

## FURTHER EVIDENCE OF THE TOXICITY OF NaCl\*

### INCREASED BLOOD PRESSURE AND MORTALITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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There are now three strains of hypertension-prone rats in which genetic factors are demonstrably involved in setting average blood pressure levels. The first strain was described by Smirk and Hall in 1958 (1), the second by Dahl et al. in 1962 (2, 3), and the third by Okamoto and Aoki in 1963 (4). Formal genetic studies indicated that blood pressure control was multigenic in all three strains (5-7). It is likely that these strains share some genes that control blood pressure but the available evidence is not consistent with the possibility that they share them all. Among a number of differences in these strains that suggest nonidentity of the gene pools controlling blood pressure, none is more evident than the response to excess dietary salt (NaCl). Both the New Zealand strain isolated by Smirk and Hall (1) and the spontaneously hypertensive rat (SHR)<sup>1</sup> separated by Okamoto and Aoki (4) develop significant hypertension without additional dietary salt, whereas the sensitive strain developed by Dahl et al. (2, 3) was originally evolved specifically on the basis of its hypertensive response to NaCl. The fact that some rats develop hypertension spontaneously on normal salt intakes led to the widespread belief that salt intake was therefore unimportant. In the aggregate, our long experience studying both clinical and experimental hypertension by modifying dietary NaCl is incompatible with such a concept. The belief that dietary NaCl is unimportant if elevations in blood pressure are insignificantly affected by increments or decrements in salt consumption represents, in our opinion, a simplistic view of such a lethal disease as hypertension. If there is evidence of increased mortality in association with increased salt intake or decreased mortality with decreased salt intake, whatever the effects on blood pressure, this is of consequence: the importance of survival in considerations affecting one of the major cardiovascular diseases is not to be underrated.

The present work was done as part of a continuing long-term study in which the relative importance of dietary NaCl and genetic makeup are being evaluated, in a variety of situations, in both clinical and experimental hypertension.

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<sup>1</sup> *Abbreviations used in this paper:* DCA, deoxycorticosterone acetate; SHR, spontaneously hypertensive rat.

The specific question was: among rats from the SHR strain, known to be (relatively) insensitive to NaCl, would there nonetheless be subtle effects not only on blood pressure but also effects of equal and possibly greater importance on mortality?

### *Materials and Methods*

All animals came from our own inbred colony of SHR (4) and were 3-wk old weanlings at the start of the experiment. The 52 males and 51 females were divided into five groups each containing approximately 10 males and 10 females, with littermates randomly distributed. Special chows, made to order by Agway, Inc., Country Foods Div., Syracuse, N. Y., containing either 0.3% NaCl ("low" salt chow) or 8% NaCl ("high" salt chow) by analysis, were fed ad lib as specified. Tap water (0.5–0.7 meq/Na/liter) was given ad lib. The control group was maintained on low salt chow constantly; the other four groups were started on high salt chow at 3, 7, 11 and 15 wk of age, respectively, having had only the low salt chow available until then.

Rats were housed in stainless steel cages, 1–5 animals/cage depending upon sex ("small" cages, 425 cm<sup>2</sup> floor space held one male or two females; "large" cages, 991 cm<sup>2</sup> floor space held four males or five females). The racks were automatically flushed at ½-h intervals (Hoeltge, Inc., Cincinnati, Ohio) with a watering system (Hardco Scientific Div., Fieldstone Corp., Cincinnati, Ohio) that also provided for drinking. The cages were in air-conditioned rooms maintained at 22°C (±1°C) and 50% relative humidity with artificial light on for 9 h and off for 15.

Blood pressures were measured at 1–4-wk intervals, depending on the severity of the hypertension. Rats were placed in a warm box (38–39°C) for approximately 10 min before being transferred to the temperature-controlled (38–39°C) plastic cabinet in which systolic blood pressure was measured by the tail cuff method without anesthesia. The animal was restrained during the blood pressure measurement by a plastic housing unit that varied with the size of the animal. Animal and housing rested on a warm bare plate with a temperature control (E & M Instrument Co., Inc., Houston, Tex.). The average of the last four readings taken at approximately 30-s intervals was used as the systolic blood pressure for each session.

Observations were continued until an animal was approximately 1-yr old (55 wk) unless it died or became chronically ill earlier. Weight was recorded with each blood pressure. A permanent weight loss in excess of 10 g from any previous maximum was considered evidence of illness (6) and only the blood pressures recorded before such a weight loss were used in the statistical analysis of data. Cumulative blood pressures (8) were used, i.e. the last blood pressure taken while a rat was still in good health was carried forward for the final group averages, without regard to whether the rat died at, e.g., 11 wk or 51 wk. Weights were not compared among groups because in our extensive experience with two other strains of rats weight has not been a significant variable in the blood pressure response (9, 10), and Okamoto (11) reports the same for the SHR strain.

Statistical analysis for difference of means was made by the analysis of variance with a special computer program. A *P* value <0.05 was considered significant, and all *P* values <0.01 were assigned that nominal value.

### RESULTS

In Fig. 1 the blood pressure data are plotted with sexes combined. Fig. 2 shows the relationship of sex and of NaCl intake to blood pressure in rats kept on low NaCl throughout their lives by a comparison with data on rats given high NaCl from weaning onwards. Fig. 3 shows the effect of NaCl on mortality.

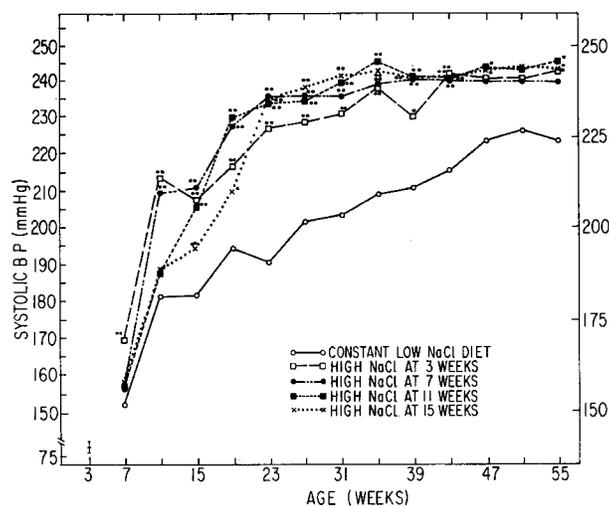


FIG. 1. The blood pressure (mean systolic  $\pm$  SE in mmHg) shown at 3 wk ( $77.4 \pm 1.38$ ) was the average of 13 females ( $77.1 \pm 2.57$ ) and 15 males ( $77.7 \pm 1.40$ ) 21–23 days of age, chosen randomly but not used thereafter in this study. Blood pressures not measured on test rats at 3 wk. \*, differs from constant low NaCl diet,  $P < 0.05$ ; \*\*, differs from constant low NaCl diet,  $P < 0.01$ . After 23 wk, all rats on NaCl with sexes analyzed separately had similar blood pressures ( $P > 0.05$ ) except in the group that started NaCl at 11 wk in which  $\sigma > \varphi$  through the 43rd wk ( $P < 0.05-0.01$ ). In low NaCl group,  $\sigma > \varphi$  after the 11th week ( $P < 0.05-0.01$ ).

The data in Fig. 1 suggest that the addition of NaCl to the diet had a significant effect on the rate of development of hypertension in the SHR; a more rapid elevation in blood pressure became evident as early as 4 wk after starting on a high NaCl diet by comparison with rats on low NaCl. This difference was generally maintained thereafter, with exceptions occurring after age 47 wk as noted in Fig. 1. By age 23 wk, the average pressures of the four salt-fed groups were similar ( $P > 0.05$ ), i.e., the effect of delaying the age at which NaCl had been started had been obliterated. After age 11 wk, among rats maintained on low NaCl (Fig. 2), females had consistently lower average pressures than males ( $P < 0.05-0.01$ ). The generally lower level of pressures for the low NaCl group with sexes combined (Fig. 1) is, therefore, to some extent misleading in that, while it correctly reflects the lower pressures of the females, it does not show the greater rise in pressure of the males: indeed, by age 27 wk, the low NaCl males had average systolic pressures similar ( $P > 0.05$ ) to those of three of the NaCl-fed groups of males and by age 39 wk to those of all four. The addition of dietary NaCl to the regimen, therefore, had no significant effect on the ultimate level of hypertension among male SHRs but accelerated the rate at which hypertension developed. Among females both the rate of its

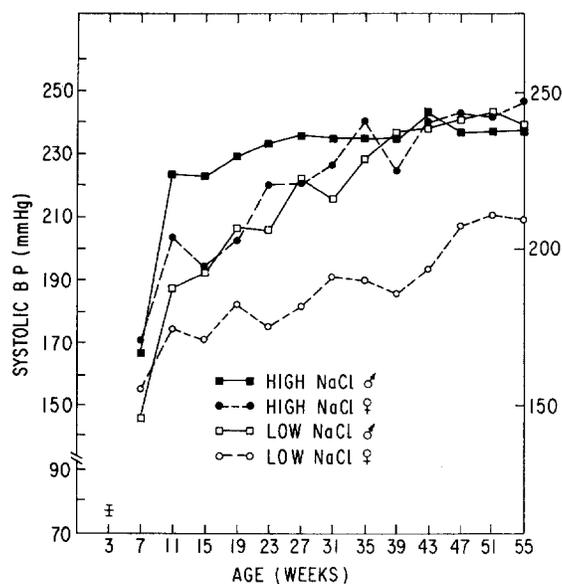


FIG. 2. See legend for Fig. 1. High NaCl diet started at 3 wk of age. After 11 wk of age, the low NaCl females had lower blood pressures than any of the other three groups ( $P < 0.05-0.01$ ). By the 27th wk, the two high NaCl groups and the low NaCl had similar pressures ( $P > 0.05$ ).

development and the level attained were increased by salt so that, ultimately, average pressures were equal to those in the males ( $P > 0.05$ ).

These modest effects on blood pressure should not be construed as indicating that the influence of NaCl was relatively benign: animals on added NaCl had a drastic increase in morbidity and mortality (Fig. 3). The 25% level of deaths, for instance, was reached as early as age 12 wk and no later than 31 wk in the four groups given extra NaCl as contrasted with age 41 wk for the control (low NaCl) group; 50% mortality occurred as early as age 15 wk and no later than 38 wk for the NaCl-fed animals but not until age 54 wk for the controls; and, finally, whereas 75% of the salt-fed rats were dead by age 46 wk, 67% of the controls were still alive at that age. No uniform correlation was found between length of time on NaCl and mortality: animals started on high NaCl at age 7 wk, for instance, generally died sooner than those started at age 3 wk. A somewhat similar observation had been made in an earlier study involving a salt-sensitive strain of Sprague-Dawley rats: NaCl addition, delayed in one group until 6 mo after weaning, gave morbidity-mortality data comparable to that of a similar group of rats in which NaCl was started 3 mo after weaning (10). The distinctly lower mortality among females as compared with males in the low salt group (eight vs. two survivors, respectively, at 55 wk) was somewhat less marked among the four salt-fed groups.

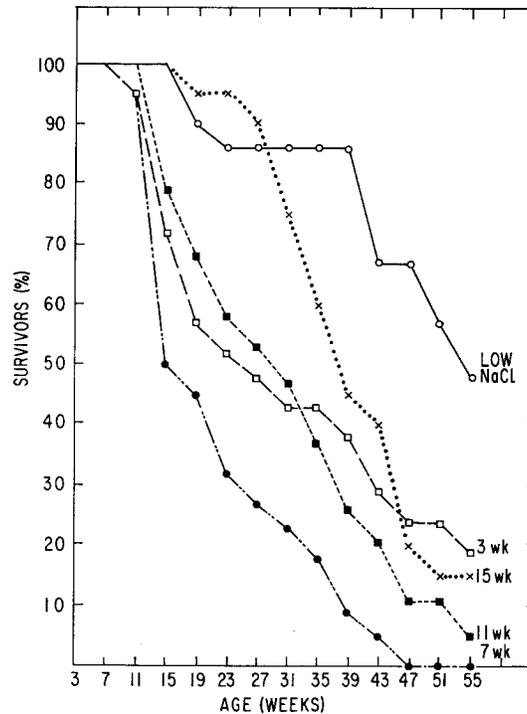


Fig. 3. Age in weeks at which high NaCl diet started

#### DISCUSSION

From this year-long study of the effect of dietary NaCl on the SHR the following conclusions emerge: (a) Salt accelerated the rate at which hypertension developed in both males and females. (b) The ultimate levels of blood pressure reached by males on either low or high salt diets were, however, similar. (c) Females reacted differently from males in this last respect: the average blood pressure of females on high NaCl ultimately became indistinguishable from males whereas the blood pressure of females on low NaCl remained significantly lower than males. (d) The added NaCl had a devastating effect on the mortality of both males and females, an effect that did not appear to be correlated directly with blood pressure per se, at least in the males. Among the low NaCl females, since both mortality and blood pressure were lower than in all other nine sets, separation of these two effects was difficult. Although the rate at which hypertension developed was generally more rapid in males than in females, it was not self-evident that this played a unique role in determining mortality: among the rats that started NaCl at age 7 wk, for instance, the blood pressure remained significantly lower in females than in males ( $P < 0.05$ ) until

age 27 wk but the mortality during that time was at least as high or somewhat higher among females.

Several earlier reports bear upon the question of the influence of NaCl on the hypertension that develops in the SHR. In the first of two papers concerned with the same male SHRs, Louis et al. (12) concluded that, although SHRs were relatively insensitive to the level of dietary sodium, rats given 4% NaCl in the diet for 10 wk had higher blood pressures than those given 1% or 0.1% NaCl. In the second paper they reported (13) that by age 32 wk the same groups of rats showed a stepwise increment in average systolic pressures, the group with the highest salt intake having the highest pressures. Barsanti et al. (14) compared the response of four female and three male SHRs given 1% saline as the sole source of fluid with controls on water: by the end of the 9th wk of study those on saline had significantly higher pressures. There are a number of reports in Okamoto's recent monograph on the SHR suggesting an increased incidence of pathological effects induced by added dietary NaCl; no effects on blood pressure per se were included (15-18). Our own experience, with salt and SHRs (Wistar derived) just outlined, as well as our much earlier reports (19, 20) involving Sprague-Dawley rats suggests that long-term consumption of NaCl probably potentiates, and may induce, pathological effects without direct relation to the level of blood pressure.

Although the hypertensinogenic effects of NaCl are now generally recognized, its possible noxious effects on blood vessels are not. Salt has been accorded little importance in the vascular disease that ultimately leads to most of the morbidity and mortality in hypertensives. Among most clinicians there is a lack of interest in a general restriction of dietary NaCl in patients with hypertension except for those individuals in whom a clear-cut fall in pressure has followed such restriction. This may be an error. Kempner (21) reported in 1948 that patients with severe hypertension often showed marked improvement in vascular retinopathy after treatment with the "rice diet" (a diet that depends for its primary effectiveness on its low sodium content [22]) even when blood pressure failed to decline significantly. In 1963 we noted (20) that we had repeatedly confirmed this phenomenon after NaCl restriction in man and have continued to do so. After observing hypertensives treated by chronic salt restriction for 25 yr we have the impression that, even among those whose pressures do not fall, the clinical course is more benign than among those who revert to or continue on their usual high salt intake.

This beneficial effect of salt restriction is admittedly difficult to prove in man. In rats, however, there can be no serious question that NaCl has an influence on morbidity, mortality, and vascular disease. In our genetically hypertension-prone strain of rats (2, 3, 6, 10, 20) the effect of dietary NaCl is devastating on life expectancy. When NaCl is used to induce hypertension, its continued consumption leads to the death of some within a few weeks and of all within a few months. And while cessation of excess salt consumption is no guarantee of longevity (10), we have repeatedly observed animals with severe salt-induced hypertension, in which death could have been firmly predicted within a few days or weeks, that continued to survive for months after salt was restricted despite the fact that blood pressure did not fall and even increased (23).

Race and Peschel found that the panarteritic lesion of rats with renal hypertension

was enhanced by supplementary NaCl (24). Kempner et al. (25) subsequently reported a marked prolongation of life-span in rats with renal hypertension on low NaCl diets. They also observed that on low NaCl "... 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." It would be unwarranted to equate the pan (or poly) arteritis so frequently observed in aging rats with a specific vascular disease in man, e.g., arterio- or atherosclerosis. Nevertheless, the fact that the development of panarteritis can be at least accelerated by NaCl in rats allows speculation that NaCl may somehow enhance the development of human arterial disease (19). Koletsky has documented the aggravating effect of NaCl on the polyarteritis that develops in rats with severe renal hypertension (26) and later (27) postulated that such vascular lesions might be "... initiated by the toxic action of salt. . . ."

In a number of studies, the earliest being those by Friedman (28) and Tobian (29, 30) and their respective associates, changes in the concentrations of electrolytes, particularly of sodium and potassium, were found in the walls of small muscular arteries, and possibly even arterioles, in hypertension. Whether such electrolyte changes play a primary role in the pathogenesis of hypertension or are effects of it is still debatable but the idea that intermittent or chronic intracellular electrolyte imbalance could invoke subtle injury in vascular tissues that might predispose to the subsequent development of chronic vascular disease seems entirely warranted. The damaging effects on tissues of intracellular disturbances of electrolytes, including sodium and potassium, are well known (e.g., 31, 32).

Meneely and his associates (33, 34) have reported in some detail the accelerated death rate associated with increased dietary NaCl among groups of rats on different intakes. Kempner also found that in rats with experimental aminonucleoside nephrosis the death rate was eight times as high among those receiving added dietary NaCl (35). Much earlier, Green et al. (36) had reported that, among rats with postdeoxycorticosterone acetate (DCA) hypertension, survival rate but not blood pressure was strikingly improved by sodium restriction. Sturtevant (37) quotes some unpublished observations of Salgado who had similar results from sodium restriction also in rats with post-DCA hypertension. Grollman and Harrison (38) found that, in rats with what apparently was relatively early renal hypertension, survival was significantly prolonged by sodium restriction. These last results are clouded (for our present purposes) by the fact that blood pressure also fell so that the influence on survival of sodium restriction cannot be clearly separated from that of lower blood pressure.

There are several reports in apparent conflict with the foregoing in that significant improvement in survival was not reported with sodium restriction. Gross (39), Hoobler (quoted by Sturtevant [37]), and Sturtevant (37) are in agreement that survival among rats with post-DCA hypertension was not improved by a low sodium intake. Tobian et al. (40) also failed to find that rats drinking 1% saline for 6 mo had an increased incidence or severity of renal hypertension and mortality was not significantly enhanced. Observations on vascular disease were not reported.

No resolution of this disagreement among others is immediately apparent. The senior author of the present study, however, after observing thousands of rats with and without NaCl restriction after the induction of either salt or renal hypertension has no doubts as to the efficacy of sodium restriction in enhancing survival.

Meneely and Ball (41) reported that some salt-fed rats had elevated serum chole-

terols and tentatively concluded that there might be a positive correlation between elevation in blood pressure and elevation in plasma cholesterol. In 1960 Dahl (19) also reported that among rats and dogs fed excess NaCl for approximately 1 and 4 yr, respectively, elevations in plasma cholesterol were frequent and sometimes marked, but no correlation between elevated pressures and elevated plasma cholesterol was evident. The cardinal role that a derangement of lipid metabolism is believed to play in the pathogenesis of atherosclerosis needs no documentation. It was speculated that increased lipid levels might be another response to a high salt intake and that "... if excessive salt ingestion is capable of elevating plasma lipids it may conceivably affect atherogenesis as well. None of the experimental studies compares in chronicity with the natural history of atherosclerosis in man, in whom slight abnormalities in lipid metabolism operating over decades might result in a significant vascular disease." (19). At that time, earlier studies by others in the chicken (42), rat (43), and rabbit (44) furnished no support for this hypothesis. Subsequently, however, it was reported (45) that the incidence of myocardial and renal infarcts as well as the deposition of fat in the aortas of rats on an atherogenic diet were increased when 1-2% saline replaced tap water for drinking, without associated changes in blood pressure. The issue is clearly unresolved at this point. In any event, it is likely that if NaCl can play a role in human vascular disease, it will turn out to be as an ancillary factor that enhances atherogenesis in the presence of a pre-existing atherogenic regimen, similar to that noted by Belliveau and Marsh (45). The Japanese, for example, have had a notably high salt intake and a high incidence of hypertension but, concomitantly, a low fat intake and a low incidence of atherosclerosis (46).

In the present study, no attempt was made to determine the pathological changes or the precise cause of death in these SHRs since our interest was directed to the question: would the addition of salt in the diet have an effect on blood pressure and/or morbidity-mortality in a strain of rats considered to be relatively insensitive to the effects of salt? In our past experience with salt-fed Sprague-Dawley rats, the cause of death was not readily apparent (10). Indeed, among the latter, the majority appeared to die from an accelerated progression of the pulmonary complications that so commonly plague rat colonies. Pathological changes in the SHR have been described in considerable detail in Okamoto's monograph (15-18) but the actual cause of death was not clear in most cases. In the same monograph, Nagaoka et al. (47) reported that spontaneous death and the moribund state did not occur until the 10th mo of age among 111 SHRs of both sexes, maintained on "standard diet." In this study, our experience with the group maintained on low NaCl chow was quite similar: significant mortality-morbidity appeared only after the 39th wk of age (Fig. 3).

The concept that dietary NaCl is inimical to blood vessels is by no means new. Indeed, F. M. Allen (48), a pioneer in the field of salt and human hypertension wrote half a century ago "... the morbidity and mortality from renal and vascular diseases would probably be reduced by a general abstinence from salt." We have mentioned the later observations and speculations of others in a similar vein. And yet well into the 8th decade of the 20 century this con-

cept is for the most part honored in the breach rather than the observance by clinicians, nutritionists, and public health workers throughout the world. We submit that a careful review of the evidence will support Allen's proposal.

#### SUMMARY

The spontaneously hypertensive rat (SHR) of Okamoto and Aoki (4) develops significant hypertension without added dietary salt. Many patients with hypertension have little or no alteration in blood pressure from increments or decrements of salt intake. Such observations have led to a widespread belief that dietary NaCl is important in hypertensives only if blood pressure changes are observed after changes in NaCl intake. Such a simplistic view of hypertension fails to take into account the possibility that morbidity and mortality might be lowered by restricting dietary NaCl, without a concomitant lowering of blood pressure.

The effect of a high NaCl intake for periods up to 1 yr has been studied in the SHR with the following conclusions: (a) NaCl accelerated the rate at which hypertension developed in both sexes. (b) The ultimate levels of blood pressure reached by males on either high or low salt diets were similar, however. (c) In females, (1) on high NaCl the average blood pressure ultimately became indistinguishable from males whereas (2) on low NaCl pressure remained significantly lower than that of males. (d) The addition of NaCl to the regimen had a devastating effect on mortality of both sexes and was not directly correlated with the level of blood pressure. Earlier reports were reviewed bearing on the possibility that NaCl is inimical to blood vessels without a necessary relationship to its hypertensinogenic effect. It was concluded that restriction of dietary NaCl would reduce morbidity and mortality in hypertension, whether or not blood pressure was reduced by such measures.

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