Collagen and Elastin Content in Canine Arteries Selected from Functionally Different Vascular Beds

By Grace M. Fischer, M.D., and Josep G. Llaurado, M.D.

ABSTRACT
The amount of collagen and elastin in normal canine arteries was determined biochemically in nine different sites. The total collagen and elastin ranges from 58 to 75% of the weight of the dry defatted artery. Reasons are adduced to propose that the expression of results as the ratio of collagen to elastin (C/E) is a useful index of the relative distensibility for the maintenance tension of arterial wall. Two arteries, carotid and renal, which are pathways to blood pressure sensors, have a statistically significantly higher C/E than the femoral and mesenteric arteries, which are pathways to regulated beds. The highest C/E was found in the coronary artery. Results are interpreted in relation to the function of the vascular beds to which the representative arterial specimens belong.

ADDITIONAL KEY WORDS connective tissue hydroxyproline coronary artery mesenteric arterial bed stiffness of arteries blood pressure regulation passive tension in arterial wall elasticity

Although connective tissue accounts for well over half of the dry weight of the arterial wall, little attention has been paid to the content of collagen and elastin in arteries. Studies relating the content of collagen and elastin in arteries to function in different vascular beds are even scarcer. However, Burton,1,2 in discussing the contribution of elastin and collagen to mechanical properties of arteries, stated that these connective tissue elements account for passive tension of the wall. These elements would thus play an important role in contributing to blood pressure regulation without the expenditure of energy.

During an investigational program to characterize control of cardiovascular function, and hence blood pressure, in physico-mathematical terms,3-5 it was believed important to obtain quantitative information on the chemical properties of vascular walls. On the basis of control systems analysis, some cardiovascular components can be grouped as (a) the system to be controlled and (b) the controlling system. Representative vessels from both parts were chosen for this study.

Previous work on the content of collagen and elastin in arteries has been mostly concerned with the aorta6-11; although Harkness et al.12 studied additional arterial sites in a few dogs. The present study attempts to correlate collagen and elastin content with function in vascular beds which exhibit a qualitatively different character in the regulation of blood pressure.

Methods
Ten adult male mongrel dogs weighing 18 to 24 kg and without any recognizable disease were killed instantaneously with a Schermer mechanical stunning apparatus applied to the occipital area. Arterial specimens of about 80 mg were immediately obtained.

CHOICE OF ARTERIAL SITES
Of arteries with controlling mechanisms, the carotid artery was chosen because it is close to the carotid sinus baroreceptors and it supplies blood to the brain. The renal artery was selected because the kidney is involved in a humoral mechanism which can regulate blood pressure.
Of arteries belonging to the controlled system, it was decided to study both the ascending and the abdominal aorta as representative large vessels, the femoral artery as a vessel feeding a skeletal muscle bed, and the mesenteric artery since the splanchnic area contains the highest percentage of blood flow of any of the major systemic vascular beds. The coronary artery site was selected because information on its connective tissue composition is lacking and because of its general importance. The base of the left coronary artery and its ventral interventricular branch were dissected by inserting a 19-gauge blunt end cannula 11.5-cm long into the coronary ostium and threading it into the artery up to the cannula hilt. This insertion allowed maneuverability of this very thin-walled vessel so that it could be freed from its adherent bed.

The exact location of the sites is shown in figure 1. In some dogs, not all arterial sites were used.

**BIOCHEMICAL DETERMINATIONS**

After removal from the animal, the arterial segments were weighed, dried for 48 hr at 100°C and reweighed; the percent of water was calculated. They were then 'defatted' by successive immersions in (a) acetone for 18 hr, (b) acetone for 6 hr, and (c) ether for 18 hr. This standard laboratory procedure extracts sufficient fat to give a reproducible dry weight, although total fat is not removed from the tissue. With this reservation and for simplicity when expressing results, the term 'dry defatted tissue' will be used. In spite of its limitations, it is believed that the inclusion of data for extracted fat may still have some comparative value among different arteries. The segments were then dried for 24 hr at 100°C and reweighed; the percent of extracted fat and water was calculated.

Collagen and elastin were separated by autoclaving each arterial specimen in 4 ml of distilled water for 18 hr at a pressure* of 10.3 to 13.8 N/cm² (15 to 20 lb/in²), decanting the supernatant, reautoclaving the residue in 4 ml of distilled water for 3 hr and combining both supernatants. Collagen is contained in the supernatant, whereas elastin remains in the residue.

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*The General Conference on Weights and Measures in formally recognizing a generalized metric International System of Units, officially designated SI, has especially repudiated the kg (force) as a unit, and adopted the newton, officially abbreviated N, as the unit of force. One newton is the force exerted by 1 kilogram (mass) when subjected to an acceleration of 1 meter per second squared. The equivalence in British-American units is 1 N = 0.2248 lb(force).

The prototype for this procedure of separating collagen and elastin was established by Neuman and Logan, who found that a third 3-hr period of autoclaving resulted in less than 1% of additional collagen extracted from arterial segments.

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**FIGURE 1**

Arterial sites analyzed for collagen and elastin: 1, left coronary artery and ventral interventricular branch; 2, ascending aorta; 3, common carotid artery; 4, abdominal aorta; 5, cranial mesenteric artery, proximal; 6, cranial mesenteric artery, distal; 7, cranial mesenteric artery, branches; 8, renal artery; and 9, femoral artery.
The amounts of collagen and elastin in arterial wall were calculated from their hydroxyproline content. Hydrolysis to hydroxyproline was accomplished by concentrating the collagen extract to 3 ml and adding 3 ml of 12N HC1, adding 6 ml of 6N HC1 to the residue containing elastin, and autoclaving both tubes for 18 hr at 15 to 20 lb/in^2 pressure. For hydroxyproline determinations the method of Neuman and Logan incorporating modifications by Martin and Axelrod was used in preliminary experiments. On some occasions, however, development of an unstable or nonspecific chromogen ensued. For this reason, some additional modifications to the above procedures were made in this laboratory: (a) Preparation of p-dimethylaminobenzaldehyde reagent. Concentrated H_2SO_4, 22.2 ml, was slowly added to 100 ml of absolute ethanol (substituting for n-propanol) in a beaker and the mixture was cooled. Separately, 6 g of p-dimethylaminobenzaldehyde was dissolved in 100 ml of absolute ethanol. Then both solutions were mixed. When stored in the refrigerator, the reagent keeps for several weeks, (b) Use of 30% H_2O_2 (Superoxol, Merck) instead of 6% H_2O_2. (c) Addition of 0.2 ml M/20 FeSO_4 instead of 0.1 ml. The method thus modified has proved to be consistently reliable.

To obtain the quantity of collagen, the hydroxyproline content was multiplied by a factor of 10. For elastin the factor was 64.1 as derived from the data by Harkness et al. Results are finally expressed as percent (w/w) of dry defatted artery constituents.

### Results

The results are presented in table 1. It should be borne in mind, however, that there are extracellular ground substance and some connective tissue cells which are not measured by the analytical method. Their contribution to the ratio of collagen to elastin is probably small as compared to that of extracellular ground substance and some connective tissue cells. Moreover, their contribution to the ratio is probably small as compared to that of extracellular ground substance. The total connective tissue defined as the sum of collagen and elastin ranges from 58 to 75% of dry defatted weight. These values are in agreement with those reported by Neuman and Logan in beef, pig, and rat aorta. The results presented in table 1 are in agreement with those reported by Harkness et al. in dogs. The method used in this laboratory is the method of Neuman and Logan incorporating modifications by Martin and Axelrod. On some occasions, however, development of an unstable or nonspecific chromogen ensued. For this reason, some additional modifications to the above procedures were made in this laboratory: (a) Preparation of p-dimethylaminobenzaldehyde reagent. Concentrated H_2SO_4, 22.2 ml, was slowly added to 100 ml of absolute ethanol (substituting for n-propanol) in a beaker and the mixture was cooled. Separately, 6 g of p-dimethylaminobenzaldehyde was dissolved in 100 ml of absolute ethanol. Then both solutions were mixed. When stored in the refrigerator, the reagent keeps for several weeks, (b) Use of 30% H_2O_2 (Superoxol, Merck) instead of 6% H_2O_2. (c) Addition of 0.2 ml M/20 FeSO_4 instead of 0.1 ml. The method thus modified has proved to be consistently reliable. To obtain the quantity of collagen, the hydroxyproline content was multiplied by a factor of 10. For elastin the factor was 64.1 as derived from the data by Harkness et al. in dogs. Results are finally expressed as percent (w/w) of dry defatted artery constituents.

### Table 1

<table>
<thead>
<tr>
<th>Artery</th>
<th>Percent of wet tissue</th>
<th>Percent of dry defatted tissue</th>
<th>Ratio Collagen/Elastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary</td>
<td>63.2 ± 1.0</td>
<td>71.5 ± 1.1</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Aorta, ascending</td>
<td>73.8 ± 0.6</td>
<td>74.0 ± 0.5</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>Carotid</td>
<td>71.1 ± 0.1</td>
<td>71.2 ± 0.1</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Aorta, abdominal</td>
<td>70.4 ± 0.4</td>
<td>70.8 ± 0.3</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Cranial mesenteric,</td>
<td>70.8 ± 0.5</td>
<td>71.6 ± 0.4</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>distal</td>
<td>71.4 ± 0.4</td>
<td>72.0 ± 0.4</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>Cranial mesenteric,</td>
<td>69.5 ± 0.6</td>
<td>73.1 ± 0.7</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>branches</td>
<td>70.4 ± 0.7</td>
<td>70.8 ± 0.7</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>Renal</td>
<td>68.0 ± 0.3</td>
<td>68.1 ± 0.3</td>
<td>0.97 ± 0.02</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation of the mean. All percent values refer to w/w.
†Number of specimens.
‡Specimens slightly dehydrated owing to unavoidably long dissection.

The amounts of collagen and elastin in arterial wall were calculated from their hydroxyproline content. Hydrolysis to hydroxyproline was accomplished by concentrating the collagen extract to 3 ml and adding 3 ml of 12N HC1, adding 6 ml of 6N HC1 to the residue containing elastin, and autoclaving both tubes for 18 hr at 15 to 20 lb/in^2 pressure. For hydroxyproline determinations the method of Neuman and Logan incorporating modifications by Martin and Axelrod was used in preliminary experiments. On some occasions, however, development of an unstable or nonspecific chromogen ensued. For this reason, some additional modifications to the above procedures were made in this laboratory: (a) Preparation of p-dimethylaminobenzaldehyde reagent. Concentrated H_2SO_4, 22.2 ml, was slowly added to 100 ml of absolute ethanol (substituting for n-propanol) in a beaker and the mixture was cooled. Separately, 6 g of p-dimethylaminobenzaldehyde was dissolved in 100 ml of absolute ethanol. Then both solutions were mixed. When stored in the refrigerator, the reagent keeps for several weeks, (b) Use of 30% H_2O_2 (Superoxol, Merck) instead of 6% H_2O_2. (c) Addition of 0.2 ml M/20 FeSO_4 instead of 0.1 ml. The method thus modified has proved to be consistently reliable. To obtain the quantity of collagen, the hydroxyproline content was multiplied by a factor of 10. For elastin the factor was 64.1 as derived from the data by Harkness et al. in dogs. Results are finally expressed as percent (w/w) of dry defatted artery constituents.
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centage, 46 to 71, but strict comparison is not possible because different arterial sites were used.

For reasons detailed in the Discussion, more functional significance is attached to the relative value of the ratio of collagen to elastin (C/E) than to the absolute percentage of either constituent. The highest C/E was found in the coronary artery, followed by the carotid and renal arteries. It is of interest that even though the cranial mesenteric and renal arteries leave the aorta in close proximity, the C/E is statistically significantly higher in the renal artery than in any of the three parts of the cranial mesenteric artery; proximal (P < 0.005 by the t-test), distal (P < 0.025) or distal branches (P < 0.01). Even more significant is the high C/E in the carotid artery as compared with all the mesenteric arterial sites. Both carotid and renal arteries have a statistically significantly higher C/E than the femoral artery (P < 0.005 and P < 0.05, respectively).

It should be noted that the composition of arteries does not merely reflect connective tissue content of the part of the aorta from which they arise, since the coronary and carotid with high C/E arise originally near the ascending aorta with low C/E. Likewise, although the section of the abdominal aorta studied was that between the mesenteric and renal arteries (fig. 1), there was a significantly different C/E between the latter two.

The lowest percentage of total connective tissue was found in the branches of the cranial mesenteric artery, these being the smallest vessels analyzed. It is of interest that the coronary artery was shown to have the lowest elastin content of all the arteries studied. It should be remarked here that the high fat content apparently present in the coronary artery was, at least partly, due to the fact that this vessel in the dog lies in a fatty bed and, in spite of the utmost care in dissecting it, probably some extraneous adipose tissue was carried over to the analytical procedure. Since the data for collagen and elastin are expressed as a percentage of dry defatted tissue, this unavoidable technical imperfection does not influence the validity of the results for connective tissue.

Discussion

A brief review of the function of the vessel wall constituents is considered necessary to evaluate the significance of these results. Smooth muscle contraction, with expenditure of energy, accounts for active tension of the vascular wall; Peterson and co-workers have extensively discussed the properties of smooth muscle and the mechanical properties of the arterial wall. However, it is also well established that the maintenance of tension without the steady expenditure of energy is one of the functions of both the elastic and collagenous fibers in the vessel wall. Furthermore, the relative roles of collagen and elastin in determining the tension-strain curves for vascular walls have been discussed in detail by Burton and Roach and Burton. According to their hypothesis: (a) the tension-strain relationship at low levels would resemble that for elastin fibers, i.e., these fibers would contribute mainly at low pressures to the elastic properties; (b) the tension-strain relationship at high levels would resemble that for collagen fibers, i.e., these fibers would be stretched only at normal and higher pressures and would have a protective supporting role. As pressure rises, the tension in the wall would rise rapidly with little further deformation (high coefficient of elasticity). In addition, elastin fibers would have the function of producing stability of the wall at low and normal blood pressure by facilitating stable graded contractions of the smooth muscle, thus tending to prevent the fundamental instability of the wall that leads to all-or-none responses (sphincter type action of smooth muscle alone). A recent study on the elastic behavior of arteries confirms that the increasing degree of stiffness, as internal pressure increases, approaches that of collagen fibers.

The question may arise as to whether arrangement as well as relative amount of collagen fibers might influence distensibility. An oblique, lacelike arrangement would allow...
more distensibility than a circumferential one. However, light and electron microscopic studies on the media of the rabbit aorta indicate that collagen fibers are indeed arranged nearly circumferentially. Thus, the amount of collagen is the all-important factor. Hypothetically, the only situation in which comparison of collagen in different beds would bear no close relationship to their relative distensibilities would be in beds having different structural arrangement of their collagen fibers. This has not yet been studied and so remains unsubstantiated.

In the light of the preceding considerations, it is proposed that the expression of collagen-to-elastin ratio may be useful in establishing an index of relative distensibility for the maintenance tension of arterial wall. The fact that the renal and carotid arteries were shown to have a high C/E would indicate that they are less distensible (stiffer). This property would serve them well in their function as obligatory pathways to sensors for blood pressure regulation. It is indeed unnecessary to elaborate here on the role of the carotid sinus. Skinner et al. have provided strong evidence that the mean blood pressure in the renal artery is the signal which influences the formation of renin by the kidney. In the present study, the specimens of carotid and renal arteries were taken proximal to the actual baroreceptor site. If the proximal vessels tend toward rigidity, especially at high pressures, the pressure in these vessels would be transmitted with less alteration to the detector, affording the possibility for more accurate counterregulation.

The mesenteric circulation plays an important role in the control of blood pressure. Not only does the splanchnic bed have the largest percentage of blood flow of any of the systemic beds, but the splanchnic circulation represents a pool that is very mobile in response to autonomic activity. Folkow et al. found that the resting level of blood flow to the intestine was as big as, or bigger than, the maximum blood flow capacity of skeletal muscle. Moreover, 30 to 40% of the blood content of intestine was expelled with norepinephrine injection (maximum sympathetic activity). The splanchnic viscera have the lowest resistance of the systemic vascular beds and may contain perhaps 35% of the total blood volume. The higher structural distensibility of the mesenteric bed, as shown by the moderate C/E, is consistent with the functional activity of this vascular bed as determined by these other investigators.

Since segmental differences have been found in the response of the mesenteric artery to drugs, especially serotonin, three different sites of mesenteric artery were included in this study. Results show that there is no marked difference in the C/E. There appears, however, to be a gradual diminution in total percentage of connective tissue as the artery extends peripherally. The lower percentage in the small branches would be consistent with a higher content of smooth muscle.

Peterson et al., after studying pressure-strain relationship, calculated the elastic modulus (E_p) in vivo for the arterial wall in several sites. Their lowest E_p (highest distensibility) was found in the thoracic aorta, whereas a higher E_p was found in the abdominal aorta, and even higher in the femoral and carotid arteries. The C/E for these arteries as reported herein follows the same order of increasing stiffness as the E_p values.

Another relevant finding is the high C/E, in fact the highest found in this work, for the coronary artery. This could be interpreted as a property destined to limit the distensibility of this vital artery. Scott et al. suggested that the gradually declining rate of fall of resistance as flow rate is increased in this artery might be accounted for as a gradual approach to the limit of distensibility of this vascular bed. Thus the relatively high collagen content of the coronary artery would provide a structural basis to the above functional observation. This biochemical stiffness occurs in an artery that is subjected to external stress, namely the force of the contracting myocardium.

The markedly low C/E ratio of the ascending aorta describes its great distensibility.
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and function as a reservoir during systole. In contrast, the abdominal aorta is much stiffer. These results are consistent with the finding of Harkness et al. that a transition in elastin content occurs about the level of the diaphragm. Since the diaphragm represents the dividing line between the protected thoracic cavity and the abdominal cavity, this is another example from which one can speculate that the external environment of an artery may also bear some relationship to its connective tissue content.

References

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doi: 10.1161/01.RES.19.2.394

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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