Quantitation of Collagen in Human Myocardium

By DONALD E. OKEN, M.D. AND ROBERT J. BOUCEK, M.D.

The collagen concentrations of the four cardiac chambers of normal hearts were determined chemically. Right ventricles and atria contained significantly higher collagen concentrations than did left ventricles and atria. The atria contained twice the collagen concentration of the ventricles. Severe atherosclerosis, hypertrophy and coronary occlusion did not necessarily increase the concentration of collagen in the left ventricle of diseased hearts.

PATHOLOGISTS frequently have described an increased proportion of collagen in microscopic sections of myocardium taken from the aged and from persons with cardiac disease. Such an increase in collagen content might alter the compliance and efficiency of the heart in much the same way that pulmonary fibrosis alters the compliance of the lung. It is the purpose of this investigation to quantitate by chemical means the amount of collagen in the myocardium of the four cardiac chambers and to correlate the collagen concentration of the myocardium with aging and heart disease.

METHOD

Human myocardium was obtained from fresh autopsy material and frozen until used. Portions of myocardium were taken from a uniform location within the right and left ventricles of 70 persons dying suddenly or violently.* The entire atria were taken from the hearts of 15 similar cadavers. There was no history of antecedent illness in most of the cases. The series contained samples of ventricle from 50 males and 20 females whose ages ranged from 22 days to 85 years. Forty-three hearts were considered normal and 27 abnormal.

Heart muscle sections, which included the entire thickness of chamber wall from endocardial to epicardial surface, each weighed approximately 1.5 Gm. The epicardium and endocardium were removed from all atrial and some ventricular samples in order to nullify and quantitate the varying ratio of endocardium to muscle mass found in chambers of different thickness.

Samples of the left and right ventricles of 7 hearts were analyzed concurrently, each series of samples containing hearts randomly selected for age and disease history. The collagen concentration of the samples was calculated from the concentration of hydroxyproline, an amino acid which is found in constant amounts in collagen but absent from muscle protein.1 After hydrolysis of the samples in 6N HCl for 16 hours at 110 C, the hydroxyproline concentration was determined colorimetrically with reproducibility within 10 per cent by the technic of Neuman and Logan.1 A gravimetric determination of collagen was made on 12 of the samples by the method of Lowry, Gilligan and Katersky2 for comparison with the chemically determined results. The nitrogen concentration of each hydrolysate and gravimetric sample was quantitated by the Conway method3 after Kjeldahl digestion,4 and the total hydroxyproline and collagen values were expressed as a percentage of the total protein in each sample.

RESULTS

When the concentration of hydroxyproline (mg./100 mg. protein) of each sample was multiplied by 7.46 (collagen contains 13.4 per cent hydroxyproline),1 collagen values were obtained which fell within 20 per cent of those obtained by the gravimetric determination. In our hands the hydroxyproline technique was considerably more reproducible and reliable than was the gravimetric technic. The quantity of collagen found by the gravimetric technic was generally lower than that derived by hydroxyproline determination, regardless of the amount of collagen present in the specimens. This was possibly because of a loss of collagen during NaOH extraction of the proteins during the gravimetric determination. Samples of left and right ventricle from which the endo- and epicardium were removed prior to hydroxyproline determination had values averaging 12 per cent less than those obtained when these samples were left intact.

Hydroxyproline Concentration of Cardiac Chambers

Ventricles. The right ventricles contained a larger hydroxyproline concentration than did

* A table containing the disease histories and data for values of all specimens analyzed may be obtained from the authors on request.

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The mean values for the entire series were 0.99 per cent and 0.74 per cent in the right and left ventricles respectively. When the samples were grouped by decades of age (fig. 1, table 2) the right ventricle values were statistically higher (p < 0.05) than those of the left ventricle in all decades except the second. The paucity of samples from the second decade precluded any statistical comparison.

Atria. The small quantity of muscle in the atria in comparison with the large amount of dense connective tissue in the epicardium necessitated the removal of the latter layer from all atria. Because removal of the epicardium from the atria of children leaves insufficient muscle for analysis, the series contained atria taken only from adults. Atrial samples were obtained from individuals whose ages ranged from 33 to 69 years.

The hydroxyproline concentrations were 1.79 ± 0.19 per cent in the left atrium and 2.24 ± 0.36 per cent in the right atrium. The difference between the hydroxyproline concentrations was statistically significant (p < 0.02).

Hydroxyproline Concentration of Ventricles from Different Age Groups

The grouping of samples of ventricle according to the age of the donors revealed no difference in hydroxyproline concentration other than that found in the first decade of life. Here (see table 1) the mean hydroxyproline value for the left ventricle, 0.82 per cent, was greater (p < 0.05) in the first decade than in any other decade except the third and seventh. In the third and seventh decades the spread of data reduced the significance below the 5 per cent confidence level. The right ventricle similarly showed a significantly higher concentration of hydroxyproline (1.30 per cent) in the first decade (table 1). The values for hydroxyproline concentration in both the right and left ventricles of the other decades were statistically equivalent.

Hydroxyproline Concentration of Ventricles from Diseased Hearts

In this study, hearts weighing more than 450 Gm. and/or having more than moderate coronary atherosclerosis were designated as abnormal. Exclusive of cases showing gross infarction, 22 hearts appeared in this category. Thirteen of these displayed severe coronary atherosclerosis; 14 hearts exhibited significant hypertrophy. In only 2 of the abnormal hearts was the hydroxyproline concentration elevated in the left ventricle. All other hearts in the disease group, with the exception of infarcted hearts, had a hydroxyproline concentration in the left ventricle similar to that of the normal heart.

Five hearts contained areas of gross myocardial infarction. Three of these had an increased hydroxyproline concentration in the sample of left ventricle assayed, 1 of them remarkably increased; but in two hearts showing gross infarction the values were normal. The latter specimens were taken far from the areas of infarction, while the former were taken...
TABLE 1.—Hydroxyproline Concentrations of Ventricles from Different Age Groups (mg./100 mg. protein)

<table>
<thead>
<tr>
<th>Decade</th>
<th>Mean values and standard deviation</th>
<th>Mean values and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1 &gt; 3</td>
<td>0.82 ± 0.18</td>
<td>0.69 ± 0.10</td>
</tr>
<tr>
<td>1 &gt; 4</td>
<td>0.82 ± 0.18</td>
<td>0.67 ± 0.15*</td>
</tr>
<tr>
<td>1 &gt; 5</td>
<td>0.82 ± 0.18</td>
<td>0.64 ± 0.10*</td>
</tr>
<tr>
<td>1 &gt; 6</td>
<td>0.82 ± 0.18</td>
<td>0.66 ± 0.14*</td>
</tr>
<tr>
<td>1 &gt; 7†</td>
<td>0.82 ± 0.18</td>
<td>0.71 ± 0.10</td>
</tr>
</tbody>
</table>

* Statistically significant difference p = <0.05.
† Elevated values from gross myocardial infarction excluded.

DISCUSSION

In 1950, Blumgart, Gilligan and Schlesinger used a gravimetric technic to determine the collagen content of 54 left ventricles. In their study no correlation could be found between the collagen content of the myocardium and the age or sex of the individuals from whom the samples were taken. They were, however, able to show an increased collagen content in 9 out of 23 hypertrophied hearts and in 7 out of 8 hearts with myocardial infarction. No assessment of the collagen content of the right ventricles or atria was reported.

In the present study the collagen content of myocardium was derived from the hydroxyproline concentration. It was shown that the relationship between the collagen values calculated from hydroxyproline concentration and those obtained by gravimetric analysis of the samples is constant. The factor 7.46 of Neuman and Logan was approximated within the wide close to, or including the infarcted myocardium. Three cases of coronary occlusion without gross infarction showed no increase in the hydroxyproline concentration in either ventricle. Of 6 cases with a past history of congestive failure only 1 showed a high concentration of hydroxyproline in the left ventricle. In only 5 of the entire group of right ventricles were the hydroxyproline values more than one standard deviation above the mean. None of these was obtained from an abnormal heart. In no case did the right ventricle hydroxyproline value differ 2 standard deviations from the mean.

Total Collagen Content in Various Chambers.

The normal adult human heart with a left ventricle weighing approximately 130 Gm. has a right ventricle weighing 70 Gm. Right and left atria weigh approximately 25 Gm. and 24 Gm. respectively. By dividing the weight of each chamber by 6.25 (protein comprises approximately 16 per cent of the mass of heart muscle) its theoretical total protein is deduced. The total protein content of each chamber multiplied by the mean hydroxyproline concentration in specimens of this series gave the following theoretical total hydroxyproline contents: left ventricle 0.15 Gm., right ventricle 0.11 Gm., left atrium 0.06 Gm., and right atrium 0.09 Gm. Multiplication of these figures by 7.46 gave total collagen contents of 1.15 Gm. and 0.83 Gm. in the left and right ventricles, and 0.45 Gm. and 0.67 Gm. in the left and right atria respectively.

Hydroxyproline Content of Males and Females.

In no age group did males and females exhibit any discernible difference in the hydroxyproline concentration of the ventricles.

TABLE 2.—Comparison Between the Hydroxyproline Concentrations of Left and Right Ventricles from Different Age Groups

<table>
<thead>
<tr>
<th>Decade</th>
<th>Hydroxyproline, mg./100 mg. protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
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<tr>
<td>6</td>
<td>0.66 ± 0.14</td>
</tr>
<tr>
<td>7</td>
<td>0.71 ± 0.10†</td>
</tr>
</tbody>
</table>

Mean values and standard deviations.
* Statistically significant difference, p < 0.05.
† Elevated values from gross myocardial infarction excluded.
limits of error found for the gravimetric technique regardless of the amount of hydroxyproline present in any sample. It seems valid, therefore, to interchange the terms hydroxyproline and collagen content after multiplying the former by 7.46.

Only 2 of 14 hearts with significant degrees of hypertrophy show an increased concentration of collagen in the left ventricle. That the ratio of collagen to total protein remains constant in hypertrophy is not, however, what would be expected if the increase in weight of the heart were due solely to an increase in muscle mass. If muscle mass alone increased the concentration of collagen in the muscle, protein would, in fact, decrease. Because concentration of collagen in left ventricular hypertrophy remains constant the total amount of collagen must actually be increased to the same degree as the increase in muscle tissue.

Coronary atherosclerosis per se does not appear to produce an increased collagen concentration in the left ventricle in the present series. Indeed, of the 6 specimens from individuals with known histories of congestive heart failure secondary to coronary artery disease, only 1 has an elevated collagen concentration in the left ventricle. Furthermore, normal values for collagen are found in 3 recent coronary occlusions without infarction and in 2 out of 5 hearts with old myocardial infarction. The specimens with infarction and increased collagen concentration were taken from within, or immediately surrounding, areas of infarction while those with normal values were obtained further from the infarcted areas. The areas of patchy myocardial fibrosis described by pathologists in severe coronary artery disease without gross infarction cannot, however, be denied. From the above findings it would appear that these areas probably do represent small areas of actual infarction rather than resulting from the generalized myocardial ischemia of coronary atherosclerosis.

The significance of the difference in collagen concentration in the 4 cardiac chambers is obscure. Neuman and Logan\(^1\) showed the collagen values for the right ventricle to be higher than those for the left in pig and beef hearts. No values for atria were given. Harman and Weber\(^4\) made no mention, however, of a difference between right and left ventricle collagen concentrations in their study of a series of 34 normal human hearts. Using the gravimetric technique for collagen determination, they showed that the atria of adults were richer in collagen than were the ventricles, while in infants and children up to 10 years of age all chambers had approximately equal collagen concentration.

In the present study we find both chambers of the right side of the heart to have a greater collagen concentration than do those of the left side. The finding of higher right than left ventricle collagen values in a stillborn 7-month premature infant (not included in this series) and in infants aged 22 days, 3 months and 5 months suggests that the collagen content of the 2 ventricles is different at birth. The atria of children were not studied in the present series, but no significant difference is seen between the atrial values obtained for middle-aged and old persons.

The finding that collagen concentration in ventricles of the first decade is higher than in those of later decades must indicate a relatively faster rate of growth of muscle than of collagen during the attainment of normal adult heart size. In this respect normal growth of the heart differs from hypertrophy where the per cent increase in muscle tissue is equal to the per cent increase in collagen.

We have been unable to show any increase in collagen concentration of the myocardium with aging or disease that might alter the compliance of the ventricles. If altered cardiac compliance is a factor in cardiac failure, it must result from either a change of physical state in a normal quantity of collagen or from an alteration in the muscle per se. The relationship between the differences in behavior of the cardiac chambers and their different collagen contents is obscure.

**SUMMARY**

The total collagen content in the myocardium of the chambers of a normal 250 Gm. heart is approximately: left ventricle 1.15 Gm., right ventricle 0.83 Gm., left atrium 0.45 Gm., right atrium 0.67 Gm.

The collagen concentration in the right heart
is considerably higher than that of the left. Atria contain more than twice the collagen percentage of the ventricles.

The increased collagen content of hearts with myocardial infarction represents circumscribed scar tissue of healing.

During the first decade of life the collagen concentration of the ventricles is greater than that in the later decades. No alteration in the collagen content of normal ventricles is evident after the third decade. During normal growth the muscle content of the heart increases at a faster rate than does the collagen.

Left ventricular hypertrophy results in an increase in the collagen content of the left ventricle to the same degree as the increase in muscle tissue.

**Summario in Interlingua**

Le contento de collageno in le massa myocardial del cameras de un normal corde de 250 g de peso attinge approximativamente le sequente totales: Ventriculo sinistre—1,15 g; ventriculo dextere—0,83 g; atrio sinistre—0,45 g; e atrio dextere—0,67 g.

Le concentration de collageno in le corde dextere es considerablemente plus alte que in le corde sinistre. Atrios contine plus que duo vices le percentage de collageno trovate in ventriculos.

Le augmentate contento de collageno in cordes con infarcimento myocardial representa circumscripita areas de tessuto cicatrice in le processus del curation.

Durante le prime decennio del vita le concentration de collageno in le ventriculos es plus alte que durante le sequente decennios.

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7. **BLOMGART, H. L., GILLIGAN, D. R., AND SCHLESSINGER, M. J.:** The degree of myocardial fibrosis in normal and pathologic hearts as determined chemically by the collagen content. Tr. A. Am. Physicians 56: 313, 1940.

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