Myosin Types in the Human Heart
An Immunofluorescence Study of Normal and Hypertrophied Atrial and Ventricular Myocardium

Luisa Gorza, Jean Jacques Mercadier, Ketty Schwartz, Lars Eric Thornell, Saverio Sartore, and Stefano Schiaffino
From the Institute of General Pathology, University of Padova, Italy, Institut National de la Santé et de la Recherche Médicale, Paris, France, and Department of Anatomy, University of Umea, Sweden

SUMMARY. Two distinct myosin heavy chain isoforms, referred to as α and β, were identified in the human heart with specific antimyosin antibodies. By indirect immunofluorescence, myosin heavy chain α was found to be a major component of atrial myosin and a minor component of ventricular myosin, while heavy chain β was found to be a major component of ventricular myosin and a minor component of atrial myosin. In the normal heart, there was marked individual variability in the proportion of ventricular myocytes reactive for heavy chain α. Atrial myocytes staining for heavy chain β were rare in the left atrium and more numerous in the right atrium, especially in the crista terminalis and in the interatrial septum. Surgical and autopic specimens from hypertrophied left ventricles of patients with mitral regurgitation showed a myosin immunoactivity pattern similar to that of normal specimens. Very rare muscle cells reactive for heavy chain α were seen in the hypertrophied left ventricles of subjects with hypertension and in the hypertrophied right ventricles of subjects with tetralogy of Fallot. A dramatic transformation of myosin heavy chain composition was observed in hypertrophied left atria of patients with mitral stenosis, with a shift to heavy chain β in a large proportion of atrial myocytes. The findings indicate that chronic exposure to hemodynamic overload can induce marked changes in the myosin heavy chain composition of human atria, whereas it affects only slightly that of the ventricles. (Circ Res 54: 694-702, 1984)

CONTRACTILE properties of muscle cells depend on the molecular structure of myosin. The notion that maximal speed of shortening is correlated with myosin ATPase activity, first established for skeletal muscle (Barany, 1967), has subsequently been extended to cardiac muscle (see Swynghedauw et al., 1976) and is now supported by direct experimental evidence based on correlated biochemical and physiological studies on normal and hypertrophied heart (see Alpert et al., 1979; Carey et al., 1979; Schwartz et al., 1981; Wikman-Coffelt et al., 1982; Ebrecht et al., 1982). Precise identification of the myosin types present in cardiac muscle cells thus provides information on the functional properties of these cells. Characterization of the isoforms of cardiac myosin heavy chain is especially important in this respect since myosin heavy chains mainly determine the level of the adenosine triphosphatase activity. Comparative studies have shown that chicken atrial and ventricular myosins differ markedly in Ca++- and actin-activated adenosine triphosphatase activity but have identical light chains (Dalla Libera et al., 1979); similarly, the V1 and V3 myosin isoforms of the rat ventricular myocardium have identical light chain complement (Hoh et al., 1978) but different actin-activated adenosine triphosphatase activity (Pope et al., 1980). Light chain-heavy chain hybridization experiments using alkali light chains of fast skeletal myosin and heavy chains of cardiac myosin, and vice versa, have clearly confirmed that the adenosine triphosphatase properties of myosin are determined largely by the heavy chains (Wagner, 1981). Progress in the study of cardiac myosin heavy chains has been especially rapid in the last few years as the result of the application of immunological methods (Sartore et al., 1978; Schwartz et al., 1978) and biochemical techniques, in particular electrophoresis in nondenaturing gels (Hoh et al., 1978) and peptide mapping of proteolytic fragments of myosin heavy chains (Flink et al., 1979; Chizzonite et al., 1982; Litten et al., 1982).

Using specific antimyosin antibodies and immunochemical procedures, we have been able to identify distinct myosin heavy chain types in the mammalian heart. The distribution of heavy chain isoforms was found to vary among atrial and ventricular muscle cells (Sartore et al., 1981; Gorza et al., 1982), and to change under conditions that are known to affect cardiac contractility, such as pressure overload or alteration in the thyroid state (Gorza et al., 1981; Sartore et al., 1981). In the rat and rabbit heart, there seems to be a close correlation...
between myosin changes visualized by antimyosin immunofluorescence in hypertrophied hearts and corresponding changes in the relative proportion of different isomyosins, as revealed by native gel electrophoresis and ATPase measurements (compare Gorza et al., 1981, with Lompré et al., 1979, for the effect of pressure overload in the rat, and Sartore et al., 1981, with Morkin, 1979, Martin et al., 1982, and Litten et al., 1982, for the effect of hyperthyroidism in the rabbit).

The results of these experimental studies prompt a reinvestigation of the myosin composition of the human heart. Available information on human cardiac myosin is scarce and limited mostly to enzymatic measurements of myofibrillar or myosin ATPase activity: the findings are often contradictory, especially regarding the myosin composition of the hypertrophied heart (e.g., compare Alpert and Gordon, 1962, with Schier and Adelstein, 1982). In this study, we have used immunofluorescence procedures to map the regional distribution of different types of myosin in the atrial and ventricular human myocardium. By the same procedures, we have also compared the distribution of specific isomyosins in various forms of cardiac hypertrophy involving either the atrial or the ventricular muscle. A correlated biochemical and immunochemical study of myosin changes in human hypertrophied ventricles has been reported in a previous paper (Mercadier et al., 1983).

**Methods**

**Tissue Sources**

Immunofluorescence studies were performed on autopic or surgical specimens from 34 different hearts. These included 17 adult normal hearts, 12 hypertrophied ventricles, and five hypertrophied atria (Table 1). Autopic specimens were obtained within 3 hours (nos. 2-4), 12 hours (nos. 6, 11, 18, 19, 25, 26), or 24 hours after death.

Samples of normal adult heart were obtained from subjects without clinical and pathological evidence of heart disease and were taken from different regions of right and left atria and ventricles. Several samples were obtained from five normal individuals who died of acute traumatic accidents (nos. 2-4, 6, 8). Small bioptic specimens from the wall of the right atrium, immediately adjacent to the atriotomy incision for cardiopulmonary bypass, were obtained from three patients undergoing cardiac surgery for coronary bypass. None of these patients showed dilation or hypertrophy of the right atrium or atrial fibrillation.

Both autopic and biopic ventricular samples were examined in hypertrophied hearts. Autopic specimens were obtained from two patients with mitral regurgitation (heart weights 450 and 820 g) and from three patients with systemic hypertension (heart weights 450–600 g). Deaths were due to postoperative heart failure and stroke, respectively, in the two groups. Surgical specimens were obtained from the hypertrophic pulmonary infundibulum of three patients undergoing primary repair of tetralogy of Fallot, and from the mitral papillary muscles of four patients undergoing valve replacement for long-standing rheumatic mitral valve disease with predominance of incompetence. These were the tips of the papillary muscles removed with the diseased valve.

Surgical specimens of hypertrophied left atrial myocardium were obtained from three patients undergoing cardiac surgery for commissurotomy or replacement of stenotic mitral valves. Specimens nos. 30 and 31 were from macroscopically hypertrophied atna: these two patients showed similar hemodynamic loading of the left atrium (mean left atrial pressure 23 mm Hg) and had normal sinus rhythm. Specimens nos. 32, 33, and 34 were from dilated and fibrotic atna: the patients had clinical evidence of atrial fibrillation and mean left atrial pressure was 10, 13, and 17 mm Hg, respectively.

**Antibodies**

The antibodies used in the present study have been described in detail elsewhere (Sartore et al., 1981; Gorza et al., 1982; Donello et al., in preparation). In brief, anti-bovine atrial myosin (anti-hP) and anti-human pectoralis myosin (anti-hP) antisera were raised in rabbits, injecting purified myosin of bovine left atrium and human pectoralis muscle, respectively. Specific IgG were obtained by affinity chromatography on the insolubilized immunogens. Anti-bA was absorbed on insolubilized bovine left ventricular myosin and anti-hP on insolubilized bovine left atrial myosin. Anti-hP was found to react selectively with type I, slow fibers in human skeletal muscle (Donello et al., in preparation).

The reactivity of the two antibodies with human atrial and ventricular myosins was determined by the immunoblot technique. Crude myosin preparations were obtained from cryosections of atrial and ventricular specimens by a procedure that will be described in detail elsewhere. The sections, about 20 μm thick, were first incubated for 1 hour in low ionic strength medium to remove soluble proteins, then extracted for 2 hours in alkaline pyrophosphate buffer (Hoh et al., 1979). The myosin samples were electrophoresed in 12.5% polyacrylamide gels, by the Laemmli buffer system (Laemmli, 1970). The procedure for electrophoretic transfer of polypeptides from SDS gels to nitrocellulose paper (Biorad) was that of Towbin et al. (1979), with the following modifications. After electrophoretic transfer, the paper strips were incubated for 2 hours at 37°C in phosphate-buffered saline, (PBS) pH 7.2 containing 10% calf serum, 0.2% gelatin, and 0.02% sodium azide. The strips were then incubated for 2 hours at 37°C with appropriate dilutions of antimyosin antibodies. After three washings in PBS containing 0.1% Tween 20, 0.1% gelatin, and 0.02% sodium azide, the strips were incubated for 1 hour at room temperature with 125I-labeled goat-anti-rabbit IgG (5 X 107 counts/min per ml) and washed again three times in the same washing solution. Air-dried strips were exposed to Kodak XAR-5 X-ray film with an intensifying screen at −80°C.

**Immunofluorescence**

The cardiac specimens were frozen in liquid nitrogen, and cryostat sections were incubated with appropriate dilutions of the specific antimyosin antibodies for 30 minutes at 37°C. The sections were rinsed for 10 minutes in PBS and incubated for 30 minutes at 37°C with fluorescein-labeled anti-rabbit IgG (Miles Lab.). Then the sections were rinsed again in PBS, fixed with paraformaldehyde 1.5% in PBS for 15 minutes, mounted with Elvanol, and
TABLE 1
Sources of Cardiac Samples Examined

<table>
<thead>
<tr>
<th>Normal samples</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Obtained from</th>
<th>Tissue examined*</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>F</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Septic shock</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>M</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Head trauma</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Head trauma</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>M</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Head trauma</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>M</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Hemorrhage in cyrtotic</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>M</td>
<td>Autopsy</td>
<td>LV</td>
<td>Head trauma</td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>F</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Hemorrhage in cyrtotic</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Traumatic hemorrhage</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>M</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Brain carcinoma</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>M</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Basilar artery thrombosis</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>F</td>
<td>Autopsy</td>
<td>LV</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>12</td>
<td>63</td>
<td>F</td>
<td>Autopsy</td>
<td>RA, LA, RV, LV</td>
<td>Hemorrhage in cyrtotic</td>
</tr>
<tr>
<td>13</td>
<td>67</td>
<td>F</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>14</td>
<td>76</td>
<td>F</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Colon carcinoma</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>F</td>
<td>Surgery</td>
<td>RA</td>
<td>Coronaropathy</td>
</tr>
<tr>
<td>16</td>
<td>54</td>
<td>M</td>
<td>Surgery</td>
<td>RA</td>
<td>Coronaropathy</td>
</tr>
<tr>
<td>17</td>
<td>61</td>
<td>M</td>
<td>Surgery</td>
<td>RA</td>
<td>Coronaropathy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypertrophied samples</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Obtained from</th>
<th>Tissue examined*</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>57</td>
<td>M</td>
<td>Autopsy</td>
<td>LV</td>
<td>Mitral regurgitation, post-op LCOS†</td>
</tr>
<tr>
<td>19</td>
<td>62</td>
<td>F</td>
<td>Autopsy</td>
<td>LV</td>
<td>Mitral regurgitation, post-op LCOS†</td>
</tr>
<tr>
<td>20</td>
<td>31</td>
<td>F</td>
<td>Surgery</td>
<td>LV</td>
<td>Mitral regurgitation</td>
</tr>
<tr>
<td>21</td>
<td>37</td>
<td>M</td>
<td>Surgery</td>
<td>LV</td>
<td>Mitral regurgitation</td>
</tr>
<tr>
<td>22</td>
<td>49</td>
<td>F</td>
<td>Surgery</td>
<td>LV</td>
<td>Mitral regurgitation</td>
</tr>
<tr>
<td>23</td>
<td>54</td>
<td>F</td>
<td>Surgery</td>
<td>LV</td>
<td>Mitral regurgitation</td>
</tr>
<tr>
<td>24</td>
<td>68</td>
<td>M</td>
<td>Autopsy</td>
<td>RV, LV</td>
<td>Hypertension, stroke</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>F</td>
<td>Autopsy</td>
<td>LV</td>
<td>Hypertension, stroke</td>
</tr>
<tr>
<td>26</td>
<td>84</td>
<td>F</td>
<td>Autopsy</td>
<td>LV</td>
<td>Hypertension, pulmonary edema</td>
</tr>
<tr>
<td>27</td>
<td>22</td>
<td>F</td>
<td>Surgery</td>
<td>RV</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>28</td>
<td>23</td>
<td>M</td>
<td>Surgery</td>
<td>RV</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>29</td>
<td>42</td>
<td>M</td>
<td>Surgery</td>
<td>RV</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>30</td>
<td>23</td>
<td>F</td>
<td>Surgery</td>
<td>LA</td>
<td>Mitral stenosis</td>
</tr>
<tr>
<td>31</td>
<td>28</td>
<td>F</td>
<td>Surgery</td>
<td>LA</td>
<td>Mitral stenosis</td>
</tr>
<tr>
<td>32</td>
<td>42</td>
<td>F</td>
<td>Surgery</td>
<td>LA</td>
<td>Mitral stenosis</td>
</tr>
<tr>
<td>33</td>
<td>42</td>
<td>F</td>
<td>Surgery</td>
<td>LA</td>
<td>Mitral stenosis</td>
</tr>
<tr>
<td>34</td>
<td>53</td>
<td>F</td>
<td>Surgery</td>
<td>LA</td>
<td>Mitral stenosis</td>
</tr>
</tbody>
</table>

* RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle.
† Postoperative low cardiac output syndrome.

Results

Myosin preparations obtained from cryosections of human left atrial and left ventricular specimens were used for immunoblotting. As shown in Figure 1, anti-bA reacted selectively with atrial myosin heavy chains (MHC), whereas anti-hP reacted selectively with ventricular MHCs. Similar results were obtained with solid-phase enzyme immunoassays: in these assays, anti-hP showed a more specific reactivity toward human ventricular myosin, compared with other antibodies previously used to study ventricular myosin of the bovine, rabbit, and rat heart (Gorza et al., 1981, 1982).

Control sections of biotic and autopic specimens of human heart incubated with preimmune IgG showed no fluorescein fluorescence. Spots of red fluorescence due to lipofuscin aggregates were often observed in the central portions of the myocytes, especially in hearts of old patients. When applied to sections of left auricular and left ventricular human myocardium anti-bA and anti-hP, myosin antibodies revealed a clearcut difference in reactivity (Figs. 2–5). Atrial muscle cells were all labeled by anti-bA and most of them were unlabeled by anti-hP, whereas ventricular muscle cells showed a reversed staining pattern. However, we found that a number...
of atrial fibers were also labeled by anti-hP, and a number of ventricular fibers were also labeled by anti-bA. This heterogeneous response was observed in both surgical and autopic specimens obtained at different times after death. Variation in antimyosin staining cannot therefore be attributed to postmortem denaturation or degradation of the myosin molecule, but must reflect real differences in myosin composition between cardiac muscle cells.

Atrial muscle cells labeled by anti-hP were rare in the left atrium (Fig. 2b), whereas they were more frequent in the right atrium (Fig. 3). They showed variable staining intensity and were either interspersed among unlabeled cells or grouped in small bundles. Labeled cells were especially numerous in the crista terminalis region and in the interatrial septum: at these sites, large bundles homogeneously composed of reactive cells were regularly observed (Fig. 3). Labeled fibers were less numerous in both autopic and surgical specimens of right auricular appendage and were very rare in the left auricular appendage. All myocytes from both right and left atrial samples were homogeneously reactive with anti-bA (Fig. 2a).

The proportion of ventricular muscle cells labeled by anti-bA was quite variable in different normal hearts. This variable response was apparently not related to age or sex. In the case of a 60-year-old man (no. 10), almost all ventricular fibers in samples from both right and left ventricle were unlabeled by anti-bA (Fig. 4a); in another case of a 67-year-old woman (no. 13), all ventricular fibers were labeled (Fig. 5b). However, most specimens gave a heterogeneous response, with a variable number of weakly reactive fibers and rare strongly reactive fibers interspersed in a mosaic pattern among a majority of...
negative fibers (Fig. 5a). There was no significant regional variation in the proportion of labeled fibers within the same heart. In particular, the human heart, unlike the bovine heart, showed no obvious difference in the distribution of reactive fibers between the right and left ventricle or between subendocardial and subepicardial regions. Occasionally, small groups of labeled fibers standing out from the surrounding unlabeled fibers and, presumably, corresponding to Purkinje fibers, were seen at the very endocardial surface (Fig. 4). All ventricular myocytes from all samples were labeled by anti-hP (Fig. 4b).

The response of the hypertrophied hearts to anti-bA myosin antibodies appeared to vary in different types of hypertrophy (Fig. 6). A heterogeneous pattern of reactivity, indistinguishable from that commonly encountered in control hearts, was observed in both the free wall and the papillary muscles from the left ventricles of patients with severe mitral regurgitation. A similar pattern was seen in surgical papillary muscles from patients with mitral regurgitation (Fig. 6a). In contrast, only very rare muscle cells labeled by anti-bA were found in the hypertrophied left ventricles of subjects with medical history of hypertension or with aortic stenosis. Interestingly, in one of these hearts (no. 24), there was a significant difference in the response to anti-bA between left and right ventricle, in that samples from the right ventricle showed a higher number of labeled fibers. Very rare reactive fibers were also seen in surgical specimens of hypertrophied pulmonary infundibulum from patients with tetralogy of Fallot (Fig. 6b). It should be stressed that the ventricular samples from normal and pathological hearts showed no difference with respect to anti-hP staining that was homogeneously strong in all myocytes from all specimens.

Striking changes in myosin immunoreactivity were found in surgical samples of the left atrium from patients with mitral stenosis. In one sample (no. 30), there was an almost complete reversal of the normal staining pattern: all atrial myocytes were strongly reactive with anti-hP, and most of them were unreactive or poorly reactive with anti-bA (Fig. 7). In another sample (no. 31), groups of fibers showing the normal staining pattern were intermingled with fibers showing a reversed staining pattern (Fig. 8). This heterogeneous response was also found in specimens from fibrillating atria, which consisted of small islands of muscle fibers embedded in fibrotic tissue. In the hypertrophied atrial samples, there was no apparent correlation between degree of hypertrophy of the muscle fibers and myosin
FIGURE 4. Normal ventricular myocardium (patient no 10) Serial sections from the endocardium of the left ventricle stained with anti-bA (panel a) and anti-hP (panel b). Only Purkinje fibers (on the right) stain brightly with both antibodies.

FIGURE 5. Normal ventricular myocardium. Sections of the left ventricular wall of patient no. 3 (panel a) and patient no. 13 (panel b) stained with anti-bA. The heterogeneous pattern of reactivity seen in panel a was also found in most other ventricular specimens, whereas the homogeneous positive reaction seen in panel b was seen only in this particular patient. In both specimens, all fibers were also labeled by anti-hP.

Discussion

This study shows that (1) different types of MCHs are present in human atrial and ventricular myocardium and are variably distributed among cardiac muscle fibers, and (2) chronic exposure to hemodynamic overload can induce dramatic changes in the MHC composition of human atria, whereas it affects only slightly that of the ventricles.

Human atrial and ventricular myosins were previously shown to differ in light chain subunit pattern and ATPase activity (Yazaki et al. 1979; Price et al., 1980). We now find that they also differ in the antigenic structure of MHCs, and that, in agreement with previous studies in other mammalian species, a number of atrial myocytes contain a ventricular-like MHC and a number of ventricular myocytes contain an atrial-like MHC. Adopting a terminology originally introduced by Hoh et al. (1979) for rat ventricular MHCs, we propose to call α-type the MHCs recognized by anti-bA, which represent a major component of human atrial myosin and a minor component of ventricular myosin, and β-type the MHCs recognized by anti-hP, which represent a major component of ventricular myosin and a minor component of atrial myosin. Identity of rabbit atrial and ventricular HCa, is suggested by biochemical and immunochemical studies (Sartore et al., 1979; Dalla Libera and Sartore, 1981; Chizzonite et al., 1981; Clark et al., 1983), whereas the relationship between atrial and ventricular HCa in the mammalian heart remains to be established.

Based on the results presented here and in a companion article (Mercadier et al., 1983), one can thus postulate the existence in the human heart of different ventricular isomyosins, corresponding to the V1 (homodimer of HCa), V2 (heterodimer), and V3 (homodimer of HCa) detected by pyrophosphate gel electrophoresis in other mammalian species (Hoh et al., 1978). The fact that only one band comigrating with rat V3 myosin is actually seen in pyrophosphate gels of human ventricular samples (Mercadier et al., 1983) may be due to intrinsic limitations of the procedure, as shown in other species (Clark et al., 1982), and to the low amount of HCa, less than...
10% in most samples, demonstrated in the ventricular myocardium by quantitative immunochemical studies (Mercadier et al., 1983; Schiaffino et al., in preparation). The specimen homogeneously composed of fibers labeled by anti-βA (Fig. 5b) was found to contain 14-24% HC₉, according to the reference system used (Mercadier et al., 1983; Schiaffino et al., in preparation). On the other hand, due to the heterogeneous distribution of HC₉ seen in most samples by immunofluorescence, it is impossible to estimate the actual concentration of HC₉ in any single labeled fiber. It is likely that some fibers contain relatively high amounts of HC₉, which might explain the finding that occasional fibers in the human ventricular myocardium show atrial-like reactivity with respect to histochemical myosin ATPase activity (Thornell and Forsgren, 1982). In the human heart, at variance with the bovine heart (Sartore et al., 1981), we found no obvious regional variation in the distribution of ventricular fibers containing HC₉.

Heterogeneity of atrial fibers in the human heart is reflected in the variable expression of a β-type MHC. Atrial fibers expressing this myosin pheno-

type showed a consistent pattern of distribution, being rare in the left auricle and particularly abundant in the crista terminalis and in the interatrial septum. These findings are in agreement with previous observations in the bovine heart (Gorza et al., 1982) and add new evidence supporting the notion that muscle cells with special properties are present in the right atrium along the pathways of preferential conduction of the electrical stimulus from the sinus node to the atrioventricular node (Sherf and James, 1979). The functional significance of the peculiar myosin composition of these atrial fibers is not known. We have speculated (Gorza et al., 1982) that, in cardiac muscle, the expression of different myosin genes may be influenced by the membrane properties of each cell type, but direct correlation between electro-physiological and immunofluorescence data on the same cell will be necessary to define the role of the various cell types. It should be stressed that, on the basis of antimyosin immunofluorescence, there is no evidence for a clearcut separation of the β-positive fibers of the crista terminalis and interatrial septum from the bulk of the atrial myocardium, fibers containing HC₈ often being mixed with unlabeled fibers at different sites.

The changes in MHC composition induced in the
Human atrial and ventricular myocardium by chronic pressure overload consist in an α-to-β transition analogous to that observed in experimental models (Lompre et al., 1979; Mercadier et al., 1981; Gorza et al., 1981; Litten et al., 1982). In the hypertrophied ventricular myocardium, this change is obscured by the considerable individual variability seen in control samples and is quantitatively negligible due to the great prevalence of HCα in the normal heart; therefore, it is presumably of limited functional relevance (see Mercadier et al., 1983). No significant change was detected in this study by immunofluorescence in ventricular specimens with hypertrophy secondary to volume overload, in agreement with previous studies on experimental models (Carey et al., 1979; Mercadier et al., 1981).

In contrast, antimyosin immunofluorescence reveals a marked redistribution of MHC isoforms in hypertrophied left atria. The α-to-β transition was apparently complete in many hypertrophied atrial fibers which showed strong immunoreactivity for HCα and no reactivity at all for HCβ. The degree of transformation was, however, variable between different specimens and even between different fibers of the same specimen. In some specimens, the majority of atrial myocytes showed a complete transformation of the immunoreactivity pattern. Preliminary immunohistochemical studies using procedures similar to those applied to quantify myosin changes in the hypertrophied ventricles (Mercadier et al., 1983) indicate that HCα is undetectable in the normal human left atria and may increase up to 90% in some hypertrophied atria (Schiaffino et al., in preparation). The transformed atria also show a reversal of the normal pattern of reactivity in sections incubated for the histochemical demonstration of myosin ATPase activity (L. Gorza, unpublished observations). The changes in atrial MHCs are accompanied by parallel changes in myosin light chains: ventricular-like myosin light chains have, in fact, been detected in hypertrophied left atria of patients with mitral stenosis (Cummins 1982). These results apparently are in contrast to a recent report that atrial hypertrophy induced in the rat by aortic banding is not accompanied by changes in the light chain pattern and in the electrophoretic mobility of myosin in nondenaturing gels (Bugaisky et al., 1983). It remains to be determined whether the different response of the rat atrial muscle is due to the type of stress inducing hypertrophy or is a species difference.

We thank Maurizio Moretto and Massimo Fabbri for excellent technical assistance and Fiorella Burgio for secretarial work.

This work was supported by Ministero della Pubblica Istruzione and Consiglio Nazionale delle Ricerche, Gruppo Cardiorespiratorio (Grants 82.02169.04 and CT82.00204.04). L. G. is a Fellow of the Anna Villa Rusconi Foundation.

Address for reprints: Dr. S. Schiaffino, Institute of General Pathology, University of Padova, Via Loredan, 16 35100 Padova, Italy. Received December 14, 1983; accepted for publication February 29, 1984.

References
Barany M (1967) ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50: 197–218
Clark WA, Chizzonite RA, Everett AW, Rabinowitz M, Zak R...
ROWN H, Stachelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and some applications. Proc Natl Acad Sci USA 76: 4350-4354

INDEX TERMS: Cardiac myosins • Immunofluorescence • Human heart • Ventricular hypertrophy • Atrial hypertrophy
Myosin types in the human heart. An immunofluorescence study of normal and hypertrophied atrial and ventricular myocardium.
L Gorza, J J Mercadier, K Schwartz, L E Thornell, S Sartore and S Schiaffino

*Circ Res.* 1984;54:694-702
doi: 10.1161/01.RES.54.6.694

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/54/6/694