

The Influence of Prenatal Salt on the Development of Hypertension by Spontaneously Hypertensive Rats (SHR) (40462)

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Ingestion of large amounts of salt by both experimental animals and humans has been associated with an accelerated rate of development of hypertension (1). Dahl (2, 3), Barsanti (4), and others (5-11) have shown that younger animals are more susceptible than older animals to the aggravating effects of salt. Spontaneously hypertensive rats (SHR), while not requiring excessive NaCl for the development of hypertension, exhibit higher than usual blood pressures when fed a high salt diet (5). In all previous studies, however, the youngest animals used have been past weaning age (3 weeks in the rat). Only 3 weeks after weaning, abnormally high blood pressures are measurable in SHR. The present experiments were therefore designed to determine the effects of a high salt diet given to mothers during pregnancy on the subsequent development of hypertension by the young. In addition, since little information is available on the effects of high salt on the pregnancies of SHR or other strains, the mothers themselves were also studied. The results show that a high salt maternal diet, when followed by high salt postnatally, produces strikingly higher blood pressures in the young as compared with young receiving high salt only postnatally. They also show that while a high salt diet does not appreciably alter pregnancy in SHR, Wistar Kyoto rats (WKY), a supposedly normotensive genetic control strain for the SHR, experience a relative hypertension during pregnancy, and this "gestational hypertension" is exacerbated by the high salt diet.

Methods. Mating females of three strains, spontaneously hypertensive rat (SHR), Wistar-Kyoto (WKY), and Sprague Dawley (CD) were used for these studies. Animals of each strain, 2-5 days pregnant, were divided into two groups which received either high or low salt chow diets and water *ad libidum*. The special chows, received from Brookhaven National Laboratories, were made to order

by Agway Inc., Syracuse, NY and contained either 0.4% NaCl (low salt chow) or 8.0% NaCl (high salt chow). At birth, litters were cross fostered to distinguish prenatal from postnatal effects. The mothers were maintained throughout the lactation period on the same diet they had received during pregnancy. After weaning, the offspring were continued on the diet of the mother who nursed them. Four groups of young for each strain resulted and were designated as follows: (a) LL: Young from *low* salt mothers, fed *low* salt postnatally. $N = 25$ SHR; 10 CD; 6 WKY. (b) HL: Young from *high* salt mothers, fed *low* salt postnatally. $N = 11$ SHR; 10 CD. (c) LH: Young from *low* salt mothers, fed *high* salt postnatally. $N = 14$ SHR; 9 CD; 6 WKY. (d) HH: Young from *high* salt mothers, fed *high* salt postnatally. $N = 22$ SHR; 18 CD; 17 WKY.

During pregnancy and lactation, females were housed in acrylic cages, one mother per cage. Weanlings were housed two to five per cage. Food and water intake during pregnancy and nursing was recorded. Mothers were weighed every 3 days through pregnancy, and after delivery pups randomly selected from each litter were weighed every 2 days. For determination of systolic blood pressures, unanesthetized animals were warmed to 39° in an animal holder (Narco-Bio Systems). Pressures were taken indirectly by a tailcuff and pneumatic pulse transducer (Narco-Bio Systems) attached to two channels of a Hewlett Packard Chart recorder. Four systolic readings taken at approximately 1 min intervals were averaged for each animal. Maternal blood pressures were recorded every 3 days. Blood pressures were obtainable only after the pups were 5 weeks old, and thereafter pup blood pressures were measured biweekly.

Results. Maternal. The high salt diet produced little change in the blood pressure patterns of mothers having successful pregnan-

cies. However, high salt increased the rate of unsuccessful pregnancies in SHR and WKY, and with unsuccessful pregnancies the usual pregnancy related drop in blood pressure in SHR was not observed. Figure 1 illustrates changes which occurred in systolic blood pressure from mid-pregnancy (8–12 days) to late pregnancy (17–20 days). SHR mothers on either high or low salt and who delivered showed a significant ($P < 0.05$) drop in blood pressure during pregnancy from hypertensive to normotensive levels. The high salt group had insignificantly higher blood pressures ($187 \text{ mm} \pm 29 \text{ mm Hg}$ —mid-pregnancy; $131 \text{ mm} \pm 18 \text{ mm Hg}$ —late pregnancy; $\bar{X} \pm \text{SD}$) when compared with those of the low salt SHR mothers ($161 \pm 16 \text{ mm Hg}$ —mid-pregnancy; $120 \pm 14 \text{ mm Hg}$ —late pregnancy). Among SHR who had reabsorbed fetuses, blood pressure was $170 \pm 22 \text{ mm Hg}$ at mid-gestation but rose to $187 \pm 14 \text{ mm Hg}$ by the expected date of delivery. The beginning of the rise in blood pressure was found in each case to follow estimated time of death of the fetuses by one to 2 days.

In the WKY groups, the low salt mothers exhibited no significant decreases in blood pressure through pregnancy ($110 \pm 14 \text{ mm Hg}$ mid-pregnancy to $98 \pm 17 \text{ mm Hg}$ —late pregnancy). High salt WKY who delivered showed a slight though insignificant increase in blood pressure ($100 \pm 10 \text{ mm Hg}$ mid-pregnancy to $110 \pm 14 \text{ mm Hg}$ —late pregnancy), thus representing the only group having successful pregnancies with an increase

(though not significant) in blood pressure. High salt WKY mothers who had unsuccessful pregnancies experienced a marked increase in systolic blood pressure ($112 \pm 9 \text{ mm}$ mid-pregnancy to $140 \pm 13 \text{ mm Hg}$ —late pregnancy).

Among CD mothers we found no significant differences in blood pressure between the high and low salt groups at any stage of pregnancy. Both groups showed a drop of about 30 mm Hg (from 85 to 55 mm Hg) from mid to late pregnancy.

High salt diet decreased successful pregnancy rates by 38% in SHR and 66% in WKY. SHR females on a low salt diet bred at one year of age ($n = 4$) or three months of age ($n = 6$) all delivered normally in terms of litter size and individual pup weight. In the SHR high salt groups, females bred at 3 months of age ($n = 6$) all delivered normal litters. SHR high salt females bred at 1 year of age ($n = 7$) tended to have abnormal pregnancies. Five of this group had partially reabsorbed fetuses (as confirmed by cesarean section) during the second week of gestation. Two high salt mothers in this older age group delivered normally.

The WKY low salt females ($n = 2$) bred at 3 months of age delivered normally. In the WKY high salt group consisting of mothers 3 months of age ($n = 6$) 4 had aborted or reabsorbed pregnancies. Two delivered normal litters.

In the CD low salt mothers 3 months of age ($n = 6$), and the CD high salt mothers 3 months of age ($n = 3$) all animals delivered normal litters.

The high salt diet depressed maternal weight gain of SHR and WKY. Litter weights were subtracted from maternal weight just before delivery to show net maternal body weight gain or loss. Low and high salt CD mothers as well as low salt WKY experienced a 35% gain in net body weight during pregnancy. Low salt SHR and high salt WKY mothers demonstrated only a 3% gain in body weight. However, high salt SHR mothers demonstrated a net loss of 1–2% in net body weight. This occurred even though high salt SHR mothers consumed two to three times the amount of chow and water as the low salt SHR, a phenomenon not observed in the other strains.

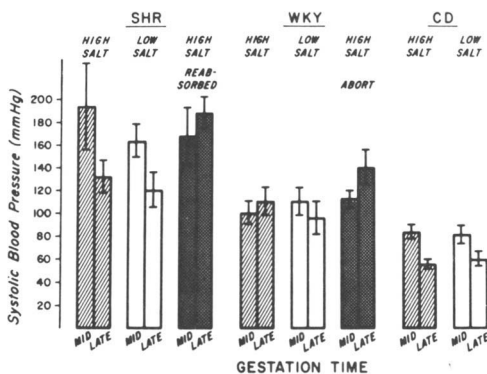


FIG. 1. Systolic blood pressures (means \pm SD) during pregnancy in the three strains (SHR, WKY, and CD) receiving either high salt (8% NaCl) or low salt (0.4% NaCl) diets. Mid-pregnancy = 8–12 days gestation. Late pregnancy = 17–20 days gestation.

Offspring. The systolic blood pressures of animals in the four diet groups of SHR young aged 5½ to 17½ weeks are shown in Fig. 2A. The group never given high salt (LL) demonstrated a rapid rise in blood pressure from 130 ± 12 mm Hg at 5½ weeks to 175 ± 12 mm Hg at 13½ weeks. Thereafter the blood pressure plateaued at 175–180 mm Hg through 17½ weeks of age. The HL group showed blood pressures slightly lower but not significantly different from those of the LL group throughout the 5½- to 17½-week age period. The blood pressures of the LH group were not significantly different from those of the LL group until 15½ weeks of age when they jumped to 198 ± 6 mm Hg, a pressure significantly higher than those of the LL and HL group. By contrast, at 11½ weeks of age and thereafter animals from the HH group (those whose mothers had received high salt and who continued on high salt postnatally) showed blood pressures significantly higher than those of all the other groups rising to 237 ± 10 mm Hg at 17½ weeks. We have not found these excessively high blood pressures

in animals of any of the other groups followed until 6 months of age. Very young animals (5½ weeks) of the HH group also showed significantly higher blood pressures (201 ± 8 mm Hg) than those of the other groups, but from 7½ to 11½ weeks blood pressures of animals in the HH group were not significantly different from those of the other groups.

The SHR HH group demonstrated high mortality and morbidity rates. 63% were dead or dying by 4 months of age. Of the 22 animals in this group eight died before 4 months of age. Critical stages occurred in their development. At 5 weeks, shortly after weaning, five animals suddenly exhibited peripheral capillary hemorrhage and died within a day. High, labile blood pressures were observed for this group. The 17 animals surviving appeared normal during the next 6–8 weeks. Symptoms of stroke in nine animals 3–4 months of age were similar to those described by Okamoto and Yamori (12) in their description of the stroke-prone SHR rat. Initial symptoms of stroke observed were motion disturbances resulting in paresis, transient episodes of repetitive lifting of paws, and ataxia. General apathy was observed near the time of death. The time-period between initial symptoms and death varied from one week to 15 weeks. Three of these animals died before 4 months of age, and only one appeared in good health at 6 months. Autopsies showed renal infarction as well as left ventricular hypertrophy. Three SHR from the HH group who survived to 20 weeks demonstrated systolic blood pressures as high as 270 mm Hg. No other animals including the SHR receiving high salt only postnatally demonstrated any of these complications and only one death occurred in SHR HL before 6 months. There have been no deaths in any other groups before this age. At the time the SHR HH offspring were dying of hypertensive sequelae, their mothers who had received the high salt diet during pregnancy were bearing subsequent litters and had blood pressures (at mid-pregnancy) averaging 200 mm Hg.

Systolic blood pressures of WKY pups from the various diet groups are illustrated in Fig. 2B. In this strain, the LL group showed a rapid increase in blood pressure (85–135

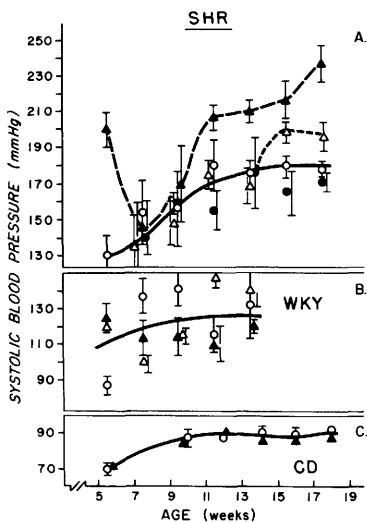


FIG. 2. Systolic blood pressures (means ± SD) in offspring of the three strains with the various diet combinations. ▲ = HH; high salt to mothers, high salt postnatally. △ = LH; low salt to mothers, high salt postnatally. ○ = LL; low salt to mothers, low salt postnatally. ● = HL; high salt to mothers, low salt postnatally. The number of animals randomly selected from each group (n) for each point is as follows: SHR HH = 10; SHR LL = 8; SHR LH = 8; SHR HL = 8; WKY HH = 8; WKY LL = 6; WKY LH = 5; CD HH = 5; CD LL = 5.

mm Hg) between 5½ and 7½ weeks of age. Thereafter blood pressure was relatively stable at 120–135 mm Hg through 17½ weeks. Throughout this age period there were no significant differences in blood pressure between the other diet groups and the LL group.

Figure 2C demonstrates the systolic blood pressures of CD pups 5½–17½ weeks old in the various diet groups. In this strain, blood pressures were 70 ± 2 mm Hg at 5½ weeks rising to 87 ± 5 mm Hg at 9½ weeks and remaining at this level through 18 weeks. There were no significant differences in blood pressure between any of the diet groups in this strain.

The high salt diet depressed the postnatal growth of the young of SHR, WKY, and CD strains. Birth weights within a given strain were identical for all diet groups. By the time of weaning both HH and LH young weighed 33% less than the LL and HL groups. This weight pattern continued through at least 6 months of age.

Discussion. The most striking finding of the present experiments is the observation that a high salt diet given to pregnant SHR can greatly aggravate the development of hypertension in the offspring. The very rapid increase in blood pressure to over 230 mm Hg at 4 months correlates with a high percentage (41%) of stroke. Although there have been reports that the tail cuff method may cause falsely high blood pressure readings (13), this method was used for both control and experimental groups. None of the other SHR diet groups showed these excessively high blood pressures. Our results show that the effects of the maternal high salt diet in SHR become manifest only when the young are fed high salt after birth; thus it is probable that developing or maturing mechanisms for the regulation of blood pressure are affected by the maternal high salt diet in such a way that animals become extremely susceptible to the effects of postnatal salt. The question arises, however, as to what the specific effect of the maternal high salt diet is. Lewis *et al.* (5) have shown that SHR fed a high salt diet (4%) show sporadic increases in serum Na concentration. Thus it is reasonable to assume that the Na concentrations presented to the placenta in the present studies (utilizing 8% Na) were at least sporadically elevated and might

have had a direct effect on fetal development. The high salt diet, on the other hand, may have produced an indirect effect on fetal development by modifying any of several functional characteristics of the mother including the hemodynamics of the placental circulation. It is possible that the higher maternal blood pressures of SHR (not significantly different for both diet groups) are involved in the higher sensitivity of the SHR offspring to prenatal salt. Whether the effect of maternal high salt on the fetus was direct or indirect, the fact remains that SHR receiving the high salt diet after birth showed the extreme aggravation of hypertension only if their mothers had received high salt during pregnancy. The fact that CD and WKY did not exhibit this effect indicates a strong genetic component for sensitivity to prenatal salt.

From an epidemiologic standpoint, it is probably significant that even the very high salt concentrations used in the present studies were not lethal to the fetuses. Furthermore, in any population, young are very likely to assume their mother's diet on weaning. The combination of high salt pre- and postnatally is therefore not an arbitrary experimental maneuver.

Body weight gain of both SHR and WKY mothers was suppressed by the high salt diet, though SHR exhibited salt appetite. This suggests that the high salt diet did not result in water retention in these animals. Blood pressures of SHR mothers on the high salt diet were slightly higher than those of low salt SHR mothers. High salt also decreased the rate of successful pregnancies in older SHR and in young WKY. Our observation that SHR on a normal diet (low salt) experience a marked drop in blood pressure during pregnancy agrees with findings of Weinberger (14). McCarty and Kopin (15) using the direct method of catheter placement in the ventral caudal artery during anesthesia did not observe such a decrease. Like Weinberger we have not observed an increase in systolic blood pressure in early pregnancy, as was reported by Lindheimer (14). A drop of about 20 mm Hg has been reported to occur in CD on a normal diet (16), and our observations concur with this. We have found also that CD on high salt experience a similar drop in

blood pressure. Douglas (17) has reported that pregnancy lowers Goldblatt hypertension in the rat.

WKY experience a high rate of unsuccessful pregnancies with the high salt diet. Even with successful pregnancy on either high or low salt, WKY did not show the drop in blood pressure characteristic of pregnant SHR and CD. WKY on the high salt diet showed a not significant increase in systolic blood pressure averaging 10 mm Hg from mid to late pregnancy. We therefore suggest that the WKY strain may prove to have a predisposition to preeclampsia of pregnancy. This deserves further investigation since no model exists which mimics all of the manifestations of the human disease (7).

Physicians have traditionally attempted to limit salt intake of pregnant patients hoping to avoid hypertensive complications in the mother. The present results indicate that this practice may have even more important implications regarding the future health of the fetus.

Summary. Spontaneously hypertensive rat (SHR) pups whose mothers receive a high salt diet during pregnancy and who continue on the high salt diet in postnatal life develop exceedingly high blood pressures (230 mm Hg before 4 months of age). This does not occur in SHR on high salt only prenatally or only postnatally nor does it occur in Wistar Kyoto (WKY) or Sprague Dawley (CD) rats. Among SHR pups on the high salt regimen both pre- and postnatally there is a greatly accelerated incidence of stroke as well as a markedly shortened life-span (36% mortality before 4 months of age). Maternal effects of the high salt diet include reduced gestational weight gain, and reduced successful pregnancies in SHR and WKY. However, in successful pregnancies maternal blood pressures in all three strains were not significantly altered by the high salt diet. The high salt diet did not affect the life span of the mothers, and

the SHR mothers of pups on high salt both pre- and postnatally outlived their offspring.

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