Ventricular Nucleic Acid and Protein Levels with Myocardial Growth and Hypertrophy

By Arthur F. Grimm, D.D.S., Ph.D., Ryo Kubota, B.S., and William V. Whitehorn, M.D.

ABSTRACT

The myocardial DNA, RNA, actomyosin, and total protein concentrations were determined in rat ventricles ranging between 251 and 1635 mg. Three phases of myocardial growth were evident. Phase 1 (ventricular weights less than 550 mg): DNA concentrations remained relatively constant with increasing ventricular weight. The increasing total DNA per ventricle suggests continuing mitotic activity. Phase 2 (ventricular weights between 550 and 1000 mg): total DNA per ventricle remained relatively constant; with ventricular growth there was a progressive decrease in DNA concentrations. This would be the phase of true myocardial hypertrophy. Phase 3: DNA concentrations continued to slowly decrease although the total DNA per ventricle increased. It would appear that this is the result of a renewed synthesis of DNA.

In phases 1 and 2, the RNA concentrations progressively decreased, although the RNA/DNA ratio was increasing. This ratio appeared to reach its maximum value by the end of phase 2 and remain relatively constant in phase 3. Thus in phase 3, the concentrations of DNA and RNA simultaneously decreased to maintain this constant ratio. The ventricular total protein and ventricular actomyosin levels did not change over this range of ventricular weights.

ADDITIONAL KEY WORDS
DNA, RNA, and actomyosin of rat myocardium myocardial postnatal growth and development myocardial hypertrophy aortic constriction

Methods

The trimmed and weighed ventricles of Sprague-Dawley male albino rats were used in these studies. Ventricles over a wide weight range...
MYOCARDIAL NUCLEIC ACID AND PROTEIN LEVELS

(276 to 1635 mg) were obtained from animals of different ages, and from animals with a subdiaphragmatic aortic constriction and their sham operated litter mates examined 5 to 7 months postoperatively. The larger ventricles of the older untreated animals and the smaller ventricles of the constricted series occupied the same weight range. Animals were further divided into smaller subgroups containing approximately equal numbers to provide points relatively evenly spaced along the ventricular weight curve. Two sets of experiments were performed. In both studies, sample values are based on duplicate chemical determinations.

In the primary study, when ventricular weights were less than 700 mg, 2 hearts were used for each sample. The ventricles were minced and divided into three portions. The water content of the myocardium was determined by drying a ventricular sample (20 to 50 mg) to constant weight at 70°C for at least 3 weeks. Actomyosin was isolated from 400 to 600 mg of ventricular muscle using essentially the method of Benson et al. In both the actomyosin isolation procedures and the DNA and RNA isolation procedures (see below), the tissues were first homogenized in a fluid volume which was dependent upon the sample weight. Since constant volume aliquots were used for all subsequent procedures, each determination was based upon an identical amount of myocardium. Total ventricular protein and actomyosin concentrations were determined by the micro-Kjeldahl technique.

DNA and RNA were isolated from another 200 to 400 mg of ventricular muscle using essentially the Schmidt-Thannhauser method as described in Methods in Enzymology, vol. IV. Tissues were homogenized in 4% ice-cold perchloric acid. After centrifugation, the precipitates were consecutively extracted with ethanol and ether-ethanol. RNA was freed from the tissue with a 1 N NaOH solution kept at 34°C for 18 hours.

After centrifugation and the removal of the RNA in the supernatant fluid, the DNA was freed from the residue by boiling in BA(ClO₄)₂. Absorbanes were determined with a Beckman DB spectrophotometer. The absorbances at 260 μg were converted to concentrations by means of standard curves based on Sigma salmon sperm DNA and Sigma yeast RNA.

Since the interpretations resulting from the preceding study were found to be partially dependent upon the range of DNA values within the population, an attempt was made to establish more precisely the true population variability. A more limited study was carried out using a slightly different isolation procedure in an attempt to reduce technically introduced variability. This study determined the myocardial DNA levels in 11 litter mate rats within a narrow range of body weights (230 to 244 g) and ventricular weights (651 to 725 mg). These determinations were made using a modified colorimetric Schmidt-Thannhauser procedure as described in Nucleic Acids, vol. II. Tissues were homogenized in 5% trichloroacetic acid and, following isolation, the DNA levels were determined colorimetrically at 595 μg with diphenylamine. The greater mean ventricular DNA concentration of this study is probably the result of using different isolation procedures.

**Results**

**VENTRICULAR PROTEIN**

Table 1 presents the data on ventricular protein concentrations over the wide range of ventricular weights. The ventricular total protein concentrations did not significantly change over this range. On the basis of the protein content of the extracted and once repurified actomyosin solution, the ventricular actomyosin concentration also did not vary.

<table>
<thead>
<tr>
<th>Ventricular weight (mg)</th>
<th>No. of animals</th>
<th>Ventricular water</th>
<th>Ventricular total protein</th>
<th>Ventricular actomyosin</th>
<th>Actomyosin/total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>342 ± 39</td>
<td>13</td>
<td>78.1 ± 1.4</td>
<td>19.8 ± 1.4</td>
<td>2.89 ± 0.41</td>
<td>14.8 ± 1.8</td>
</tr>
<tr>
<td>476 ± 34</td>
<td>12</td>
<td>77.5 ± 0.9</td>
<td>19.8 ± 0.8</td>
<td>2.94 ± 0.18</td>
<td>15.1 ± 1.0</td>
</tr>
<tr>
<td>620 ± 40</td>
<td>12</td>
<td>76.9 ± 1.2</td>
<td>21.0 ± 1.8</td>
<td>2.77 ± 0.30</td>
<td>13.3 ± 1.9</td>
</tr>
<tr>
<td>816 ± 71</td>
<td>14</td>
<td>76.4 ± 1.0</td>
<td>21.5 ± 0.9</td>
<td>2.99 ± 0.29</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>932 ± 93</td>
<td>7</td>
<td>76.4 ± 1.4</td>
<td>20.3 ± 0.8</td>
<td>2.88 ± 0.11</td>
<td>14.2 ± 0.8</td>
</tr>
<tr>
<td>1098 ± 22</td>
<td>12</td>
<td>76.7 ± 0.8</td>
<td>20.7 ± 1.2</td>
<td>2.83 ± 0.22</td>
<td>13.8 ± 1.3</td>
</tr>
<tr>
<td>1181 ± 15</td>
<td>9</td>
<td>77.7 ± 1.1</td>
<td>19.7 ± 1.0</td>
<td>2.92 ± 0.34</td>
<td>14.7 ± 1.7</td>
</tr>
<tr>
<td>1257 ± 32</td>
<td>9</td>
<td>77.8 ± 1.5</td>
<td>20.3 ± 0.5</td>
<td>2.92 ± 0.20</td>
<td>14.4 ± 0.9</td>
</tr>
<tr>
<td>1402 ± 97</td>
<td>11</td>
<td>77.1 ± 1.2</td>
<td>20.7 ± 1.3</td>
<td>2.97 ± 0.17</td>
<td>14.4 ± 1.1</td>
</tr>
</tbody>
</table>

Mean values ± sd.

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Myocardial Nucleic Acid Content

<table>
<thead>
<tr>
<th>Phase</th>
<th>Ventricular weight (mg)</th>
<th>No. of animals</th>
<th>DNA concentration (in g/mg of wet ventricular weight)</th>
<th>RNA concentration (in g/mg of wet ventricular weight)</th>
<th>RNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>342 ± 476</td>
<td>13</td>
<td>1.34 ± 0.01</td>
<td>4.44 ± 0.38</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>476 ± 620</td>
<td>12</td>
<td>1.31 ± 0.28</td>
<td>4.11 ± 0.58</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>516 ± 81</td>
<td>14</td>
<td>0.85 ± 0.16</td>
<td>3.86 ± 0.25</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>927 ± 1105</td>
<td>10</td>
<td>0.81 ± 0.24</td>
<td>3.58 ± 0.50</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>1105 ± 1178</td>
<td>16</td>
<td>0.69 ± 0.19</td>
<td>3.35 ± 0.41</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>1178 ± 1254</td>
<td>10</td>
<td>0.66 ± 0.14</td>
<td>3.05 ± 0.23</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>1254 ± 1393</td>
<td>9</td>
<td>0.64 ± 0.14</td>
<td>3.15 ± 0.32</td>
<td>4.9</td>
</tr>
<tr>
<td>8</td>
<td>1393 ± 1393</td>
<td>12</td>
<td>0.62 ± 0.14</td>
<td>3.06 ± 0.33</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Mean values ± sd.

* Sigma salmon sperm deoxyribonucleic acid used as standard.
† Sigma yeast ribonucleic acid used as standard.

VENTRICULAR DNA

The concentration of DNA in ventricles of varying weights is presented in Table 2. It can be seen that DNA per milligram of ventricle decreased with increasing ventricular weights. These data are graphically presented in Figure 1. On the basis of this curve, three phases of DNA concentration may be distinguished: In phase 1, which ends with ventricular weights of about 550 mg, the DNA per milligram of ventricle shows little change. Therefore, with increasing ventricular weight, the DNA per ventricle is increasing markedly over this range. The DNA per ventricle is presented in Figure 2.

In phase 2, the ventricles are characterized by a marked decrease in the concentration of DNA per milligram of ventricle. This is the result of the almost constant level of DNA per ventricle.

In phase 3, which begins with rat ventricular weights over 1000 mg, the DNA per milligram of ventricle decreases while the total DNA per ventricle increases with ventricular growth. However, during phase 3, a 26% increase in ventricular weight (1105 to 1393 mg) is accompanied by only a 13% increase in DNA per ventricle. As a result DNA per milligram of ventricle continues to decline.

VENTRICULAR RNA

The concentration of RNA per milligram of ventricle for ventricles of varying weights is presented in Table 2. A graphical presenta-
Mean total ventricular DNA-ventricular weight relationships.

- = normal animals-different age series; △ = aortic constricted and sham operated animals-same age series.

In phases 1 and 2, the RNA per milligram of ventricle progressively decreases and the total RNA per ventricle increases with ventricular growth. In phase 3, the RNA per milligram of ventricle remains at a relatively constant level.

An examination of the DNA concentrations in Table 2 reveals a relatively broad scatter of points about the means, the standard deviation being 20 to 30% of the mean. This variability agrees with that obtained by others. In the more limited study on 11 animals (with body weight 238 ± 4 grams; ventricular weight 689 ± 23 mg) technical variability was greatly reduced. The DNA concentration was 2.13 μg/mg of ventricle and the SD was 0.11 or about 5% of the mean, a value similar to that described by Gluck et al. It appears that the true population variability in DNA content of rat ventricles is small.

Discussion

The relatively constant concentration of DNA in phase 1 and the marked increase in total DNA per ventricle during this period strongly suggests that mitotic activity is continuing and supports the concept that myocardial growth in this phase is due to both hyperplasia and hypertrophy. This would correspond to the stage of myocardial growth in the human infant in which mitotic figures have been described and in which the total number of nuclei increases along with the increase in the length, width, and number of the muscle fibers. Phase 1 probably also includes the enlarged hearts of anemic piglets in which Widdowson and McCance found normal concentrations of protein and nucleic acid.
with growth and concluded that there had been a cellular hyperplasia. Recently Rumyantsev,\textsuperscript{17} on the basis of labeling with thymidine-H\textsc{3}, demonstrated proliferation of rat nuclei through the 15th to 20th postnatal day; this period would lie within phase 1.

The data of phase 2 indicate a relatively constant amount of DNA per ventricle during this period. The actual measured values of DNA per milligram of ventricle during this phase closely approximate a curve calculated on the assumption of a constant amount of DNA per ventricle (dashed line of Fig. 1). It would thus appear that, during phase 2, little or no new DNA is being produced. This is the phase of true myocardial hypertrophy. It is probable that the upper limits of normal ventricular growth in the rat are included in this phase. These data are in agreement with the observations of Petersen and Baserga\textsuperscript{18} on growing mice. These workers were also able to demonstrate that increases in DNA content per ventricle are the result of nuclear division. Phase 2 probably corresponds to the phase of increased muscle fiber size\textsuperscript{19} and decreased nuclear/cytoplasmic volume\textsuperscript{1} in the human. It would include the normal post-infantile heart and might also include the “physiologic hypertrophied” heart of Linzbach.\textsuperscript{4, 5}

Since man, unlike the rat, is not a continually growing species, the human heart probably reaches its maximum size in young adults and, with the absence of disease, remains at this size during the remainder of life. Thus, human myocardial DNA concentration can be expected to decrease as the heart weight reaches its maximum and stable value in early adulthood. From this point the DNA concentration can be expected to remain relatively constant. The studies of Wüst\textsuperscript{20} support this view.

The total DNA per ventricle increases again in phase 3 while DNA per milligram of ventricle decreases at a slower rate than was the case in phase 2. It would appear, therefore, that myocardial growth may again be the result of hyperplasia as well as hypertrophy. Phase 3 probably corresponds to the histologic picture described by Linzbach\textsuperscript{4, 5} for the “pathologically hypertrophied” heart, a heart characterized by a fiber and nuclear hyperplasia and the presence of swollen and enlarged nuclei. The DNA relationships found in this phase agree with those reported by Nowy and Frings\textsuperscript{21} for enlarged rabbit hearts produced as the result of an experimentally induced aortic insufficiency. In these hearts, an organ weight increase of 83\% was accompanied by a 46\% increase in total DNA and a correspondingly decreased ventricular DNA concentration. Similarly, following aortic stenosis in dogs, Kletke and Sydow\textsuperscript{13} found an organ weight increase of 49\%, a total DNA increase of 21\% and a correspondingly decreased ventricular DNA concentration.

In phases 1 and 2, ventricular growth is characterized by increasing total RNA per ventricle and by progressively decreasing concentrations of RNA per milligram of ven-
tricle (Table 2, Figs. 3 and 4). The shape of the curve suggests that a single continuous process is operative during this period. Since the ventricles in these groups were from animals of differing ages, the decline in RNA concentrations with ventricular growth might be related either to the ventricular size or to the age of the specimens. On the basis of these results, this question must remain unanswered. A progressive reduction in myocardial RNA concentration with age has been described for the rat by Wulff and Freshman. Wüst showed similar reductions in human myocardial RNA concentration. In phase 3, the RNA concentration remains at a relatively constant level over the range of ventricular weights examined. This is reflected by a change in the slope of this portion of the total RNA per ventricle curve. The more nearly constant RNA concentrations in phase 3 may represent a true change from the pattern seen in phases 1 and 2: there may be a stimulus for the maintenance of the RNA concentration in these greatly enlarged hearts. However since the ventricles in this group were from animals of nearly the same age, if RNA concentrations are dependent on animal age, the nearly constant RNA concentration in phase 3 may be simply the result of the similar animal ages. A choice between these alternatives cannot be made on the basis of the experimental findings.

Relatively normal RNA concentrations have been described for hypertrophied hearts in the dog and in the rabbit. Some months after production of an aortic stenosis. However, myocardial RNA concentration first markedly increases following aortic constriction and then slowly returns to the normal level.

The ratio of concentrations of RNA and DNA increases during phases 1 and 2 and reaches a constant maintained level in phase 3. If it is assumed that a certain minimal DNA concentration is necessary to maintain RNA concentrations, such a maximal RNA/DNA ratio would be expected. In phase 3 the DNA synthesis was inadequate to maintain DNA concentrations and, since the RNA/DNA ratio was maintained, RNA concentrations fell simultaneously. It is tempting to speculate that this limiting relationship may contribute to the changes in protein synthesis which have been reported in myocardial hypertrophy and subsequent failure. Other reports, however, have been contradictory. Moreover total and contractile myocardial protein concentrations in this and previous studies show no change. Until further experimental evidence is obtained, the functional significance of the described changes in myocardial nucleic acid levels must remain in question.

References


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