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# Composition of Glycosaminoglycans from Aortas from Deoxycorticosterone-Induced Hypertensive Pigs[1] (42141)

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*Abstract.* Hypertension was induced in young male pigs by subcutaneous implantation of deoxycorticosterone acetate in a Silastic rubber carrier. Hemodynamic variables were periodically monitored. Six to eight weeks after implantation, the animals were necropsied and aortas were dissected. Glycosaminoglycans (GAG) were isolated from aortas from hypertensive pigs and from normotensive controls. Individual glycosaminoglycans in mixtures were fractionated and quantitated by chromatography on Dowex 1 $Cl^-$ column. No differences were noted in total glycosaminoglycan concentration between hypertensive and control animal aortas; the differences in individual GAG in aorta media-adventitia between the groups were not statistically significant. The relative proportions of heparan sulfate and dermatan sulfate in aorta intima were significantly greater ($p < 0.05$) in hypertensive aortas than in normotensive intima. These two glycosaminoglycans increased in hypertensive animals at the expense of chondroitin 4-sulfate and hyaluronic acid. There was no difference in chondroitin 6-sulfate between the groups. A possible explanation of changes of glycosaminoglycans in hypertension because of altered lysosomal activity is suggested. 

It is well recognized that hypertension is one of the major risk factors for cardiovascular disease and for acceleration of atherosclerosis (1). Chronic elevation of blood pressure regardless of etiology produces arterial wall thickening and endothelial injury that initiates the development of atherosclerotic lesions. Considerable information is now available on arterial wall connective tissue changes in experimental models of atherosclerosis (2) but data on connective tissue changes in aorta in models of hypertension are limited (1, 3, 4).

Hypertension can be induced in experimental animals by a variety of methods including mineralocorticoid treatment (5). Higher levels of mineralocorticoids produce disturbances in electrolyte and water metabolism and may influence arterial wall mesenchyme through fluid content and via elevated blood pressure. Proteoglycans influence the metabolism of electrolytes and water (6) and as an important constituent of arterial wall matrix it is important to know their changes in hypertension. In the present study hypertension was induced in pigs by 11-deoxycorticosterone acetate treatment and arterial wall glycosaminoglycan (GAG) composition of the hypertensive pigs was studied in comparison to normotensive animals.

**Materials and Methods.** *Animals.* Young male pigs (2–3 months old) were obtained from a farm near Ann Arbor, Michigan. Each animal was housed in a metabolic cage (4 × 4 feet) and provided with Purina Pig Growena and tap water *ad libitum*. Daily measurements of food were taken. Each pig received approximately 150–175 meq Na/day in the diet.

The animals received instrumentation for monitoring hemodynamic variables: a left thoracotomy for implantation of arterial flowprobe, or the placement of occlusive catheters in the jugular vein and carotid

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artery. Details of these procedures were described earlier (7–9).

After 2 to 3 weeks of stable baseline measurements six experimental animals received a subcutaneous implant of either 11-deoxycorticosterone acetate (DOCA, 100 mg/kg, Sigma Chemical Co.) in Silastic rubber carrier (Dow Corning, 1 part DOCA to 2 parts Silastic) and one pig received DOCA-free Silastic implant. Six to eight weeks after implantation the pigs were necropsied and aortas were dissected, immediately frozen, and shipped to New Orleans in dry-ice pack for GAG studies.

Six control animals used in the study did not undergo the treatment.

*Glycosaminoglycan studies.* The aortas were thawed and the intima stripped from the aortas by fine dissection under magnification (invariably about a third of the inner media was included in the intimal preparation). Although this technique does not provide a precise line of demarcation of intima from media, this method of splitting aortas in many earlier studies (10–14) gave reproducible results. The residual tissue was considered as media-adventitia. The tissues were finely minced, dry-defatted over several changes of acetone at 4°C, air-dried, and stored at 5°C in a vacuum desiccator until used for GAG isolation. Dry-defatted bovine aortic intima available in the laboratory from other studies was used for quality control.

*Isolation of glycosaminoglycans.* GAG were isolated in duplicate from each tissue by a method described earlier (15). The procedure involved treatment of the tissue with 2% NaOH at 24°C for 48 hr followed by papain digestion for 48 hr at 65°C. The hydrolyzate was exhaustively dialyzed against distilled water and the small amount of precipitate formed was separated by centrifugation. The clear supernatant was concentrated to a small volume and an aliquot of the concentrated supernatant was analyzed for uronic acid by the method of Dische (16). The remainder of the solution was used for chromatography to quantitate individual GAG.

*Chromatography of glycosaminoglycans.* GAG were fractionated and quantitated by chromatography on a Dowex 1 $Cl^-$ column by a previously described procedure (17). Prior to chromatography GAG were precipitated from solution by adding 4 vol of ethanol and sodium acetate. The precipitated GAG were separated from ethanol by centrifugation, dissolved in 10 ml distilled water, and applied on a Dowex 1 $Cl^-$ (200–400 mesh) column (0.6 × 35 cm). The column was eluted with a NaCl–$MgCl_2$ gradient (0.2 to 3.0 *M*). The effluent from the column was analyzed by an automated orcinol–$H_2SO_4$ reaction in the Technicon sugar chromatography system (Technicon Instruments Corp., Tarrytown, N.Y.).

This procedure resolves mixtures of GAG from aorta into hyaluronic acid, heparan sulfate, and chondroitin sulfate–dermatan sulfate peaks. The areas under the peaks are proportional to their concentrations. Chondroitin sulfates and dermatan sulfate elute together from the column as a single peak. Fractions corresponding to this peak are collected from the sample outlet of the manifold using a fraction collector, pooled, dialyzed against distilled water, and analyzed for chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate by the enzymatic method of Saito *et al.* (18). This procedure of analysis was found reproducible and the results were comparable to those obtained by manual chromatography followed by analysis of individual fractions for characterization. Replicate analyses showed a mean deviation of 2% from the average for hyaluronic acid, 4% for heparan sulfate, 7% for chondroitin 6-sulfate, 10% for chondroitin 4-sulfate, and 5% for dermatan sulfate.

**Results.** All animals survived until termination of the experiment. No major clinical complications were noted in the animals, but one developed chronic arthritis and two developed abscesses. All pigs gained weight approximately 0.5 kg/day. Their mean arterial pressure before treatment and at the termination of the experiment along with their body weights and ages are reported in Table I. DOCA treatment resulted in an increase in the mean arterial pressure in these animals from a mean value of 98.8 to 131.2 mm Hg. In the Silastic-treated pig there was no change in the mean arterial pressure after the treatment. The control animals were somewhat younger and weighed less than the DOCA-treated animals at the termination of the experiment but their mean arterial pressure

TABLE I. MEAN ARTERIAL BLOOD PRESSURE OF PIGS, PRE- AND POST-DOCA TREATMENT

| Animal | Age (months) | Weight (kg) | Mean arterial pressure (mm Hg) | |
|---|---|---|---|---|
| | | | Pretreatment[a] | Posttreatment[b] |
| DOCA-treated group | | | | |
| 1 | 7 | 81.1 | 99.1 | 134.6 |
| 2 | 5 | 49.8 | 100.0 | 135.0 |
| 3 | $5\frac{1}{2}$ | 65.4 | 101.6 | 136.8 |
| 4 | 7 | 77.9 | 100.9 | 129.2 |
| 5 | 7 | 74.2 | 92.3 | 117.6 |
| 6 | $4\frac{1}{2}$ | 39.6 | —[c] | 134.0 |
| Silastic-treated pig | | | | |
| 7 | 6 | 79.4 | 116.0 | 114.6 |
| Control group[d] | | | | |
| 8 | 4 | 38.2 | 100.0 | |
| 9 | 3 | 29.6 | 98.0 | |
| 10 | 4 | 45.3 | 103.0 | |
| 11 | 3 | 37.0 | 102.0 | |
| 12 | 3 | 36.4 | 100.0 | |
| 13 | 3 | 23.0 | 97.0 | |

[a] Ten day average value.
[b] Average of last 5 days' value.
[c] Not determined.
[d] These animals did not receive any treatment. Mean arterial pressure reported was average of readings on the last 5 days prior to sacrifice.

was similar to the pretreatment values of the DOCA group.

The composition of GAG in intima media and media-adventitia of the aortas from the animals is shown in Table II. As we noted earlier, in man and in several animal species (12, 19, 20), the concentration of total uronate in intima media was greater than in media-adventitia in these animals (4 mg vs 2.3 mg/g dry-defatted tissue). There was no difference among the groups in the total GAG concentration either in intima media or in media-adventitia. Dowex 1 $Cl^-$ column chromatography profiles of GAG from intima media of three DOCA-treated and three control pigs are shown in Fig. 1. In all the samples the GAG resolved into three peaks. Peak 1 represented hyaluronic acid, peak 2, heparan sulfate, and peak 3, chondroitin sulfates and dermatan sulfate.

Relative proportions of individual GAG quantitated from the chromatograms and by enzymatic analyses of chondroitin sulfates and dermatan sulfate mixtures are shown in Table II. Intima media preparations from control animals had a mean proportion of 8.5% hyaluronic acid, 14.1% heparan sulfate, 24.2% chondroitin 4-sulfate, 19.2% dermatan sulfate, and 33.9% chondroitin 6-sulfate in their GAG. DOCA-treated animals had significantly ($P < 0.05$) greater proportions of heparan sulfate and dermatan sulfate in their intima media preparations than in the preparations from the controls. These two GAG increased at the expense of hyaluronic acid and chondroitin 4-sulfate in the DOCA-treated group. The same index of changes in these GAG persisted even when the concentrations were calculated as microgram per milligram tissue. No difference was noted in chondroitin 6-sulfate proportion among these groups. The GAG composition of DOCA-free Silastic-treated pig aorta resembled that of control animals.

Mean relative proportions of individual GAG in media-adventitia preparations of control animals were 5.5% hyaluronic acid, 11.7% heparan sulfate, 22.7% chondroitin 4-sulfate, 31.5% dermatan sulfate, and 28.6% chondroitin 6-sulfate. There were no differences in the relative proportions of these GAG in media-adventitia preparations from

TABLE II. GAG COMPOSITION OF AORTAS FROM HYPERTENSIVE AND NORMOTENSIVE PIGS

| | Intima-media | | | | | | Media-adventitia | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % of total GAG | | | | | | % of total GAG | | | | |
| Animal | Total uronate (mg/g tissue)[a] | HA[b] | HS | C4-S | DS | C6-S | Total uronate (mg/g tissue)[a] | HA | HS | C4-S | DS | C6-S |
| DOCA-treated group | | | | | | | | | | | | |
| 1 | 3.8 | 3.7 | 19.6 | 12.0 | 32.1 | 32.6 | 2.4 | 6.8 | 9.3 | 24.0 | 34.7 | 25.2 |
| 2 | 4.4 | 4.3 | 18.4 | 12.9 | 33.7 | 30.7 | 2.6 | 4.1 | 16.8 | 23.7 | 26.8 | 28.6 |
| 3 | 4.0 | 2.1 | 24.6 | 14.9 | 27.6 | 30.8 | 1.8 | 6.3 | 14.8 | 19.1 | 31.7 | 28.1 |
| 4 | 3.9 | 6.1 | 21.9 | 18.4 | 25.7 | 27.9 | 3.0 | 3.7 | 15.4 | 22.0 | 35.3 | 23.6 |
| 5 | 4.1 | 2.4 | 25.0 | 14.6 | 28.6 | 29.4 | 2.2 | 4.2 | 19.3 | 19.8 | 37.9 | 18.8 |
| 6 | 5.0 | 6.2 | 24.9 | 11.9 | 24.1 | 32.9 | 2.0 | 2.8 | 10.2 | 22.8 | 33.4 | 30.8 |
| Mean | 4.2 | 4.13 | 22.4 | 14.1 | 28.6 | 30.7 | 2.33 | 4.7 | 14.3 | 21.9 | 33.3 | 25.9 |
| ±SE | 0.18 | 0.72 | 1.18 | 1.0 | 1.50 | 0.77 | 0.18 | 0.64 | 1.58 | 0.83 | 1.55 | 1.75 |
| Silastic animal | | | | | | | | | | | | |
| 7 | 5.3 | 8.2 | 19.2 | 20.8 | 18.6 | 33.2 | 2.5 | 6.3 | 17.2 | 21.6 | 27.7 | 27.2 |
| Control group | | | | | | | | | | | | |
| 8 | 4.2 | 7.3 | 12.3 | 24.2 | 16.7 | 39.5 | 2.1 | 2.4 | 12.7 | 20.9 | 33.5 | 30.5 |
| 9 | 3.6 | 6.5 | 15.6 | 21.8 | 23.9 | 32.2 | 2.3 | 5.2 | 11.9 | 21.8 | 29.4 | 31.7 |
| 10 | 3.8 | 12.1 | 19.7 | 24.0 | 17.4 | 26.2 | 1.8 | 9.3 | 12.3 | 19.6 | 35.9 | 22.9 |
| 11 | 3.6 | 13.7 | 9.5 | 27.8 | 18.4 | 30.6 | 1.9 | 6.7 | 13.0 | 24.2 | 33.6 | 22.5 |
| 12 | 4.8 | 6.8 | 14.0 | 22.3 | 21.7 | 35.2 | 2.6 | 5.9 | 9.9 | 23.4 | 31.0 | 29.8 |
| 13 | 4.2 | 4.8 | 13.7 | 25.0 | 16.8 | 39.7 | 2.8 | 3.4 | 10.6 | 26.3 | 25.7 | 34.2 |
| Mean | 4.0 | 8.5 | 14.1 | 24.2 | 19.2 | 33.9 | 2.3 | 5.5 | 11.7 | 22.7 | 31.5 | 23.6 |
| ±SE | 0.19 | 1.44 | 1.39 | 0.88 | 1.21 | 2.16 | 0.16 | 1.0 | 0.50 | 0.99 | 1.48 | 1.96 |
| Significance ($P<$)[c] | NS | 0.05 | 0.05 | 0.02 | 0.05 | NS | NS | NS | NS | NS | NS | NS |

[a] Acetone dry-defatted tissue.

[b] Abbreviations = HA—hyaluronic acid; HS—heparan sulfate; C4-S—chondroitin 4-sulfate; DS—dermatan sulfate; C6-S—chondroitin 6-sulfate; NS—not significant.

[c] $P$, statistical significance by Student's $t$ test.

among DOCA-treated, Silastic-treated, and control animals.

Although DOCA-treated animals are older than control animals there were no correlations between age and total GAG or specific GAG.

**Discussion.** Several studies have now shown that arterial wall GAG composition is altered in hypertension (1, 4). These studies utilizing DOCA-induced hypertension in pigs substantiate those findings in yet another experimental model. In the aorta, proteoglycans are synthesized by both endothelial and smooth muscle cells (21–26). Since endothelial cells synthesize mostly hyaluronic acid and heparan sulfate while smooth muscle cells synthesize predominantly chondroitin sulfates and dermatan sulfate, the current observations suggest vascular responses to the stress of hypertension is largely related to endothelial cell activity in the intact animal. Subjecting cardiovascular tissue to hypertension also promotes proliferation of arterial smooth muscle cells (27, 28). This process in turn elaborates connective tissue components including proteoglycans (1). Schmidt *et al.* (29) noted greater incorporation of $^{35}S$ into proteoglycans in cultures of smooth muscle cells from hypertensive rats than from normotensive rats. Similar observation was noted in uninephrectomized rats in which a greater $^{35}S$ uptake by sulfated proteoglycans than in controls was noted (3) but such studies do not clarify the contribution of intimal (endothelial layer) or the vascular wall and smooth muscle cell responses. Hollander *et al.* (30) also observed an augmented synthesis of GAG in hypertensive and atherosclerotic vessels without elaborating specific changes of GAG or of the nature of change within the aorta, intima, or media. In the present study we did not observe increased total concentration of GAG in aortas from hypertensive pigs over controls. The total content of GAG per unit of tissue was not altered but the distribution of GAG was clearly

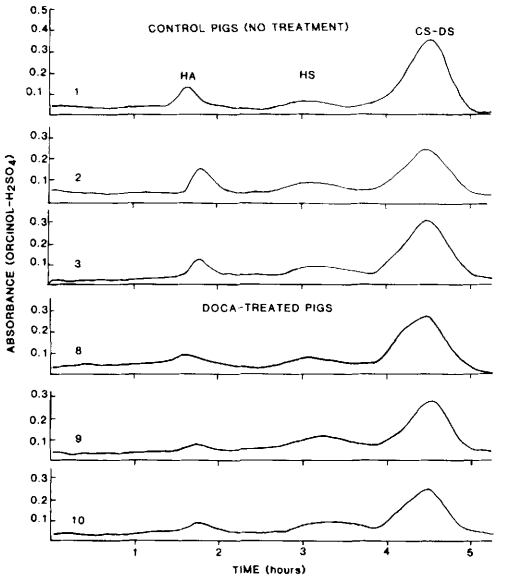


FIG. 1. A composite illustration of Dowex 1 $Cl^-$ column chromatography profiles of GAG from intima media of aortas from DOCA-treated and control pigs. Two hundred fifty to three hundred microgram uronate material from each sample was used for chromatography. Uronate was monitored by orcinol-$H_2SO_4$ reaction in Technicon sugar analyzer. Numbers on chromatograms correspond to animal number in Tables I and II. As examples, three preparations from each of DOCA-treated and control (untreated) animals are shown in the figure. Silastic-treated control animal (not shown) resembled untreated controls.

changed. In this experimental model, it is difficult to point out the effect of DOCA per se on GAG metabolism. Treatment of DOCA invariably induces hypertension in experimental animals and this is a well-established model. It is possible that in aortas under hypertensive stress increased synthesis of proteoglycans might have occurred but some proteoglycans might have also been preferentially catabolized. Increased activities of $\beta$-glucuronidase and $\beta$-*N*-acetylglucosaminidase have been demonstrated in hypertensive aortas (31). The increased activities of these enzymes suggest an increased degradation of selected GAG, hyaluronic acid, and chondroitin sulfates in hypertensive aortas.

It is apparent that arterial wall connective tissue responds to hypertension and eventually vessel wall physical and chemical characteristics change. Of the GAG occurring in aorta only heparan sulfate and dermatan sulfate are resistant to hyaluronidase. It is interesting to note that only these two GAG increased in hypertensive aortas over those in controls. Whether these GAG are disproportionately synthesized is not known. Deficient activities of enzymes like $\alpha$-iduronidase and idurono-sulfatase result in increased amounts of heparan sulfate and dermatan sulfate. Increased concentration of these GAG is also noted in human as well as experimental atherosclerosis (2). Although the present study cannot explain the precise reason for accumulation of these GAG in hypertensive aortas, it is likely that arterial cells from hypertensive pigs have altered characteristics of lysosomal enzymes as shown by the studies of Wolinsky and Fowler (32). Deficiency of certain specific enzymes, e.g., cholesterol esterase (33) in smooth muscle cells of atherosclerotic lesions has also been reported. Studies of Hollander *et al.* (30) in nonhuman primates indicate that GAG in the involved arteries accumulate both extracellularly as well as intracellularly similar to certain lysosomal deficiency diseases. The current observations suggest in hypertension alteration of physical properties of aorta, e.g., stiffness (34), may result in part to variable responses of lysosomal enzyme changes and to disparate concentrations of GAG.

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