The Electrolytes of Arterial Wall in Experimental Renal Hypertension

By Louis Tobian, M.D.

With technical assistance of Lois White

In rats with renal hypertension, smooth muscle cells in the wall of the aorta appear to have more water, sodium, potassium, magnesium and phosphorus per unit of cell solids than similar cells in normotensive rats. When the blood pressure of a few hypertensive rats dropped to normal as a result of a low sodium diet, the potassium and phosphorus in the arterial cells also appeared to decrease to normal levels. These studies give indications of the compositional changes in arterial smooth muscle cells that are associated with renal hypertension.

In a previous study of the arterial wall in rats with renal hypertension, the concentrations of sodium and potassium per unit of dry weight were found to be elevated above the levels found in rats that were normotensive despite similar kidney manipulations. These changes were not present in brain or skeletal muscle tissue. Since the sodium content of the arterial wall was elevated while the chloride content was not, it seemed likely that the excessive sodium content was probably intracellular in location. However, the potassium results were more difficult to interpret. They could be explained alternatively as an increase in intracellular potassium per unit of cell solids, or as a great increase of the intracellular relative to the extracellular phase. To resolve this question and to get further data on the phosphorus and magnesium content of the arterial wall in renal hypertension, the experiments described below were carried out. The effect of a low sodium diet in rats with renal hypertension was also studied.

Methods

Renal hypertension was produced in male Sprague-Dawley rats by placing a figure-of-eight ligature around one kidney and removing the contralateral kidney 1 week later. Six months following these operations the blood pressure was carefully measured on at least two occasions. The hypertensive rats were divided into 2 groups: those eating regular rat chow (3 per cent sodium), and those on a low sodium diet, containing .003 per cent sodium and all essential vitamins and minerals. Rats on the low sodium diet remained healthy and active. After 5 weeks on either diet, the blood pressures of all rats in both groups were again carefully ascertained. The rats were then sacrificed; their aortas were removed in a cold room (4 C.) immediately after death, blotted free of blood, weighed on a torsion balance, then dried from the frozen state and reweighed. The dried aortas were extracted with .05 N LiOH at room temperature for 4 days. Lipids were not removed by extraction since the solvents might denature the proteins normally soluble in weak alkali, and past experience indicates that the lipid content of the aorta is similar in normotensive and hypertensive rats. Aliquots of the LiOH extract were analyzed for sodium and potassium using flame photometry. Chloride was determined by the potentiometric method of Kolthoff and Kuroda. Magnesium was determined by the method of Kunkel, Pearson, and Schweigert. Total phosphorus was analyzed by Lowry's micro-method. Protein content of the LiOH extract was determined by the method of Hoch and Vallee. According to the principle described by Lilienthal and associates, tissue proteins that are soluble in dilute alkali at room temperature were considered an index of the amount of intracellular protein in the tissue. Collagen and elastin are not soluble under these conditions. This type of analysis is particularly useful in tissues that might have a varying mixture of muscle cells and connective tissue. It should be pointed out, however, that the protein soluble in dilute alkali represents only a fraction of the total intracellular protein. Skeletal muscle and heart muscle, both being tissues in which most of the solids are intracellular, were lyophilized and subsequently extracted with .05 N LiOH. The protein that went into solution was
only about one third of the total estimated intracellular protein.

Blood pressure was determined by the Friedmann microphonic method after gently heating the rat. The mean blood pressure in Sprague-Dawley rats was found to be 121 mm Hg with a standard deviation of 10 mm Hg. Out of 55 normal rats that were tested, no normal rat had a blood pressure higher than 143 mm Hg. A blood pressure above 150 mm Hg was considered a hypertensive level. Rats with the most severe hypertension had blood pressure readings around 230 mm Hg. The average blood pressure of the hypertensive group was 180 mm Hg. Operated rats with pressures less than 131 mm Hg were placed in the "operated normotensive" group. This group constituted the best control for determining whether changes in tissue composition were related to hypertension per se.

The system of chemical dissection devised by Hastings and Eichelberger was used in calculating the amount of extracellular sodium; it assumes that all the chloride in the wall of the aorta is extracellular and in the same concentration as in an ultrafiltrate of plasma. The extracellular water was not similarly estimated since Manery and Hastings pointed out that this type of calculation is not applicable to tissues containing abundant amounts of connective tissue. The calculation of extracellular sodium itself is most likely in absolute error, but is useful in comparing the relative amounts of extracellular sodium in the wall of the aorta in normotensive and hypertensive rats. Manery, Danielson, and Hastings pointed out that while connective tissue was an integral part of the extracellular phase, it nevertheless had more chloride and less sodium per liter of tissue water than an ultrafiltrate of plasma. For this reason the method of chemical dissection which works so well with many tissues, e.g., skeletal muscle, can not be accurately applied to tissues of the type that contain an abundant quantity of connective tissue. In photomicrographs of the rat aorta, the cells appear to occupy about two fifths of the wall of the aorta, a much greater proportion than is indicated by the classic chemical dissection.

Results

Table 1 shows a comparison of the hypertensive and operated normotensive rats in regard to the composition of the wall of the aorta. In the former the water content per 100 Gm. of solids was 10 per cent and the amount of sodium per 100 Gm. of aorta solids was 15 per cent higher than in the latter group. Both differences were significant and confirm the observations in an earlier series. However, also confirming previous work, the chloride content of the aorta wall in this group was virtually the same as in the operated normotensive group. Only one fifth of the difference in sodium content between normotensives and hypertensives can be accounted for as an increase in extracellular sodium. The remaining four fifths of the excess sodium in the aortas of the hypertensive group is probably located intracellularly.

The predominantly intracellular substances can be related to the protein soluble in dilute alkali. As seen in table 1, the wall of the aorta in the hypertensive rats had 37 per cent more

<table>
<thead>
<tr>
<th>Rats normotensive despite renal operations</th>
<th>No. of rats</th>
<th>H2O</th>
<th>NePr</th>
<th>Na</th>
<th>NcPr</th>
<th>Cl</th>
<th>K</th>
<th>Mg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gm./100 Gm. TS</td>
<td>mEq/100 Gm. TS</td>
<td>mEq/100 Gm. TS</td>
<td>mEq/100 Gm. TS</td>
<td>mEq/100 Gm. NePr</td>
<td>mEq/100 Gm. NePr</td>
<td>mEq/100 Gm. NePr</td>
<td>mEq/100 Gm. NePr</td>
</tr>
<tr>
<td>Rats with renal hypertension</td>
<td>16</td>
<td>282</td>
<td>10.6</td>
<td>41.2</td>
<td>36.6</td>
<td>28.3</td>
<td>120.3</td>
<td>12.1</td>
<td>49.2</td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>±4.3</td>
<td>±0.5</td>
<td>±1.3</td>
<td>±3%</td>
<td>+2%</td>
<td>+37%</td>
<td>+10%</td>
<td>+21%</td>
</tr>
<tr>
<td>p value of difference between the two means</td>
<td></td>
<td>.002*</td>
<td>.01*</td>
<td>.02*</td>
<td>.27</td>
<td>.0006*</td>
<td>.001*</td>
<td>.04*</td>
<td>.50</td>
</tr>
</tbody>
</table>

* A difference considered significant since there is less than one chance in twenty that the difference is due to chance.

± = standard error of the mean
NePr = noncollagen protein
TS = total solids
NcPr = extracellular sodium

**Table 1.—Mean Content of Electrolytes in the Wall of the Aorta in Rats with Renal Hypertension**

672 ARTERIAL WALL ELECTROLYTES IN EXPERIMENTAL HYPERTENSION
TOBIAN

potassium, 21 per cent more magnesium, and 36 per cent more phosphorus/100 Gm. of non-collagen protein than aortas of the operated normotensive rats. All of these differences were statistically significant. Related to units of dry weight, the differences between the hypertensive rats and the operated normotensive rats were not so striking. The potassium in the aorta wall, however, was significantly greater in the hypertensive rats. The phosphorus and magnesium per unit of total aorta solids were not significantly increased in the hypertensive rats compared to operated normotensive ones. The actual relative content of these substances in the cells of the aorta wall would seem to be most validly indicated by using the alkali-soluble protein as a basis, since it is a good index of intracellular protein.

Four of the 16 hypertensive rats had a moderate degree of azotemia. However, the same alterations in the composition of the aorta were found. As seen in table 1, the wall of the aorta in the hypertensive rats had 16 per cent less alkali-soluble protein/100 Gm. of total solids than the aortas of the operated normotensive group. This was a significant difference. Though there may have been some cellular hypertrophy in the walls of the hypertensive aortas, the data suggests that it was outstripped by a hyperplasia of connective tissue fibers, leaving the percentage of intra-cellular solids lower in the hypertensive aortas than in the normotensive ones. Essentially similar concentrations of sodium and chloride were found in the serum of both groups. Thus, in operated normotensive and hypertensive rats the serum concentrations (meq./L.) of sodium were respectively 147.6 and 148, and of chloride, 103 and 103.1.

**Effect of Low Sodium Diet.** Nine of the rats with renal hypertension survived a 5 week period during which time their diet contained only .003 per cent sodium. Six had no lowering of blood pressure. In 3 the arterial blood pressure fell to normal levels. The number of rats in these 2 contrasting groups is admittedly small, and only very striking differences could possibly be meaningful and significant. However, just such differences were observed in regard to the potassium and phosphorus content per unit of non-collagen protein in the aorta wall. Table 2 gives the individual analytical results in each rat of both groups. Rats whose arterial pressure fell to normal while on a low sodium diet showed a striking 25 per cent reduction in both the potassium and phosphorus in the aorta wall, down to levels seen in the operated normotensive group. There was no overlapping of values in the 2 groups. Analyzed statistically according to the method recommended by Arkin and Colton for handling small samples, there is about 1 change in 200 (p = .005) that the difference between the means of these 2 groups is due to chance. This holds true for both the potassium and phosphorus values. The 2 groups did not show this striking difference in regard to the water, sodium, or magnesium content in the wall of the aorta. The question as to whether real differences of water, sodium and magnesium are present will have to await the analysis of a larger series of rats.

<table>
<thead>
<tr>
<th>TABLE 2.—Content of Potassium and Phosphorus in the Aortas of Individual Rats with Varying Blood Pressure Responses to a Low Sodium Diet</th>
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</thead>
<tbody>
<tr>
<td>Six hypertensive rats whose blood pressures did not fall while on a low sodium diet</td>
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<tr>
<td></td>
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<tr>
<td>101</td>
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<td>105</td>
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<td>108</td>
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<td>121</td>
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<td>124</td>
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<td>130</td>
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<td>Mean values...</td>
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*NcPr = noncollagen protein.*

Discuss,

From data presented it would appear that the cells in the aortic wall of rats with renal hypertension contain more water, sodium, potassium, magnesium, and phosphorus per unit of cell solids than those of normotensive rats with similar kidney operations. The cells in question are smooth muscle cells in the...
main, as they are present in greatest abundance in the wall of the rat aorta.

One can only speculate about the importance of this altered cell composition in the whole hypertensive process. The increased water content per unit of solids in the arterial wall, if present in the very small arteries, could conceivably encroach somewhat on the size of the arterial lumen and thereby increase peripheral resistance to flow. This possibility has been discussed previously, and receives some support from the finger plethysmographic studies of Mendlowitz and Meyer. The passive structural narrowing of digital vessels deduced from their studies is quite compatible with a waterlogging of the walls of small vessels with a consequent decrease in lumen size. This, of course, does not preclude additional narrowing from increased vasomotion such as is strongly suggested by the studies of Greisman and Mendlowitz and associates, on hypertensive subjects.

The possible effects of changes in the intracellular cations on membrane potential and on actomyosin reactions in arterial smooth muscle have been discussed previously.

**SUMMARY**

A hundred rats had one kidney excised and the other compressed with a figure-of-eight ligature. Some developed hypertension; others did not. The hypertensive rats were compared with the rats that remained normotensive despite the operations.

Significant data was obtained suggesting that the cells in the aortic wall in the hypertensive group contained more water, sodium, potassium, magnesium and phosphorus per unit of cell protein than those of the operated normotensive group.

The aortas of the hypertensive rats had significantly less "noncollagen" protein per gram of total solids than the aortas of the operated normotensive rats.

Nine hypertensive rats were placed on a diet very low in sodium. When a rat's blood pressure fell to normal on the diet, the potassium and phosphorus per unit of "noncollagen" protein also fell to normal levels. If the rat's blood pressure remained elevated, this drop in potassium and phosphorus did not occur; the potassium and phosphorus remained at the higher levels characteristic of rats with renal hypertension.

**ACKNOWLEDGMENT**

The authors are indebted to Dr. J. H. Shaw for help in making the low sodium diet.

**SUMARIO IN INTERLINGUA**

In 100 rattos un ren esseva excidite, le altre comprimit per un ligatura cruciate. Certe animales disveloppava hypertension; alteres non. Le rattos hypertensive esseva comparate con le rattos que havevaremanite normotensive in despecto del operation.

Esseva obtenite datos significative que indicava que le cellulas del pariete aortic in le gruppo hypertensive contineva—in comparation con le gruppo normotensive ben que operate—plus alto nivellos de aque, natrium, kalium, magnesio, e phosphoro in proportion al proteina cellular.

Le aortas del rattos hypertensive haveva significativemente plus basse valores de proteina "non-collagenic" per gramma de solidos total que le aortas del operate sed normotensive rattos.

Un gruppo de 9 rattos hypertensive esseva subjicite a un dieta a bassissime contento de natrium. Quando le pression sanguine de un ratto con iste dieta se reduceva a vlaores normal, etiam le proportion de kalium e phosphoro a proteina "non-collagenic" se reduceva al norma. In casos in que le pression de sanguine del ratto remaneva elevate, le mentionate reduction de kalium e phosphoro non occurreva; le kalium e le phosphoro remaneva al nivellos elevate caracteristic de rattos con hypertensión renal.

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