Distribution of Myosin Isozymes within Single Cardiac Cells  
An Immunohistochemical Study

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SUMMARY. Isozymes of myosin have been localized with respect to individual cardiac myocytes in hearts from 3-week-old, adult controls, and adult hypophysectomized rats, and in cultured cardiac cells. For this purpose, affinity-purified antibodies reacting specifically with the heavy chains of each of the two major myosin isozymes of adult rat heart, V1 and V3, were used. The distribution of the two isomyosins was determined by double immuno-labeling of the same cell, V1 myosins being revealed by rhodamine and V3 myosins by fluorescein. A procedure is described which allows optimum immunological visualization of the myosin filaments of rod-shaped isolated myocytes. It was found that the response of the cardiac cells to the two antimyosins varied depending on the state of the animal. In 3-week-old rats, all cells were stained with the anti-V1, and almost none with the anti-V3 myosin. In the hypophysectomized animals, on the contrary, all cells were stained with the anti-V3 and none with the anti-V1. A mixed pattern of reactivity was observed in adult controls since 50% of the cells reacted with the anti-V1, 10% with the anti-V3, and 40% with both antibodies. In the latter case, the distributions of V1 and V3 reactivities were homogeneous throughout the cell, and absolutely superimposable. The same double reactivity and homogeneous repartition were observed in cultured cells. These findings indicate that myocytes from adult rat myocardium are heterogeneous in terms of their isomyosins content and show for the first time that two isomyosins can coexist and be equally distributed in one cardiac cell. These observations are relevant to the regulation of individual heart cell contractility. (Circ Res 52: 200–209, 1983)
which involved preparation of antibodies that distinguish specifically V1 and V3 and double labeling of each myocyte. The results indicate that V1 and V3 either are segregated among different cells, or coexist in the same myocyte and are then identically distributed in the myofibrils.

Methods

Reagents

Reagent grade chemicals were obtained from the following sources: piperazine-N,N'-bis(2-ethane sulfonic acid) (PIPES), N-2-hydroxy-ethylpiperazine N'-2-ethane sulfonic acid (HEPES), Brij 58 (polyoxyethylene 20 cetyl ether) from Sigma; collagenase type 1 from Worthington Biochemical Corporation; CNBr-activated Sepharose 4B from Pharmacia; fluorescein isothiocyanate-labeled goat anti-rabbit IgG (FITC) and tetramethylrhodamine isothiocyanate-labeled goat anti-rabbit IgG (TMRITC) from Cappel Laboratories; products for the purification of cells and Nonidet P40 (NP40) from Fluka; Triton X-100 from Prolabo; analar formaldehyde from BDH chemicals LTD. All other compounds used were purchased from standard chemical suppliers.

Animals

Wistar rats (Ifacredo) were used throughout this study. Normal animals were 3- or 8-week-old rats. Hypophysectomized animals were operated at 10 weeks of age and the rats were killed 6-10 weeks after surgery.

Myocyte Isolation from Whole Hearts

The rats were killed by a blow to the head, and the hearts were rapidly excised and placed in room temperature Krebs-Ringer bicarbonate buffer (KRB buffer) which contained the following (mM) = NaCl, 118.5; NaHCO3, 14.5; KCl, 2.5; KH2PO4, 1.18; MgSO4, 1.18; and glucose, 11.1). The aorta was rapidly dissected out and attached to the canula of a Langendorff perfusion apparatus. Ventricular myocytes were isolated by a procedure similar to that of Cutilella et al. (1977). Briefly, the heart was perfused for 4 minutes with KRB buffer. Twenty-five milligrams of collagenase (at least 125 U/ml) and 500 mg of bovine serum albumin (BSA) were added to 50 ml of the same buffer, and this solution was recirculated at a coronary flow rate of one drop per second for 15 to 25 minutes. When the heart was “soft,” perfusion was performed with KRB buffer made 1 mM EGTA but without collagenase and BSA. The heart was then removed and freed from the atria. The ventilates were cut into small fragments and cells were dispersed by a gentle homogenization in Sorvall polycarbonate tubes with a Teflon pestle in 1 mM EGTA KRB buffer. Purification was achieved by 3 washes in the same buffer and centrifugation at 55 g during 3 minutes on 30 ml of this medium made 3% Ficoll. The last pellet was resuspended in 1 ml of 1 mM EGTA KRB buffer, and an aliquot of the myocytes was counted in a Malassez cell (0.2 mm depth).

Myocyte Culture

Procedures for culture of cardiac myocytes were essentially similar to those previously described (Schiaffino et al., 1977; Cantini et al., 1980). In brief, muscle cell suspensions were prepared from minced newborn rat ventricles by trypsin treatment and plated on gelatin-coated glass coverslips placed in plastic Petri dishes. The culture medium was a 1:3 mixture of Parker medium 199 and Dulbecco’s modified Eagle medium supplemented with 10% horse serum and 1% chick embryo extract. Cultures were grown at 37°C in a humidified air atmosphere containing 7.5% CO2.

Electrophoresis of Native Myosins

Aliquots from purified cells were rapidly frozen in liquid nitrogen. Myosins were analyzed in non-denaturing conditions on polyacrylamide gels as in Hoh et al. (1978) and Lompré et al. (1979).

Preparation and Characterization of Antibodies

Antisera to rat cardiac myosin were prepared as previously described by injecting native cardiac-heavy meromyosin from 3-week-old rats into guinea pigs (Schwartz et al., 1977). Antisera against myosins isolated from the slow-contracting skeletal muscle guinea pig soleus were prepared in rabbits according to the method of Pierobon-Bormioli et al. (1981). The antisera were purified by affinity chromatography on insolubilized myosins. For this, pure V1 and pure V3 myosins were prepared essentially according to the method of Offer et al. (1973) from 3-week-old and hypophysectomized rat hearts, respectively, and guinea pig soleus myosin was obtained as in the study by Gorza et al. (1981). Myosins were coupled to CNBr-activated Sepharose 4B, following the procedure recommended by Pharmacia, and the specific immunoglobulins were eluted from the immunoaffinity columns by 0.2 M glycine buffer, pH 2.8. Antisera to rat cardiac myosin were first adsorbed on the V3 column, and the unretained fraction was adsorbed on the V1 column. The immunoglobulins eluted from this V1 column were the anti-V1 antibodies. As for the antisera to guinea pig soleus myosin, they were adsorbed on the guinea pig soleus myosin column, and the eluted fraction constituted the anti-V3 antibodies.

The specificity of the antibodies towards the heavy chains of pure V1 and pure V3 myosins has been demonstrated elsewhere (Schwartz et al., 1982). It was shown by enzyme-linked immunosorbent assays and immunoblots on electrophoretically purified myosins that (1) only the heavy chain portion of the myosin molecule is recognized and (2) the anti-V1 and the anti-V3 immunoglobulins recognize only the V1 and V3 form, respectively. A typical immunoblot is shown in Figure 1.

Cell Fixation Procedures

Various fixation procedures were tested.

In procedures A and B, cells were first sedimented, then fixed and permeabilized. Twenty-five microliters of the cell suspension (10^6 cells/ml) were transferred into a glass chamber, according to the method of Karsenti et al. (1978a), and sedimented onto coverslips for 30 minutes at 2,500 g at +15°C. Cells were fixed in 3.5% formaldehyde in phosphate-buffered saline (PBS) (10 mM phosphate buffer, 150 mM NaCl, 2.5 mM KCl, pH 7.2) for 10 minutes at room temperature. The coverslips then were washed quickly in PBS and treated either with methanol at 20°C for 4 minutes, followed by acetone for 2 minutes at ~20°C (procedure A) (Weber et al., 1976), or with acetone for 5 minutes at ~20°C (procedure B) (Weber et al., 1974; Welsh et al., 1979) then air-dried before being rehydrated in PBS.

In the second type of procedures (C to F), cells were first permeabilized with a detergent treatment. The various treatments with detergents were as follows. Procedure C: cells were treated with saponin (Eckert 1980) diluted in the...
The following controls were performed: (1) cells were incubated with the two secondary antibodies without prior incubations with purified antimyosin immunoglobulins; (2) antibodies raised against V1 or V3 were preadsorbed for 1 hour on purified myosin V1 or V3 coupled to CNBr-Sepharose 4B before being added to the cells. In all these conditions, only a low level of background fluorescence was detected. Cells were also incubated with one of the two antibodies (anti-V1 or anti-V3) and then incubated with the two labeled immunoglobulins. The only observed staining was that corresponding to the first antibody (anti-V1 or anti-V3) added to the incubation medium. This indicated that anti-V1 or anti-V3 immunoglobulins reacted specifically with their respective labeled immunoglobulins, without any contaminating cross-reactions.

Immunocytochemistry of Cell Cultures

Cells cultured on coverslips for 2-7 days were washed in PBS, fixed 5 minutes in acetone at -20°C, and air dried. The air-dried cells were reacted with rabbit anti-V3 myosin or guinea pig anti-V1 myosin antibodies for 30 minutes at 37°C, washed in PBS, and treated with fluorescein-conjugated goat anti-rabbit IgG or with rhodamine-conjugated anti-guinea pig IgG. For double label immunofluorescence, cultures were treated with guinea pig antibodies to V1 myosin followed by rhodamine-conjugated goat anti-guinea pig IgG, then with rabbit antibodies to V3 myosin followed by fluorescein-conjugated goat anti-rabbit IgG. Non-immune rabbit and guinea pig sera were used for control. In addition, we performed appropriate tests in order to determine whether anti-rabbit IgG and anti-guinea pig IgG did not cross-react with the heterologous IgG. Cultures treated with rabbit anti-V3 myosin followed by rhodamine anti-guinea pig IgG were completely negative, whereas cultures treated with guinea pig anti-V1 myosin followed by fluorescein anti-rabbit IgG showed a very weak staining. For double-label experiments, fluorescein-conjugated goat anti-rabbit IgG was incubated with an equal volume of guinea pig nonimmune serum for 2 hours at room temperature and then centrifuged at 10,000 g for 10 minutes prior to staining. The antibodies cross-reactive with heterologous IgG present in the fluorescein conjugate were completely eliminated by this absorption.

Results

Preparation of Isolated Cardiac Myocytes

As seen in Table 1, both myocyte yield and percentage of rod-shaped cells varied, depending on the state of the animal. During normal growth (at 3 and 8 weeks of age), the number of myocytes isolated from one heart increased, together with heart weight. Out of both cell populations, 40-60% of the total maintained their rectangular shapes, and these data were in good agreement with those of Nag et al. (1977) and Robinson et al. (1981). When hearts from hypophysectomized animals were used, the recovery was only half that of those from normal animals. Moreover, most of the cells rounded up during the isolation procedure, and the quantification of rod-shaped cells indicated that only 10-20% of the total remained cylindrical. Since hearts from hypophysectomized animals always were processed together and under exactly the same experimental conditions as...
is myosins in Single Cardiac Myocytes

TABLE 1

Purification of Isolated Cardiac Myocytes

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Heart wt (g)</th>
<th>Number of cells/heart (X 10^6)</th>
<th>Percentage of rod-shaped cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Week-old rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.37</td>
<td>2</td>
<td>52</td>
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<td>2</td>
<td>0.41</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>0.49</td>
<td>5.2</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>0.45</td>
<td>3.1</td>
<td>45</td>
</tr>
<tr>
<td>8-Week-old rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.86</td>
<td>5.6</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
<td>6</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>7.3</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>6.7</td>
<td>44</td>
</tr>
<tr>
<td>Hypophysectomized rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.75</td>
<td>1.2</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>2.5</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>0.79</td>
<td>2.2</td>
<td>22</td>
</tr>
</tbody>
</table>

* Approximately 300 cells were counted per heart to determine the percentage of rod-shaped cells.

FIGURE 2. Phase contrast image of myocardial cells isolated from a 3-week-old rat. Note the typical cross-striation of myosin fluorescence in the rod-shaped cells and the disorganized structure of the rounded cells. (Bar = 50 μm).

the controls, the reasons why their myocytes were less disaggregated and more damaged are not clear. One can only postulate that hormonal deficiency modified the sensitivity of membrane transport systems and intracellular structures to external ions and dissociating enzymes. It should be pointed out that conditions for optimal release of rod-shaped myocytes from controls have been determined empirically (cf. Dow et al., 1981a, 1981b, for reviews), and it is not so surprising that they are no more optimal in pathological situations.

Under phase contrast microscopy, rod-shaped myocytes from both normal (Fig. 2) and hypophysectomized (not shown) animals appeared elongated, and their lengths ranged from 80 to 150 μm. They showed characteristic shapes of isolated living cardiac muscle cells with alternating light and dark transverse banding, thus fulfilling the morphological criteria of intact cells (Sommer and Johnson, 1979).

Procedures for Immunofluorescence Microscopy of Isolated Cardiac Myocytes

Complete accessibility of the myofilaments to antimyosin immunoglobulins and maintenance of intracellular integrity are prerequisites for accurate studies of myosin distribution within isolated cells. Current procedures for immunofluorescent localization of myosin have been documented in detail for rather flat cells, but not for cells as thick as cardiac myocytes (10-35 μm width). We have thus tested several experimental conditions, in which the fixation technique and the permeabilization of the cell toward the immunoglobulins varied, alone or in combination. These studies were performed only on rod-shaped cells, since the rounded cells, which are damaged, are non-specifically stained by the antibodies. Results are summarized in Table 2. Procedures A and B were first used because they permitted the clear visualization of actin (Lazarides and Weber, 1974; Karsenti et al., 1978b) and myosin (Weber and Groeschel-Stewart, 1974) filaments in nonmuscle cells and in myocytes from primary heart cells cultures (Masse and Harary, 1981). After the fixation step is achieved by formaldehyde treatment, sarcosomal lipids are extracted at low temperature by organic solvents, which renders the plasma membrane permeable to the antibody molecules (Lazarides and Weber, 1974). In our hands, these procedures allowed neither maintenance of cell shape, nor homogeneous labeling of myosin.

The experimental steps then were reversed: the permeabilization was performed before fixation of the cell, and various detergents were used to partially destroy the plasma membrane. Saponin was tested in procedure C, NP40 in procedure D, and Brig 58 in procedure E. None of these procedures gave sufficiently good patterns of myosin organization. Finally, the non-ionic detergent Triton X-100 was tested (procedure F). The resulting preparation showed no structural abnormalities within the limits of light microscopy and a myosin framework typical of intact striated muscles (see Figs. 3-5). Procedure F was thus selected.

TABLE 2

Procedures Tested for the Labeling of Cells with Purified Antimyosin Immunoglobulins

<table>
<thead>
<tr>
<th>Test</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation procedures</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Maintenance of cell shape*</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Immuno-labeling of myosin</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+ +</td>
</tr>
</tbody>
</table>

* Maintenance of cell shape was evaluated by comparison of the percentage of rod-shaped-cells in the glass chamber before and after the fixation step: −, less than 10%; +, approximately 50%; ++, 90-100%.
Visualization of V1 and V3 Myosins in Isolated Myocytes

The response of the cardiac myocytes to the two anti-myosins showed striking differences depending on the source of the cells. Myocytes from 3-week-old animals displayed an uniform pattern of reactivity, all cells being strongly stained by the anti-V1 myosin, and not at all by the anti-V3 myosin (Fig. 3; Table 3). Within the cell, the

FIGURE 3. Double indirect immunofluorescence micrograph of a myocyte isolated from a 3-week-old rat heart. Incubation was performed with anti-V1 myosin labeled with TMRITC antibodies (part a) and with anti-V3 myosin labeled with FITC antibodies (part b). The staining is positive only with anti-V1 myosin and not with anti-V3 myosin. (Bar = 10 μm).

FIGURE 4. As in Figure 3, except that the myocyte was from an hypophysectomised rat; the cell was stained only with anti-V3 myosin (part b). (Bar = 10 μm).

FIGURE 5. As in Figure 3, except that the myocytes were from a 8-week-old rat; cells were incubated with anti-V1 myosin (parts a, c, and e) and anti-V3 myosin (b, d, f). In parts a and b, example of a myocyte stained with both antibodies. Note that the reactive fibers exhibit the same overall morphology. In parts c and d, cells were stained only with anti-V3 myosin myosin, whereas in parts e and f, cells were stained only with anti-V1 myosin. (Bar = 10 μm).
fluorescence was regularly distributed and formed arrays roughly perpendicular to the long axis. These stained bands were interrupted by nonreactive thin striations. Note that the fluorescence is diffuse in some areas of the cell. This is only a technical artifact due to the thickness of the myocyte, which is composed of superimposed rows of myofibrils. Under the microscope, the striation can be clearly seen in those diffuse areas by changing the focus.

An opposite label was observed with myocytes from hypophysectomised rats (Fig. 4; Table 3). All cells were stained only by the anti-V3 myosin, and not at all by the anti-V1 myosin. Here, also, the fluorescence was intermittent and oriented perpendicular to the long axis.

Myocytes from adult normal rats exhibited three types of labeling (Fig. 5; Table 3). Approximately 40% of the cells reacted with both types of antibodies, 50% were labeled only with anti-V1 myosin, and the remaining 10% only with anti-V3 myosin. In the cells stained by both antibodies, the reactive fibers exhibited the same overall morphology and periodicity. This structural relationship was confirmed by superimposing the negative of the photography of one staining (V1) and the positive of the other (V3) (Fig. 6).

Electrophoresis of Native Myosins

Figure 7 illustrates the electrophoretic pattern of the myosins of myocytes purified from the three types of hearts considered above. As already described by Hoh et al. (1978) and Lompré et al. (1981), 3-week-old animals contained one band referred to as V1, and hypophysectomized animals showed only V3 isoform. Adult normal rat ventricular myosin was composed mostly of V1, with trace amounts of V2 and V3. These patterns were qualitatively in complete agreement with the findings of the immunofluorescence observations.

Visualization of V1 and V3 Myosins in Cell Cultures

Spontaneously beating muscle cells were observed in cultures from newborn rat ventricle during the first 1 or 2 days, and increased in number during subsequent days. Cultures processed for immunofluorescent staining with anti-V1 or anti-V3 myosin antibodies were found to contain numerous reactive cells, many of which showed a typical striation pattern with

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**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Percentage of cells labeled with</th>
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<tbody>
<tr>
<td></td>
<td>Anti-V1 myosin</td>
</tr>
<tr>
<td>3-Week-old rats</td>
<td>(4)*</td>
</tr>
<tr>
<td>8-Week-old rats</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Hypophysec-</td>
<td>(3)*</td>
</tr>
<tr>
<td>tomized rats</td>
<td></td>
</tr>
</tbody>
</table>

Approximately 1000 cells were counted for each heart, which corresponds to two coverslips.

* Four and three experiments were performed, respectively.
FIGURE 8. Culture from newborn rat ventricular myocardium after 4 days in vitro. Indirect immunofluorescence with anti-V3 myosin. Two labeled muscle cells are present in this field. The round unstained regions correspond to the cell nuclei. Staining is almost exclusively restricted to the myofibrils with clear sarcomeric distribution. A bands are positive, I bands (short arrow) negative. Note that the central zone of the A bands (M band = large arrow) is less reactive. (Bar = 10 μm).

labeled A bands. At higher magnification, it was apparent that the immunoreactivity for both V1 and V3 was not homogeneous throughout the A band, the central region, corresponding to the M region, showing often weaker staining (Fig. 8). Variations in the intensity of staining between different muscle cells in the same culture were also frequently observed with both antimyosins (Fig. 9). However, a quantitative evaluation of the different muscle cell populations was not possible, as muscle cells could not be classified in discrete classes with respect to staining intensity; rather, both in terms of V1 and V3, immunoreactivity there was apparently a continuous spectrum ranging from weakly to strongly reactive cells. A large number of completely unreactive cells, presumably fibroblasts, also were present in the cultures. Double staining with antibodies to V1 and V3 myosins revealed that most muscle cells synthesized both myosin types (Fig. 10). The two myosins appeared to have a similar distribution, the same myofibrils in double-labeled cells reacting with both antimyosins. A number of muscle cells in double-labeled cultures showed weaker staining with either anti-V1 or anti-V3 myosin antibodies, with a tendency for those cells that were less reactive with one antimyosin to be more reactive with the other. A more detailed account of the pattern of reactivity of cultured muscle cells with different anti-myosin antibodies will be presented elsewhere.

Discussion

The results of this study indicate that adult rat ventricular myocardium is composed of three types of myocytes: one type contains only the myosin heavy-chain isoform V1, the other type contains only the isoform V3, and the third contains both V1 and V3. These data are consistent with earlier observations which showed a regional variation in the ventricular distribution of one isomyosin in the rat, the rabbit, and the cow (Schiaffino et al., 1980; Sartore et al., 1981; Gorza et al., 1981). We show also that, within single myocytes, V1 and V3 heavy chains are identically distributed and that this homogeneous organization of the thick filament occurs in adult heart cells.

Localization of myosin isozymes by immunocytochemistry requires antibodies highly specific for one or the other isoform. The specificities of our affinity-purified antibodies toward V1 or V3 heavy chains had previously been assessed by various sensitive procedures: immunodiffusion, immunofluorescence on tissue heart sections (Gorza et al., 1981), direct and competitive enzyme-linked immunoassays, and immunoblots with pure V1 and pure V3 myosins (Schwartz et al., 1982, and Fig. 1). The selective stainings of myocytes from 3-week-old and hypophysectomized animals (Figs. 3 and 4) provided additional evidence for antibody specificity. Moreover, the staining pattern of myofibrils from cultured cells (Fig. 8) clearly showed that only the A band was stained, and that, within this A band, the center, i.e., the M region was less reactive. In isolated myocytes, this nonreactive M region was not visualized (Fig. 3). A simple explanation is that isolated myocytes are more or less contracted, and that, under these conditions, the resolution of the immunofluorescence technique is not sufficient to distinguish a thin unreactive zone. Free access of the antibodies to the myofilaments is also imperative to use this immunological approach suc-
cessfully. The assays which we have performed (Table 2) emphasized the difficulties of labeling sarcomeric structures of myocytes with procedures currently used in immunofluorescence studies. Labeling of the cells after permeabilization of the membrane with Triton X-100 and fixation yielded preparations in which the stained myofilaments appeared arranged in a regular matrix all along the cell. This arrangement was seen with both types of myosin antibodies (Figs. 3 and 4), which clearly indicated that our experimental conditions induced appropriate myosin labeling.

We have tried to quantify the relative proportions of V1, V3 and V1 + V3 myocytes from the ventricular preparations (Table 3). It is possible that such an evaluation gives a distorted image of real ventricular composition since only rod-shaped cells were kept for the study (Table 1). Moreover, the yield of these cells was markedly lower in hearts from hypophysectomized animals than those from controls (20 and 50%, respectively). However, it seems unlikely that a selection has occurred in our experiments: 3-week-old and hypophysectomized animals produced V1 and V3 myocytes, respectively, and the quantitative data of Table 3 are those that would be expected from the immunofluorescence data of Gorza et al. (1981) and from the pyrophosphate gel analysis of isolated myosins (Hoh et al., 1978; Lompre et al., 1981; and Fig. 7). An interesting point is the comparison of V1 and V3 contents of the whole ventricular tissue when analyzed either by immunohistochemistry or by electrophoretic procedures. Only small amounts of V2 and V3 are seen in the gels (Fig. 7), whereas almost 50% of the cells are brightly stained with anti-V3 myosin (Table 3). As a matter of fact, immunological labeling cannot be considered as quantitative since (1) light intensity differs among the two markers, fluorescein and rhodamine, (2) even with one marker, variations in intensity can hardly be quantitated, and (3) the affinity of both types of antibodies towards their respective myosin isozymes probably is very different. On the other hand, pyrophosphate gels certainly give a slight underestimate of V3 content: (1) V2 and V3 are resolved on these gels, and V2 is supposed to be an heterodimer composed half of V1 heavy chain, and half of V3 heavy chain (Hoh et al., 1979; Chizzonite et al., 1982), and (2) the gels are stained with Coomassie blue, which is far less sensitive than the immunological labeling, and which hardly allows the detection of 1% V3 (unpublished observations). Nevertheless it appears that one of the main advantages of immunocytochemistry, i.e., its ability to detect trace amounts of antigens within a tissue section, is also its main pitfall, if quantitative information is required.

This is the first demonstration of the coexistence of two myosin heavy chain isoforms in the same cardiac cell and of their analogous distribution inside the cell. Interestingly, this structural linkage is found both in whole hearts and in cultured cells, which strongly suggests that it is a general feature of the sarcomeric organization of cardiac tissue. The nature of the double-labeling technique makes it unlikely that subtle differences in isozyme distribution could be detected. Silverstein and Lowey (1981) have found that the myosin light chain isozymes of chicken skeletal muscle are located on the same filament. We have found that V1 and V3 myosin heavy chains are distributed throughout all cardiac sarcomeres. Whether they are located on the same filament remains to be determined. Our experiments do not allow us to determine whether or not the double-labeled cells contain a natural V2 heterodimer or V1 and V3 homodimers closely mixed within one myofibril. Recently, two myosin heavy chain genes coding for proteins of different lengths have been identified in the adult rat ventricular myocardium (Mahdavi et al., 1982). It is unlikely that these proteins correspond to the V1 and V3 isoforms, but rather, to two closely related V1 myosins. If this interpretation is correct, our study suggests that three genes can be expressed in the same cell in vivo. The factors responsible for the variable expression of the myosin heavy chains in different
ventricular cells are not yet completely understood (see Schiaffino et al., 1980; Sartore et al., 1981; Gorza et al., 1981; Mercadier et al., 1981). The existence of cardiac muscle cell heterogeneity in culture could also be related to the presence of conducive tissue cells, as previously suggested for cultured chicken cardiac muscle (Cantini et al., 1980). Further studies are now in progress in order to characterize more precisely the myosin immunoreactivity of cultured myocytes with respect to the age of the animal from which the cultures derive, the time of growth in vitro, and the composition of the culture medium.

V1 and V3 forms differ markedly in their enzymatic activities (Hoh et al., 1978; Pope et al., 1980; Lompre et al., 1981; Litten et al., 1982), and a positive correlation exists between myosin distribution and maximum velocity of shortening of papillary muscle (Schwartz et al., 1981; Ebrecht et al., 1982). This correlation is similar to that previously found by Barany (1967) in skeletal muscles. The present observations would suggest that, in spite of their functional differences, V1 and V3 myosins must operate in vivo within the constraints of a single cell. Recently, cAMP regulation of cardiac isomyosins has been proposed (Winegrad et al., 1983). If this hypothesis is true, it is an attractive explanation of the means by which, in one myocyte, a specific isozyme could be selected for force generation.

The findings reported here do not provide sufficient information to speculate on the regulation of cardiac contraction at the filament level; however, with the development of immunological methods to localize different isomyosins, we are now beginning to acquire a more complete understanding of the complex interactions involved in cardiac contraction. Moreover, the approach presented here holds promise for characterizing isolated cardiac myocytes for structural and functional studies: it is possible to measure mechanical parameters at the level of a single cardiac cell (De Clerck et al., 1981), and our method allows one to characterize the same cell in terms of its isomyosin content.

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