Experimental Atheromatosis:
Acid Mucopolysaccharide Content of the Aorta

By Alfred Jay Bollet, M.D., Chuni Wang, M.D., and David Adlersberg, M.D.

Histochemical and chemical studies of the acid mucopolysaccharide content of the aorta were made in rabbits fed cholesterol for up to seven months. Colloidal iron staining suggested an increase in acid mucopolysaccharide content of the aortas with mild atheromatous lesions but not when severe lesions were present. Chemical determination, however, revealed no increase in the concentration of acid mucopolysaccharide in early lesions, but did reveal a significant increase in the more severely atheromatous aortas of rabbits fed cholesterol for 6 months or more.

Investigation of the role of the ground substance in the formation of atheromatous lesions has been stimulated by the observation that the pathological deposits in human xanthomatosis and experimental atheromatosis occur in structures rich in connective tissue, including vessels wall, tendons and skin. Histochemical observations have suggested that an increase in the acid mucopolysaccharide concentration occurs in the aorta and dermis during the process of atheroma and xanthoma formation in cholesterol-fed rabbits. The administration of cortisone has been found to retard atheroma formation in cholesterol-fed rabbits despite the enhanced elevation of serum lipids occurring in these animals. Hyaluronidase counteracts this effect of cortisone on atheroma formation. These observations suggested that changes in mucopolysaccharides are of importance in atheroma formation and may play a role in the pathogenesis of this lesion.

The histochemical technics which have been used in the study of acid mucopolysaccharides are not entirely specific. With the recent development of a convenient chemical method of measurement of the concentration of acid mucopolysaccharides in tissue samples, it seemed advisable to determine the extent and timing of the changes in the acid mucopolysaccharide concentration in the aorta in experimental atheromatosis.

Methods

Rabbits were fed 1 Gm. of cholesterol daily without an additional fat vehicle, and were sacrificed after 1 to 7 months of feeding. Eight rabbits, who received Purina chow without cholesterol supplements served as controls. Plasma cholesterol, cholesterol esters, phospholipid, neutral fat and total lipid were determined at two week intervals during the course of the experiments and at the time of sacrifice. Serum cholesterol and esters were determined by Sperry-Schoenheimer method, phospholipids by the Sperry modification of the Subbarow technic; total lipids by the method of Bloor. Patty acids were calculated by subtraction.

The aortas and other large arteries were examined for atherosclerosis. The degree of atheroma formation was quantitated on a 1 to 4 plus basis. A small segment of the base of each aorta was removed for histological studies which included routine hematoxylin and cosin stain, periodic acid-Schiff stain, and the Rinehart modification of Hale colloidal iron stain for acid mucopolysaccharides. The remainder of each aorta was analyzed chemically for acid mucopolysaccharides by the method described previously. Both carbazole and orcinol methods were used to determine the uronic acid.
EXPERIMENTALATHEROMATOSIS

Table 1

Plasma Lipid Levels of Control and Cholesterol-Fed Rabbits. Figures Given Are Mean and Standard Deviation of the Mean

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rabbits</th>
<th>Duration of Cholesterol Feeding (days)</th>
<th>Aortic atherosclerosis</th>
<th>Cholesterol total (mg./100 ml.)</th>
<th>Cholesterol esterified (mg./100 ml.)</th>
<th>Phospholipid (mg./100 ml.)</th>
<th>Neutral fat (mg./100 ml.)</th>
<th>Total Lipid (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>59± 25.4</td>
<td>45± 19.7</td>
<td>97± 30</td>
<td>244</td>
<td>400± 182</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>34</td>
<td>0 to +</td>
<td>909± 180</td>
<td>664± 122</td>
<td>542± 186</td>
<td>867</td>
<td>2318± 922</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>62-63</td>
<td>+</td>
<td>1313± 236</td>
<td>928± 185</td>
<td>600± 91.9</td>
<td>1236</td>
<td>3148± 229</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>90-100</td>
<td>++</td>
<td>1147± 621</td>
<td>793± 402</td>
<td>564± 269</td>
<td>1070</td>
<td>2771± 1480</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>186-500</td>
<td>++ to +++</td>
<td>777± 372</td>
<td>540± 239</td>
<td>370± 141</td>
<td>579</td>
<td>1726± 953</td>
</tr>
</tbody>
</table>

*This fraction was calculated by subtraction; no standard deviation is given.
†Determined in four animals.

Results

The levels of plasma lipids observed in each group of animals are given in table 1. A marked rise in total cholesterol, phospholipid, and total lipid was observed in animals fed cholesterol for 1 month or longer; these levels were relatively stable during continued feeding. As was seen previously, the increase in phospholipid was proportionately less than of cholesterol.

The degree of atheroma formation observed in each aorta is shown in figure 1. Early changes were observed in animals fed cholesterol for 1 or 2 months. Moderate to severe lesions were observed in animals fed 3 months or longer.

Histochemical observations revealed only traces of staining with colloidal iron in the intima of the aortas of control animals. A marked increase in colloidal iron staining was noted in sections of the aortas of animals fed cholesterol for 2 months who had only 1 plus atheromatosis. The aortas of those animals who had 3 or 4 plus atheromatosis showed distinctly less blue-staining by the colloidal iron procedure. The changes observed in the aortas closely resembled those seen in the dermis of animals treated in similar fashion.

A graphic presentation of the results obtained by chemical determination of the concentration of acid mucopolysaccharide fraction extracted from each aorta. Results are expressed as milligrams of uronic acid in mucopolysaccharide per 10 Gm. of dried, fat-free tissue.

Table 2

Acid Mucopolysaccharide Concentration of Aortas of Control and Cholesterol-Fed Rabbits. Figures Given Are mg. of Uronic Acid in Acid Mucopolysaccharide Fraction Per 100 Gm. of Dried Fat-Free Tissue. Results Are Given for Both Carbazole and Orcinol Methods of Determination. Groups Correspond to Those Given in Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Uronic Acid (mg./100 Gm.)</th>
<th>S.E.M.*</th>
<th>No. of obs.</th>
<th>Mean Carbazole method (mg./100 Gm.)</th>
<th>S.E.M.</th>
<th>No. of obs.</th>
<th>Mean Orcinol method (mg./100 Gm.)</th>
<th>S.E.M.</th>
<th>No. of obs.</th>
<th>Ratio carbazole/orcinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>341</td>
<td>± 38.9</td>
<td>8</td>
<td>292</td>
<td>± 11.6</td>
<td>4</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>342</td>
<td>± 25.7</td>
<td>5</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>264</td>
<td>± 18.0</td>
<td>7</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>341</td>
<td>± 50.7</td>
<td>5</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>515</td>
<td>± 36.2</td>
<td>8</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of mean.
†Number of observations.
mucopolysaccharide concentrations than normal. After 3 months of feeding, the mean value was again the same as in the control animals; only animals fed 6 months or longer had significantly elevated levels. A greater increase in acid mucopolysaccharide concentration was found using the carbazole method of determination than using the orcinol method, resulting in a fall in the ratio of carbazole to orcinol values.

When the acid mucopolysaccharide concentration was compared to the severity of the atheromatosis, it was found that a few of the animals with moderate atheromatosis and all but one of those with severe atheromatosis had increased levels. A mean value of 517 mg. per cent was found by the carbazole method of determination in the group with 3 plus atheromatosis; this differed significantly from the finding in control animals with a p value of less than .001. Similar, but less marked changes were found using the orcinol method of determination, resulting in a fall in the ratio of carbazole to orcinol values.

Discussion

Several acid mucopolysaccharides are present in aortic tissue. Heparitin sulfate is one of the most abundant; chondroitin sulfates A and B and hyaluronic acid are present in smaller amounts. The total concentration of acid mucopolysaccharides is very high in the aorta compared to most other tissues. Histochemical observations suggest that these compounds are located principally in the intima. Study of alterations in the concentration of mucopolysaccharides in the aorta would seem to be particularly pertinent in disease principally affecting the intima.

The histochemical observations made with the Rinehart modification of the colloidal iron stain for mucopolysaccharides duplicated previously reported findings of an increase in colloidal iron staining early in atheroma formation, with less marked changes in more severe atheromatosis. The results obtained with the chemical method of determination of acid mucopolysaccharide concentration are in distinct contrast to the histochemical observa-
tions made on the same aortas. Whereas aortas with 1 and 2 plus atheromatosis showed marked increase in colloidal iron staining, there was no change or a slight fall in the chemical values found for acid mucopolysaccharide concentration. On the other hand, aortas of animals fed cholesterol for 6 months or more, who had 3 and 4 plus atheromatosis, showed only a slight increase in colloidal iron staining but had a marked increase in acid mucopolysaccharide concentration by chemical determination.

A decrease in the ratio of carbazole to orcinol values for the uronic acid content of the acid mucopolysaccharide fraction of the aorta occurred in animals with severe atheromatosis. This observation suggests that a qualitative change in this fraction may have occurred, since the acid mucopolysaccharides in the aorta vary in their color development with these reagents. Heparitin sulfate gives high ratios, chondroitin sulfate B low ratios, and hyaluronate and chondroitin sulfates A gives ratios close to unity. 

These results suggest that alterations in the mucopolysaccharides and lipids of the aorta are related. The fact that increase in the concentration of mucopolysaccharide determined by chemical methods occurred only in the more advanced lesions suggests that these changes are secondary to the deposition of lipid. The increase in mucopolysaccharide concentration may be part of the intimal reaction to the deposition of lipid. These findings do not support the suggestion that alterations in the mucopolysaccharides of the aorta play a primary role in the pathogenesis of atheromatosis.

The discrepancy between the chemical and histochemical observations is of interest, and suggests a need for study of the factors which can influence the affinity of tissue for colloidal iron, as well as the specificity of that staining procedure for acid mucopolysaccharide.

Summary

Histochemical and chemical studies of the acid mucopolysaccharide content of the aorta were made in rabbits fed cholesterol for up to 7 months. Colloidal iron staining suggested an increase in acid mucopolysaccharide content of the aortas with mild atheromatous lesions but not when severe lesions were present. Chemical determination, however, revealed no increase in the concentration of acid mucopolysaccharide in early lesions, but did reveal a significant increase in the more severely atheromatous aortas of rabbits fed cholesterol for 6 months or more. The results with the chemical method of assay suggest that alterations in mucopolysaccharide concentration of the aorta are secondary to the lipid deposition.

Summary in Interlingua

Studios histochemic e chimic del contenio aortic de mucopolysaccharido acide esseva effectuate in conilios recipiente cholesterol dietari duraiite periodos de usque a 7 menses. Tinctorium a ferro colloido suggesteva le occurrentia de un augmento del contenio aortic de mucopolysaccharido acide quando leo lesiones atheromatose esseva presente sed non in le presentia de sever lesiones de ille genero. Tamen, determinaciones chimic revelava nullo augmento del concentration de mucopolysaccharido acide in lesiones precoce, sed—del alto lato—illlos rereeva le presentia de significativa tal augmentos in le plus severemente atheromatose aortas de conilios tractate con cholesterol duraiite 6 menses o plus. Le resultatos obtentite con methodos chimic de essayage suggere quo alterationes in le concentrationes aortic de mucopoly saccharido es secundari al deposition de lipid.

References

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Circ Res. 1960;8:88-92
doi: 10.1161/01.RES.8.1.88

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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