Effect of Sodium and Calcium on Vascular Reactivity

By THOMAS ZSOTÉR, M.D. AND MICHAEL SZABO, M.D.

A study was made in the rat of variations in sodium and calcium content of the diet as possible factors influencing vascular reactivity. The latter was estimated by the sensitivity of the mesoappendix vessels to epinephrine.

For many years evidence has accumulated on the role played by ions in various functions of the organism, particularly hemodynamic. Potassium deficiency is known to cause certain changes in the electrocardiogram, hypotension and a state similar to heart failure. In our experiments, hypotension was found to be associated with decreased sensitivity to epinephrine of the mesoappendix vessels of rats kept on a diet low in potassium, a change which could be restored to or toward normal by the addition of potassium to the diet.

The effect of sodium on blood pressure is well known. Ambard and Volhard and many other authors, particularly Kempner, have shown the efficacy of low sodium diets in the treatment of hypertension. A relationship between hypertensive vascular disease and sodium has been inferred by Tobian and Binion from their demonstration of abnormally high sodium concentration in the vessels of hypertensive patients. Furthermore, such patients eliminate sodium more rapidly than normotensive controls, provided that their renal function is normal. Sodium plays a decisive role in the development of the vascular lesions and hypertension associated with the administration of desoxycorticosterone and in the evolution of experimental renal hypertension. Sapirstein et al. found hypertension in rats given 2 per cent saline to drink. (We could not confirm these findings in our earlier experiments.) Others have found that a high intake of sodium is sufficient to cause histologic changes in the heart, kidney and vessels as well as hypertension.

Much less is known about the action of calcium than of sodium on blood vessels and blood pressure. However there have been reports of hypertension following intravenous administration of calcium salts and of vascular changes, similar to periarteritis nodosa, caused by large doses of calcium and vitamin D.

Despite the large number of publications on the effect of sodium and calcium on circulation, one cannot conclude that these ions, like potassium, have a decisive action on the peripheral circulation and vascular reactivity. Many of the observations cited above have to do with the studies of blood pressure, which is the result of the interplay of so many factors that changes are difficult to interpret. Others have studied histologic alterations resulting from experimental manipulations so violent as to have no importance in clinical practice. We considered it of interest therefore to examine by means of the rat mesoappendix preparation the effect of sodium and calcium on the reactivity of peripheral vessels.

Methods

Rats of a single, stable strain, weighing between 60 and 70 Gm. at the beginning of the experiment were used. The following diets were administered for a period of 10 to 15 weeks: In 25 rats, a high sodium diet (basic diet + 2 Gm. sodium per 100 Gm. diet); in another 25 animals, a low sodium diet (without sodium chloride in the salt mixture); in another 13, a low calcium diet (without calcium lactate in the salt mixture); and 25 control animals on the basic diet alone. A sixth group on the basic diet, and animals on the high calcium diet (basic diet + 2 Gm. calcium per 100 Gm. diet) in another 13, a low calcium diet (without calcium lactate in the salt mixture); and 25 control animals on the basic diet alone. A sixth group on the basic diet, and animals on the high calcium diet, received one drop of AT 10 (dihydroteachysterol) in solution every second day starting at the ninth week of the experiment. The basic diet consisted of 61 per cent starch, 18 per cent casein, 8 per cent yeast, 8 per cent oil,
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1 per cent cod liver oil and (in control diet) 4 per cent of salt mixture of Sos. Differences between the various experimental diets lay only in the salt mixture. On the low sodium diets the sodium chloride was deleted from the salt mixture, conversely in the high sodium diets 2 per cent of the total diet consisted of sodium. Similarly the low calcium diets or high calcium diets were prepared by the deletion of calcium lactate from the salt mixture or by the addition of 2 per cent calcium as calcium chloride.

The animals were weighed once a week. Blood pressure was recorded weekly under light ether anesthesia using the tail plethysmographic method of Byrom and Wilson. Electrocardiograms were recorded in 3 animals of each group prior to administration of the experimental diet, and again after 6 and 12 weeks. Urinary sodium, calcium, potassium and chloride were determined once a week in 2 animals chosen at random from each group. Urine samples were collected over a period of 12 to 16 hours. Serum electrolytes were determined by a modification of the method of Kramer and Tisdall and chloride by Rusznyak's procedure. Serum protein levels were estimated every 2 weeks using a refractometer.

After the various diets had been administered for 10 to 15 weeks, the epinephrine threshold, as represented by the response of the mesoappendix vessels, was determined. A modification of the method of Chambers and Zweifach was used. This method differs from the original in that the epinephrine threshold is the concentration of epinephrine solution which, when applied topically, causes a reversible stoppage of blood flow rather than a mere narrowing of vessels. Furthermore, at least 2 to 3 vessels were observed in each rat, anesthetized with sodium salt of X-methyl-5, 5-allyl-isopiopylbarbituric acid (Alenal). Following the determination of the epinephrine threshold, histologic examination of the heart, lung, kidney and adrenal was made on 4 to 6 rats of each group.

**RESULTS**

*Effect of Sodium.* The high-sodium and the sodium deficient diets were equally palatable to the rats since their weight gains were the same as those of the controls. At the end of 12 weeks the mean weight of the animals in all 3 groups was about 140 Gm., an increase of approximately 75 Gm. from the start of the experiment. A marked increase in water intake was noted in rats on a high sodium diet.

Changes in blood pressure were not observed in the animals on either high or low sodium intakes. Our data do not confirm reports of elevation of blood pressure caused by a high sodium intake. Our results indicate that healthy rats with good renal function are able to compensate for the effect on blood pressure of a moderately increased sodium intake. The electrocardiogram showed a heart frequency of 400 to 500/min. and generally a right axis deviation. When high sodium diets were administered for 12 weeks, relative shortening of the electric systole (Q-T interval) was seen. The serum protein levels were between 5.7 and 6.3 per cent at the start of the experiment and did not change during the experimental period.

The urinary sodium and chloride values were slightly decreased by the low sodium diet as compared to the controls, while potassium and calcium values were not affected. Urine sodium output of the rats on a high sodium diet increased to as much as 100 mg./day, which is 5 times as great as that seen in the controls. Chloride output was also markedly increased but potassium output was slightly reduced. The serum levels were not significantly elevated (an average of 350 mg. per cent compared to 340 for controls), thus demonstrating that the organism is able to preserve normal serum sodium levels for a relatively long period of elevating the renal excretion of sodium.

After the experimental diets had been administered for 10 to 15 weeks observations were made on the responses of the vessels to epinephrine. Whereas simple microscopic investigations of the mesoappendix vessels did not show any obvious changes in circulation between the different groups of animals, tests of sensitivity to epinephrine revealed a dif-
TABLE 1.—Epinephrine Thresholds of Mesoappendix Vessels (Sodium Study)*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total no of vessels</th>
<th>1/1000</th>
<th>1/10,000</th>
<th>1/100,000</th>
<th>1/1,000,000</th>
<th>1/10,000,000</th>
<th>1/100,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>High sodium</td>
<td>45</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>4</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>14</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Low sodium</td>
<td>71</td>
<td>5</td>
<td>17</td>
<td>18</td>
<td>13</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

*Figures represent number of vessels responding to specified concentration of epinephrine.

ference. The median epinephrine threshold of 52 vessels in control animals was 1:500,000; this is the concentration which caused a reversible stoppage of the circulation. The corresponding value for 45 vessels from animals on a high sodium diet was 1,200,000,000 and that for 71 vessels from animals on a low sodium diet was 1:100,000. This indicates that the vessels of the mesoappendix of rats on a high sodium intake were 20 times as sensitive to epinephrine as those of animals on a low sodium diet.

Table 1 represents the scatter of individual values. For statistical analysis we have grouped our results into 3 categories to avoid overemphasis of $\chi^2$ values (and statistical difference) which might be due to the small number of vessels observed in some of the classes. We have taken as normal vascular sensitivity the median value of the control animals, i.e., 1:500,000 epinephrine concentration. The remaining classes are the threshold values lower and higher, respectively, than the median value of the controls. The $\chi^2$ value on this basis is 39.94 ($p < 0.01$) when the animals on a high sodium and low sodium diet are compared. The difference between the epinephrine threshold of animals on a low sodium diet ($p = 0.01$ to 0.02) and those on a high sodium diet ($p < 0.01$) are also significant when compared with the controls.

After determination of the epinephrine threshold, 0.25 ml physiologic saline was injected into the tail vein. Physiologic solution of KCl or glucose was used as a control injection. After a 15 min. period the epinephrine thresholds were again determined. Although KCl or glucose did not cause any change in vessel reactivity, a slight augmentation of epinephrine sensitivity was observed after NaCl. This was most marked in rats maintained on the low sodium diet.

We believe the importance of these findings to be in the observation that increases of epinephrine sensitivity were detected in animals on a high sodium intake, and decreases in others in a low sodium intake, even though these diets did not cause any morphologic changes detectible by histologic examination.*

Effect of Calcium. The growth of animals on a low calcium diet was equal to that of the controls, but it was poor in those on a high calcium diet. Furthermore, after 8 weeks of the experiment, when AT 10 was administered, the weight of these animals definitely decreased. This decrease in growth is probably explained by the poor consumption of the experimental diet observed in the animals of this group.

The only group in which a change in blood pressure was observed was that on a high calcium diet. However, this elevation of blood pressure became evident only when AT 10 was administered. Following this a slight but permanent elevation in blood pressure was seen (averages from 73 to 102 mm. Hg; these values, measured indirectly in the tail under light ether anaesthesia, are appreciably lower than those obtained by direct

*These examinations were made through the kindness of Dr. Gabriel Lusztig.
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TABLE 2.—Epinephrine Thresholds of Mesappendix Vessels (Calcium Study)*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total no of vessels</th>
<th>1/100,000</th>
<th>1/10,000,000</th>
<th>1/1,000,000,000</th>
<th>1/10,000,000,000</th>
<th>1/1,000,000,000,000</th>
<th>1/10,000,000,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium rich</td>
<td>28</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
<td>-</td>
<td>3</td>
<td>14</td>
<td>20</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Calcium low</td>
<td>29</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* Figures represent number of vessels responding to specified concentration of epinephrine.

Dietary puncture). The only obvious change in the electrocardiogram was a relative shortening of the Q-T interval similar to that previously described in rats consuming a high sodium diet. No changes were seen in serum protein level in any of the animals.

The rats on a low calcium diet had urinary sodium, potassium, chloride and calcium values which were essentially the same as those of control animals as were serum sodium, potassium and calcium levels. (In these rats a mobilization of calcium from the bones seems probable.) However, in rats which had received a high calcium diet and AT 10, a remarkable change in electrolyte metabolism was seen. The urine calcium excretion was 3 to 5 times greater than in the controls, and these values were associated with an elevated chloride and a decreased potassium and sodium excretion. The serum calcium increased to an average of 16.6 mg. per cent in contrast to a mean value of 10.6 for the control group. Each of these changes of electrolyte metabolism was less marked in rats which had received only AT 10 with the control diet.

The animals on a low calcium diet for 10 to 15 weeks showed the same average epinephrine threshold as animals on the control diet. The median effective concentration for 29 vessels from the low calcium group was 1:500,000. A remarkable contrast was seen in animals on a high calcium intake whose median epinephrine threshold was 1:4,000,000. It may be seen from table 2 that, when one considers the variation in values, the increase in epinephrine sensitivity of the vessels in this group compared to that of rats on calcium deficient diet is significant. The $X^2$ is 20.09 and $p < 0.01$. The epinephrine sensitivity of rats consuming AT 10 with the control diet was not elevated as much as it was in rats which had received a high calcium diet, median value in this group being 1:1,000,000.

The injection of 0.25 ml. of physiologic saline, caused a slight increase in epinephrine sensitivity, but injections of solutions of KCl or glucose had no effect. Histologic examination of selected animals from each group did not reveal any significant change in the vessels of parenchymal organs.

DISCUSSION

In these experiments a high sodium diet or a high calcium diet increased the epinephrine sensitivity of the blood vessels, while a diet deficient in sodium caused a decrease. No changes were observed following the low calcium diet. We feel that the significance of these data is emphasized by the fact that such changes in vessel reactivity occurred following the administration of diets which do not cause any morphologic changes in vessels, and did not influence either the growth of the animals or their blood pressure. (An exception to this was the slight elevation of blood pressure seen in those animals on a high calcium diet supplemented with AT 10.)

It is interesting that Na$^+$ and Ca$^{++}$ and—as we found in previous work—also K$^+$ influence vascular reactivity in the same direction. This is particularly pertinent when one considers the many data on the antagonism...
between Na⁺, K⁺ and Ca++] on other phenomena. However, it is known from other studies that these electrolytes may work in the same direction.17,18

Our experiments do not explain the mechanism by which Na⁺ and Ca++] act on the vessels. The diets cause no morphologic changes in the heart muscle and have no effects on the electrocardiogram, so that we cannot attribute our results to an action on the heart. It does not seem probable that the action is mediated by the adrenal cortex.19 It is known that adrenalectomy results in decreased epinephrine sensitivity on the part of blood vessels. Since a low sodium diet causes increase in width of the zona glomerulosa it would appear unlikely that the decreased sensitivity following sodium deficiency can be explained through an adrenal mechanism. We feel the likely explanation lies in a direct effect of electrolytes on vascular contractility.

The increased epinephrine sensitivity of animals in which the Na⁺ intake is elevated, and decreased sensitivity when it is lowered, probably is pertinent to the role of Na⁺ in the regulation of blood pressure. These findings parallel the deleterious effect of increasing Na⁺ intake in various experimental hypertensions, and the efficacy of Na⁺ withdrawal in the treatment of hypertensive patients. However, before reaching such definite conclusions one must bear in mind that epinephrine sensitivity does not imply a priori the same change of reactivity to other vasoactive materials. Similarly, one must bear in mind that the mesoappendix vessels of the rat may not be truly representative of other vascular territories of other species.

**Summary**

The following experimental diets were administered to rats: high sodium, low sodium, high calcium with the addition of AT 10, low calcium, basic control diet, and basic diet with AT 10. Weight gain, blood pressure, electrocardiogram and electrolyte metabolism were observed.

After 10 to 15 weeks, the epinephrine sensitivity of the mesoappendix vessels was increased in the rats receiving a diet high in either Na⁺ or Ca+++, and decreased in those receiving a Na deficient diet. A low Ca+++ diet did not cause any change in epinephrine sensitivity.

**Summary in Interlingua**

Le sequente typos de dieta experimental esseva administrate a rattos laboratorial: (1) Dieta a alte contento de natrium, (2) dieta a basse contento de natrium, (3) dieta a alte contento de calcium con supplemento de AT 10, (4) dieta a basse contento de calcium, (5) dieta basil de controlo, e (6) dieta basil con supplemento de AT 10. Esseva observe (1) le augmento del peso corporeo, (2) le pression de sanguine, (3) le electrocardiogramma, e (4) le metabolismo electrolytic.

Post 10 a 15 septimanas, le sensibilitate a epinephrina mesurate in le vasos del mesoappendice esseva augmentate in le rattos nutrite con dietas a alte contento de natrium o de calcium. Illo esseva reducece in le rattos nutrite con dietas a basse contento de natrium. Le dieta a basse contento de calcium non alterava le sensibilitate a epinephrina.

**References**

7. SAPFIRESTEIN, L. A., BRANDT, W. L., AND
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