Cellular Hyperplasia and Hypertrophy in Cardiomegalies Induced by Anemia in Young and Adult Rats

By Joachim F. Neffgen and Borivoj Korecky

ABSTRACT

In our previous studies a twofold cardiomegaly associated with a proportional increase in cardiac DNA was found in rats subjected to a low-iron diet after weaning. To localize the newly synthesized DNA, control and anemic rats 24—34 days of age were given three intraperitoneal injections of \(^3\)H-thymidine (1 μc/g body weight; 22c/mM) at 2-hour intervals. The radioautographs were prepared from sections of the left ventricle stained with PAS-hematoxylin. Between 26 and 34 days of age, significantly larger numbers of labeled nuclei were found in both muscle (31–160/1000) and nonmuscle cells, (29–139/1000) in the hearts of anemic rats as compared to controls (11–19/1000 and 6–35/1000, respectively). When 80-day-old rats were given a low-iron diet, a cardiomegaly of approximately 50% developed. A nonsignificant increase in total cardiac DNA and a small significant increase in labeled cardiac nonmuscle nuclei (16–35/1000) was observed in the anemic groups. We conclude that hyperplasia of cardiac muscle and nonmuscle cells is responsible for the increased cardiac mass if cardiomegaly is induced during early postweaning life, while cardiomegaly induced in adult life gives rise to hypertrophy of cardiac muscle cells and hyperplasia of cardiac nonmuscle cells.

KEY WORDS

cardiac muscle nuclei radioautography \(^3\)H-thymidine quantitative histology cardiac nonmuscle nuclei

It is well known that cardiomegaly may develop during postnatal life if the heart is subjected to an increased afterload. However, some controversy still exists concerning the extent to which cardiac cell hypertrophy or hyperplasia occurs in conditions leading to the increase in cardiac mass.

A number of investigators induced cardiomegaly in adult animals by aortic banding or by producing aortic insufficiency and observed in the enlarged hearts an increase in total DNA but a slight decrease in its concentration (1–3). These findings would indicate both hypertrophy and hyperplasia of cardiac cells.

Radioautography demonstrated that the cells undergoing hyperplasia are almost exclusively nonmuscle cells (4, 5). A different result was obtained in our previous experiments in which cardiomegaly was caused by iron deficiency anemia (6, 7). We subjected rats to a diet low in iron after weaning (21 days) and observed a roughly twofold increase in heart weight at about 100 days of age. Despite this increase in mass, the number of myocardial fibers per unit cross-sectional area remained normal (6) and the concentration of DNA did not differ from the control values (7). These results, which are supported by those of Widdowson and McCance (8), suggest hyperplasia of cardiac muscle cells if cardiomegaly is induced by anemia shortly after weaning. The differences in findings between our studies and those mentioned above may be due either to different experimental conditions (aortic banding as compared to iron deficiency anemia), or to a difference in the ages of the
animals at which cardiomegaly was induced, rather than in the techniques.

To study the effect of age, we induced cardiomegaly by anemia caused by a diet low in iron in young and adult rats and compared the extent of hypertrophy and that of hyperplasia of cardiac muscle and nonmuscle cells in the two age groups. The heart weights and the concentrations of cardiac DNA, RNA, and protein were determined in both groups and compared to littermate controls. The newly formed cardiac DNA, an indicator of new cell formation, was localized after injections of [methyl-3H] thymidine by examining radioautographs of left ventricles.

Methods

**EARLY POSTWEANING STUDIES**

Male Wistar rats, weaned at 21 days of age, were used. At weaning, pairs of male littermates were divided randomly into groups which were killed at different ages during the early postweaning period. Each group consisted of an experimental and a control subgroup with equal numbers of animals in the two subgroups. For each member in the experimental subgroup there was a corresponding littermate in the control subgroup. Anemia was produced in the experimental animals by feeding a modified milk diet (7) and distilled water. The control animals received an identical diet supplemented with iron and copper salts and ordinary tap water. After death by decapitation the hearts were removed and separated into atria, left and right ventricles, and septa as described by Fulton et al. (9). Left ventricular and total heart weights of the anemic animals were expressed as absolute weights and as percent deviations from the expected weights of control animals of identical body weights. The newly formed cardiac DNA, an indicator of new cell formation, was localized after injections of [methyl-3H] thymidine by examining radioautographs of left ventricles.

Three experiments were done on anemic and control animals. The first experiment was made to determine the effect of low-iron diet upon hemoglobin concentration and heart weight during the early postweaning period. Anemic and control groups were killed at the ages of 20, 25, 30, and 40 days. The hearts were removed and dissected manually into the plot of left ventricular and total heart weights against body weights of our control animals.

In a second experiment the concentrations of cardiac DNA, RNA, and protein were determined in one control group 20 days of age and in three groups of control and anemic rats killed at 24, 29-33, and 45 days of age. DNA, RNA, and total protein were extracted from left and right ventricles by a modification of the Schmidt-Thannhauser technique (10). DNA was determined by the diphenylamine method (11), RNA by the orcinol reaction (12), and protein by the Lowry method (13). The results in the first and second experiments were evaluated by analysis of variance.

In the third experiment control and anemic groups were injected intraperitoneally with [methyl-3H] thymidine ([3H]-thymidine) when 24-30 and 32-34 days old (1 μc/g body weight; 22.6 c/mM; 1 mc/ml; diluted 1:2 in normal saline). Three injections were given at 2-hour intervals and the animals were killed 2 hours after the last injection. To avoid possible effects due to diurnal variations, all injections were begun at 9-10 A.M. The hearts were removed and dissected as described above. Radioautographs were made from sections of the left ventricles stained with PAS-hematoxylin according to Belanger and Leblond (14, 15).

The relative numbers of labeled cardiac muscle and nonmuscle nuclei were determined by examining a total of 1000 nuclei per section. To facilitate the counting a ruled grid of 25 divisions was inserted into the ocular of a light microscope at a 10 × 63 magnification. Although the fields containing approximately 50 nuclei each were selected randomly, an attempt was made to get equal representation from all parts of the section. Fields showing major blood vessels or parts thereof were disregarded. After application of an inverse sine transformation \(\sin^{-1}\sqrt{F}\) (16), the results were evaluated statistically by Student’s t-test.

**ADULT ANIMAL STUDIES**

Male Wistar rats were weaned and divided randomly into experimental, control, and normal groups when 21 days old. The control and experimental groups were kept at hemoglobin levels of 10-11 g% until 70 days old by a low-iron diet (17) supplemented occasionally with a standard laboratory diet. Keeping the iron stores in the body partially depleted facilitated the induction of severe anemia at a later stage but did not produce cardiomegaly itself. At 70 days of age, supplementation with the standard laboratory diet was discontinued in the experimental groups (from here on referred to as anemic groups) and consequently their hemoglobin levels fell below 5 g% within 30 to 50 days. The control group, on the other hand, was maintained at 10-11 g% hemoglobin. Another group of animals (normal) was given only the standard laboratory diet after weaning. The design of this study is summarized in Figure 1 and displayed in terms of changes of blood hemoglobin levels. All animals...
were killed by decapitation and their hearts were dissected as described earlier. Left ventricular and total heart weights were expressed as absolute weights and as percent deviations from their expected values, which were obtained from regression equations previously determined for normal animals of the same colony: weight of left ventricle (mg) = 1.51 body weight (g) + 167.15, (18); heart weight (mg) = 2.14 body weight (g) + 295.10, (19).

In the first experiment on the adult animals, the left ventricular concentrations of DNA, RNA, and protein were determined in control animals killed 104–157 days of age, in three groups of anemic animals killed at 104, 115, 135–157 days of age and in normal animals killed between 104 and 157 days of age. Otherwise the procedures were the same as described earlier.

In our second experiment on adult animals, 3H-thymidine was injected intraperitoneally using the same methods as described in the early postweaning study. Four groups of anemic animals were given three intraperitoneal injections after their hemoglobin levels had been below 5 g% for 1, 3, 5, and 7 days. In addition to the anemic groups, one control and one normal group were subjected to identical treatments. The techniques used to prepare and to evaluate radioautographs of the left ventricles were the same as described in the early postweaning study. The data were statistically analyzed by analysis of variance, using an inverse sine transformation \( \sin^{-1}\sqrt{P} \) (16).

**Results**

**EARLY POSTWEANING STUDIES**

The results of the first experiment are shown in Table 1. A decrease in blood hemoglobin content in the anemic animals to values as low as 4 g% was associated with a marked retardation in body growth. At the same time these animals developed cardiomegaly up to 60%. No significant differences in

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**Table 1**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Hemoglobin (g%)</th>
<th>Body wt (g)</th>
<th>Heart wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td>20</td>
<td>9.0 ± 0.3</td>
<td>50</td>
</tr>
<tr>
<td>Control (5)</td>
<td>25</td>
<td>11.5 ± 0.4</td>
<td>63</td>
</tr>
<tr>
<td>Anemic (5)</td>
<td>25</td>
<td>7.5* ± 0.6</td>
<td>60</td>
</tr>
<tr>
<td>Control (5)</td>
<td>30</td>
<td>13.0 ± 0.5</td>
<td>88</td>
</tr>
<tr>
<td>Anemic (5)</td>
<td>30</td>
<td>4.8† ± 0.4</td>
<td>67*</td>
</tr>
<tr>
<td>Control (5)</td>
<td>40</td>
<td>15.7 ± 0.3</td>
<td>173</td>
</tr>
<tr>
<td>Anemic (5)</td>
<td>40</td>
<td>4.0† ± 0.6</td>
<td>103†</td>
</tr>
</tbody>
</table>

Mean values = s.e. Heart weight % shows the difference from expected values of hearts of control animals of identical body weight (see text). Significant differences between corresponding control and anemic groups: *P < 0.05; †P < 0.01. Number of animals is given in parentheses.
**TABLE 2**

Concentration of DNA, RNA and Protein in Left Ventricle of Control and Anemic Young Rats

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body wt (g)</th>
<th>Left ventricle (mg) (%)</th>
<th>Concentration (mg/g)</th>
<th>RNA/DNA</th>
<th>Prot/DNA</th>
<th>Total DNA (µg)</th>
<th>Total RNA (µg)</th>
<th>Total Prot (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td>20</td>
<td>50</td>
<td>120</td>
<td>3.20 ± 0.07</td>
<td>= 0.19 ± 2</td>
<td>= 0.09 ± 3</td>
<td>1.58 ± 1</td>
<td>56 = 19</td>
</tr>
<tr>
<td>Control (11)</td>
<td>24</td>
<td>66</td>
<td>189</td>
<td>2.83 ± 0.16</td>
<td>= 0.09 ± = 3</td>
<td>= 0.05 ± = 3</td>
<td>1.19 ± 3</td>
<td>60 = 40</td>
</tr>
<tr>
<td>Anemic (7)</td>
<td>24</td>
<td>59</td>
<td>260*</td>
<td>+ 54%*</td>
<td>2.55 ± 0.07</td>
<td>= 0.07 ± = 2</td>
<td>1.36* ± 0.03</td>
<td>60 ± = 3</td>
</tr>
<tr>
<td>Control (24)</td>
<td>29-33</td>
<td>88</td>
<td>268</td>
<td>2.59 ± 0.04</td>
<td>= 0.14 ± = 3</td>
<td>= 0.02 ± = 1</td>
<td>1.12 ± 0.8</td>
<td>60 ± 22</td>
</tr>
<tr>
<td>Anemic (24)</td>
<td>29-33</td>
<td>71*</td>
<td>= 364*</td>
<td>+ 62%*</td>
<td>2.30* ± 0.08</td>
<td>= 0.05 ± = 2</td>
<td>1.31* ± 0.04</td>
<td>62 ± 24</td>
</tr>
<tr>
<td>Control (6)</td>
<td>45</td>
<td>213</td>
<td>356</td>
<td>1.93 ± 0.04</td>
<td>= 0.04 ± = 2</td>
<td>= 0.04 ± = 2</td>
<td>1.38 ± 0.8</td>
<td>80 ± 27</td>
</tr>
<tr>
<td>Anemic (6)</td>
<td>45</td>
<td>128*</td>
<td>458f</td>
<td>+ 86%*</td>
<td>1.84 ± 0.05</td>
<td>= 0.05 ± = 2</td>
<td>1.59f ± 0.07</td>
<td>79 ± 91</td>
</tr>
</tbody>
</table>

Mean values ± s.e. Left ventricle % shows the difference from expected values of left ventricles of control animals of identical body weight (see text). Significant differences between corresponding control and anemic groups: *P < 0.01; †P < 0.05. Number of animals is given in parentheses. Prot = protein.
TABLE 3
Incorporation of \( ^3 \)H-Thymidine into Left Ventricles of Control and Anemic Young Rats

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. animals</th>
<th>Labeled muscle cells/1000</th>
<th>Labeled nonmuscle cells/1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>5</td>
<td>64 ± 23</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>61 ± 17</td>
<td>80 ± 35</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
<td>35 ± 16</td>
<td>160 ± 27*</td>
</tr>
<tr>
<td>27-30</td>
<td>8</td>
<td>49 ± 14</td>
<td>119 ± 26*</td>
</tr>
<tr>
<td>32-34</td>
<td>8</td>
<td>11 ± 5</td>
<td>31 ± 11*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>51 ± 18</td>
<td>18 ± 10</td>
</tr>
<tr>
<td>Anemic</td>
<td></td>
<td>58 ± 17</td>
<td>85 ± 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 ± 12</td>
<td>139 ± 19*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 ± 14</td>
<td>95 ± 19*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 ± 3</td>
<td>29 ± 9*</td>
</tr>
</tbody>
</table>

Mean values ± SE of control and anemic rats of the early postweaning period. Significant differences between control and anemic animals: *P < 0.01; †P < 0.02.

myocardial water content between anemic (78.6%) and control hearts (78.2%) were observed.

Concentration of DNA, RNA, and Protein.
—The biochemical data from left ventricles of control and anemic animals are shown in Table 2. Significant differences in left ventricular weight and total DNA were found between anemic and control animals. At 24 days of age the anemic animals showed a 37% increase in absolute weight of left ventricles and a 20% increase in total left ventricular DNA. At 29-33 days of age the absolute weights of the left ventricles had increased by 46% and the total DNA by 30%, at 45 days of age these values were 35% and 33%, respectively. Changes in total RNA and protein were parallel to changes in total DNA. The DNA concentrations of left ventricles of control and of anemic animals decreased with age. Although left ventricular DNA concentrations in the anemic animals were lower than those in the corresponding controls, this difference was significant only between 29 and 33 days of age. The ratios RNA/DNA in the anemic animals were significantly higher than those of control ventricles in all age groups studied. The increase in right ventricular weights and the changes in the concentrations and total amounts of DNA, RNA, and protein in the right ventricles were similar to those in the left ones.

Incorporation of \( ^3 \)H-thymidine.—The incorporation of \( ^3 \)H-thymidine into muscle and nonmuscle cells of control left ventricles decreased during this period. In left ventricles of anemic animals, however, the incorporation into both types of cells increased until 26 days of age. The thymidine indexes decreased in older anemic groups but remained significantly higher than in the controls. No statistical differences were found between the thymidine indexes of left ventricular muscle and of nonmuscle cells in any of the groups investigated.

ADULT ANIMAL STUDIES
The design of these experiments is summarized in Figure 1 in terms of changes of blood hemoglobin levels.

Concentration of DNA, RNA, and Protein.
—Table 4 shows that a decrease in the hemoglobin levels from 10.7 g% (control) to approximately 4 g% (anemic) caused a significant increase in the weight of left ventricles. In all instances the changes in left ventricular weight reflected the changes in total heart weight. The water content of the hearts in the anemic groups did not differ from the normal or control groups and ranged between 78.5 and 78.1%.

Changes in left ventricular DNA, RNA, and protein content were particularly marked in the anemic animals 135-157 days of age. An increase in absolute left ventricular weight (as compared to the control value) of 27% \( P < 0.01 \) was accompanied by an 18% decrease in DNA concentration \( P < 0.01 \), by a significant increase in total RNA \( P < 0.05 \) and in RNA/DNA \( P < 0.01 \); however, total DNA...
The young rats used in this study were given a normal or low-iron diet at a time of rapid growth, as may be seen from the changes in body weights in Table 1. The withdrawal of iron during this period of life caused a quick fall in blood hemoglobin levels and a simultaneous increase in heart weights.

The labeling indexes of cardiac muscle cells and cardiac nonmuscle cells were essentially the same in each type of animal (anemic or control) and each age group studied. If one accepts the thymidine index as an indicator of de novo cell formation (an assumption dealt with below), then the cardiomegaly in our young anemic animals can to a significant extent be attributed to hyperplasia of cardiac muscle and cardiac nonmuscle cells. Also, it is not likely that the increase in DNA content is due to a redistribution of cardiac muscle cells, since the labeling indexes of cardiac muscle cells were essentially the same in each type of animal (anemic or control) and each age group studied.

The incorporation of 3H-thymidine into cardiac muscle and cardiac nonmuscle cells was particularly high between the ages of 20 and 30 days, i.e., during the first 10 days of the stimulated heart growth as shown by the thymidine labeling indexes in Table 3.

The labeling indexes of cardiac muscle cells were essentially the same in each type of animal (anemic or control) and each age group studied. The data in Table 2 indicate that the cardiomegaly in the young anemic animals was accompanied by a significant increase in synthetic cardiac DNA. This increased DNA synthesis was particularly high between the ages of 20 and 30 days, i.e., during the first 10 days of the stimulated heart growth as shown by the thymidine labeling indexes in Table 3.

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would seem that the proliferating cardiac muscle and nonmuscle cells contributed to the increased cardiac mass proportionately. In addition to a marked increase in total cardiac DNA, a significantly lower value of its concentration was observed in anemic animals 29-33 days old. Thus, in addition to hyperplasia of cardiac cells, some degree of cell hypertrophy may have occurred during the early development of cardiomegaly. However, no significant difference in DNA concentration between control and enlarged hearts was found in anemic animals 45 days old. This indicates that the increase in total DNA and thus in the numbers of cardiac cells had become proportional to the increase in cardiac mass, while the size of the cells in the enlarged hearts was essentially equal to the corresponding controls. The present data agree with previous findings obtained under similar experimental conditions in rats (7) and in pigs (8).

Earlier studies in our laboratory proved it difficult to produce severe nutritional anemia and thus cardiomegaly in normal adult rats, presumably due to significant iron stores in these animals. However, this difficulty may be overcome if the animals' iron stores are kept low before feeding the iron-deficient diet. In our adult animal studies the formation of iron stores was prevented by giving barely enough iron to maintain the hemoglobin concentration of blood at about 10-11 g%. When the adult rats were finally subjected to the diet containing practically no iron, severe anemia and a cardiomegaly of 48-62% developed in these animals within 4-10 weeks. Control animals, maintained at approximately 10-11 g% hemoglobin, did not develop cardiomegaly, as indicated by their left ventricular and total heart weights expressed as percent differences from the expected values (Tables 4 and 5). In addition to showing no significant cardiac enlargement, our control animals seemed similar to normal animals (15-17 g% hemoglobin) with respect to left ventricular concentrations of DNA, RNA, and proteins as well as the RNA-DNA and protein-DNA ratios.

A cardiomegaly of 52% in the anemic adult animals, 135-157 days of age, was associated with a significant decrease in left ventricular DNA concentration and a significant increase in RNA/DNA, indicating that cell hypertrophy was partly responsible for the increased cardiac mass (Table 4). However, at the same time total left ventricular DNA was slightly elevated in the enlarged hearts. Although this increase was not statistically

<table>
<thead>
<tr>
<th>TABLE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incorporation of ^3H-Thymidine into Left Ventricle of Control, Anemic, and Normal Adult Rats</strong></td>
</tr>
<tr>
<td><strong>Age (days)</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Control (5)</td>
</tr>
<tr>
<td>Anemic (8)</td>
</tr>
<tr>
<td>Anemic (5)</td>
</tr>
<tr>
<td>Anemic (5)</td>
</tr>
<tr>
<td>Anemic (5)</td>
</tr>
<tr>
<td>Normal (9)</td>
</tr>
</tbody>
</table>

**Mean values ± SE. Significant differences between control and other groups:** *P < 0.01; †P < 0.05. Heart weight % shows the difference from expected values of hearts of normal animals of identical body weight (see text). Number of animals is given in parentheses. Hb = hemoglobin.
significant, it may well reflect the proliferation of cardiac nonmuscle cells (connective tissue and endothelial cells), as the thymidine indexes in Table 5 seem to indicate. The \(^{3}H\)-thymidine was injected into the adult anemic animals during a period of rapid increase in heart weights. Although the proportion of label increased significantly over the control values, the thymidine indexes did not exceed 35/1000 and the label was almost exclusively confined to nonmuscle cells (Table 5), a finding in good agreement with similar works by other investigators (4, 5, 20–22). Therefore we may conclude that the cardiomegaly produced in our adult rats encompassed both hypertrophy of cardiac cells and proliferation of cardiac nonmuscle cells.

In discussing our data on young and adult rats up to this point we have accepted the thymidine indexes as indicators of de novo cell formation. We assumed, therefore, that a cell synthesizing new DNA during interphase would divide eventually and would pass on this DNA to its daughter cells. In the light of work on metabolic DNA, such an assumption must be considered with some caution. Pelc (23) believes that cells, including cardiac muscle cells, form metabolic DNA after the last mitosis as a first step toward differentiation. He states that, in mice, as much as 36–43% of the total DNA may constitute a labile, metabolic DNA, the remainder being a stable fraction. According to some workers (24) this metabolic DNA contains additional copies of genetic DNA which are active in differentiated cells as working DNA, presumably in the transcription of the base sequence of DNA to RNA. The metabolic DNA would thus protect the genetic DNA, which is called upon only when the metabolic DNA has to be renewed or repaired.

It is possible that a part of the increased total DNA in our type of cardiomegaly was caused by increased metabolic DNA formation, polyploidy, or both. Our previous studies of cardiomegaly of early onset showed however, that cell size in the muscle tissue of enlarged hearts of anemic animals is essentially the same as in normal cardiac muscle tissue (6). Thus the increased total DNA and thymidine indexes together with the increased total RNA and protein which accompanied the increase in cardiac mass in our young anemic animals can be best explained by cellular hyperplasia. In cardiomegaly in the adult rat it is difficult to assess the extent to which metabolic DNA or polyploidy could have contributed to the increase in label. We believe that since the label was found almost exclusively in nonmuscle cells, an increased metabolic DNA or polyploidy is probably not the explanation for the increase in \(^{3}H\)-thymidine incorporation. Our conclusions are supported by studies of Grove et al. (25) who showed that in rats polyploidy is of little significance in cardiomegaly due to aortic banding.

Our work differs from other studies dealing with cardiomegaly in one important aspect. Instead of stimulating heart growth in only one particular period of life, cardiomegaly was induced in young as well as in adult animals by the same technique. The results show that the presence of cardiac cell hypertrophy, hyperplasia, or both in cardiomegaly may well be a function of the age at which cardiomegaly is induced, as was suggested by Rakusan and Poupa (26). It appears that hypertrophy of cardiac cells and proliferation of cardiac nonmuscle cells are the predominant mechanisms of heart growth when cardiomegaly is induced by anemia in adult rats. Various other studies, despite the use of different experimental models, confirm this position (1–5, 20–22). However, if cardiomegaly is induced during early life at a time when both muscle and nonmuscle cells in the heart have the ability to proliferate in the normal animal (27), then a proportional increase in the number of muscle and nonmuscle cells may form the basis of the increase in cardiac mass. Again, it seems that an examination of the relevant literature supports this concept (7, 8, 28–30).

We believe that much of the apparent conflict of opinion, i.e., hyperplasia vs. hypertrophy in cardiomegaly, may be due to a failure to recognize that the response of the
cardiac cell is dependent on the age at which cardiomegaly is produced.

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References


Cellular Hyperplasia and Hypertrophy in Cardiomegalies Induced by Anemia in Young and Adult Rats

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