# Changes in Ribonucleic Acid and Protein Synthesis during Induced Cardiac Hypertrophy*

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ABSTRACT: Cardiac hypertrophy was induced in rats by controlled constriction of the aorta. Increase in heart:body weight ratio was paralleled by an increase in RNA synthesis measured in isolated nuclei. Template activity of chromatin and changes in pattern of labeled proteins were studied at various stages of hypertrophy. At 2 days after constriction of the aorta (before there was any change in heart:body weight or RNA synthesis), chromatin activity was slightly increased and there were substantial changes in labeling of soluble but not of residue proteins. At 7 days (when heart:body weight and RNA synthesis were increasing), chromatin activity was substantially increased and there were changes in labeling of both soluble and residue proteins. At 15 days (when hypertrophy was essentially complete and RNA synthesis was apparently stabilized at a higher level), chromatin activity was only slightly above control and there were few changes in soluble proteins but substantial changes in residue proteins. We interpret these results to indicate that cardiac hypertrophy involves an extensive series of changes in the pattern of protein synthesis; in this respect it differs substantially from the testosterone-induced hypertrophy of skeletal muscle. The changes (and increase in RNA synthesis) can be attributed only in part to greater availability of genes to specify the synthesis of various kinds of RNA.

It is well established that hypertrophy of the left ventricle is induced when cardiac work load is increased by controlled constriction of the aorta (for review, see Meerson, 1969). There is general agreement that hypertrophy involves a substantial increase in RNA synthesis which leads to an increase in RNA content of hearts, but any new DNA synthesis is restricted to nonmuscular cells in the heart (Grimm *et al.*, 1970; Grove *et al.*, 1969a,b; Morkin and Ashford, 1968). Moroz (1967) found that the increased synthesis of protein observed in hypertrophy (Gudbjarnason *et al.*, 1964) could be attributed to increased ribosome content of the hearts, an observation in agreement with the general increase in RNA synthesis in isolated nuclei found by Nair *et al.* (1968) and the increased labeling of nucleoli (Rumyantsev, 1966). However, there is apparently no striking difference in the base composition or labeling pattern of RNA during cardiac hypertrophy (Nair *et al.*, 1968; Fanberg and Posner, 1968; Koide and Rabinowitz, 1969), so it seems unlikely that the increased RNA synthesis is restricted to ribosomal RNA.

We (Breuer and Florini, 1965; Florini and Breuer, 1966) have reported that testosterone and growth hormone have substantial effects on RNA and protein synthesis as measured in subcellular preparations from rat skeletal muscle. Consequently, we were very interested in the reports of Meerson *et al.* (1968), Beznak (1952), and Morkin *et al.* (1968) that castration and hypophysectomy delayed or prevented cardiac hypertrophy in response to aortic constriction. Although these studies did not include any demonstration that administration of testosterone or growth hormone restored the lost activity, it seemed possible that work-induced cardiac hypertrophy might be similar to the hormone-induced growth of skeletal muscle. Meerson (1969) suggested that the increased RNA synthesis in cardiac hypertrophy might result from gene activation, but apparently this suggestion has not been tested by experimental study of the template activity of chromatin. This report describes our characterization of early macromolecular changes observed when cardiac hypertrophy was induced by controlled constriction of the aorta.

## Materials and Methods

*Materials.* Male albino rats of a Sprague–Dawley derived strain were obtained from Russell Miller Farms, Cazenovia, N. Y. Animals were allowed at least 1 week to adapt to our quarters; aortic constrictions were performed on animals weighing 250–300 g. Food and water were provided *ad libitum* except in the 13-day hypertrophy experiments, which were done on a pair-feeding basis. Unlabeled nucleoside triphosphates were purchased from P-L Biochemicals, [$^3$H]UTP (11.0 or 17.8 Ci/mmole) from Schwarz BioResearch, salmon sperm DNA from Calbiochem, phosphocreatine from General Biochemicals, RNA polymerase (*Escherichia coli*) from Biopolymers, ampholites from LKB, NCS and phosphors from Amersham-Searle, and [$^{14}$C]leucine from New England Nuclear. [$^3$H]Leucine was prepared in our laboratory as described by Florini (1964). Acrylamide and bisacrylamide were purchased from Eastman; both were recrystallized shortly before use as described by Loening (1967).

*Aortic Constriction.* The technique for aortic constriction was derived from that described by Mallov and Alousi (1967). A rat was placed face up on the operating table and kept under anesthesia by placing a beaker of ether-soaked cotton near the nostrils. A midline incision 3 to 5 cm long was made from the clavicle downward, and intestines and spleen were pushed to the left to expose the left kidney, adrenal gland, and renal and adrenal veins. The abdominal aorta was

* From the Zoology Department and Biochemistry Committee, Syracuse University, Syracuse, New York 13210. *Received August 31, 1970.* This investigation was supported by a grant from the Heart Association of Upstate New York and Grant HE-11551 from the U. S. Public Health Service. Portions of this work are from a thesis submitted by Frances L. Dankberg in partial fulfillment of the requirements for the Ph.D. degree, Biochemistry Committee, Syracuse University, Syracuse, N. Y.

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exposed and freed of connective tissue on all sides at a location above the renal vein near the adrenal gland. Surgical thread was drawn under the aorta, a 1.0-mm (diameter) wire placed alongside the artery, and aorta and wire tied tightly together with the thread. The wire was removed, leaving the artery constricted to a diameter of 1.0 mm. Sham operations were done by identical procedures, except that no knot was tied in the thread. The abdominal muscle incision was stitched with surgical thread and the skin incision clamped with 9-mm Autoclips. With practice, the operation could be completed in 3–5 min with a survival rate of about 90%.

*Determination of Changes in Heart:Body Weight Ratio.* Hypertrophy is defined as an increase in weight of the heart independent of an increase in body weight; therefore it is measured as an increase in the ratio of heart weight to body weight. (For the purposes of this paper, heart weight refers to the wet weight of the ventricles.) The per cent change in this ratio in comparisons of rats with aortic constriction and corresponding sham-operated controls was calculated in the following manner. Animals were pair fed for the 13-day experiments so that weight gain was equal in both groups; for shorter experiments, animals were paired for comparisons on the basis of final body weight. The ratio of heart weight to body weight was calculated for each pair of animals in the experiment, and the per cent change in this ratio was calcutlaed for each pair.

*Isolation of Nuclei from Rat Hearts.* Nuclei were isolated by a procedure derived from that of Nair *et al.* (1967). Rats were killed under chloroform, hearts were rapidly removed and trimmed of atria and connective tissue, and the ventricles rinsed with water, weighed, and placed in ice-cold medium B (0.32 M sucrose–0.003 M $MgCl_2$). All subsequent operations were done at 4° unless otherwise specified. The ventricles were minced and homogenized in the Polytron Model 10 OD homogenizer (setting 3, 45 sec, followed by setting 8, 45 sec) in 3 volumes of medium B. The homogenate was filtered through one layer of flannelette and then made 0.25 M in sucrose by dilution with $H_2O$. The diluted homogenate was layered over medium B equal in volume to the original homogenate and centrifuged 10 min at 700*g* in a Sorvall RC2-B centrifuge. The supernatant was discarded and the pellet suspended in 2.4 M sucrose–0.001 M $MgCl_2$ by very mild homogenization (Polytron, setting 0.5, 30 sec); the volume of 2.4 M sucrose used for this homogenization was 3.8 times the original wet weight of the ventricles. The preparation was centrifuged at 65,000*g* 1.5 hr in the SB-283 rotor of an International B-60 ultracentrifuge. For this run, the temperature was maintained at 12° rather than the customary 4°; this increase in temperature decreased the viscosity of the sucrose and gave a substantial increase in yield of the nuclei with no apparent adverse effect on their purity or activity. The nuclear pellet was dissolved in 2–3 ml of medium B by very gentle homogenization with a Potter-type homogenizer.

The DNA content of each nuclear preparation was determined using the diphenylamine test as modified by Burton (1956), with salmon sperm DNA used as standard.

*Assay of RNA Synthesis in Isolated Nuclei.* The assay conditions were adapted from Nair *et al.* (1967). A final volume of 0.5 ml contained 50 μmoles of Tris-HCl, pH 8.5, 2.5 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol, 5 μmoles of creatine phosphate, a few crystals (about 50 μg) of creatine kinase, 3 μmoles of NaF, 0.4 μmole each of GTP, ATP, and CTP, 1.0 μCi of [$^3$H]UTP, and 0.1 ml of a suspension of heart nuclei containing 0.1–0.3 mg of DNA. The reaction was started by addition of the nuclei; incubations were 15 min at 37° without shaking. The reaction was terminated by addition of 5 ml of ice-cold 10% (w/v) trichloroacetic acid containing 0.1 M sodium pyrophosphate. Samples were allowed to stand several hours at 0°, and precipitates collected by centrifugation for 2 min at 2600 rpm in a Sorval GLC-1 tabletop centrifuge. The pellets were homogenized in 1% trichloroacetic acid using a ground-glass homogenizer. The precipitates were again removed by centrifugation, resuspended using a Vortex mixer and a conical spatula, and washed twice more with 5 ml of 1% trichloroacetic acid, 1 ml of ethanol–ether (3:1), and 1 ml of ether. The precipitates were air-dried and incubated overnight at room temperature with 0.2 ml of NCS to solubilize the RNA; the solution was washed into counting vials with 10 ml of toluene–phosphor containing 4 g/l. of 2,5-diphenyloxazole and 50 mg/l. of 1,4-bis-[2-(4-methyl-5-phenoloxazolyl)]. Radioactivity was determined as described below. Results were calculated as pmoles of UMP from [$^3$H]UTP incorporated into RNA per mg of DNA in the nuclei incubated; all incubations were in triplicate.

*Preparation and Assay of Chromatin.* Chromatin was prepared from ventricles of 3–5 rats by the procedure described by Baieve and Florini (1970), except that all homogenizations after initial homogenization of the tissue were done with minimum force; Polytron setting of 1 for 5 sec gave better yields of chromatin in the preparations from heart. DNA content of the chromatin was estimated from absorbancies at 260 and 280 nm, and these values were then used to establish dilutions for immediate assays of template activity of the chromatin preparations. Subsequent determinations of DNA content by the diphenylamine procedure (Burton, 1956) were done to establish the amounts of chromatin DNA actually present in the assay tubes. Template activity of chromatin was determined as described by Breuer and Florini (1966). In all cases, duplicate assays were done at each of three chromatin concentrations. Results were consistently linear with DNA concentration, demonstrating that chromatin was the limiting component of the reaction under our conditions. Results were calculated as μmoles of [$^3$H]UMP incorporated into RNA per μg of DNA incubated; each value thus represented the mean of six assays at three chromatin concentrations.

*Analyses of Proteins Labeled in Isolated Perfused Hearts.* Injection of radioactive amino acids in intact rats gave very little labeling of heart muscle proteins; apparently most of the labeled precursor was very rapidly removed from the circulation by other organs such as the liver and kidneys. Consequently, we utilized perfusion techniques to obtain useful levels of labeling of heart proteins. The apparatus and techniques were those described by Morgan *et al.* (1961). Hearts were perfused by a cannula inserted into the aorta; each heart was perfused with 100 ml of Krebs–Ringer buffer containing 180 mg% glucose and amino acids (except leucine) at three times the concentration normally found in rat plasma (Jefferson and Korner, 1967). Hearts from sham-operated rats were labeled with [$^3$H]leucine (3.3 μCi/ml) and those from rats with aortic constrictions with [$^{14}$C]leucine (1.0 μCi/ml); in both cases, total leucine content was adjusted to 40 pmoles/ml by appropriate addition of unlabeled leucine. Under these conditions, rat hearts continue to incorporate amino acids into protein for 3–4 hr (S. Geary, unpublished results); our preparations were incubated for 2 hr. At the end of the incubation, labeled hearts were blotted, washed briefly in unlabeled buffer, and ventricles from the two hearts mixed, minced, and homogenized in 3 ml of medium

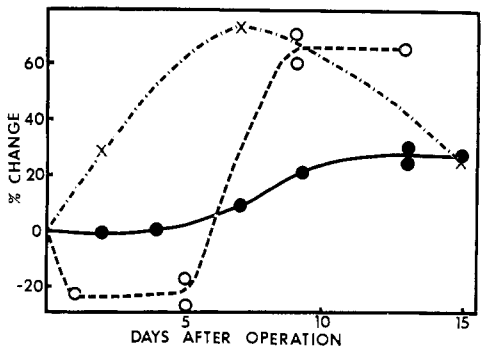


FIGURE 1: Time course of effects of aortic constriction in rat heart. Aortas were constricted and chromatin, nuclei, and ventricles isolated and assayed as described under Materials and Methods. The per cent changes were calculated as 100 times (constricted minus control)/control for each quantity; in all cases comparisons were made between preparations isolated and assayed strictly in parallel on the same days, as unavoidable variations in precursor specific activity, RNA polymerase preparations, etc., caused some changes in absolute activity from one preparation to another. Heart:body weight is designated by solid circles, RNA synthesis in isolated nuclei by open circles, and template activity of chromatin by X's.

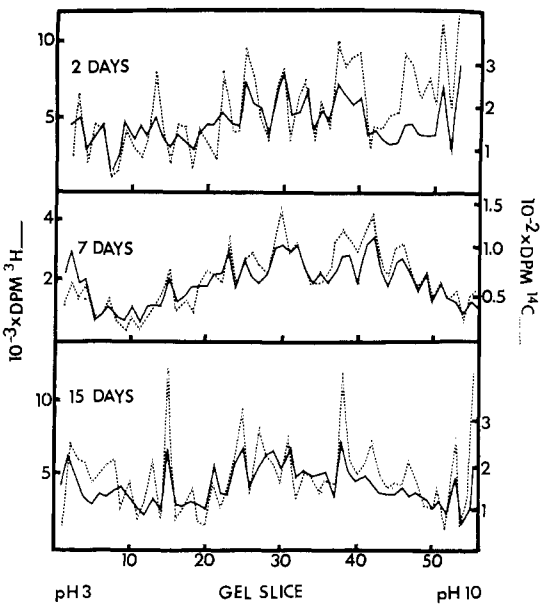


FIGURE 2: Pattern of labeling in soluble proteins in hearts at various times after aortic constriction. Proteins were labeled by perfusion of isolated hearts; labeled proteins were separated and analyzed by isoelectric focusing in polyacrylamide using ampholite range pH 3–10; details of these procedures are presented under Materials and Methods. Dotted lines indicate $^{14}C$ disintegrations per minute in proteins from hearts of animals with aortic constriction; solid lines indicate $^{3}H$ disintegration per minute in proteins from hearts of sham-operated animals. Points are omitted for clarity of presentation; radioactivity was determined in each numbered gel slice.

A (Florini and Breuer, 1966) using the Polytron homogenizer (setting #2, 30 sec). Proteins were separated and analyzed as described by Florini (1970); soluble proteins (147,000*g* supernatant) were separated by isoelectric focusing in 5% polyacrylamide gels containing 6 M urea and residue proteins (147,000*g* pellets solubilized in 9 M urea) by disc electrophoresis in 2.2% polyacrylamide gels containing 9 M urea. Isoelectric focusing of residue proteins was done in 2.6% polyacrylamide with all solutions containing 9 M urea; essentially this technique is a combination of the Catsimpoolis (1969) procedure for isoelectric focusing with our method (Florini and Brivio, 1969) for disc electrophoresis of myosin.

*Radioactivity Determinations.* In all cases, radioactivity was determined in a Nuclear-Chicago Unilux II three-channel liquid scintillation counter equipped with an external standard. Window and attenuator settings were arranged to exclude $^{3}H$ from the B channel; under the conditions of these experiments, $^{3}H$ was counted at approximately 30% and $^{14}C$ at approximately 65% efficiency. The counter was calibrated for variation of counting efficiency with external standard channels ratio over a wide range of counting efficiencies; quadratic equations were fitted to 10-point calibration curves for $^{3}H$ in the A channel and for $^{14}C$ in both the A and B channels. Data from each sample were analyzed to determine channels ratio, counting efficiencies, and disintegrations per minute for each isotope. A Programma 101 computer was used for these calculations.

## Results

*Changes in RNA Synthesis Following Aortic Constriction.* Figure 1 depicts the sequence of changes in template activity of chromatin, RNA synthesis by isolated nuclei, and heart:body weight ratio at various times after constriction of the abdominal aorta in normal male rats weighing 250–300 g. Our observations on activity of isolated nuclei are in essential agreement with those of Nair *et al.* (1968), although the procedure we used for aortic constriction resulted in a somewhat longer lag period before increases in nuclear activity or heart:body weight ratio were observed. Induction of hypertrophy by aortic constriction caused a substantial increase in template activity of chromatin soon after the operation; these increases occurred before there was any apparent increase in heart:body weight. Comparisons within Figure 1 show that template activity of chromatin increased before RNA synthesis in nuclei was stimulated, and template activity ultimately decreased while nuclear RNA synthesis remained elevated. These data indicate that the changes in observed RNA synthetic activity of nuclei can be attributed only partially to changes in availability of genes for transcription.

To investigate the suggestion (R. Chalkley, personal communication) that apparent differences in activity of chromatin preparations might be a trivial manifestation of differences in protease content of the preparations (Panyim *et al.*, 1968; Stellwagen *et al.*, 1968) which could increase the apparent template activity of chromatin by hydrolyzing proteins which block substantial portions of the DNA, we preincubated chromatin preparations under the conditions used for our assays of template activity. At the end of the 15-min preincubation period, RNA polymerase and nucleoside triphosphates were added and the incubation continued another 15 min. Under these conditions, there was no difference in effects on chromatin preparations from the sham-operated or aortic-constricted animals; preincubation caused a slight (10%) and consistent decrease in template activity of all the preparations. Preincubation experiments were done on chromatin showing a large difference (7 day) and a relatively small difference (15 day) between treated and control animals; in all cases the difference was not changed by the preincubation. Thus we are reasonably confident that the observed differences in template activity

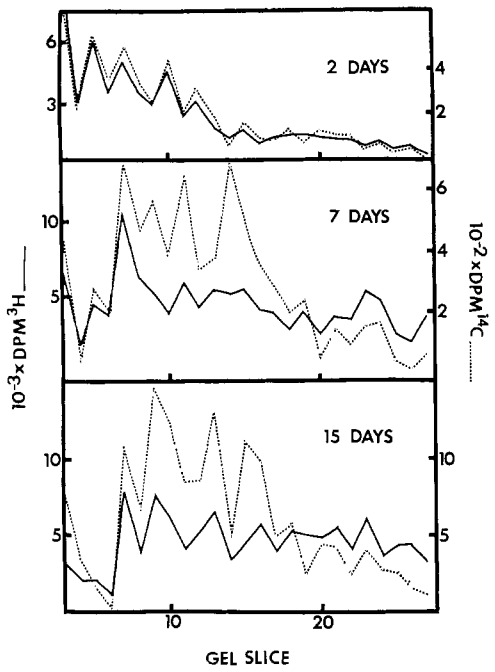


FIGURE 3: Analysis by disc electrophoresis of pattern of labeling in residue proteins in hearts at various times after aortic constriction. Proteins were labeled and analyzed as described under Figure 2, except that proteins were analyzed by disc electrophoresis in 2.2% polyacrylamide gels; all solutions contained 9 M urea.

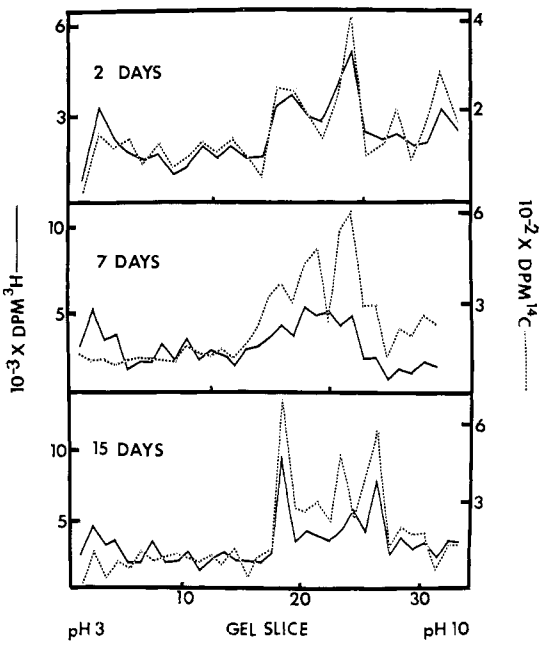


FIGURE 4: Analysis by isoelectric focusing of pattern of labeling in residue proteins in hearts at various times after aortic constriction. Proteins were labeled and analyzed as described under Figure 2, except that all solutions contained 9 M urea and the samples were subjected to isoelectric focusing in 2.6% polyacrylamide.

do not result from simple changes in protease content of the chromatin preparations.

*Effect of Aortic Constriction on Pattern Synthesis in Isolated Hearts.* The changes in template activity of chromatin observed during cardiac hypertrophy are similar to the increases in template activity of chromatin in skeletal muscle following testosterone administration (Breuer and Florini, 1966). In that case, the additional sequences made available for transcription were either highly redundant or were involved in control processes, as there was no detectable qualitative change in the proteins synthesized in muscle of castrated rats after administration of testosterone (Florini, 1970). A similar investigation of the proteins synthesized in cardiac hypertrophy gave drastically different results. Two days after constriction of the aorta, when there was no significant change in heart:body ratio or in nuclear RNA synthesis, and only a relatively small increase in template activity of chromatin, a striking change in the population of soluble proteins labeled in isolated perfused hearts was observed, as shown in Figure 2. The differences are particularly evident in proteins with isoelectric points in the range pH 7–10; both qualitative and relative quantitative differences are apparent. Similar comparisons 7 and 15 days after aortic constriction revealed less extensive differences in the population of proteins synthesized; at these times, substantial differences in template activity of chromatin, RNA synthesis in nuclei, and heart:body weight ratios were observed.

Analyses of the residue proteins—defined as proteins insoluble in dilute buffer but soluble in 9 M urea—revealed a different aspect of the process. In this case (Figure 3), there was little or no difference 2 days after aortic constriction, but very substantial differences 7 and 15 days after the operation. The greatest differences were observed at rates of migration approximately equal to those of the large subunits of myosin (Florini and Brivio, 1969), although quantitative limitations of the technique prevented inclusion of unlabeled myosin markers to establish unequivocally the identity of the proteins which showed such extensive differences 7 and 15 days after constriction of the aorta.

Disc electrophoresis is not completely satisfactory for analysis of residue proteins because there is substantial precipitation of proteins in a thin band at the top of the gel, where the sample is very highly concentrated at the beginning of the run. (The first slice in each gel is not plotted in Figure 3 because of the presence of this precipitate.) This introduces uncertainties in interpretation of the results, as the nature of the labeled proteins lost in the precipitate cannot be known with any certainty, although it does seem reasonable to assume that there would be no preferential loss of either $^{14}$C- or $^3$H-labeled proteins. To avoid any such uncertainties, we (Florini *et al.*, 1971) have devised methods for isoelectric focusing of residue proteins in 9 M urea in 2.6% gels. Results of analyses of the residue proteins by this technique are presented in Figure 4. These observations strongly reinforce the data presented in Figure 3; there was little change in residue proteins 2 days after constriction of the aorta, but substantial changes at 7 and 15 days. It should be emphasized that in isoelectric focusing, the protein sample is present homogeneously throughout the gel at the beginning of the run, so there is no loss of sample due to precipitation when the protein is concentrated as it is about to enter the column. Accordingly, we consider isoelectric focusing to be the preferable method for analysis of residue proteins.

## Discussion

In spite of obvious parallels in changes in RNA and protein synthesis, the growth of heart muscle stimulated by constriction of the aorta was strikingly different from the growth of skeletal muscle stimulated by testosterone. In

both cases, there were substantial increases in template activity of chromatin (compare Figure 1 and Breuer and Florini, 1966), but testosterone stimulated a general increase in synthesis of all proteins in skeletal muscle (Florini, 1970), whereas aortic constriction caused selective increases in labeling of certain proteins at specific times after the operation (Figures 2–4). The results presented in this paper indicate that increasing the work load of the heart caused synthesis of a number of new soluble proteins within 2 days of the operation (Figure 2), possibly by making additional genes available for transcription, but there was no general increase in RNA synthesis in isolated nuclei (Figure 1); the lack of hypertrophy at this early stage is consistent with the lack of change in synthesis of residue proteins—presumably at least partially myosin—at this time (Figures 3 and 4). At 7 days, when RNA synthesis and heart:body weight (Figure 1) were increasing, there was a major change in synthesis of residue proteins (Figures 3 and 4), a substantial increase in template activity of chromatin (Figure 1), and a lesser difference in the kinds of soluble proteins synthesized (Figure 2). Finally, at 15 days, when hypertrophy was essentially complete under our conditions, RNA synthesis and "myosin" formation were still elevated, although template activity was again approaching that of control hearts and there was little difference in synthesis of soluble proteins. Thus these results indicate a series of early "adaptive" changes in soluble heart proteins, followed by specific increases in synthesis of proteins associated with the contractile apparatus.

Evaluation of these suggestions obviously requires consideration of the validity of the techniques used in these experiments. It is often assumed but rarely demonstrated that isolated nuclei are useful indicators of the amount of RNA synthesized in the intact tissue; Roeder and Rutter (1970b) have recently reported the results of careful studies on developing sea urchin eggs; they conclude that isolated nuclei provide an accurate indication of the rate of RNA synthesis in the intact animal. Several groups (Paul and Gilmour, 1968; Bekhor *et al.*, 1969; Tan and Miyagi, 1970) have demonstrated that chromatin *in vitro* specifies RNA molecules indistinguishable from those synthesized in the intact animal, although the value of these observations is decreased by the inherent limitations of RNA–DNA hybridization studies in animal systems (Melli and Bishop, 1969). We have shown in this paper that the quantitative differences in activity of our chromatin preparations are not attributable to simple variations in destruction of chromosomal proteins by contaminating proteases during the assays. Technical necessity has forced us to assume that the same proteins are synthesized in heart muscle *in vivo* and during perfusion *in vitro*—or at least that hearts from treated and control rats generate identical artifacts during perfusion. The overall metabolic stability of hearts during perfusion, and the labeling of very large proteins with isoelectric points similar to that of myosin provide some reassurance; presumably the formation of a peptide chain the size of the large subunit of myosin (mol wt about 200,000) would represent a very substantial challenge to an *in vitro* system. Unfortunately, the technical limitations which forced adoption of the perfused heart system for these studies also preclude the obvious experiment to establish its validity; we have been unable to obtain sufficient labeling of heart proteins *in vivo* to allow detailed comparison with proteins labeled by perfusion *in vitro*.

Our results are generally consistent with published studies on induced cardiac hypertrophy. Like Nair *et al.* (1967, 1968), we find a substantial increase in RNA synthesis in isolated nuclei; this increase roughly parallels the increase in heart:body weight. In our studies, the descending aorta was constricted just above the renal artery; Nair *et al.* constricted the ascending aorta at a point nearer the heart. This latter technique gave more rapid increases in heart:body weight and RNA synthesis than occurred in our animals, but in other respects the systems seem similar. Constriction of the abdominal aorta may also decrease blood flow through the kidneys and adrenals; this could account in part for the longer lag period observed in this system. In addition, reduced perfusion of kidneys and adrenals may cause the apparent reduction in RNA synthesis in nuclei measured 1 and 5 days after constriction of the aorta (see Figure 1), although the significance of this small change (about 20% below sham-operated controls) is questionable. We originally chose to use the technique of Mallow and Alousi (1967) because it involves a relatively simple surgical procedure; the time course of hypertrophy we observed is quite similar to their results.

Meerson (1969) suggested that hypertrophy may involve increased activity of genes in the synthesis of RNA. Our studies on chromatin show that this increase accounts for only part of the increase in RNA synthesis. However, the available evidence (Nair *et al.*, 1967, 1968; Koide and Rabinowitz, 1969; Fanburg and Posner, 1968) reveals no apparent changes in the kinds of RNA synthesized during cardiac hypertrophy. The techniques used for those analyses (sucrose gradients, base composition, and nearest neighbors) would reveal only very substantial changes in the populations of RNA synthesized; we have seen no studies of RNA synthesis in hypertrophy utilizing disc electrophoresis, competitive hybridization, or column chromatography.

Moroz (1967) reported that the increase in protein synthesis in cardiac hypertrophy (Gudbjarnason *et al.*, 1964) could be attributed to increased ribosome content of the microsome fraction of cardiac muscle. This is generally consistent with the increased RNA content of cardiac muscle during hypertrophy (Koide and Rabinowitz, 1969) but a simple increase in ribosomes would not account for the substantial differences in protein profiles that we observed (Figures 2–4). We suggest that some additional kinds of mRNA are specified by the parts of the genome which become available for transcription shortly after constriction of the aorta; the resultant shift in kinds of soluble proteins synthesized (Figure 2) may account for the very substantial metabolic changes observed early in hypertrophy (Meerson, 1969). Possibly these represent the "early" proteins which Posner and Fanburg (1968) postulated as being required for the increase in RNA synthesis. Of course, the polymerase I fraction described by Roeder and Rutter (1970a) to be specifically involved in synthesis of ribosomal RNA is an obvious choice as an essential "early" protein, but thus far there is no information on the isoelectric behavior of the solubilized RNA polymerases from muscle, so we cannot identify the proteins which exhibit differential labeling in hypertrophy. In addition, the large increase in ribosome content of cardiac muscle during hypertrophy suggest that a portion of the DNA sequences which become available for transcription may specify the synthesis of ribosomal RNA.

## Acknowledgments

We are grateful to Dr. Samuel Mallov for training us in the techniques of aortic constriction and heart perfusion. We thank Stephen Geary for doing the heart perfusions.

The method used for isoelectric focusing of the double-labeled proteins was developed by Barbara Battelle and Rosaria Brivio. Careful technical assistance at all stages of this project was provided by Ruth Caverhill.

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