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)

IN disease states characterized by altered loading conditions, the heart adapts primarily by hypertrophy and increased muscle performance to fulfill its task of pumping blood according to the metabolic requirements of the organism. In contrast, the mechanism of hypertrophy in primary cardiomyopathies is poorly understood. Since myosin is the major contractile protein of the cardiac muscle cell producing force and shortening, it has been argued that changes in its isoform composition may account for the altered contractile properties in hypertrophy (Wikman-Coffelt et al., 1979; Cummins, 1983). Myosin has a hexameric structure consisting of two heavy chains (HC) and two pairs of light chains (LC) in the 200,000 and 20,000 D molecular weight range, respectively. Various isoforms of both LC and HC are known to occur. Thus, the LC of atrial and ventricular tissue differ in their apparent molecular weights as assessed by sodium dodecyl sulfate (SDS) gel electrophoresis in all mammalians, including man (Srihari et al., 1982a, 1982b). In early developmental stages, ventricular tissue contains a fetal type-1 LC which resembles the atrial LC-1 (ALC-1) besides the normally occurring ventricular LC-1 (VLC-1). This additional fetal LC disappears soon after birth (Price et al., 1980; Whalen et al., 1982; Tuchschmid et al., 1983). This ALC-1 was found to persist, however, in ventricles of infants with congenital malformations associated with increased work load (Tuchschmid et al., 1983). Conversely, a ventricular-like LC-2 partially replacing the normal ALC-2 was detected in human atrial tissue exposed to augmented intracavitary pressure (Cummins, 1982). Whether such changes in LC composition have a functional significance is not yet clear.

With respect to myosin HC, in principle, two types exist in cardiac muscles, $\alpha$ and $\beta$ (Mercadier et al., 1981, Chizzone et al., 1982). These two types of HC have been shown in animals to form myosin molecules composed either of an $\alpha,\alpha$-homodimer or
an αβ-heterodimer, or a ββ-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 (ββ-homodimer) and only few amounts of the V-1 species (αα-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 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 chlordiazepoxide (Librium), given perorally 1 hour before examination, all patients underwent right- and left-heart catheterization, biplane left ventricular angiography, 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 silhouettes 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-angiog 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 angiography). Moreover, peak and end-diastolic circumferential wall stress [p6 and diast. S, respectively, in dynes/cm²] were calculated according to the method proposed by Gaasch et al. (1972) and left ventricular muscle mass index [LMM 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-
Peptide Maps of Isolated Myo9in 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 ethylenedinitrilo 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-acrylamide (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 Ultrace 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 21,000 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 quantitated.

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. 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.

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>ΔP (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></td>
<td></td>
<td></td>
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</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></td>
<td>94 ± 30</td>
<td>61 ± 7</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></td>
<td>100 ± 27</td>
<td>66 ± 12</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></td>
<td>60 ± 11</td>
<td>212 ± 39</td>
<td>59 ± 13</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></td>
<td>174 ± 45</td>
<td>35 ± 12</td>
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</tbody>
</table>

### Table 2

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>MNSER (vol/s)</th>
<th>Vpm (muscle lengths/s)</th>
<th>max dP/dt (mm Hg/s)</th>
<th>dynes•10⁹/cm²</th>
<th>pS</th>
<th>diast. S</th>
<th>LMMI (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>4</td>
<td>2.3 ± 0.6⁴‖</td>
<td>1.5 ± 0.5⁵</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</td>
<td>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</td>
<td>1.9 ± 0.4</td>
<td>1.4 ± 0.1†</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</td>
<td>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; † hyper trophy 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>Table 3</th>
<th>Muscle Fiber Diameter, Nonmuscle Tissue Content, Total Amount of Myosin Light Chain 1 and Relative Amount of Atrial Myosin Light Chain 1</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>Muscle fiber diameter (μm)</td>
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<tr>
<td>---------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>9</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>11</td>
</tr>
<tr>
<td>Volume overload</td>
<td>16</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 ± 1 so. Significances, with \( P < 0.03 \) different from: * all other groups; † controls; ‡dilated cardiomyopathy; §hypertrophic cardiomyopathy; ¶pressure overload; ‡volume overload (modified \( \bar{t} \)-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

<p>| Table 4 |</p>
<table>
<thead>
<tr>
<th>Correlations between Ventricular Tissue Content of Atrial-Like Myosin Light Chain 1 and Various Parameters of Ventricular Load and Function in Various States of Hypertrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees of Freedom</td>
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<tr>
<td>ALC-1 vs. peak stress</td>
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<tr>
<td>log ALC-1 vs. peak stress</td>
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<td>log ALC-1 vs. mean normalized systolic ejection rate</td>
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<tr>
<td>log ALC-1 vs. peak measured velocity of fiber shortening</td>
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<tr>
<td>log ALC-1 vs. peak stress and mean normalized systolic ejection rate</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress and peak measured velocity of fiber shortening</td>
</tr>
<tr>
<td>log ALC-1 vs. peak stress, mean normalized systolic ejection rate, and peak measured velocity of fiber shortening</td>
</tr>
<tr>
<td>Mean values of 4 hypertrophy groups of log ALC-1 vs. peak stress</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-
It can therefore be concluded that the genuine VLC-animal cardiac overload model (Moalic et al., 1984). in a near stoichiometric relationship to myosin as well. This defined molar relationship between the tropomyosin seems to be preserved at least in the muscles including the myocardium (Katz, 1970; Fa-tigati and Murphy, 1984), tropomyosin stays quantitatively, as were the LC. The amount of both 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; Fa-tigati and Murphy, 1984), and since the myocardial ratio of myosin to actin is about 1:5 (Frederiksen et al., 1978; Kozlovskis 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 isoform. 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 isoform 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, immunochromatographic studies on myosin HC from human ventricular tissue indicate that, besides V-3, the V-1 isoform also is present in small quantities ranging between 1% and 16%. Their proportion does not change in hypertrophy, however (Mercadier et al., 1983; Gorza et al., 1984). This situation differs from the one in small animals where the ventricular V-1 myosin with the α-HC isoform changes toward V-3 myosin with the β-HC and a lower ATPase activity at increased work loads (Alpert et al., 1979; Lompre et al., 1979; Mercadier et al., 1981).

Since the active site of myosin is comprised in its HC, and since the LC-1 associated with it has no influence on its activity in vitro (Wagner and Weeds, 1977; Wagner and Ginger, 1981; Tobacman and Adelstein, 1984), it is not surprising that no changes in ATPase activity were found when pure myosin from hypertrophic ventricles was examined, even when activated by actin (Schier and Adelstein, 1982; Mercadier et al., 1983). Nevertheless, it has repeatedly been claimed that myofibrillar ATPase activity from hypertrophic human hearts is lower than normal (Leclercq et al., 1976; Swinghedauw et al., 1977). Thus, if the contractile machine of the sarcomere were responsible for diminished myocardial contractility, some change in its protein composition would be expected. The change in LC-1 isoform composition observed in this study could therefore be of importance, and represent a key explanation for the above-cited findings made in intact myofibrils.

Since the total amount of LC-1 remained constant relative to tropomyosin in all disease states as well as the controls, and since each myosin molecule is associated with two moles of LC-1, one with each head portion (Pfister et al., 1974), our results indicate that in total tissue homogenates LC-1 is always in a slight molar excess to myosin of about 0.8. Because, in heart muscle, the LC have a slower turnover than the HC (Zak, 1977), such a surplus may be necessary in order to allow all myosin HC to combine with the appropriate LC-1 to form intact molecules. Thus, the switch in LC-1 isoform expression from VLC-1 to ALC-1 without a concomitant increase in total LC-1 in certain types of hypertrophy implied that, under these conditions, a corresponding fraction of myosin has to be bound to ALC-1.

The fetal LC-1 in human ventricles which disappears within the first few months after birth has the same electrophoretic properties as the genuine atrial ALC-1 (Price et al., 1980; Cummins, 1982), as well as the ALC-1-like protein described in adult hypertrophied ventricle (Tuchschmid et al., 1983). Peptide analyses indicate homology and probably identity between the fetal LC-1 and atrial ALC-1 (Cummins, 1982). It is then very likely that the fetal LC-1 is the same protein 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 crossbridges to actin (Prince et al., 1981; Yamamoto and Sekine, 1983).

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