and five with dilated cardiomyopathy) by means of a Millar 7F-micromanometer-angio catheter advanced into the left ventricle through a transseptally introduced 11.5 F Brockenbrough guiding catheter. The micromanometer was calibrated by superimposing its tracing with the conventional pressure tracing (Hess et al., 1981). This approach permitted the calculation of various indices of contractility, such as the maximal rate of left ventricular pressure rise (max dp/dt in mm Hg/sec), peak measured velocity of contractile element shortening (Vpm in muscle lengths/sec), and mean normalized systolic ejection rate (MNSER in end-diastolic volumes/sec; the ejection time was obtained from aortic pressure tracings recorded during angangiography). Moreover, peak and end-diastolic circumferential wall stress [pS and diast. S, respectively, in dynes/cm², calculated according to the method proposed by Gash et al. (1972)] and left ventricular muscle mass index (LMMI in g/cm³, estimated by the method of Rackley et al. (1964)) were determined.

Technical Aspects of Myocardial Biopsies

Left ventricular tissue samples were either taken at autopsy (four cases), surgery (12 cases), or during cathe-
terization (36 cases). Thus, in the 36 patients who were evaluated by high-fidelity pressure measurements, 4–5 endomyocardial catheter biopsies were obtained by means of the King's College biopsyte introduced into the left ventricle through the Brockenbrough guiding catheter (Hess et al., 1981). This approach permitted repeated biopsies to be taken from similar regions of the anterolateral wall. In general, one-catheter biopsy was used for biochemical analysis, whereas two to three pieces were subjected to morphometry. In some patients, multiple-catheter biopsies were examined biochemically and in two of 36 patients, additional biopsy material was gained intraoperatively. At surgery, cylindric transmural tissue pieces were taken from the anterior wall by means of a Travenol biopsy needle. Finally, the tissue probes obtained at autopsy originated also from the anterior wall of the left ventricle.

**Determination of Muscle Fiber Diameter and Nonmuscle Tissue Content**

The tissue samples were fixed immediately in ice-cold 2% glutaraldehyde containing 0.1 M phosphate buffer, pH 7.4, and were kept for at least 24 hours. Subsequently, the samples were postfixed for 2 hours in 2% osmium tetroxide containing 0.2 M S-collidin buffer. After dehydration in a graded series of ethanol, the tissue probes were treated with propylene oxide and embedded in Epon-812. Semithin sections of 0.5 and 1.0 µm were stained with toluidine blue or azure-II plus methylene blue, respectively, for examination by light microscopy. Muscle fiber diameter and nonmuscle tissue content were determined by morphometric methods (Weibel et al., 1966; Schwarz et al., 1982) using a grid providing intersection points. In each biopsy, 500–1000 points were counted. Muscle fiber diameter was calculated from at least 100 individual measurements (Kunkel et al., 1978).

**Electrophoretic Resolution of Myosin Light Chains**

Prior to processing, tissue samples were stored at −20°C or lower. After homogenization of 1–10 mg of tissue, the protein concentration was determined according to the method of Lowry et al. (1951). One-dimensional polyacrylamide gel electrophoresis in the presence of SDS was carried out according to the method of Laemmli and Favre (1973), by using 3% stacking and 12.5% separating gels. For two-dimensional gel electrophoresis, isoelectric focusing was carried out first according to the method of O'Farrell (1975), using a (4:1, vol/vol) mixture of ampholines (1.75%) of the pH ranges 5–8 and 3.5–10, respectively, on 3.64% tube gels. Thereafter, one-dimensional gel electrophoresis was performed as described above. The proteins were stained in 0.25% Coomassie Brilliant Blue R-250. Destaining of gels and determination of pH gradient in isoelectric focusing gels were done by the method described by Srihari et al. (1981).

**Peptide Maps of Isolated Myosin Heavy Chains**

For isolation of myosin HC, the tissue samples were homogenized in 10 volumes of 40 mM sodium pyrophosphate (pH 7.5) containing 2 mM dithiothreitol and 5 mM ethylenebis(oxyethylenenitriilo)tetraacetic acid (Rupp, 1981). Prior to electrophoresis, 4 mM N-ethylmaleimide were added to the homogenate containing 200–400 µg of protein (Schaub et al., 1984) and loaded on gels of 3% acrylamide and 0.24% bis-acrylylcystamine (Hansen, 1976) with chamber and gel buffers pH 8.4, according to the method of Weeds (1976). Gels then were stained with Coomassie Brilliant Blue R-250, and were soaked for 15 minutes in 20% methanol, followed by 15 minutes in the chamber buffer. The bands representing myosin HC were excised and the gel slices dissolved by the addition of 3–4% β-mercaptoethanol (Schaub et al., 1984). Before digestion, 150 mM NaCl and 0.03 mM MgCl₂ were added, and the pH was adjusted to 6.5 with phosphate buffer (Whalen et al., 1979). Eighty to 100 µg of myosin HC per sample were digested with either proteinase from Staphylococcus aureus V8 (EC 3.4.21.19) (Miles, Elkhart) or papain (EC 3.4.22.2) (Boehringer, Mannheim). The molar ratio of both proteinases to myosin HC ranged from 0.001 up to 0.05, based upon the molecular weights of 27,700 D for the proteinase V8 and 23,350 D for papain. Digestion was performed for 30 minutes at 37°C, and the reaction was stopped by the addition of phenylmethylsulfonyl fluoride in about 100-fold molar excess in the case of proteinase V8, and by heating the samples for 3 minutes at 100°C in the case of papain. The protein digest was then resolved on one-dimensional gel electrophoresis in SDS. The peptide bands were stained with Coomassie Brilliant Blue R-250 and, after destaining, in many cases were stained again with silver (Wray et al., 1981) in order to increase the sensitivity for visualization of peptides occurring in small quantities.

**Evaluation of One- and Two-dimensional Gel Patterns**

Stained one- and two-dimensional gels and positive films thereof were scanned by densitometric equipment (LKB 2202 Ultrascan Laser Densitometer, LKB-Produkter AG; Shimadzu Dual-Wavelength TLC-Scanner CS-930, Shimadzu Corp.). With purified tropomyosin for calibration, the measurements yielded a linear relationship from 0.5–10 µg of protein per spot. In this way, the myosin LC were quantified in each two-dimensional gel relative to tropomyosin. No allowance was made for possible differences in dye uptake by these proteins. The calculation of the molar relationship was based upon the chemical molecular weights of 21,000 D for LC-1, 18,000 D for LC-2, and 66,000 D for tropomyosin. However, the apparent molecular weights of the LC in SDS gel electrophoresis are different, amounting to 26,000 D and 21,000 D for the ventricular VLC-1 and VLC-2, and to 27,400 D and 22,700 D for the atrial ALC-1 and ALC-2, respectively (Srihari et al., 1982a). Since the myosin HC and, at times, the actin could not be resolved reliably in the isoelectric focusing run, they were not quantified.

**Statistics**

The results are expressed as mean ± 1 SD. To evaluate the statistical significance of differences between the various groups, one-way analysis of variance (SPSS program) and, if appropriate, modified t-tests and Bonferroni method were applied (Wallenstein et al., 1980). Multiple regression analysis was used to examine correlations between various parameters.

**Results**

**Hemodynamic and Angiographic Data**

Patients were assigned to groups of primary (hypertrophic and dilated cardiomyopathy) and secondary hypertrophy (pressure or volume overload due to aortic stenosis or aortic insufficiency, respectively), coronary heart disease and 'controls' accord-
ing to the typical hemodynamic and angiographic findings (Braunwald and Aygen, 1963; Goodwin and Oakley, 1972; Roskamm et al., 1972; Krayenbuehl et al., 1979; Huber et al., 1981) (Tables 1 and 2). High-fidelity left ventricular pressure measurements were performed only in the patients with primary and secondary forms of hypertrophy.

In the 16 cases with aortic stenosis, the mean pressure gradient across the aortic valve amounted to 74 ± 17 mm Hg (range 43–103 mm Hg) and the mean aortic valve area to 0.8 ± 0.4 cm². Clinical symptoms were present 0.5–5 years before the invasive examination. Six patients were treated with digoxin (0.25 mg daily) for 0.5–5 years.

In the cases with aortic insufficiency, the regurgitant fraction averaged 60 ± 11% (range 41–71%) and the end-diastolic volumes were markedly enlarged averaging 212 ± 39 ml/m² (range 134–256 ml/m²). In all cases, left ventricular muscle mass index was greatly increased, amounting to 217 ± 34 g/m², and "contractility indices" were somewhat reduced. No other associated valvular lesions of hemodynamic relevance were present in any of the patients. Five of 11 patients were treated with digoxin.

Two patients with hypertrophic cardiomyopathy had an obstructive form of the disease (pressure gradient across the outflow tract at rest 30 and 58 mm Hg, respectively), and two had asymmetric septal hypertrophy without obstruction. Left ventricular filling pressure was markedly increased in all four patients. True left ventricular muscle mass, as assessed by the respective index value (LMMI), certainly has been underestimated, since the method of calculation does not account for the highly asymmetric form of hypertrophy present in these cases. All four patients complained about anginal symptoms and/or dyspnea for 2–5 years before the invasive investigation. One patient without obstruction was on digoxin medication during the previous 6 months.

The five patients with dilated cardiomyopathy showed increased left ventricular end-diastolic volumes and low ejection fractions. All indices of contractility were clearly diminished, whereas both peak and end-diastolic circumferential wall stress were greatly increased. All patients had suffered from shortness of breath and atypical chest pain for several months. Two of five patients were treated with digoxin for more than 6 months.

All patients with coronary heart disease had multiple vessel lesions. In addition five of nine patients had experienced a myocardial infarction 1–5 years prior to the investigation. Nevertheless, the basic

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>AP (mm Hg)</th>
<th>Valve area (cm²)</th>
<th>Reg. frac. (%)</th>
<th>EDVI (ml/m²)</th>
<th>EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7</td>
<td>48 ± 16</td>
<td>3.1 ± 0.2</td>
<td>148 ± 28</td>
<td>9 ± 5</td>
<td>101 ± 62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>9</td>
<td>57 ± 7</td>
<td>2.6 ± 0.7</td>
<td>130 ± 23</td>
<td>12 ± 4</td>
<td>94 ± 30</td>
<td>61 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>4</td>
<td>42 ± 12</td>
<td>3.3 ± 0.6</td>
<td>156 ± 37</td>
<td>20 ± 5</td>
<td>100 ± 27</td>
<td>66 ± 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume overload</td>
<td>11</td>
<td>47 ± 11</td>
<td>3.6 ± 0.6</td>
<td>136 ± 17</td>
<td>15 ± 10</td>
<td>60 ± 11</td>
<td>212 ± 39</td>
<td>59 ± 13</td>
<td></td>
</tr>
<tr>
<td>Pressure overload</td>
<td>16</td>
<td>52 ± 14</td>
<td>3.4 ± 0.8</td>
<td>206 ± 18</td>
<td>13 ± 5</td>
<td>74 ± 17</td>
<td>0.8 ± 0.4</td>
<td>8 ± 7</td>
<td>115 ± 29</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>5</td>
<td>50 ± 8</td>
<td>3.1 ± 0.6</td>
<td>121 ± 17</td>
<td>14 ± 8</td>
<td>174 ± 45</td>
<td>35 ± 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI = cardiac index; LVSP = left ventricular peak systolic pressure; LVEDP = left ventricular end-diastolic pressure; AP = mean pressure gradient across aortic valve; reg. frac. = aortic regurgitant fraction; EDVI = left ventricular end-diastolic volume index; EF = left ventricular ejection fraction.

* Hemodynamic data available in three of seven controls. Shown are mean values ± 1 so. Significances, with P < 0.05 different from: † all other groups; ‡ pressure overload, hypertrophic cardiomyopathy, coronary heart disease, and controls (modified t-tests and Bonferroni method).

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>MNSER (vol/s)</th>
<th>Vpm (muscle lengths/s)</th>
<th>max dP/dt (mm Hg/s)</th>
<th>dyne·10⁸/cm²</th>
<th>pS</th>
<th>diast. S</th>
<th>LMMI (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>4 3.2 ± 0.6‡</td>
<td>1.5 ± 0.5f</td>
<td>1946 ± 923‡</td>
<td>322 ± 55*</td>
<td>51 ± 23</td>
<td>127 ± 23</td>
<td></td>
</tr>
<tr>
<td>Volume overload</td>
<td>11 1.8 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>1475 ± 279</td>
<td>448 ± 111</td>
<td>69 ± 51</td>
<td>217 ± 34‡</td>
<td></td>
</tr>
<tr>
<td>Pressure overload</td>
<td>16 1.9 ± 0.4</td>
<td>1.4 ± 0.1f</td>
<td>2176 ± 285†</td>
<td>463 ± 51</td>
<td>48 ± 22</td>
<td>201 ± 42</td>
<td></td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>5  1.5 ± 0.5</td>
<td>0.9 ± 0.3</td>
<td>1111 ± 353</td>
<td>465 ± 47</td>
<td>56 ± 24</td>
<td>145 ± 39</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MNSER = mean normalized systolic ejection rate; Vpm = peak measured velocity of contractile element shortening; max dP/dt = maximum rate of pressure rise; pS = peak circumferential wall stress; diast. S = end-diastolic circumferential wall stress; LMMI = left ventricular muscle mass index. Shown are mean values ± 1 so. Significances, with P < 0.05 different from: * all other groups; ‡ dilated cardiomyopathy; † hypertrophic cardiomyopathy (modified t-tests and Bonferroni method).
hemodynamic findings were within normal limits. Symptomatology dated back to between 6 months and 10 years. In all cases, transmural biopsies from left ventricular myocardium perfused by nonstenosed arteries were taken at surgery.

Since four of seven biopsy specimens considered as control samples were obtained from patients who died in traffic accidents, no hemodynamic data are available. None of them, however, had a history of heart disease and they all showed normal cardiac anatomy at necropsy. The other three control patients underwent heart surgery for atrial septal defect of the secundum type. They had large left-to-right shunting which amounted to between 60% and 70% of pulmonary blood flow. In each case, left ventricular hemodynamics were normal.

Muscle Fiber Diameter and Nonmuscle Tissue Content

The results summarized in Table 3 indicate that in all disease groups (data not available for cases with coronary heart disease), muscle fiber diameter was increased about twice and nonmuscle tissue content 4–5 times above control levels. This increase in nonmuscle tissue content is corroborated by the findings of Hess et al. (1979a, 1979b) and of Pearlman et al. (1982). Only Schwarz and coworkers (1980) did not find a similar increase of nonmuscle tissue content in patients with aortic valve disease. It may be noteworthy that the mean fiber diameter is largest in volume overload, even larger than in pressure overload. Again, our findings are in agreement with values from the literature (Ashley, 1945; Arai et al., 1968; Jones et al., 1975; Linzbach, 1976; Astorri et al., 1977; Hess et al., 1979b).

It may be surprising that both fiber diameter and nonmuscle tissue content are quite different from normal, but do not differ in the four disease groups studied. These latter groups, however, can be distinguished from one another by their hemodynamic and angiographic characteristics (Tables 1 and 2).

The growth in fiber diameter does not seem, on the average, to more than double. This may support the hypothesis that any further increase in hypertrophy must occur by amitotic muscle cell division rather than by continuous increase in cell size (Linzbach, 1976; Ferrans, 1984).

Myocardial Isoforms of Myosin

Myosin Light Chain Composition

The most significant result in two-dimensional electrophoresis of total left ventricular tissue homogenates was the occurrence of an additional peptide which appeared above the VLC-1 in certain forms of cardiac hypertrophy (Fig. 1). Coelectrophoresis with normal atrial tissue showed that this peptide and ALC-1 exhibited identical electrophoretic properties. However, its isoelectric point was marginally more acidic and its apparent molecular weight 6% larger than for VLC-1 (Srihari et al., 1982a; Tuchschmid et al., 1983). Based on quantitative densitometry, on the average, the largest amount of this ALC-1-like protein was found in dilated cardiomyopathy, intermediate in pressure overload and smaller in volume overload (Table 3). In controls, it was absent, or a faint spot was seen, never exceeding 0.8% of total LC-1. In the cases with hypertrophic cardiomyopathy, it was equally low or absent. Finally, in the cases with coronary heart disease it varied between 0 and 3.5% of total LC-1.

Although there is considerable individual scatter, the group with dilated cardiomyopathy differed significantly from all other groups when analyzed by careful statistical procedures. Both volume and pressure overload groups differed significantly from control and hypertrophic cardiomyopathy groups, but there was no statistical significance between them and the coronary heart disease group. Partitioning of the coronary heart disease group into a subgroup of five cases with and a subgroup of four cases without prior myocardial infarction yielded an

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle fiber diameter (μm)</th>
<th>Nonmuscle tissue content (%)</th>
<th>Total LC-1 (mol/mol TM)</th>
<th>ALC-1 (% LC-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>15 ± 2</td>
<td>4 ± 2</td>
<td>2.7 ± 0.4</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>30 ± 5†</td>
<td>20 ± 6†</td>
<td>2.5 ± 0.3</td>
<td>1.3 ± 1.3†</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>34 ± 4††</td>
<td>27 ± 9†</td>
<td>2.5 ± 0.3</td>
<td>2.9 ± 1.3†§</td>
</tr>
<tr>
<td>Volume overload</td>
<td>28 ± 3†</td>
<td>23 ± 10†</td>
<td>2.7 ± 0.7</td>
<td>12.1 ± 8.4*</td>
</tr>
<tr>
<td>Pressure overload</td>
<td>16 ± 5†</td>
<td>25 ± 7†</td>
<td>3.1 ± 0.7</td>
<td>6.4 ± 5.0†§</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>28 ± 3†</td>
<td>23 ± 10†</td>
<td>2.7 ± 0.7</td>
<td>12.1 ± 8.4*</td>
</tr>
</tbody>
</table>

Abbreviations: total LC-1 = total amount of light chain 1 in moles per mole of tropomyosin; ALC-1 = amount of atrial light chain 1 relative to total light chain 1. Shown are mean values ± s. Significances, with P < 0.05 different from: * all other groups; † controls; ‡ dilated cardiomyopathy; § hypertrophic cardiomyopathy; †† pressure overload; ‡‡ volume overload (modified t-tests and Bonferroni method).
FIGURE 1. Two-dimensional electrophoresis of total tissue homogenate from left ventricular myocardium. Gels are presented with the basic pH range in isoelectric focusing (IEF) to the left and decreasing molecular weights from top to bottom in SDS electrophoresis. Details are given in the region of actin (A) and the myosin LC Panel a: normal ventricle of a woman, 55 years old with only minimal traces of ALC-1 (arrow); panel b: ventricle of a 42-year-old woman with dilated cardiomyopathy. Note the very large portion of ALC-1 (arrow). TM: α-tropomyosin with traces of β-tropomyosin on top; VLC-1 and VLC-2: ventricular myosin LC; Coomassie brilliant blue staining.

ALC-1 content of 1.9 ± 1.5% and only 0.6 ± 0.6%, respectively.

In single cases of dilated cardiomyopathy and pressure overload, the ALC-1 content amounted up to 20–30% without affecting the total amount of LC-1, which remained remarkably constant relative to tropomyosin content in all cases, despite large variations in this additional ALC-1. In two cases (one with pressure overload, the other with hypertrophic cardiomyopathy), the blinded analysis of an endomyocardial biopsy sample and a second tissue probe obtained at surgery a few weeks later revealed identical ALC-1 contents. Moreover, analysis of tissue from endo-, mid-, and epicardial layers performed in two transmural biopsy specimens yielded exactly the same results for ALC-1 and total LC-1 content (Table 4).

The ventricular type 2 light chain (VLC-2) was in most cases partially phosphorylated, giving rise to a double spot in two-dimensional electrophoresis (Fig. 1). An additional atrial-like type-2 LC was never seen above the VLC-2 in any of the tissue samples. VLC-2 had a tendency to be reduced variably below stoichiometric amounts, compared to VLC-1. It has

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Degrees of freedom</th>
<th>Variance ratio</th>
<th>t-value for predictor variables</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALC-1 vs. peak stress</td>
<td>23</td>
<td>0.509</td>
<td>0.71</td>
<td>0.15</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress</td>
<td>23</td>
<td>4.997</td>
<td>2.24</td>
<td>0.42</td>
</tr>
<tr>
<td>log ALC-1 vs. mean normalized systolic ejection rate</td>
<td>25</td>
<td>2.347</td>
<td>1.53</td>
<td>0.29</td>
</tr>
<tr>
<td>log ALC-1 vs. peak measured velocity of fiber shortening</td>
<td>23</td>
<td>0.973</td>
<td>0.98</td>
<td>0.20</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress and mean normalized systolic ejection rate</td>
<td>22</td>
<td>3.090</td>
<td>1.41, 1.07</td>
<td>0.47</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress and peak measured velocity of fiber shortening</td>
<td>21</td>
<td>2.115</td>
<td>1.62, 1.54</td>
<td>0.41</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress, mean normalized systolic ejection rate, and peak measured velocity of fiber shortening</td>
<td>20</td>
<td>1.914</td>
<td>1.08, 1.20, 0.09</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean values of 4 hypertrophy groups of log ALC-1 vs. peak stress</td>
<td>3</td>
<td>19.686</td>
<td>4.44</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Abbreviations: log ALC-1: logarithm of atrial-like light chain 1 content. Calculations by multiple regression analysis (significance better than 0.05 marked in brackets).
been reported that this LC-2 type characteristic of myosins with low ATPase activity always yields variable recoveries in different extraction procedures (Weeds, 1976). Therefore, its quantification proved to be erratic.

Myosin Heavy Chain Composition

For examination of the peptide patterns of isolated myosin HC, it is essential to perform the limited proteolysis in SDS under carefully controlled conditions. In each experiment, there were several digestions with varying concentrations of the proteinases in relation to the amount of the HC needed to be performed. Samples from cases with pressure overload and hypertrophic cardiomyopathy always were processed strictly in parallel with control samples. Only parallels with the same amount of HC, digested to the same extent, yielded meaningful comparison of their peptide maps. Digestion performed in solution, as described in Methods, produced greater reproducibility than when performed within the gel matrix (Cleveland et al., 1977). Various degrees of digestion allowed to compare the peptides separately in the higher (80–200 kD), middle (30–100 kD), and lower (10–50 kD) molecular weight range. In this way, over 200 peptide bands could be resolved clearly and compared between control and disease cases. No reproducible qualitative differences could ever be detected in the one-dimensional peptide maps between the hypertrophic cases and controls. Only minute quantitative variations in some of the minor peptide bands were observed (Fig. 2). Our results are in agreement with the findings of Schier and Adelstein (1982).

Correlations between Myosin Light Chain Isoforms, Clinical, Hemodynamic, and Angiographic, and Morphometric Parameters

With respect to the clinical manifestation of the disease, it may be noteworthy that in patients with pressure overload and dilated cardiomyopathy, where the highest amounts of ALC-1 were found, the ALC-1 content tended to be lower the longer symptoms were apparent (Fig. 3a). This may either reflect a reversion of the occurrence of this additional ALC-1 with prolongation of the disease, or indicate that patients with a longer history were those who survived longer due to a milder course of the disease. Digoxin administration per se did not seem to influence the amount of ALC-1 present in the tissue (Fig. 3b).

To test the hypothesis that the appearance of ALC-1 in hypertrophied tissue might be a consequence of the increased workload imposed on the ventricle and/or reflect a decrease in contractile function, the amount of ALC-1 present in the tissue was related to the various hemodynamic and angiographic parameters indicative of either load or muscle performance. However, no distinct correlation could be established between ALC-1 content and any single functional parameter. Nevertheless, the various plots suggested higher tissue content of ALC-1 at increased peak circumferential wall stress whereby the relationship appeared to be exponential (Fig. 4). Thus, in relating log ALC-1 content to peak circumferential wall stress, a significant correlation was found, even though the regression coefficient is low (Table 4). No other functional parameter was correlated with the log ALC-1 content. Combination of any single or multiple functional parameters with peak circumferential wall stress did not improve the relationship substantially. However, comparison of the mean values of log ALC-1 content of the various
hypertrophy groups with the respective mean values of peak circumferential wall stress yielded a significant correlation with an \( r \) value of 0.96. This correlation, which holds for disease groups with high peak wall stress as well as for the low wall stress characteristic for hypertrophic cardiomyopathy, is supported by inclusion of the corresponding values for nonhypertrophic hearts (Fig. 5). It is emphasized that the biochemical parameter differentiates more clearly between the different disease groups than peak circumferential wall stress alone (Tables 2 and 3).

Neither muscle fiber diameter nor the percentage of nonmuscle tissue as determined by morphometry was related to ALC-1 content.

**Discussion**

The present study documents a shift in ventricular myosin LC-1 isoform composition in various forms of primary and secondary hypertrophy. In relation to augmented work load, ventricular VLC-1 becomes partially replaced by increasing amounts of an atrial-like LC-1 reaching up to 20–30% of total LC-1. The highest contents of this ALC-1 were found in patients with dilated cardiomyopathy; in-

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**Figure 3.** Tissue content of atrial myosin light chain 1 (ALC-1) expressed in percent of total myosin light chain 1 (LC-1) and duration of clinical symptoms (left panel) and medication with digoxin (0.25 mg/d) (right panel) in patients with pressure overload (black dots) and with dilated cardiomyopathy (open squares). Whereas the amount of ALC-1 tends to decline the longer the duration of symptoms, no difference is observable between patients on chronic digoxin medication and those not receiving the drug.

**Figure 4.** Relationship between peak circumferential wall stress as an indicator of the workload imposed to the ventricle and tissue content of atrial-like myosin light chain-1 (ALC-1) expressed in percent of total light chain 1 (LC-1) and plotted in a linear fashion (left panel) and in a logarithmic fashion (right panel) in the four disease groups with primary or secondary hypertrophy. The distribution of the individual points in the left panel suggests an exponential relationship. By logarithmic transformation of ALC-1 content (right side) a linear correlation (\( r < 0.05 \)) results, although the correlation coefficient \( r = 0.42 \) is weak due to substantial scatter. Since ALC-1 content was close to zero in three cases with hypertrophic cardiomyopathy (filled triangles), values of \(-1.0\) were chosen arbitrarily for the ALC-1 reflecting a minimal amount of ALC-1 of 0.1% of total LC-1. Open squares: dilated cardiomyopathies; filled circles: pressure overload; open circles: volume overload; filled triangles: hypertrophic cardiomyopathies.
termediate levels were found in patients with pressure or volume overload due to aortic stenosis or regurgitation, respectively. This latter finding has been published previously in a meeting report (Schaub et al., 1984). As in controls, this ALC-1 was virtually absent, in cases with hypertrophic cardiomyopathy and in patients with coronary heart disease without myocardial infarction. It was, however, slightly increased in patients who had already experienced such an event.

In all disease groups examined, the total amount of LC-1 did not change, and remained in the same proportion to tropomyosin as in normal myocardium. Tropomyosin was found to be the most reliable reference protein, since it was always resolved quantitatively, as were the LC. The amount of both myosin HC and actin varied somewhat due to aggregation when high-loading doses of total tissue homogenates were used. Since tropomyosin stays in a fixed molar ratio to actin of 1:6 in all mammalian muscles including the myocardium (Katz, 1970; Fatigati and Murphy, 1984), and since the myocardial ratio of myosin to actin is about 1:5 (Frederiksen et al., 1978; Kozlovsik et al., 1984), tropomyosin stays in a near stoichiometric relationship to myosin as well. This defined molar relationship between the three major contractile proteins myosin, actin and tropomyosin seems to be preserved at least in the animal cardiac overload model (Moalic et al., 1984).

It can therefore be concluded that the genuine VLC-1 indeed becomes gradually replaced by ALC-1 in certain forms of hypertrophy. The reason Klotz and coworkers (1982) did not find changes in the primary structure of LC-1 in their preparation of myosin from two hypertrophied human left ventricles, compared to myosin from healthy controls, may be technical in nature and related to the small amounts of ALC-1 present which may have escaped from detection in amino acid sequence studies.

Even though the graded distribution of this ALC-1 between different disease groups proved to be statistically significant, its large variations within each group may raise several questions about the specificity of this new finding. Thus, it might be argued that ALC-1 could be a variable degradation product of the genuine VLC-1. This is unlikely, because it has a higher apparent molecular weight and, consequently, migrates more slowly than VLC-1. Furthermore, it exhibits disease-specific occurrence. Inhomogeneity in tissue distribution of LC-isoforms, on the other hand, would be an alternative explanation for its scattering. Unfortunately, no information on regional LC-isoform distribution in human ventricular tissue is available at present. The following considerations, however, vote against such an interpretation. (1) Determination of LC-isoform composition in endo-, midwall, and epicardial layers of the left ventricle performed in two cases yielded exactly the same result. (2) The analysis (done without knowledge of the source) of LC-isoform composition in biopsy specimens and surgically excised tissue of two patients, one with hypertrophic cardiomyopathy and one with pressure overload, as well as in multiple biopsies taken from several other cases, revealed identical relative amounts of ALC-1. (3) Despite monotonous muscle fiber enlargement and increase in nonmuscle tissue content in all disease groups, the ALC-1 content varied significantly but independently of these morphometric aspects. Thus, the increased ALC-1 content seems to represent indeed a molecular characteristic of certain types of ventricular hypertrophy.

Provided the hypertrophy process is related in some way to the appearance of this new ALC-1 in ventricular tissue, one would expect that the content of ALC-1 parallels either functional or morphological alterations, or both, seen under these conditions. However, no single hemodynamic or angiographic parameter permits an accurate assessment of either the load imposed to the ventricle, or its contractile state. Due to the complex geometry of the ventricle and its cyclic function, wall stress as a measure of ventricular load varies in each myocardial segment and during contraction and relaxation. Correspondingly, shortening function of the muscle may differ from one region to the other as well. Thus, it is even more surprising that, despite this, a good correlation was obtained by relating the log of ALC-1 content to peak circumferential wall stress. ALC-1 isoform expression therefore seems to depend primarily on the workload imposed on the ventricle. In reality, this relation may be much more complex. Considering the characteristics of the various disease
groups, the appearance of ALC-1 in ventricle permits better distinction than any single hemodynamic parameter.

The finding of increased amounts of ALC-1 in normal myocardium of patients with coronary heart disease who had experienced an infarction implies that the surviving myocardium hypertrophies consecutively to the reduction in muscle mass following infarction. While long-term medication of digoxin had no visible effect on the ALC-1 content it seems worth mentioning that in cases with pressure overload and dilated cardiomyopathy, its occurrence was more pronounced in earlier stages of the disease. This could mean that in the early phase of overload, the myocardial tissue responds in an effort to redress the hemodynamic imbalance but becomes exhausted and loses its capacity to compensate as the malignant stimulus of hypertrophy continues, reflected by the inability to keep up further the production of the temporarily expressed ALC-1 isofrom. An atrial-like light chain 2 was never observed in hypertrophied ventricular tissue. In contrast, neither muscle fiber diameter nor nonmuscle tissue content permitted a distinction between primary and secondary forms of hypertrophy, a finding which is in accordance with the literature (Astorri et al., 1977; Hess et al., 1979a; 1979b; Schwarz et al., 1980, 1981a, 1981b).

The presence of appreciable amounts of a new HC species can also be excluded, since no changes were found in the primary structure of the myosin HC in all disease groups as judged from the peptide patterns. The electrophoretic methods employed would definitely have permitted the detection of any single additional peptide originating from another HC isofrom amounting to or exceeding 5% of total protein. These results are in agreement with the findings of Schier and Adelstein (1982). However, theoretically, the peptide patterns of ventricular HC could represent a mixture of HC isoforms. Since no quantitative differences were detected between normal and pathologically altered tissue, it has to be postulated that such a mixture remains constant under all conditions. Indeed, immunochcmical studies on myosin HC from human ventricular tissue indicate that, besides V-3, the V-1 isofrom also is present in small quantities ranging between 1% and 16%. Their proportion does not differ between normal and pathologically altered tissue, it indicates homology and probably identity of the fetal LC-1 and atrial ALC-1 (Cummins, 1982). It is then very likely that the fetal LC-1 is the same peptide which under pathological conditions occurs also in adult ventricle. In cases with congenital malformations associated with pressure overload of the right ventricle, the fetal ALC-1-like protein persists, amounting up to one-third of total LC-1 (Tuchschmid et al., 1983). Thus, increased load operating on the ventricular wall seems to be the common denominator for retardation of the developmental transition from ALC-1 to the tissue-specific VLC-1, as well as for the retrograde transition observed in adult ventricle in certain disease states. The coupling of these transitions in ventricular type-1 LC to a functional parameter indicates the involvement of this myosin subunit in the contractile process. In fact, in skeletal muscle, the type-1 LC has been implicated in the binding of the myosin cross-bridges to actin (Prince et al., 1981; Yamamoto and Sekine, 1983).
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Relationship between myosin isoenzyme composition, hemodynamics, and myocardial structure in various forms of human cardiac hypertrophy.

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