Biochemical Correlates of Cardiac Hypertrophy: I. Experimental Model; Changes in Heart Weight, RNA Content, and Nuclear RNA Polymerase Activity

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Biochemical Correlates of Cardiac Hypertrophy

I. EXPERIMENTAL MODEL; CHANGES IN HEART WEIGHT, RNA CONTENT, AND NUCLEAR RNA POLYMERASE ACTIVITY

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ABSTRACT

Cardiac hypertrophy occurred in mature rats after producing supravalvular aortic stenosis with a specially designed silver clip. For 2 weeks following this procedure, heart weight, body weight, and RNA content of the myocardium were serially determined. Heart weight and RNA content increased within 24 hours of aortic banding, reaching a maximal level in 2 days and remaining elevated during the 2 weeks of observation. Nuclei were isolated and purified from heart muscle homogenates, and changes in RNA polymerase activity following aortic banding were determined. The nearest neighbor frequency of the bases of the RNA synthesized by the polymerase from nuclear preparations was identical in both the banded animals and the sham-operated controls. Both groups could thus be compared on the basis of the enzyme assay. RNA polymerase activity in nuclei from the hearts of banded rats rose rapidly when compared with the activity in sham-operated rats; peak values were reached on the second day, the earliest detectable change being around 12 hours. The increase in RNA polymerase activity represents one of the earliest biochemical events that take place in the myocardium following aortic banding.

ADDITIONAL KEY WORDS **ventricular water content** **aortic banding** **DNA** **base analysis of RNA** **nearest neighbor frequency analysis of RNA** **isolation of heart nuclei** **RNA polymerase** **rat**

■ The mechanisms by which physiological stimuli such as pressure or volume overload of the heart produce cardiac hypertrophy are not clear. Of particular interest is the manner by which increased cardiac work leads to the synthesis of nucleic acids and protein in the myocardium. It is now thought from experimental evidence in bacterial and mammalian systems that DNA-dependent RNA polymerase plays a key role in the control of protein synthesis. An increase in protein synthesis is preceded by the following events: (1) transcription of de-repressed segments of DNA template by RNA polymerase with resultant increase in the synthesis of RNA; (2) translation of the "message" present in the newly produced RNA by the ribosomal assemblage to form polypeptide chains having specific amino acid sequences; and (3) self-assembly of protein subunits with the formation of a protein that has a characteristic secondary and tertiary structure.

Our laboratory is investigating the biochemical events that occur during experimental cardiac hypertrophy. This communication presents the details of the experimental model and the data relating to the changes in heart weight and RNA content of the myocardium following supravalvular aortic constriction. The sequential changes in the activity of the

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DNA-dependent RNA polymerase, which is responsible for the synthesis of cellular RNA, were measured in isolated cardiac nuclei. To evaluate the results of these assays, the nearest neighbor frequency of the bases in RNA synthesized by isolated nuclei from hypertrophied hearts was compared with controls. Our studies indicate that one of the earliest events following aortic banding is an increase in the RNA polymerase activity of the ventricular myocardium.

Material and Methods

LIST OF ABBREVIATIONS

PCA = perchloric acid
PEP = phosphoenolpyruvate
PK = pyruvate kinase
ATP = adenosine triphosphate
GTP = guanosine triphosphate
CTP = cytosine triphosphate
UTP = uridine triphosphate
AMP = adenosine monophosphate
GMP = guanosine monophosphate
CMP = cytosine monophosphate
UMP = uridine monophosphate

Production of Experimental Cardiac Hypertrophy.—We used mature female rats of the Sprague-Dawley strain weighing 200 to 220 g. They were fed on Purina chow ad libitum. Left ventricular hypertrophy was produced by a standard procedure in which the ascending aorta was banded by a circular clip made of silver wire 0.85 mm thick. For rats weighing 200 to 210 g the clips were made from 6.25-mm lengths of wire: for rats weighing over 210 g the pieces of wire were 0.25 mm longer. To produce sufficient aortic constriction, it is important that the ends of the clip close as shown in Figure 1 (*b*). The slight crossover of the two ends of the clip also prevents the band from becoming loose.

The rats were anesthetized intraperitoneally with Brevital (sodium methohexital), 30 to 35 mg/kg body weight. Positive-pressure respiration was produced by passing 95% O_2-5% CO_2 through an oral tube at the rate of 3 liters/min with intermittent compression of the nose and mouth. Abdominal distention was prevented by a binder. Following thoracotomy in the fourth left intercostal space, the ascending aorta was dissected free and the silver clip fitted over it. Sham-operated litter mates matched for body weight served as controls. A similar dissection was performed around the aorta but no clip was introduced, and the chest wall was rapidly closed. The mortality rate was about 30%, the chief cause of death being acute pulmonary edema in the immediate postoperative period. Fewer than 1 out of 200 rats died after the first day of the operation.

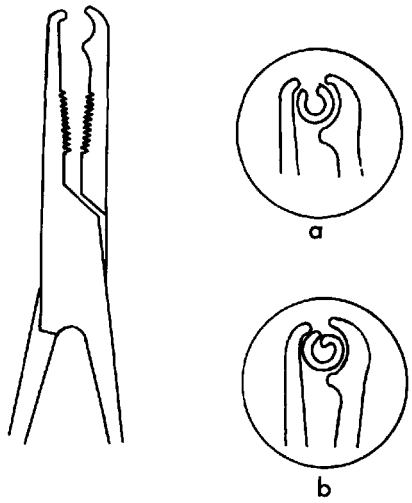

FIGURE 1

Instrument used for banding the ascending aorta. The ends of a pair of artery forceps were modified to hold silver clips made from 6.5-mm lengths of wire. The internal area of the band is approximately 1.6 mm². The band should be interlocked as shown in b *and not as in* a.

Determination of Heart Weight and Nucleic Acid Content.—Body weights were recorded at the time of surgery and again before the animals were killed by rapid decapitation. The hearts were quickly excised, blotted, and weighed. The left ventricles were minced and divided into samples for the determination of dry and wet weight, RNA and DNA content, and tissue nitrogen content. Left ventricular dry weight was obtained by drying a weighed amount of tissue (about 100 mg) in a vacuum oven at 80°C for 1 week. Tissue nitrogen was estimated in a separate sample by the Kjeldahl micromethod.

The DNA and RNA content of the mycardium were determined by a modification of the method of Maggio, Siekevitz and Palade (1). A sample of myocardium (50 to 100 mg) was ground in 0.25 ml of ice-cold 0.5N PCA using a mortar and pestle and a small amount of sea sand (reagent grade, Merck). The homogenate was diluted to 3 to 4 ml with 0.5N PCA and allowed to stand in ice for 1 hour. After low speed centrifugation (2,000 × g)

for 3 minutes in a refrigerated centrifuge, the precipitate was washed once with 2 to 5 ml of ice-cold 0.5N PCA, twice with 2 to 5 ml of absolute ethanol and finally with 2.5 ml of ether. The precipitate was dried under a tungsten lamp. RNA and DNA were extracted from the dried samples in 4 ml of 0.5N PCA at 70°C for 20 minutes. The extraction was repeated and the combined extracts were used for the estimation of RNA by the orcinol method (2) and DNA by the diphenylamine method (3). Calf thymus DNA (Sigma) and yeast RNA (Sigma) were used as standards.

Isolation of Nuclei.—Nuclei were isolated from heart muscle homogenates by differential centrifugation using 2.2M sucrose. Details of the method for isolation of purified nuclear fractions from heart muscle have been published previously (4). The fractionation procedure is based on the method published by Widnell and Tata (5) and Chauveau et al. (6).

RNA Polymerase Assays.—DNA-dependent RNA polymerase activity in nuclear preparations was assayed according to the methods of Weiss (7) and Widnell and Tata (5).

System I (Mg^{2+}-activated reaction).—The 0.5-ml incubation mixture contained: 50 μmoles of tris-HCl buffer pH 7.5, 2.5 μmoles $MgCl_2$, 10 μmoles of freshly neutralized glutathione, 5 μmoles of PEP (Sigma), 10 μg of PK (Sigma), 3 μmoles of NaF, 0.4 μmoles each of GTP, CTP, and ATP (Sigma), 0.024 μmoles of UTP-α^{32}P (specific activity 200 to 300 μc/μmole, Schwarz BioResearch) and 0.1 ml of nuclear suspension containing 100 to 200 μg DNA. In some experiments the labeled nucleotide was in the tritiated form (^{3}H-CTP or ^{3}H-UTP, specific activity 200 to 300 μc/μmole).

System II (Mn^{2+}, NH_4^+-activated reaction).—The 0.5-ml incubation mixture contained: 50 μmoles of tris-HCl buffer pH 7.5, 2 μmoles of $MnCl_2$, 300 μmoles of $(NH_4)_2SO_4$ adjusted to pH 7.5 with NH_4OH, 5 μmoles of PEP, 10 μg PK, 0.4 μmoles each of GTP, CTP, and ATP, 0.024 μmoles of UTP-α^{32}P and 0.1 ml of nuclear suspension containing 100 to 200 μg of nuclear DNA. In some experiments the labeled nucleotide was ^{3}H-CTP or ^{3}H-UTP, specific activity 200 to 300 μc/μmole.

All incubations were carried out in duplicate in a Dubnoff shaker at 37°C. The blanks contained 5 ml of 0.5N PCA added at zero time. The period of incubation was 5 minutes, during which the rate of incorporation of labeled nucleotide into RNA was linear in both systems I and II (Fig. 2). Incorporation of labeled nucleotide into RNA was linearly related to enzyme concentration in both systems (Fig. 3).

The method for the extraction of radioactive RNA was as previously described (4). Samples were counted in a liquid scintillation counter (Packard) using Bray's solution (8).

Nearest Neighbor Frequency Analyses of the Bases in RNA.—The method of Josse et al. was followed (9). The details of this procedure as applied to RNA extracted from RNA polymerase reactions have been published earlier (4). The hydrolyzed nucleotides were separated and estimated by anion exchange chromatography as described by Katz and Comb (10).

Results

Changes in Heart Weight.—The heart weights of banded animals are compared to those of sham-operated litter mates in Figure 4. A small increase in heart weight was already present within 24 hours after aortic

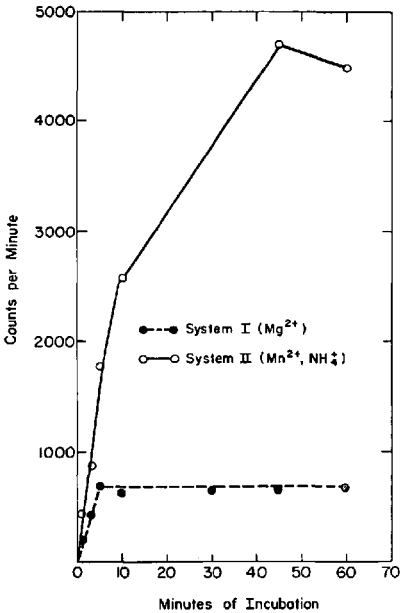

FIGURE 2

Time course of incorporation of ^{3}H-UMP into RNA. In system I a plateau was reached after 5 minutes of incubation. Note the twofold stimulation by $(NH_4)_2SO_4$ at 5 minutes, and the fivefold stimulation at 45 minutes, of incubation.

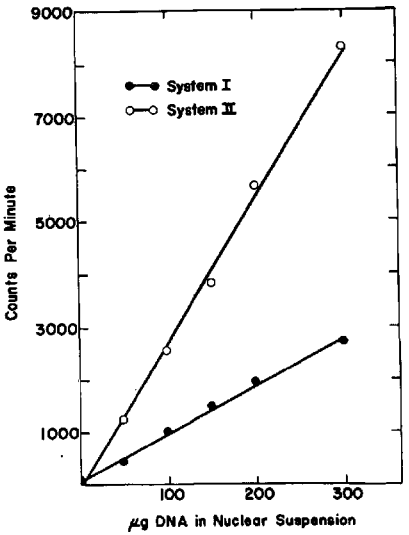

FIGURE 3

Relation between enzyme concentration (as indicated by the amount of DNA in the nuclear suspension) and enzyme activity in assay systems I and II. ^{3}H-CTP was used as the labeled nucleoside triphosphate. Incubation was for 5 minutes at 37°C.

banding. From the first day on, the differences in heart weight were highly significant (Fig. 4). In this series, the average weights of hearts exceeded those of the sham-operated ones by 150 mg. The evidence indicates that there is a rapid relative increase in heart weight during the first 2 or 3 days and that this difference is maintained but not substantially increased as the animals continue to grow.

There was a considerable scatter of the values of the heart weights of the banded rats. The extent of hypertrophy varied from 10% to 60% above control values. This scatter was probably due to the critical nature of the aortic stenosis; small differences in the degree of narrowing may result in much greater differences of the degree of hypertrophy achieved. When the bands were examined at autopsy it was found that those with areas varying from 1.4 mm^2 to 1.8 mm^2 invariably produced significant cardiac hypertrophy. Bands with areas smaller than 1.2 mm^2 produced death from left ventricular failure and pulmonary edema. The margin between the degree of constriction required to produce hypertrophy or heart failure is therefore quite narrow.

The data presented in Figures 4 and 5 show results of a series of experiments performed by the same surgeon under rigorously controlled conditions. Two other series of similar size yielded identical results with respect to heart weight and increase in RNA content. Minor variations in technique produced small but systematic differences in the magnitude of the changes observed. For illustrative purposes the data for one of the series are given here.

Ventricular water content was estimated in 127 hearts (47 with aortic banding, 60 sham-operated controls, and 20 unoperated rats). The values were 75.88 ± 1.59 mg/100 ml (SD) in the banded rats, 75.94 ± 1.55 mg/100 ml in sham-operated controls, and 75.57 ± 0.86 mg/100 ml in unoperated litter mates. These differences in water content are not statistically significant; therefore, the changes in cardiac weight following aortic banding are not secondary to changes in myocardial water

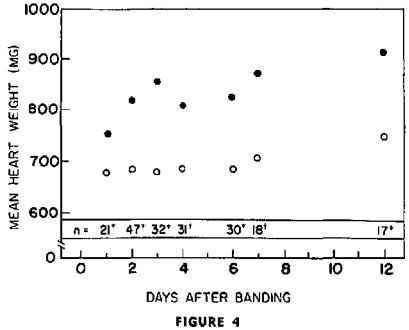

FIGURE 4

*Changes in heart weight following surgery in banded rats (solid circles) and in sham-operated rats (open circles). *$P < 0.005$; †$P < 0.05$.*

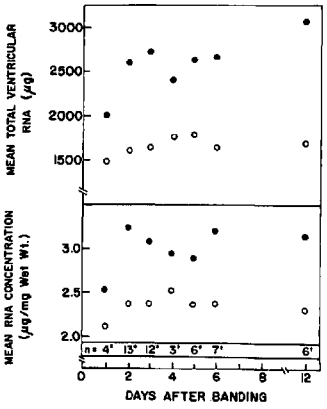

FIGURE 5

*Changes in RNA concentration and total ventricular RNA at various times after surgery in banded rats (solid circles) and sham-operated rats (open circles). *$P < 0.005$; †$P < 0.05$; x $P < 0.20$.*

content. The increases in heart weight correlate very well with increases in tissue nitrogen as estimated by the Kjeldahl micromethod.

Changes in RNA Concentration and Total Ventricular RNA.—The RNA content of the heart increases rapidly during the early phase of cardiac hypertrophy (11-22). An increase in both RNA concentration per milligram of wet weight and total ventricular RNA was observed within 24 hours after aortic banding (Fig. 5). Maximal increases in RNA concentration (30%) and total ventricular RNA (65%) were seen by the second day after the production of acute supravalvular aortic stenosis. The RNA concentration and total ventricular RNA reached a plateau value thereafter and remained elevated for at least 12 days (Fig. 5). During the first 2 weeks following aortic banding the increase in RNA concentration paralleled the increase in heart weight, as seen in Figure 6. Hearts with the greatest hypertrophy had the greatest increment in RNA content. The relationship between heart weight and RNA content is accentuated, of course, when total ventricular RNA is plotted against the percent increase in heart weight.

Four to 8 weeks after banding of the aorta, heart weight and total RNA content remained significantly greater than in controls. However, the heart weight did not increase progressively in this relatively small series of animals. The aortic stenosis might have been expected to pose a more severe hemodynamic problem with increased growth of the animal and thus lead to more extreme degrees of hypertrophy. Even more striking is the observation that there was only a small increase in RNA content. Total ventricular RNA remained minimally elevated 2 months after aortic banding, but the RNA concentration per milligram of wet weight remained at or even fell below control values (Fig. 7). In all rats the bands were intact at the time of death.

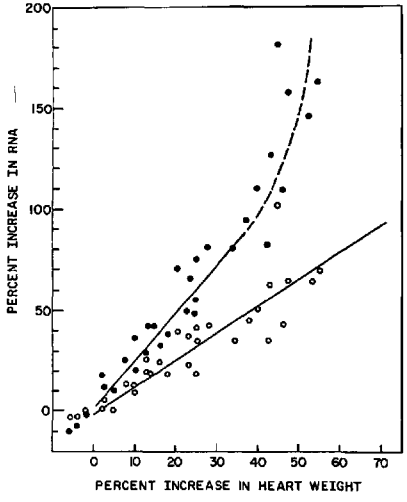

FIGURE 6

Relation between the percent increment in RNA concentration and in heart weight after aortic banding. Data are expressed as percent increase over sham-operated controls. Determinations were made 2 to 8 days after banding. Open circles = changes in RNA concentration and solid circles = changes in total ventricular RNA.

RNA Polymerase System.—The increase in myocardial RNA content following hypertrophy may be secondary to increased synthesis of RNA or to a decreased breakdown of the existing RNA, or possibly to a combination of the two. To evaluate the mechanism of the increase in RNA, the activity of the enzyme responsible for RNA synthesis, RNA polymerase, was studied. RNA polymerase activity may be assayed in intact nuclei isolated from tissues. In such preparations the enzyme is bound to the nuclear DNA. Under suitable conditions the RNA polymerase activity may be a good indicator of the physiological activity of the enzyme in vivo. A change in the activity of the enzyme may represent changes in the template activity of DNA rather than the amount of enzyme present. Since changes in the template activity of DNA may be one of the very early steps in the evolution of cardiac hypertrophy, a systematic evaluation of nuclear RNA polymerase activity and of its conditions of assay was carried out.

A method for the isolation of purified nuclear preparations from heart muscle according to the techniques used by Widnell and Tata (5) and Chauveau et al. (6) has been developed in our laboratory. The properties of the RNA polymerase system in nuclear preparations and the characterization of the product synthesized by the enzyme under conditions of low and high ionic strength have been previously published (4).

The requirements of the RNA polymerase system are shown in Table 1. All four nucleoside triphosphates are required for the reaction. Optimal incorporation occurs in the presence of SH groups and an energy-generating system. The addition of $(NH_4)_2SO_4$ at high ionic strength stimulates RNA synthesis. At an ammonium sulfate concentration of 300 μmoles/ml, 1.5- to 2-fold stimulation is regularly obtained at the end of 5 minutes of incubation at 37°C. It can be seen from Figure 2 that in system I, RNA synthesis reached a plateau after 5 minutes of incubation. Incorporation in system II continued

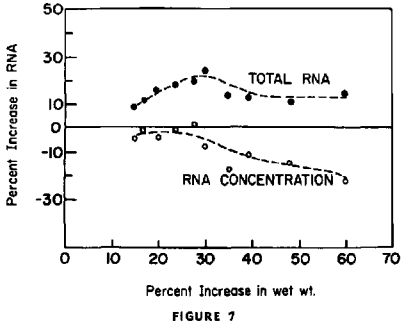

FIGURE 7

Relation between the increase in RNA content and increase in heart weight 4 to 8 weeks after aortic banding. The data are expressed as percent change from the value of sham-operated controls. Note the decline in RNA concentration during the late phase of cardiac hypertrophy.

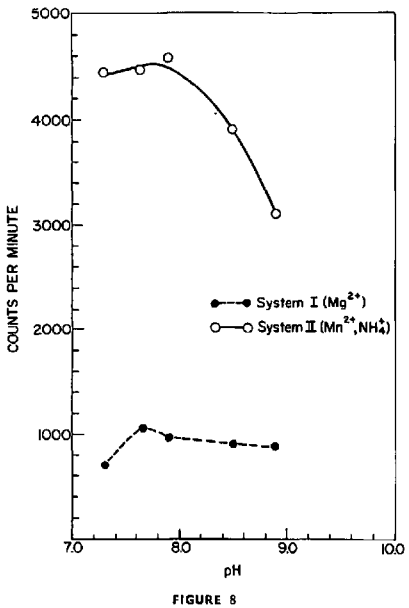

FIGURE 8

Effect of pH on RNA polymerase activity in assay systems I and II. Tris-HCl buffer was used throughout. Incubation was for 5 minutes at 37°C.

TABLE 1
Requirements for Assay of Nuclear RNA Polymerase Activity

	$\mu\mu$moles ^{3}H-CMP incorp./mg DNA/5 min	
	System I	System II
Complete system	195	280
Minus PEP and pyruvate kinase	105	196
Minus glutathione	92	235
Minus GTP	13	10
Plus 2 μg actinomycin D	42	62
Plus 10 μg actinomycin D	15	27

The compositions of the incubation mixtures used in system I (Mg^{2+}-activated reaction) and system II (Mn^{2+}, NH_4^+-activated reaction) are given in Methods.

almost linearly, so that at the end of 40 minutes an apparent 5-fold stimulation of RNA synthesis was present.

The stimulatory effect is sensitive to pH. The pH optima for the two systems are given in Figure 8. At pH values above 8.0, there was a sharp reduction in the incorporation of the radioactive label into RNA in system I. The concentration of the divalent cations is also critical; the optimal value for Mg^{2+} in system I is 2.5 μmoles/ml, and the optimal value for Mn^{2+} in system II, is 2 μmoles/ml. Finally, both systems are stimulated by addition of sulfhydryl groups in the form of glutathione.

Nearest Neighbor Frequency Analyses of RNA.—If the assays of RNA polymerase activity in hypertrophied hearts and in the controls are to be compared, it is necessary to be sure that the product of the enzyme reaction is identical in the two cases. In an earlier communication we have shown that at relatively low ionic strengths (system I), the RNA synthesized has the composition of ribosomal RNA or its precursor, whereas in the presence of high concentrations of $(NH_4)_2SO_4$ (system II) the RNA is similar to complementary RNA (DNA-like RNA) in its composition (4). Table 2 gives the percentage of 2′3′ nucleotide monophosphates obtained after alkaline hydrolysis of RNA synthesized by nuclear preparations from hypertrophied hearts and sham-operated controls.

These data show that when system I is used the RNA has a nearest neighbor frequency similar to ribosomal RNA, i.e. UMP is most often adjacent to GMP (over 30%); next in frequency it is adjacent to UMP itself, then to CMP and finally to AMP. In system II the nearest neighbor frequency pattern differs. Now UMP is most frequently adjacent to UMP itself, and then to GMP and CMP about equally. The same nearest neighbor frequency patterns are found in the RNA hydrolysates from the polymerase reactions with nuclei from hypertrophied hearts. Thus there is no significant difference in the products of the polymerase reactions and the validity of the assay system appears to be established.

Changes in RNA Polymerase Activity during Cardiac Hypertrophy.—Assays of RNA polymerase activity were carried out using nuclear preparations from the hearts of rats

TABLE 2
Nearest Neighbor Frequency Analysis

Assay system	% Total ^{32}P recovered in 2′, 3′ nucleoside monophosphate			
	AMP	GMP	UMP	CMP
I				
Control	12.1	32.7	28.4	26.8
Hypertrophy	12.4	30.8	33.5	23.3
II				
Control	18.3	24.8	31.0	25.9
Hypertrophy	18.4	24.2	33.6	23.8

The above data were obtained as follows: Nuclei were isolated from five rat hearts following aortic constriction for 6 days and from five sham-operated controls. Incubations were carried out in duplicate, using pooled nuclei, and the RNA product was hydrolyzed and assayed for base composition as described in Methods.

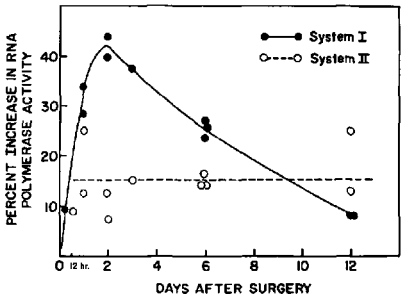

FIGURE 9

Time course of changes in nuclear RNA polymerase activity following aortic banding. Each point represents the result obtained from duplicate assays using nuclei from two pooled hearts. Data are expressed as percent changes over values obtained from sham-operated controls.

with ventricular hypertrophy and of their sham-operated litter mates. Assays were carried out in duplicate at 12 hours, at 1 to 7 days, and at 12 days after banding of the aorta. A detectable increase in enzyme activity was seen 12 hours after aortic constriction; maximal increases were obtained on the second day (system I, Fig. 9). A gradual decline in activity then occurred until at the end of a week there was a minimal difference between operated animals and sham-operated controls. With assay system II, there was a relatively slight (10 to 15%) increase in the incorporation of label into RNA using nuclear preparations from hypertrophied hearts; this increment was relatively constant from 12 hours to the seventh day.

It would appear from these results that one of the earliest changes in response to aortic banding is a rise in RNA polymerase activity of the nuclei. Whether this increase in activity is present only in the nuclei of connective tissue and vascular elements which proliferate during the early phase of cardiac hypertrophy or in both muscle nuclei and nuclei from other tissue is not known as yet. Attempts to fractionate nuclei according to their cellular origin have not yet been successful.

Thus, 12 hours following aortic banding, there is a demonstrable increase in the in vitro ability of isolated nuclei to synthesize RNA which has a predominant base composition similar to ribosomal RNA or its precursor. It could well be that intracellular changes occur more rapidly.

Discussion

Model System.—In this paper the experimental model of banding of the ascending aorta has been used for the study of cardiac hypertrophy. The method has been standardized and it offers certain advantages. First, the response to aortic banding occurs rapidly, with the result that maximal hypertrophy is present by the second or third postoperative day. Second, it is possible to carry out experiments with radioactive isotopes in the rat which may not be possible in larger animals because of the expense. A disadvantage of the method is that considerable variability in the degree of hypertrophy is obtained, and several animals must be used in each experimental set. Small changes in the degree of aortic constriction can impose significantly different work loads on the heart, with resulting variations in the synthesis of nucleic acids and proteins. Within 48 hours, cardiac weight increased by an average value of 30% and RNA content by about 65%. The heart weight, RNA content, and RNA concentration appeared to reach a plateau by the second or third day.

RNA Polymerase Activity.—The enzyme responsible for the synthesis of RNA is nuclear DNA-dependent RNA polymerase. Following aortic banding, the myocardium reacts swiftly with an increase in the activity of polymerase. This is one of the earliest biochemical manifestations of hypertrophy. The increase in activity may be due to one or more of the following processes: (a) increase in the rate of synthesis and in the content of RNA polymerase, (b) activation of the enzyme like that in the transformation from a dimeric to a monomeric state (24), (c) removal of an inhibitor, (d) stimulation by cytoplasmic factors (25), (e) derepression of the DNA

template according to the model suggested in bacterial systems by Jacob and Monod (26).

The increased activity of the enzyme in assay system I suggests that additional nuclear DNA sites may be made available for the transcription of RNA. Liao has shown that an extract having RNA polymerase activity can be obtained in soluble form from isolated nuclei in much larger amounts than would be predicted from the activity of intact nuclei (personal communication, Dr. Shutsung Liao). The enzyme therefore appears to be present in excess in nuclei. The availability of active DNA template rather than amount of enzyme probably limits RNA polymerase activity in the intact cell. We have previously shown that system I reflects mainly the synthesis of ribosomal RNA or its precursor. The product is the same in nuclei obtained from hypertrophied hearts. It seems likely that in hypertrophied hearts more DNA sites coding for ribosomal RNA become active templates for RNA synthesis.

In system II the high concentration of $(NH_4)_2SO_4$ unmasks the DNA template so that the product is complementary to DNA in its base composition. Only minimal increases in RNA polymerase activity are noted under these circumstances. A simple hypothesis is that the assay may reflect the amount of RNA polymerase present. If this is true, then the amount of enzyme is not the limiting factor in the process of cardiac hypertrophy since only a minimal increase is seen. However, this conclusion is tentative in view of the complexity of the RNA polymerase reaction at high ionic strength. For example, $(NH_4)_2SO_4$ inhibits nucleases; pH and ionic strength markedly alter template activity (27); and high ionic strength inhibits free RNA polymerase activity.

These results are consistent with the hypothesis that the mechanism of increased protein and nucleic acid synthesis in cardiac hypertrophy involves a repressor-derepressor system similar to that originally postulated by Jacob and Monod (26) and recently characterized in E. coli (28) and in the λ phage system (29). One may speculate that the physiological stimulus leads to the formation or accumulation of a metabolite that acts functionally as a derepressor. Segments of DNA are thereby made available for transcription by RNA polymerase. Enhanced synthesis of messenger, ribosomal and transfer RNA, and subsequently of proteins and other cellular components follows.

The order and time course of the changes in RNA polymerase activity observed after aortic banding are similar to those seen in the liver of rats after partial hepatectomy (30) or after treatment with triiodothyronine (31), or to the changes in the rat prostate gland following testosterone treatment (32). An increase in RNA concentration in the left ventricle is detectable as early as 24 hours following aortic banding. Increased RNA polymerase activity was found 12 hours after banding. The data is insufficient to state definitely that the increased activity of RNA polymerase in the in vitro nuclear system precedes the increment of myocardial RNA content. However, the data strongly suggests that the elevation of myocardial RNA content in vivo is a consequence of increased RNA synthesis in the cell.

Measurement of RNA synthesis with radioactive precursors confirm that the rates of ribosomal and RNA synthesis are increased by 3-4 fold following experimental aortic banding (T. Koide and M. Rabinowitz, unpublished results). Changes in the rate of RNA degradation after aortic banding have not yet been reported. Most of the newly synthesized RNA is ribosomal, but changes in messenger RNA and transfer RNA also occur (19, 33). In the early phases after banding, there is a rapid increase in RNA content (μg/mg wet weight) and total ventricular RNA. The extent of the increase depends on the intensity of the stimulus. The rapid rise in RNA soon levels off. With continued increase in heart weight there may be a "dilution" effect resulting in a lower than normal content per unit of tissue (mg wet weight). The increment in total cardiac RNA may also diminish.

TABLE 3

Summary of Changes in Heart Weight and RNA Content in Experimental Cardiac Hypertrophy

Reference	Mode of producing hypertrophy	Animal	Duration	% Change in heart wt	RNA Control values (μg/mg wet wt)	% Change in left ventricular RNA	
						Concentration (μg/mg wet wt)	Total
Nowy & Frings (11)	constrict ascending aorta	mature rabbit	6 months	+51%	3.22*	+6%	+60%
Rossi & Mor (22)	constrict ascending aorta	mature rabbit	8 da	not given	2.2*	+46%	not given
			4 mo			−10%	
Meerson & Ramenskaya (14)	constrict ascending aorta	mature rabbit	7 da	+75%	not given	+22%	+110%
			50 da	+50%		+5%	+57%
Gluck et al. (18)	constrict ascending aorta	puppy	8 da	not given	5.5*	+545%	not given
Kleitke & Sydow (23)	constrict ascending aorta	mature dog	1½-2 yr	+48%	0.7	−11%†	+31%†
Grimm et al. (20)	constrict abdominal aorta	mature rat	5-7 mo	+100%	4.09	−20%†	+55%†
Hoyle et al. (13)	diet-induced anemia	mature rat	120 da	+51%	2.63	0	+50%
Korecky & French (21)	diet-induced anemia	mature rat	120 da	+100%	2.00	0	+100%
Present series	constrict ascending aorta	mature rat	1-12 da	+30%	2.2	+30%	+65%
			4-8 wk	+40%		−12%	+12%

*Recalculated.

†Analyses done on total ventricular muscle.

The differences in published values for the RNA content of hypertrophied hearts probably reflect differences in the degree of hypertrophy and in the time interval between measurements and application of the stimulus. Values for changes in RNA concentration per milligram of tissue and total ventricular RNA in experimental cardiac hypertrophy reported by several workers are summarized in Table 3. In the studies of Gluck et al. (18) as well as in the present study, the increase in RNA concentration per milligram of net weight is striking because of the relatively acute nature of the work load and the early time periods of analysis. In late studies, in which myocardial RNA has been analyzed after a period of several months or even years (e.g., those of Kleitke et al. [23] or Grimm et al. [20]), a fall in RNA concentration is seen. In spite of the fall in RNA concentration, total ventricular RNA is still elevated.

The increased RNA content correlated well with the degree of cardiac hypertrophy in the early stages of the process. Animals with the largest increase in heart weight also had the most marked increment in RNA content. Since approximately 80% of cardiac RNA is ribosomal, a relationship between the number of ribosomes and rate of cardiac growth is apparent. This result is consistent with an observation of Moroz (34) that microsomes isolated from hypertrophied hearts several days after aortic banding synthesize proteins more actively than controls. The increased activity is largely due to an increased content of RNA and of ribosomes. Changes in polysome pattern could not be detected. In experiments using perfused rat hearts, however, changes in polysome profile and presumably in messenger RNA synthesis were observed after acutely increasing cardiac work (35). The increased protein synthesis therefore probably results from two factors: the synthesis of more ribosomes and the utilization of new messenger RNA by existing ribosomes.

The increased synthesis of RNA following aortic banding may be prevented by selective inhibitors of DNA-dependent RNA synthesis, for example by actinomycin D (36). Meerson et al. (37) and Zühlke et al. (38) have previously shown that actinomycin D blocks RNA and protein synthesis in animals with experimental aortic stenosis. Interpretation of these experiments is complicated by the generalized effect of actinomycin D, which may lead to anorexia and extreme weight loss (39). The reports suggest that inhibition of RNA synthesis or of protein synthesis in the early stages of cardiac hypertrophy may result in the development of cardiac failure. Studies with selective inhibitors of RNA and protein synthesis are now in progress in our laboratory. Our experimental model, which produces significant changes in RNA and protein synthesis within 24 hours, should be useful in separating the specific effects of inhibitors on the cardiac hypertrophy from nonspecific systemic effects of these agents.

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