Maternal influences on cardiovascular pathophysiology

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Abstract. Elevated blood pressure (BP) is of special clinical significance because of its association with pathophysiologies such as heart disease, renal failure, and stroke. We described the development of a protocol for use with hypertensive rats in which prepubertal exposure to a high salt (8% NaCl) diet results in a pathophysiological syndrome including rapid increase in BP, failure to maintain normal weight gain, renal damage, cerebrovascular lesions, and early mortality. These phenomena are described for the inbred spontaneously hypertensive rat (SHR), and for reciprocal F_1 hybrids of a cross between SHR and the Dahl salt-sensitive (SS/Jr) inbred strain. The study with reciprocal F_1s revealed striking effects of maternal environment on pathophysiological response to a high salt diet. F_1s nurtured by SHR mothers weighed less at 35 days of age, and after exposure to the high salt diet suffered more rapid BP increases, greater incidence of stroke, body weight loss, and mortality, than F_1s nurtured by SS/Jr dams. These results suggest that maternal mediation of the nutritional status of the animal may play an important role in determining susceptibility to elevated BP and subsequent pathophysiology associated with exposure to a high salt diet. The implication of these findings for human hypertension is briefly discussed.

Key words. Reciprocal cross; F_1 hybrids; maternal environment; salt-induced hypertension; stroke; pathophysiology; SHR; SS/Jr.

Introduction

Elsewhere in this review series, the contribution of the preweaning environment to the level of blood pressure (BP) reached in adult life has been systematically discussed[18,22]. McCarty and co-workers have demonstrated that cross-fostering of spontaneously hypertensive rats (SHR)[23] or salt-sensitive (SS/Jr) rats[25] at birth to the relevant normotensive controls, results in their expressing a lower BP in adult life[9,19]. The decreased BP is not due to the fostering process, per se, because the BP of SHRs and SS/Jrs raised by their own mothers does not differ from BP of animals fostered to mothers of their own strain. In addition, Myers and colleagues have demonstrated a significant correlation between the frequency of specific kinds of maternal behaviors and the mean adult BP of offspring exposed to those behaviors[20,21].

These results should be considered in the context of a wealth of psychobiological research in the late 1950s and early 60s which established that alterations of the postnatal environment, including cross-fostering, had effects on a variety of behaviors which were observable in adult life[13,17,26]. The current research extends the relevance of the preweaning maternal environment to fundamental physiological processes, and has focussed interest on the importance of early development in the expression of hypertension.

In their reviews, McCarty and Myers have restricted their discussion to the effects of the maternal environment on BP per se. However, concern with hypertension in humans rests on its association with heart disease and other circulatory disorders including stroke: in other words, the pathophysiological sequelae of elevated BP. Our own studies have emphasized the influence of the maternal environment on these pathophysiological consequences, and this will be the topic of our review. This emphasis is intended to show that variations in maternal environment have an impact on a broad spectrum of health-related processes.

Research paradigm and background studies

Protocol used to study the pathophysiological consequences of elevated blood pressure

Even in the genetic models of hypertension that are most frequently studied, the life-threatening, pathophysiological consequences of elevated BP, such as stroke or renal disease, are not usually seen until an animal has been exposed to high arterial pressure for many months. In order to permit pathophysiology to be studied within a short period of time, we adapted a protocol similar to that originally used by Lewis K. Dahl to select for high and low BP-response to placement on a high salt (HS) diet containing 8% NaCl[10]. The diet we used was a modified form of the standard maintenance diet for rats at Harlan Industries; further details on the composition of the diet are available elsewhere[5].

Beginning at 35 days of age, systolic blood pressure (SBP) is measured via the indirect tail-cuff method on 2–3 consecutive days. The procedure in use in our laboratories produces reliable estimates of SBP when compared with directly recorded arterial pressure[1,4]. At the time of the baseline measures, rats are randomly assigned from within litters (split-litter design) to the HS or LS dietary conditions. SHR rats are exposed to the HS diet beginning at approximately 37 days of age. Following

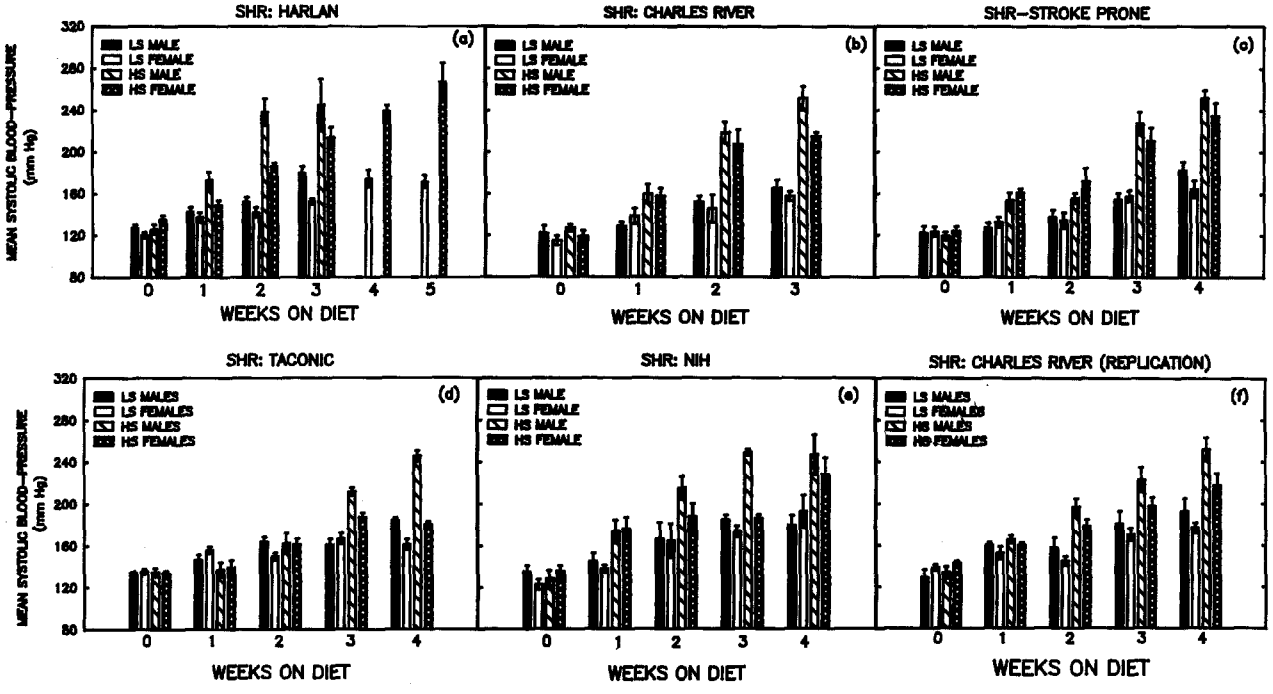

Figure 1. Mean systolic blood pressure, + / − SEM, in 4 different SHR-derivations and the stroke-prone SHR (SHR-SP). After rearing on a low salt (LS) diet containing 0.3% NaCl, male and female SHRs (N = 5–8 per gender diet sub-group) from Harlan, Charles River (2 replications), Taconic, NIH and the SHR-SP derivations were placed on a high salt (HS) diet containing 8% NaCl when they were 37 days old. The HS diet produced a significant increase in systolic blood pressure in all groups compared to low salt controls. Mortality or debilitation resulted in a reduction of the N in some groups. If this was greater than 2/6 or 33%, the experiment was terminated. (From Blizard, Peterson and Adams[5]).

collection of baseline data and dietary assignment, SBP and BWT are measured weekly. Brains, hearts, and kidneys are variously sampled at death or sacrifice.

This dietary challenge results in large increments in BP within 2–6 weeks, is accompanied after 2–4 weeks by failure to gain body weight (BWT), subsequently BWT loss, and culminates in pathological changes in the kidney and brain. As might be anticipated, animals frequently succumb to the adverse effects of the pathophysiological lesions. None of the preceding changes are seen in SHRs maintained on a low salt (LS) laboratory chow containing approximately 0.3% NaCl. A detailed description of the pathophysiological consequences of the HS diet is provided below.

Effects of high salt diet on BP and pathophysiology in SHR

BP increases in HS rats. Our studies were initially undertaken with SHRs obtained from the colony maintained by Harlan Industries (Indianapolis, IN). Later, because of interest in the possibility of sub-line differentiation among the various SHR stocks[24], the same paradigm was applied to other commercial and non-commercial SHR colonies. Figure 1 shows that male and female SHRs from several major commercial suppliers of SHR and from the non-commercial NIH SHR-N and SHR-SP (stroke-prone SHR) colonies, exhibited significant increases in SBP within 3–5 weeks of exposure to the HS diet[5]. Males were influenced more quickly than females in SHRs from most colonies, but females usually reached the same very high BP (220–240 mm Hg) at later sampling points. The numbers of rats tested from each supplier were relatively small, but replications with Harlan and the Charles River SHRs suggested that the week-by-week changes in SBP induced by the HS diet were highly reliable[5].

Weight loss. Initially, BWT gain of SHRs exposed to the HS diet is similar to that seen in LS controls. However, within a few weeks of exposure to the HS diet, BWT fails to increase as much as that seen in LS controls, and, subsequently, BWT loss is observed. In males, failure to gain BWT occurs after approximately 2–3 weeks exposure to the HS diet, and BWT loss occurs shortly thereafter; females exhibit the same syndrome, but at a slower rate. Figure 2 displays the pattern of BWT gain/loss in SHRs from the different colonies of the SHR.

Mortality and its association with SBP. As previously noted, mortality as a result of maintenance on the HS diet is significant, and is associated both with elevations in SBP and BWT loss. As an example, in the study with Harlan SHRs described above, males reached approximately 240 mm Hg after 2–3 weeks on the HS diet, and 50% mortality occurred after 22 days exposure. In females, the same level of BP was reached within 4–5 weeks and 50% mortality occurred at 6 weeks. Thus, gender-dependent patterns of BP-increase on the HS diet were closely correlated with gender-dependent patterns of mortality. In addition, the relationship between elevat-

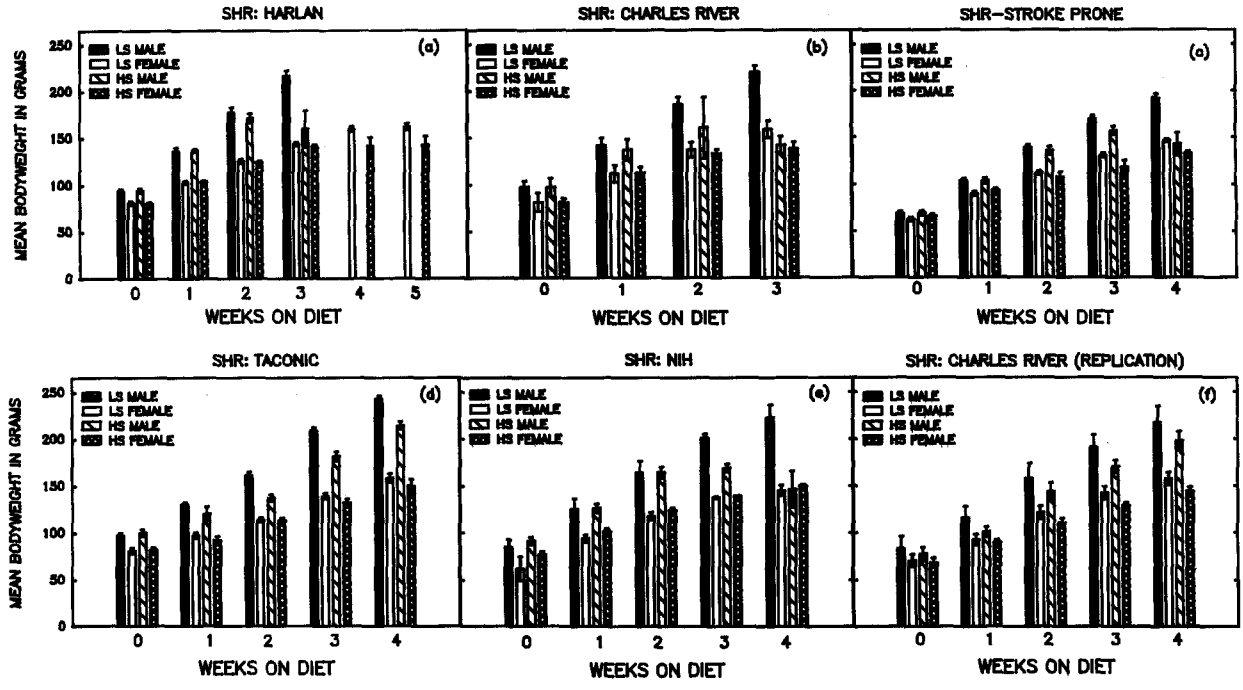

Figure 2. Mean body weights + / − SEM of male and female SHR-SPs and SHRs from Taconic, Charles River, and Harlan colonies maintained on a low salt (LS) diet containing 0.3% NaCl, or on a high salt diet (HS) containing 8% NaCl from 37 days of age. Beginning at about the 2nd or 3rd week after placement on the HS diet, there was an attenuation of the normally-occurring increase in body weight. In later weeks, as blood pressure increased to very high levels, body weight decreased. As with blood pressure, body weight changes due to the HS diet were seen sooner in males than females. (From Blizard, Peterson and Adams[5]).

Table 1. Effects of a high salt (8% NaCl) diet (HS) on systolic blood pressure (SBP) in mm Hg, and body weight (BWT) in Harlan SHR rats, and the change Δ (+ / − SE) in SBP and BWT from low salt control group means[1]

	Weeks on the high sodium diet									
	1		2		3		4		5	
Females	SBP	Δ	SBP	Δ	SBP	Δ	SBP	Δ	SBP	Δ
All rats	149	12	186	44	214	61	239	65	267	95
Subgroup dying[2]	157	19	191	49	228	75	250	76	285	113
Subgroup surviving	141	3	182	40	201	48	228	54	248	76
	BWT	Δ	BWT	Δ	BWT	Δ	BWT	Δ	BWT	Δ
All rats	104	2	125	− 1	141	− 3	142	− 18	143	− 19
Subgroup dying	103	0	125	− 1	140	− 3	139	− 21	131	− 31
Subgroup surviving	106	3	124	− 2	142	− 2	145	− 15	155	− 7
Males	SBP	Δ	SBP	Δ	SBP	Δ	SBP	Δ	SBP	Δ
All rats	171	30	239	86	246	66	N/A	N/A	N/A	N/A
Subgroup dying[3]	168	25	240	87	207	27	N/A	N/A	N/A	N/A
Subgroup surviving	178	35	238	85	271	91	N/A	N/A	N/A	N/A
	BWT	Δ	BWT	Δ	BWT	Δ	BWT	Δ	BWT	Δ
All rats	137	0	172	− 6	160	− 57	N/A	N/A	N/A	N/A
Subgroup dying	136	− 1	172	− 6	160	− 57	N/A	N/A	N/A	N/A
Subgroup surviving	138	1	172	− 6	160	− 57	N/A	N/A	N/A	N/A

[1] Refer to figure 1 for week 0 (baseline) SBP means. The data are a subset from Blizard, Peterson, and Adams[5].
[2] 50% of the females died at a mean of 39 days on the HS diet; the remaining were sacrificed following 44 days on the HS diet.
[3] 50% of the males died at a mean of 22 days on the HS diet; the mean SBP for week 3 is based on two males resulting in an asymmetry between means for All rats vs the two subgroups. The remaining males were sacrificed following 23 days on the HS diet; thus, data are not available (N/A) for males during the fourth and fifth sampling points.

ed BP and mortality was also seen within groups. Table 1 shows an association between SBP and mortality in the Harlan SHR females (n = 6) that had been placed on the HS diet at 37 days of age: SBP of the three females which died after 5–6 weeks on the HS diet was considerably higher than the three females which survived past 6 weeks. The magnitude of BP-elevations shortly after exposure to the HS diet were also predictive of subsequent mortality. Females which eventually died after approximately 5–6 weeks on the HS diet had SBPs of nearly

20 mm Hg above LS controls after 1 week on the HS diet, whereas HS females surviving beyond 6 weeks showed little increase (3 mm Hg) above LS controls at this point in the experiment.

Association of body weight and mortality. The pattern of BWT loss on the HS diet was also correlated with mortality in female SHRs in the Harlan experiment. BWT of HS females did not differ noticeably from LS controls in the first 4–5 weeks of exposure to the HS diet; thereafter, BWT of HS females which died did not keep pace with that of LS females, whereas BWT of surviving HS females matched the BWT of LS females (table 1).

Stroke. Hemorrhagic stroke was an important cause of death in SHRs on the HS diet. In the study of Harlan SHRs, neurologically confirmed hemorrhagic stroke was seen in 33% of rats maintained on the HS diet. Bleeding was telencephalic in location (see fig. 3), and, in at least one case, the damage was bilateral. Stroke was accompanied by several physical and behavioral characteristics such as a wobbly gait and microhemorrhages around the eyes during the early stages. Subsequently, retardation or loss of the righting reflex occurred and rats' coats became increasingly matted and spiky. In the terminal stages we observed seizure activity, loss of vibrissae movements and loss of pain response. A more detailed description of the kind of behavioral changes seen in these animals has been provided elsewhere [3]. No behavioral or neurological evidence of stroke was observed in any rats maintained on the LS diet.

Cardiac hypertrophy. As might be expected of animals experiencing major elevations in BP, Harlan SHRs exposed to the HS diet had significantly larger hearts ($\bar{X} = 1.05$ g and 0.8 g in males and females respectively) than SHRs remaining on the LS diet ($\bar{X} = 0.81$ g and 0.68 g). The differences between the HS and LS groups were even greater when heart weight was corrected for BWT. These differences were measured after 3 weeks of exposure to the HS diet in males, and after 6 weeks exposure in females, that is, when the increase in BP had reached excessive levels in the two groups.

Renal pathology. A sampling of kidneys obtained from approximately half of the HS rats were studied by Dr Samy S. Iskandar of the Department of Pathology of Bowman Gray School of Medicine, and showed clear signs of vascular and glomerular lesions, whereas no lesions were present in a sample of LS rats. The lesions varied in severity from focal fibrinoid necrosis with marked disruption of the arterial wall, thrombosis, and granulomatous arteritis (fig. 4, a and b). Glomerular lesions were also noted, consisting of mesangial hypercellularity, and focal glomerulosclerosis of both segmental and global distribution. In rats with pronounced vascular lesions, intraglomerular thrombosis was also present.

Association between renal pathology and high BP. The relationship between elevated BP and kidney pathology was examined in another study of Harlan SHRs in which rats were placed on an HS diet and sacrificed when SBP had reached moderate (170 mm Hg), or excessive levels of SBP (225 mm Hg)[6]. Confirming the pronounced gender difference in BP response to the HS diet seen in earlier experiments, figure 5 shows that males reached both criteria approximately twice as fast as females. 24 h before sacrifice, in collaboration with Dr Z. K. Shihabi of the Department of Pathology of Bowman Gray School of Medicine, we measured urinary protein excretion (UPE) in these rats, and kidneys were examined for the patterns of pathology described above[6]. Table 2

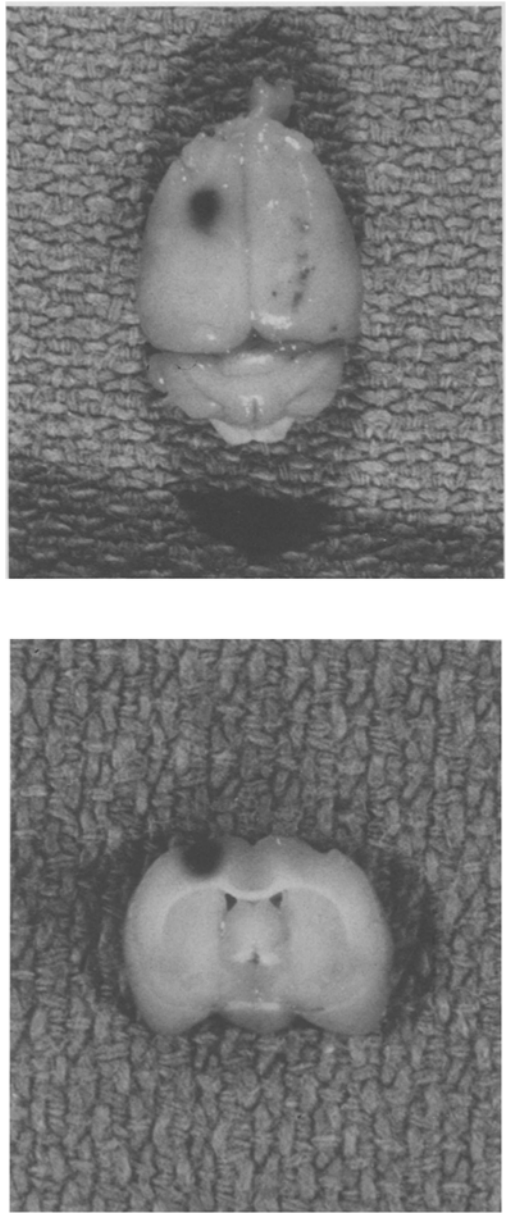

Figure 3. Example of hemorrhagic stroke in a SHR male rat. Damage was always telencephalic and usually unilateral. a) The top panel shows the external appearance of the hemorrhage in the left hemisphere. b) The bottom panel shows the same lesion from a coronal view. (From a subject in Blizard, Peterson and Adams [5]).

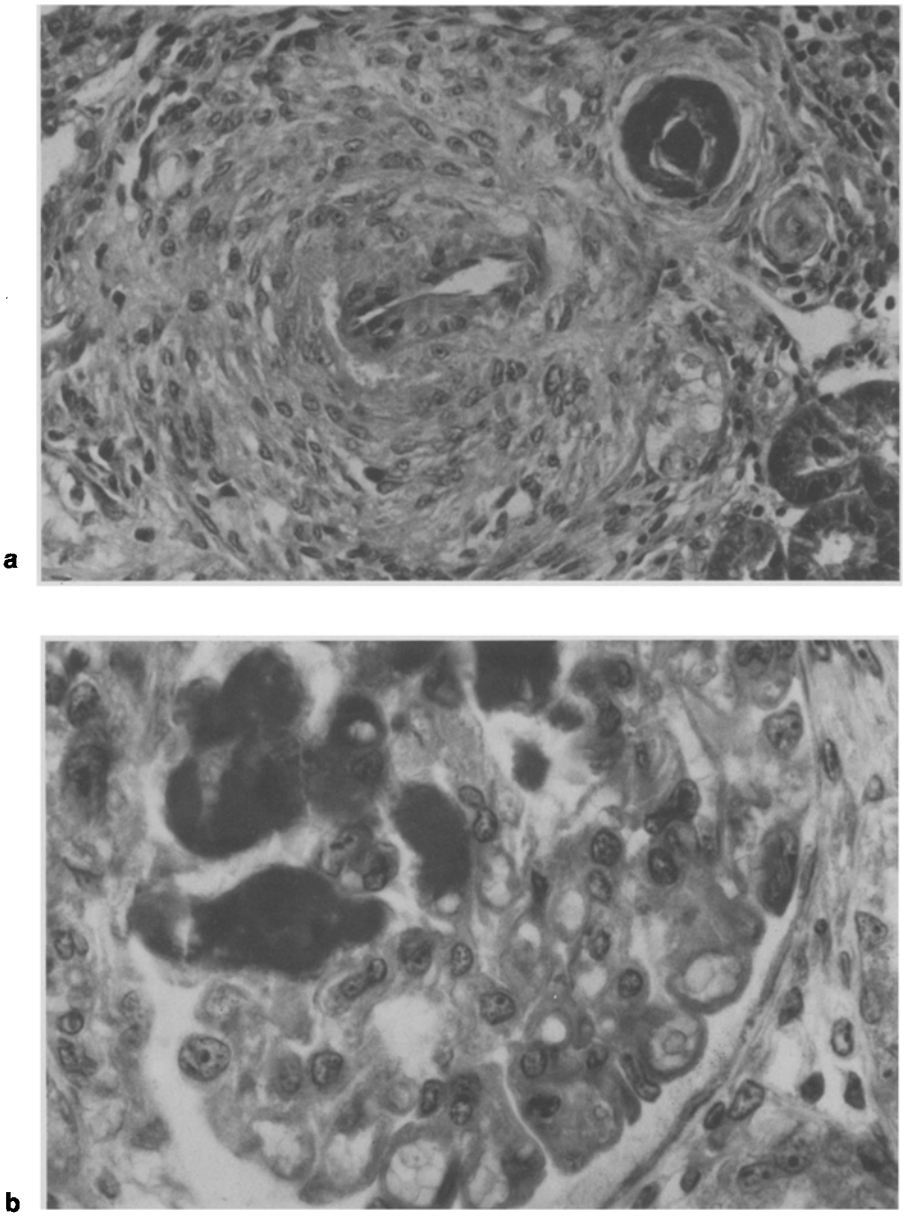

Figure 4. Examples of renal lesions. a) The top panel illustrates fibrinoid necrosis in an arteriole (right), and the adjacent angiocentric granulomatous reaction (Verhoff trichrome, ×156). b) The bottom panel shows a glomerulus with moderate mesangial expansion and focal thrombosis (Verhoff-trichrome, × 500). (From Blizard, Peterson, Iskandar, Shihabi and Adams[6]).

Table 2. 24-hour urinary protein excretion and renal vascular pathology in Harlan SHR rats maintained on low (LS) or high (HS) salt diets[1]

	Males		Females	
	Urinary protein (mg/24 h)	Renal pathology (severity)	Urinary protein (mg/24 h)	Renal pathology (severity)[2]
LS group	1.05 +/− 0.17	0	1.83 +/− 0.47	0
HS (moderate BP)	12.53 +/− 1.77	0.83	3.74 +/− 0.26	0.2
HS (excessive BP)	69.62 +/− 6.73	2.9	24.9 +/− 6.57	2.0

[1] Data reprinted from Blizard, Peterson, Iskandar, Shihabi and Adams[6].
[2] Renal vascular pathology was rated on a scale from 0 to 4.

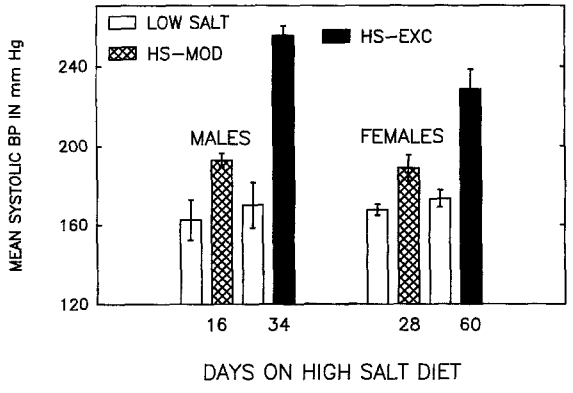

Figure 5. Mean systolic blood pressure in male and female Harlan SHRs after placement on a high salt diet on day 37. HS-MOD: rats sacrificed after moderate BP increases; HS-EXC: rats sacrificed after excessive BP-increases; LS = low salt controls. (From Blizard, Peterson, Iskandar, Shihabi and Adams[6]).

shows that UPE and renal pathology were significantly greater in HS rats than control LS rats, and both were significantly higher in HS rats which had excessive SBP than in HS rats which had moderate increases in SBP. Again, gender differences were a prominent feature of the findings: males had markedly higher UPE and more severe pathology than females.

The higher UPE and renal pathology did not appear to be a simple consequence of elevated SBP. Although there was an association between SBP and renal pathology across groups, there were no significant correlations between SBP and UPE levels or between SBP and renal pathology within groups. In addition, males had more severe renal pathology and greater UPE than females in HS groups despite the fact that observations on males and females were made when BP increments due to the HS diet were similar.

High salt diet in the SHR: Pathophysiological overview. These results show that several of the prominent sequelae of elevated BP in humans can be produced in SHR rats by placing them on an HS diet in early life. Although detailed pathophysiological studies of stroke and renal damage were limited to Harlan SHRs, the same general trends of BP-elevation, BWT loss, neurological symptoms, etc., were observed in SHRs and SHR-SPs from all colonies. Thus, it is likely that our results with the Harlan SHR indicate the general pathophysiological consequences of an HS diet for the SHR, rather than a specific susceptibility of the Harlan SHR colony. The protocol we used has the principal advantage that it produces several of the pathophysiological consequences of elevated BP within a short period of time. This permits the relevant organ systems to be individually studied, but also allows the interrelationships between the various processes, elevated BP, renal pathology, stroke, etc., to be assessed. The application of this protocol to studying the effects of maternal environment on pathophysiological processes will now be described.

Maternal studies

Reciprocal cross methodology for studying maternal influences

The maternal environment was studied by means of the reciprocal F_1 methodology[1,3]. In a reciprocal cross a female of one inbred strain is mated with the male of the other strain, and vice versa. The two groups of F_1 offspring resulting from reciprocal crosses are genetically identical except for males who differ with regard to the derivation of their X and Y chromosomes. Because the two reciprocal F_1 groups develop in different pre-natal and post-natal environments, maternal factors are likely contributors to any differences that appear between them (fig. 6). The reciprocal cross design was thoroughly discussed by Broadhurst in his efforts to show that maternal effects did not account for behavioral differences between the Maudsley strains[7], and has been recently reviewed by Blizard[2]. Compared to cross-fostering, one advantage of the reciprocal cross design is that no direct manipulation of the pups or the maternal environment is required. A potential disadvantage is that the pre- and post-natal maternal influences are confounded. However, this confound can be overcome if one includes a cross-fostering manipulation to separate these possibilities[15]. Although it is primarily intended for investigating maternal influences, the reciprocal cross design is also useful in looking for X-linked characteristics[2].

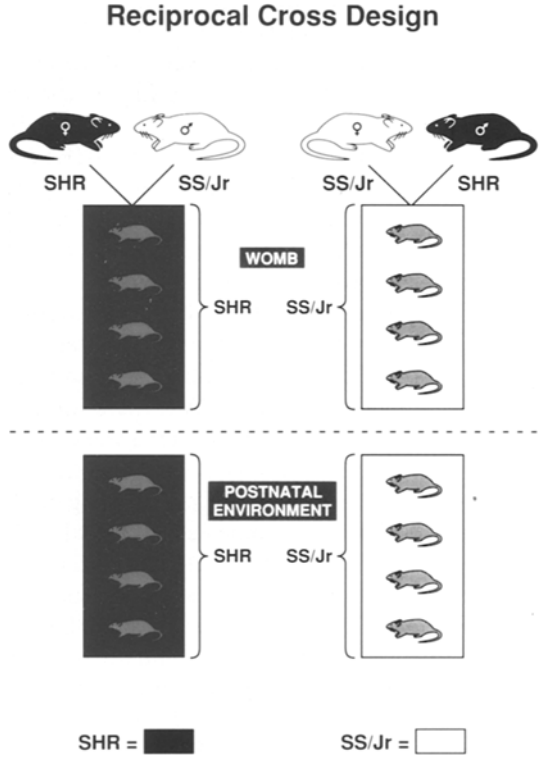

Figure 6. The reciprocal cross design used for the study of pre- and post-natal maternal influences in SHR × SS/Jr F_1s. F_1s nurtured pre- and post-natally by SHR mothers were designated F_1-H. F_1s nurtured pre- and post-natally by SS/Jr mothers were designated F_1-J. (Adapted from J. A. Gray, The Psychology of Fear and Stress, Cambridge 1987).

Reciprocal cross of SHR and SS/Jr strains

Typically, crosses are made between two strains which are the 'opposite' products of directional selection; that is, between SHR and and its normotensive control, the WKY, and between SS/Jr and its normotensive control, the SR/Jr, and so on. The usual purpose is to discover how much of the phenotypic expression of a given trait can be attributed to differences in the maternal environments provided by the two strains. However, in our cross we examined the genetic and maternal contributions to hypertension by crossing two strains which both had high BP. We reasoned that we would be more likely to see pathophysiology in reciprocal F_1s if their BP was in the hypertensive range. Crosses of a hypertensive strain with its normotensive control might not result in appropriate BP levels for the pathophysiological effects of an HS diet to be demonstrated. As depicted in figure 6, we crossed SHR males with SS/Jr females to obtain F_1s raised by SS/Jr mothers (designated F_1-Js), and SS/Jr males with SHR females to obtain F_1s raised by SHR mothers (designated F_1-Hs).

As with our previous studies in SHR, half of the rats within litters of each reciprocal cross remained on the LS diet on which they were reared (controls), the other half were allocated to receive the HS diet beginning between 35 and 37 days. After baseline SBP determinations, the SBP and BWT measures were obtained on a weekly schedule via the indirect tail-cuff methodology, and were supplemented by direct measures of mean aterial pressure from conscious animals in adult life. The surgical procedure used to implant the cannula and the computerized monitoring procedure are described elsewhere [1].

High salt diet, BP and pathophysiology in F_1 hybrids: An overview. In general, F_1 hybrids of the SHR × SS/Jr cross exposed to the HS diet exhibited the same progression of symptoms previously found to occur in the inbred SHRs. Significant increases in BP relative to LS controls occurred in all reciprocal F_1 X gender groupings after 3 weeks on the HS diet ($p < 0.01$). In most groups, rats weighed significantly less than LS controls after 4 weeks of exposure to the HS diet. In addition to the large increases in BP and alterations in BWT, there was a 45% level of mortality across all groups after 8 weeks of exposure to the HS diet. Stroke was a major contributor to mortality with a 36% incidence of stroke being observed across all groups during the same period. Renal pathology, similar to that seen in inbred SHRs exposed to the HS diet was also present in 75% of the kidney samples available from HS rats. These results clearly show that the F_1 hybrid derived from a cross of SHR and SS/Jr strains in an excellent model for pathophysiological studies.

Maternal influence on increments in BP due to a high salt diet. Figure 7 shows that female F_1-Hs (F_1s reared by SHR dams) exhibited greater absolute SBP following 3 weeks of exposure to HS diet (182 mm Hg vs 150 mm Hg, $p < 0.01$) than F_1-J females (reared by SS/Jr dams). This was clearly the result of exposure to the HS diet because

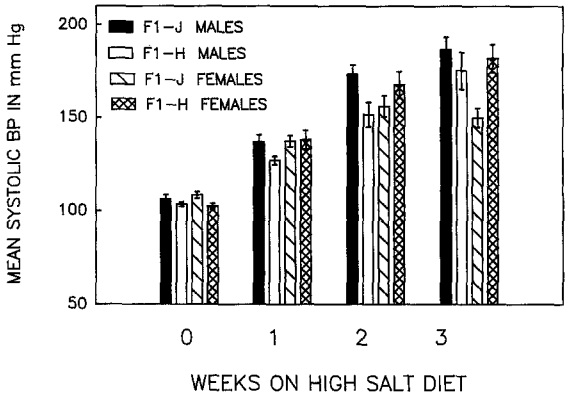

Figure 7. Mean systolic blood pressure in two reciprocal F_1 hybrids when exposed to a high salt (HS) diet (8% NaCl) at 37 days of age. At baseline, there were no differences among reciprocal F_1s, but F_1-H females exhibited a significantly higher SBP after 3 weeks on the HS diet than F_1-J females. Differences between the male reciprocal F_1s were not statistically significant. (Data are reclassified from Adams and Blizard [1]).

there was no difference in SBP of reciprocal F_1 females raised on the LS diet. (This latter comparison, obtained with the indirect tail-cuff procedure, was later confirmed with direct arterial recordings[1]). Although HS F_1-J females exhibited a slower rise in SBP than F_1-H females, F_1-J females ultimately exhibited excessive SBP (X = 199 mm Hg) after 10 weeks on the HS diet.

Males in the two reciprocal F_1 groupings had similar BP when maintained on the LS diet throughout the experiment, and, although both reciprocal F_1s showed an increase in BP following placement on the HS diet, the magnitude of the increase did not differ between them.

Maternal influences on body weight loss due to a high salt diet. F_1-H females exposed to the HS diet exhibited a slower increase in BWT than HS F_1-J females such that the former group did not gain BWT between the 2nd and 4th weeks of exposure to the HS diet whereas F_1-Js did. Figure 8 shows that 4 weeks of exposure to the HS diet resulted in BWT of F_1-Hs being 27% lower than their LS controls, whereas there was no deficit in HS F_1-J females relative to their LS controls. BWT of F_1 males on the HS diet was lower than that of LS controls, but there was similar decrement in BWT in the reciprocal F_1s.

Maternal influence on incidence of hemorrhagic stroke. As previously noted, 45% of animals across all HS groups died within 8 weeks of placement on the HS diet. Stroke was associated with early death; 81% of the rats dying within the first 8 weeks of exposure to the HS diet showed evidence of stroke whereas only 15% of those dying after that time had hemorrhagic stroke [3].

Exposure to the SHR maternal environment resulted in a higher incidence of hemorrhagic stroke. Across reciprocal F_1 groupings in the first 8 weeks of exposure to HS, of the 13 rats which died suffered hemorrhagic stroke, 9 were F_1-H, and only 4 were F_1-Js. Overall, 9/17 F_1-H rats were demonstrated to have suffered cerebral hemorrhage (53%), whereas this pathology was only seen in

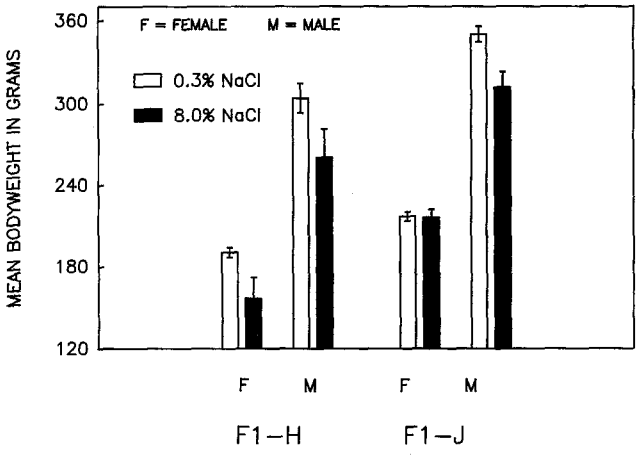

Figure 8. Maternal influence on the effect of high salt diet on body weight (BWT) at 65 days of age (4 weeks' exposure to HS diet) in F_1s. F_1-Hs weighed less than F_1-Js at 35 days of age before placement on the high salt diet as well depicted here at 65 days. BWT of reciprocal F_1 females significantly differed in response to exposure to HS: F_1-Hs weighed 27% less than their LS controls whereas F_1-J females did not differ across diet groups. Reciprocal F_1 males exposed to the HS diet showed the same pattern of weight gain relative to their respective LS controls. (Data are reclassified from Adams and Blizard [1]).

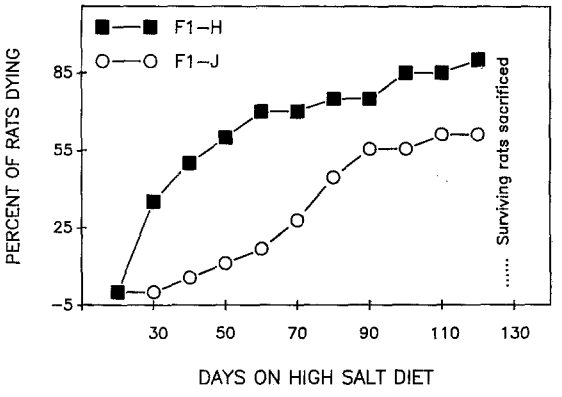

Figure 9. Plot of incidence of mortality in the reciprocal F_1 groupings as a function of days on the 8% NaCl diet. Combining data across males and females within these groupings, F_1-H rats (nurtured from conception to weaning by spontaneously hypertensive mothers) succumbed earlier to the adverse effects of the HS diet than F_1-Js (raised by Dahl salt-sensitive mothers). (Data from Blizard, Challa, Iskandar, El-Tamawy and Adams [3]).

4/19 (24%) of F_1-Js ($p < 0.05$). The above is likely an underestimate of the differences between the two groups because the two earliest deaths (after just 20 and 21 days on the HS diet) in F_1-H females were not detected soon enough to collect the brains.

Rats which died of stroke had a mean of 226 mm Hg compared to a mean of 200 mm Hg for rats not exhibiting signs of cerebral hemorrhage. However, as previously noted, because F_1-J females eventually reached SBP levels as high as F_1-Hs but had a lower incidence of stroke, it appeared that the absolute level of BP per se was not the only contributor to the differences in stroke observed between F_1-H and F_1-J females.

Maternal influences on mortality. Figure 9 shows the results of the maternal influences on pathophysiology as it affected mortality of the reciprocal hybrids. Overall, F_1-Hs on the HS diet lived for a significantly shorter time than F_1-Js surviving a mean of 52 days vs 80 days exposure to the HS diet. Thus, increased BP (in females), decreased BWT, and occurrence of hermorrhagic stroke were predictive of earlier mortality in the F_1-H rats.

Gender-specific patterns of mortality differed in the two F_1s. Female F_1-H HS rats were quicker to die than male F_1-Hs; after 8 weeks only 2 of 10 females were living whereas 5 of 10 males had survived. In F_1-Js on the HS diet most rats of both sexes survived to 8 weeks (78% for both, 7 of 9), but soon after that time male F_1-Js began to die more quickly than females. After 12 weeks on the HS diet only 2 of 9 males were living, whereas 6 of 9 F_1-J females survived until all rats were sacrificed after some 120 days on the HS diet.

Renal pathology. Renal samples were obtained for histological processing [6] from rats (n = 20) which were sacrificed when death was imminent, at the end of the study, or from carefully monitored rats which had died recently. (Histological procedures necessitated securing tissues shortly after death). In 15 cases severe renal damage was present similar to the type previously described in studies with the SHR. The mean BP of rats with kidney damage was 228.5 mm Hg whereas it was 190.8 for the 5 rats free of kidney damage. The presence of kidney damage was a necessary, but not sufficient, indicator of stroke. In the 7/15 cases where stroke was observed, and renal tissue was also collected, all rats had moderate to severe vascular lesions. However, renal damage was seen independently of stroke in 8 cases. The association between higher BP and higher incidence of stroke in F_1-Hs, and the relationship between these two variables and renal damage suggests that maternal environment might also impact on renal pathology. However, because of the sampling restrictions in relation to the renal tissues mentioned earlier, we had too few rats from the respective groups to show a direct association between maternal environment and renal pathology.

Pre-experimental BWT as a predictor of pathophysiology. Initial BWT, prior to assignment to the HS diet, was associated with susceptibility to pathophysiological changes in response to HS. Table 3 shows that within HS groups there was a tendency for rats which died earlier to weigh slightly less at baseline (35–37 days) and have greater increases in BP after 2 weeks. BWT at 35 days of age was also predictive of incidence of stroke: in three of the four reciprocal cross X sex groupings, rats which suffered stroke were on average 20 g lighter at 35 days, than those rats not exhibiting evidence of stroke. However, in the fourth group (F_1-J males) the opposite pattern was discovered: two of the heaviest males were victims of stroke. The effects of the maternal environment on BWT were long lasting; F_1-H rats which were continously maintained on the LS diet continued to have significantly lower BWT than F_1-Js until at least 150 days of age.

Table 3. Association of rate of rise in blood pressure in mmHg, stroke, and 35-day body weight (BWT) in g in reciprocal F hybrids raised by SHR mothers (F_1-H) or SS/Jr mothers (F_1-J)[1]

	F_1-H Early deaths	Survivors	F_1-J Early deaths	Survivors
Male rats				
N	5	5	5	4
X BP rise in 2 weeks +/− SEM	66.7 (15.7)	35.5 (4.9)	76.8 (7.1)	55.0 (7.8)
Median days on HS diet until death	32	120	60	85
% stroke	80%	0%	60%	25%
X BWT at 35 days +/− SEM	105.8 (7.4)	129.8 (9.2)	151.6 (7.9)	154.2 (9.6)
Female rats				
N	5	5	3	6
X BP rise in 2 weeks +/− SEM	87[2] (7.2)	54.8 (9.7)	58.3 (5.4)	43.0 (8.8)
Median days on HS diet until death	26	58	56	120
% stroke	100%[3]	40%	66%	16%
X BWT at 35 days +/− SEM	87.6 (3.2)	101.4 (6.7)	118.7 (8.4)	129.3 (7.7)

[1] Early death animals were the first half of each group to die, survivors were the latter half to die or among those sacrificed after 120 days on the HS diet. Only 3 F_1-J females died spontaneously before sacrifice. Data are reclassified from Adams and Blizard, in press[1], and Blizard, Challa, Iskandar, El-Tamawy and Adams, 1990[3].
[2] Significantly different from F_1-J female groups, $p < 0.05$.
[3] Only 3 brains of the 5 rats in this group were available for analysis.

Unfortunately, dividing the F_1 X sex groupings into subgroups in the manner depicted in table 3, resulted in too few subjects for statistical significance except as noted in the table. However, the consistency of the relationship between 35-day BWT, and pathophysiological susceptibility within the HS groups is compelling from a clinical perspective.

The association between pre-experimental BWT and HS-induced pathophysiology was also consistent across groups. F_1-H females had the lowest BWT at 35–37 days, the highest increment in BP during the first 2 weeks exposure to the HS diet, the highest percentage of stroke incidence, and the greatest mortality rate whereas the F_1-J females had the lowest values on most of these characteristics.

Discussion

As noted earlier, McCarty and Myers and their respective associates have shown that the post-natal environments of SHR and SS/Jr hypertensive rats strains contribute to the level of BP reached in adult life. If genetically hypertensive pups are raised by the relevant control mothers (WKY in the case of SHR; SR/Jr in the case of SS/Jr) a lower level of BP is reached in adult life than if the pups are raised by a mother from their own strain. Our results extend the relevance of the maternal environment to the pathophysiological consequences of elevated BP associated with exposure to a high salt diet. Our findings demonstrated that exposure to the SHR maternal environment (as compared to the SS/Jr maternal environment) resulted in major differences in pathophysiology among hybrid offspring. When exposed to the HS diet, F_1-H animals which were raised by SHR mothers exhibited a greater incidence of stroke and a higher rate of mortality than F_1-Js (raised by SS/Jr mothers). This effect was more pronounced in females, where F_1-Hs also had swifter elevations in BP and greater BWT loss than F_1-Js following exposure to the HS diet.

McCarty and Myers implicated the post-natal maternal environment as the mediator in the expression of BP in adult life. As previously noted, our reciprocal cross design does not distinguish between the contributions of pre- and post-natal maternal environments. Thus, we are not able to specify which phase of maternal life modifies the pathophysiological effects on the HS diet. Because of the results reported elsewhere in this series, it would appear that the post-natal maternal environment is a likely contributor to adult BP. This is also supported by the findings of Dene and Rapp who found no evidence of pre-natal factors influencing adult BP as a result of intrauterine transfers between the SS/Jr and SR/Jr strains[11]. Nevertheless, pre-natal factors cannot be ruled out. There is evidence indicating the influence of pre-na-

tal factors on a variety of adult behaviors in laboratory rodents[27]. Thus, it will be important, in future research, to identify which of these two developmental phases is responsible for maternal influences on pathophysiology, or, indeed, if both are.

Body weight as a correlate of pathophysiological risk

Between-group differences in BWT and pathophysiology. We observed that maternal environment produced correlated variations in BWT and pathophysiology. When raised on the LS diet, F_1-H rats (reared by SHR dams) were significantly lighter at 35–37 days of age than their F_1-J counterparts. This difference between reciprocal F_1 groupings persisted throughout the study in LS controls. The differences in pre-experimental BWT between reciprocal F_1s were associated with the BP-elevating and pathophysiological consequences of the HS diet. F_1-H rats suffered greater BP elevation in 2–3 weeks (in females) and were more likely to suffer stroke and die when placed on the HS diet.

Within-group differences in BWT and pathophysiology. Individual differences in 35-day BWT within the reciprocal F_1 X sex groupings were also associated with risk for pathophysiology. As noted, *within* three of the above groupings there was a tendency for rats with lower pre-experimental BWT to die sooner and to have suffered hemorrhagic stroke. These results are consistent with a study by Dene and Rapp in which lower BWT at weaning was also found to be associated with a higher rate of mortality in SS/Jr rats exposed to an HS diet[12]. In their experiment, variations in BWT were produced by manipulating litter size. This suggests that post-natal environmental influences may be partly responsible for the association between BWT and mortality, but does not rule out a potential contribution of pre-natal maternal influence on BWT.

Role of elevated blood pressure in pathophysiology

The maternal effect on BWT loss, and pathophysiology in F_1 females appeared to be associated with the rate of rise, rather than the absolute level of BP. F_1-J females eventually reached similar BP levels to F_1-Hs, but did not experience the same level of mortality and pathophysiology. In an earlier study with SHRs, we also observed that, rather than being correlated with absolute BP, differences between male and female SHRs in the degree of urinary protein excretion and renal pathology were associated with the rate of rise of BP in these groups[6]. It would appear that a rapid increase in BP in an *immature* animal may be a particularly important feature underlying the renal and cerebrovascular damage observed in these studies.

Maternal X F_1 genotype interaction

What is it about the SHR maternal environment that has such deleterious effects when hybrid offspring are challenged by HS diet? One possibility may be that the SHR mothers are unable to provide F_1s with the nutritional input needed to cope with the HS challenge. Such an hypothesis needs to be carefully calibrated in light of the fact that SHR dams can provide appropriate levels of nutrition for SHR pups to cope with this same challenge; that is, in comparisons between F_1-H pups (reared by SHR dams) and SHR pups reared by their own mothers, the F_1 pups reared by SHR dams suffered more severe pathophysiology and mortality than inbred SHR pups with SHR dams[1]. This raises the possibility that we are not observing a maternal effect that is observed in all kinds of mother/litter interactions, but rather a maternal X pup genotype interaction. As demonstrated by Myers and colleagues, the phenotype of pups may determine the nature of the maternal response. In their example, SHR × WKY F_1 pups induced SHR-like maternal behavior from WKY dams[20]. Thus, it is possible that in our study SHR × SS/Jr F_1 pups presented stimulation which resulted in different maternal responses in SS/Jr and SHR dams. Whatever its nature, it resulted in differences in 35-day BWT, and this was associated with other pathophysiological effects of the HS diet. It would be useful to compare maternal behavior of SHR and SS/Jr dams when nursing F_1 hybrids versus inbred animals in future studies.

Typically, the reciprocal F_1 methodology is used to evaluate the contribution that the maternal environment of two inbred strains makes to the phenotypic expression of a specific character. Along these lines, the research of McCarty suggests that the maternal behaviors idiosyncratic to each hypertensive strain makes a substantial contribution to phenotypic expression of BP in adult life. Our results, on the other hand, do not fit into this category of maternal influences. There were no differences between the effects of the two maternal environments on BP when animals were raised on the LS diet. In addition, as discussed above, the deleterious effects of the SHR maternal environment on the pathophysiological effects of the HS diet on F_1 females did not mimick the effects of HS in SHR females. Our results show that the different maternal environments of SHR and SS/Jr mothers respond quite differently to the stimulus of hybrid offspring. Thus, there is clearly another class of maternal influences to consider other than those that are usually explored. Using inbed mice, the research of Carlier, Roubertoux and associates, has provided several examples of maternal effects on the one hand, and pup effects on the other in a variety of developmental parameters[8]. The experimental paradigms and approaches that they have used provide the hypertension field with some innovative strategies for studying mother/pup interaction effects.

Sex differences. Males in the two reciprocal crosses differ in the origin of their X and Y chromosomes; F_1-H males receive their X from SHR and Y from SS/Jr, and vice versa for F_1-males. Thus, when male F_1s differ and female F_1s do not, the reciprocal cross design permits identification of X-linked characters. Interpretation of our results are complicated by a sex X maternal environment

interaction indicating that reciprocal F_1 males differed *less* in their pathophysiological response to the HS diet than reciprocal F_1 females. Moreover, the degree to which males differed was in the opposite direction of the female difference. That is, F_1-H males survived longer than F_1-H females when exposed to HS. In F_1-J rats, most females continued to maintain their health beyond 12 weeks on the HS diet whereas most F_1-J males died. One interpretation of this gender difference is that F_1 males and females may stimulate differential treatment by the SHR vs SS/Jr mothers in a manner which influences their response to the physiological challenge of HS diet. Alternatively, recent studies have attempted to account for sex differences in SBP of SHRs via Y-chromosomal mediation of elevations in SBP observed in F_1s[14]; thus the possible influence of both X or Y chromosomal factors needs to be considered when explaining maternal influences and gender-dependence of maternal influences in reciprocal F_1s.

Conclusion

According to the results of our studies, being nurtured in utero or during the preweaning period by an SHR mother significantly increased the pathophysiological risk associated with maintenance on a HS diet during adolescence. Pathophysiology was seen in kidney and brain, but may have affected other major organ systems. The net effect of the various lesions was devastating, resulting in inability to maintain BWT, convulsant activity, and early death. The protocol we used was designed to produce pathophysiological lesions within a short period of time so that they could be conveniently studied. Whether the maternal effects, such as those demonstrated with this protocol, could also influence susceptibility to pathology over longer time periods when BP is rising more gradually needs to be examined. This is especially relevant when considering extrapolations of these findings to man, where many decades may intervene between mother/infant interactions and pathophysiological insult.

In our study, there is strong evidence that being raised by an SHR mother resulted in lower BWT of hybrid offspring at 35 days. Taken together with Myers' findings indicating a correlation between BWT gain between the 10th and 16th day of life and adult BP, there is a strong possibility that the maternal environment/pathophysiology connection, as well as the maternal environment/BP connection is importantly associated with the nutritional state of the animal. In our study, animals that had lower BWT at day 35 tended to exhibit greater increases in BP during the first two weeks of exposure to the HS diet, and were more susceptible to pathophysiological insult than their heavier mates. One possibility is that lower BWT renders animals less fit to cope with the adverse effects of the HS diet. A second is that lighter animals are at an earlier stage of development and that animals in a more immature physiological state may be less able to adapt to the HS diet.

Unpublished data from our laboratory provide some support for this interpretation in that Harlan SHRs which were started on the HS diet at approximately 100 days did not exhibit BP-increases as rapidly as the subjects of this experiment and did not suffer the same pathophysiological symptoms.

Our speculations about mechanisms of how maternal effects are mediated are necessarily very general in nature. Our various studies have unearthed important phenomena but are not yet sufficiently advanced to entertain specific mechanisms. Nevertheless, the next stage of research will need to consider the fact that maternal influences have their impact at a time when major neural, endocrinological, and physiological systems are undergoing important transitions. Some of the systems that may be altered by variations in the maternal environment are discussed by Kirby and Johnson in their contribution to this multi-author review series[16]. Future research would do well to assess the contributions of specific physiological systems to maternal effect on BP and pathophysiological effects of an HS diet.

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Regulation of sodium and body fluid homeostasis during development: Implications for the pathogenesis of hypertension

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Abstract. The spontaneously hypertensive rat (SHR) is an important animal model of human essential hypertension. During the first month of life, increased retention of sodium is present in the SHR which appears to be mediated by the renin-angiotensin system. The present review will discuss the role that increased activity of the renin-angiotensin system plays in sodium/body fluid regulation during early development. It is hypothesized that disordered regulation of sodium/body fluid homeostasis during this stage leads to pathological cardiovascular regulation in adulthood. Through an understanding of the relationship between sodium/body fluid balance in the young and cardiovascular function in the adult insights may be gained into both the pathological state of hypertension and the critical role played by early development in shaping homeostatic mechanisms in adulthood.
Key words. Renin-angiotensin; development; hypertension; SHR.

The present review will explore the role that activity of the renin-angiotensin system (RAS) during preweaning development may play in the etiology of high blood pressure in the spontaneously hypertensive rat (SHR). The period of early development has been shown to be a critical age for establishing set points of both humoral and neurotransmitter regulation in adulthood. Investigations by Ira Black and his associates on the development of the autonomic nervous system led to the proposal that preweaning development serves as a stage of 'modulation' in which activity of the system acts to determine adult function[2]. This hypothesis also appears to be appropriate for the influence of preweaning environmental variables upon adult cardiovascular homeostasis.

Manipulations limited to the preweaning period of development produce permanent reductions in arterial pressure in several animal models of high blood pressure, or hypertension. For example, rearing of genetically hypertensive rat pups by normotensive foster mothers results in 20–25% reductions in adult blood pressure[22]. Therefore, research on the role of developmental factors in hypertension is presently challenged to identify the physiological mechanisms that mediate permanent changes in arterial pressure. The results from such studies will first provide critical information on the pathological state of hypertension, and second, insights will be gained into the broader question of how early development can shape adult function.

Two systems that play critical roles in the maintenance of cardiovascular and body fluid homeostasis are the sympathetic nervous system and the renin-angiotensin system (RAS). In the adult animal, their influences have been well characterized and a wide variety of tools are available with which to selectively intervene in each system. This provides a valuable framework for investigations in the developing animal. In addition, previous studies[16, 17, 23, 24, 35, 37, 38] have demonstrated that the sympathetic nervous system and RAS begin to show adult-like characteristics of central neural control during the preweaning period of development. Thus, the role of