Ventricular Nuclei-DNA Relationships with Myocardial Growth and Hypertrophy in the Rat

By Arthur F. Grimm, D.D.S., Ph.D., Luis de la Torre, Ph.D., and Michael La Porta, Jr., D.D.S.

ABSTRACT

Rat ventricles, ranging from 306 to 1999 mg, were obtained from normal animals of different ages and from animals subjected to chronic aortic constriction. The concentration of DNA in the paired ventricles (right and left) was found to be closely related to the concentration of nuclei in the left ventricular papillary muscles. Muscle nuclei represented only 10 to 15% of this population of nuclei. In young animals (phase 1), the total ventricular content of muscle nuclei, nonmuscle nuclei, and of DNA were increasing with ventricular growth. In the adult rat (phase 2), the total ventricular content of DNA and of both muscle and nonmuscle nuclei remained relatively constant with ventricular growth. In the enlarged hearts (phase 3), there was a further increase in total ventricular DNA but there was no further increase in the total number of muscle nuclei. Spectrophotometric studies (Feulgen stain), showed that 88% of the muscle nuclei belonged to a single ploidy class (probably diploid). No relation could be demonstrated between the extent of nuclear polyploidy and the weight of the ventricles. It was concluded that polyploidy was not a significant factor in the increased total DNA of phase 3. The increased ventricular DNA of phase 3 was explained by the proliferation of the nonmuscle nuclei.

ADDITIONAL KEY WORDS myocardial nuclei nuclear volume nuclear activity nuclear ploidy Feulgen spectrophotometry aortic constriction papillary muscle myocardial hypertrophy myocardial postnatal growth and development

 Shortly after birth, mitotic activity of cardiac muscle cells appears to become rare or even absent (1-6). This observation has led to examinations of the myocardial DNA relationships during cardiac growth and enlargement (7-13).

In an earlier study (7), the DNA, RNA, actomyosin, and total protein concentrations were determined for a wide range of rat paired (right and left) ventricular weights. Three phases of growth were described: In phase 1 (hearts from young animals with ventricular weights of less than 550 mg), DNA concentrations remained relatively constant with increasing ventricular weights. The increasing total DNA in the paired ventricles (right and left) suggested continuing mitotic activity. This conclusion is supported by the histologic demonstration of both DNA labeling (with 3H-thymidine and autoradiography) and of mitotic division in young animals (1, 2, 4-6). In phase 2 (ventricular weights were between 550 and 1000 mg), total DNA per the paired ventricles remained relatively constant; with ventricular growth there was a progressive decrease in DNA concentrations. This finding can be explained by the reduced rate of cardiac mitotic activity in older animals (1, 2, 5, 10). This hypertrophic phase probably includes the range of weights of normal adult rat paired ventricles (a reexamination of this data suggests that the
upper limits of phase 2 should be expanded to include ventricular weights between 550 and 1200 mg). In phase 3 (ventricular weights exceeding 1200 mg), DNA concentrations remained relatively constant as the total DNA in the paired ventricles, again increased. Several reasonable possibilities exist to explain this increase: (a) there may have been mobilization and entrapment of nucleated cells from the circulation; (b) there may have been an increase in the amount of DNA per nucleus (an increase in the state of ploidy). Kompmann et al. (14), from cytophotometric measurements of nuclear DNA, have described significant degrees of nuclear polyploidization in normal and hypertrophied human hearts; (c) there may have been increased mitotic activity of the cardiac muscle cells or any of the other ventricular cell types. Indeed, proliferation of the muscle nuclei (15) and of the interstitial cells (16) of the heart has been described during the development of cardiac hypertrophy after experimental aortic stenosis.

The present histologic investigation was made to determine the actual cellular mechanisms responsible for the described DNA relationships, using some of the experimental material from the previous study (7).

**Methods**

Sprague-Dawley male albino rats were used in these studies. As previously described (7), two experimental approaches were used to obtain a wide range of ventricular weights. In the first approach, hearts were obtained from normal animals of different ages and body weights; in the second, hearts were obtained from animals with a subdiaphragmatic-suprarenal aortic constriction and from their sham-operated litter mates examined 5 to 7 months postoperatively. The aortic constriction was produced by: (a) placing a rod 1.1 mm in diameter next to the aorta of rats weighing 150 to 250 g, (b) completely occluding the descending aorta with a subdiaphragmatic-suprarenal aortic constriction and from their sham-operated litter mates examined 5 to 7 months postoperatively, (c) removing the rod. This procedure, which should produce an aortic constriction approximately equal to the diameter of the rod, resulted in about 50% mortality after surgery.

The three experimental groups (A, normal animals of different ages, body weights, and ventricular weights; B, sham-operated animals; C, animals with aortic constriction) were further subdivided into groups 1 and 2 on the basis of ventricular weights. The specimens were selected so that some of the larger ventricles of group A, subgroup 2, were similar in weight to the smaller ventricles of group B, subgroup 1, and some of the larger ventricles of group B, subgroup 2, were similar in weight to the smaller ventricles of group C, subgroup 1. Since there were no apparent experimental differences between groups in the regions of overlap, the results are treated as a continuum.

DNA concentrations were determined in 200 to 400 mg of the paired ventricular muscle using essentially the Schmidt-Thannhauser method, as described by Korn (17). The total DNA in paired ventricles was calculated from the DNA concentrations and the ventricular weights. At the termination of the experiment, a single left ventricular papillary muscle was tied in situ to an applicator stick, freed from the ventricle, and fixed with 10% formalin for 2 hours. Following standard dehydration and paraffin embedding procedures, sections 15μ thick were cut with a Leitz No. 1300 large-base sledge microtome. Thirty papillary muscles were selected from specimens of the preceding study (7) to provide specimens distributed relatively evenly according to their ventricular weights. Feulgen stained sections were prepared from this group of 30 muscles using essentially the method described by Leuchtenberger (18). Each of these muscles was derived from a separate animal. An hydrolysis time of 8 minutes was chosen as the optimum for this material following preliminary studies. The nuclear volume and DNA content were determined for 30 muscles using essentially the method described by Leuchtenberger (18). Each of these muscles was derived from a separate animal. An hydrolysis time of 8 minutes was chosen as the optimum for this material following preliminary studies. The nuclear volume and DNA content were determined for 30 muscles using essentially the method described by Leuchtenberger (18). Each of these muscles was derived from a separate animal. An hydrolysis time of 8 minutes was chosen as the optimum for this material following preliminary studies.
VENTRICULAR NUCLEI-DNA RELATIONSHIPS

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Ventricular wt (mg)</th>
<th>Terminal body wt (g)</th>
<th>DNA Concentration (µg/mg)</th>
<th>DNA content ventricular total (µg)</th>
<th>All nuclei</th>
<th>Muscle nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>6</td>
<td>378 ± 31</td>
<td>1.45 ± 0.15</td>
<td>550</td>
<td>1894 ± 143</td>
<td>107 ± 15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>647 ± 43</td>
<td>1.08 ± 0.16</td>
<td>830</td>
<td>1361 ± 165</td>
<td>130 ± 40</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>7</td>
<td>782 ± 40</td>
<td>1.07 ± 0.15</td>
<td>820 (5)</td>
<td>1038 ± 91</td>
<td>121 ± 18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>1084 ± 30</td>
<td>0.85 ± 0.10</td>
<td>1140 (5)</td>
<td>871 ± 57</td>
<td>143 ± 15</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>6</td>
<td>1248 ± 19</td>
<td>0.78 ± 0.06</td>
<td>980</td>
<td>924 ± 103</td>
<td>175 ± 26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>1425 ± 43</td>
<td>0.79 ± 0.11</td>
<td>1128</td>
<td>1029 ± 107</td>
<td>219 ± 23</td>
</tr>
<tr>
<td></td>
<td>Extremely large heart</td>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td>1757</td>
<td>532 ± 18</td>
</tr>
</tbody>
</table>

See text for description of group classification. Values are means ± SE. Number in parentheses is number of specimens when less than that of number of animals.

(unpublished observations), a correction factor of 2 was used in the following calculation:

\[
\text{Estimated total number of nuclei} = \frac{(\text{nuclear count per section area}) \times (\text{ventricular wt in mg})}{(\text{2}) \times (3.3 \times 10^{-3})}
\]

A computer analysis was used to determine the population distribution of the DNA content per nucleus. This employed a multiple gaussian curve decomposition program by the gaussian transform-least squares method.

Results

DNA AND NUMBERS OF NUCLEI

Table 1 presents the mean values and the SE of the DNA concentrations in the ventricles and the concentrations of nuclei in the left ventricular papillary muscles. Both determinations were carried out on material from the same hearts. The DNA concentrations and the total nuclear concentrations are presented in Figure 1. DNA concentrations fell from a high level in phase 1 to a much lower value in phase 2. From the end of phase 2 through phase 3, DNA concentrations were relatively constant. These results are identical with those previously described (7). The concentration of the total nuclei, which also fell during phases 1 and 2, was relatively constant from the middle of phase 2 through phase 3. The nuclear concentration is expressed as numbers of nuclei counted per standard field (0.22 x 10^6 µ^2). In the group with the largest ventricles (group C-2), there may be a suggestion of an increase in the total nuclear concentration. However, this increase only approaches the 5% significance level. The largest of the ventricles (1999 mg) substantially exceeded the size of any other specimen in group C-2 and was consequently treated independently. Since this very large ventricle had an even greater concentration of total nuclei, the increase in nuclear concentration with the very large ventricular weights (group C-2 and the 1999-mg specimen) may be more...
FIGURE 1

Relationships between ventricular weight and concentrations of ventricular DNA and papillary muscle total nuclei. ◆ = normal animals; ▲ = sham-operated animals; ▲ = animals with aortic constriction.

than a coincidental occurrence. Particular attention should be directed to the almost identical shape of the ventricular DNA concentration curve and the nuclear concentration curve. It would appear that the histologically determined nuclear concentration in the left ventricular papillary muscle closely reflected the DNA concentration of the paired ventricles.

The total DNA and the total number of nuclei for the paired ventricles are presented in Table 1 and Figure 2. As previously described, phase 1 was characterized by an increase in the total ventricular DNA, phase 2 by a relatively constant level of ventricular DNA, and phase 3 by a renewed synthesis of DNA. The shape of the curve for the total number of nuclei for the paired ventricles appears almost identical with the total DNA curve.

Figure 2 also presents the relationship between the ventricular weights and the number of muscle nuclei. In phase 1, both muscle and nonmuscle nuclei are probably increasing in total number. The increase in the number of muscle nuclei from phase 1 to phase 2 was statistically significant (A-1 vs. A-2, P < 0.05; A-1 vs. A-2, B-1, B-2, P < 0.01). However, in ventricles greater than 550 mg, there was no statistically significant further increase in the total number of muscle nuclei. The number of muscle nuclei was found to represent approximately 10 to 15% of the total number of nuclei. (For reasons that are not clear, this value is less than that described by Morkin and Ashford [16] who reported that muscle nuclei compose approximately 25% of the total nuclei in 200 g female rats.)

The absolute number of nonmuscle nuclei also increased in phase 1, was relatively constant in phase 2 but, in contrast to the muscle nuclei, markedly increased in phase 3. The increase in total nuclei in phase 3 is primarily due to the increased number of nonmuscle nuclei.

MUSCLE NUCLEI: DNA CONTENT AND NUCLEAR VOLUME

Figure 3 presents the frequency distribution of the DNA contents in muscle nuclei expressed in arbitrary units. By the computer analysis, the skewed curve here can be best
explained by the presence of a second curve of low magnitude whose peak mean value occurs at twice that of the primary curve. The dashed lines represent such a curve; the hatched areas represent the contribution of the second curve. On this basis, 88% of the nuclei are contained within a single ploidy class which will be assumed to be the diploid class (2n), and an additional 11% were within the tetraploid class (4n). It can be calculated that the presence of higher ploidy classes (above 2n) only increases the mean DNA per nucleus to about 110 to 115% of the diploid value. A relation between ventricular weight and the degree of polyploidy could not be demonstrated.

Table 2 presents the relation between the amount of DNA (degree of ploidy) in a muscle nucleus and the volume of that muscle nucleus. Values are selected from those nuclei which could be segregated into distinct ploidy classes: nuclei with DNA contents below 8 arbitrary units are compared with those above 12; values in the overlap region of the two curves of Figure 3 were not included in the analysis. There is a statistically highly significant increased nuclear volume in the poly-ploid nuclei. Indeed in the predominantly tetraploid nuclei, the mean muscle nuclear volumes are approximately twice that of the diploid nuclei.

As seen in Table 1, mean muscle nuclear volume increased during phase 2, an increase which was not related to the degree of nuclear ploidy. Accordingly, a statistical comparison was made between the volumes of the muscle nuclei from ventricles greater and less than 900 mg. Table 3 shows that this increase (approximately 25%) in mean nuclear volume was highly significant.

**Discussion**

In phase 1, the increasing total DNA in paired ventricles (7) is accompanied by an increasing total number of both muscle and nonmuscle nuclei. This is almost certainly the result of continuing mitotic activity in the young animal, a conclusion supported by the demonstration of mitotic figures in both hearts of infants and young rats (2, 6, 20).

In phase 2, which probably includes the range of ventricular weights that occur during the period of normal adult life in the rat, there are relatively stable levels of both total nuclei and total DNA over a wide range of ventricular weights. Phase 2 is a period of almost pure hypertrophy. During this phase, the constant number of muscle nuclei is being diluted by the increasing muscle cytoplasmic volumes. Since it is probable that the increased cytoplasmic volumes of the muscle

**Table 2**

<table>
<thead>
<tr>
<th>DNA Content (arbitrary units)</th>
<th>No. of nuclei</th>
<th>Mean vol. (μ³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 7</td>
<td>937</td>
<td>181</td>
</tr>
<tr>
<td>≥ 12</td>
<td>755</td>
<td>168</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Wt. of paired ventricle (mg)</th>
<th>No. of nuclei</th>
<th>Mean vol. (μ³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;900</td>
<td>330</td>
<td>139</td>
</tr>
<tr>
<td>&gt;900</td>
<td>480</td>
<td>202</td>
</tr>
</tbody>
</table>
cells do impose increased functional demands on the constant number of muscle nuclei, the larger muscle nuclear volumes found in the larger ventricles (>900 mg) may simply reflect this elevated nuclear activity. This is not unlikely since nuclei of active cells are often larger than those of similar inactive cells (21). The slight rise in total DNA and total nuclei in phase 2 appears to be due to a slow continuing proliferation of nonmuscle nuclei.

In phase 3, there were increasing amounts of DNA. However, after phase 1, the number of muscle nuclei remained relatively constant. Previous studies, which have described the apparent absence of mitotic activity in cardiac muscle nuclei and their dilution with growth, have also concluded that there is a relatively constant number of muscle nuclei in the adult growing or enlarged heart (1, 3, 10).

In contrast to these conclusions, there have been reports of muscle nuclear labeling in the heart subsequent to the administration of tritiated thymidine. These results have been supported by reports of apparent labeled mitotic figures in muscle nuclei. The usual interpretation is that these findings demonstrate either continuing mitotic activity or else the process of polyploidization (22, 23).

However, Walker and Adrian (1), who studied and counted only labeled nuclei cut in cross section, found no labeled muscle nuclei in 21- and 30-day-old mice. They concluded that reports of labeled muscle nuclei are based on the presence of nonmuscle nuclei superimposed on the muscle fibers. Another explanation has been proposed by Pelc (24, 25) who has concluded that there is a regular renewal of some of all of the DNA in nondividing nuclei (metabolic DNA). Either of the latter may provide the explanation for the reported presence of labeled nuclei in the heart when muscle nuclear multiplication is absent.

In the present study, there was no real evidence for a significant degree of polyploidy; only about 10% of the muscle nuclei were polyploid. This finding is similar to that reported for the normal and hypertrophied human heart by Capers (26), and for the growing mouse (10). Furthermore, since no relation could be demonstrated between ventricular weights and the number of polyploid nuclei, it is concluded that there is no significant role for polyploidy in the increasing total DNA of phase 3. The increasing total ventricular DNA levels of phase 3 are due to the proliferation of nonmuscle nuclei. A similar conclusion has been drawn by Morkin and Ashford (16) and by Grove et al. (27). In histologic studies, these authors have described a proliferation of nonmuscle nuclei in hypertrophied rat hearts.

Since polyploidy was not an important factor in the present studies, the ventricular DNA should be directly related to the number of ventricular nuclei. Petersen and Baserga (10) using a nuclear DNA content of $7 \times 10^{-12}$ g demonstrated such a relationship in the mouse. Using the same nuclear DNA content per nucleus and multiplying it by the number of nuclei ($121 \times 10^6$ at a ventricular weight of 782 mg) the ventricular DNA should be equal to $847 \mu g$, a value in very good agreement with the 820 µg actually obtained. Indeed for the six groups, this calculated DNA \[\text{Number of nuclei} \times (7 \times 10^6)\] was 116 ± 19% of the measured value.

In contrast to these findings, Kompmann et al. (14) have described a very substantial amount of polyploidy in the muscle nuclei of human hypertrophied hearts. This very interesting report, if confirmed, may demonstrate a species difference between the rat and man either in their capacity for polyploidy or in the extent of possible hypertrophy. However, it should be restated that in the present study no relation could be demonstrated between the ventricular weight and the degree of ploidy.

A significant relation was found between the degree of muscle nuclear ploidy and the muscle nuclear volumes in the present study. Similar increases in nuclear volumes with polyploidy have been reported for other tissues (28, 29).

In the largest hearts, there may be evidence for an additional change in the nuclear DNA pattern with growth. The group of the largest hearts and the observations in the one
extremely large heart may suggest that, with very large hearts, nuclear proliferation may increase to a point where nuclear concentrations could be similar to levels found in the young small heart. This may provide an explanation for the findings of Korecky and French (30) who produced extremely large hearts by iron deficiency anemias and found essentially normal concentrations of ventricular DNA. The site of this proliferation should be of particular interest since the present study would suggest that the predominant response is a massive proliferation of nonmuscle nuclei. The question remains as to the type or types of nonmuscle nuclei which do proliferate in phase 3. The possible proliferation of the blood vessels in hypertrophy (3, 31) suggests that there may be a proliferation of the endothelial and allied tissue nuclei. Recently, Buccino et al. have reported an increased collagen content in cat hearts hypertrophied by means of a pulmonary artery constriction (32). This report would suggest that there may also be proliferation of the cardiac fibroblasts. Proliferation of both connective tissue and endothelial cells has been described during the acute phases of cardiac hypertrophy (16). Further investigations appear to be required to determine the type or types of proliferating nonmuscle nuclei in phase 3.

The dilution of muscle nuclei is a striking feature of this type of study. Since polyploidy is apparently not a significant factor, this muscle nuclear dilution also represents a dilution of the nuclear material (DNA) of the muscle cells. The ventricular concentration of muscle nuclei fell from a value of \(36 \times 10^3/\text{mg}\) at a ventricular weight of 378 mg to a value of \(17 \times 10^3/\text{mg}\) at a ventricular weight of 1425 mg (and a value of \(9 \times 10^3/\text{mg}\) at the 1999 ventricular weight). Though this extreme two- to fourfold dilution of the muscle nuclear material might be expected to have functional significance, it must be emphasized that experimental evidence which can ascribe functional changes in the myocardium to this nuclear dilution is presently lacking.

Acknowledgment
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