Myofibrillar Mass in Rat and Rabbit Heart Muscle

CORRELATION OF MICROCHEMICAL AND STEREOLOGICAL MEASUREMENTS IN NORMAL AND HYPERTROPHIC HEARTS

By Ernest Page, Philip I. Polimeni, Radovan Zak, Judy Earley, and Margaret Johnson

ABSTRACT

A sequestered fraction of myofibrillar magnesium presumably bound to thin filaments during polymerization of actin was used to determine myofibrillar mass in rat left ventricles and rabbit hearts. Myofibrillar volume was also determined by stereological measurements on electron micrographs of rat ventricles. Myofibrillar Mg (1.11 mmoles/kg dry ventricle) can be determined in glycerinated ventricle either by measuring 28Mg-inexchangeable Mg or Mg remaining after extraction with EDTA, KCl, and a nonionic detergent. These measurements, combined with the Mg content of myofibrillar suspensions similarly extracted (3.2 mmoles/kg protein), indicated that myofibrillar mass was ~347 g myofibrillar protein/kg dry ventricle. In rabbit hearts, increases in myofibrillar Mg per unit dry weight were as follows: auricular appendage < right ventricle < left ventricle ≈ interventricular septum. After production of left ventricular hypertrophy in rats by constriction of the ascending aorta, both myofibrillar Mg and volume increased within 24 hours. After 10 or more days, myofibrillar Mg and myofibrillar volume increased proportionately more than tissue dry mass and cell volume, and the ratio of mitochondrial volume to cell volume decreased.

KEY WORDS

myofibrillar magnesium myofibrillar mass ventricular hypertrophy myofibrillar volume myofibrillar changes mitochondrial volume

When heart muscle undergoes hypertrophy, the contributions of myofibrillar and mitochondrial mass to the total weight of the tissue may change. Although the myofibrillar proteins can be extracted and purified from homogenates of heart muscle, there are few reliable quantitative estimates of myofibrillar mass. The reason is that the procedures used for extraction may lead to an incorrect estimation, because the extraction is incomplete and contaminants from other cellular structures remain associated with the extracted components. These difficulties notwithstanding, the measurement of myofibrillar mass is crucial for the quantitative description of cardiac hypertrophy.

For the present work we developed a new microchemical technique to estimate myofibrillar mass in heart muscle and compared the results obtained with this technique in normal and hypertrophic left ventricles (1) with stereological measurements on electron micrographs. The microchemical technique,
which is based on the determination of traces of magnesium sequestered in the myofibril, has the advantage that it is applicable to human material obtained at autopsy as well as experimental and biopsy material.

Methods

DETERMINATION OF MYOFIBRILLAR MASS FROM THE AMOUNT OF SEQUESTERED MYOFIBRILLAR MAGNESIUM

In one set of experiments, glycerinated unhomogenized left ventricles, for which the dry weight before glycerination was known, were minced; their membranous constituents and their content of $^{28}$Mg-exchangeable Mg were removed by a treatment which solubilizes membranous contaminants and extracts exchangeable Mg bound to myofibrils and other structures. A determination of the Mg content of the glycerinated ventricle after this treatment yields a figure for the sequestered myofibrillar Mg content per unit dry weight of unglycerinated ventricle. In a parallel set of experiments myofibrils were isolated from a homogenate of rat ventricle by differential centrifugation. The isolated myofibrils were treated so as to remove all except the sequestered Mg content per unit of myofibrillar protein. It will be shown that the procedure used to extract glycerinated ventricles (1) did not extract any inexchangeable myofibrillar Mg and (2) extracted all nonmyofibrillar Mg and all $^{28}$Mg-exchangeable myofibrillar Mg. The figures for the Mg contents of isolated myofibrils and glycerinated, extracted ventricles were then combined to give the myofibrillar mass, as follows:

$$M = \frac{M_{\text{gr}}}{M_{\text{dm}}},$$

where $M$ is myofibrillar mass in grams of myofibrillar protein per gram dry weight of unglycerinated ventricle; $M_{\text{gr}}$ is Mg content of unglycerinated ventricle in $\mu$moles per gram dry ventricle, and $M_{\text{dm}}$ is Mg content of myofibrils in $\mu$moles per gram myofibrillar protein.

Determination of Sequestered Mg in Isolated Myofibrils.—Myofibrils were isolated from ventricular homogenates by two procedures (A and B), each of which yielded a myofibrillar preparation which appeared free of membranous contamination on electron microscopic examination of osmium-fixed pellets stained with uranyl acetate and lead hydroxide.

For procedure A, 200-g female Sprague-Dawley rats were injected intraperitoneally with heparin. They were then stunned and their hearts were cut out and immersed immediately in an ice-cold buffered solution (pH 7.2) whose millimolar composition was sucrose 300, KCl 10, and Tris 50 (pH 7.2); for this and other Tris buffers the pH was adjusted with HCl. After a third change of solution, the ventricles were minced as finely as possible with a small pair of iris scissors. The mince was spun down for 10 minutes at 18,800 g in a refrigerated Sorvall centrifuge and the supernatant fluid was discarded. The pellet from each half of ventricular wall was resuspended in 20 ml of a solution whose composition was EDTA 5mM, KCl 100 mM, and Triton X-100 1%. This suspension was shaken at 0°C for 16-40 hours. The suspended particles were then spun down at 27,000 g, and the pellet was washed three times by resuspending it in a solution whose composition was EDTA 5mM, KCl 0.1mM, and Tris 0.01mM (pH 7.2).

The final pellet was transferred to a quartz crucible, dried in a vacuum oven at 90°C, ashed at 550°C in a muffle furnace. The Mg content was determined by atomic absorption spectrophotometry as described elsewhere (2). For the half of the left ventricle used for glycerination, the dry weight before glycerination was calculated from the wet weight before glycerination using the ratio of dry weight to wet weight determined on the half of the ventricle which was not glycerinated.

It was found that the extraction with Triton was complete after shaking for 16 hours at 0°C. However, shaking for an additional 24 hours did not change the Mg content per gram unglycerinated dry weight.

Determination of Sequestered Mg in Isolated Myofibrils.—Myofibrils were isolated from ventricular homogenates by two procedures (A and B), each of which yielded a myofibrillar preparation which appeared free of membranous contamination on electron microscopic examination of osmium-fixed pellets stained with uranyl acetate and lead hydroxide.

For procedure A, 200-g female Sprague-Dawley rats were injected intraperitoneally with heparin. They were then stunned and their hearts were cut out and immersed immediately in an ice-cold buffered solution (pH 7.2) whose millimolar composition was sucrose 300, KCl 10, and Tris 50, and EDTA 5. The atria and connective tissue were trimmed off, and the heart was rinsed several times. All subsequent procedures were carried out at 0°C. The hearts were first minced finely with scissors and then homogenized with an M.S.E. blade and flask attached to a Virtis homogenizer. The homogenate was centrifuged at 700 g in a Sorvall model RC2B refrigerated
centrifuged, and the supernatant fluid was discarded. The pellet was resuspended in the previously described buffer solution containing, in addition, 0.02% Triton. After straining through cheesecloth, 8 ml of the homogenate were layered over 2 ml of a solution (pH 7.2), whose molar composition was sucrose 0.8, EDTA 0.001, and Tris 0.1, in a centrifuge tube. The tube was centrifuged for 10 minutes in a swinging bucket rotor at 40 g. After centrifugation, the mitochondria were discarded; this left a preparation of myofibrils still contaminated to a variable extent with fragmented mitochondrial, sarcotubular, and sarcotubular membranes. The pellet was resuspended and stirred gently for 15 minutes in a solution (designed to solubilize the membranous contaminants) whose composition was Triton X, EDTA 0.001M, and Tris 0.01M (pH 7.2). The myofibrillar protein concentration during this procedure was 4 mg protein/ml suspension. The detergent was then removed by centrifuging the suspension at 3000 g and washing the resuspended pellet three times with Triton-free buffer. The exposure to Triton was repeated once or twice until the resulting pellet was almost white (i.e., free of hem-containing mitochondrial fragments and containing no identifiable membranes on electron microscopic examination). Samples of a suspension of this pellet were then used to determine the protein and Mg contents. Protein was measured by the method of Lowry et al. (3).

Procedure B was previously developed and described by one of us (R.Z.) for studies of protein synthesis (4), adenosinetriphosphatase activity, and protein turnover (5). Although the method is more elaborate, we wanted to examine the Mg content of a preparation which was pure on electron microscopic examination and for which the chemical and enzymatic properties are well defined. The procedure involves five steps: relaxation of the muscle, homogenization, washing, treatment with detergent, and removal of nuclei, all carried out at 0°C (4).

**Determination of Mg Exchange in Glycerinated Ventricle.**—After rinsing out the glycerol, eight glycerinated halves of left ventricles were shaken for 48 hours at 3°C with a 28Mg-labeled solution containing 150 mKCl, 0.1 mM MgCl₂, and 5 mM Tris (pH 7.2). These ventricles were not minced. An additional ten ventricles were similarly shaken for 72 hours. At the end of the equilibration the ventricles were rinsed several times for a total of 45 minutes at 3°C with Tris buffer (pH 7.2); this rinse removed isotopic and nonisotopic Mg at the surface or in solution in the water associated with the muscle. The ventricles were then shaken for 2 hours with a solution containing lanthanum, hydrochloric acid, and perchloric acid, which served as both the extractant and blank for atomic absorption spectrophotometry (2). The gamma radiation in 1-ml aliquots of this solution and in 1-ml aliquots of the 28Mg-labeled incubation medium was then counted separately (in triplicate) in the well of a scintillation spectrometer (Packard Instruments model 3001), and the chemical content of Mg in both of the above samples was measured as usual by atomic absorption spectrophotometry. The radioactivity of all samples exceeded 5000 counts per 2-minute counting interval.

**Experiments on Rabbit Hearts.**—To examine the distribution of myofibrillar Mg in different cardiac chambers, rabbits were stunned and their hearts were rapidly excised and blotted. Blocks of tissue were immediately cut out with a razor blade, weighed, and processed for the determination of wet weight, dry weight, and sequestered myofibrillar Mg. Samples were obtained from the right and left ventricles, interventricular septum, and auricular appendages; both auricular appendages were removed in entirety.

**Experiments on Hypertrophic Left Ventricle.**

**Experimental Design.**—Female Sprague-Dawley rats weighing approximately 200 g were lightly anesthetized by intraperitoneal injection with sodium methohexital (38 mg/kg) and their left hemithorax was cut open to expose the heart and ascending aorta. The aorta was constricted with a silver band by the method of Nair et al. (1) and the left hemithorax was immediately closed with metal clips. Litter mates matched before the operation with respect to body weight were similarly operated on, except that no band was placed around the aorta. From 24 to 60 days after the operation the animals with banded aortas and their sham-operated controls were stunned by a blow on the head, and their hearts were excised. The hearts were either prepared for electron microscopic examination or used for various microchemical measurements.

**Stereological Measurements.**—Hearts were fixed as previously described (6) by perfusing them with fixative at room temperature on the Langendorff cannula. The hearts of animals with constricted aortas were taken for fixation only if the left ventricles were obviously selectively thickened. The fixative was an isosmotic electrolyte solution containing 60 mM osmium tetroxide buffered to pH 7.2. The great vessels, atria, right ventricle, and septum were discarded, and blocks of tissue from the subepicardial portions of the left ventricular wall were chosen for further processing. No attempt was made at more specific selection of sampling sites from the left ventricular wall, except that the apex was discarded to avoid contamination with fat or with septal tissue.
TABLE 1

Comparison of the Amount of Residual Mg in Glycerinated Rat Ventricles after Extraction with Triton-EDTA-KCl with the Amount of 48Mg-Inexchangeable Mg

<table>
<thead>
<tr>
<th>Residual Mg after extraction with</th>
<th>mmoles Mg/kg dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Triton, 5 mm EDTA, 150 mM KCl</td>
<td>1.11 ± 0.01 (33)</td>
</tr>
<tr>
<td>48 hours</td>
<td></td>
</tr>
<tr>
<td>28Mg-inexchangeable Mg</td>
<td>1.13 ± 0.05 (8)</td>
</tr>
<tr>
<td>72 hours</td>
<td>1.13 ± 0.07 (10)</td>
</tr>
<tr>
<td>48 + 72 hours</td>
<td>1.13 ± 0.04 (18)</td>
</tr>
</tbody>
</table>

Number of ventricles is in parentheses.

*Mg content referred to dry weight of the unglycerinated ventricle (see text).

The material was dehydrated, embedded in Araldite, and sectioned on an LKB ultramicrotome. Slightly oblique, ultrathin cross sections giving a gray interference pattern were stained either with the permanganate-lead stain of Reedy (7) or with uranyl acetate and examined on a Siemens IA electron microscope. The microscope was calibrated for each cassette of 11 plates with a Fullam carbon replica cross-grating (21,600 lines/cm). The criterion for an acceptable cross section was the finding of the classical hexagonal arrays of thick and thin filaments in the electron micrographs of the myofibrils and the absence of any longitudinal striation.

The stereological techniques and analysis applied to the electron micrographs have been described in detail elsewhere (8, 9).

Microchemical Measurements.—After excision of the heart, the combined left ventricular wall and interventricular septum were placed in a tared, covered crucible and immediately weighed on an analytical balance. The left ventricular wall was then cut away from the septum and divided into three roughly equal parts, each of which was also weighed. One part was glycerinated for subsequent determination of sequestered myofibrillar Mg; the second was dried overnight at 90°C in a vacuum oven and reweighed for determination of the dry weight; the third was extracted in a lanthanum-hydrochloric acid-perchloric acid solution for determination of total Mg.

Unless otherwise indicated, values in the tables and text are means ± se.

Results

Measurements on Normal Hearts

Amount of Mg Extracted from Glycerinated Rat Ventricles with EDTA-KCl-Triton.—The method of estimating myofibrillar mass used in this paper is based on the premise that the Mg content of the glycerinated ventricles after treatment with Triton-KCl-EDTA corresponds to the Mg bound to the thin filament during the polymerization of actin (see Discussion). On the basis of this premise it can be predicted that a fraction of the ventricular Mg content should be inexchangeable with 28Mg. This fraction should

Table 1 shows the Mg content of left ventricles treated to remove nonmyofibrillar Mg and all myofibrillar Mg except that not extractable with Triton-EDTA-KCl. The Mg content of glycerinated ventricles is expressed as mmoles Mg per kilogram dry weight of unglycerinated ventricle; this unit of reference permits comparison of the residual Mg after various methods of extraction with the total Mg content of unglycerinated ventricles (41.4 mmoles/kg dry weight, N = 10). For the hearts of 200-g female Sprague-Dawley rats these quantities vary surprisingly little. Electron micrographs of glycerinated ventricles exposed only to 150 mM KCl and not extracted with Triton show a very large amount of mitochondrial membranes, although mitochondrial morphology is, of course, distorted by glycerination. The Mg content of 18 such glycerinated ventricles was 2.54 ± 0.06 mmoles/kg dry ventricle; this is 2.3 times the amount of Mg which remains after extraction with Triton-EDTA-KCl (Table 1). Extraction with EDTA alone gave results essentially identical to those obtained after extraction with 150 mM KCl alone, the residual Mg content being 2.6 ± 1.0 mmoles/kg dry weight (N = 4). It is therefore clear that even after glycerination mitochondrial membranes and matrix contain tightly bound Mg which cannot be removed by 5 mM EDTA or 150 mM KCl; instead it is necessary to extract the membranes (and thereby, the membrane-bound Mg) by selectively solubilizing them with Triton X-100.

Comparison of the Amount of Mg in Rat Ventricles after Extraction with Triton-EDTA-KCl with the Amount of 48Mg-Inexchangeable Mg.—The method of estimating myofibrillar mass used in this paper is based on the premise that the Mg content of the glycerinated ventricles after treatment with Triton-KCl-EDTA corresponds to the Mg bound to the thin filament during the polymerization of actin (see Discussion). On the basis of this premise it can be predicted that a fraction of the ventricular Mg content should be inexchangeable with 28Mg. This fraction should
equal the Mg content remaining after extraction with Triton-KCl-EDTA provided that the extraction removes all nonmyofibrillar Mg and all exchangeable myofibrillar Mg and does not extract any inexchangeable myofibrillar Mg.

Table I shows that the content of $^{28}$Mg-inexchangeable Mg is indeed exactly equal to that of the residual Mg after the extraction of glycerinated ventricles with KCl-EDTA-Triton. The table also shows that the exchange is effectively complete after 48 hours.

Exploratory experiments in which suspensions of myofibrils isolated by procedure A were incubated for 0.5–12 hours with $^{28}$Mg indicate that no measurable radioactivity remains bound to the myofibrils after extraction with Triton-EDTA-KCl.

Mg Content of Isolated Purified Rat Cardiac Myofibrils.—Table 2 presents the Mg contents of the myofibrillar fractions isolated and purified by procedures A and B. The mean myofibrillar Mg contents obtained in multiple preparations by both procedures are similar, but both show a rather large dispersion. In addition, we have carried out an experiment in which one of two weighed and roughly equal portions of a preparation of pooled left ventricles was used for extracting the total myofibrillar protein content and the other was glycerinated and used for determining the residual Mg content after extraction with KCl-EDTA-Triton. Table 2 shows that the Mg content per unit of myofibrillar protein thus obtained is similar to that of isolated myofibrils. The resultant value for myofibrillar mass was $-347$ mg myofibrillar protein/g dried ventricle.

Sequestered Myofibrillar Mg in Different Parts of the Rabbit’s Heart.—The rat heart is too small to permit comparisons of the sequestered myofibrillar Mg content in different parts of the heart. We therefore analyzed different portions of the rabbit heart after weighing, glycerination, and extraction with KCl-EDTA-Triton. Table 3 shows that the myofibrillar Mg content per unit unglycerinated dry weight is largest in the left ventricular myocardium and interventricular septum, smaller in the right ventricular myocardium, and smallest in the atria.

MEASUREMENTS ON HYPERTROPHIC RAT LEFT VENTRICLES

Stereological Measurements.—Table 4 summarizes the results of stereological measurements on hypertrophic left ventricles and their sham-operated controls at intervals of between 10 and 50 days after operation. The table shows that 10 days after aortic constriction there is a significant increase in the fraction of cell volume occupied by myofibrils ($P<0.005$) and a significant decrease in the corresponding fraction occupied by mitochondria ($P<0.01$). Both of these changes become much more pronounced 50 days after aortic constriction. After 45 days the myofibrillar volume fraction is elevated, but that for mitochondria is anomalous in being unchanged from control volumes.

Microchemical Measurements.—The presence of hypertrophy was determined by comparing the dry weight of the left ventricle plus interventricular septum from rats with banded aortas with the corresponding weight from sham-operated controls. The body

### TABLE 2

*Estimation of Myofibrillar Mass in Rat Left Ventricle*

<table>
<thead>
<tr>
<th>Myofibrillar Mass</th>
<th>Mg content (mmoles Mg/kg protein)</th>
<th>Calculated myofibrillar mass (g myofibrillar protein/kg dry ventricle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated myofibrils, procedure A</td>
<td>3.2 ± 0.2 (6)</td>
<td>347</td>
</tr>
<tr>
<td>Isolated myofibrils, procedure B</td>
<td>3.5 ± 0.3 (4)</td>
<td>317</td>
</tr>
<tr>
<td>Sequestered Mg content of glycerinated ventricle/myofibrillar protein extractable from ventricle</td>
<td>3.2 (1)</td>
<td>347</td>
</tr>
</tbody>
</table>

Number of preparations is in parentheses.
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TABLE 3

Amount of Sequestered Myofibrillar Mg in Different Parts of the Rabbit's Heart

<table>
<thead>
<tr>
<th>Part of the Heart</th>
<th>(mmoles Mg/kg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auricular appendage</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Right ventricular wall</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>Left ventricular wall</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td>1.00 ± 0.04</td>
</tr>
</tbody>
</table>

Results are from determinations on five hearts.

weight of the control and experimental animals differed by 2 g or less on the day of operation. We arbitrarily defined hypertrophy as an increase of 8% or more in dry weight of the ventricle relative to that of the control, and we discarded without analysis ventricles which showed an increase in dry weight of less than 8%. We chose to measure the septum together with the left ventricular wall because it was possible to isolate the two components as an easily reproducible unit. This unit will hereafter be referred to in this section as "left ventricle." The choice of the dry weight (rather than the wet weight) as the unit of reference was based on our observation that in animals with banded aortas the ventricular wet weight may increase without a detectable concomitant increase in dry weight, particularly in the first 1–2 days after aortic constriction. In Figure 1 the sequestered myofibrillar Mg content of the left ventricles is plotted against the ventricular dry weight for both control and hypertrophic ventricles. The data are cumulative for all ventricles and have not been treated to take into account matching of control animals and those with banded aortas with respect to body weight. It is apparent that sequestered myofibrillar Mg content increases with increasing left ventricular dry weight. In an exploratory experiment, the Mg content of myofibrils from hypertrophic ventricles was found to be 3.4 mmoles/kg myofibrillar protein, a value which does not differ significantly from that of 3.2–3.5 for normal rat left ventricles (Table 2). Since the sequestered Mg content per unit of myofibrillar protein is the same in normal and hypertrophic ventricles, the increase in the left ventricular content per kilogram dry ventricle indicates that ventricular hypertrophy is associated with an increase in myofibrillar mass. The statistical analysis of the cumulative data on the sequestered myofibrillar Mg content of hypertrophic control and ventricles banded for 1–60 days is given in Table 5.

To obtain a more clear-cut result and a smaller scatter of the data it is essential to compare the sequestered myofibrillar Mg content of each hypertrophic ventricle with that of the ventricle from its own control (taken from the same litter of rats and matched with respect to body weight). In the

TABLE 4

Stereological Measurements on Hypertrophied and Control Rat Ventricles

<table>
<thead>
<tr>
<th>Days after operation</th>
<th>Type of operation</th>
<th>Myofib vol Cell vol</th>
<th>Mito vol Cell vol</th>
<th>Wt on day of operation (g)</th>
<th>Wt on day of cardiectomy (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>banded</td>
<td>0.53 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>210</td>
<td>224</td>
</tr>
<tr>
<td>10</td>
<td>sham</td>
<td>0.46 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>208</td>
<td>214</td>
</tr>
<tr>
<td>21</td>
<td>banded</td>
<td>0.54 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>240</td>
<td>264</td>
</tr>
<tr>
<td>21</td>
<td>sham</td>
<td>0.47 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>239</td>
<td>255</td>
</tr>
<tr>
<td>45</td>
<td>banded</td>
<td>0.53 ± 0.01</td>
<td>0.36 ± 0.01</td>
<td>200</td>
<td>247</td>
</tr>
<tr>
<td>45</td>
<td>sham</td>
<td>0.46 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>199</td>
<td>248</td>
</tr>
<tr>
<td>50</td>
<td>banded</td>
<td>0.57 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>207</td>
<td>262</td>
</tr>
<tr>
<td>50</td>
<td>sham</td>
<td>0.50 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>209</td>
<td>270</td>
</tr>
</tbody>
</table>

All values are the result of processing eight prints, as described in reference 8, obtained from a single heart. Myofib vol and mito vol refer to the volumes of myofibrils and mitochondria, respectively, sham refers to sham-operated rats, and banded refers to rats with banded aortas.

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days after aortic constriction it significantly exceeds unity, indicating that myofibrils are being accumulated in excess of other components of the dry weight. This finding is consistent with the stereological observations which showed that myofibrils make up an increasing proportion of cellular volume in hypertrophic ventricles 10 or more days after aortic constriction.

**Discussion**

**SEQUESTERED MYOFIBRILLAR MAGNESIUM**

The principle used in this paper to determine myofibrillar mass takes advantage of the binding of Mg to the filamentous actin in the thin filaments of the myofibrils. It is based on the finding by Oosawa et al. (10), Drabikowski and Strzelecka-Golszewksa (11), and Weber et al. (12) that Mg bound to filamentous actin during the polymerization of globular actin is in exchangeable against calcium and other divalent cations. Since the Mg concentration in the intracellular solution is normally very much higher than that of calcium or other divalent cations, the divalent cation sequestered when actin filaments are laid down in embryonic or postnatal muscle cells is predominantly Mg. Moreover, it has recently been shown in glycerinated skeletal muscle cells that this fraction of cellular Mg is in exchangeable with $^{28}$Mg (2). The finding of a sequestered and in exchangeable fraction of Mg in mammalian heart muscle is therefore not surprising.

The use of the sequestered myofibrillar Mg as an indicator of myofibrillar mass is based on the assumption that the amount of Mg thus bound per unit of myofibrillar protein should not vary as the total myofibrillar mass increases or decreases. In support of this assumption, we have been able to show that (1) the sequestered myofibrillar Mg content in rats with banded aortas to myofibrillar Mg in sham-operated rats is significantly greater than unity at all measured intervals after the production of aortic constriction. This finding suggests that the sequestered myofibrillar Mg content, and therefore myofibrillar mass, increases progressively as ventricular dry weight gets larger with duration of hypertrophy. It is of interest to determine whether the myofibrillar Mg content of hypertrophic ventricles increases in proportion to the increase in left ventricular dry weight.

Table 5 also gives the ratio of (myofibrillar Mg in rats with banded aortas + myofibrillar Mg in sham-operated rats) to (dry weight in rats with banded aortas + dry weight in sham-operated rats) for individual pairs of ventricles at different times after constriction of the ascending aorta. If the sequestered myofibrillar Mg content increases in the same proportion as ventricular dry weight, the ratio should be equal to 1.00. In fact 10 or more right half of Table 5 the ratio of myofibrillar Mg in rats with banded aortas to myofibrillar Mg in sham-operated rats is significantly greater than unity at all measured intervals after the production of aortic constriction. This finding suggests that the sequestered myofibrillar Mg content, and therefore myofibrillar mass, increases progressively as ventricular dry weight gets larger with duration of hypertrophy. It is of interest to determine whether the myofibrillar Mg content of hypertrophic ventricles increases in proportion to the increase in left ventricular dry weight.

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TABLE 5
Effects of Duration of Aortic Constriction on Rat Left Ventricular* Dry Weight and on Myofibrillar Magnesium Content

<table>
<thead>
<tr>
<th>Days post-op</th>
<th>MF Mg (mmoles/kg)</th>
<th>LV DW (g)</th>
<th>LV DW banded/LV DW sham</th>
<th>MF Mg banded/MF Mg sham</th>
<th>MF Mg banded/LV DW sham</th>
<th>MF Mg banded/MF Mg sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banded</td>
<td>1</td>
<td>1.18 ± 0.05 (6)</td>
<td>0.133 ± 0.005</td>
<td>1.12 ± 0.01</td>
<td>1.13 ± 0.04</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>Sham</td>
<td>1</td>
<td>1.14 ± 0.01 (8)</td>
<td>0.117 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banded</td>
<td>5</td>
<td>1.07 ± 0.02 (5)</td>
<td>0.161 ± 0.005</td>
<td>1.24 ± 0.05</td>
<td>1.26 ± 0.06</td>
<td>1.01 ± 0.02</td>
</tr>
<tr>
<td>Sham</td>
<td>5</td>
<td>1.07 ± 0.02 (4)</td>
<td>0.128 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banded</td>
<td>10</td>
<td>1.27 ± 0.04 (3)</td>
<td>0.19 ± 0.03</td>
<td>1.27 ± 0.04</td>
<td>1.34 ± 0.08</td>
<td>1.12 ± 0.01</td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>1.13 ± 0.04 (5)</td>
<td>0.121 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banded</td>
<td>25</td>
<td>1.37 ± 0.03 (5)</td>
<td>0.151 ± 0.005</td>
<td>1.23 ± 0.02</td>
<td>1.33 ± 0.04</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>Sham</td>
<td>25</td>
<td>1.23 ± 0.02 (4)</td>
<td>0.127 ± 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banded</td>
<td>60</td>
<td>1.20 ± 0.04 (4)</td>
<td>0.18 ± 0.02</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>1.13 ± 0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>60</td>
<td>1.04 ± 0.02 (5)</td>
<td>0.139 ± 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DW = dry weight, MF = myofibrillar, and LV = left ventricle.

*In this table left ventricular refers to the entire left ventricular wall plus the interventricular septum.

†Ratios represent comparisons between paired ventricles from litter mates matched to within 1g with respect to body weight; the ratios were then averaged to give the tabulated values. All other data are pooled (unpaired) values.

the sequestered myofibrillar Mg will provide a useful index of the myofibril content of heart muscle under conditions like hypertrophy, atrophy, or destruction of myocardial cells. Indeed, the method should be applicable to material obtained at postmortem examination since Krames and Page (13) have shown that the usual formaldehyde fixation does not significantly lower the Mg content of heart muscle. The prerequisite for the use of the method would seem to be the preservation of striations (and therefore of polymerized myofibrillar actin) as seen in light or electron micrographs. Conversely, changes in the myofibrillar protein content which precede the laying down of polymerized actin filaments would not be detected, and changes involving the depolymerization of the thin filaments would result in a decrease in the content of sequestered myofibrillar Mg.

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hours or less in response to the hypertrophic stimulus. During the first 5 days after banding of the aorta the sequestered myofibrillar Mg content increases proportionately with the dry weight. After 10 or more days the increases in myofibrillar mass (Table 5) and myofibrillar volume (Table 4) exceed those of the dry weight and of cell volume, respectively. An increase in fractional myofibrillar volume and an associated decrease in fractional mitochondrial volume have also been described in rat left ventricles hypertrophied after prolonged experimental hypertension (14). Meerson (15) has reported that constriction of the ascending aorta in rabbits causes a decrease in the left ventricular content of myofibrillar protein 2 days after operation and an increase after 1, 6, and 6 months. The net increase in myofibrillar mass in response to constriction of the ascending aorta in the rat results from an increase in the rate of myofibrillar synthesis and a decrease in the rate of degradation of the myofibrillar proteins (16, 17). If, as suggested for rabbit hearts by Hatt et al. (18) and Meerson (15) there is a phase of increased myofibrillar degradation early after aortic constriction, this phase in the rat heart is presumably either over or counterbalanced by an even more marked increase in the rate of myofibrillar synthesis by the time our earliest (24-hour) samples were taken for analysis of sequestered Mg content. In this connection, Schreiber et al. (19) have shown that the isolated perfused guinea pig heart subjected to ventricular overload incorporates 14C-lysine into myosin at an increased rate as early as 3 hours after the aortic pressure is raised.

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ERNEST PAGE, PHILIP I. POLIMENI, RADOVAN ZAK, JUDY EARLEY and MARGARET JOHNSON

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