Biochemical Correlates of Cardiac Hypertrophy

V. LABELING OF COLLAGEN, MYOSIN, AND NUCLEAR DNA DURING EXPERIMENTAL MYOCARDIAL HYPERTROPHY IN THE RAT

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ABSTRACT

The incorporation of [2, 3-3H]proline into collagen hydroxyproline and into noncollagenous protein was measured during development of cardiac hypertrophy produced by surgical constriction of the ascending aorta in rats. Heart weight increased sharply during the first 4 days after aortic banding and then slowly rose to a plateau. Free intracellular proline concentration remained unaltered 2 days after banding and increased by 38% on the fifth postoperative day. Specific radioactivity of free proline 3 hours after injection of [3H]proline was elevated by 40% on the second postoperative day but was unchanged on the fifth day. Incorporation of [3H]proline into collagen hydroxyproline peaked sharply on the second day after aortic constriction (550% above control) in one experiment, and on the fourth day (330% above control) in another. The peak labeling of noncollagenous protein by [3H]proline (100% over control) was less than that of collagen. To further evaluate the differences in synthetic response of muscle cells and interstitial cells to increased work load, incorporation of radioactive precursors into myosin, collagen, nuclear DNA, and noncollagenous protein were measured simultaneously, after aortic constriction. Collagen labeling was maximal either on day 2 or 4, depending on the degree of hypertrophy. Labeling of myosin and noncollagenous protein reached a plateau on day 4. Incorporation of [3H]thymidine into nuclear DNA peaked on day 7. Thus both muscle cells and connective tissue cells respond independently during cardiac hypertrophy with an increased synthesis of specific proteins.

KEY WORDS interstitial cells experimental aortic constriction noncollagenous protein muscle cells proline thymidine hydroxyproline

The rapid enlargement of adult rat heart that follows experimentally produced pressure overload results from hypertrophy of muscle fibers and hyperplasia of connective tissue cells. The increased activity of connective tissue cells in cardiac hypertrophy is well documented both by radioautography and by determination of mitotic indexes. Cardiac enlargement produced by systemic hypertension (1) or by increased resistance to blood flow produced by constriction of the ascending aorta (2-4) results in marked labeling, with [3H]thymidine, of otherwise inactive connective tissue nuclei. The mitotic indexes of interstitial cells of hypertrophied hearts were found to be ten times higher than in controls (5). Both the mitotic activity and the degree

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of DNA labeling are proportional to the degree of hypertrophy (4, 5).

Nuclear DNA synthesis in enlarging adult hearts is almost exclusively confined to the interstitial tissue. The very rare labeling of muscle cell nuclei with \([^{3}H]\)thymidine seen in radioautographs of the hypertrophic heart (4, 6) may be related to the increased polyploidization of existing nuclei. The frequency of polyploid nuclei is considerably increased in pathological specimens of hypertrophied human hearts (7, 8) but only a relatively small increase in polyploidy is seen in experimentally produced cardiac hypertrophy (5, 9).

A substantial increase in collagen content has been noted in hearts enlarging in response to constriction of the ascending aorta (4) or of the main pulmonary artery (10). Collagen is believed to be produced only by connective tissue cells. It is apparent therefore that synthetic activities of both muscle and connective tissue cells are stimulated in adaptive cardiac growth.

It is the purpose of this paper to present information concerning the significance of connective tissue hyperplasia and increased collagen synthesis in the development of cardiac hypertrophy. We examined the time courses of enhancement of some of the synthetic activities of connective tissue cells and muscle cells following experimental aortic constriction in the rat. Connective tissue hyperplasia was measured by incorporating \([^{3}H]\)thymidine into purified nuclear DNA. Collagen biosynthesis in connective tissue cells was estimated by following the incorporation of radioactive proline into tissue hydroxyproline, since collagen is the only protein in mammalian tissue containing significant amounts of hydroxyproline. Collagen hydroxyproline is derived from proline that is hydroxylated after its incorporation into protocollagen, the polypeptide precursor of collagen (11). Muscle cell synthetic activity was monitored by measuring the incorporation of labeled amino acids into myosin.

Our results show that the labeling of collagen is several times as great by the second postoperative day. The maximal labeling of collagen exceeds that of the bulk of noncollagenous protein and myosin, and precedes that of nuclear DNA by several days. After this early burst of activity, a moderately increased rate of labeling was noted as long as 1 month after aortic constriction. Preliminary results of this study have been published (12).

**Materials and Methods**

**PRODUCTION OF EXPERIMENTAL CARDIAC HYPERTROPHY**

The ascending aortas of female Sprague-Dawley rats weighing 195–205 g were constricted with silver bands as previously described (13). Rats with sham operations served as controls. The animals were killed at varying times after operation. Hearts were removed, rinsed in ice-cold 0.01M Tris buffer, pH 7.5, containing 0.25M sucrose, 0.01M KCl, 0.1M Na-EDTA, and 10 USP units heparin/ml, and the atria and aortas were trimmed off. The hearts were then blotted, weighed, and analyzed as described below.

**ADMINISTRATION OF RADIOISOTOPES**

Radioisotopes were administered intraperitoneally at varying times after aortic constriction. In experiments comparing the synthesis of collagen and noncollagenous protein, each animal received 50 \( \mu \)C of \([2,3^{3}H]\)proline unless otherwise noted. For the simultaneous measurement of labeling of collagen, myosin, and DNA, 50 \( \mu \)C of \([2,3^{3}H]\)proline, 10 \( \mu \)C of \([U-^{14}C]\)lysine, and 100 \( \mu \)C of [methyl-\( ^{3}H\)]thymidine were administered together. Preliminary experiments showed that when \([^{3}H]\)proline and \([^{3}H]\)thymidine were injected individually, the DNA fraction contained no radioactivity derived from \([^{3}H]\)proline, and that the fraction used to assay for hydroxyproline contained no detectable radioactivity derived from \([^{3}H]\)thymidine. Similarly, label derived from \([^{14}C]\)lysine was not present in significant amounts in the hydroxyproline or DNA fractions. The labeling of myosin and that of noncollagenous protein were also compared using \([^{3}H]\)proline. Animals were killed 3 hours after injection of the radioisotopes since incorporation into the components was comparable 3, 6, and 12 hours after injection.

**DETERMINATION OF AMOUNT AND RADIOACTIVITY OF COLLAGEN**

The hearts were frozen in liquid nitrogen, pulverized, and extracted twice in hot 5% trichloroacetic acid (TCA) by a procedure in which over 95% of the tissue collagen is recovered (14). An equal volume of concentrated HCl was added to an aliquot of TCA extract and the
solution was hydrolyzed in an autoclave overnight. The content and radioactivity of hydroxyproline in the hydrolysate were determined by methods previously described (15, 16), with slight modification. Hydroxyproline in the hydrolysate was oxidized to pyrrole. The pyrrole was extracted into toluene, and samples were taken for pyrrole determinations and for assay of radioactivity in a Packard Tri-Carb liquid scintillation counter. [3H]Toluene standards were counted simultaneously to convert cpm to dpm. Under the conditions of these experiments, pyrrole recovery was proportional to the amount of hydroxyproline in the hydrolysate of TCA extract. The collagen content of the heart was calculated from a standard curve prepared from hydrolysates of calf skin gelatin. Since cardiac DNA and protein concentrations do not change significantly during the first 2 weeks after aortic constriction (5), radioactivity determinations are expressed as dpm per 100 mg tissue. Collagen content was also measured by the method of Newman and Logan (17) as previously described (4).

DETERMINATION OF NONCOLLAGENOUS PROTEIN RADIOACTIVITY

The residue remaining after extraction with hot 5% TCA was left overnight at 4°C in 0.1 N NaOH. After centrifugation at 3000 × g, the supernatant fluid was collected and neutralized with 1 N HCl, and 0.1 volume of 50% TCA was added. After 30 minutes in ice, the precipitate was collected and washed three times with 5% TCA at room temperature. The residue was extracted for 30 minutes at 80-90°C with 5% TCA, washed with acetone, and then with absolute ethanol. The residue was dried under an infrared lamp and dissolved in formic acid. A sample was evaporated to dryness in a vacuum desiccator and then dissolved in 0.2M KCl, 0.01M Tris-HCl buffer, pH 7.8, and 0.25 mM Na-thioglycolate. The myofibrils were washed twice in the solution containing 0.01M KCl, 0.05M Tris-HCl, pH 7.5, and 0.25 mM Na-thioglycolate. Myosin was extracted as follows: the myofibrils were suspended in a solution which contained, after the addition of KCl, 8 mM K-phosphate buffer, pH 7.8, 1 mM Na-EDTA, 5 mM Mg-ATP, and 0.25 mM Na-thioglycolate; 2M KCl was then added gradually to a final concentration of 0.6M. The suspension was homogenized by 10 strokes in a Dounce homogenizer, left in ice for 20 minutes and centrifuged 2 hours at 160,000 × g. The top two-thirds of the supernatant were removed and dialyzed against 0.01M KCl, 0.25 mM Na-thioglycolate. The precipitated myosin was collected by centrifugation and dissolved in 0.2M KCl, 0.01M Tris-HCl buffer, pH 7.8. Approximately 20 mg of myosin was adsorbed on a 15 × 2 cm DEAE-cellulose column and eluted by a linear KCl gradient (20). The fractions containing myosin were combined and the 35-45% (NH₄)₂SO₄ fraction was collected. The purified myosin was washed three times with 5% TCA at room temperature and counted by the procedure described above for noncollagenous protein.

The purity of myosin was evaluated by SDS-polyacrylamide electrophoresis, and by analysis of sensitivity of ATPase activity to Mg²⁺ and Ca²⁺. In addition, myosin was purified after adding to the homogenate possible contaminating proteins (sarcoplasmic proteins, actin, and tropomyosin-troponin complex) which had been labeled with [carboxyl-¹⁴C]-chloromercuribenzoate (21).
DETERMINATION OF NUCLEAR DNA RADIOACTIVITY

The crude nuclear fraction was suspended in 0.15M NaCl, 0.01M EDTA, 0.05M Na-phosphate buffer, pH 6.5, and extracted with an equal volume of phenol. DNA was precipitated with ethanol, washed with 75% ethanol, dissolved in standard sodium citrate solution (0.15M NaCl, 0.015M Na citrate) and digested with RNAase and pronase. The nuclear DNA was separated from remnants of mitochondrial DNA, after denaturation and renaturation by CsCl equilibrium centrifugation, and precipitated by TCA. Its radioactivity was measured in the liquid scintillation counter and its deoxyribose content was determined colorimetrically by the diphenylamine reaction. The details of these procedures were given previously (22).

ESTIMATION OF EXTRACELLULAR SPACE

The extracellular space was assumed to be equal to the volume of distribution of sulfate (23, 24). For determination of the extracellular space in vivo, Na$_2$H$^3$SO$_4$ (5 μc/100 g body weight) was injected intravenously immediately after the animals had been nephrectomized under ether anesthesia. After an equilibration period of 2 hours (25), the animals were killed and the hearts removed, blotted, weighed, and placed in 3 ml of 0.5N NaOH and heated overnight in a water bath at 45°C to dissolve the tissue. Protein was precipitated from a 1-ml sample by adding an equal volume of 10% TCA.

Blood was collected in beakers containing heparin at the time the animals were killed. The plasma was separated by centrifugation and diluted fivefold with water. Protein was precipitated by adding an equal volume of 10% TCA. Samples (0.2-0.5 ml) of plasma and heart TCA supernatants were added to 15 ml of Bray's scintillator and counted in a Packard Tri-Carb liquid scintillation counter. An internal standard was used to correct for quenching.

The sulfate space was calculated according to the following formula: sulfate space (μl/100 g wet weight of heart) = count/min g$^{-1}$ wet weight of heart × weight count/min μl$^{-1}$ of plasma

The S$^3$S counts in plasma were corrected for Donnan factor of 0.95 (26).

TOTAL TISSUE WATER

A small portion (50-100 mg) of heart muscle was placed in a previously weighed crucible, weighed, to constant weight in an oven at 100°C, and the tissue water was calculated from the loss in weight of the tissue and expressed as μl/g wet weight.

DETERMINATION OF INTRACELLULAR PROLINE CONCENTRATION AND SPECIFIC RADIOACTIVITY

[$^3$H]Proline was injected intravenously (25 μc/100 g body weight), and the rats killed after 3 hours. The hearts were removed, frozen, pulverized, and extracted three times with 1 ml of 5% TCA, as described above for the study of collagen. Plasma was collected and prepared for liquid scintillation counting and counted as described for the study of extracellular space. Total proline was determined in the heart TCA extracts and the plasma TCA supernatant fluids by the method of Troll and Lindsley (27).

The intracellular concentration of proline was calculated by the following formula:

$$C_I = \frac{C_T - F_0 \times C_E}{F_1}$$

Where:

- $C_I$ = Intracellular proline concentration (μl/g wet weight of heart)
- $C_T$ = Total proline concentration (μl/g wet weight of heart)
- $F_0$ = Dilution factor for plasma
- $C_E$ = Extracellular proline concentration (μl/g wet weight of heart)
- $F_1$ = Dilution factor for tissue

Effect of banding on recovery of radioactive proline as collagen hydroxyproline and in noncollagenous protein. Animals were each injected with 10 μc [U-$^3$C] proline on day 7 after banding, and with 50 μc [2,3-$^3$H]proline on all other days. The height of the bars represents mean radioactivity recovered in hearts from rats with sham operations (white bars) and banded (black bars) rats; vertical lines represent 2 s.e. of the means. The number in each group is given at the base of the bars. Each difference between corresponding control and experimental groups is significant by Student's t-test with a P value of 0.01 or less. Here and in other figures, HYPRO = hydroxyproline, NCP = noncollagenous protein, PRO = proline.
where \( C_t \) = concentration in intracellular water in \( \mu \)moles/100 ml; \( C_e \) = concentration in extracellular fluid in \( \mu \)moles/100 ml; \( F_e \) = fraction of the tissue water that is extracellular; and \( F_t \) = fraction of the tissue water that is intracellular.

**Results**

**TIME COURSE OF LABELING OF COLLAGEN AND NONCOLLAGENOUS PROTEIN**

The relative rates of synthesis of collagen and noncollagenous protein during development of cardiac hypertrophy were estimated by comparing the incorporation of \([H]proline\) into collagen hydroxyproline with its incorporation into noncollagenous protein. Two sets of experiments were carried out. In the first, surgical aortic constriction was performed at various times (2, 4, 7, and 30 days) before isotope administration and death (Fig. 1). In the second, surgery was performed on the same day and the animals were killed at intervals of 1 to 7 days later (Fig. 3). The results of the first experiment are shown in Figure 1. Maximal incorporation of radioactive proline into collagen occurred on the second day (565% above control values), the earliest time studied. Incorporation was still considerably elevated (245% above control value) on the thirtieth day after surgery. The labeling of noncollagenous protein with \([H]proline\) was maximal on the second day and remained elevated on the fourth and seventh days (Fig. 1). Maximal noncollagenous protein labeling was only 160% greater than control values, compared with a 565% elevation observed in collagen labeling.

The degree of increment in labeling of collagen hydroxyproline varied directly with the extent of cardiac hypertrophy indicated by increase in heart weight (Fig. 2). There was considerable variation in the extent of cardiac hypertrophy in each group. Differences in animal weight as well as other unknown and uncontrolled variables may contribute to this variation. To minimize these factors, we banded a large group of animals closely matched for weight on a single day. Incorporation of \([H]proline\) into collagen hydroxyproline and noncollagenous protein was then measured 1 to 7 days after banding. As seen in Figure 3, collagen labeling averaged about

![Figure 2](http://circres.ahajournals.org/)

*Correlation of heart weight with collagen labeling. Individual data from the experiments in Figure 1 are plotted. Open circles = sham operations; solid circles = banded; solid lines are the best fit lines to the equation for a straight line.*

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Time course of collagen and NCP labeling after banding. The mean radioactivity recovered in rats with sham operations (open circles, n = 1-3) and banded (solid circles, n = 3-5) animals is plotted. Vertical lines represent 2 SE of the means. Where comparisons between banded and control animals are possible, the differences are significant by Student's t-test with a P value of 0.02 or less.

EXTRACELLULAR SPACE AND INTRACELLULAR PROLINE POOL

Since changes in the level of amino acid incorporation into proteins can be greatly affected by changes in the size and specific activity of the precursor pool, estimations of free proline specific activity and concentration in the heart muscle were made 2 and 5 days after aortic constriction. It should be pointed out that this measurement represents values for both cardiac muscle cells and connective tissue cells.

The total tissue water of the rat heart averaged 777 ± 1.4 μliter/g in both controls and banded animals. Cardiac hypertrophy resulted in a significant increase in extracellular fluid as measured by 35S at both 2 days (24% increase) and 5 days (17% increase) after banding. The possibility that this increase may have been the result of increased permeability of the myocardium to SO₄²⁻ ions under conditions of cardiac hypertrophy has not been eliminated. Accordingly, all calculations of proline intracellular concentrations in banded animals were made using extracellular sulfate space values obtained in the controls. Calculations of intracellular proline pools in banded rats using sulfate space values obtained with banded animals give values for total intracellular proline concentration 7% greater than those presented in Table 1. Calculations of specific activity of intracellular proline on the same basis resulted in values 3% lower than those presented in Table 1.

There was no increase in intracellular proline 2 days after banding, but by day 5 a 38% increase in total intracellular proline had occurred. Specific activity of intracellular proline was 40% greater than in controls on day 2 after banding, and was slightly less than
TABLE 1

<table>
<thead>
<tr>
<th>Operation</th>
<th>No. of rats</th>
<th>Hypertrophy (%)</th>
<th>Extracellular space (g)</th>
<th>Intracellular proline pool (μmol/100 ml cell H2O)</th>
<th>Specific radioactivity (dpm X 10^-9/imole of proline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham, 2 days</td>
<td>8</td>
<td>29 ± 5</td>
<td>247 ± 10</td>
<td>5</td>
<td>93.64 ± 4.05</td>
</tr>
<tr>
<td>Banded, 2 days</td>
<td>8</td>
<td>29 ± 5</td>
<td>307 ± 10</td>
<td>4</td>
<td>95.93 ± 5.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P &lt; 0.02)</td>
<td></td>
</tr>
<tr>
<td>Sham, 5 days</td>
<td>10</td>
<td>41 ± 7</td>
<td>270 ± 9</td>
<td>4</td>
<td>80.50 ± 5.49</td>
</tr>
<tr>
<td>Banded, 5 days</td>
<td>8</td>
<td>41 ± 7</td>
<td>315 ± 13</td>
<td>5</td>
<td>124.14 ± 8.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P &lt; 0.01)</td>
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</table>

Intracellular proline was calculated on the basis of extracellular space values obtained in controls (sham operations). Percent hypertrophy was calculated by comparison of ratios of heart weight to body weight of banded and control rats.

*P > 0.05.

In hearts 5 days after banding (Table 1). It is evident from these data that the changes in proline incorporation into myocardial collagen resulting from aortic constriction are much larger than the changes in the specific radioactivity of intracellular free proline.

**TIME COURSE OF LABELING OF COLLAGEN, MYOSIN, NUCLEAR DNA, AND NONCOLLAGENOUS PROTEIN**

We wished to further assess differences in the temporal responses in the stimulation of synthetic activities of muscle cells and of connective tissue cells during cardiac hypertrophy. Incorporation of labeled precursors into collagen, myosin, nuclear DNA, and noncollagenous protein were measured simultaneously. At varying times after aortic constriction, sets of animals received [2, 3-^3H]proline to measure collagen labeling, [U-^14C]lysine for myosin and noncollagenous protein, and [methyl-^3H]thymidine to measure nuclear DNA labeling, and were killed 3 hours later. The hearts were divided into groups (2 or 3 hearts per group) according to whether they exhibited moderate hypertrophy (10-25% weight gain) or severe hypertrophy (more than 25% weight gain). Hearts showing less than 10% increase in weight were discarded. Hearts of rats with sham operations served as controls (4 hearts per sample). The time course of collagen labeling depended on the degree of hypertrophy (Fig. 4). In hearts with severe hypertrophy, collagen labeling was maximal at 2 days and remained elevated to the same extent at 4 or 7 days after banding. In hearts with moderate hypertrophy, collagen labeling peaked sharply (over 10 times control value) on the fourth day. In light of the observation that the enhancement of collagen synthesis is correlated with the degree of hypertrophy (Fig. 2), peak collagen synthesis in severely hypertrophied hearts possibly occurred before our first measurement on the second day after banding. Incorporation of [^3H]thymidine into DNA peaked at 7 days after aortic banding (Fig. 4) when it was 600% greater than in controls. On the eleventh day, the DNA specific radioactivity was less than the maximal value observed. DNA labeling in moderately hypertrophied hearts paralleled that in severely hypertrophied hearts, although at a lower level.

The time courses of labeling of myosin and of noncollagenous protein with [^14C]lysine were similar. The extent of labeling was proportional to the increase in heart weight, and a plateau of incorporation was reached at about 4 days after banding (Fig. 4). Identical patterns of labeling of noncollagenous proteins and of myosin were obtained by using either lysine or proline as precursors. The elevated incorporation observed in hypertrophied hearts was of the same magnitude in each case. The ratios of specific radioactivity of noncollagenous proteins to myosin were identical in control and banded animals with...
both precursors (Table 2). Thus the same response to banding of the two protein fractions was observed with both proline and lysine.

**TABLE 2**
Comparison of the Labeling Pattern of Noncollagenous Proteins (NCP) and Myosin with Proline and Lysine

<table>
<thead>
<tr>
<th>Operation</th>
<th>Hypertrophy (%)</th>
<th>Specific radioactivity NCP</th>
<th>Specific radioactivity myosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proline</td>
<td>Lysine</td>
</tr>
<tr>
<td>Sham, 2 days</td>
<td>24 and 27</td>
<td>1.56 ± 0.10</td>
<td>1.06 ± 0.07</td>
</tr>
<tr>
<td>Banded, 2 days</td>
<td>32</td>
<td>1.50 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Sham, 4 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banded, 4 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham, 5 days</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Banded, 5 days</td>
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Two separate experiments were performed, one using [3H]proline (N = 3) as precursor and a second with [14C]lysine (N = 6). Data are expressed as means ± SE. Percent hypertrophy was calculated as for Table 1.
In our previous report, the increase in heart weight resulting from aortic constriction occurred primarily during the first 2-3 days (13). In the present experiments, heart weight increased relatively little on the first day after banding, sharply from day 2 to day 4, and then gradually to day 7, the latest time point measured (Figs. 3 and 4).

Total collagen did not increase substantially until the seventh day after banding, when it was elevated by 81% (Fig. 5). Mean total tissue collagen on day 11 was only 104% higher than in the control. Collagen concentration (collagen weight/wet weight tissue) fell during the early period of cardiac hypertrophy, when the heart weight increased and total tissue collagen remained relatively constant. After the fifth day following aortic banding, collagen concentration was elevated compared to the controls.

Discussion

The labeling of heart components was measured at varying times after the induction of cardiac hypertrophy by aortic banding. Appropriate radioactive precursors were injected intraperitoneally, and 3 hours later the animals were killed and the radioactivity in tissue fractions was determined. There was an early, sharp peak of labeling of collagen, 400-600% greater than in controls, between 2 and 4 days after surgery. The extent and timing of the peak depended on the degree of hypertrophy. An increase in the capacity for collagen labeling persisted until 30 days after surgery, the latest time studied. The peak of collagen labeling coincided with the onset of collagen accumulation in the enlarging heart. The bulk of the increase in heart collagen content occurred between days 4 and 7 of hypertrophy. Later, the rate of increase diminished.

The shapes of the curves of labeling of collagen and of nuclear DNA as a function of time after onset of hypertrophy are similar. The peak of the increase in collagen synthesis, however, is greater than that of DNA, and precedes it by about 3 days. The curves of labeling of myosin and noncollagenous protein (which is about 30% myosin) are unlike those of collagen and DNA, rising moderately within the first 2 to 4 days to a plateau approximately 100% greater than control.

A possibility that must be considered is that the differences observed in labeling patterns of myosin, collagen, and nuclear DNA represent changes in specific activities in the precursor pool (radioactive proline, lysine, or thymidine), rather than changes in their rates of synthesis. With regard to DNA we have previously shown that the rate of entry of free thymidine into heart and its subsequent turnover were not changed after aortic constriction (4). Moreover, the time course of labeling of nuclear DNA coincides closely with measurements of mitotic activity (5), and the number of nuclei labeled as measured by radioautography (2-4).

The problem is more complex, however, with respect to amino acid incorporation into proteins. A moderate increase (40%) in [3H] proline specific radioactivity 2 days after aortic constriction returned to normal values at the fifth postoperative day. This change is small in comparison to the three- to tenfold increase in collagen labeling observed the first
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2-4 postoperative days. Moreover, Kimata et al. (28) reported no change in [3H]lysine specific activity after aortic constriction in the rabbit. There are, however, other uncertainties involved in interpretation of data on incorporation even when specific activities of intracellular free amino acid are measured. The immediate protein precursor is not the free amino acid but aminoacyl transfer RNA, and several studies have suggested that total specific radioactivity of intracellular free amino acid may not necessarily represent the immediate precursor (29, 30). Another complication in the interpretation of our data is that myosin and collagen are being synthesized in different cell types, i.e., muscle and connective tissue cells, leaving open the possibility of nonparallel changes in precursor pool in the two cell types. Despite these limitations, considering the magnitude of the changes observed, it is likely that the differences in labeling patterns of collagen and myosin represent differences in patterns of synthesis.

The early increase in free proline specific activity and later increase in total proline pool is of interest because of previous observations that the rate of transport of aminoisobutyric acid is increased in hypertrophic heart (31) and also in passively stretched rabbit papillary muscle (32).

A number of possible mechanisms which might explain the observed increase in collagen labeling following aortic banding require consideration. The increased tension resulting from volume overload could be followed by an increased synthesis of collagen. A number of tissues (see ref. 33 for review), such as the uterus stretched by wax, bone subjected to the pull of a muscle, and wounds healing under tension, respond to tension by an increased synthesis of collagen. Schreiber et al. (34), however, have presented evidence that is somewhat in disagreement with our findings and opposed to the above interpretation. They found no increase in incorporation of [14C]proline into collagen hydroxyproline of hearts that were perfused under increased pressure. Incorporation of [14C]lysine into myosin was enhanced, however, by an increase in perfusion pressure.

Another possibility is that collagen or other hydroxyproline containing polypeptides are specifically involved in regulation of muscle growth, as suggested by in vitro studies of myogenesis (35). It is also possible that aortic constriction leads to relative cardiac ischemia that may serve as a stimulus for collagen synthesis. Proliferation of collateral arterioles, accompanied by increased DNA synthesis, follows gradual constriction of coronary arteries (36). The synthesis of collagen associated with small vessels might be similarly enhanced.

Another mechanism that could lead to increased collagen synthesis during cardiac hypertrophy is that of scarring following cardiac necrosis. No light microscopic evidence for necrosis, following aortic constriction, was observed in rat heart (37), although occasional infarcts were noted in rabbit heart (38). A statistical analysis of the spatial distribution of connective tissue nuclei, however, demonstrated that the nuclei were arranged more irregularly after hypertrophy than before it. This observation was compatible with the possibility that connective tissue cells developed in areas of scattered necrosis (4). By electron microscopy, scattered cell necrosis and degeneration of myofibrils were noted during the first few days after aortic constriction (6). Hatt et al. (39) described dislocation of Z-bands, fragmentation of myofibrils, and disappearance of actin filaments in rabbit hearts that had failed acutely following marked stenosis of the ascending aorta. In an alternate interpretation of these morphological changes, Bishop and Cole (40) suggested that the Z-band was altered by a newly developing sarcomere. Thus, there is disagreement regarding the positivity and interpretation of anatomical evidence for cardiac necrosis following experimental production of aortic stenosis.

Biochemical studies have failed to demonstrate changes in myocardial protein metabolism in the hypertrophic heart that are consistent with extensive myofibrillar damage.

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The turnover of the total myocardial protein was unchanged during hypertrophy (41). The rate of degradation of myosin was not altered by the gradual development of hypertrophy (28) and was actually decreased in more acute hypertrophy (42). Our observation that the peak of collagen labeling precedes that of nuclear DNA labeling suggests that, during hypertrophy, collagen synthesis does not occur initially in new connective tissue cells that have replaced damaged muscle cells.

At least four combinations of changes in heart size and tissue collagen have been observed, depending on the experimental procedure used. (1) Cardiac hypertrophy accompanied by an increase in tissue collagen concentration has been observed following repeated injections of isoproterenol to 300-g rats for 15 days (43), following constriction of the ascending aorta in 200-g rats (4, this report), 20–50 days following constriction of the main pulmonary artery of the adult cat (10); 5 months after constriction of the abdominal aorta of the weanling rat (43); and, 6 weeks after the onset of exposure of rats 2½ months old to physical stress (although no changes were observed when rats were 8 months old when stress adaptation was begun [43]). Crane and Dutta (1) observed that treatment of unilaterally nephrectomized rats with desoxycorticosterone resulted in cardiac hypertrophy accompanied by proliferation of connective tissue cells, but did not measure heart collagen. (2) Cardiac hypertrophy unaccompanied by a change in collagen concentration (i.e., the collagen increased in proportion to the heart enlargement) was observed in autopsy specimens of human hearts with left ventricular hypertrophy secondary to coronary atherosclerosis, rheumatic heart disease, renal disease, and chronic lung disease (44). Oken and Boucek (45) observed similar changes in 12 of 14 human hearts enlarged as a result of coronary atherosclerosis. In two of the hearts they studied, however, the collagen concentration was increased in association with cardiac hypertrophy. (3) Cardiac hypertrophy with no change in heart collagen content (and therefore decreased collagen concentration) was observed after 15 days of administration of thyroxine to 200-g rats (43) and in rats 3–10 weeks of age that had developed sideropenic anemia secondary to dietary deficiency (43). (4) Increased collagen concentration unaccompanied by cardiac hypertrophy was observed after long-term (320 days) exposure of 200-g rats to a simulated altitude of 5000 m (43).

The collagen produced during the latter phase of hypertrophy could alter heart function. The property of preventing distention of blood vessels has been attributed to collagen (see ref. 33). Excessive distention of the heart and the resultant inefficient muscle contraction might be similarly prevented. On the other hand, it is possible that an increased amount of collagen in the hypertrophied heart might interfere with the muscle contraction. Decreased contractility has been observed in papillary muscles of hypertrophied cat hearts (46). It is possible to produce myocardial hypertrophy with or without increased collagen (see review above). A comparison of the mechanical properties of such preparations might help to elucidate the role of collagen in altering heart muscle function.

References


Biochemical Correlates of Cardiac Hypertrophy: V. LABELING OF COLLAGEN, MYOSIN, AND NUCLEAR DNA DURING EXPERIMENTAL MYOCARDIAL HYPERTRPHY IN THE RAT

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