Quantitative Analysis of the Development of Experimental Atherosclerosis in the Dog

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With the assistance of Almorris Lynch

ONE THEORY of atherosclerosis states that in the development of that disease, intact lipoproteins containing cholesterol pass from plasma into arterial walls.\(^1\)\(^2\) If this is true, then one might expect the concentrations of cholesterol appearing in arterial walls in the development of atherosclerosis to correlate with the rates of entrance into arterial walls of a simple plasma protein like albumin. Albumin is known to enter the aortic wall of the dog with a gradient of rates.\(^3\) It enters fastest proximally and progressively less rapidly down the length of the aorta. Thus the aortic wall of the dog seemed to be a suitable place to look for the possible correlation. The present study was designed to determine whether such a correlation does, in fact, exist there.

Method

Adult mongrel dogs of both sexes, weighing between 9 and 16 Kg., were used in this study. Twenty normal dogs served as controls. After blood samples had been obtained, they were sacrificed and the aorta dissected. In the dissection, the aorta from the valve ring to the brachiocephalic artery was separated into the outer and the inner side of the arch. Each of these sites was split into an inner layer containing intima and media, a middle layer consisting of media, and an outer layer consisting of media and adventitia. The small portion of aorta from which the brachiocephalic and left subclavian arteries arise was discarded. This section was also discarded in our previous studies on albumin\(^3\) and labeled cholesterol.\(^4\) The descending thoracic aorta below the left subclavian artery was divided into upper, middle, and lower thirds. Each of these was split into 2 layers: an inner layer of intima and media, and an outer layer of media and adventitia. The small portion of aorta from which the brachiocephalic and left subclavian arteries arise was discarded. This section was also discarded in our previous studies on albumin\(^3\) and labeled cholesterol.\(^4\) The descending thoracic aorta below the left subclavian artery was divided into upper, middle, and lower thirds. Each of these was split into 2 layers: an inner layer of intima and media, and an outer layer of media and adventitia. The abdominal aorta was also separated into the same 2 layers. The outer ascending aorta and arch were called site 1; the inner ascending aorta and arch, site 2; the upper, middle, and lower thirds of the thoracic descending aorta, sites 3, 4, and 5, respectively; and the abdominal aorta, site 6. The cholesterol concentrations of the sera and the aortic sites were determined by methods previously described.\(^4\)

The results were expressed on a wet weight basis. Twenty-one dogs were fed 2 Gm. of thiouracil and 10 Gm. of cholesterol daily. The cholesterol was dissolved in hydrogenated vegetable oil and added to a commercial dog food. In the final mixture, protein supplied 19%, fat 46%, and carbohydrate 35% of the dietary calories. Sera were obtained periodically from the dogs before their daily feeding and were analyzed for cholesterol. Fifteen of these experimental dogs were sacrificed after 26 to 41 (average 33) days and 6 after 129 to 181 (average 150) days of feeding thiouracil and cholesterol. The aortas were dissected and analyzed for cholesterol, as described above. Three dogs were given 2 Gm. of thiouracil by mouth daily and a diet containing no added cholesterol. Sera were obtained once a week and analyzed for cholesterol. These dogs were sacrificed at from 157 to 172 days and the aortas dissected and analyzed for cholesterol.

Statistical Analysis of the Data

For statistical analysis, data from individual sites were sometimes combined to give the cholesterol concentrations in larger areas. The differences between the concentrations in the areas considered were determined for each control and experimental aorta. In all significance tests the p values corresponding to the absolute values for the t's were used.

Let \(d\) be the mean difference between any 2 sites in the control aortas and \(D\) the mean difference between those sites in the experimental aortas. The descending thoracic aorta below the left subclavian artery was divided into upper, middle, and lower thirds. Each of these was split into 2 layers: an inner layer of intima and media, and an outer layer of media and adventitia. The abdominal aorta was also separated into the same 2 layers. The outer ascending aorta and arch were called site 1; the inner ascending aorta and arch, site

\[
 t = \frac{(D-d)}{\sqrt{SE_a^2 + SE_b^2}}
\]

was used. Since the variances from the control and experimental aortas were different, they were not pooled in the computations. Degrees of freedom were computed from a formula for estimating them when the variances are different.\(^5\)

Circulation Research, Volume VIII, September 1960 1023

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Received for publication May 6, 1960.
Table 1
Mean Concentrations* of Cholesterol in the Aortas of Twenty Normal Dogs

<table>
<thead>
<tr>
<th>Site</th>
<th>Inner</th>
<th>Middle</th>
<th>Outer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25</td>
<td>1.48</td>
<td>1.90</td>
</tr>
<tr>
<td>2</td>
<td>1.21</td>
<td>1.46</td>
<td>1.43</td>
</tr>
<tr>
<td>3</td>
<td>1.39</td>
<td></td>
<td>1.51</td>
</tr>
<tr>
<td>4</td>
<td>1.47</td>
<td></td>
<td>1.60</td>
</tr>
<tr>
<td>5</td>
<td>1.51</td>
<td></td>
<td>1.59</td>
</tr>
<tr>
<td>6</td>
<td>1.77</td>
<td></td>
<td>1.13</td>
</tr>
</tbody>
</table>

*Concentration is expressed as milligrams of cholesterol per gram of aorta.
†Sites and layers are defined in the text.

Results
The data obtained in this study are presented as averages. In general, there was a good deal of variation in the data and the regularities to be described were not always seen in individual dogs.

The mean aortic cholesterol concentrations of the 20 control dogs are printed in Table 1. The mean concentrations for the inner layer formed a gradient. The concentration was least in the ascending aorta and rose progressively along the length of the aorta. The mean difference between the concentration in the inner layer of the ascending aorta and that in the inner layer of the descending thoracic aorta was significant at the 0.4 per cent level. The mean difference between the concentration in the inner layer of the descending thoracic aorta and that in the inner layer of the abdominal aorta was significant at the 0.1 per cent level.

The average plasma cholesterol concentration of the control dogs and of the other dogs prior to the experimental regimen was 60 mg. per 100 ml. In the dogs fed thiouracil and cholesterol, the plasma concentration of cholesterol rose in a roughly linear fashion to an average of 1,060 mg. per 100 ml. at the seventh week. Thereafter, it averaged 820 mg. per 100 ml.

The mean cholesterol concentration was computed for each site in the dogs fed thiouracil and cholesterol for an average of 33 days. From these values were subtracted the mean control values for the corresponding sites to obtain the mean increment for each site. These increments are printed in Table 2. The mean increments in the inner aortic layer formed a gradient. The increment was greatest in the ascending aorta and progressively less down the length of the aorta. The mean increment in the ascending aorta was 6 times that in the abdominal aorta. The difference in the mean increment in the inner layer of the ascending aorta is significantly different from that increment at site 3 at the 1 per cent level. The difference in the mean increment in the inner layer at site 3 is significantly different from the increment in the abdominal aorta at the 5 per cent level.

In the dogs sacrificed late in the course of the experiment (at an average of 150 days) a gradient in the inner layer no longer existed. Instead, in each dog the cholesterol increment in the inner layer of the abdominal aorta exceeded that in the inner layer of the thoracic aorta.

None of the dogs sacrificed early in the course of the experiment had macroscopic atherosclerotic lesions of the aorta. Four of the dogs sacrificed late showed macroscopic lesions of the aorta. These consisted of small atheromatous plaques around the intercostal...
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Table 2
Mean Increments of Cholesterol Concentrations* in the Aortas of Dogs Fed Cholesterol and Thiouracil

<table>
<thead>
<tr>
<th>Time on experimental regimen and number of dogs</th>
<th>25-41 days (15 dogs)</th>
<th>120-181 days (8 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Inner</td>
<td>Middle</td>
</tr>
<tr>
<td>1</td>
<td>1.21</td>
<td>.58</td>
</tr>
<tr>
<td>2</td>
<td>.71</td>
<td>.38</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
<td>.36</td>
<td>.33</td>
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<tr>
<td>5</td>
<td>.23</td>
<td>.66</td>
</tr>
<tr>
<td>6</td>
<td>.16</td>
<td>.26</td>
</tr>
</tbody>
</table>

*Increment in cholesterol concentration equals the mean concentration in the experimental dogs minus the mean concentration in the control dogs and is expressed as milligrams of cholesterol per gram of tissue.

ostia of the lower third of the descending thoracic aorta and of mild to moderate plaques in the abdominal aorta.

The average plasma cholesterol in the dogs given thioracil, but no cholesterol, was 171 mg. per cent. The cholesterol concentrations of their aortas fell within the range for the control aortas.

Discussion

The data demonstrate that a direct correlation exists between the concentrations of cholesterol appearing in the inner aortic wall in the development of experimental atherosclerosis and the rates of entrance of labeled albumin into the inner aortic wall of the normal dog. This is shown in figure 1, where these rates of entrance of albumin and the concentrations of cholesterol accumulated in the inner aortic wall early in the course of the atherogenic regimen are plotted. In our first work on albumin,3 we determined the rates of entrance into only the first 5 aortic sites. We have since determined the rates of entrance into the inner layer of all 6 sites. These latter values were the ones used in the construction of figure 1.

It is reasonable to suppose that if 2 proteins enter the aortic wall by a similar mechanism and 1 of them shows a gradient, then the other will also. Thus, the similarity of gradients reported here is predicted by the concept that in atherogenesis intact low-density lipoproteins bearing cholesterol enter the aortic wall by a mechanism similar to that by which albumin enters. This similarity of gradients therefore supports that concept of atherogenesis. The alternative view that cholesterol is synthesized in the aortic wall appears less attractive, since it does not explain the similarity of gradients.

Albumin was the first protein shown to enter the aortic wall with a gradient of rates.5 Later, studies on the passage of labeled cholesterol into the aortic wall of the normal dog suggested that high-density lipoproteins enter the inner aortic wall with a similar gradient of rates.4 The present data support the view that the low-density lipoproteins present in the plasma of dogs receiving thioracil and cholesterol also exhibit this gradient. The mechanism of origin of the gradient is not known. We previously suggested a possible mechanism. That hypothesis has not been tested.

Despite the faster initial deposition of cholesterol in the thoracic aorta, later in the course of the atherogenic regimen the cholesterol increment in the abdominal aorta was greater than any increment in the thoracic aorta. The cholesterol present at any site at a given time is the difference between the amount deposited there and that which has been removed from the site. Since there is evidence that cholesterol entered the abdominal aorta more slowly than it did the thoracic aorta, it appears probable that its eventual
greater concentration in the abdominal aorta was due to extremely slow removal from that site.

However, this alone cannot explain the time-course of the accumulation of cholesterol in the abdominal aorta. The cholesterol increment at a mean of 150 days on the experimental regimen was much more than 5 times that at a mean of 33 days on the regimen. This indicates that the rate of accumulation of cholesterol in the abdominal aorta must have increased during the course of the experimental disease. In part, this may have been due to the fact that during most of the first 33 days, the plasma cholesterol concentration was below the average value maintained thereafter. This alone is not adequate to explain the magnitude of the difference between the early and late concentrations of cholesterol in the abdominal aorta. It appears likely that either the characteristics of the plasma lipoproteins changed with time, or that the accumulation of lipid in the abdominal aorta changed the permeability to lipoprotein of that portion of the aorta.

It is well known from anatomic studies that experimental canine atherosclerosis is more pronounced in the abdominal aorta than it is in the thoracic aorta. In man and chicken, spontaneous atherosclerosis also is more marked in the abdominal than in the thoracic aorta. However, aortic lesions need not develop more rapidly in the abdominal aorta; in the rabbit and in the chicken, experimental atherosclerosis afflicts the thoracic aorta more than it does the abdominal aorta.

Our data demonstrate the gradient of cholesterol concentration existing in the inner layer of the aorta of the normal dog. The detection of this gradient in the dog depends on the division of the aorta into layers. We have analyzed our data on the basis of the entire thickness of the aortic wall, as well as on the basis of layers. This analysis shows the concentration of cholesterol along the length of the thoracic aorta to be almost constant and slightly higher than in the abdominal aorta. This result is similar to that of Norcia et al. who studied the concentration of cholesterol in the entire thickness of the canine aortic wall. They found that the cholesterol concentration decreases down the length of the aorta. Gradients of cholesterol concentration similar to that that we have described for the inner layer of canine aorta were found by Werthessen et al. for the entire thickness of the aortic walls of normal swine and cattle.

It is possible that the similarity between the distribution of cholesterol in the normal and in the atherosclerotic canine aorta may arise through similar mechanisms. That is, just as the predominance of abdominal lesions in atherosclerosis may be due to the slower removal of cholesterol from that site, so the higher cholesterol concentration in the abdominal portion of the normal aorta's inner layer may be due to the slower removal of what small amounts of cholesterol enter there. However, the alternative possibility exists that the cholesterol concentration at each site reflects the cellular and fibrillar concentrations there.11

Summary

Atherosclerosis was produced in dogs by feeding them thiourea and cholesterol. The increments in cholesterol concentration at sites along the length of the aortas of these dogs were determined by subtracting from the value for each site the corresponding value obtained from normal dogs. After about 1 month on the experimental regimen the cholesterol increments along the length of the aorta formed a gradient. The increment was greatest in the proximal aorta and progressively less down the length of the aorta. After about 5 months on the regimen, the gradient no longer existed. Instead, the cholesterol increment in the abdominal aorta exceeded that in the thoracic aorta. Albumin is known to enter aortic wall with a gradient of rates that is similar to the gradient of cholesterol increments early in the course of experimental atherosclerosis.

The foregoing facts are consistent with the following theory: In the development of atherosclerosis, intact low-density lipoproteins containing cholesterol enter the aortic wall with a gradient of rates. They enter fastest...
in the proximal aorta and progressively less rapidly down the length of the aorta. However, cholesterol is removed from the abdominal aorta much more slowly than from the thoracic aorta. Thus, the increment in cholesterol concentration in the abdominal aorta eventually exceeds that in the thoracic aorta.

Acknowledgment

We are grateful to Dr. Samuel Greenhouse for advice concerning the statistical analysis of the data.

Summario in Interlingua

Atherosclerosis esseva producite in canes per le administration diotari de thiouracil e cholesterol. Le augmentos del concentration de cholesterol a varie sitios al longo del aortas del assi-tractate canes essova determinate per subtraher ab le valor pro omne sito individual le correspondente valor trovate in canes normal. Post circa un mens de le dieta experimental, le augmentos de cholesterol al longo del aorta formavan un gradiente. Le augmento esseva maximal in le aorta proxima e declinava progressivamente in sitos plus distal. Post circa 5 menses del dieta, le gradiente habeva disprisite. In loco de illo, le augmento de cholesterol in le aorta abdominal excedeva illo in le aorta thoracica. Il es cognosceque le albumina entra in le pariete aortica secondo un gradiente de valores que es simile al gradiente del augmentos de cholesterol precescente in le curso de atherosclerosis experimental.

Le supra-reportate factos es de accordo con le sequente theorisa: In le developpamento de atherosclerosis, intnete lipoproteinas de basse densitate e a contento de cholesterol entra in le pariete aortica secondo un gradiente de valores. Lor entrata es le plus rapide in le aorta proximal e deveni progressivamente minus rapide al longo del curso del aorta. Tamen, cholesterol es eliminete ab le aorta abdominal plus lenetamente que ab le aorta thoracica. Assi, in le curso del tempore, le augmento del concentration de cholesterol in le aorta abdominal excede le correspondente augmento in le aorta thoracica.

References

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Circ Res. 1960;8:1023-1027
doi: 10.1161/01.RES.8.5.1023

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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