

Arterial wall metabolism in experimental hypertension of coarctation of the aorta of short duration

William Hollander, ... , Melvin Farmelant, Irving M. Madoff

J Clin Invest. 1968;47(5):1221-1229. <https://doi.org/10.1172/JCI105811>.

Research Article

Coarctation of the mid-thoracic aorta was surgically produced in mongrel dogs which were sacrificed from 4-12 wk after the operation. As compared to the findings in control animals, the sodium, chloride, and water content of the hypertensive portion of the coarcted thoracic aorta was significantly elevated, whereas the electrolyte and water content of the relatively normotensive portion of the coarcted aorta was normal. The sodium, potassium, and water content of the pulmonary artery, skeletal muscle, and cardiac muscle of the coarcted dog was not altered. These observations suggest that an elevated arterial pressure may influence the electrolyte and water composition of the arteries.

The arterial pressure also may influence the content and synthesis of acid mucopolysaccharides (MPS) in the arteries since the content of sulfated MPS and the incorporation of injected radiosulfate into sulfated MPS were significantly increased in the hypertensive portion of the coarcted thoracic aorta but were significantly reduced in the relatively normotensive ("hypotensive") portion of the coarcted aorta. The observed increase in MPS may have been a factor directly responsible for the increase in the sodium content of the hypertensive aorta since MPS can act as polyelectrolytes and bind cations.

Although the arterial pressure may influence certain metabolic functions in the arteries, it did not appear to have a direct effect on the arterial lipids since the [...]

Find the latest version:

<https://jci.me/105811/pdf>



Arterial Wall Metabolism in Experimental Hypertension of Coarctation of the Aorta of Short Duration

WILLIAM HOLLANDER, DIETER M. KRAMSCH, MELVIN FARMELANT, and
IRVING M. MADOFF

*From the Robert Dawson Evans Memorial Department of Clinical Research,
University Hospital, and the Department of Medicine, Boston University
School of Medicine, Boston, Massachusetts 02118*

ABSTRACT Coarctation of the mid-thoracic aorta was surgically produced in mongrel dogs which were sacrificed from 4–12 wk after the operation. As compared to the findings in control animals, the sodium, chloride, and water content of the hypertensive portion of the coarcted thoracic aorta was significantly elevated, whereas the electrolyte and water content of the relatively normotensive portion of the coarcted aorta was normal. The sodium, potassium, and water content of the pulmonary artery, skeletal muscle, and cardiac muscle of the coarcted dog was not altered. These observations suggest that an elevated arterial pressure may influence the electrolyte and water composition of the arteries.

The arterial pressure also may influence the content and synthesis of acid mucopolysaccharides (MPS) in the arteries since the content of sulfated MPS and the incorporation of injected radiosulfate into sulfated MPS were significantly increased in the hypertensive portion of the coarcted thoracic aorta but were significantly reduced in the relatively normotensive (“hypotensive”) portion of the coarcted aorta. The observed increase in MPS may have been a factor directly responsible for the increase in the sodium content of the hypertensive aorta since MPS can act as polyelectrolytes and bind cations.

Although the arterial pressure may influence certain metabolic functions in the arteries, it did

Received for publication 25 September 1967 and in revised form 4 December 1967.

not appear to have a direct effect on the arterial lipids since the lipid content of the hypertensive and of the relatively normotensive portions of the coarcted aorta were comparable to the values found in the normal aorta.

INTRODUCTION

It has been shown by a number of workers that the content of sodium and water is elevated in the arteries of animals with different forms of experimentally induced hypertension (1–5). These forms of hypertension include Goldblatt’s hypertension (renal), deoxycorticosterone hypertension, and adrenal regeneration hypertension. Tobian and Binion also have reported an abnormally high content of sodium and water in the renal arteries of hypertensive patients (6). Whether these changes in salt and water represent a cause or an effect of the hypertension has not been established. Since previous studies have suggested that the level of arterial pressure, per se, may influence certain metabolic functions (7–10), we decided to study the metabolism of the arteries in experimentally induced coarctation of the aorta, a condition in which the blood pressure is high above the coarcted site and relatively normal below this site (11).

The results of the present study suggest that the level of arterial blood pressure may influence the metabolism of salt, water, and acid mucopolysaccharides in the arteries. The studies also indicate that these metabolic changes may occur with-

out accompanying changes in the aortic lipid content of the aorta.

METHODS

Procedure. Coarctation of the thoracic aorta was produced in 13 adult mongrel dogs. The dogs were anesthetized with sodium pentobarbital and pulmonary ventilation was maintained with an intratracheal tube and a mechanical insufflator. A surgical incision was made anteriorly and laterally along the fifth left intercostal space. The thoracic aorta was exposed and completely occluded by a Derra clamp at its mid-portion for 15 min. During this time the walls of the occluded aorta were stenosed by sutures for a distance of about 2.5 cm. A supporting band of nylon was drawn around the coarcted portion and sutured without further constriction of the vessel. The stenosis caused approximately a 75–90% decrease in the cross-sectional area of the aorta. After completion of the operation a thrill was palpable in the thoracic aorta distal to the coarctation. Most of the dogs developed transient ischemic paralysis of the hind legs which disappeared in 2–3 days after the coarctation operation.

The blood pressure was determined preoperatively and postoperatively in the forelegs during the waking state by the cuff method of Wilson and Clarke (12). On the day of sacrifice the carotid and femoral intra-arterial blood pressures were measured simultaneously during anesthesia with strain gauge transducers and recorded on a Sanborn Polyviso, (Sanborn Co., Waltham, Mass.). In five dogs the thoracic aortic blood pressure was measured immediately before and after coarcting the aorta.

The coarcted dogs and the 13 matched control dogs were maintained on a standard diet of Purina dog chow with daily supplements of ground beef. The dogs were sacrificed with intravenous pentobarbital from 4–12 wk after the surgical coarctation. At 24 hr before sacrifice, ³⁵S-labeled sodium sulfate (specific activity 175 mc/mmole) was injected intravenously in a dose of 14 μ c/kg of body weight. The autopsy findings indicated that none of the dogs had developed complicating atherosclerosis.

The thoracic aorta was dissected and removed immediately after sacrifice. Segments of the descending thoracic aorta proximal to the coarctation and distal to the coarctation were excised and analyzed separately. The same thoracic aortic segments were analyzed in the matched control dogs. The adventitia was stripped from the arterial segments to be analyzed. The fresh specimens were blotted of adhering blood, weighed, and then frozen.

Electrolyte and water measurements. The sodium, potassium, chloride, and water contents of the arterial segments were determined according to the method of Tobian and Fox (13). The samples of arterial wall were dried in vacuo to a constant weight. After drying they were reweighed to determine the water content and dry weight. The dry arteries were then extracted with known volumes of 0.75 N HNO₃. Aliquots of the extract were analyzed for sodium and potassium by flame photometry and for chloride by the micromethod of Lowry, Roberts,

Leiner, Wu, and Farr (14). Samples of pulmonary artery, left ventricular myocardium, and foreleg skeletal muscle were similarly analyzed.

Acid mucopolysaccharide (MPS) measurements. The acid mucopolysaccharides (MPS) in the arterial wall were determined by a slight modification of the method of Sirek, Schiller and Dorfman (15). The arterial segments were minced, defatted with successive washings of acetone and diethyl ether, and dried in a vacuum desiccator. Weighed samples of arterial tissue were suspended in 0.1 M acetate buffer, pH 5.5, containing 0.005 M cysteine-HCl and 0.005 M disodium ethylenediaminetetraacetic acid. Crystalline papain,¹ 2 mg/g of dried tissue was added to the samples which were refrigerated for 15 hr and then incubated in a shaking incubator for 6 hr at 60°C. Whenever necessary, additional papain in a dose of 0.2 mg/g of dried tissue was introduced at the end of 3 hr of incubation to assure complete solubilization of the tissue. The digested tissue was dialyzed at 4°C against running tap water for 24 hr and then against distilled water for another 24 hr. Trypsin,² 2.5 mg/g of dried tissue, was added to the dialyzed samples, which again were dialyzed in 0.1 M phosphate buffer of pH 7.8 for 4 days. Trichloroacetic acid was added to achieve a final concentration of 10% at 0°C. The supernatant was dialyzed against distilled water at 4°C for 24 hr and passed through a column of Dowex 50-X8 (200–400 mesh) to remove trace amounts of protein. The MPS were precipitated from the effluent in 0.038 M sodium chloride by forming a complex with excess cetavlon,³ 10 mg/mg of MPS. The precipitate was allowed to incubate at 37°C for 1 hr, after which time approximately 20 mg of celite⁴ per mg of MPS was added. The samples were stirred, kept at 4°C for 15 hr, and centrifuged in a Lourdes centrifuge at 12,000 rpm for 30 min at 4°C. The supernatant was carefully decanted and the MPS were recovered from the insoluble cetavlon complex by the method of Kaplan and Meyer (16). The precipitate was dissolved in a 40% ethanol solution containing 6.25% sodium acetate, after which the alcohol concentration was increased to over 80%. The resultant precipitate was isolated by centrifugation at 4°C and dried in a vacuum desiccator. The isolated MPS were dissolved in water and analyzed for uronic acid, hexosamine, and sulfate. The recovery of standard amounts of chondroitin sulfate and heparin for the entire method varied from 96–102%. An aliquot of the isolated MPS also was passed through a column of Dowex I-X2 (100–200 mesh) in the chloride form. The column was washed with water and the hyaluronic acid was separated from the MPS by elution with 0.8 M NaCl. The sulfated MPS were then eluted with increasing concentrations of NaCl to a maximum of 4 moles/liter. The eluates were analyzed after dialysis for 24 hr against distilled water. Approximately 90% of the uronic acid applied to the column was recovered.

¹ Worthington Biochemical Corp., Freehold, N. J.

² Worthington Biochemical Corp.

³ Eastman Organic Chemicals, Rochester, N. Y.

⁴ Johns-Manville, Lampoc, Calif.

The hexosamine and uronic acid contents of the MPS were determined by the method of Boas (17) and Dische (18), respectively. The sulfate content was determined after hydrolysis of the isolated MPS with 4 N HCl at 100°C for 15 hr, by precipitation with ¹³⁸Ba-labeled barium chloride standards. The methods used were similar to those used by Miller, Hlad, Levine, Holmes, and Erlick for plasma (19). After the hydrolysate was evaporated to dryness and dissolved in 1 ml of distilled water, 1 ml of 95% ethanol and 1 ml of a solution of known barium chloride concentration containing 0.18 N HCl was added. The mixture was kept at 5°C for at least 18 hr before centrifugation. The concentration of the barium did not exceed the sulfate concentration by more than a factor of 3 to insure that the error of measurement of unprecipitated ¹³⁸Ba was kept to a minimum. After refrigeration and centrifugation, the counting rate of 2 ml (or two-thirds) of the supernatant was determined and the amount of sulfate calculated as follows:

$$SO_4 = I - 1.5 S/I \times M$$

Where SO_4 is moles of sulfate in sample, I is initial counting rate of the barium solution used, S is counting rate of two-thirds (or 2 ml) of the supernatant, M is moles of barium standard added to the sulfate sample.

The recovery of sulfate from ammonium sulfate standards containing $2.0\text{--}6.2 \times 10^{-4}$ mole/liter of sulfate averaged $98.8 \pm 4\%$. The recovery of sulfate in a nonalcoholic solution was variable and did not exceed 50%. Standards of sodium phosphate added to the sulfate standard in amounts three to nine times that of the sulfate did not alter the recovery of the sulfate. The phosphate standards alone were not precipitated by the acidified barium chloride solution.

A separate aliquot of the isolated MPS was assayed for ³⁵SO₄ radioactivity after acid hydrolysis and evaporation of the HCl. The residue was dissolved in 1 ml of water and the radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer using a Hyamine-Triton-toluene system.⁵

All radioactive samples were counted to a statistical accuracy of at least 5% (20). Variations in counting between duplicate samples were not greater than 4%.

Lipid measurements. The arterial tissue was minced and extracted into chloroform-methanol, 2:1 (v/v), according to the method of Folch, Lees, and Stanley (21). Aliquots of the minced tissue were dried to a constant weight and all the results were expressed in terms of the dry tissue weight. Aliquots of the chloroform-methanol extract were used to determine cholesterol by the method of Schoenheimer and Sperry (22), triglycerides by the method of Van Handel and Zilversmit (23), phospholipid by the method of Youngberg and Youngberg (24), and total fatty acids by the method of Albrink (25).

⁵ 1 ml of 1.5 M Hyamine 10-X, 5 ml of toluene A and Triton X-100 mixture 2:1 (v/v), and 12 ml of toluene A. Toluene A was prepared as follows: 4.0 g of 2,5-diphenyloxazole, and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, were made up to 1 liter with toluene.

RESULTS

A diagram of the surgically coarcted aorta of dog is shown in Fig. 1. Based on the blood pressure changes after the coarctation, the portion of the descending thoracic aorta proximal to the coarctation was designated the "hypertensive" segment, and the portion of the thoracic aorta distal to the coarctation was designated the "hypotensive" segment or the relatively normotensive segment. The composition and metabolism of these segments were compared with the corresponding aortic segments of the paired control dogs, particularly since the metabolic activity of the aorta at these sites appeared to be different under normal conditions. The data shown in Tables II and IV indicate in the normal dog that the mean content of sodium, potassium, chloride, water, acid mucopolysaccharides, and cholesterol was higher in the upper thoracic aorta than in the lower thoracic aorta.

Arterial blood pressure. Immediately after the surgical narrowing of the thoracic aorta, the thoracic aortic blood pressure proximal to the coarctation rose on the average of 32/14 mm Hg above control values. In the thoracic aorta distal to the coarctation, the blood pressure fell on the average of 19/4 mm Hg and resulted in a marked reduction of the pulse pressure below the coarcted site (Fig. 2). After the coarctation, the cuff systolic blood pressure in the forelimbs progressively rose and reached a definite hypertensive level in 1 wk after the operation. At this time the cuff systolic blood pressure was 210 ± 9 SE as compared to the preoperative value of 147 ± 4 SE.

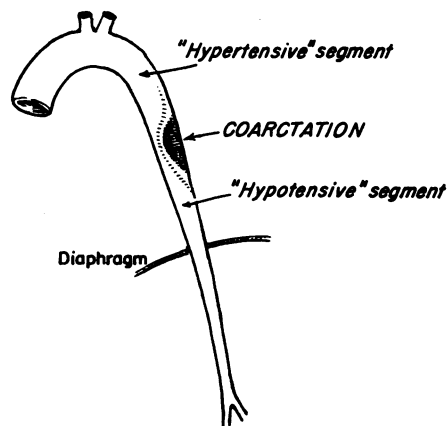


FIGURE 1 Diagram of coarcted aorta of dog.

TABLE I
Comparison of Carotid and Femoral Arterial Pressures in Control and Coarcted Dogs at Time of Sacrifice

	No. of studies	Body wt	Carotid arterial pressure				Femoral arterial pressure			
			Systolic	Diastolic	Pulse	Mean	Systolic	Diastolic	Pulse	Mean
Control dogs	13	kg 24.4* ±1.5	158 ±4.4	100 ±3.9	58 ±1.8	120 ±4.0	160 ±4.4	100 ±3.6	60 ±2.0	120 ±3.7
Coarcted dogs	13	25.3 ±1.0	199 ±6.3	137 ±5.3	62 ±4.4	158 ±5.2	130 ±3.0	108 ±4.1	22 ±2.4	115 ±3.7
Mean difference		+0.9 ±1.8	+41 ±7.7	+37 ±6.5	+4 ±4.8	+38 ±6.5	-30 ±5.3	+8 ±5.5	-38 ±3.1	-5 ±5.2
<i>P</i>		>0.6	0.01	<0.01	>0.3	<0.01	<0.01	>0.1	<0.01	>0.4

* Values represent the mean and standard error.

The intra-arterial pressures simultaneously measured in the carotid and femoral arteries of the control and coarcted dogs at the time of sacrifice are shown in Table I.

In comparison with the arterial pressures of the control dogs, the coarcted dogs developed a significant elevation of blood pressure in the carotid artery and presumably in the thoracic aorta proximal to the coarctation. The rises in carotid, systolic diastolic, and mean blood pressures averaged 41, 37, and 38 mm Hg above control levels, respectively. In the femoral artery and presumably in the thoracic aorta distal to the coarctation, the systolic blood pressure fell significantly. The decrease in femoral systolic blood pressure averaged 30 mm Hg below control values, while the diastolic

blood pressure showed a slight and insignificant rise. As a result of these changes, there was a striking decrease of 38 mm Hg in the femoral arterial pulse pressure (Fig. 3) without a significant change in the mean femoral arterial pressure. Similar decreases in systolic and pulse pressures were assumed to occur in the thoracic aorta distal to the coarcted site and hence this position of the aorta is referred to as the "hypotensive" aortic segment.

Body weight, serum sodium, and potassium. The body weights of the control and coarcted dogs were comparable (Table I). The serum sodium and potassium levels in the control and experimental dogs also were not significantly different ($P > 0.3$). The serum sodium and potassium in mEq/liter averaged 147 ± 1.1 and 4.7 ± 0.1 SE,

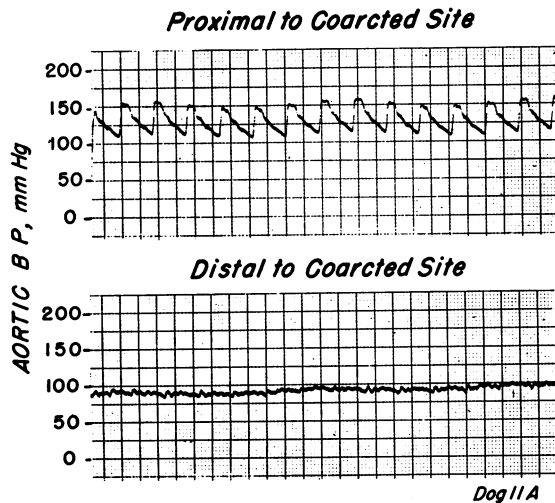


FIGURE 2 Aortic blood pressure (BP) immediately after surgical narrowing of the thoracic aorta of a dog.

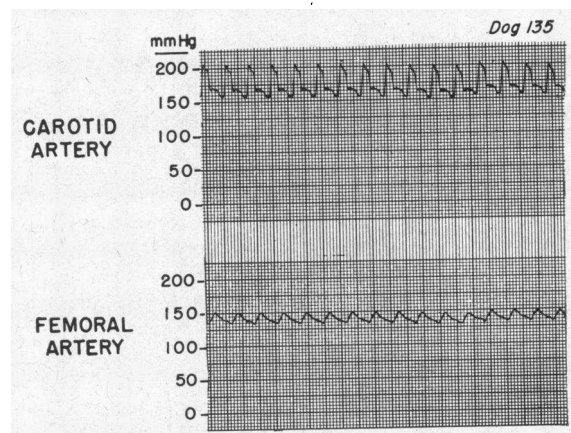


FIGURE 3 Carotid and femoral arterial pressures simultaneously measured in a dog with coarctation of the aorta of 10 wk duration.

respectively, in the control dogs and 147 ± 1.2 and 4.6 ± 0.1 SE, respectively, in the coarcted dogs.

Electrolyte and water content of the aorta. The sodium, potassium, chloride, and water contents of the aorta of coarcted dogs and of the corresponding aortic segments of control dogs are compared in Table II. The hypertensive portion of the coarcted aorta contained significantly more sodium and chloride than did the control aorta. The in-

creases in sodium and chloride content averaged 18.2 and 25.0% above control values, respectively. The water content of the hypertensive segment of the coarcted aorta also increased significantly although the increase was only 4.3% above the control values. The differences in the potassium content of the "hypertensive" and control aortas were slight and insignificant. In contrast with the changes in the hypertensive segment of the co-

TABLE II
Comparison of Electrolyte, Water, and Acid Mucopolysaccharide (MPS) Content of Aorta of Control and Coarcted Dogs

	No.	Normal dog, control upper aorta	Coarcted dog, "hypertensive" upper aorta	Mean difference	P	Normal dog, control lower aorta	Coarcted dog, "hypertensive" lower aorta	Mean difference	P
Electrolytes									
Na, $\mu\text{Eq/g dry wt}$	13	296* ± 8	350 ± 10	+54 ± 13	<0.01	282 ± 8	282 ± 7	0 ± 11	>0.9
Cl, $\mu\text{Eq/g dry wt}$	10	220 ± 10	275 ± 14	+55 ± 17	<0.01	203 ± 15	225 ± 11	+22 ± 19	>0.2
K, $\mu\text{Eq/g dry wt}$	13	163 ± 5	166 ± 5	+ 3 ± 8	>0.7	136 ± 6	127 ± 5	- 9 ± 8	>0.1
H ₂ O, mg/g wet wt	13	705 ± 5	735 ± 5	+30 ± 7	<0.01	678 ± 8	676 ± 8	- 2 ± 11	>0.8
Total MPS									
Uronic acid, mg/g dry wt	13	2.96 ± 0.16	3.92 ± 0.23	+ 0.96 ± 0.28	<0.01	2.41 ± 0.15	1.83 ± 0.09	- 0.58 ± 0.18	<0.01
Hexosamine, mg/g dry wt	13	2.55 ± 0.09	3.46 ± 0.21	+ 0.91 ± 0.23	<0.01	2.12 ± 0.09	1.61 ± 0.09	- 0.51 ± 0.13	<0.01
SO ₄ , mg/g dry wt	10	1.45 ± 0.06	1.84 ± 0.09	+ 0.39 ± 0.11	<0.01	1.31 ± 0.09	1.01 ± 0.06	- 0.30 ± 0.11	0.02
Sulfated MPS									
Uronic acid, mg/g dry wt	7	2.71 ± 0.18	3.77 ± 0.21	+ 1.06 ± 0.27	<0.01	2.29 ± 0.19	1.68 ± 0.13	- 0.61 ± 0.23	0.04
SO ₄ , mg/g dry wt	7	1.44 ± 0.09	1.86 ± 0.14	+ 0.42 ± 0.17	0.05	1.33 ± 0.07	1.07 ± 0.07	- 0.26 ± 0.10	0.04
³⁵ SO ₄ , % dose $\times 10^{-3}/g$ dry wt	7	3.00 ± 0.29	5.20 ± 0.66	+ 2.20 ± 0.73	0.02	2.61 ± 0.21	1.50 ± 0.20	- 1.11 ± 0.29	0.01
SO ₄ , SA, ³⁵ SO ₄ /SO ₄	7	2.07 ± 0.16	2.80 ± 0.23	+ 0.73 ± 0.28	0.04	2.02 ± 0.14	1.41 ± 0.07	- 0.61 ± 0.15	0.01
Hyaluronic acid									
Uronic acid, mg/g dry wt	7	0.26 ± 0.04	0.28 ± 0.08	+ 0.02 ± 0.08	>0.7	0.21 ± 0.05	0.18 ± 0.06	- 0.03 ± 0.08	>0.7

* Values represent the mean and standard error.

arcted aorta, the contents of sodium, chloride, potassium, and water in the "hypotensive" segment of the coarcted aorta were not significantly different from those of the normal aorta.

Electrolyte and water content of other tissues. The sodium, potassium, and water contents of the pulmonary artery, skeletal muscle and cardiac muscle are compared in normal and coarcted dogs in Table III. The electrolyte and water contents of these tissues were not significantly different in normal and coarcted dogs.

Acid mucopolysaccharides of the aorta. The MPS contents of the coarcted aorta and the normal aorta are compared in Table II. The total MPS content of the aorta is expressed in terms of its major constituents: hexuronic acid, hexosamine, and sulfate in milligram per gram of dried defatted tissue. As compared with the control values, the hexuronic acid, hexosamine, and sulfate contents of the total MPS in the hypertensive

aorta were significantly increased by about 27–35%. The isolated sulfated MPS, as indicated by their sulfate and uronic acid contents, increased by a similar percentage, whereas the hyaluronic acid content, which constituted less than 10% of the total MPS, showed no significant change. Thus the increase of the total MPS content in the hypertensive segment of the coarcted aorta was due to an increase in the sulfated MPS.

The MPS in the "hypotensive" aortic segment of the coarcted dogs also changed significantly. The MPS content of hexuronic acid, hexosamine, and sulfate in this portion of the aorta showed decreases of about 22–24% below control values. A decrease of sulfated MPS appeared to account for the reduction of the total MPS since the isolated sulfated MPS decreased by a similar percent below control values, whereas the hyaluronic acid content showed no significant change.

The incorporation of $^{35}\text{SO}_4$ into aortic MPS at 24 hr after the intravenous administration of the radiosulfate is summarized in Table II. The amount of $^{35}\text{SO}_4$ converted into sulfated MPS was significantly higher in the hypertensive portion of the coarcted aorta than in the control aorta by about 73%. Since the sulfate content of the MPS increased by about half as much as the radiosulfate incorporation, the calculated specific activity of the sulfated MPS was significantly higher in the "hypertensive" aorta than in the control aorta by about 40%. In contrast to these findings, the mean incorporation of radiosulfate and the specific activity of the sulfated MPS were significantly lower in the "hypotensive" portion of the coarcted aorta than in the control aorta by about 42 and 30%, respectively.

MPS of other tissues. As shown in Table III, the uronic acid (MPS) contents of the pulmonary artery and cardiac muscle were not significantly different in normal and coarcted dogs.

Lipid content of the aorta. The lipid content of the normal and coarcted aorta are compared in Table IV. The contents of cholesterol, phospholipids, triglycerides, and total fatty acids in the hypertensive and relatively normotensive portions of the coarcted aorta and in the corresponding segments of the control aorta were not significantly different. The plasma cholesterol levels also were not significantly different in the control and coarcted dogs. The mean plasma cholesterol in mg/

TABLE III
Comparison of Electrolyte, Water, and MPS (Uronic Acid)
Content of Skeletal Muscle, Cardiac Muscle and
Pulmonary Artery of Control and
Coarcted Dogs

	No.	Normal dog	Coarcted dog	Mean difference	P
Skeletal muscle					
Na, $\mu\text{Eq/g dry wt}$	13	96* ± 4	100 ± 6	+4 ± 7	>0.4
K, $\mu\text{Eq/g dry wt}$	13	325 ± 17	326 ± 15	+1 ± 22	>0.9
H ₂ O, mg/g wet wt	13	703 ± 13	710 ± 8	+7 ± 5	>0.6
Heart muscle					
Na, $\mu\text{Eq/g dry wt}$	10	141 ± 7	149 ± 9	+8 ± 11	>0.4
K, $\mu\text{Eq/g dry wt}$	10	318 ± 9	314 ± 6	-4 ± 10	>0.6
H ₂ O, mg/g wet wt	10	748 ± 6	745 ± 6	-3 ± 8	>0.7
Uronic acid (MPS), mg/g dry wt	10	0.45 ± 0.02	0.45 ± 0.02	0 ± 0.02	>0.9
Pulmonary artery					
Na, $\mu\text{Eq/g dry wt}$	10	373 ± 10	372 ± 17	-1 ± 20	>0.9
K, $\mu\text{Eq/g dry wt}$	10	202 ± 7	191 ± 10	-11 ± 12	>0.3
H ₂ O, mg/g wet wt	10	738 ± 5	734 ± 6	-4 ± 8	>0.6
Uronic acid (MPS), mg/g dry wt	8	1.94 ± 0.13	2.02 ± 0.18	+0.08 ± 0.22	>0.7

* Values represent the mean and standard error.

TABLE IV
Comparison of the Lipid Content of Aorta of Control and Coarcted Dogs

	No.	Normal dog, control upper aorta	Coarcted dog, "hypertensive" upper aorta	Mean difference	P	Normal dog, control lower aorta	Coarcted dog, "hypertensive" lower aorta	Mean difference	P
Cholesterol, mg/g dry wt	13	5.8* ±0.4	5.2 ±0.4	-0.6 ±0.5	>0.2	4.6 ±0.2	4.4 ±0.2	-0.2 ±0.3	>0.5
Triglycerides, mg/g dry wt	13	30.1 ±3.0	26.3 ±6.2	-3.8 ±6.9	>0.6	50.8 ±10.8	52.1 ±10.3	+1.3 ±15.0	>0.4
Phospholipids, mg/g dry wt	13	18.8 ±1.3	20.6 ±1.9	+1.8 ±2.3	>0.4	15.0 ±1.1	14.8 ±1.0	-0.2 ±1.4	>0.8
Total fatty acids, μ Eq/g dry wt	13	199 ±26	174 ±24	-25 ±35	>0.4	338 ±48	343 ±40	+5 ±62	>0.9

* Values represent the mean and standard error.

100 ml was 182 ± 13 SE in the control dogs and 184 ± 14 SE in the coarcted dogs.

DISCUSSION

The present findings in experimental coarctation of the aorta indicate that the content of sodium, chloride, and water in the hypertensive segment of the coarcted aorta was increased significantly, whereas the content of salt and water in the relatively normotensive portion of the coarcted aorta was normal. The pulmonary artery and skeletal and heart muscle of the coarcted dog also were found to contain normal amounts of sodium and water. These observations suggest that a high level of arterial pressure may operate to increase the sodium and water content of the aorta. It, therefore, is conceivable that the reported increases in sodium, chloride, and water content of the arteries in certain forms of hypertension may be largely the result and not necessarily the cause of the hypertension.

In addition to the height of the arterial pressure, other changes, such as in the metabolism of mineralocorticoids and mucopolysaccharides, may occur in different forms of hypertension and influence the salt and water content of the arteries. These factors may account for the differences in the magnitude of changes in electrolytes and water reported in hypertensive arteries (1-5). In the current study, the increases in the sodium content of the hypertensive portion of the coarcted aorta appeared to be comparable to those reported in other forms of experimental hypertension, whereas

the increases in the water content were smaller, and those in the chloride content were generally higher than those previously reported. The potassium content, which did not change in the coarcted aorta, has been found to be normal or increased in the arteries of animals with other forms of experimental hypertension.

Although the increment of sodium was greater than that of water in the hypertensive portion of the coarcted aorta, it is unlikely that an increase in the osmolarity occurred in this aortic segment since the sodium and potassium content of the plasma and of the aorta distal to the coarctation did not change. Therefore, it would be reasonable to assume that much of the excess sodium in the hypertensive aorta was bound or stored in a non-ionizable form. There is evidence from in vitro studies (26, 27) that both intracellular sodium and extracellular sodium in the normal artery is present in a bound form as well as in an ionizable form. The exact location of aortic sodium was not established in the present study. However, the extra sodium in the hypertensive portion of the coarcted aorta might be present mainly in the extracellular space since the increases in the chloride content were comparable to those in the sodium content. This interpretation requires additional support since the extracellular location of chloride has not been established in the arteries as it has been in the skeletal muscle.

A number of workers (28-30) have shown that acid mucopolysaccharides, which are extracellular components of the connective tissue and can act

as polyanions, are capable of binding or storing appreciable amounts of sodium and other cations in an osmotically inactive form. There also is some evidence (31) that the acid mucopolysaccharide, hyaluronic acid, may influence the water content of the tissue because of its high hydrodynamic specific volume. In view of these observations, it is possible that the increase in the sodium content in the hypertensive portion of the coarcted aorta was not a primary event but resulted mainly from an increase in the sulfated acid mucopolysaccharide content of the aorta. It likewise is possible that the absence of a significant change in the hyaluronic acid content of the coarcted aorta might account for the lack of a striking increase in the water content of the aorta.

In addition to acid mucopolysaccharides, there likely are other factors which operate to control the salt and water composition of the coarcted aorta since the sodium content of the aorta distal to the coarctation did not change with the fall in the acid mucopolysaccharide content in this portion of the aorta. Phospholipids, particularly those that have a net negative charge at physiologic pH levels, have the ability to bind cations (32, 33). Although phosphatides may influence the cation composition of the arteries, the content of phospholipids in the coarcted aorta below as well as above the coarcted site was not significantly altered. Nucleic acids and nucleotides are other classes of compounds which can act as polyanions (34) and, hence, also may influence the cation composition of the tissues.

The metabolism of arterial acid mucopolysaccharides also may be influenced by arterial pressure since the content of mucopolysaccharides was increased in the hypertensive portion of the aorta above the coarcted site, whereas it was decreased in the aortic segment below the coarcted site where the systolic and pulse pressures were reduced. These changes in the aortic acid mucopolysaccharides appeared to be due mainly to changes in the sulfated acid mucopolysaccharides, which constituted over 90% of the aortic mucopolysaccharides in the normal and coarcted aorta. The studies of Sirek et al. (15) indicate that chondroitin sulfate A and C are the major acid mucopolysaccharide components in the normal canine aorta with hyaluronic acid being present in negligible amounts.

The changes in the acid mucopolysaccharide

content of the coarcted aorta were associated with similar changes in the incorporation of radiosulfate into sulfated acid mucopolysaccharides at 24 hr after the intravenous administration of the radio-sulfate. These observations suggest that the arterial pressure may influence the mucopolysaccharide content in the aorta by altering the rate of synthesis of the sulfated acid mucopolysaccharides. Studies on the turnover rate of acid mucopolysaccharides in the aorta (35) have shown that the maximal incorporation of radiosulfate into acid mucopolysaccharides occurs in about 24 hr after the administration of the sulfate and is significantly correlated with the turnover rate of the sulfated acid mucopolysaccharides.

ACKNOWLEDGMENTS

We are grateful for the technical assistance of Marilyn Colombo and Margaret Sullivan.

This paper was supported in part by grants HE-07299 and HE-0536-13 from the U. S. Public Health Service and by the U. A. Whitaker Fund.

REFERENCES

1. Tobian, L., Jr., and J. Binion. 1954. Artery wall electrolytes in renal and DCA hypertension. *J. Clin. Invest.* **33**: 1407.
2. Daniel, E. E., and O. Dawkins. 1957. Aorta and smooth muscle electrolytes during early and late hypertension. *Am. J. Physiol.* **190**: 71.
3. Tobian, L., and P. D. Redleaf. 1958. Ionic composition of the aorta in renal and adrenal hypertension. *Am. J. Physiol.* **192**: 325.
4. Koletsky, S., H. Resnick, and D. Behrin. 1959. Mesenteric artery electrolytes in experimental hypertension. *Proc. Soc. Exptl. Biol. Med.* **102**: 12.
5. Jones, A. W., E. O. Feigl, and L. H. Peterson. 1964. Water and electrolyte content of normal and hypertensive arteries in dogs. *Circulation Res.* **15**: 386.
6. Tobian, L., Jr., and J. T. Binion. 1952. Tissue cations and water in arterial hypertension. *Circulation.* **5**: 754.
7. Perry, H. M., Jr., and H. A. Schroeder. 1955. Depression of cholesterol levels in human plasma following ethylenediamine tetracetate and hydralazine. *J. Chronic Disease.* **2**: 520.
8. Deming, Q. B., M. E. Hodes, A. Baltazar, J. G. Edreira, and S. Torosdag. 1958. The changes in concentration of cholesterol in the serum of hypertensive patients during antihypertensive therapy. *Am. J. Med.* **24**: 882.
9. Hollander, W., and W. E. Judson. 1957. Electrolyte and water excretion in arterial hypertension. I. Studies in non-medically treated subjects with essential hypertension. *J. Clin. Invest.* **36**: 1460.
10. Hollander, W., and W. E. Judson. 1958. Electrolyte and water excretion in arterial hypertension. II. Stud-

- ies in subjects with essential hypertension after anti-hypertensive drug treatment. *Circulation*. 17: 576.
11. Hollander, W., I. M. Madoff, D. Kramsch, and S. Yagi. 1964. Arterial wall metabolism in experimental hypertension of coarctation of the aorta. *Hypertension*. 13: 191.
 12. Wilson, R. B., and T. J. Clarke. 1964. Indirect measurement of systolic blood pressure in dogs by the use of xylol pulse indicator. *J. Am. Vet. Med. Assoc.* 144: 981.
 13. Tobian, L., and A. Fox. 1956. The effect of norepinephrine on the electrolyte composition of arterial smooth muscle. *J. Clin. Invest.* 35: 297.
 14. Lowry, O. H., N. R. Roberts, K. Y. Leiner, M.-L. Wu, and A. I. Farr. 1954. The quantitative histochemistry of brain. I. Chemical methods. *J. Biol. Chem.* 207: 1.
 15. Sirek, O. V., S. Schiller, and A. Dorfman. 1964. Acid mucopolysaccharides in aortic tissue of the dog. *Biochem. Biophys. Acta.* 83: 148.
 16. Kaplan, D., and K. Meyer. 1962. The fate of injected mucopolysaccharides. *J. Clin. Invest.* 41: 743.
 17. Boas, N. F. 1953. Method for the determination of hexosamines in tissues. *J. Biol. Chem.* 204: 553.
 18. Dische, Z. 1947. A new specific color reaction of hexuronic acids. *J. Biol. Chem.* 167: 189.
 19. Miller, E., C. J. Hlad, S. Levine, J. H. Holmes, and H. Erlick. 1961. The use of radioisotopes to measure body fluid constituents I. Plasma sulfate. *J. Lab. Clin. Med.* 58: 656.
 20. Loevinger, R., and M. Berman. 1951. Efficiency criteria in radioactivity counting. *Nucleonics*. 9: 26.
 21. Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226: 497.
 22. Schoenheimer, R., and W. M. Sperry. 1934. A micro-method for the determination of free and combined cholesterol. *J. Biol. Chem.* 106: 745.
 23. Van Handel, E., and D. B. Zilversmit. 1957. Micro-method for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* 50: 152.
 24. Youngburg, G. E., and M. V. Youngburg. 1930. Phosphorus metabolism. I. A system of blood phosphorus analysis. *J. Lab. Clin. Med.* 16: 158.
 25. Albrink, M. J. 1959. The microtitration of total fatty acids of serum, with notes on the estimation of triglycerides. *J. Lipid Res.* 1: 53.
 26. Headings, V. E., P. A. Rondell, and D. F. Bohr. 1960. Bound sodium in artery wall. *Am. J. Physiol.* 199: 783.
 27. Friedman, S. M., and C. L. Friedman. 1963. Ionic basis of vascular response to vasoactive substances. *Can. Med. Assoc. J.* 90: 167.
 28. Kulonen, E. 1953. On the relation of hyaluronic acid to the water and electrolyte metabolism. *Acta. Physiol. Scand.* 27: 82.
 29. Farber, S. J., and M. Schubert. 1957. The binding of cations by chondroitin sulfate. *J. Clin. Invest.* 36: 1715.
 30. Aldrich, B. I. 1958. The effects of the hyaluronic acid complex on the distribution of ions. *Biochem. J.* 70: 236.
 31. Rienits, K. G. 1960. The acid mucopolysaccharides of the sexual skin of apes and monkeys. *Biochem. J.* 221: 211.
 32. Gravin, J. E., and M. L. Karnovsky. 1956. The titration of some phosphatids and related compounds in a non-aqueous medium. *J. Biol. Chem.* 239: 4066.
 33. Hokin, L. E., and M. R. Hokin. 1963. Phosphatidic acid metabolism and active transport of sodium. *Federation Proc.* 22: 8.
 34. Shack, J., R. J. Jenkins, and J. M. Thompsett. 1953. The interaction of ions and desoxyribose nucleic acid of calf thymus. *J. Biol. Chem.* 203: 373.
 35. Hauss, W. H., G. Junge-Hülsing, and H. J. Holländer. 1962. Changes in metabolism of connective tissue associated with ageing and arterio- or atherosclerosis. *J. Atherosclerosis Res.* 2: 50.