AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 215, No. 6, December 1968. *Printed in U.S.A.*

Myocardial DNA synthesis in experimental cardiac hypertrophy

EUGENE MORKIN AND THOMAS P. ASHFORD
Departments of Medicine and Surgery, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215

MORKIN, EUGENE, AND THOMAS P. ASHFORD. *Myocardial DNA synthesis in experimental cardiac hypertrophy.* Am. J. Physiol. 215(6): 1409–1413. 1968.—Although mitotic activity is rarely observed in adult cardiac muscle, the increase in DNA content of the heart during development of cardiac hypertrophy is nearly proportionate to the increase in heart weight. In this study myocardial DNA synthesis was measured and the cell types involved identified in 200- to 220-g rats following either aortic coarctation (19 animals) or sham operation (9 animals). Rats were injected 1, 2, 3, 7, and 10 days after operation with thymidine-methyl-^{3}H and sacrificed 4 hr later. Hearts were divided into right ventricle (RV) and left ventricle plus septum (LV) and weighed; blocks of tissue were removed for autoradiography, and the remainder analyzed for nucleic acids and radioactivity. The results indicated: *1*) LV weight increased 23% by the 3rd day, after which it remained constant; *2*) LV RNA concentration increased 17% while DNA concentration decreased 10%; *3*) LV thymidine-^{3}H incorporation into DNA increased three- to fivefold between the 2nd and 7th days, then returned nearly to control; *4*) similar, but smaller changes were observed in RV nucleic acid concentration and DNA synthesis; *5*) autoradiographs revealed 99% of labeling occurred in connective tissue and blood vessel endothelial nuclei, with 1–2% of these nuclei labeled in control LV and 4–8% in hypertrophied LV. The increase in myocardial DNA content during development of cardiac hypertrophy represents an increase in DNA synthesis which normally occurs in the interstitial cells of the heart.

heart; ventricular hypertrophy; myocardial nucleic acid content

THE HEART RESPONDS to a chronic increase in work load by an increase in muscle mass. Since mitotic figures are rarely observed in adult cardiac muscle (17) and muscle fiber diameter is increased in hypertrophied hearts (11), this increased muscle mass is thought to represent primarily an enlargment of individual cardiac cells without an accompanying increase in nuclei (26). However, determination of the nucleic acid concentration of hypertrophied hearts consistently has revealed a smaller decrease in DNA concentration than would be predicted from the increased heart weight in the absence of DNA synthesis (7, 8, 27).

The purpose of the present study was to measure myocardial DNA synthesis following aortic coarctation and to identify the cell types in which synthesis occurs. The results indicate that myocardial DNA synthesis, as measured by thymidine-^{3}H incorporation, is increased markedly between the 2nd and 7th days after operation, returning to control levels by the 10th day. Autoradiographs show that DNA synthesis is limited almost entirely to the nuclei of interstitial and connective tissue cells and does not occur to any significant extent in muscle nuclei.

METHODS

Myocardial DNA synthesis was investigated in 200- to 220-g adult female CD rats (Charles River Breeding Laboratories, North Wilmington, Mass.) following either placement of a constricting silk ligature about the abdominal aorta (19 animals) or sham operation (9 animals). In order to assure a reasonably uniform degree of constriction, the constricting tie was placed over a blunt no. 22 needle (1 mm diam) which was then removed, leaving a constriction equal to the diameter of the needle. Before and after operation rats were maintained on a diet of Purina rat chow and water.

Rats with and without aortic constriction were injected intraperitoneally with 1 μc/g body weight thymidine-methyl-^{3}H (15.6 c/mmole) at 1, 2, 3, 7, and 10 days and were sacrificed 4 hr later. Injections were made at the same time each morning to eliminate the influence of possible diurnal variations in DNA synthesis. The rats were killed by a blow on the head, the hearts rapidly excised, chilled in crushed ice, divided into right ventricle and left ventricle plus septum, and weighed on a torsion balance in a cold room at 4 C. Blocks of tissue were removed from the free wall of the left ventricle midway between base and apex for histological examination and the remainder homogenized in cold saline for separation into RNA and DNA fractions by a modification of the Schmidt-Thannhauser pro-

cedure (25). The tissue suspension was precipitated with an equal volume of cold 10% trichloroacetic acid (TCA) and resuspended three times in cold 5% TCA and once in 90% ethanol containing 2% Na acetate. RNA was freed by incubation in 1 N NaOH for 4 hr at 37 C. After acidification of the digest DNA was extracted from the precipitate by heating at 90 C for 30 min in 5% TCA. RNA was determined by the orcinol reaction (1), and DNA by Burton's modification of the diphenylamine test (4), using yeast RNA and thymus DNA as standards. Duplicate nucleic acid determinations agreed within ± 5%. Radioactivity in the acid-alcohol washings and in the DNA fraction were determined in duplicate by liquid scintillation counting in Bray's solution (3) and corrected for background radioactivity and quenching. Sufficient counts were accumulated to reduce the counting error to less than ± 4%.

For microscopic examination and autoradiography, tissue was fixed in phosphate-buffer glutaraldehyde (pH 7.4) and embedded in epoxy resin, Epon (16), from which 0.4-μ sections were cut on a Porter-Blum microtome. Autoradiographs were prepared as described by Caro (5), using Ilford K.5 emulsion and stored in a light-tight cabinet for 4 weeks. After developing, the sections were stained with filtered methylene blue. The relative frequency of nuclei of different cell types and the distribution of radioactivity between them was determined in each animal by examining the nuclei contained in 100 high-power fields.

Blood pressure measurements were made under light ether anesthesia in a separate group of rats 5 days after either sham operation or aortic coarctation by insertion into the carotid artery of a glass cannula attached through a short length of plastic tubing to an electric strain gage (Statham P23 Db). Tracings were made on an oscillographic recorder (Electronics for Medicine).

RESULTS

Blood pressure measurements. Measurement of carotid arterial blood pressure on the 5th postoperative day in four sham-operated rats revealed an average blood pressure (mean ± SE) of 125 ± 5/87 ± 5, 108 ± 5 mm Hg, whereas in nine rats with coarctation the pressure averaged 153 ± 5/110 ± 5, 132 ± 4 mm Hg. An average increase of 25 mm Hg in mean blood pressure ($P < .01$) was produced, indicating only a moderate increase in left ventricular work. Overt signs of left ventricular failure were generally absent.

Ventricular weight, nucleic acid concentration, and DNA synthesis. Changes in ventricular weight, nucleic acid concentration, and thymidine-^{3}H incorporation following aortic coarctation and sham operation are shown in Table 1. Sham-operated rats sacrificed during the 1st week after operation showed no systematic difference in ventricular weight, nucleic acid concentration, or thymidine-^{3}H incorporation, and have been summarized as a single control group. As shown in the upper graph in Fig. 1*A*, there was a rapid increase in left ventricular (LV) weight following coarctation; by the 3rd postoperative day LV weight was increased 23% as compared with controls ($P < .01$).

Concomitant with the increase in LV weight, LV RNA concentration increased 17% ($P < .01$), remaining approximately at this level for the remainder of the period of observation. On the contrary, LV DNA concentration decreased about 10% during the phase of rapid increase in ventricular weight. However, by the 10th day after operation continuing DNA synthesis returned the DNA concentration nearly to normal level.

As shown in the lowermost graph in Fig. 1*A*, there was little change in LV DNA synthesis, as measured by the rate of thymidine-^{3}H incorporation into DNA, until the 2nd postoperative day. The rate of DNA

TABLE 1. *Ventricular weight, nucleic acid concentrations, and thymidine-^{3}H incorporation after sham operation and aortic coarctation*

State	Post-operative Day	Body Wt, g	Left Ventricle				Right Ventricle			
			Ventricular wt/body wt, mg/100g	Concn of wet weight tissue, μg/mg		Thymidine-^{3}H incorporation, dpm × 10^3/mg DNA	Ventricular wt/body wt, mg/100 g	Concn of wet weight tissue, μg/mg		Thymidine-^{3}H incorporation, dpm × 10^3/mg DNA
				RNA	DNA			RNA	DNA	
Sham	1–7 (9)	211.1 ± 4.2	228.5 ± 3.6	3.67 ± .07	1.43 ± .08	49 ± 10	65.8 ± 2.5	3.46 ± .10	1.71 ± .07	36 ± 5
Coarctation	1 (4)	207.4 ± 2.8	237.9 ± 4.2	3.65 ± .10	1.47 ± .12	39 ± 2	74.0 ± 5.4	3.42 ± .09	1.72 ± .17	25 ± 3
	2 (4)	207.8 ± 8.4	242.9 ± 5.6	3.83 ± .06	1.45 ± .07	115 ± 39	64.4 ± 6.0	3.83 ± .06	1.81 ± .10	99 ± 33
	3 (4)	197.3 ± 2.3	281.6 ± 6.9	4.31 ± .05	1.37 ± .08	120 ± 8	75.9 ± 2.6	3.96 ± .15	1.67 ± .10	131 ± 43
	7 (3)	217.5 ± 12.1	278.0 ± 10.4	3.96 ± .17	1.28 ± .06	300 ± 21	68.2 ± 2.1	3.49 ± .19	1.61 ± .06	130 ± 34
	10 (4)	219.2 ± 5.4	273.0 ± 8.0	4.11 ± .06	1.40 ± .05	47 ± 24	67.5 ± 2.2	3.66 ± .11	1.71 ± .06	26 ± 9

Values are means ± SE. Numbers in parentheses are numbers of animals.

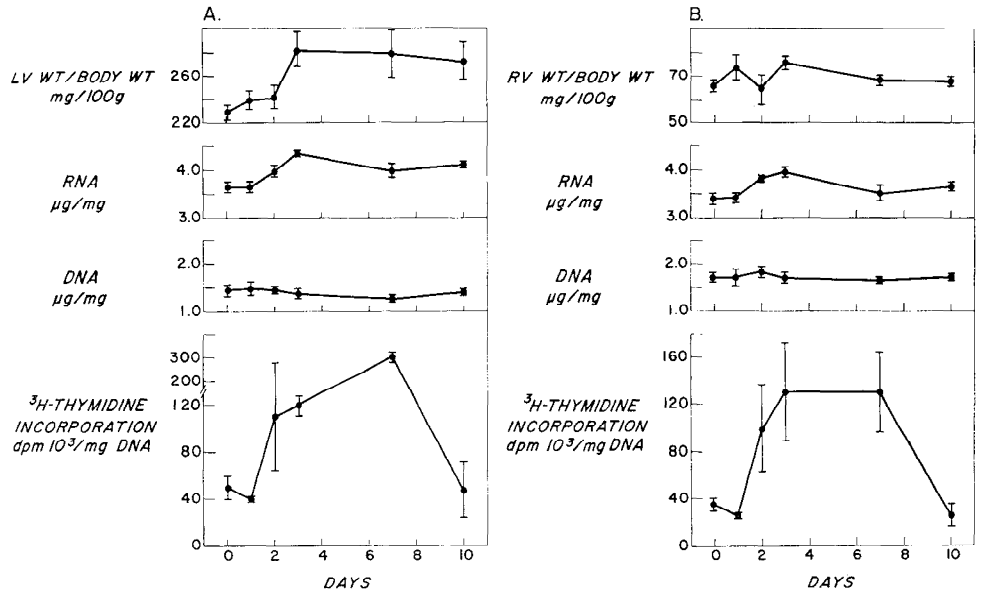

FIG. 1. Changes in left ventricular (*A*) and right ventricular (*B*) weight, nucleic acid concentration, and thymidine-^{3}H incorporation following aortic coarctation. Results for sham-operated animals are plotted at zero time. Values are means ± SE.

TABLE 2. *Ventricular acid-alcohol soluble radioactivity after thymidine-^{3}H injection*

State	Postoperative Day	Radioactivity, dpm/mg	
		LV	RV
Sham	1–7 (9)	1,313±64	1,202±70
Coarctation	1 (4)	1,230±44	947±28
	2 (4)	1,356±97	1,072±105
	3 (4)	1,434±40	1,319±22
	7 (3)	1,445±154	1,194±118
	10 (4)	1,637±145	1,389±100

Values are means ± SE. Numbers in parentheses are numbers of animals. LV = left ventricle, RV = right ventricle. Radioactivity is expressed per mg wet weight of ventricular tissue.

synthesis was markedly increased between the 2nd and 7th postoperative days, averaging three to five times control ($P < .01$). By the 10th postoperative day DNA synthesis had returned toward control level.

Interestingly, RV nucleic acid concentration and thymidine-^{3}H incorporation underwent similar, but smaller changes than those observed in LV (Fig. 1*B*). Increased RV weight and RNA concentration have been reported previously following left ventricular overload (7), and apparently are due to elevation of left ventricular filling pressure and pulmonary arterial and right ventricular pressures (28).

In order to be certain that alterations in DNA synthesis during development of cardiac hypertrophy were not related to differences in absorption of injected thymidine-^{3}H, measurements were made of the acid-alcohol soluble radioactivity representing thymidine and its derivatives taken up by the myocardium, but not incorporated into DNA during the 4-hr labeling period. As shown in Table 2, radioactivity in the acid-alcohol washings from RV and LV of control rats were similar ($P > .05$). Over the 10-day period of study in the ventricles of animals with coarctation there was a small increase in acid-alcohol soluble radioactivity which was statistically insignificant ($P > .05$). These changes may reflect alterations in size of DNA precursor pools (13); in any case, they are an unlikely explanation for the changes observed in DNA synthesis as the increase in acid-alcohol soluble radioactivity was much less than the increase observed in DNA synthesis, and was maximum on the 10th postoperative day when DNA synthesis had returned toward control level.

Localization of myocardial DNA synthesis. Autoradiographs from hypertrophied and control ventricles revealed that labeling is confined almost entirely to the nuclei of endothelial and connective tissue cells (Fig. 2, *A* and *B*). There was no increase over background in labeling of possible extranuclear sites (mitochondria) of DNA synthesis. An analysis of the distribution of thymidine-^{3}H among myocardial cell types is shown in Table 3. In the sham-operated group connective tissue and blood vessel endothelial nuclei comprised 73% of the total nuclei observed, whereas muscle nuclei represented only 27% of the total. Over 99% of labeling occurred in connective tissue and blood vessel endothelial nuclei, with only a rare labeled muscle nucleus encountered. In left ventricles from control animals 1.8% of connective tissue nuclei and 1.2% of blood vessel endothelial nuclei were labeled. Occasionally, a white blood cell with nuclear labeling was observed within a myocardial blood vessel; these represented only a fraction of a percent of the labeled nuclei observed and have not been included in Table 3.

The relative number of endothelial, connective tissue, and myocardial cells seen during the first 10 days following aortic coarctation was not significantly different from that seen in sham-operated animals. However,

animals sacrificed on the 3rd and 7th postoperative days had about a fourfold increase (P between .05 and .01) in labeling of interstitial nuclei; no labeled muscle nuclei were encountered. By the 10th postoperative day labeling of interstitial nuclei had returned to control level. These changes in nuclear labeling during development of hypertrophy corresponded closely with determinations of thymidine-^{3}H incorporation into the DNA fraction of these ventricles (Fig. 1*A*).

DISCUSSION

These results indicate that an increase in myocardial DNA synthesis in the rat during development of hypertrophy following aortic coarctation represents an increase in the normal synthesis occurring in interstitial cells and does not reflect synthetic activity within myocardial cells. This conclusion is consistent with observations during growth in the rat that after the first few weeks of life DNA synthesis in the heart is limited to supportive tissues (21, 23). Thymidine labeling of adult rat cardiac muscle also has not been observed after myocardial injury (22) or cardiac hypertrophy associated with deoxycortisone-induced hypertension (6). However, mechanisms have been proposed whereby the DNA content of cardiac muscle nuclei might increase without mitotic activity. Linzbach (14, 15) reported an increase in the number of cardiac muscle nuclei in hypertrophied hearts which he attributed to "amitotic" division; Sandritter and Scomazzoni (24) found an increased DNA content of muscle nuclei from hypertrophied hearts (polyploidy) as measured by Feulgen staining and microphotometry. Since there was no evidence of DNA synthesis by cardiac muscle in the present study, neither mechanism seems likely to have been responsible for the observed increase in DNA content. It is conceivable that failure to label cardiac muscle nuclei might be explained by an inability of these cells to utilize exogenous thymidine. However, this possibility seems unlikely as a rare labeled muscle nucleus was seen, and exogenous nucleosides are utilized by cardiac muscle nuclei in RNA synthesis (21).

Rats with aortic coarctation injected with thymidine-^{3}H 1 day after operation showed no increase in DNA synthesis over control values whereas protein (28) and RNA synthesis (12) recently have been shown to be increased within a few hours after coarctation. A delay in the onset of DNA synthesis also has been noted in renal tubular cells after unilateral nephrectomy (10), and in liver parenchymal cells following partial hepatectomy (9). Since entirely different cell lines and stimuli are involved, this similarity may be fortuitous, but it is tempting to speculate that common factors may determine the number of cells entering the phase of DNA synthesis.

The analysis of autoradiographed sections of myocardium (Table 3), in addition to localizing DNA synthesis, also revealed an interesting predominance of interstitial cells (73%) as compared to muscle cells (27%) both in normal and hypertrophied hearts. Since the nucleus of each mature cell contains approximately the same diploid complement of DNA (2), it would appear that three-fourths of myocardial DNA is of connective tissue and endothelial origin. Hence, changes in the DNA content of the heart can be ascribed readily to alterations in the rate of DNA synthesis by interstitial nuclei. This predominance of interstitial nuclei also suggests the need for caution in interpretation of changes in activity of nuclear enzymes prepared from whole heart homogenates (19); careful consideration should be given to the extent to which such preparations reflect the function of interstitial or myocardial cells.

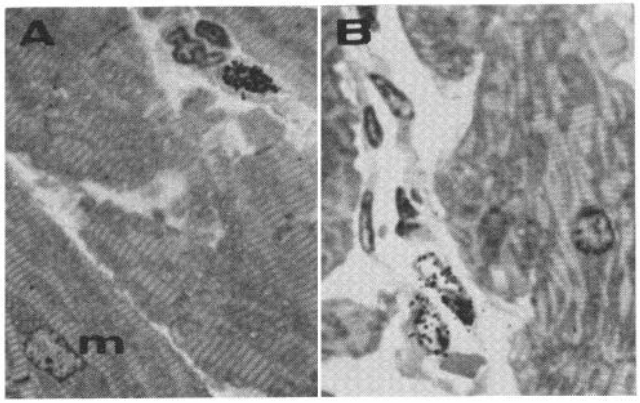

FIG. 2. Labeled fibroblast nucleus (*A*) and capillary endothelial nuclei (*B*) in hypertrophied left ventricle after injection with thymidine-^{3}H. m = muscle nucleus. Methylene blue staining. (× 1,320.)

TABLE 3. *Analysis of the distribution of thymidine-^{3}H labeling of myocardial nuclei*

State	Postoperative Day	Muscle Nuclei			Connective Tissue Nuclei			Blood Vesel Endothelial Nuclei		
		% Total	% Labeled	Mean grain count	% Total	% Labeled	Mean grain count	% Total	% Labeled	Mean grain count
Sham	1–7 (3)	27 ± 4	0.1	23	35 ± 1	1.8 ± 0.5	27 ± 8	38 ± 1	1.2 ± 0.6	18 ± 6
Coarctation	1–2 (4)	28 ± 1	0		30 ± 1	1.8 ± 0.5	32 ± 9	42 ± 1	0.6 ± 0.3	27 ± 14
	3–7 (4)	24 ± 1	0		34 ± 1	7.9 ± 1.4	30 ± 8	42 ± 1	5.3 ± 2.1	19 ± 7
	10 (3)	25 ± 1	0		34 ± 3	2.1 ± 1.0	26 ± 9	41 ± 1	1.1 ± 0.5	11 ± 5

Values are means ± SE. Numbers in parentheses are numbers of animals.

The research was supported by grants from the Life Insurance Medical Research Fund, the Greater Boston Chapter of the Massachusetts Heart Association (no. 787), and the National Institutes of Health (HE-06316). The paper was presented, in part, at 7th Annual Meeting of American Society for Cell Biology, Denver, Colorado, 13–15 November, 1967.

Received for publication 27 February 1968.

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