Biochemical Correlates of Cardiac Hypertrophy: III. CHANGES IN DNA CONTENT; THE RELATIVE CONTRIBUTIONS OF POLYPLOIDY AND MITOTIC ACTIVITY

David Grove, K. G. Nair and Radovan Zak

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Biochemical Correlates of Cardiac Hypertrophy

III. CHANGES IN DNA CONTENT; THE RELATIVE CONTRIBUTIONS OF POLYPLOIDY AND MITOTIC ACTIVITY

By David Grove, B.S., K. G. Nair, M.D., Ph.D., and Radovan Zak, Ph.D.

ABSTRACT

DNA concentration in the left ventricle of 3-month-old rats 3 to 12 days after aortic constriction was similar to the values obtained in sham-operated litter mates (1.38 ± 0.03 μg/mg wet weight). The total DNA content of the heart was thus increased proportionally to its enlargement. In chronic hypertrophy (2 to 4 months after aortic constriction), the DNA content was increased to a significantly less extent than in short-term hypertrophy. The percentage of polyploid nuclei in 12- to 13-week-old rats increased 1 to 3 weeks after aortic constriction from a normal value of 1.8% to 4.2%. In 17- to 18-week-old rats, polyploidy was present in 3.9% of the nuclei 10 days after banding as compared with 1.6% in normal rats. Mitotic figures were localized almost exclusively outside muscle cells. Mitotic indices in hypertrophied hearts were ten times higher than in controls during the first 3 weeks after aortic constriction.

ADDITIONAL KEY WORDS
left ventricle mitotic figures experimental aortic constriction rats

■ Enlargement of the heart may be due to diffuse enlargement of individual cells of a tissue, or to an increase in cell numbers, or both. Measurement of tissue DNA is one method of distinguishing between the two processes, since the total DNA in the organ should remain unchanged when cells increase in size, but should increase when the number of cells increases. In experimentally induced work overload of the left ventricle, such as is produced by aortic banding, increase in the size of cells should result in a fall of the DNA concentration per milligram of heart tissue since the increase in heart weight would dilute the constant DNA content. An unchanged or increased DNA concentration would indicate DNA synthesis and the formation of new nuclei and probably of new cells.

Information on the changes in DNA content of the ventricular myocardium in cardiac enlargement is conflicting. Some investigators report a decrease in the concentration of DNA (mg DNA per unit weight of tissue) with no change in total DNA content of the heart (1-4). Others describe a moderate decline in DNA concentration with increase in total DNA content (5, 6). A third group claims that there is no change in DNA concentration and that the total increase in DNA content is proportional to the increase in cell mass (1, 3, 7-11). Finally, a fourth group has described a definite increase in both DNA concentration and total DNA content of the heart during cardiac enlargement (12, 14).

This and the accompanying paper reexam-

From the Department of Medicine of the Pritzker School of Medicine of the University of Chicago, Departments of Biochemistry and Physiology, and the Argonne Cancer Research Hospital (operated by the University of Chicago for the United States Atomic Energy Commission), Chicago, Illinois 60637.

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ine the process of DNA synthesis in heart muscle during cardiac hypertrophy.[1] We have studied the changes in myocardial DNA during the first 12 days after aortic constriction with the aim of determining the changes in DNA content and concentration during hypertrophy, and the cellular origin of the changes.

We confirm that cardiac DNA content rises significantly after acutely produced cardiac hypertrophy. Possible causes for this increase are: (1) increased mitotic activity in muscular elements; (2) increased mitotic activity in nonmuscular elements; (3) polyploidization of existing nuclei; (4) infiltration by inflammatory cells; (5) changes in cytoplasmic DNA, i.e., mitochondrial DNA. The first four processes are considered in this paper.

Materials and Methods

Supravalvular aortic stenosis was produced in female Sprague-Dawley rats weighing 200 to 220 g (12 to 13 weeks old) as described by Nair et al. (15). Both normal and sham-operated litter mates served as controls. Rats were killed at intervals of 2 to 12 days after operation. Hearts were excised, trimmed to remove connective tissue and the atria, blotted thoroughly to remove excess blood, and weighed. The left ventricle and septum were dissected from the right ventricle and prepared for biochemical or histological analysis.

Least-square regression lines of heart weight versus body weight in sham-operated controls were drawn. The extent of hypertrophy was determined by comparing the observed heart weight with the value indicated by the appropriate plot for a control animal of the same age and weight.

The DNA content of the left ventricle was determined 2 to 12 days after operation by grinding a 50- to 100-mg piece in 0.5N perchloric acid with a small quantity of sea sand (Reagent grade, Merck) in a mortar and pestle. Nucleic acids were extracted as previously described (15). DNA determinations were done in duplicate by the diphenylamine method of Burton (16).

Sections for microspectrophotometry and for the counting of polyploid nuclei and mitotic indices were prepared as follows: The left ventricle was opened by a longitudinal cut, fixed 24 hours in phosphate-buffer neutral formalin (pH 6.9), dehydrated, and embedded in paraffin. Sections 14μ thick were cut at a right angle to the mid-point of the long axis of the ventricle. Sections were stained by Feulgen's method and mounted under oil of refractive index 1.568, which rendered the cytoplasm virtually invisible. Measurements of nuclear light absorbance were made by the two-wavelength microspectrophotometric method (17) using a microspectrophotometer built by Dr. Hewson Swift.[2] Microscopic fields in which nuclei were to be scored for polyploidy corresponded to points on a preselected grid. For each heart studied, 1,000 or more muscle cell nuclei in fields containing muscle bundles oriented parallel to the plane of the section were scored visually as diploid (2c) or polyploid (4c or more) (17). Only nuclei of muscle cells were scored for polyploidy since nuclei of nonmuscular elements presented a technical problem.

The accuracy with which muscle nuclei could be identified in Feulgen-stained sections was estimated as follows: Sections stained with Harris' hematoxylin and eosin B, and Feulgen-stained sections, were prepared from each of six hearts. Two thousand nuclei were counted and classified as muscle or connective tissue cell nuclei in each section. The correlation coefficient was determined for the regression line relating the relative frequencies of muscle nuclei found in the two counts.

Whether polyploid muscle nuclei were distributed in sections according to the predictions of the Poisson distribution was tested by means of chi-square. Sections from ten hearts were used. The total number of polyploid nuclei observed was divided by the total number of fields (area each field = 0.0202 mm^2) to give the value of the Poisson λ. From this value the expected frequency of fields containing 0, 1, 2, or 3 or more polyploid nuclei, based on the total number of fields observed, was calculated, and compared by chi-square with the frequencies observed.

Mitotic indices (number of mitoses observed per 10,000 nuclei) were determined by counting 25,000 or 50,000 total nuclei per heart in sections from left ventricular muscle of several hypertrophied and control hearts. Mitotic figures from metaphase to early telophase were scored as mitoses. When necessary, sections were remounted under oil of unmatched refractive index to facilitate identification of cell types.

[1] In this and the accompanying paper the term "hypertrophy" is used to signify cardiac enlargement, secondary to the experimental procedure, without any implication that it causes enlargement of individual cells.

[2] Department of Biology, University of Chicago.

TABLE 1

Changes in DNA Concentration and Total DNA Content in Left Ventricle after Aortic Constriction

Experiment	Number of rats	DNA*			
		μg/mg tissue	P	μg/ventricle	P
		Short-Term Hypertrophy			
Sham-operated	40	1.38 ± 0.03		$6.7 \times 10^2 \pm 10$	
Aortic constriction					
Moderate hypertrophy (19%)†	30	1.41 ± 0.04	< 0.5	$8.0 \times 10^2 \pm 19$	< 0.001
Gross hypertrophy (44%)	10	1.51 ± 0.05	< 0.5	$10.3 \times 10^2 \pm 30$	< 0.001
		Chronic Hypertrophy			
Sham-operated	18	1.22 ± 0.03		$7.0 \times 10^2 \pm 16$	
Aortic constriction					
Moderate hypertrophy (31%)	8	1.02 ± 0.02	< 0.2	$7.6 \times 10^2 \pm 23$	< 0.05
Gross hypertrophy (55%)	6	0.86 ± 0.03	< 0.1	$7.6 \times 10^2 \pm 28$	< 0.05

All rats were banded at the age of 3 months. Short-term hypertrophy: animals were killed 3 to 12 days after banding. Chronic hypertrophy: animals were killed 2 to 4 months after banding.

*Means ± SE are given.†The percentage of hypertrophy was determined by comparison of wet weights of banded hearts with those of sham-operated litter mates of similar body weight. P = probability of chance occurrence.

Sections stained with hematoxylin and eosin were used to evaluate the possibility of infiltration of inflammatory cells.

Results

From 3 to 12 days following aortic banding, there was a considerable increase in total cardiac DNA content (Table 1 and Fig. 1). The DNA concentration per milligram of heart tissue remained similar to control values, although an increase or decrease was occasionally observed.

When the changes in total ventricular DNA and DNA concentration were plotted against the change in heart weight following aortic constriction, two results were apparent: (1) There was no consistent fall in DNA concentration. (2) Total ventricular DNA increased significantly above the values obtained for the control hearts (Fig. 1).

In chronically hypertrophied hearts 2 to 4 months after aortic constriction the total DNA in the left ventricle was 10% higher than in sham-operated controls of the same age. Total DNA content in greatly enlarged hearts was less marked after chronic hypertrophy than after short-term hypertrophy. The reduction in DNA content between the two periods under study was about 25%. Although there was also a tendency toward the decrease in DNA concentration per milligram of heart tissue in chronic hypertrophy, and especially in gross hypertrophy, the changes were not statistically significant.

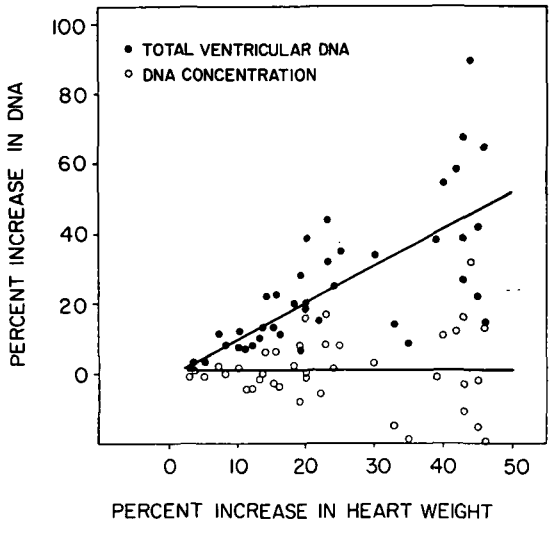

FIGURE 1

Relation of changes in DNA concentration, and total ventricular DNA, to changes in heart weight after aortic constriction. Data are expressed as percentage of increase over sham-operated controls. Determinations were made 2 to 12 days after banding. The slope of the lines were calculated by the method of least squares.

POLYPLOIDY

We wished to determine the degree of polyploidy specifically in muscle cell nuclei. We were able to select muscle cell nuclei

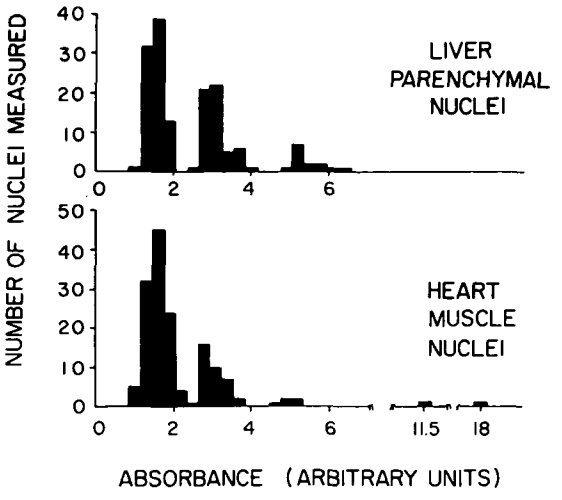

FIGURE 2

Absorbance values for Feulgen-stained rat liver cells and heart muscle cell nuclei. (The numbers of nuclei of each type measured were not proportional to their frequency in the tissue.)

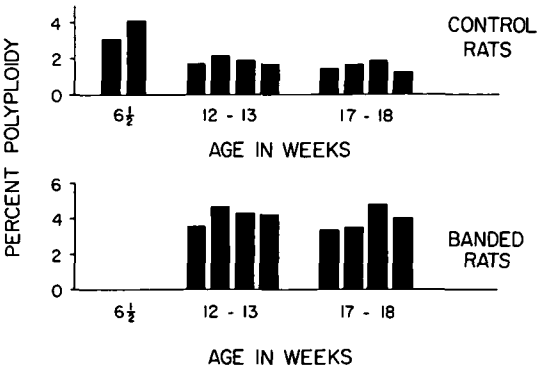

FIGURE 3

Polyploidy frequencies in heart muscle cell nuclei in banded and control rats. Rats 12 to 13 weeks old were killed 1 to 3 weeks after operation. Rats 17 to 18 weeks old were killed 10 days after banding.

accurately for the study of polyploidy in Feulgen-stained sections. The correlation coefficient for the regression of "percentage of muscle nuclei in Feulgen-stained sections" upon "percentage of muscle nuclei in hematoxylin and eosin-stained sections" was 0.98.

The histogram of absorbance values for Feulgen-stained heart muscle cells was polymodal (Fig. 2), indicating the presence of diploid and polyploid nuclei. Several nuclei of high, but indeterminate, degrees of polyploidy were also observed. Absorbance measurements included in this figure were made on nuclei of one hypertrophied and two normal hearts, stained together. Measurements of liver parenchymal nuclei are included for comparison (Fig. 2).

The frequencies of polyploid nuclei were determined from clearly identified heart muscle cells in sections from animals of different ages. The following groups of animals were examined: (1) 6 weeks old, two unoperated controls; (2) 12 to 13 weeks old, one sham-operated control, three normal unoperated controls, and four banded animals with cardiac hypertrophy ranging from 27% to 67% above control animals. Animals were killed 1 to 3 weeks after operation. (3) 17 to 18 weeks old, one sham-operated, three normal controls, and four animals with 32% to 51% cardiac hypertrophy killed 10 days after aortic constriction.

Hypertrophy was accompanied by an increase in the frequency of polyploid nuclei of the muscle cells (Fig. 3). Hypertrophied hearts of 12- to 13-week-old rats showed 4.2 ± 0.11 (SE) % polyploid muscle nuclei compared to 1.89 ± 0.08% in controls. Here in the 17- to 18-week-old group, hypertrophied hearts had 3.9 ± 0.2% polyploid muscle nuclei,

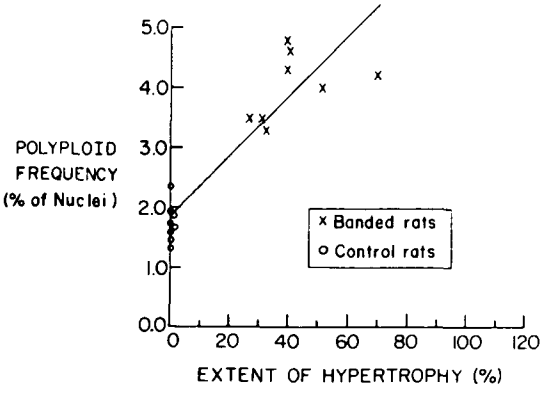

FIGURE 4

The relative frequency of polyploid muscle nuclei as a function of the extent of hypertrophy. The correlation coefficient, r, is 0.91 and is significantly larger than zero: for the null hypothesis that $r = 0$, $P < 10^{-3}$.

TABLE 2

Observed Distribution of Polyploid Muscle Cell Nuclei Compared with the Distribution Predicted by the Poisson Distribution

Number of polyploid nuclei per field i	Number of fields observed to contain i polyploid nuclei f_i	Total number of polyploid nuclei in class i $i \times f_i$	Poisson predictions of numbers of fields containing i nuclei f_p	$(f_i - f_p)^2/f_p$
0	1686	0	1677.2928	0.04520
1	330	330	347.1663	0.84882
2	44	88	35.9283	1.81340
3 or more	3	9	2.6126	0.05745
TOTAL	2063	427	2063.0000	$\chi^2 = 2.76487$
	$\lambda = 427/2063 = 0.20698$			$P > 0.20$, 2 df

compared to control hearts with $1.56 \pm 0.06\%$. The increases are statistically significant ($P<0.01$ in each case). When "percentage of polyploid nuclei" was plotted as a function of "percentage of hypertrophy," the correlation coefficient, r, was $+0.91$. The correlation coefficient was shown to be significantly different from zero by the Z-test ($P< 10^{-7}$, $n = 16$; see Fig. 4). Thus, there was a rather regular correlation between the degree of hypertrophy and the relative frequency of polyploid muscle cell nuclei in these experiments. The distribution of polyploid nuclei in microscope fields did not differ significantly from that predicted by a fitted Poisson distribution, when compared by chi-square with two degrees of freedom (Table 2).

INFILTRATION BY INFLAMMATORY CELLS

Inflammatory cells (so-called round cells) made up 0.2% to 0.3% of the nuclear population in both control and hypertrophied hearts. Significant infiltration of inflammatory cells was not observed in the hypertrophied hearts.

MITOTIC INDICES

The mitotic indices of hypertrophied hearts averaged ten times higher than those of controls (Table 3). The mitoses were found in cells other than muscle cells. In only one instance out of eighty-six was a mitosis observed in a cell that could not be distinguished clearly from muscle cells. The increase in mitotic index in hypertrophied hearts was statistically significant. The correlation coefficient of the regression line of the mitotic index as a function of degree of hypertrophy was + 0.90 (Fig. 5).

TABLE 3

Mitotic Indices of Control and Hypertrophied Hearts

Control group (n = 7)	Banded group (n = 7)
0.00	1.00
0.19	2.39
0.19	2.40
0.38	2.40
0.40	2.93
0.40	3.97
0.40	4.74
AVERAGE 0.28	2.80
SE 0.06	0.46

Mitotic index = mitoses/10,000 nuclei.

By t-test, $P < 0.0025$ (one tail). The experimental animals were studied 2 days after aortic banding.

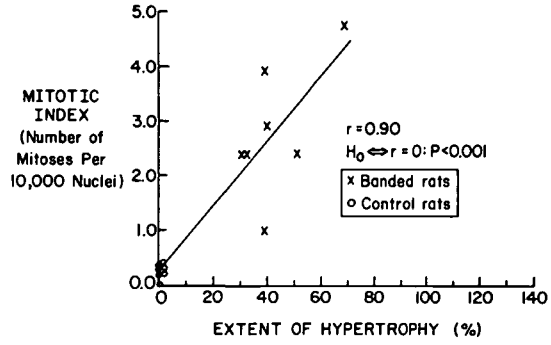

FIGURE 5

The mitotic index as a function of the extent of hypertrophy. The correlation coefficient, r, is significantly larger than zero: for the null hypothesis (H_0) that $r = 0$, $P < 10^{-3}$.

TABLE 4

DNA Changes in Short-Term and Chronic Myocardial Hypertrophy

Reference	Animal	Part of the heart	Time after operation	Type of operation	Wet wt. change (%)	RNA change (conc.) (%)	DNA change (%) (Conc.)	DNA change (%) (Total)
			Short-Term Hypertrophy					
1	Rabbit	LV	8 days	AA	N.G.	+ 46	+18(NS)	
3	Rabbit	LV	7 days	AA	+84	+ 30	− 2(NS)	+80
14	Puppy	LV	4 days	AA	N.G.	+164	+70	
18	Rat	LV	5 days	AA	+30	+100	+15(NS)	+50
11	Rat	LV + RV	7 days	AA	30-50	+ 30	− 2(NS)	+40
10	Rat	LV	4 days	AA	+23	+ 17	− 4(NS)	+18
This paper	Rat	LV	3-12 days	AA	+30	+ 32	+ 2(NS)	+32
			Chronic Hypertrophy					
1	Rabbit	LV	4 mo.	AA	+25	− 10(NS)	−25	0
2	Rabbit	LV	6 mo.	AA	+51	+ 6	−20	+21
3	Rabbit	LV	6 mo.	AA	+100	+ 2(NS)	−72	−44
13	Dog	LV	2.5 yr.	AA	+53	+ 35	+30	+98
5	Dog	LV	2.5 yr.	AA	+48	− 11(NS)	−15	+26
6	Rat	LV + RV	5-7 mo.	AbA	N.G.	0		
This paper	Rat	LV	3-4 mo.	AA	+28	− 10	−13	+12

LV = left ventricle; LV + RV = left and right ventricle; AA = constriction of ascending aorta; AbA = constriction of abdominal aorta; NS = not significant; N.G. = not given by authors.

Discussion

DNA CONTENT IN HYPERTROPHY

Our results clearly indicate that the total DNA content of cardiac tissue rises during the acute phase of cardiac hypertrophy. Otherwise there would have been a "dilution effect," i.e., the DNA concentration would have fallen with increasing hypertrophy. Instead, DNA concentration did not change significantly.

The results obtained by different investigators are summarized in Table 4. It is evident that the data pertaining to changes in DNA concentration should be analyzed according to the duration and intensity of the hypertrophy. The age and maturity of the cardiac tissues must also be taken into account. During the acute phase of cardiac hypertrophy the DNA content appears to change proportionally to the increase in heart weight so that there is no increase in DNA concentration although the total DNA content is increased. RNA concentration in this period is markedly increased. The very large increase in DNA concentration noted by Gluck and collaborators (14) may result partly from their use of very young, rapidly growing animals.

Four months or more after constriction of the aorta the situation differed. RNA, if still increased, was barely above control levels. Most reports agree that the DNA concentration in chronically hypertrophied hearts is below control values while total DNA content is still increased, although to a lesser extent than in short-term hypertrophy.

That cardiac hypertrophy is accompanied by a more or less proportional increase in the DNA concentration has also been reported by other laboratories in different situations such as DOCA-induced hypertension (12) and in nutritional anemia (7-9). A change in total DNA was not detected in the hearts of anemic animals however (4).

MECHANISM OF DNA INCREASE

The mechanism of increase in DNA content has been the subject of systematic study in our laboratory. No evidence for infiltration by inflammatory cells was noted and therefore increase in DNA content through such a process is eliminated.

The frequency of polyploid nuclei was the next subject of study (19). In autopsy specimens from human hearts Sandritter and Scomazzoni (20), as well as Kompman et al. (21) found that normal human hearts have an appreciable population of polyploid nuclei and that the frequency of nuclei with a high degree of polyploidy (8c, 16c, and 32c) increases markedly with myocardial hypertrophy. In hypertrophied rabbit hearts, Arutyunov (22) showed that both the DNA concentration and the DNA content per nucleus increased 7 to 10 days after operation. In rats, the DNA content per nucleus was reported by Chernukh et al. (23) to remain stable for 4 to 7 days after banding, to decrease by 15% at 21 days, and then to increase by 22% three months after aortic banding. Capers (24), however, found no change in DNA content of the nuclei of enlarged human hearts. No change in the frequency of occurrence of polyploid muscle nuclei in hypertrophied rat heart was reported by Mirakyan and Rumyantsev (25). Perhaps these differences are due to variations in the technique of estimation. We have employed the rigorous criteria of microspectrophotometry, using the method of Swift (17). We observed more than a doubling of the frequency of polyploid nuclei. This increase, however, can account for no more than 1% of the increase in DNA concentration of the myocardium since muscle nuclei comprise 26% or less of the total heart nuclei. In the present study no evidence was found for variation in the DNA content of individual diploid or tetraploid nuclei with hypertrophy. Therefore, although polyploidy increased in our model, it is quantitatively of little significance.

The development of polyploidy and its significance are as yet unclear. Sandritter et al. (20) and Kompmann et al. (21) consider polyploidy as a step in what is termed an amitotic process of nuclear division. Beam and King (26) suggested a mechanism whereby successive mitoses in single liver cells produce cells with two stable polyploid nuclei. If such a mechanism were responsible for the production of polyploid muscle nuclei, these nuclei

should appear in pairs. That they were distributed in a manner not significantly different from the predictions of a "fitted" Poisson distribution suggests that most of them are produced by random DNA replication in single nuclei, and not by the double mitosis proposed by Beam and King.

The correlation coefficients for the changes in mitotic index and percentage of polyploid muscle nuclei with hypertrophy are both positive and significantly different from zero (Figs. 4 and 5). Whether polyploidization of the muscle nucleus population can increase indefinitely with extreme degrees of hypertrophy in rat hearts remains to be learned, although Sandritter and Scomazzoni (20) found very extensive polyploidization in pathologically hypertrophied human hearts.

MITOTIC INDEX

Mitotic figures are conspicuously absent in the adult human heart, whereas they are abundant in the heart of the newborn rat (27 and D. Grove, unpublished observation). The observations of Klinge and Stocker (27) indicate that in the rat, maturity of heart muscle cells in terms of lack of mitotic activity is manifest by 3 months of age. Our studies were done on mature female rats in the acute phase of cardiac hypertrophy. Mitotic indices (number of mitoses per 10,000 nuclei), as determined by systematic counting of 25,000 or 50,000 nuclei per left ventricle, were on the average ten times higher than the corresponding indices in control rats. In no instance could we find a mitotic figure in a muscle cell. The mitotic activity was restricted to nuclei of nonmuscular elements such as connective tissue cells in the interstitium and nuclei of blood vessels. Thus the bulk of DNA synthesis is associated with mitotic activity in the less differentiated elements of the cardiac tissue. This process will be discussed in more detail in the accompanying publication.

Acknowledgments

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