Effect of Diet on Experimental Hypertension and on the Development of Polyarteritis Nodosa in Rats

By Walter Kempner, M.D., Ernst Peschel, M.D., and Bernard Black-Schaffer, M.D.

Rats with experimental renal hypertension were fed various diets differing in sodium and protein content. Marked prolongation of life was achieved by rice diet. Polyarteritis nodosa developed, as a result of the experimental procedures, in 23.5 per cent of the animals. Its incidence was closely correlated to sodium ingestion: 4 per cent in rats on low sodium intake, 36 per cent in rats on normal or slightly higher sodium intake. Protein intake showed no effect on incidence of polyarteritis but distinct effect on survival time. Except for one rat where a sarcoma destroyed almost all kidney tissue, no animal on rice diet developed polyarteritis nodosa.

The present investigation grew out of a study of the influence of diet on hypertension and vascular disease. It had been established in man that a strictly enforced rice diet tends to restore normal tension and to cause regression of visible vascular lesions in the retina. In order to obtain quantitative data on the effects of various dietary components, experimental hypertension was produced in rats.

It was noted in the course of the experiments, an observation frequently made by other investigators, that severe hypertension in rats was often accompanied by polyarteritis nodosa. This has been considered analogous to the arteriolonecrosis of malignant hypertension in man.

Methods

Female rats of Osborne-Mendel strain were operated on when they attained a body weight of 75 grams; fascia and adrenal gland were stripped from one kidney but not removed, and the kidney enclosed in a latex capsule slightly greater in capacity than the kidney. The mouth of the capsule was drawn about the renal hilus by a thread, care being taken to avoid constricting the renal vessels or the ureter. One week later, the contralateral kidney was removed after stripping but retaining the fascia with the adrenal gland; the ureter and the blood vessels were ligated. The animals by this time were 40 to 50 days old.

Systolic blood pressure was measured in the unanesthetized animal once or twice a week by an apparatus similar to the Chittum, Hill and Grimson modification of the Skeggs and Leonards apparatus. The values listed in Table 2 are based on averages of the last four blood pressure figures before the death of the animal (excessively high or low values occurring only within the last two days before death were not included). Each figure represents the average of five successive measurements, which usually did not differ by more than 5 mm. Hg.

The rats were maintained, until one week after unilateral nephrectomy, on dog chow and tap water. By this time hypertension was established, and the animals could be separated into groups with comparable ranges of blood pressure.

Five diets represented decreasing sodium intake in the order given: dog chow, bread, meat, peas, and rice (table 1). These comprised the major groups. All five groups were divided into four sub-groups, receiving a) no salt addition; b) potassium chloride (50 mg. daily); c) sodium chloride (100 mg. daily); and d) sodium chloride + potassium chloride (100 and 50 mg., respectively, daily). The salt solutions were given in special 5 cc. containers which had to be emptied before tap water was permitted ad libitum. All diets were supplemented with vitamins: per day and per rat thiamine 40 γ; riboflavin 100 γ; niacin 500 γ; pyridoxin 40 γ; calcium pantothenate 150 γ; choline 7 mg.; ascorbic acid 2.5 mg.; β-carotene 200 γ; vitamin D 5 USP units; α-tocopherol 500 γ. Control, unoperated rats kept on the same diets and salt solutions showed that the vitamin supplements were adequate to insure normal growth and health.

Received for Publication: August 24, 1954.
Diet and Experimental Hypertension

Table 1.—Sodium and Protein Consumption in Five Diet Groups of Rats with Experimental Hypertension (Average daily intake)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sodium (mg.)</th>
<th>Protein (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dog chow</td>
<td>80</td>
<td>5.2</td>
</tr>
<tr>
<td>2. Bread</td>
<td>67</td>
<td>1.5</td>
</tr>
<tr>
<td>3. Meat</td>
<td>12.4</td>
<td>7.9</td>
</tr>
<tr>
<td>4. Peas</td>
<td>7.6</td>
<td>2.4</td>
</tr>
<tr>
<td>5. Rice</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 2.—Blood Pressure and Survival Time of Hypertensive Rats on Various Diets without and with Addition of Sodium Chloride

<table>
<thead>
<tr>
<th>Diet</th>
<th>Without Additional NaCl</th>
<th>With Additional NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Systolic Blood Pressure (mm. Hg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dog chow</td>
<td>216 ± 35 (8)</td>
<td>226 ± 24 (5)</td>
</tr>
<tr>
<td>2. Bread</td>
<td>220 ± 28 (9)</td>
<td>194 ± 32 (9)</td>
</tr>
<tr>
<td>3. Meat</td>
<td>197 ± 29 (15)</td>
<td>189 ± 21 (17)</td>
</tr>
<tr>
<td>4. Peas</td>
<td>196 ± 24 (13)</td>
<td>204 ± 33 (10)</td>
</tr>
<tr>
<td>5. Rice</td>
<td>170 ± 52 (24)</td>
<td>194 ± 29 (22)</td>
</tr>
</tbody>
</table>

(b) Survival Time (Days After Nephrectomy)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Without Additional NaCl</th>
<th>With Additional NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dog chow</td>
<td>40 ± 25 (8)</td>
<td>37 ± 18 (5)</td>
</tr>
<tr>
<td>2. Bread</td>
<td>36 ± 13 (9)</td>
<td>35 ± 27 (9)</td>
</tr>
<tr>
<td>3. Meat</td>
<td>45 ± 31 (15)</td>
<td>27 ± 20 (17)</td>
</tr>
<tr>
<td>4. Peas</td>
<td>110 ± 98 (13)</td>
<td>39 ± 27 (10)</td>
</tr>
<tr>
<td>5. Rice</td>
<td>218 ± 153 (24)</td>
<td>71 ± 43 (22)</td>
</tr>
</tbody>
</table>

Results

Hypertension developed in all animals with kidney encapsulation and contralateral nephrectomy. The systolic blood pressures of the unoperated rats varied between 90 and 120 mm. Hg. and were independent of food and salt intake. Among the operated rats, pressure rose in 4 to 7 days after nephrectomy to 160 to 200 mm. Hg. The hypertension was associated with rather severe renal damage. Some animals showed not only infarction of large areas of the kidney but suppurative perinephritis as well. At best, when infarction was absent, there was always a marked thickening of the renal capsule, which was associated with atrophy of subjacent nephrons, particularly at the poles. All these changes were the result of chronic perinephritis due to the presence of the latex capsule. The degree of impairment of renal function is not known. However, blood urea nitrogen determinations carried out on a number of rats indicate that, in the last weeks before death, renal insufficiency with rising urea concentrations was a frequent occurrence. Urea nitrogen values were most elevated in hypertensive rats on high-protein diets, and were only rarely increased in hypertensive rats on rice.

No difference was found between the subgroups to which potassium chloride alone was administered and those receiving no salt supplement; nor between the subgroups receiving potassium chloride and sodium chloride and those receiving sodium chloride alone. Therefore, subgroups a and b, and c and d, respectively, were combined, thus reducing the subgroups to two, one without and one with NaCl supplementation.

Table 2 (a) shows the blood pressures (averages) of the rats on the various diets without and with additional sodium chloride. Only the animals without sodium chloride addition in group 5 (rice) have a blood pressure average which differs significantly from that of the other groups. The average, 170 mm. Hg., includes the blood pressures of 10 rats which were the only ones in the entire series showing a return to normotensive levels. Fig. 1 illustrates the distribution of the individual blood pressures in the various diet groups. Statistical analysis showed that the differences of the averages are without significance (p > 0.05) for all groups except for group 5 without sodium chloride (p between 0.05 and 0.02) and for group 3 with sodium chloride (p < 0.01). The slightly lower blood pressure average for this latter group is most likely due to the very short survival time.

Table 2 (b) shows the survival times (averages) of the rats on the various diets. Even those animals in the rice group without additional salt which remained hypertensive, lived longer than those in the other groups; this is evident from the survival average of 218 days which, on statistical analysis, is...
highly significant \((p < 0.01)\). In addition to this group, rats on peas without additional salt showed a survival time average which differs significantly from that on normal rat food (dog chow), \(p\) being between 0.05 and 0.02. The differences between the other averages are without statistical significance \((p > 0.05)\).

The animals fed rice without additional sodium chloride lived approximately six times as long as the animals on the two unsupplemented diets with high original NaCl content. The addition of sodium chloride to these diets (dog chow and bread) did not alter blood pressure and survival time. The influence of the addition of salt to the diets originally low in salt, i.e., meat, peas, and rice, on the survival time was not statistically significant in the meat group \((p > 0.05)\); it was significant in the peas group \((p\) between 0.05 and 0.02), and highly significant in the rice group \((p < 0.01)\). Rats on meat, even without additional salt, had a short survival time, comparable to that of rats on diets high in salt.

Of the 132 rats which were examined microscopically, 31 \((23.5\) per cent) showed the typical panarteritis of polyarteritis nodosa. The lesions were recognized grossly in the mesentery of some of the animals (Figure 2) and were histologically identical with those repeatedly described in the literature. Morphologic details and pathogenesis are discussed in a preceding paper\(^6\). The frequency of involvement of various arteries was as follows:
Table 3.—Incidence of Polyarteritis Nodosa in Hypertensive Rats, in Relation to Diet Groups

<table>
<thead>
<tr>
<th>Diet</th>
<th>Without Additional NaCl</th>
<th>With Additional NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rates without Polyarteritis</td>
<td>Rates with Polyarteritis</td>
</tr>
<tr>
<td>Original salt content high:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dog Chow</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2. Bread</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Original salt content low:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Meat</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>4. Peas</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>5. Rice</td>
<td>23</td>
<td>1</td>
</tr>
</tbody>
</table>

mesenteric, 17; coronary, 13; peripheral (axillary or femoral), 9; pancreatic, 6; renal, 2; lung, 2; root of aorta, 1; hepatic, 1; adrenal, 1; mesovarium, 1.

Table 3 shows the distribution of the cases of polyarteritis among the diet groups. Food groups 1 and 2 contain about one per cent of sodium chloride; this represents the usual salt intake of rats. Group 1 diet is also high in protein. The sodium chloride supplement increased the daily sodium chloride consumption in both groups by 50 to 70%; the additional NaCl intake did not seem to have any effect on the incidence of polyarteritis, but the number of animals is too small to be of statistical significance. Among the 31 animals on these two diets, either with or without sodium chloride addition, 15 (48 per cent) showed polyarteritis nodosa. Of the 52 animals on the naturally low NaCl diets, groups 3, 4 and 5, only 2 (4 per cent) developed polyarteritis nodosa. In contrast, 14 of 49 rats (29 per cent) on the same diets with additional NaCl developed the lesion.

In the two single animals on low-salt diet (Table 3, groups 4 and 5), in which polyarteritis nodosa occurred, it apparently began very much later than in the rats on a high salt intake; their survival time was 364 and 344 days, respectively, after nephrectomy.

The one animal on a rice and tap water diet who died with polyarteritis nodosa 344 days after nephrectomy developed a sarcoma, presumably arising from the latex-enclosed, thickened fibrous capsule of the remaining kidney. Almost all of the kidney was replaced by the sarcoma, and the rat was practically arenal. The kidneys of all other members of groups 4 and 5 showed the changes already described and attributed to the manipulations and the latex capsule.

Discussion

The etiology of polyarteritis nodosa is still uncertain in spite of intensified interest during the past 15 years which has led to the reporting of numerous instances of the lesion in man and its discovery and/or production in several genera of animals. Since the lesion was first described by von Rokitansky in 1852 and by Kussmaul and Maier in 1866, etiologic importance has been attributed to various factors. Several reviews dealing with this subject have appeared recently.

As is well known, polyarteritis nodosa, in older rats, is a spontaneously occurring disease. Thus, Wilens and Sproul found it in 9.7 per cent of 487 animals. In no instance was the rat younger than 500 days. The authors state that 32 of their 47 polyarteritis animals showed renal lesions.

The rats of the present experiment, at the time of death, varied in age from 57 to 737, averaging 131 days. Only 3 animals among those which developed polyarteritis were older than 200 days, the age of the oldest being 414 days and the average age of the affected animals being 111 days. This age speaks against any significant incidence of spontaneously occurring renal lesions or of spontaneously occurring polyarteritis nodosa as reported by Wilens and Sproul. Furthermore, the overall incidence of 23.5 per cent polyarteritis nodosa in the animals of this report is significantly higher than the 9.7 per cent of Wilens and Sproul in old rats. Both facts indicate that the lesions found in the present series are a result of the experimental procedures.

Various factors may have been responsible for the development of polyarteritis nodosa in our rats: infection, high salt intake, hypertension, and renal dysfunction. Infection may have been present in many of the animals since no attempt at complete asepsis was made during the operations. Table 3 shows that sodium
chloride intake was a decisive factor in the development of polyarteritis in the present series. To our knowledge, no observations have been reported on the occurrence of polyarteritis in animals on a restricted sodium intake; in all instances mentioned, the salt intake was at least normal. In a recent study on vascular lesions in alloxan-poisoned rats, Chute, Orr, O'Brien and Jones frequently found polyarteritic lesions when the diet contained 5 to 10 per cent sodium chloride. This represents a daily intake of approximately 750 to 1500 mg. of sodium chloride. The lesions developed more slowly and were less severe on the 5 per cent sodium chloride intake. In our series, the daily sodium chloride consumption considered a high salt intake was between 100 and 300 mg.; no distinct difference was noted within this range in the incidence and the period of time required for the development or the severity of the lesions. Hypertension was present in all of our animals. Its severity was not necessarily correlated with the development of polyarteritis nodosa. As was previously stated the blood urea determinations in a number of rats showed the existence of uremia in many of them. At death, severe injury of the kidney was histologically present in every case.

In their interesting work, Chute, Orr, O'Brien and Jones make the following statement: "The vascular lesions (periarthritis nodosa and fibrinoid degeneration) are produced by the action of sodium chloride in those cases in which the kidneys had been damaged by alloxan." The rats with the highest incidence of vascular lesions and the highest blood pressure average (177 mm. Hg.) are found in their experiment in which 10 per cent sodium chloride was given and where the most severe renal damage occurred.

It would seem from this as well as from our work that renal damage and hypertension in the presence of normal or higher sodium chloride intake are decisive factors in the pathogenesis of fibrinoid degeneration and polyarteritis nodosa of the rat.

Rats with renal hypertension had a short survival time on a diet of meat. Control rats tolerated this diet rather well, although they too had an elevated blood urea. The natural salt intake is low on this diet; therefore, the short survival time of the hypertensive rats on meats must be attributed to a factor other than sodium.

The hypertension in our experimental animals was due to renal dysfunction but was not necessarily associated with azotemia. From our data it is hardly possible to separate the role of hypertension from that of other manifestations of renal dysfunction in the pathogenesis of the polyarteritis nodosa.

A certain degree of correlation between hypertension and polyarteritis was present only in group 5 of our animals. In the other groups on the low salt diets (groups 3 and 4), the incidence of polyarteritis nodosa was much lower than in those on the high-salt diets even though the average blood pressure figures were comparable. Apparently, then, hypertension cannot be the only cause of the vascular lesion. It may be argued that the rat like man has no critical blood pressure level separating the benign from the malignant forms of hypertension.

The results of these experiments are compatible with the concept of polyarteritis nodosa as the rat analogue of arteriolonecrosis in malignant hypertension and demonstrate the effectiveness of the rice diet in the control of the vascular sequelae of renal hypertension which cause the death of the animal.

One animal listed in Table 3, second column, deserves further mention. This rat, on rice and tap water diet, surviving 344 days, with the hypertension reversed to normotension for many months, developed a sarcoma, presumably arising from the latex-enclosed, thickened fibrous capsule of the remaining kidney. As a consequence of invasion, almost all of the kidney was destroyed. The long survival of this animal is an additional indication of the effectiveness of the rice diet in the treatment of chronic renal disease and the prevention of uremia.

**SUMMARY AND CONCLUSIONS**

Marked prolongation of life in rats with experimental hypertension may be achieved by the rice diet. Polyarteritis nodosa develops in many
animals with experimental renal hypertension. The results of the present experiments are compatible with the concept of polyarteritis nodosa as the rat analogue of arteriolonecrosis in malignant hypertension in man.

The incidence of polyarteritis nodosa in rats with experimental renal hypertension is closely correlated to sodium ingestion. Of 52 rats on a low sodium intake, 4 per cent had polyarteritis; of 80 rats on a high sodium intake (though only moderately high: 40 to 120 mg. daily), 36 per cent had polyarteritic lesions.

Among the groups on low sodium diets without salt addition, the incidence of polyarteritis nodosa was negligible, no matter whether a diet high in protein of animal or vegetable origin (meat, peas), or low in protein (rice) was given. However, there was a great difference with regard to survival time: on meat, the average was 45 days, on peas 110 days, on rice 218 days.

No animal on the strict rice diet showed polyarteritis nodosa except one which, after a survival time much longer than the average, developed a sarcoma, arising from the experimentally damaged fibrous capsule of the kidney. Only after destruction of almost all of the kidney did recurrence of hypertension and polyarteritis nodosa develop.

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Circ Res. 1955;3:73-78
doi: 10.1161/01.RES.3.1.73
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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