PREVENTION OF VASCULAR LESIONS BY CHONDROITIN SULFATE A IN THE CORONARY ARTERY AND AORTA OF RATS INDUCED BY A HYPERVITAMINOSIS D, CHOLESTEROL-CONTAINING DIET

L. M. MORRISON, G. S. BAJWA, R. B. ALFIN-SLATER and B. H. ERSHOFF

Institute for Arteriosclerosis Research, Loma Linda University School of Medicine, Culver City, Calif. 90230 and the University of California School of Public Health, Los Angeles, Calif. 90024 (U.S.A.)

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SUMMARY

Severe lesions in the coronary arteries and aortas occurring primarily in the media and consisting of degeneration, calcification, plaque formation, metachromasia and the presence of intracellular and extracellular stainable lipid material present mainly in the areas of the damaged media were induced within 6 weeks in young adult rats fed a purified diet supplemented with 1.5% cholesterol, 0.5% cholic acid and 1.25 million U.S.P. units of vitamin D₂ per kg of ration. Such lesions were noted in the aortas of 17 of 18 male rats as well as 16 of 16 female rats and the coronary arteries of all rats fed the above diet.

Lesions of the aorta were completely prevented in 18 of 18 male rats and were present in only 5 of 18 female rats fed a similar ration supplemented with chondroitin sulfate A at a 1% level in the diet. Lipid-containing coronary lesions were present in only 3 of 18 male rats and 3 of 18 female rats fed the latter diet. The protective effects of chondroitin sulfate A administration indicated above were not accompanied by a reduction in plasma and liver cholesterol or liver total lipids compared to that of rats fed a similar diet without the chondroitin sulfate A supplement.

Key words: Aortic atherosclerosis – Coronary atherosclerosis – Plasma and liver lipids

INTRODUCTION

Considerable data are available indicating that administration of chondroitin

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sulfates, which are widely distributed in mammalian, fish and fowl connective tissues, have an inhibitory effect on the development of atherosclerosis. Kurita in 1955 reported that intravenous injections of 5 mg/kg of body weight daily of chondroitin sulfate C inhibited atherosclerosis in cholesterol-fed rabbits. Ohdo found that sodium chondroitin sulfate inhibited the formation of atheromatous aortic lesions in cholesterol-fed cockerels when administered orally at a level of 20 mg/kg of body weight per day. Murata found that daily intravenous injections of 5 mg/kg of body weight of a chondroitin polysulfate prepared by sulfation of chondroitin from shark cartilage inhibited the severity of cholesterol-induced atherosclerosis in rabbits. Morrison et al. observed that when chondroitin sulfate A (CSA) was administered for 9 months at a level of 10 mg daily by subcutaneous injection to squirrel monkeys (Saimiri sciurea) fed a diet consisting of 1.5% cholesterol, 20% butter and 78.5% ground Purina Monkey Chow the severity of atheromatous aortic lesions was substantially less than that of animals fed a similar diet but which did not receive the CSA treatment. More recently, Morrison et al. found that rats exposed to a single dose of 600 r total body X-irradiation and subsequently fed a highly purified diet containing 1% cholesterol, after 12 weeks of feeding exhibited a high incidence of lipid-containing coronary lesions resembling atherosclerosis. Non-irradiated rats fed a similar diet did not show such lesions nor did X-irradiated rats fed a cholesterol-free diet. The incidence and extent of lipid deposition in the coronary arteries of X-irradiated, cholesterol-fed rats was significantly reduced by the oral administration of CSA at a 0.4% level in the diet. Bajwa et al. have recently described an experimental procedure which was highly effective for the rapid induction of severe lesions in the aorta and coronary arteries of rats occurring primarily in the media and consisting of degeneration, calcification, plaque formation, metachromasia and the presence of intracellular and extracellular stainable lipid material. Young adult rats fed a highly purified diet supplemented with 1.5% cholesterol, 0.5% cholic acid and 1.25 million U.S.P. units of vitamin D3 (viosterol) per kg of diet developed such lesions within an experimental period of 6 weeks. In the present communication data are presented indicating that the occurrence of lipid-containing aortic and coronary lesions in rats fed the above diet was largely prevented by the concurrent administration of CSA at a 1% level in the diet.

METHOD

The basal ration used in this study was a highly purified diet consisting of sucrose, 61%; casein*, 24%; cottonseed oil, 10%; salt mixture**, 5%; and the following vitamins per kg of diet; thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100

* Vitamin-Free Test Casein, General Biochemicals, Chagrin Falls, Ohio.

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mg; ascorbic acid, 200 mg; biotin, 1 mg; folic acid, 10 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; vitamin B₁₂, 150 μg; 2-methyl-1,4-naphthoquinone, 5 mg; choline chloride, 2 g; vitamin A, 5000 U.S.P. units; vitamin D₂, 560 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. Fifty-four male rats of the Long-Evans strain which were 52 to 56 days of age and averaged 145.4 g in body weight (range 141 to 155 g) and 54 female rats of the Long-Evans strain which were 54 to 61 days of age and averaged 146.5 g in body weight (range 140 to 158 g) were selected for the following experiment. Animals were separated into 5 groups of comparable weight (3 groups of 6 rats of each sex; and 2 groups of 18 rats of each sex). These were fed the diets indicated in Table 1. Group I was fed the basal ration. Group II was fed the basal ration + 1.5% cholesterol + 0.5% cholic acid. Group III was fed the basal ration + 1% CSA*. Group IV was fed the basal ration + 1.5% cholesterol + 0.5% cholic acid + 1.25 million U.S.P. units of vitamin D₂ per kg of diet. Group V was fed the basal ration + 1.5% cholesterol + 0.5% cholic acid + 1% CSA + 1.25 million U.S.P. units of vitamin D₂ per kg of diet. Supplements were incorporated in the diet in place of an equal amount of sucrose. Groups I, II and III consisted of 12 rats each; groups IV and V of 36 rats each. Animals were kept in metal cages with raised screen bottoms (3 rats per cage) and were provided the above diets and water ad libitum. The animals were fed daily and all food not consumed 24 h after feeding was discarded. The rats were weighed weekly during the course of the experiment.

After 6 weeks of feeding the surviving rats (2 of the 108 rats in the experiment died during the course of feeding) were anesthetized with sodium pentobarbital, and blood was withdrawn from the heart into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of THOMPSON et al., and total and free cholesterol were determined on liver and plasma by a modification of the method of Schoenheimer and Sperry as reported by NIEFT and DEUEL. Total lipids were determined gravimetrically on an aliquot of the liver extract. At necropsy the hearts and aortas were fixed in 10% buffered formalin. The hearts were divided into 3 parts consisting of the apex, middle and basal portions; the aortas were cut transversely at the arch and the mid thoracic level. Frozen sections of the above were prepared, cut at 16–20 μ in thickness and stained with Oil-Red-O and hematoxylin. Ten cross sections were prepared through each part of the heart and through the arch and thoracic portions of the aorta. The tissues remaining after preparation of the frozen sections were prepared for paraffin embedding in the routine manner, sectioned at 6 μ in thickness, and consecutive sections were stained with hematoxylin and eosin, von Kossa stain, Masson’s Tri-Chrome stain, and Weigert’s elastic tissue stain. Three

* Chondroitin sulfate A was prepared from bovine tracheal cartilage obtained from a commercial source and purified in our laboratory. Analysis of this material showed a typical chondroitin sulfate A infra-red spectrophotometric absorption curve. Optial rotation determinations gave values of [α]₂₅° = -24°; the nitrogen content was 3.95% average. Sulfur content was 2.3% average.
TABLE 1

EFFECTS OF CHONDROITIN SULFATE A ON PLASMA AND LIVER CHOLESTEROL AND LIVER TOTAL LIPID LEVELS OF RATS FED A HYPERVITAMINOSIS D,
ATHEROGENIC DIET

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>No. of animals per group</th>
<th>Initial body wt.</th>
<th>Final body wt.</th>
<th>Plasma cholesterol</th>
<th>Liver cholesterol</th>
<th>Liver total lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>free (mg/100 ml)</td>
<td>total (mg/100 ml)</td>
<td>free (mg/g)</td>
</tr>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-atherogenic diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (basal)</td>
<td>6</td>
<td>145.3</td>
<td>362.0</td>
<td>23.9 ± 4.7b</td>
<td>80.2 ± 10.6</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Group II (basal + cholesterol + cholic acid)</td>
<td>6</td>
<td>145.3</td>
<td>371.5</td>
<td>43.1 ± 13.6</td>
<td>163.4 ± 24.8</td>
<td>6.2 ± 1.6</td>
</tr>
<tr>
<td>Group III (basal + 1% CSA)</td>
<td>6</td>
<td>145.3</td>
<td>365.7</td>
<td>20.2 ± 3.8</td>
<td>69.0 ± 14.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Atherogenic diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV (basal + cholesterol + cholic acid + vit. D_2)</td>
<td>18</td>
<td>145.6</td>
<td>131.1</td>
<td>49.1 ± 10.7</td>
<td>226.0 ± 21.7</td>
<td>7.0 ± 1.5</td>
</tr>
<tr>
<td>Group V (idem + 1% CSA)</td>
<td>18</td>
<td>145.6</td>
<td>158.9</td>
<td>58.8 ± 13.2</td>
<td>223.8 ± 24.8</td>
<td>6.4 ± 1.6</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-atherogenic diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (basal)</td>
<td>6</td>
<td>146.5</td>
<td>240.8</td>
<td>23.7 ± 4.4</td>
<td>77.0 ± 7.9</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Group II (basal + cholesterol + cholic acid)</td>
<td>6</td>
<td>146.5</td>
<td>259.6</td>
<td>47.1 ± 9.2</td>
<td>216.7 ± 28.0</td>
<td>8.5 ± 2.7</td>
</tr>
<tr>
<td>Group III (basal + 1% CSA)</td>
<td>6</td>
<td>146.5</td>
<td>229.3</td>
<td>18.8 ± 4.4</td>
<td>69.5 ± 14.7</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Atherogenic diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV (basal + cholesterol + cholic acid + vit. D_2)</td>
<td>18</td>
<td>146.5</td>
<td>110.3e</td>
<td>58.7 ± 16.1</td>
<td>265.1 ± 75.6</td>
<td>7.6 ± 2.1</td>
</tr>
<tr>
<td>Group V (idem + 1% CSA)</td>
<td>18</td>
<td>146.5</td>
<td>138.2</td>
<td>62.9 ± 11.5</td>
<td>270.4 ± 70.2</td>
<td>8.9 ± 1.7</td>
</tr>
</tbody>
</table>

* Animals were sacrificed after 6 weeks of feeding. Body weight figures reported are average.

b Standard deviation.

* Two animals in this group died during the course of the experiment. Data are based on the surviving 16 animals.
sections stained with each of the above stains were prepared of each portion of the heart and aorta of each rat. The slides were then studied for pathological findings without knowledge of the groups from which they were obtained.

RESULTS

Body weight

The final body weight of rats in the various groups is indicated in Table 1. Rats fed the diets containing 1.25 million U.S.P. units of vitamin D₂ per kg of diet (groups IV and V) weighed substantially less at the termination of the experiment than did rats fed the diets not containing this supplement (groups I, II and III). Whereas rats in the latter groups showed a continuous weight increment during the course of the experiment, those in groups IV and V gained weight during the first week of feeding, lost weight during the subsequent 2 to 3 weeks and thereafter in general plateaued in weight although several rats in each of these groups continued to lose weight and several regained some of the weight they had lost. The final body weight of both male and female rats fed CSA in conjunction with the toxic dose of vitamin D₂ (group V) was slightly elevated over that of rats fed a similar diet with CSA omitted (group IV). In general the weight loss induced by the toxic vitamin D₂ supplement was greater in the case of female rats than in males. All rats survived the experimental period of 6 weeks with the exception of 2 female rats in group IV.

Food consumption was not determined for rats in the various groups. It was grossly apparent, however, that rats fed the diets containing 1.25 million U.S.P. units of vitamin D₂ per kg of diet (groups IV and V) ate substantially less than did rats fed the diets not containing this supplement (groups I, II and III).

Plasma and liver cholesterol and liver total lipid

Terminal plasma and liver cholesterol levels of rats fed the basal ration + 1.5 % cholesterol + 0.5% cholic acid (group II) were considerably elevated over that of rats fed the basal ration alone (group I) or the basal ration + 1 % CSA (group III). A still further increment in plasma and liver cholesterol levels occurred in rats fed the basal ration + 1.5 % cholesterol + 0.5% cholic acid + 1.25 million U.S.P. units of vitamin D₂ per kg of diet (group IV). No notable differences in plasma and liver cholesterol were observed between rats fed the latter diet and a similar ration supplemented with 1 % CSA (group V). Rats fed the cholesterol-containing diets (groups II, IV and V) also showed a highly increased increment in liver total lipid levels over that of rats fed the unsupplemented basal ration (group I) or the basal ration + 1 % CSA (group III). No appreciable differences in liver total lipid levels were observed between the various groups fed the cholesterol-containing diets. Similar findings were observed in both male and female rats. Results are summarized in Table 1.

Microscopic appearance of aorta and coronary arteries

In agreement with previous findings⁸ rats fed the basal purified ration + 1.5 % (continued on p. 112)

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Fig. 1. Section of the arch of the aorta from a rat fed the basal ration + 1.5%, cholesterol + 0.5%, cholic acid + 1.25 million U.S.P. units of vitamin D₃ per kg of diet (group IV). Note the medial degeneration and changes in the thickness of the aortic wall. Oil-red-O and hematoxylin stains, x 60.

Fig. 2. Section of the thoracic portion of the aorta of a rat in group IV. Note extensive calcification of the damaged media. Von Kossa stain, x 60.

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Fig. 3. Section of the arch of the aorta of a rat in group IV. Note the massive areas of medial degeneration and the subendothelial plaque consisting of proliferating mesenchymal cells and cellular material. Note also the presence of an intra-medial plaque. Oil-red-O and hematoxylin stains, × 60.

Fig. 4. Section of the arch of the aorta of a rat in group IV. Note extensive lipid stainable material (dark staining material) involving about 2/3 of the media. This lesion was given a grade of 3. Oil-red-O and hematoxylin stains, × 60.

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cholesterol + 0.5% cholic acid + 1.25 million U.S.P. units of vitamin D$_{3}$ per kg of diet (group IV) developed extensive lesions of the aorta and coronary arteries within an experimental period of 6 weeks. In the aorta the major changes occurred in the media and consisted of degeneration (Fig. 1), calcification (Fig. 2), plaque formation (Fig. 3), metachromasia, lysis and fragmentation of the elastic lamina and in addition the presence of intracellular and extracellular stainable lipid material particularly marked in the areas of medial injury and plaque formation but also present in the intima (Fig. 4). The above changes were most marked in the arch of the aorta and in

### TABLE 2

**EFFECTS OF CHONDROITIN SULFATE A ON THE INCIDENCE AND DISTRIBUTION OF LIPID-CONTAINING LESIONS IN THE AORTA AND CORONARY ARTERIES OF RATS FED A HYPERVITAMINOSIS D, CHOLESTEROL-CONTAINING DIET**

No pathologic lesions were observed in the aorta and coronary arteries of either male or female rats fed the unsupplemented basal ration (Group I), the basal ration + 1.5 % cholesterol + 0.5 % cholic acid (Group II) or the basal ration + 1% CSA (Group III). Data for groups, I, II and III were based on 6 male and 6 female rats per group.

<table>
<thead>
<tr>
<th>Hypervitaminosis D, cholesterol-containing diet</th>
<th>without CSA (group IV)</th>
<th>with CSA (group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male rats</td>
<td>female rats</td>
</tr>
<tr>
<td>Number of animals per group at time of autopsy$^a$</td>
<td>18</td>
<td>16$^b$</td>
</tr>
<tr>
<td>Incidence of aortic lesions (%)$^c$</td>
<td>94.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Percent of animals per group showing lesions in the following parts of the aorta and score per affected rat:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intracardiac</td>
<td>44.4</td>
<td>87.5</td>
</tr>
<tr>
<td>(2.0)$^d$</td>
<td>(2.0)$^d$</td>
<td></td>
</tr>
<tr>
<td>arch</td>
<td>88.9</td>
<td>100.0</td>
</tr>
<tr>
<td>(2.1)$^d$</td>
<td>(2.7)$^d$</td>
<td></td>
</tr>
<tr>
<td>mid thoracic</td>
<td>66.7</td>
<td>93.8</td>
</tr>
<tr>
<td>(1.3)$^d$</td>
<td>(1.6)$^d$</td>
<td></td>
</tr>
<tr>
<td>Incidence of coronary lesions (%)$^e$</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Average number of arteries affected per cross section in the following parts of the heart:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>4.2</td>
<td>2.7</td>
</tr>
<tr>
<td>middle</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td>apical</td>
<td>2.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

$^a$ The experiment was terminated after 6 weeks of feeding.

$^b$ Two of the rats in this group died during the course of the experiment. Data in this group are based on the surviving 16 animals.

$^c$ Decrease in the incidence of aortic lesions in both male and female rats of group V compared to those of group IV: $p < 0.001$.

$^d$ The severity of lesions was evaluated on the basis of a scale ranging from 0 to 4.

$^e$ Decrease in the incidence of lipid-containing coronary lesions in both male and female rats of group V compared to those of group IV: $p < 0.001$.

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Fig. 5. Section of the arch of the aorta of a rat fed the basal ration + 1.5% cholesterol + 0.5% cholic acid + 1.25 million U.S.P. units of vitamin D₂ per kg of diet + 1% chondroitin sulfate A (group V). Note the normal appearance of the intima, media and adventitia in contrast to Figs. 1-4. Hematoxylin and cosin stains, x 96.

Fig. 6. Section of the arch of an aorta of a rat in group V. Note the normal appearing elastic lamina. Weigert's stain, x 96.
descending order in the mid-thoracic and intracardiac portions. These changes were completely prevented in male rats by the concurrent administration of CSA at a 1% level in the diet (group V) (Figs. 5 and 6) and to a lesser but very marked degree in female rats as well. The decrease in the incidence of aortic lesions in both male and female rats of group V compared to those of group IV was statistically significant: $p < 0.001$ (Table 2). No pathologic changes were observed in the aorta of rats fed diets without the toxic vitamin D$_2$ supplement (groups I, II and III).

The principal changes in the coronary arteries of rats in group IV occurred in the media and consisted of degeneration, calcification, metachromasia, plaque formation and the presence of intracellular stainable lipid material (Fig. 7). The medial changes were often so severe as to result in complete loss of cellular details and structure. The elastic membrane showed extensive fragmentation, duplication and disorientation and was often absent over large areas. The lipid was present primarily in the areas of the damaged media, the plaque and subintima. The above lesions were noted in all parts of the heart but were particularly prevalent in the basal portion. A marked reduction in the incidence and severity of such lesions occurred in rats fed a diet similar to that administered to the above group but which was supplemented with 1% CSA (group V) (Figs. 8 and 9). Lipid-containing coronary lesions were present in only 3 of 18 male rats and 3 of 18 female rats fed the latter diet. The decrease in the incidence of lipid-containing coronary lesions in both male and female rats of group V compared to those of group IV was statistically significant: $p < 0.001$ (Table 2). An additional 3
Fig. 8. Section of the proximal coronary artery of a rat in group V. Note that the intima, media and adventitia are well preserved and normal in appearance in contrast to findings in Fig. 7. Oil-red-O and hematoxylin stains, × 96.

Fig. 9. Section of the proximal coronary artery of a rat in group V. Note normal appearing and well preserved internal elastic membrane. Weigert's stain, × 96.
male and 4 female rats on the latter diet had medial lesions (not containing lipid-stainable material) which were primarily focal and were confined in large part to the basal portion of the heart. No pathologic changes were observed in the coronary arteries of rats fed the basal ration alone (group I) or the basal ration supplemented with cholesterol and cholic acid (group II) or 1% CSA (group III). The incidence and distribution of lipid-containing lesions in the aorta and coronary arteries of rats in the various groups are summarized in Table 2.

DISCUSSION

In this study severe lesions in the aorta and coronary arteries occurring primarily in the media and consisting of degeneration, calcification, plaque formation, metachromasia and the presence of intracellular and extracellular stainable lipid material were induced within six weeks in young adult rats fed a purified diet supplemented with 1.5% cholesterol, 0.5% cholic acid and 1.25 million U.S.P. units of vitamin D$_2$ per kg of ration (group IV). A striking reduction in the incidence and severity of such lesions occurred in rats fed a similar diet plus 1% CSA (group V). Comparable findings in respect to vascular lesions were noted in male and female rats fed the above diets although female rats exhibited a greater loss in body weight than was the case for males. Pathologic lesions of the aorta and coronary arteries did not occur in rats fed the unsupplemented basal purified ration (group I) or the basal ration + 1.5% cholesterol + 0.5% cholic acid (group II) or the basal ration + 1% CSA (group III).

It is of interest that the protective effect of orally administered CSA indicated above was not associated with any detectable effect of this preparation in lowering plasma and liver cholesterol and liver total lipids. Plasma and liver cholesterol and liver total lipid levels of rats fed the hypervitaminosis D, cholesterol-containing diet + CSA (group V) were just as elevated over that of rats on the basal ration (group I) as were values for rats fed the hypervitaminosis D, cholesterol-containing diet without CSA (group IV). Present findings that the protective effects of orally administered CSA as indicated above were not correlated with a reduction of elevated plasma and liver cholesterol and liver total lipid levels are in agreement with previous findings that the anti-atherosclerotic activity of orally administered CSA in X-irradiated, cholesterol-fed rats was also unaccompanied by an attendant reduction in lipid levels.

Previous reports by Morrison et al.$^9,10$, Branwood et al.$^11$, Rutstein et al.$^12$ and others have described the effects of chondroitin sulfate A and other acid mucopolysaccharides in "clearing" lipids and lipoproteins out of arterial and other connective tissue cells in organ and tissue cultures. Selye et al.$^13$ have described the preventive action of chondroitin sulfate A in inhibiting the deposition of calcium in the connective tissue of rats in the Selye systems of calciphylaxis and calcergy.

Bazin and Delaunay$^{14}$ demonstrated the effectiveness of chondroitin sulfate A
in preventing the inflammatory reactions induced by polyvinyl sponge implantations in the connective tissues of rats.

The senior author and associates have previously shown the increased synthesis of mRNA and DNA in connective tissue cells of tissue cultures induced by chondroitin sulfate A as a result of increased rate and stimulation of cellular metabolism\textsuperscript{15}. Increased rates of lipid and fatty acid turnover have similarly been demonstrated by chondroitin sulfate A in tissue culture systems as well as in segments of human and rabbit aorta by MORRISON, QUILLIGAN, MURATA et al.\textsuperscript{9,10}.

Electronmicroscopic studies by SCHJEIDE et al.\textsuperscript{16} in tissue culture systems have shown chondroitin sulfate A properties of cellular repair and regeneration as described in different models and preparations by BAZIN AND DELAUNAY\textsuperscript{14}, JUDD AND WEXLER\textsuperscript{37} and others.

Similar control experiments have been performed and repeated in which the hypervitaminosis D, cholesterol-containing diet was employed as a model for comparative assays of activities against coronary and aorta vascular deposition of lipids and lesions described under RESULTS above. Various acid mucopolysaccharides, heparinoids and chondroitin 4 and 6 sulfates (CSA and C)*, derived from bovine cartilage, shark and whale cartilage were tested. In the latter trials, maximum inhibition of coronary artery and aorta vascular lesions was consistently found with chondroitin-4-sulfate (CSA) prepared in our laboratory from bovine nasal septum and tracheal cartilage in a series of controlled experiments\textsuperscript{16}.

No data are available as to the modus operandi whereby CSA exerts its protective effects in this investigation. Since plasma and liver cholesterol and total liver lipid levels were not reduced in rats administered CSA in conjunction with the hypervitaminosis D, cholesterol-containing diet, it would appear that the protective effects of CSA were not due to its preventing the absorption of dietary cholesterol or its promoting an increased excretion of this lipid from body tissues, at least as evidenced by plasma and liver cholesterol levels. In view of the molecular weight of CSA\textsuperscript{**} it would seem unlikely that significant amounts of this material would be absorbed intact through the intestinal wall. It is possible, therefore, that the activity of CSA under conditions of the present experiment was due to effects exerted in the intestinal tract. The possibility that the activity of CSA may be due not to the entire molecule per se but to some constituent part thereof that may be released in the intestinal tract and absorbed therefrom has not been excluded.

Unpublished studies from this laboratory indicate that considerable variation exists in the activity of different CSA preparations when tested under conditions of the present experiment despite the similarity on the basis of chemical analyses of the various preparations tested. Further studies are indicated to determine to what extent differences in processing procedures or the species, physiologic state of the animal or

\* The estimated molecular weight of the chondroitin sulfate A employed in the present experiment based on its sedimentation constant was 29,500.

\** In this paper the older classification acid mucopolysaccharide is employed although the newer term glycosaminoglycan is meant.

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type of cartilage from which CSA was prepared were responsible for the diverse results obtained.

REFERENCES

14 Bazin, S. and A. Delaunay, Personal communication from the Dept. of Experimental Pathology, Pasteur Institute, Paris.
16 Schjeide, O. A. and L. M. Morrison, Coronary Heart Disease and the Mucopolysaccharides (Glycosaminoglycans), Thomas, Springfield, Ill., In press.