Ribonucleic Acid Synthesis in Experimental Cardiac Hypertrophy in Rats

I. CHARACTERIZATION AND KINETICS OF LABELING

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ABSTRACT

Cardiac hypertrophy was induced in rats by constriction of the aortic arch. Hearts from rats with aortic constriction increased in weight about 30% above those of control rats by 7 days after operation. RNA concentration increased between 24 and 48 hours after constriction and, thereafter, returned toward the control level. DNA concentration of hearts from rats with aortic constriction remained unchanged from that of sham-operated rats. Labeling of heart RNA with $^{32}$P was increased relative to that of sham-operated rats within the first 4 hours after aortic constriction. However, RNA labeling, relative to the labeling of AMP derived from ATP, increased above that of sham-operated rats only after 4 hours of aortic constriction and rose to maximum or near maximum values by 8 hours after operation.

Various methods of extraction of RNA showed that the increase in labeling involved all the major species of RNA (28S, 18S, and 4S), and no evidence was found for the selective stimulation of a minority species. The nucleotide base composition of the various species of heart RNA of rats with aortic constriction was not significantly different from that of normal rats during the 4- to 8-hour period after operation. There was no terminal labeling of sRNA in hearts of either normal animals or those with aortic constriction.

ADDITIONAL KEY WORDS: aortic constriction, extraction of heart muscle RNA, $^{32}$P labeling of RNA, AMP obtained from ATP, fractionation of RNA, heart muscle sRNA, base composition of RNA

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RNA synthesis during rapid growth has been examined in several mammalian tissues (1-8). The regenerating rat liver after partial hepatectomy has been studied in greatest detail (9-16). Although there have been several reports concerning changes in total RNA concentration in the hypertrophying heart (17-21), little attention has been directed to the early more specific changes in RNA synthesis during the process of hypertrophy. We have induced cardiac hypertrophy by constricting the aortic arch of rats and have characterized the kinetics and pattern of stimulation of RNA produced during the first several hours after constriction. These data provide a background for further studies on the regulation of RNA synthesis in the hypertrophying heart.

Methods

Animals.—Male albino rats obtained from the Charles River Laboratories were fed chow without restriction and used when 180 to 220 g in weight except where otherwise indicated.

Chemicals.—Phenol, 8-OH quinoline, bentonite, diphenylamine, and orcinol were purchased from the Fisher Scientific Company. Sodium dodecyl sulfate, apyrase (potato), yeast RNA (type XI), and calf thymus DNA were obtained from the Sigma Chemical Company. Carrier-free $[^{32}P]$...
H$_3$PO$_4$ was obtained from the New England Nuclear Corporation. AG 50W-X4 (400 mesh) in the H$^+$ form was purchased from Bio-Rad Laboratories.

**Operative Procedure.**—Animals were anesthetized with ether. A midline incision extending from the middle of the sternum into the neck was made, and the upper one-third to one-half of the sternum was split to expose the superior mediastium. The lobes of the thymus were separated, and the aorta was freed and raised into the incision. A needle was pressed against the aorta and 00 nylon thread was tied tightly around the aorta and needle just distal to the innominate artery to occlude the aorta. The needle was quickly withdrawn thus permitting the rapid re-establishment of circulation through the aorta. Sham-operated rats underwent the same procedure but no tie was made. An 18-gauge needle was ineffective in producing cardiac hypertrophy. A 19-gauge needle produced too high a mortality to be practical. A needle was therefore constructed midway in size between 18 and 19 gauge, with an external diameter of 1.170 mm, and this provided satisfactory aortic constriction. At appropriate times, rats were decapitated, and the hearts were rinsed and frozen between slabs of dry ice. The lobes of the thymus were separated, and the aorta was freed and raised into the incision. The sternum was split to expose the superior mediastinum, and the aorta was torn from the middle of the sternum into the neck was added only during the second phenol extraction phase was separated in a refrigerated centrifuge at 10,000 X g for 10 minutes and was treated with 10% NaCl and stored at 0 to 2°C for 48 hours.

**Preparation of Soluble RNA.**—RNA was extracted as above, except that SDS (at 0.5%) was added only during the second phenol extraction and no treatment with DNase was introduced. The RNA was purified by dissolving in 28 Na acetate and precipitating with 3.5 volumes of 95% ethanol. This procedure was repeated, and the final RNA precipitate was dissolved in 4 ml of 10% NaCl and stored at 0 to 2°C for 48 hours. The insoluble RNA was removed, and

A procedure adapted from that of Hatt (24) was used to remove the DNA extracted with the RNA. The white precipitate was dissolved in 3.0 ml of medium containing 0.01 M Tris (pH 7.2), 0.02 M MgSO$_4$, and 10 μg/ml DNase I (Worthington) and allowed to stand at room temperature for 15 minutes. Ice-cold distilled water (7 ml) was added, and the mixture was made 0.5% with respect to SDS. After the addition of 5 ml of 88% phenol, the mixture was shaken mechanically on ice for 15 minutes. The aqueous upper phase was separated in a refrigerated centrifuge at 10,000 X g for 10 minutes and was treated with sodium acetate and 95% ethanol, as before, to precipitate the nucleic acids.

RNA was freed of oligodeoxyribonucleotides by the following procedure. The precipitate was dissolved in 2 ml of 28 sodium acetate (pH 5.2), and an equal volume of 4 M potassium acetate was added. Ice-cold 50% ethanol was added in drops while mixing to a final concentration of 25%. The solution, usually cloudy at this point, was kept at —20°C for 30 minutes, and the precipitate, collected by centrifugation, was subjected to the above procedure a second time. The final precipitate was washed three times with 95% ethanol and then dissolved in an appropriate volume of 28 sodium acetate (pH 5.2).

This procedure removed not only oligodeoxyribonucleotides but also a variable amount of RNA with a sedimentation velocity of 4S. This when RNA is precipitated from 28 sodium acetate with final concentrations of ethanol > 25%, considerably more 4S material is seen on zone centrifugation of the final RNA specimen.
the supernatant was treated with 2.5 volumes of 95% ethanol. The precipitate which formed was washed with ethanol and dissolved in 2 ml of 2% Na acetate and 2 ml of 4 M K acetate was added. Ethanol was added to a concentration of 20%, and the mixture was allowed to stand at —20°C for 10 minutes. The precipitate which formed was removed by centrifugation. The supernatant was made 70% in ethanol and allowed to stand at —20°C for 30 minutes, resulting in a precipitate which we have referred to in Figure 7 as soluble (s) RNA.

Preparation of AMP from Tissue.—Frozen hearts were thawed and homogenized in ice-cold 0.3N HClO₄ (5 to 10 g homogenate). The homogenate was centrifuged at 0°C at 12,000 x g for 10 minutes, and the supernatant was brought to pH 6.5 with KOH; the KClO₄ precipitate was removed by filtration through a 0.45 μMillepore filter. The clear filtrate was made 0.1 M in Na succinate and 0.5% in CaCl₂; and after the addition of 0.5 mg/ml of potato apyrase, the solution was incubated for 20 minutes at 30°C, made 0.3N in HClO₄, and rapidly chilled. This procedure was adapted from that described by Krishnan (27).

The precipitate was removed by centrifugation at 1000 x g for 10 minutes, and about 2 ml of charcoal suspension (100 mg charcoal/ml of 0.1N HCl) was added to the clear supernatant. The mixture was shaken at room temperature for 10 minutes, and the charcoal was separated by centrifugation at 1000 x g for 10 minutes, washed once with distilled water, and eluted with 20 ml of ethanol:NH₄OH:water (66:33:1.5 by volume) by shaking at room temperature for 1 hour and filtering through a 0.45 μMillepore filter. The clear filtrate was dried in a vacuum desiccator, and the residue was dissolved in 2 ml of distilled water. AMP was isolated by passing the sample through a Dowex-1 formate column according to the technique of Bergkvist and Deutsch (28).

The adequacy of this fractionation method was checked with standards of nucleotide solutions, and the purity of the AMP peak was attested to by the constancy of the specific activity across the symmetrical peak eluted with 0.1 M formic acid. As a further check, the AMP was concentrated after elution and, when chromatographed in isobutyric acid:H₂O:cone. NH₄OH (66:33:1.5 by volume) (29), was found to migrate at the same rate as standard AMP. AMP prepared in this manner represents the AMP portion of tissue ATP since the amount of free AMP in muscle is negligible (30).

Charcoal (Norit A) was activated by the following treatment. It was suspended in 2N HCl (1 g/20 ml), and the suspension was degassed under vacuum. After the bubbling ceased, the charcoal was allowed to settle, and the supernatant was aspirated. This procedure was repeated, and the charcoal was suspended in H₂O (1 g/30 ml), stirred thoroughly, and allowed to settle. The supernatant was again aspirated, and the slurry was poured onto a Buchner funnel containing a Whatman no. 4 filter and dried with suction. The charcoal was then suspended in 0.1N HCl at a concentration of 100 mg/ml. This preparation was used to adsorb nucleotide. The efficiency of recovery of adsorbed nucleotide from charcoal was about 70% and represented the only part of the procedure where significant loss of nucleotide occurred.

Analysis of RNA by Sucrose Density Gradient.—The method of Britten and Roberts (31) as adapted by Munro et al. (32) was used. RNA, 150 to 250 μg in 0.2 to 0.3 ml of 2% sodium acetate, was applied to a 5 to 20% sucrose gradient, the sucrose for this gradient having been dissolved in 2% sodium acetate (pH 5.2). The material was centrifuged at 39,000 rpm for 3 hours in a SW-25 Spinco rotor, then the bottom of the tube was punctured with a no. 22 needle, and sequential samples were collected in tubes containing distilled water.

Base Analysis of RNA.—Each animal with aortic constriction was injected with approximately 1 μc of [³³P] H₃PO₄ at 4 hours after operation and 4 hours later was killed. Normal control animals were labeled for a period of 4 hours before they were killed. Hearts were immediately frozen on dry ice, extraction and purification of RNA was carried out on the combined hearts of each group as described above. The pooled RNA from each group of animals was fractionated on a 5 to 20% sucrose gradient. An aliquot from each tube was taken for measurement of optical density and counting, and the gradient was subdivided into fractions noted in Table 3. Tubes of each fraction were pooled, and 300 to 500 μg of yeast RNA was added as a carrier. RNA of the fraction was then precipitated on ice by making the aliquot 3% in trichloroacetic acid. The precipitate was separated by centrifugation, washed twice with ether, and dried. Hydrolysis was carried out in 0.4 ml of 0.3N KOH for approximately 1.6 hours at 20°C. After the addition of 0.5 ml of 0.25 M NH₄OH formate (pH 4.1), the pH was adjusted with perchloric acid to 4.1 to facilitate handling. The KClO₄ precipitate was removed, and the sample was applied to an AG 50W-X4 (400 mesh) column in the H⁺ form. Separation of the 4 nucleotides was carried out as described by Blattner and Erickson (33) with the use of 0.25 M NH₄OH formate (pH 4.1) for elution. The eluate was collected in fractions and counted as described below. The counts in each of the four nucleotides were added together and, for each nucleotide, were expressed as percent of the
sum of the counts of the 4 nucleotides. Reproducible separation and distribution of nucleotides was demonstrated by repeated runs of the same sample.

**Measurement and Radioactivity of RNA, AMP, and Nucleotides after Hydrolysis of RNA.**—RNA from the gradient samples and AMP from the Dowex-1 formate column were measured by determining optical density at 260 nm in a Beckman DB spectrophotometer. RNA, AMP, and fractionated nucleotide solutions were counted in vials containing 5% naphthalene, 0.7% 2,5-diphenyloxazole (PPO), and 0.005% 1,4-bis(5-phenyloxazoyl) benzene (POPOP) in xylene in a Packard tri-carb liquid scintillation counter. The volumes of fluid used produced no significant quenching of the counts. The efficiency of counting was better than 95% for 3P.

Specific activity for total RNA was determined by dividing the sum of the radioactive counts by the sum of optical densities of the entire gradient. Specific activity for 28S RNA was obtained by dividing the sum of the counts by the sum of the optical densities at the peak-points of the 28S peak.

**Measurement of Specific Activity of Inorganic Phosphorus in the Heart after Labeling with [32P]H3PO4.**—Hearts labeled, as described above, with [32P]H3PO4 were homogenized (5 to 10%) in 0.3N perchloric acid. After removal of nucleotides with charcoal as described above, inorganic phosphorus was precipitated by adjusting the pH of the extract to 8.0 with 0.3N Ba(OH)2. The precipitate was washed several times with H2O, and dissolved in 0.1N HCl. Inorganic phosphorus was determined by the Fiske and Subbarow method (34), and an aliquot was taken for determination of radioactivity.

**Results**

**Effect of Constriction on Heart Weight**

Changes in heart weight after aortic constriction and sham operation are illustrated in Figure 1. During the first 4 days after aortic constriction, there was an increase in body weight with no change in heart weight for control rats and the heart weight/100 g body weight ratio decreased to a greater extent than during the remainder of the period studied. In rats with aortic constriction there was little increase in this ratio during the first 4 days, while from 5 to 7 days after operation there was an abrupt rise in heart weight/100 g body weight. For the first 4 days following operation, both the lack of change in heart weight/100 g body weight of rats with aortic constriction and the more rapid decrease in heart weight/100 g body weight of sham-operated rats probably resulted from postoperative catabolism. The process of hypertrophy appeared to begin from the first day as indicated by the more linear plot of percent increase above values of heart weight/100 g body weight in sham-operated rats (Fig. 1). A new steady state is reached for both groups 7 days after operation and is characterized by a constant decline in the heart weight-body weight ratio during the ensuing three weeks of observation. A similar gradual decline in heart weight relative to body weight as animals increase in size has been reported by Grimm et al. (21).
RNA SYNTHESIS IN THE HYPERTROPHING HEART

Changes in total and concentration of heart RNA and DNA in rats with aortic constriction. RNA and DNA of pooled groups of hearts were measured as indicated in Methods. Total RNA and DNA were determined by multiplying concentration of RNA or DNA by the average heart weight of the group. The number of animals at each time is noted at the bottom of the figure. Open circles = hearts from rats with constricted aortas; X = hearts from sham-operated rats.

**COMPARISON OF RNA AND DNA CONCENTRATION AND CONTENT IN RATS WITH AORTIC CONSTRICTION OR SHAM OPERATION**

RNA concentration in hearts of rats with aortic constriction rose abruptly between the first and second day after aortic constriction and remained approximately 30% above that of hearts from sham-operated rats during the first 7 postoperative days (Fig. 2). After the first 7 days, during which time there was a rapid increase in heart weights of rats with aortic constriction (Fig. 1), RNA concentrations in the two groups again approximated one another. Total RNA content per heart of sham-operated rats remained quite constant during the 28-day postoperative period. In contrast, there was a 100% increase in RNA per heart in animals with aortic constriction during the first 7 postoperative days, after which the rate of accumulation decreased, and the total amount per heart remained relatively constant over the next 21 days.

DNA concentration per heart remained approximately the same for hearts from both rats with aortic constriction and sham-operated rats and decreased gradually during the 28-day period. Total DNA per heart, however, increased about 40%, similar to the increase in heart weight, during the first 7 days after aortic constriction. The increase was both less abrupt and less extensive than that for RNA. After the first 7 days, total DNA content per heart from rats with aortic constriction remained constantly elevated above that from the heart of sham-operated rats.

**TIME COURSE OF AMP LABELING**

As shown in Figure 3, there was a linear increase in the labeling of AMP for at least 8 hours following the introduction of [3H]AMP.
TABLE 1
Labeling of Inorganic Phosphorus of Hearts of Rats with Aortic Constriction and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Specific activity of Pi (counts/mi Constricted</th>
<th>Specific activity of Pi (counts/mi Sham)</th>
<th>Ratio of specific activity (Constricted/Sham)</th>
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<tbody>
<tr>
<td>2 hours</td>
<td>346,000</td>
<td>238,000</td>
<td>1.48</td>
</tr>
<tr>
<td>24 hours</td>
<td>278,000</td>
<td>214,000</td>
<td>1.3</td>
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<tr>
<td>7 days</td>
<td>392,000</td>
<td>309,000</td>
<td>1.28</td>
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</table>

Groups of 3 to 5 animals were injected with 2 μCi [14C]H3PO4 at the denoted times after operation. Four hours later, the rats were killed, and the hearts were pooled and extracted for inorganic phosphorus as noted in Methods.

TABLE 2
Time Course of Labeling of RNA

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Exp. no.</th>
<th>Type of operation</th>
<th>No. animals in group</th>
<th>Specific activity of 28S RNA (counts/min/OD)</th>
<th>Specific activity of total RNA (counts/min/OD)</th>
<th>Specific activity of AMP (counts/min/OD)</th>
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<td>5 minutes</td>
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<td>C</td>
<td>4</td>
<td>556</td>
<td>747</td>
<td>4650</td>
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<td></td>
<td></td>
<td>S</td>
<td>4</td>
<td>396</td>
<td>431</td>
<td>3160</td>
</tr>
<tr>
<td>2 hours</td>
<td>1</td>
<td>C</td>
<td>5</td>
<td>860</td>
<td>1090</td>
<td>3740</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>4</td>
<td>450</td>
<td>715</td>
<td>2850</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>4</td>
<td>660</td>
<td>930</td>
<td>3120</td>
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<td></td>
<td>S</td>
<td>3</td>
<td>350</td>
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<td>2030</td>
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<td>4 hours</td>
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<td>5</td>
<td>1172</td>
<td>1590</td>
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<td></td>
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<td>435</td>
<td>2500</td>
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<td>4</td>
<td>960</td>
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<td>335</td>
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<td>4</td>
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<tr>
<td>20 hours</td>
<td>1</td>
<td>C</td>
<td>3</td>
<td>1120</td>
<td>1061</td>
<td>3800</td>
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<td>S</td>
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<td>48 hours</td>
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<td>3</td>
<td>1040</td>
<td>972</td>
<td>3740</td>
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<td>S</td>
<td>3</td>
<td>390</td>
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<tr>
<td></td>
<td>2</td>
<td>C</td>
<td>3</td>
<td>1170</td>
<td>1593</td>
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<td></td>
<td>S</td>
<td>3</td>
<td>550</td>
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<td>7 days</td>
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<td>C</td>
<td>3</td>
<td>1485</td>
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<td>8300</td>
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<td>S</td>
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<td>544</td>
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<td>3</td>
<td>690</td>
<td>710</td>
<td>4630</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>3</td>
<td>385</td>
<td>487</td>
<td>2940</td>
</tr>
</tbody>
</table>

Animals underwent either constriction of the aorta (C) or sham operation (S). At postoperative times noted in the Table, each animal was injected with 2 μCi/g body weight of [14C]H3PO4 at 4 hours later and killed. Hearts from each group of animals were pooled and extracted for RNA and AMP. RNA was fractionated and analyzed as noted in Methods. Specific activity was obtained by dividing the sum of counts/min by the sum of optical densities for either total RNA or for the three highest points of the 28S RNA peak of the gradient. AMP, obtained from an aliquot of the corresponding group of hearts used for RNA determination, was analyzed as noted in Methods.
RNA SYNTHESIS IN THE HYPERTROPHYING HEART

H₈PO₄. The extent of labeling leveled off by 12 hours and began to fall between 16 and 20 hours. It should be noted that the specific activities at 4 hours in sham-operated rats are very close to normal values. In contrast, the specific activities of AMP from all but one of the rats with aortic constriction are above the values from normal and sham-operated rats. The higher specific activity of AMP from the hearts of these animals is probably due in great part to the higher specific activity of inorganic phosphorus in hearts from rats with aortic constriction as compared to hearts from sham-operated rats (Table 1).

TIME COURSE OF STIMULATION OF RNA LABELING AFTER AORTIC CONSTRICTION

To determine the kinetics of RNA labeling after aortic constriction, labeling of RNA from hearts of rats with aortic constriction was compared with that from hearts of sham-operated rats at various times after operation. A 4-hour labeling period was chosen to obtain sufficient labeling of RNA and to discern labeling of specific species of RNA. Specific activity of the 28S fraction was measured as an index of ribosomal RNA labeling. The results of the study which include both RNA and AMP labeling from the same group of hearts are recorded in Table 2.

Stimulation of RNA labeling following aortic constriction increased very rapidly within the first few hours (Fig. 4). Total RNA and 28S RNA are depicted in the figure, but the rate of rise in stimulation is about the same for all species of RNA indicating no early stimulation of a particular RNA species. Without correction for AMP specific activity, an increase in stimulation was seen within the first 4 hours after operation. This reached a maximum during the labeling period from 4 to 8 hours after which there was a gradual decline in stimulation during the next 7 days of the study. When a correction was made for AMP specific activity, there was a lag period of about 4 hours after constriction before the stimulation of RNA labeling occurred. (The percent stimulation with AMP correction was calculated by subtracting the RNA-AMP ratio of sham-operated animals from that of animals with aortic constriction and then dividing the result by the RNA-AMP ratio of sham-operated animals and multiplying by 100. The units for RNA and AMP are counts/min/OD.) A lag period of comparable duration appeared to obtain if inorganic phosphorus specific activity was used to correct RNA specific activity. The increased labeling in the first 4 hours may be due to increased nucleotide labeling and not actual stimulation of RNA synthesis. Except for the lag in time of onset, the same general pattern of stimulation was seen, with a maximum at 4 to 8 hours, when corrections are made for AMP.

PATTERN OF RNA LABELED DURING THE 4-TO-8-HOUR INTERVAL AFTER CONSTRICTION

Figures 5 and 6 demonstrate the fractionalization of RNA labeled during the period 4 to
Patterns of heart RNA labeled from 4 to 8 hours after operation and extracted without SDS in rats with aortic constriction and sham-operated rats. A total of 7 rats with aortic constriction and 8 rats with sham operation were injected with 2 μg/g body weight of \[^{32}P\]HPO₄ at 4 hours after operation and were killed 4 hours later. Each of these two groups of hearts was cut up and subdivided into two groups, one of which was extracted for RNA in the absence and the other in the presence of SDS as noted in Methods. The RNA was purified and analyzed by sucrose gradient fractionation. Specific activities were calculated by dividing the counts in each fraction by the OD of that fraction. Solid circles = optical density; open circles, connected by dashed line = counts/min; open triangles = specific activity.

8 hours after operation, RNA from the hearts separated into three distinct peaks on a 5 to 20% sucrose density gradient. We have previously demonstrated that these correspond in sedimentation characteristics to the major RNA species from liver (35).

When RNA was extracted without SDS, specific activities remained constant for the heavier species of RNA (28S and >18S), while specific activities of the lighter species (<18S) were considerably higher. The specific activity of RNA across the entire gradient was about 90% higher for hearts from rats with aortic constriction than for those from sham-operated rats (Fig. 5).

When RNA was extracted in the presence of SDS, a different labeling pattern was seen (Fig. 6). The point of lowest specific activity was at the 28S peak. The >28S and <18S species had comparable high specific activities. The specific activity of the 28S area was similar to that of RNA extracted without SDS. There was no increase in total RNA specific activity with SDS. The specific activity of total RNA of sham-operated rats

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RNA SYNTHESIS IN THE HYPERTROPHYING HEART

was 677 counts/min/OD<sub>290</sub> without SDS and 633 counts/min/OD<sub>290</sub> with SDS; that of rats with aortic constriction was 1298 counts/min/OD<sub>290</sub> without SDS and 1080 counts/min/OD<sub>290</sub> with SDS. All species of RNA from hearts of rats with aortic constriction extracted with SDS had specific activities about 70 to 80% higher than those from hearts of sham-operated rats.

For a comparison of heart RNA from both groups of animals during a period of labeling shorter than 4 hours, a higher dose of <sup>32</sup>P<sub>H</sub>P<sub>4</sub> was given at 7 hours after operation, and the rats were killed after a labeling period of 1 hour. As demonstrated in Figure 7, the heterogenous species of RNA labeled during this period were stimulated consequent to constriction.

EFFECT OF HOT PHENOL EXTRACTION ON RNA LABELING

Hot phenol has been shown to effect a more complete release of RNA from some mammalian tissues (36-39). Extraction of heart muscle RNA with phenol at 65°C yielded RNA of higher specific activity than extraction with phenol at 0°C. The constriction-sham RNA specific activity ratio was similar for both types of extraction (1.9 at 0°C and 2.05 at 65°C) indicating that hot phenol (65°C) extraction did not release species of RNA which might have been selectively stimulated consequent to aortic constriction.

Labeling of Soluble RNA

Soluble RNA, prepared as noted in Methods, separated on a sucrose gradient as a symmetrical peak in the 4S area (Fig. 8). Soluble RNA prepared in this manner was labeled to about the same extent as 28S RNA. Following aortic constriction, it was stimulated to the same extent as 28S RNA. The specific activity of soluble RNA increased 67% as compared to an increase of 60% for that of 28S RNA during a 4- to 8-hour postoperative period. Material with a higher specific activity than that of soluble RNA was present in the 4S area on sucrose gradient analysis of both the 10% NaCl insoluble RNA fraction and the small fraction which was insoluble in K acetate-25% ethanol (see Methods). This probably represents breakdown products from more highly labeled, heavier, RNA (4). Without additional purification of the 4S material, the impression would be gained that soluble RNA is more highly labeled than 28S RNA.

Base Analysis of Heart RNA

RNA labeled in the 4- to 8-hour period after constriction was analyzed for nucleo-
Comparison of Base Composition of RNA from Hearts of Normal Rats and Rats with Aortic Constriction

<table>
<thead>
<tr>
<th>RNA fraction</th>
<th>Type of animal</th>
<th>CMP</th>
<th>AMP</th>
<th>GMP</th>
<th>UMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;28S</td>
<td>N</td>
<td>21.8 ± 2.7</td>
<td>19.3 ± 1.8</td>
<td>29.4 ± 0.5</td>
<td>29.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>32.5 ± 2.9</td>
<td>14.6 ± 1.8</td>
<td>28.1 ± 1.3</td>
<td>32.0 ± 1.7</td>
</tr>
<tr>
<td>28S</td>
<td>N</td>
<td>22.6 ± 2.5</td>
<td>15.5 ± 0.5</td>
<td>31.2 ± 1.4</td>
<td>30.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>32.9 ± 3.2</td>
<td>13.4 ± 0.9</td>
<td>30.9 ± 0.6</td>
<td>31.0 ± 1.9</td>
</tr>
<tr>
<td>18S</td>
<td>N</td>
<td>18.5 ± 2.4</td>
<td>21.8 ± 2.6</td>
<td>25.5 ± 2.8</td>
<td>34.2 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>21.9 ± 3.0</td>
<td>18.1 ± 2.4</td>
<td>20.1 ± 0.4</td>
<td>33.7 ± 3.4</td>
</tr>
<tr>
<td>4-18S</td>
<td>N</td>
<td>17.5 ± 1.9</td>
<td>19.8 ± 0.7</td>
<td>26.9 ± 1.5</td>
<td>30.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>19.7 ± 2.2</td>
<td>20.3 ± 3.1</td>
<td>22.5 ± 1.0</td>
<td>37.4 ± 3.9</td>
</tr>
<tr>
<td>4S</td>
<td>N</td>
<td>17.5 ± 1.7</td>
<td>19.0 ± 0.1</td>
<td>23.3 ± 1.5</td>
<td>38.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>21.8 ± 2.3</td>
<td>20.2 ± 0.7</td>
<td>22.0 ± 2.6</td>
<td>30.3 ± 4.8</td>
</tr>
<tr>
<td>sRNA</td>
<td>N</td>
<td>24.5</td>
<td>13.8</td>
<td>24.4</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>20.3</td>
<td>17.9</td>
<td>27.0</td>
<td>34.6</td>
</tr>
</tbody>
</table>

Base analysis of RNA was carried out as noted in Methods. Three sets containing 4 to 6 rats in each set were used for base analyses where standard errors are calculated. (N = normal rats, C = rats with aortic constriction.)

RNA synthesis in the heart following aortic constriction, as in regenerating liver after partial hepatectomy (11), increases soon after the operative procedure. In the present studies, the increased RNA labeling within 4 hours of aortic constriction may reflect an increase in specific activity of the precursor as estimated by the specific activity of AMP derived from tissue ATP. The increased specific activity of AMP in this early period appears to be determined, in large part, by the increase in specific activity of inorganic phosphorus (Table 1). A comparable approach to precursor specific activity has been used by others (4). The adequacy with which this determination reflects precursor specific activity depends upon the degree of heterogeneity of cellular ATP as well as the extent to which the specific activity of AMP from ATP compares to that of the other nucleoside monophosphates derived from their corresponding nucleoside triphosphates. Subsequent to the first 4 hours, the increase in RNA labeling can be only partially accounted for by an increase in AMP labeling. Since RNA labeling is maximally stimulated 4 to 8 hours after constriction, it was of particular interest to examine the pattern of labeling of RNA in this period. RNA was
extracted with and without SDS and analyzed by sucrose gradient centrifugation. Though the specific activity of total RNA extracted with SDS is no higher than that extracted without SDS, the pattern of labeling differs. The presence of SDS results in the recovery of highly labeled, heavy (>28S) RNA. In the absence of SDS, relatively more radioactivity is associated with the lighter RNA species. It is possible that SDS facilitates the extraction of a heavier more highly labeled species (>28S) which may be nuclear in location (36, 37) or that it preserves from degradation to smaller molecules those species which are ordinarily easily extracted. With constriction, there is a generalized stimulation of RNA labeling throughout the gradient both in the presence and absence of SDS. Extraction with hot phenol, though increasing the specific activity of the RNA recovered, does not demonstrate a more remarkable stimulation of RNA labeling consequent to constriction.

The 4S RNA obtained after phenol extraction and sucrose density fractionation is heterogeneous, probably due to contamination of sRNA by breakdown products of heavier species (4). Thus, sRNA was isolated with the use of 10% NaCl. A further purification step (i.e., precipitation of contaminants from 2 M K acetate by 25% ethanol) was found to produce a more uniformly labeled material, whose specific activity closely compares to ribosomal RNA. As in the rat prostate (4), there is no evidence for terminal labeling of sRNA from either normal or hypertrophying hearts.

Subsequent to aortic constriction, there is a stimulation of labeling of all RNA species including ribosomal RNA. Generalized stimulation of RNA synthesis has been demonstrated in regenerating liver (16), in the liver of adrenalectomized animals stimulated with cortisol (4, 5, 16), in the seminal vesicles of castrated rats stimulated with testosterone (4, 5), and in the uterus of ovariectomized animals treated with estrogen (1). Our observations are fully in accord with the observations of Muro (40) who concluded that a major factor determining increased protein synthesis, leading to cardiac hypertrophy, is an early increase in the number of ribosomes in the hypertrophying heart muscle.

A qualitative change in RNA, if large enough, could conceivably be observed as a change in base composition. Indeed, in a recent study, Meerson and colleagues demonstrated an alteration in hypertrophying hearts of the base composition of total RNA determined chemically (41). In our system the base composition of RNA labeled during the 4- to 8-hour interval after constriction shows little difference from that obtained from nonhypertrophying hearts. Thus, we have been unable to demonstrate a qualitative change in any major species of RNA stimulated subsequent to aortic constriction. The possibility of a minor alteration in the character of messenger RNA cannot be excluded until more sensitive methods for isolation and study of messenger RNA are available.

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