Biochemical Correlates of Cardiac Hypertrophy

II. INCREASED RATE OF RNA SYNTHESIS IN EXPERIMENTAL CARDIAC HYPERTROPHY IN THE RAT

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ABSTRACT

Cardiac hypertrophy was produced in rats by banding the ascending aorta. Increases in heart weight and RNA content occurred within 48 hours, indicating that hypertrophy develops rapidly. The total RNA content and the specific radioactivity of RNA labeled with $^3$H-uridine were determined. The rate of RNA synthesis was estimated from these data and from the specific radioactivity of the myocardial uridine nucleotide pool. The rate of RNA synthesis in hearts of banded animals averaged four times greater than controls on the second postoperative day and about two to three times greater on the fourth to fifth postoperative days. An expansion of the myocardial uridine nucleotide pool was observed in banded rats, but the size of the myocardial adenosine nucleotide pool was not changed. No changes in base composition of 28S, 18S, or 4S RNA were observed following cardiac hypertrophy.

ADDITIONAL KEY WORDS

myocardial nucleotides
uridine nucleotide pool
experimental aortic constriction
base composition of RNA

Following the production of cardiac hypertrophy there are parallel increases in heart weight and protein content (1-5). In experimental cardiac hypertrophy enhancement of the rate of protein synthesis is indicated in two ways; by an increased incorporation of radioactive amino acids administered to the intact animal into myocardial protein (6) and by an augmented incorporation of labeled amino acids into protein by microsomes isolated from hypertrophied hearts (7). Since RNA participates in protein synthesis in the form of messenger RNA, ribosomal RNA, and transfer RNA, changes in the rate of nucleic acid synthesis would also be expected.

An increase in the myocardial RNA content has been shown repeatedly in various types of experimental cardiac hypertrophy (2-5). The increased RNA content may be due to a faster rate of synthesis, or to a slower rate of degradation of RNA, or to a combination of these two processes. Recently, Nair and associates (8) in our laboratory demonstrated increased activity of DNA-dependent RNA polymerase in nuclei isolated from hypertrophied myocardium of rats following banding of the ascending aorta. This enzyme is responsible for cellular RNA synthesis (9).

The purpose of this study was to estimate directly the rates of RNA synthesis in hearts at different stages of cardiac hypertrophy. Measurements were made of $^3$H-uridine incorporation into 28S, 18S, and 4S species of RNA. The relative rates of myocardial RNA synthesis before and after aortic constric
were estimated using measurements of the specific activity of the myocardial uridine nucleotide.

**Materials and Methods**

**SURGICAL AND EXPERIMENTAL PROCEDURE**

Left ventricular hypertrophy was produced in 61 female Sprague-Dawley rats, weighing 210 to 235 g. The animals were anesthetized by intravenous injection of sodium methohexital (Brevital sodium, Eli Lilly Co.), 9 mg/animal. A silver clip was applied around the ascending aorta using a standard technique previously described in detail (8). Positive-pressure breathing was maintained with a mixture of 95% oxygen and 5% carbon dioxide during the open-chest operation. Rats were killed by decapitation at either 2 days or 4 to 5 days after operation. Hearts were quickly excised, rinsed in ice-cold 0.01M Tris chloride (pH 7.2), blotted on filter paper, weighed, frozen in a dry ice-methyl cellosolve mixture, and stored at -20°C until analyzed. The large blood vessels and atria were cut off before weighing.

**LABELING OF RNA WITH RADIOISOTOPES**

Carrier-free 32P-labeled orthophosphate was purchased from Mallinckrodt Nuclear Corp., and generally labeled 3H-uridine (specific activity 2 mec/μmole) was obtained from New England Nuclear Corp. Either 0.5 to 2.0 mc of 32P or 0.1 to 0.5 mc of 3H-uridine was injected into the tail vein 30 minutes to 24 hours before the animal was killed. Injections were made under light ether anesthesia.

**DETERMINATION OF NUCLEIC ACID CONTENT**

Nucleic acids were determined on 50-mg aliquots of myocardium obtained from the left ventricular free wall near the apex. The tissue was homogenized in 2 ml of ice-cold 0.7n perchloric acid using a tightly fitting, glass Potter-Elvehjem homogenizer. The homogenate was transferred quantitatively into a small centrifuge tube, allowed to stand on ice for 1 hour, and centrifuged at 2000 x g for 5 minutes at 4°C. The precipitate was washed once with 2 ml of 0.5n perchloric acid. RNA and DNA were extracted in 0.5n perchloric acid at 80°C for 20 minutes (10). The extractions were twice repeated and the extracts, whose total volume was 5 ml, were combined. DNA in the combined supernatant was determined by Burton's modification of the diphenylamine reaction (11). RNA was determined by the orcinol reaction (13); the absorption resulting from DNA interference was subtracted from the total color developed.

**EXTRACTION OF RNA**

For density gradient and base composition analysis, RNA was extracted with a phenol-m-cresol mixture and sodium 4-aminosalicylate as described by Kirby (13), except that all procedures were done at 4°C. Usually two hearts were combined for one determination. To remove DNA, the ethanol-precipitated RNA was treated with 20 μg of electrophoretically pure DNase (Sigma Chemical Co.) for 30 minutes at 37°C in 2 ml of 0.01M Tris chloride (pH 7.2) and in the presence of 0.02M MgSO4 (14). The solution was deproteinized at 4°C for 10 minutes with 8 ml of 6% sodium 4-aminosalicylate and 5 ml of phenol-m-cresol mixture (13). After centrifugation at 5,000 x g for 10 minutes at 4°C, the RNA was precipitated with 2 volumes of absolute ethanol and kept at -20°C for 1 hour. Degraded DNA was removed from the precipitate by two additional precipitations with 25% ethanol at high salt concentration (14). The final precipitate was washed once with absolute ethanol and dissolved in 0.5 ml of 0.1m sodium acetate, 10-3m EDTA, pH 5.2. RNA was extracted from rat liver in the same way.

**SUCROSE DENSITY GRADIENT ANALYSIS OF RNA**

Approximately 200 μg of RNA were layered on 5.0 ml of a 5 to 20% linear sucrose gradient containing 0.01m sodium acetate, pH 5.2, 10-3m EDTA. The tubes were centrifuged at 25,000 rpm for 15 hours at 4°C in a Spinco SW 39-L rotor using a Spinco Model L II ultracentrifuge. Twenty-seven to twenty-eight fractions were obtained by piercing the bottom of the gradient tubes. Optical density was measured at 260 mp with the Zeiss M4QIII spectrophotometer after addition of 0.4 ml of water to each fraction. Absorbancy of blank fractions was subtracted from each reading. 32P was counted using 2-inch aluminum planchettes in the Beckman Low Beta low background counter. RNA labeled with tritiated uridine was precipitated with 0.7n perchloric acid after adding 100 μg of carrier yeast RNA. The precipitate was collected on a millipore filter (diameter 2.5 mm, pore size 0.45 μ) and was washed with 80 ml of 0.5n perchloric acid and 5 ml of 80% ethanol. The filter was dried and counted in 15 ml of toluene scintillator (0.5% 2,5-diphenylloxazole, 0.05% phenyl-oxazolyl-phenyl-oxazolyl-phenyl in toluene) using a Packard Tri Carb liquid scintillation counter.

**BASE COMPOSITION ANALYSIS**

The base composition of ribosomal RNA was analyzed using RNA labeled for 24 hours with 32P. Fractions of 28S, 18S, and 4S RNA obtained from sucrose density gradient centrifugation were combined, and the RNA was precipitated with

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0.5N perchloric acid. The precipitate collected by centrifugation was hydrolyzed at 37°C for 17 hours in 0.1 ml of 0.3N potassium hydroxide. The hydrolysate was applied to Whatman No. 3MM paper, and electrophoresis was done at 2,500 v for 3 hours in 0.2M potassium acetate, pH 3.5 (15). The ultraviolet absorbing spots were eluted with 0.1N hydrochloric acid, dried on aluminum planchettes, and counted in the Beckman Low β-II counter.

SEPARATION OF MYOCARDIAL NUCLEOTIDES

The pool size and specific radioactivity of myocardial uridine nucleotide were determined in hearts of banded and sham-operated animals. Rats were injected intravenously with 100 μCi of 3H-uridine. Ventricular tissue was homogenized in 3 ml of ice-cold 0.7N perchloric acid with a glass Potter-Elvehjem homogenizer. After centrifugation at 2,000 x g for 5 minutes at 4°C, the precipitate was re-extracted with 1.5 ml of cold 0.5N perchloric acid. The combined supernatant fluid was heated in a boiling water bath for 30 minutes to convert the nucleoside polyphosphates into nucleoside 5'-monophosphates. After cooling in ice, the solution was neutralized with potassium hydroxide and the precipitate of potassium perchlorate was removed by centrifugation at 2,000 x g for 5 minutes. The supernatant fluid was diluted to 50 ml with a solution containing 1 X 10⁻²M of ammonium formate and 2 X 10⁻⁵M of ammonium hydroxide, and the diluted mixture was applied to a 0.8 cm² X 1.3 cm column of an anion exchange resin (Dowex AG 1-x8 in the formate form). Adenosine monophosphate (AMP), cytosine monophosphate, and guanosine monophosphate were eluted with 100 ml of 0.1M formic acid after the column was washed with 20 ml of water. Uridine monophosphate was eluted in seven 10-ml fractions with 0.04M ammonium formate and 0.004M formic acid (16). The purity of the uridine monophosphate was verified by paper electrophoresis. Optical density was determined at 250, 260, and 280 μM, and 1-ml aliquots were counted in 15 ml of Bray’s solution (17) with a Packard Tri Carb liquid scintillation counter. The amount of 5'-AMP was calculated using the optical densities at the three wavelengths to correct for contamination with cytosine monophosphate and guanosine monophosphate (16).

STATISTICAL ANALYSIS

Data were analyzed for statistical significance using Student’s t-test.

ESTIMATION OF THE RATE OF RNA SYNTHESIS

The calculation of the rate of RNA synthesis was made using the following equation modified from Sheppard (18):

\[ \epsilon = \frac{a_{RNA} \times w_{RNA} \times \frac{1}{f_{RNA}} - k}{\text{area} \times \text{time}} \]

where \( \epsilon \) is the index of RNA synthesis (arbitrary units per hour), \( a_{RNA} \) is the specific radioactivity of RNA after 24 hours of labeling (μCi/mg), \( w_{RNA} \) is the total amount of RNA in the heart (mg), area \( \text{I}_t \) is the integrated area under specific radioactivity-time curve of uridine phosphate from 0 to 24 hours (μCi/hour/μg uridine monophosphate), \( f_{RNA} \) is the fraction of uridine monophosphate in RNA by weight, and \( k \) is the constant to correct for uncontrolled variables.

The units for the rate of RNA synthesis are considered to be arbitrary. They are useful to compare the relative rate of RNA synthesis in experimental and control animals.

Results

BODY WEIGHT AND RNA CONTENT

Significant increases in ventricular RNA content and ventricular weights of operated rats compared to sham-operated controls were observed 2 days and 4 to 5 days after banding of the ascending aorta (Table 1). There were no differences in body weight between operated and sham-operated animals at the time the animals were killed. The ratio of heart weight to body weight was therefore increased. The average increase in ventricular weight in 2 days was 14% and in 4 to 5 days was 25%. Ventricular weight exceeded control values by more than 10% in 11 of 20 animals on the second postoperative day; by the fourth to fifth days 34 of 41 banded animals had increments of ventricular weight greater than 10%.

Experimental animals showed elevations in both RNA content per mg wet weight of cardiac tissue and in total ventricular RNA. Changes were already evident 2 days after surgery. The data showing increments in cardiac RNA after banding are similar to those obtained in a larger series reported previously (8).

TIME COURSE OF °P INCORPORATION INTO RNA

The time course with which different species of RNA become labeled with °P on the fourth to fifth postoperative day was followed by sucrose density gradient centrifugation analysis (Fig. 1). These data also yield information concerning the types of
TABLE 1
Effect of Banding the Ascending Aorta on Heart Weight and RNA Content

<table>
<thead>
<tr>
<th></th>
<th>Two Days after Operation</th>
<th>Four to Five Days after Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Sham-operated</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ventricular weight (mg)</td>
<td>219 ± 2</td>
<td>653 ± 9</td>
</tr>
<tr>
<td>RNA (μg/mg tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA (mg/heart)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P†</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± se are given.

*Difference in values between paired sets of banded and sham-operated rats.
†Probability of chance occurrence.
‡Not significant.

FIGURE 1
Pattern of labeling of myocardial RNA with 32P. Sucrose density gradient profiles of RNA, 1 hour and 24 hours after labeling with 32P for banded and sham-operated hearts. Radioactivity was normalized to values for the injection of 1 mc of 32P per rat. Optical density at 260 μm (solid line), counts per minute (open circles), and specific radioactivity (x’s).

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RNA being synthesized. After a 1-hour pulse of $^{32}$P, the radioactivity profile did not follow the optical density profile of carrier ribosomal 28S and 18S RNA or of transfer 4S RNA. There was significant radioactivity and an elevated specific radioactivity in the region heavier than 28S in both banded and sham-operated controls. The region of high specific radioactivity may reflect synthesis of heavy messenger RNA or of ribosomal-precursor RNA. There was also a high level of radioactivity in the lighter (4 to 10S) region. The labeled material probably represents predominantly transfer RNA but may include some degraded messenger RNA. After a 4-hour period of labeling with $^{32}$P, the radioactivity closely followed optical density in the entire region heavier than 10S. Specific radioactivity remained higher in the 4S region. The elevation of specific radioactivity in the 4S region compared to the 18S and 28S regions became less marked with time, disappearing in 24 hours. At this time radioactivity coincided exactly with absorbancy.

Labeling of 1S to 28S RNA increased almost linearly during the first 24 hours and was twice as high in banded hearts as in sham-operated controls at any labeling time (Fig. 2). Specific radioactivity of 4S RNA increased more rapidly in the first several hours probably due to terminal nucleotide exchange in sRNA. The rate of increase then diminished gradually, and at 24 hours the specific radioactivity was the same as that of ribosomal RNA. The specific activity of 4S RNA was also about twice as great in operated animals as in sham-operated controls.

MEASUREMENT OF THE SIZE AND SPECIFIC ACTIVITY OF THE URIDINE NUCLEOTIDE POOL

The pattern of labeling RNA with $^3$H-uridine was similar to that for $^{32}$P. On the basis of the data for $^{32}$P incorporation, RNA was labeled with $^3$H-uridine for 24 hours in both operated animals and sham-operated controls. To estimate rates of RNA synthesis, it was necessary to measure the size and specific activity of the nucleotide precursor pool. For convenience, the entire uridine nucleotide
pool was converted to uridylic acid by acid hydrolysis prior to analysis. The assumption is made that all uridine nucleotides are essentially in equilibrium.

The specific radioactivity of the myocardial uridine nucleotide pool is plotted against time after injection of $^3$H-uridine in Figure 3. In sham-operated hearts the specific activity rose rapidly, reaching a peak within 1 hour. It remained at a high level for 4 hours and then declined exponentially with a half time of approximately 6 hours. In banded hearts a sharp peak of specific activity was seen within 1 hour. This peak was followed by an exponential decline with a half time of about 5 hours. The specific radioactivity of the uridine nucleotide pool in banded hearts was higher than in sham-operated controls during the first 2 hours of labeling, but was considerably lower at 24 hours. The patterns were similar at 2 days and at 4 to 5 days. The integrated 24-hour specific activity of the uridine nucleotide pool was 15% lower in hearts from banded rats than in controls at 2 days, and 5% lower at 4 to 5 days.

Two days after aortic banding, the size of the uridine nucleotide pool was markedly elevated (Table 2). The concentration per mg wet tissue increased by 50%, and total uridine nucleotide per heart was approximately 100% above control values. Four to five days after surgery, the elevations were still present but were only half as great. The expansion of the uridine nucleotide pool in hearts from banded animals was substantially greater than the decrease in specific radioactivity of the pool. The increase in uridine nucleotide pool appears to be a sensitive response to aortic banding, since it was present in all but one of 12 banded animals, whereas an increase in RNA concentration was present only in 7 of 11 animals on the second postoperative day.

It is also of note that the adenosine nucleotide pool did not expand in hypertrophied hearts. Since the total adenosine nucleotide per heart remained unchanged, the increase in heart weight in banded animals led to a decrease in the content of adenosine nucleotide per mg wet weight of tissue.

**BASE COMPOSITION OF RNA**

Base compositions of 28S, 18S, and 4S RNA in hearts of banded and sham-operated rats were determined from the distribution of $^{32}$P after 24 hours of labeling. As is shown in Table 3, the base composition of any species of RNA did not significantly differ in hearts of banded and sham-operated animals.

**RATE OF RNA SYNTHESIS**

The rate of RNA synthesis was estimated in duplicate for the second and for the fourth to fifth postoperative days. The data are...
shown in Table 4. Integrated specific radioactivity of the uridine nucleotide pool was measured in separate animals. The rate of RNA synthesis in hearts of banded animals averaged four times higher than values in hearts of matched sham-operated controls on the second postoperative day; the increment in the rate of RNA synthesis was less remarkable (two to three times control values) 4 to 5 days after operation.

Discussion

INCREASED RATE OF RNA SYNTHESIS AFTER BANDING OF THE AORTA

Several biochemical events accompany the production of experimental cardiac hypertrophy. In the model system used in this study, significant increases in the RNA, DNA, and protein content of the heart are apparent within 24 to 48 hours after banding of the thoracic aorta. In a study previously reported from our laboratory, the activity of DNA-dependent RNA polymerase in nuclei isolated from hearts of banded animals was sharply increased compared to the activity of nuclei from sham-operated animals (8). RNA polymerase activity reached a peak in the second postoperative day and subsided thereafter, but still remained elevated on the twelfth

### Table 2

<table>
<thead>
<tr>
<th>Number</th>
<th>Sham-operated</th>
<th>Banded</th>
<th>Banded - sham-operated*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two Days after Operation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine nucleotide pool</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>(µmoles/g)</td>
<td>0.20 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.31 ± 0.03</td>
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<tr>
<td>(µmoles/heart)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Adenosine nucleotide pool</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
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<td>12</td>
<td>12</td>
</tr>
<tr>
<td>(µmoles/g)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(µmoles/heart)</td>
<td>12</td>
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Four to Five Days after Operation

<table>
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<th>Banded</th>
<th>Banded - sham-operated*</th>
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</thead>
<tbody>
<tr>
<td>Uridine nucleotide pool</td>
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<td></td>
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</tr>
<tr>
<td>Number</td>
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<tr>
<td>(µmoles/g)</td>
<td>0.23 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>(µmoles/heart)</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<tr>
<td>Adenosine nucleotide pool</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Number</td>
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<td>10</td>
<td>10</td>
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<tr>
<td>(µmoles/g)</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>(µmoles/heart)</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tbody>
</table>

Mean values ± SE are given.

* Differences between paired sets of banded and sham-operated rats.
† Not significant.

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cytosine</th>
<th>Adenine</th>
<th>Guanine</th>
<th>Uridine</th>
<th>A+C</th>
<th>A+U</th>
<th>G+U</th>
<th>G+C</th>
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</thead>
<tbody>
<tr>
<td>Heart (sham-operated)</td>
<td>32.1 ± 3.0</td>
<td>21.8 ± 0.9</td>
<td>32.1 ± 0.4</td>
<td>14.0 ± 1.8</td>
<td>1.17</td>
<td>0.56</td>
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<tr>
<td>Heart (banded)</td>
<td>31.2 ± 0.5</td>
<td>19.7 ± 2.8</td>
<td>32.2 ± 0.3</td>
<td>17.0 ± 2.5</td>
<td>1.03</td>
<td>0.58</td>
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<tr>
<td>Liver</td>
<td>29.3 ± 0.3</td>
<td>19.0 ± 0.3</td>
<td>36.2 ± 0.5</td>
<td>15.6 ± 0.5</td>
<td>0.93</td>
<td>0.53</td>
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<tr>
<td>Heart (sham-operated)</td>
<td>29.7 ± 0.3</td>
<td>22.0 ± 1.1</td>
<td>27.5 ± 1.0</td>
<td>20.8 ± 0.4</td>
<td>1.07</td>
<td>0.75</td>
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<tr>
<td>Heart (banded)</td>
<td>32.1 ± 1.1</td>
<td>21.9 ± 1.8</td>
<td>29.7 ± 2.8</td>
<td>16.3 ± 3.3</td>
<td>1.17</td>
<td>0.62</td>
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<tr>
<td>Liver*</td>
<td>26.2</td>
<td>22.3</td>
<td>31.4</td>
<td>20.2</td>
<td>0.94</td>
<td>0.74</td>
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</tr>
</tbody>
</table>

Base compositions are expressed as moles percent. Mean values ± SE of three determinations are given. A = adenine, C = cytosine, G = guanine, U = uridine.

* Only one determination was done.
TABLE 4
Rates of RNA Synthesis in Hearts of Banded and Sham-Operated Rats

<table>
<thead>
<tr>
<th></th>
<th>Ventricular weight</th>
<th>Total ventricular RNA (mg)</th>
<th>Labeling of RNA (muc/mg)</th>
<th>Integrated labeling of uridine phosphate (muc/hr/μg UMP)</th>
<th>Rate of RNA synthesis (units/hr/heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Two Days after Operation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated</td>
<td>0.69</td>
<td>1.56</td>
<td>17.8</td>
<td>19.8</td>
<td>6.7</td>
</tr>
<tr>
<td>Banded</td>
<td>0.71</td>
<td>2.03</td>
<td>46.1</td>
<td>16.2</td>
<td>26.8</td>
</tr>
<tr>
<td><strong>Four to Five Days after Operation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated</td>
<td>0.69</td>
<td>1.45</td>
<td>6.9</td>
<td>11.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Banded</td>
<td>0.82</td>
<td>2.52</td>
<td>10.8</td>
<td>10.8</td>
<td>12.1</td>
</tr>
</tbody>
</table>

Mean values of two experiments are shown. In each experiment two hearts were pooled. Data are normalized for injection of 1 μg of 3H-uridine per rat. UMP = uridine monophosphate.

day. The data presented in this paper present additional direct evidence for enhanced synthesis of ribosomal RNA in hearts of banded animals. The rate of RNA synthesis was maximal 2 days after banding of the aorta and was still elevated, although to a lesser extent, at 4 to 5 days. Thus the time course of stimulation of RNA synthesis is similar to the pattern of elevation of RNA polymerase after aortic banding.

Most of the new RNA synthesized is ribosomal and transfer RNA. The RNA synthesis precedes and accompanies the increased protein synthesis. Evidence for increased synthesis of messenger RNA was not obtained in this study. Schreiber et al., however, have presented data suggesting increased messenger RNA synthesis in perfused rat hearts subjected to an increased pressure load (19). In the less acute stages of hypertrophy, a correlation between the quantity of ribosomal RNA and rate of protein synthesis appears to be present. This correlation has also been observed in other mammalian systems (20).

The calculation of rate of RNA synthesis involves several assumptions and must be considered an approximation. However, the data on the specific activity of the precursor pool allow us to draw conclusions that are sounder than if we relied solely on the incorporation of labeled precursor into RNA. We have shown in this work that marked changes in precursor pool size may occur in experimental animals. Undetected changes in specific activity of the precursor pool in experimental animals could lead to spurious calculated changes or could obscure significant changes in the rate of RNA synthesis.

**EXPANSION OF URIDYLYC ACID POOL**

Recently the uptake of pyrimidine bases into the intracellular pool of *Escherichia coli* B/1 was shown to be limited by the rate of utilization of the base during the exponential phase of growth; in this microorganism the pool size was therefore kept constant (21). Our finding that the uridine nucleotide pool size in myocardium increases in cardiac hypertrophy shows that the observation made in *E. coli* does not directly apply to all species. Several observations indicate that the conversion of 3H-uridine to uridine nucleotides is enhanced in hearts of banded animals. Despite the doubling of the size of the uridine nucleotide pool, the specific radioactivity 1 to 2 hours after injection of 3H-uridine was higher in hearts of banded animals than in controls. The total amount of 3H-uridine entering the uridylic acid pool was also increased in hearts of banded animals. This is shown by the relatively small decrease of the integrated 24-hour specific radioactivity in banded hearts relative to the marked increase in pool size. Increased utilization of uridine nucleotide for RNA synthesis is also suggested by the somewhat more rapid decay of...
specific radioactivity in banded hearts in the face of a much enlarged pool size.

It is possible that a step in the conversion of uridine to uridine triphosphate or in the synthesis of uridine nucleotide may in part control the rate of RNA synthesis. This possibility is supported by the fact that the expansion of the uridine nucleotide pool is seen so early after banding of the aorta. An enlargement of the pool size was present in some animals even when RNA and heart weight had not yet increased. The uridine nucleotide pool as defined in this study is, however, a complex one which includes uridine diphosphate glucose and uridine diphosphate acetyl glucosamine; conclusions about the nature of the expansion in pool size may therefore be premature. Possibly changes in polysaccharide metabolism lead to enlargement of the uridine nucleotide pool. The expansion of pool size did not include all nucleotides, since the size of the adenosine nucleotide pool did not change.

Visioli and his collaborators (22) did not find any marked change in the myocardial uridine nucleotide pool in rats after severe and repeated physical exercise. Perhaps a transient change in pool size was not detected since the stimulus used to produce hypertrophy was a chronic one.

**BASE COMPOSITION ANALYSIS**

Meerson and his collaborators (23) reported changes in the base composition of myocardial RNA 4 days after banding the ascending aorta of the dog. Since the fraction of uridine monophosphate in RNA influences the calculation in the rate of RNA synthesis, base composition analyses of RNA from experimental and control hearts were done. The \(^{32}P\) labeling technique revealed no difference in the base composition of RNA from banded hearts relative to that of hearts from sham-operated controls. Despite limitations of the method, significant changes in base composition probably would have been detected. Our data do not explain the marked changes in base composition of RNA in cardiac hypertrophy reported by Meerson et al. (23).

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