Myosin Isoenzymic Changes in Several Models of Rat Cardiac Hypertrophy

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SUMMARY We studied the effect of chronic mechanical overloading on the isoenzymic composition of rat cardiac myosin in several experimental models: aortic stenosis (AS), aortic incompetence (AI), aortocaval fistula (ACF), overload of the non-infarcted area after left coronary ligation (INF), and overload of the spontaneously hypertensive rats (SHR). Samples of the left and right ventricles were isolated from these hearts, and myosins were analyzed by electrophoresis in non-dissociating conditions. The myosin isoenzymes were called VI, V2, and V3 in order of decreasing mobility, according to the nomenclature of Hoh et al. Controls of the Wistar and Wistar Kyoto (WKY) strains were almost exclusively VI. A slow age-dependent shift toward V3 was observed in the left ventricles of adult Wistar rats, which at 30 weeks of age (body weight 600 g) contained approximately 15% of this form. In all models of cardiac hypertrophy, an isoenzymic redistribution was observed with a significant increase in V3. The level of V3 was statistically correlated with the degree of hypertrophy in the AS, (n = 11, r = 0.8, P < 0.003), the AI (n = 14, r = 0.88, P < 0.001), and the AS + AI (n = 14, r = 0.89, P < 0.001) but not in the ACF (n = 16, r = 0.48). The isoenzymic changes could account for the decreases in both myosin ATPase activity and cardiac contractility described previously in our laboratory and by others. They also demonstrate that changes in myosin isoenzymes represent a general response of the rat heart, to chronic mechanical overloading.


HYPERTROPHY is a fundamental adaptive response of the heart to an imposed work overload. The intrinsic contractile state of such hypertrophied hearts has been the subject of considerable and often contradictory investigation. It is generally admitted that the velocity of shortening of cardiac fibers is modified to variable degrees, depending on the inciting stimulus: for example, it is enhanced in response to thyroxin intoxication (see Morkin, 1979), and markedly depressed in chronic pressure induced hypertrophies (see Maughan et al., 1979). The close relationship between the shortening velocity of these hearts and their myosin ATPase activity has recently been mentioned by Scheuer and Bahn (1979) in a comprehensive review: increased performance is associated with increased ATPase activity and, conversely, hypotrophic states are accompanied by depressed activity.

Isoenzymic changes in myosin, which can account for the enzymatic alterations, have been described recently in two models of cardiac hypertrophy. In response to thyroxin administration, euthyroid rabbits (see Morkin, 1979) and hypophysectomized rats (Hoh et al., 1978) both synthesize an isoenzyme of high ATPase activity. We have found, in rats, that, in gross cardiac hypertrophy induced by aortic stenosis or aortic incompetence, an isoenzyme exhibiting a low activity is predominant (Schwartz et al., 1978; Lompré et al., 1979). In the rat, these isoenzymes (VI and V3 according to the nomenclature of Hoh et al.) display different electrophoretic mobilities under non-dissociating conditions (Schwartz et al., 1978; 1980; Lompré et al., 1979). The close relationship between the shortening velocity of these hearts and their myosin ATPase activity has recently been mentioned by Scheuer and Bahn (1979) in a comprehensive review: increased performance is associated with increased ATPase activity and, conversely, hypotrophic states are accompanied by depressed activity.

Isoenzymic changes in myosin, which can account for the enzymatic alterations, have been described recently in two models of cardiac hypertrophy. In response to thyroxin administration, euthyroid rabbits (see Morkin, 1979) and hypophysectomized rats (Hoh et al., 1978) both synthesize an isoenzyme of high ATPase activity. We have found, in rats, that, in gross cardiac hypertrophy induced by aortic stenosis or aortic incompetence, an isoenzyme exhibiting a low activity is predominant (Schwartz et al., 1978; Lompré et al., 1979). In the rat, these isoenzymes (VI and V3 according to the nomenclature of Hoh et al.) display different electrophoretic mobilities under non-dissociating conditions (Hoh et al., 1978; Lompré et al., 1979). Observations made on myosins composed almost exclusively of one form or the other have shown that V1 and V3 differ, in addition to their charge and their enzymatic activity (V1 > V3), in the antigenic structure and the peptide maps of their heavy chains. These conclusions were based on differences in micro-complement fixation performed with antibodies specific to the native or denatured configurations of the heavy chains (Schwartz et al., 1978; 1980; Lompré et al., 1979) and on one- and two-dimensional peptide maps obtained after chemical or proteolytic cleavage (Hoh et al., 1979; Klotz et al., 1981; Schwartz and Whalen, unpublished observations). In the rabbit, as well, structural differences have been found between the heavy chains of cardiac myosins obtained from normal and hyperthyroid animals (see Morkin, 1979).

The present study was undertaken to determine
whether, in rats, the synthesis of the isoenzyme of low activity V3 occurred also in other models of chronic mechanical overloading, and whether these isoenzymic changes were correlated with the increase in cardiac mass. For this purpose, mechanical overload of various types was produced in rats. The myosin isoenzymes of the corresponding hearts were investigated by their migration in gel electrophoresis (Hoh et al., 1978; d’Albis et al., 1979).

**Methods**

**Experimental Groups and Surgical Procedures**

This study included 65 operated male Wistar rats and 15 controls, plus eight spontaneously hypertensive rats (SHR) of Wistar Okamoto strain and seven Wistar Kyoto normotensive controls (WKY). The rats were housed in air-conditioned quarters in group cages with no more than six rats per cage and were provided food (Extralabo M 25 pellets) and tap water ad libitum.

Surgical procedures were carried out under anesthe-

Constriction of the aorta (AS) was performed in 11 rats as described by Cutulletta et al. (1978) for the ascending aorta, except that the partially occluded neck hemoclip was positioned around the upper part of the abdominal aorta. Aortic incompetence (AI) was induced in 14 rats by the method of Jouannot et al. (1973): a stiff polyethylene catheter was introduced in the right carotid artery and the patency of the fistula was assessed by the appearance of the vena cava which was stretched, and the inside of the vein washed with Ringer’s solution. This incision allowed us to reach the medial common wall and a microsurgical suture (9-0 Ethilon, Ethicon) was passed through this double layer. The suture then was pulled so that the common wall protruded in the middle of the phlebotomy (Fig. 1). This protruding area was resected under the suture with microscissors and the vein closed with an overcast seam. Clamps were removed and the patency of the fistula was assessed by the appearance of the vena cava which was stretched, reddish, and pulsatile. The abdominal incision was closed and the rat was allowed to recover. Since it has been impossible to standardize the size of the

Left ventricular infarction was induced in 10 rats (INF) by a method similar to those previously described (see Pfeffer et al., 1979a): each rat was anesthetized with ether and a left thoracotomy was performed. The heart was exteriorized by gentle pressure on the right side of the thorax. The left coronary artery was ligated between the pulmonary artery and the left atrium. The heart was returned to its position and the thorax rapidly closed after pressing on it to empty the remaining air from the pleural cavity.

In all these groups, the animals were killed at various times after surgery (3 to 28 weeks, see Table 1).

In 16 rats an aortocaval fistula (ACF) was produced by slightly modifying the procedures of Attel et al. (1979) and Flaim et al. (1979): the aortocaval pedicle was reached through an abdominal midline incision and, under a dissecting microscope, the part between the origin of the renal arteries and the aortic bifurcation was carefully dissected from the posterior layers. Small vascular bulldog clamps were positioned on the two vessels from the right to the left, one over the aortic bifurcation and the other beneath the origin of the renal arteries. By a right-to-left rocking motion of approximately 90° of the two clamps, the vena cava was brought above the aorta. A longitudinal 2- to 3-mm-long incision was made on the vein with a razor blade fragment and the inside of the vein washed with Ringer’s solution. This incision allowed us to reach the medial common wall and a microsurgical suture (9-0 Ethilon, Ethicon) was passed through this double layer. The suture then was pulled so that the common wall protruded in the middle of the phlebotomy (Fig. 1). This protruding area was resected under the suture with microscissors and the vein closed with an overcast seam. Clamps were removed and the patency of the fistula was assessed by the appearance of the vena cava which was stretched, reddish, and pulsatile. The abdominal incision was closed and the rat was allowed to recover. Since it has been impossible to standardize the size of the

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of rats</th>
<th>Duration of overload (wks)</th>
<th>Age at study (wks)</th>
<th>Body wt at study (g)</th>
<th>Ventricular wt at study (g)</th>
<th>Hypertrophy (%)</th>
<th>(Ventricular wt/ body wt) × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>6</td>
<td>8–13</td>
<td>300–450</td>
<td>0.80–1.10</td>
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<td></td>
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<tr>
<td>AS</td>
<td>9</td>
<td>15–30</td>
<td>490–680</td>
<td>1.20–1.60</td>
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<td>AI</td>
<td>7</td>
<td>3–14</td>
<td>265–400</td>
<td>0.73–1.58</td>
<td>27–100</td>
<td></td>
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<td>AI + AI</td>
<td>4</td>
<td>25–26</td>
<td>489–700</td>
<td>2.20–2.40</td>
<td>40–86</td>
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<tr>
<td>AS + AI</td>
<td>6</td>
<td>5–23</td>
<td>370–440</td>
<td>1.30–1.80</td>
<td>28–72</td>
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<tr>
<td>AS + AI</td>
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<td>30–36</td>
<td>530–670</td>
<td>1.55–2.50</td>
<td>8–93</td>
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<td>ACF</td>
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<td>11–29</td>
<td>580–650</td>
<td>1.65–1.87</td>
<td>63–116</td>
<td></td>
<td></td>
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<tr>
<td>INF</td>
<td>16</td>
<td>12</td>
<td>517–709</td>
<td>1.45–2.32</td>
<td>25–97</td>
<td></td>
<td></td>
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<tr>
<td>INF</td>
<td>7</td>
<td>3–6</td>
<td>270–378</td>
<td>0.83–1.30</td>
<td>14–66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>8</td>
<td>52</td>
<td>270±9*</td>
<td>0.81±0.03</td>
<td>Z3±0.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>8</td>
<td>14–16</td>
<td>430–535</td>
<td>1.06–1.44</td>
<td>0–25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as ranges or as mean ± 1 SEM(*) AS = aortic stenosis AI = aortic incompetence ACF = aortocaval fistula INF = left ventricular infarction WKY = Wistar Kyoto SHR = spontaneously hypertensive rats of Wistar-Okamoto strain
fistula, the overload was presumed to be highly variable, and all animals of this group were killed approximately at the same time after surgery (12 weeks).

The 15 controls were unoperated rats of the same strain, kept under identical housing conditions as the operated rats. In order to fit approximately the age and weight of the operated animals, 8- to 30-week-old rats were used [body weight (BW) from 300 g to 680 g].

The male spontaneously hypertensive rats (SHR) of the Wistar Okamoto strain and their controls of the Wistar-Kyoto strain (WKY) were a gift of Prof. P.Y. Hatt and were taken from a breeding stock originally provided from Iffa Credo (Paris). They were housed in Prof. P. Y. Hatt’s animal house, and killed at 12 months of age.

All animals were killed by a blow to the head. They were weighed (BW) and the heart was rapidly excised. Atria and great vessels were removed. The ventricles were opened, washed with saline, blotted dry, and weighed (ventricular weight, VW). For some experiments, the right ventricle was dissected along its septal insertion from the rest of the ventricular mass. The ventricles were frozen in liquid nitrogen within 2 minutes and stored at -20°C.

The myocytes were prepared from isolated hearts and perfused with hyaluronidase (0.1% wt/vol) and collagenase (0.05% wt/vol) according to Cutilletta et al. (1977). The cells were stored at -20°C in the presence of 50% (vol/vol) glycerol.

Estimation of Heart Hypertrophy

The degree of hypertrophy as a result of AS, AI, AS + AI, and ACF was calculated using a regression curve BW/VW established in 152 controls from the same strain (Berson and Swynghedauw, 1973). For each rat, the theoretical heart weight was: ThW (g) = \((2.02 \times BW) + 197\) \(\times 10^{-3}\). The percentage of hypertrophy was \((VW - ThW) \times 100 / ThW\).

For the rats that underwent an experimental myocardial infarction, no reliable means of expressing hypertrophy of the noninfarcted area could be found. Only the rats showing evidence of a large ventricular infarction (scar assessed by marked thinning and pale color of the wall, >7 mm in diameter) were kept for the study and the percentage of hypertrophy was calculated as above.

For the SHR and WKY, the VW/BW ratios were calculated.

Analytical Procedures

Crude tissue extracts were obtained from samples of about 100 mg crashed in liquid nitrogen and extracted for 20 minutes at +4°C with 4 volumes (wt/vol) of a slightly modified Guba’s solution (Guba et al., 1968) [0.3 M KCl, 0.1 M PO₄, 0.05 M PO₄, 0.001 M MgCl₂, 0.001 M P₂O₅, 0.1% (wt/vol) Azide Na, 1% (vol/vol) 2-mercaptoethanol]. The homogenate was centrifuged 20 minutes at +4°C and 30,000 g and the supernatant stored at -20°C in 50% (vol/vol) glycerol.

Polyacrylamide gel electrophoresis in non-dissociating conditions was performed in a Pharmacia apparatus (GE-411) which allows thermostating and recirculation of the buffer between the anodic and the cathodic reservoirs, according to Hoh et al. (1978), except that the running buffer contained, in addition to 0.02 M Na₃P₂O₅ and 10% (vol/vol) glycerol, 0.001 M EDTA, 0.01% (vol/vol) 2-mercaptoethanol, pH 8.5 (d’Albis et al., 1979). Cylindrical polyacrylamide gels (60 mm × 5 mm) were prepared with 3.88% (wt/vol) acrylamide, and 0.12% (wt/vol) N,N'-methylene bisacrylamide. Each gel was loaded with approximately 1-2 μg of myosin, i.e., 30 μl of a 40-fold dilution of the crude extract with 0.01 M Na₃P₂O₅, 50% (vol/vol) glycerol, traces of bromophenol blue, pH 8.5. Temperature of the electrophoresis buffer was maintained between +1 and +3°C, and gels were run at a constant voltage of 14 V/cm for 20 to 24 hours.

Staining and destaining of the gels were carried...
out according to Hoh et al. (1978) in a Hoefer Scientific Instruments apparatus. Densitometric tracings of the gels were obtained with a Gilford Spectrophotometer model 240 equipped with a Hewlett-Packard 70-44A multirecorder tracing table. The relative amount of each isoenzyme was calculated from the height of each peak. Results were expressed as percentages of V3. Reproducibility of the whole procedure, calculated according to Henry (1964) was equal to 9% (16 pairs of duplicates). Results were expressed as the mean ± SE. When two groups of data were compared, statistical comparisons were carried out by Student's t-test. Regression lines were calculated using the method of least squares. Statistical significance was considered to be \( P < 0.05 \).

**Results**

**Magnitude of Hypertrophy**

Table 1 shows that the experimental devices we used allowed us to obtain a wide range of hypertrophies, up to approximately 120%. The most important increases in cardiac mass were obtained with the two-step procedure using AS and AI. The degree of hypertrophy was not related to the duration of hemodynamic overload, which was probably due to the technical difficulties in imposing overloads reproducible from one animal to the other. The INF group also displayed significant degrees of hypertrophy (up to 66%) despite the important myocardial defect induced by the infarction itself. This indicated that the remaining tissue was, in fact, more hypertrophied than was reflected in our calculated values. In the SHR group, the VW/BW ratio was significantly increased, as compared with their WKY controls (\( P < 0.001 \)).

Signs of heart failure (dyspnea, prostration of the animal, ascitis, pleural effusion) were never noted, except in a few animals exhibiting more than 100% hypertrophy. None of the INF displayed any of these signs.

**Myosin Isoenzymic Patterns**

Gels typical of the LV of control animals and of rats submitted to each type of overload are shown in Figure 2. In controls, the predominant form was V1, in close agreement with Hoh et al. (1978) and with previous observations from our laboratory made on sham-operated animals of the same rat strain (Lompré et al., 1979). An isoenzyme redistribution in favor of the slow migrating form V3 was observed in all the models we studied. The electrophoretic mobility of each isoenzyme (V1, V2, and V3) was identical in controls and in experimental animals of the Wistar strain as well as in the SHR and WKY rats.

Electrophoresis of myosins obtained from myocytes of a control heart (BW 500 g) and a hypertrophied heart (AS, 96% hypertrophy, approximately same BW) are shown in Figure 3. Both patterns were in close agreement with those observed above with the whole ventricular tissue, and this established that the isoenzymic changes occurred in the muscle cells themselves.

**Relationship between the Degree of Hypertrophy and Isoenzymic Shift**

A significant correlation was found (Fig. 4) between left ventricular V3 and the degree of heart hypertrophy for AS (\( n = 11, r = 0.61, P < 0.05 \)), for AI (\( n = 14, r = 0.88, P < 0.001 \)), and for AS + AI (\( n = 14, r = 0.68, P < 0.01 \)), and this showed that the increase in ventricular mass and the appearance of V3 were simultaneous. A very important reversal of the isoenzymic pattern was observed for the bulky hearts (approximately 60% of V3 for 100% hypertrophy), in close agreement with our previous observations. In contrast, the correlation was not significant for the ACF (\( n = 16, r = 0.46 \)), and the extent of the isoenzymic shift was small: out of 13 hypertrophied hearts, values between 35 and 40% were observed in only three animals, whereas all others contained between 15 and 22% V3, which is
Control AS

**Figure 3** Gel electrophoresis in non-dissociating conditions of native myosins from myocytes of a control and a hypertrophied rat heart (aortic stenosis, 96% hypertrophy).

equal to or only slightly different from controls of the same body weight (see Fig. 5). No hypertrophy was found in three animals, possibly because the fistula was spontaneously obstructed: two of them exhibited a normal isoenzymatic pattern, and the third one contained 20% V3, which is slightly above controls.

An equivalent correlation seemed to exist in the INF, but was not statistically significant ($n = 10$, $r = 0.6$, $0.05 < P < 0.1$). The average amount of V3 was $29 \pm 2\%$, which represented a mean 3-fold increase as compared to controls of the same body weight. As compared to the WKY, an approximately 4-fold increase in V3 was observed in the SHR, whose LV myosin contained $35 \pm 2\%$ V3 ($P < 0.001$).

LV-RV Differences

During growth (Fig. 5) the amount of the slow migrating form V3 progressively increased in the LV of control animals, whereas the amount of the fast form was depressed in a parallel manner. The increase in V3, though small, was significantly correlated with the increase in BW ($n = 15$, $r = 0.73$, $P < 0.01$). In contrast, no significant relationship was found in the RV, whose V3 seemed to remain approximately constant ($n = 9$, $r = 0.2$). LV of 1-year-old WKY rats contained slightly less V3 than did 6-month-old Wistar controls (9% and 16%, respectively).

Table 2 shows the differences in the amounts of V3 between left and right ventricles in controls and in some experimental animals, as a function of the amount of V3 in the left ventricle: all right ventricles contained less V3 than did the left. In the ACF

**Figure 4** Relationship between degree of ventricular hypertrophy and the associated changes in left ventricular myosin isoenzyme V3 amount for AS ($r = 0.61$, $P < 0.06$), AI ($r = 0.88$, $P < 0.001$), AS + AI ($r = 0.69$, $P < 0.01$) and ACF ($r = 0.46$, NS).

**Figure 5** Age-dependent changes in the amount of myosin isoenzyme V3 left (●) and right (○) ventricles of control rats of the Wistar strain. The linear regression was significant for the left ventricle ($r = 0.73$, $P < 0.01$) but not for the right ($r = 0.20$).
group, the LV-RV difference was small and of the same order of magnitude as for the elder controls. In the AS, AS + AI and INF, LV V3 increased before RV V3: RV V3 was only 15%, when LV V3 was already about 35%. Further increase in LV V3 was accompanied by a similar increase in the RV, and the LV-RV differences were small.

For the SHR, the approximately 4-fold increase in LV V3 as compared with the WKY controls (see above) was accompanied by an equivalent increase in RV V3, \((P < 0.001)\), and thus, the LV-RV difference was slightly increased (Fig. 6).

![Figure 6](https://example.com/figure6.png)

**Figure 6** Comparison of myosin isoenzyme V3 amounts in right (RV) and left (LV) ventricles of Wistar-Kyoto rats (WKY), and spontaneously hypertensive Wistar-Ohamato rats (SHR). Values represent mean ± 1 se. 

**TABLE 2** Comparison of the Amounts of V3 (%) in Left and Right Ventricles from Normal and Hypertrophied Rat Hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls (n = 9)</th>
<th>ACF (n = 15)</th>
<th>AS, AS + AI (n = 9)</th>
<th>INF (n = 10)</th>
</tr>
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<tbody>
<tr>
<td>LV</td>
<td>LV - RV</td>
<td>LV - RV</td>
<td>LV - RV</td>
<td>LV - RV</td>
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<tr>
<td></td>
<td>5</td>
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<td>40</td>
<td>7</td>
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LV = left ventricle; n = number of animals, LV-RV = difference between left and right ventricles. The rest of the notation is as in Table 1.

**Discussion**

From this study, it appears that, in the rat, synthesis of a cardiac myosin isoenzyme V3 is a general process occurring in cardiac myocytes with hypertrophy due to chronic mechanical overload (Fig. 2). Recent observations from our laboratory (Lompré et al., 1979) have shown that myosins obtained from gross cardiac hypertrophies, which displayed a low Ca\(^{2+}\) ATPase activity as compared with myosins from control hearts, contained predominantly the V3 form. On the other hand, hypothyroid rats also contain a V3 form of low activity (Hoh et al., 1978; Pope et al., 1980), and there is now evidence from one- and two-dimensional peptide maps that it is the same isoenzyme that is synthetized in AS, AS + AI, aging and hypothyroidism (Klotz et al., 1981; Schwartz and Whalen, unpublished observations). It thus seems reasonable to assume that the V3 band we found in ACF, INF, and SHR is also the same. Since it is generally admitted that contractile element shortening capacity is correlated with the ability of myosin to hydrolyze ATP (Barany, 1967; Delcayre and Swynghedauw, 1975), our findings thus provide a molecular basis for declines in both ATPase activity and myocardial contractility previously observed in the same animal species in similar models, i.e., aortic banding or aortic regurgitation (Berson and Swynghedauw, 1973; Jouannot et al., 1975; Schwartz et al., 1978), left ventricular infarction (Pfeffer et al., 1979a), and genetic hypertension (Pfeffer et al., 1979b; Medugorac, 1980; Mayr et al., 1980; Lecarpentier, unpublished observations).

The statistical correlation between the amount of V3 and the degree of hypertrophy in the AS, AI, and AS + AI groups (Fig. 4) is in close agreement with our previous findings using an immunochromatographic approach (Schwartz et al., 1978). This shows that, in these types of overloaded rat hearts, an increase in protein synthesis is accompanied by the synthesis of a different myosin form. It is not clear whether or not such a relationship also exists in the other groups. In the ACF, the relationship was not significant and the shift seemed less pronounced (Fig. 4). One explanation for this smaller increase in V3 would be that, by plotting total ventricular-hypertrophy against LV V3, we have overestimated the degree of hypertrophy of the LV in this model as compared with the others. To compensate for this overestimation, the origin of the abscissa, for the ACF, may be considered to be shifted to the right (Fig. 4), and, under these conditions, the magnitude of the isoenzymic shift would be equivalent to that of the other models for equivalent LV hypertrophies. Another possibility is that, in this kind of overload, V3 is not synthesized to the same extent or with the same time course as in the other models. This would be consistent with data of several authors showing, for other animal species, a normal or only slightly modified ATPase activity and con-
tractile function after atroventricular block (Turina et al., 1969); atrial septal defects (Cooper et al., 1973; Carey et al., 1979), and aortocaval fistulas (see Ross, 1974). In this hypothesis, the moderate isoenzymic shift we observed in the ACF might be due to the fact that the hearts we studied were overloaded more severely and for a longer period than in the above reports. Indeed, contractility was found to be impaired in one dog with a large chronic fistula (Ross 1974) and in several dogs submitted to an aortocaval shunt over a 12-week period (Pinsky et al., 1979).

In the INF group, our data suggested that V3 percentage and the degree of hypertrophy were also related, although this relation was not statistically significant. Further investigations with a more precise evaluation of the tissue hypertrophy of the non-infarcted ventricular area may give a better answer to this question.

Our results in the control Wistar group revealed a small and progressive age-dependent increase in the percentage of V3 in the LV (Fig. 5). Hoh et al. (1978) had also observed this phenomenon, but these authors did not analyze RV and LV separately. Although both sets of data are qualitatively in agreement, the maximal value of LV V3 never exceeded 15% in our study (Fig. 5), whereas it reached 30% in the report by Hoh et al. for animals of similar ages. It is difficult to compare precisely the two groups of controls, since the BW were not reported by Hoh et al. One might only hypothesize that housing conditions, and especially physical activity, interfere with the isoenzymic pattern. This points out the need for control animals, not only of the same age and weight, but also bred under the same conditions as the experimental ones. In the same way, it is possible that the small differences that we observed between the WKY and the Wistar controls (Figs. 5 and 6) were also due to different housing conditions, although the hypothesis of strain differences cannot be ruled out. The amount of V3 which we found in the 22- to 30-week-old animals (Fig. 5) is insufficient to induce a detectable decrease in the overall enzymatic activity of the whole myosin. However, this is probably the beginning of the process which leads, in older animals, to decreases in both ATPase activity of myosin and velocity of shortening of the cardiac muscle (Alpert et al., 1968; Chesky and Rockstein, 1977).

It has long been known that decreases in ATPase activity and myocardial contractility also occur after chronic mechanical overload in other animal species, such as rabbits (see Maughan et al., 1979), dog (Wikman-Coffelt et al., 1978), cats (Chandler et al., 1967; Skelton and Sonnenblick, 1974; Carey et al., 1978), and humans (Alpert et al., 1962; Leclercq et al., 1976; Strauer and Burger, 1980). Recent data indicate that adult animals of these species (especially rabbits) contain a predominant isoenzyme of the V3 type, whereas young animals contain both V1 and V3 (Hoh and Yeoh 1979; Lompré et al., in press). In view of these findings, decreases in ATPase activity could be due (1) to the use of very young animals at the time of surgical procedure, (2) to the synthesis of a yet unknown isoenzyme of lower ATPase activity but displaying a charge analogous to that of V3, or (3) to biochemical mechanisms other than isoenzymic change. This remains to be determined.

We can only speculate on the mechanisms underlying the isoenzymic shift in the rat species. Pressure overload recently has been proposed as an obligatory trigger for the decrease in myosin ATPase activity in the cat (Carey et al., 1979). Indeed pressure overload could be one of the triggers that all models of our study—except perhaps the ACF—have in common. This process would also be involved in the AI which may be considered as a combination of pressure and volume overload, and in the LV of aging rats, since the increase in the higher frequency terms of the aortic input impedance induces a pressure overload on this ventricle (Yin et al., 1980). However, it is probable that other triggers also exist, for example, in the ACF or in the right ventricles of the SHR which also display significant increase in V3 (Fig. 6).

It is interesting to note that, except for young rats (Fig. 5), all RV studied here contained less V3 than the corresponding LV. In all experimental models except the ACF, the LV-RV differences should be explained by the fact that overload of the LV was always the primary event, the RV being affected only later. The difference was more surprising for the ACF since, theoretically, both ventricles were equally affected. The slight differences we have found might be due either to a difference in the stress imposed on each ventricular wall for apparently equivalent overloads, or to the RV contractile reserve postulated by Wikman-Coffelt et al. (1979). Nevertheless, it appears that isoenzymic changes can be a selective and sensitive regional response.

The present findings show that myosin isoenzymic redistribution is, like hypertrophy, a general response of the rat heart to sustained mechanical overload. Together with the data of Hoh et al. (1978), this strongly suggests that the type of myosin isoenzyme present is an important determinant of the contractile activity of the cardiac muscle. There is evidence to suggest that, in other states associated with modified contractile performance, changes in myosin form also occur (see Scheuer and Bahn, 1979; Malhotra et al., 1979). Further investigation of the molecular events which trigger the transformation of cardiac myosin may thus bring new insights into the biochemical basis of heart failure.

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