Sodium Restriction and Reserpine Administration in Experimental Renal Hypertension

A Correlation of Arterial Blood Pressure Responses with the Ionic Composition of the Arterial Wall

By Paul D. Redleaf, M.D. and Louis Tobian, M.D.

An increase of intracellular potassium and sodium content in the wall of the aorta occurs in experimental renal hypertension. The elevated potassium tends to revert toward normal when blood pressure is again lowered with reserpine. The efficacy of rigid restriction of dietary sodium intake in decreasing blood pressure proved quite limited. The development of renal hypertension in the face of rigid sodium restriction further demonstrates that dietary sodium plays no more than an enhancing role in the pathogenesis of renal "ischemic" hypertension.

Restriction of dietary intake of sodium is often but not always of value in severe hypertension in humans, and the mechanism by which it sometimes lowers blood pressure remains obscure. The demonstration that the renal arteries of hypertensive patients contain an increased amount of sodium suggested that the effect of a diet low in sodium might be to reverse this "chemical lesion." In several types of experimental hypertension in the rat, an elevation of both sodium and potassium in the aortic wall has been demonstrated. The following studies were undertaken, therefore, to delineate further the relationships between dietary intake of sodium, compositional changes in the aorta, and blood pressure.

Experiment I: Effect of Restriction of Dietary Sodium

Because different types of renal hypertension might respond differently to variations in dietary sodium, two types of renal hypertension were studied simultaneously.

Method

A silver ribbon clip was applied around one renal artery of male Wistar rats weighing 120 to 240 Gm. and the contralateral kidney was removed. This operation places all renal tissue behind a clip, and no kidney tissue is subjected to increased blood pressure. A smaller silver clip was applied to one renal artery of a second group of rats, in which the contralateral kidney was left intact. In this situation, the "untouched" kidney is subjected to increased blood pressure.

All rats ate Purina laboratory chow for 16 weeks following the operation. "Initial" blood pressures were then measured under light ether anesthesia by a microphonic method. Each operated group was then divided into two subgroups matched for blood pressure levels. One subgroup was placed on a "synthetic" diet of low sodium content; the other subgroup received the same diet to which sodium chloride had been added to give a final sodium concentration of 0.4 per cent. After 35 days on the "synthetic" diet, all rats in the group which had one kidney removed and the other clipped were killed. In order to determine whether more prolonged restriction of sodium would result in a greater fall in blood pressure, the group with one kidney clipped and the other intact continued on the "synthetic" diet for a total of 56 days. In addition, two groups of unoperated control Wistar rats of the same weight were each given one of the two "synthetic" diets for a period of 35 days.

All blood pressure measurements represent the

*Twenty per cent casein, 70 per cent sucrose, 5 per cent corn oil, 5 per cent sodium-free salt mixture with supplementary vitamins. The sodium content of the diet was .0023 per cent by analysis.
Table 1.—Effect of Diet on Blood Pressure, Serum Electrolytes, and Composition of the Aorta in Operated and Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean blood pressure (mm. Hg)</th>
<th>Mean serum electrolytes (mEq./L.)</th>
<th>Mean composition of aorta (mEq./100 Gm. dry solids)</th>
<th>Corrected Xa/Cl ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Clipping plus nephrectomy; added Na (8)</td>
<td>174 ± 20</td>
<td>184 ± 20</td>
<td>140.3 ± .74</td>
<td>5.6</td>
</tr>
<tr>
<td>Clipping plus nephrectomy; low Na (14)</td>
<td>182 ± 24</td>
<td>174 ± 23</td>
<td>139.0 ± .89</td>
<td>5.7</td>
</tr>
<tr>
<td>Clipping alone; added Na (17)</td>
<td>164 ± 35</td>
<td>168 ± 26</td>
<td>141.9 ± .38</td>
<td>5.3</td>
</tr>
<tr>
<td>Clipping alone; low Na (28)</td>
<td>165 ± 33</td>
<td>158 ± 25</td>
<td>139.5 ± .44</td>
<td>5.8</td>
</tr>
<tr>
<td>Unoperated controls; added Na (8)</td>
<td>105 ± 6</td>
<td>111 ± 8</td>
<td>141.2 ± .56</td>
<td>4.9</td>
</tr>
<tr>
<td>Unoperated controls; low Na (9)</td>
<td>108 ± 8</td>
<td>109 ± 6</td>
<td>139.8 ± .52</td>
<td>5.3</td>
</tr>
</tbody>
</table>

( ) number of rats; [ ], the range of blood pressure in a group; ±, standard deviation; ( = ), standard error of the mean; $, significant difference between the two dietary groups (p < .05).

The results show that rats receiving the diet with added sodium showed a slight increase in mean blood pressure, whereas mean blood pressure decreased

### RESULTS

**Blood Pressure.** As indicated in table 1, rats receiving the diet with added sodium showed a slight increase in mean blood pressure, whereas mean blood pressure decreased.
slightly in operated rats given the diet low in sodium. However, no unequivocally hypertensive rat (blood pressure over 140 mm Hg) had a fall in blood pressure to levels below 135 mm Hg. Combining the rats with both types of renal hypertension, we compared the change in blood pressure resulting from the two diets. The changes, although in the anticipated direction, fell short of statistical significance (p = .11).

**Serum Electrolytes.** Serum sodium was slightly lower in each group on the low intake of sodium than in the comparable group consuming added salt (table 1). Only in the group which had had clipping alone, however, was this difference significant (p < .001).

Serum potassium was slightly higher in each of the groups on a sodium-poor diet compared with similar rats eating the diet with added salt. For two of the three comparisons this difference was statistically significant (p < .005).

Serum chloride was not significantly different in the two dietary groups.

**Composition of Aorta.** There was a greater content of total sodium in the wall of the aorta in each of the groups consuming added salt than in comparable rats given a diet low in sodium. This was significant (p < .04) for both types of operated rats as well as the unoperated rats.

Although the amount of potassium in the aorta was higher in each of the groups consuming added sodium than in comparable rats given a diet low in sodium, this was not statistically significant.

The amount of chloride in the aorta decreased in rats whose dietary sodium was restricted. In two of the three groups, this finding had statistical significance (p < .03).

Because sodium and chloride in the aorta changed in parallel fashion, the corrected Na:Cl ratio of the aorta did not differ significantly among the dietary subgroups. This suggests that the decrease in sodium content of the aorta induced by a diet low in sodium involved primarily reduction of the size of the extracellular compartment, and that the amount of intracellular sodium was not altered appreciably.

In the group that underwent clipping of one kidney with contralateral nephrectomy, all rats became hypertensive with a blood pressure over 138 mm Hg at the end of the experiment. In these hypertensive groups, both with and without added salt, the content of sodium and potassium and the corrected Na:Cl ratio in the wall of the aorta were all significantly greater (p < .004) than that found in the appropriate unoperated control group. Moreover, rats with the most severe high blood pressure had higher sodium and potassium levels and higher Na:Cl ratios in the aortic wall than rats with moderate hypertension.

In the rats that had the clipping without contralateral nephrectomy, 22 per cent never developed blood pressures above 133 mm Hg and many were within the normal range. These operated rats with little or no hypertension were few in number, but had a lower sodium and potassium content and a lower Na:Cl ratio in the wall of their aortas than truly hypertensive rats with a similar operation and diet.

Uremia occurred in only one of the 22 rats which had had one kidney removed and a clip applied around the opposite renal artery. The composition of the aorta in this rat did not differ appreciably from that of nonuremic rats with similar blood pressures. This apparent lack of influence of uremia on the composition of the aorta has been noted previously.2

**Experiment II:**

**Further Studies on Sodium Restriction**

Because of the failure of restriction of dietary sodium to lower arterial blood pressure significantly in experiment I, additional experiments were performed to determine whether other conditions would make a diet low in sodium more effective in reducing blood pressure. If restriction of sodium is efficacious in this regard, its ability to depress blood pressure should be most apparent when such dietary restriction is begun early after the establishment of hypertension, or if it is begun in the prehypertensive stage.
TABLE 2.—Blood Pressure of Rats Placed on Varying Intakes of Sodium 26 Days after Clipping of One Renal Artery

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. rats</th>
<th>Blood pressure*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Immediately before &quot;synthetic&quot; diet</td>
</tr>
<tr>
<td>Added sodium</td>
<td>8</td>
<td>135 ± 31</td>
</tr>
<tr>
<td>Low sodium</td>
<td>16</td>
<td>138 ± 33</td>
</tr>
</tbody>
</table>

*Group mean, mm. Hg; ±, standard deviation; ( ), standard error of the mean.

METHODS

In each of 24 male Wistar rats weighing 112 to 278 Gm. a clip was placed around one renal artery, as in experiment I. The rats received Purina laboratory chow and tap water. After 26 days, 13 rats had blood pressures over 130 mm. Hg. At this time, 16 of the original group of 24 rats were placed on the "synthetic" diet low in sodium; 8 of the original 24 rats, matched for blood pressure with the preceding group, received added sodium (0.4 per cent) in the "synthetic" diet. Blood pressures were measured at 32 and 35 days after institution of the "synthetic" diet. A diet of laboratory chow was then reinstituted, and blood pressures were again determined 3 weeks later.

In experiment I, hypertension developed uniformly when rats with a clip on one kidney and the opposite kidney removed were given laboratory chow. Accordingly, 29 male Wistar rats weighing 108 to 215 Gm. were placed on the "synthetic" diet low in sodium and given distilled water to drink. Four days later, a clip was applied around one renal artery and the contralateral kidney was removed. Blood pressures were determined after 8 weeks and again after 15 weeks.

RESULTS

Table 2 compares the effect of the two diets of different sodium content on the blood pressure of rats which had had one renal artery clipped 26 days before the "synthetic" diet was instituted.

Every rat given the diet containing 0.4 per cent sodium had a rise in blood pressure. For the group, this averaged 33 mm. Hg. Among rats given a diet low in sodium, two rats had a fall in blood pressure (from 199 to 161, and from 142 to 126 mm. Hg). The remaining 14 rats had a final blood pressure equal to or higher than their initial blood pressure. The mean rise in blood pressure in this group, 14 mm. Hg, was significantly less than the rise which occurred in the group receiving added salt, however (p < .03). The rats eating the sodium-deficient diet remained quite healthy.

Three weeks after resumption of the diet of laboratory chow, blood pressures were again similar in both groups, with a group mean of 157 mm. Hg for rats formerly receiving 0.4 per cent sodium, and a group mean of 160 mm. Hg for rats formerly on a diet low in sodium.

Table 3 shows blood pressures at 8 and 15 weeks after clipping plus nephrectomy in the group receiving a low intake of sodium throughout the experiment. These are compared with blood pressures recorded at similar intervals in rats treated identically except for the fact that laboratory chow and tap water had been given throughout this period.

The mean blood pressure was significantly lower in rats which received the diet low in sodium than in rats which received chow, at both 8 and 15 weeks after operation (p = .05 and p < .001, respectively). Three of the 18 rats which survived on a low intake of sodium for 3½ months remained unequivocally normotensive throughout (blood pressure < 120 mm. Hg). Six additional rats had slightly elevated blood pressures (126 to 136 mm. Hg) at 8 weeks, but these had returned to normotensive levels at 15 weeks. However, 5 rats demonstrated marked hyper-
## Table 4.—Effect of Reserpine or Saline on Blood Pressure and Composition of Aorta in Rats with Clip around One Renal Artery

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mean blood pressure (mm. Hg)</th>
<th>Mean composition of aorta (mEq./100 Gm. dry solids)</th>
<th>Corrected Na/Cl ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Na</td>
</tr>
<tr>
<td>Initial BP &gt; 140 mm. Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>119</td>
<td>122</td>
<td>27.41</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td>(± .39)</td>
</tr>
<tr>
<td>Reserpine</td>
<td>121</td>
<td>82</td>
<td>27.09</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td></td>
<td>(± .62)</td>
</tr>
<tr>
<td>Initial BP &lt; 140 mm. Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>119</td>
<td>122</td>
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<td></td>
<td>(± .62)</td>
</tr>
</tbody>
</table>

( ) Number of rats; ± standard error of the mean.

### Experiment III: Reserpine in Renal Hypertension

The limited antihypertensive potency of restriction of sodium shown in experiments I and II prompted our use of reserpine in order to correlate the chemistry of the artery wall with reversal of hypertension. Reserpine nearly always produced substantial decreases in the blood pressure of hypertensive rats.

#### METHODS

Forty-one male Wistar rats weighing 122 to 208 Gm. had a clip applied around one renal artery; the other kidney was untouched. Seven or 8 weeks later, blood pressures were determined. At this time, half the rats began receiving daily injections of reserpine, 10 µg./100 Gm. subcutaneously, as a 0.005 per cent solution in 0.9 per cent saline. The rest of the rats, matched for blood pressure with the preceding group, were injected with the saline vehicle alone. Blood pressures were measured on the seventh and tenth day after injections had been begun, in each case 24 hours following the most recent injection. The two determinations were averaged to give the final blood pressure. Immediately following the last blood pressure determination, the rats were killed and the analytic procedures outlined in experiment I were repeated.

A smaller group of unoperated rats was similarly injected with reserpine for 10 days, and compared with an unoperated control group which had received saline injections.

#### RESULTS

The administration of reserpine resulted in a mean decrease in blood pressure of 40 mm. Hg in the whole group of operated rats with varying degrees of hypertension.

Table 4 shows the blood pressure before and after treatment in reserpine-injected and saline-injected rats. We have omitted 3 rats which had decreases in blood pressure of less than 20 mm. Hg as a result of reserpine. Thus all tabulated reserpine-treated rats had a significant fall in blood pressure in contrast to control animals, whose blood pressures...
remained unchanged while receiving saline injections for 10 days.

The treated and control groups were well-matched for blood pressure initially. Any differences between the two groups in regard to the composition of the aorta could be attributed, therefore, to the administration of reserpine.

Regardless of the pretreatment blood pressure, a significant decrease occurred in the amount of potassium in the aortas of rats receiving reserpine \((p = .02)\). The content of sodium and chloride was not altered appreciably.

Serum electrolytes were not altered as a result of reserpine.

Six unoperated rats responded to daily administration of reserpine with a mean blood pressure drop of 25 mm. Hg. The composition of the aorta in this small group was virtually identical with that in a control group of unoperated rats receiving saline injections.

**DISCUSSION**

**Blood Pressure Responses.** Grollman and Harrison\(^8\) first reported the hypotensive effect of rigorous restriction of the intake of sodium in rats rendered hypertensive by compression of renal parenchyma, with or without contralateral nephrectomy. Twelve animals in their series showed a mean fall in arterial blood pressure from 175 mm. Hg to less than 130 mm. Hg within 4 days after beginning a dialyzed diet.

Handler and Bernheim,\(^9\)\(^10\) using a diet low in sodium, were able to lower blood pressure of subtotally nephrectomized or post-choline-deficiency hypertensive rats. In their experience, similar lowering could be produced by restriction of the intake of protein. Indeed, in rats consuming a diet high in protein, drastic salt restriction resulted in only slight drops in blood pressure.\(^11\)

By means of a rice diet, Kempner and associates lowered blood pressure to normal in 40 per cent of a group of rats with hypertension produced by encapsulating one kidney in latex and removing the contralateral kidney.\(^12\) However, the mean blood pressure of this entire group remained approximately 50 per cent above the upper limit of normal. Furthermore, the reduction in the intake of protein on a rice diet appeared in this study to be as important as the reduction in intake of sodium in lowering blood pressure and prolonging survival.

In a previous study from this laboratory, restriction of sodium for 5 weeks resulted in a fall of arterial blood pressure to normal in but 3 of 9 rats, which had been made hypertensive by a figure-eight ligation and contralateral nephrectomy 6 months earlier.\(^13\)

In the present experiments, a very low intake of sodium tended to lower blood pressure. The fact that this drop in blood pressure was small in experiment I indicates that the intake of sodium there was of much less importance quantitatively than other factors acting to sustain the hypertension. Furthermore, although continuous rigorous restriction of sodium produced an average lowering of blood pressure in experiment II, hypertension nevertheless developed in a considerable number of rats while they were consuming a diet very low in sodium. This study in particular tends to minimize the importance of dietary intake of sodium in the pathogenesis of renal hypertension.

The responsiveness of hypertension to the restriction of sodium intake may be species specific, since it does not occur in the dog.\(^14\)\(^15\) It is also possible that Wistar rats, which we utilized, are not as responsive to a lowering of sodium intake as other strains used by other investigators. Another possibility is that only certain types of renal hypertension may respond, hypertension following narrowing of the renal artery being among the relatively unresponsive group.

**Chemical Alterations in the Aorta.** The increase in the amount of sodium and of potassium in the aorta of hypertensive rats in the two types of hypertension studied here is similar to that found in two other types of renal hypertension,\(^2\)\(^3\) "adrenal regeneration" hypertension,\(^3\) desoxycorticosterone hypertension,\(^2\)\(^16\) and "postdesoxycorticosterone" hypertension.\(^5\) The increment of
potassium is clearly intracellular, and is not accounted for by muscular hypertrophy.\textsuperscript{15} The increase in "corrected sodium: chloride ratio" in the aorta in the present study strongly suggests that much of the increment of sodium in the hypertensive aortas is also intracellular. The increased amounts of sodium and potassium per unit of dry weight of aorta strongly indicate that the aorta also contains an increased amount of water. Such a "waterlogging" of the arterial walls could conceivably produce a significant decrease of the lumen size in the very small arteries. The possible significance of these findings in relation to the increased peripheral resistance in hypertension has received comment elsewhere.\textsuperscript{1-3, 15, 16}

The present study indicates that the amount of sodium in the diet is directly correlated with the amount of sodium in the aorta. However, the fact that sodium and chloride in the aorta decreased in parallel fashion during the restriction of dietary sodium suggests that extracellular sodium was being influenced primarily. In a previous study\textsuperscript{17} the chloride content of the aorta was not changed by varying the intake of sodium. However, the group on a high intake of sodium in that experiment consumed laboratory chow, whereas the group on a low sodium intake ate a "synthetic" diet. Thus there were other differences in the diet besides the amount of sodium, and this may account for the different behavior of chloride ion in the two experiments. The low intake of sodium in the present study mainly reduced the content of extracellular sodium and did not greatly alter the increased amount of intracellular sodium that is characteristic of hypertensive rats. This relative lack of influence on intracellular sodium may account for the small lowering of arterial blood pressure in these experiments.

The direct relationship between tissue sodium and dietary intake of sodium has been discussed by other workers in regard to other tissues.\textsuperscript{18-20}

The decrease in serum sodium and increase in serum potassium which resulted from a diet low in sodium has been noted previously,\textsuperscript{15} and requires no additional comment.

The experience with reserpine requires some caution in interpretation. The mechanism through which the rauwolfia alkaloids lower arterial blood pressure is not fully understood, but is probably related to an action on the central nervous system, increasing parasympathetic outflow and decreasing sympathetic outflow.\textsuperscript{21} However, the extent of autonomic innervation of the aorta in the rat is not known. Consequently, we cannot gauge the extent to which the compositional changes in the aorta reflect directly changes in neurogenic tone following reserpine. Furthermore, direct and indirect humoral effects of reserpine on the aorta are unknown. It remains significant, whatever the ultimate interpretation, that the characteristic increase of intracellular potassium in the aorta which occurs in hypertension tends to revert toward normal pari passu with a decrease in blood pressure following administration of reserpine.

**SUMMARY**

The hypertension which develops in rats following constriction of one renal artery, with or without removal of the opposite kidney, is accompanied by an increase in the amount of potassium and of sodium in the wall of the aorta. These changes, which parallel the severity of the hypertension, are similar to our findings in other types of hypertension in the rat.

The effect of restriction of the intake of sodium upon the blood pressure and the composition of the aorta was studied in both types of renal hypertensive rats. Restriction of sodium was of limited efficacy in reducing arterial blood pressure. Only when begun early could a statistically significant effect on blood pressure be demonstrated. A significant reduction in the amount of extracellular sodium in the aorta occurred as a result of such a diet. However, we could not demonstrate a significant change in the potassium content or the Na:Cl ratio, both of which are characteristically elevated in experimental hypertension. The fact that re-
striction of dietary sodium did not greatly alter intracellular sodium or potassium may be related to its limited efficacy in lowering blood pressure.

Experimental renal hypertension can actually develop despite the most rigorous restriction of salt intake. This demonstrates that dietary sodium intake plays no more than a secondary or enhancing role in the pathogenesis of this type of hypertension.

A decrease in the blood pressure of hypertensive rats as a result of the administration of reserpine can be correlated with a decrease in the previously elevated amount of potassium in the wall of the aorta. This is further evidence that the compositional changes in the hypertensive aorta are intimately associated with the level of arterial blood pressure, and may be indicative of similar changes occurring in the walls of arterioles.

SUMMARY IN INTERLINGUA
Le hypertension que se disveloppa in rattos post le constriction de un arteria renal—sin o con ablation del ren al latere opposite—es accompaniate de un augmento del quantitate de kalium e de natrium in le pariete del aorta. Iste alterationes que es parallel al severitate del hypertension es simile a nostre constatationes in altere typos de hypertension in rattos.

Le effecto de un restriction del ingestion de natrium super le pression sanguinee e super le composition del aorta esseva studiate in rattos con ambe typos de hypertension renal. Le restriction del natrium habeva un efficacia limitate in reducir le pression sanguinee arterial. Il esseva solmente in casos in que le restriction del natrium habeva essite initiate precocemente que le effecto super le pression sanguinee esseva statisticamente significative. Grados significative de reduction in le concentration del natrium extracellular in le aorta esseva constatabile como resultado de un tal dieta. Tamen, nos non succedeva a demonstrar ulle alteration significative in le contento de kalium o in le proportion Na:Cl, que ambes exhibi un elevation characteristic in hypertension experimental. Le facto que le restriction de natrium dietari non alterava grandemente le nivello intracellular de natrium e kalium es possiblemente relationate a su limitate efficacia in reducir le pression sanguinee.

Hypertension renal experimental pot de facto occurrer in despecto del plus rigorose restriction del ingestion del sal. Isto demonstra que le ingestion dietari de natrium ha solmente un rolo secundari o promotori in le pathogenese de iste typo de hypertension.

Un reduction del pression sanguinee de rattos hypertensive como resultado del administration de reserpina pot esser correlazione con un reduction del previemente elevate concentration de kalium in le pariete del aorta. Isto prova additionalmente que le alteration compositional in le aorta hypertensive es associate intimemente con le nivello del pression de sanguine arterial e indica possiblemente simile alterationes que occurre in le parietes del arteriolas.

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