As discussed elsewhere in this issue, beginning with David Barker’s insight and original observations leading to the Fetal Origins of Adult Disease hypothesis (1), epidemiologic studies have strongly suggested that an adverse intrauterine environment may lead to adult hypertension (2–5). However, not all reports have been able to document such an association (6), and the epidemiologic studies have been criticized on the basis that it may be impossible to account for all confounding variables (7). Moreover, human epidemiology does not provide cause–effect relationships. Therefore, experimental models serve two purposes. First, they should determine unequivocally, by eliminating the variation in genetic background and other risk factors, whether prenatal factors are capable of programming adult hypertension (“proof of principle”) and, if so, whether the characteristics of the hypertension are consistent with the experience in humans. Second, they are the key to unraveling the mechanisms involved. In this review, we discuss studies on animal models of prenatally programmed hypertension as they pertain to these two objectives. In the process, we incorporate published studies selectively to serve the goals of this discussion, and we apologize to the many investigators in the field whose work is not quoted.

Is Adult Hypertension Programmable in Experimental Animals by the Prenatal Environment? (“Proof of Principle”)

Experimental Species

It has been shown convincingly that a variety of nutritional manipulations or other variations in prenatal environment during pregnancy can program later-life hypertension in sheep, rats, pigs, and guinea pigs (8–20). The largest body of information has been gathered from rats and sheep, two species with very different lengths of gestation, maturity state of the newborn, and adult characteristics, strengthening the argument that the programming is not species specific.

Nature of Prenatal Insult

Because the original human reports linked prenatally programmed hypertension to intrauterine growth restriction, most experimental protocols have aimed at duplicating the fetal growth impairment. The prenatal manipulations used can generally be divided into three categories: (1) Maternal nutritional deficiencies; these have entailed either restriction of total food intake (“global food restriction”) during all or part of pregnancy or restriction of a particular nutrient, usually protein, throughout or during a specific time window of pregnancy; (2) maternal glucocorticoid treatment; and (3) interference with placental function.

Maternal Diet.

Global Food Restriction. Woodall and colleagues (17,18) reported that allowing rats only 30% of a normal food intake throughout pregnancy programmed the offspring for hypertension. We have shown that an even more modest 20 to 30% reduction in maternal food intake during the latter part of pregnancy is effective in inducing hypertension in the offspring (21). In contrast, others have reported that a 50% reduction in food intake during the second half of pregnancy did not cause hypertension in female rat offspring (22); the reason for the discrepancy is not clear. In the sheep, a 15% reduction in maternal food intake during the first half of gestation caused an increase of approximately 10 mmHg in mean arterial pressure in the offspring at 80 to 85 d of age (23). Thus, global food restriction for all or part of gestation can lead to increased BP in the offspring, but it is not clear whether the important factor in these diets is the overall reduction in calories or the reduction in a specific nutrient.

Protein Restriction. Reduction of maternal protein intake while maintaining a normal total caloric intake has been used extensively in the rat. “Normal” dietary protein level has usually been set at approximately 19% protein (21% casein), and the restricted levels have ranged from 12% (“mild”) to 9% (“modest”) to 5% (6% casein, “severe”) of total caloric intake. All three levels of restriction during pregnancy have been re-
ported to increase offspring arterial pressure in adulthood, although the effects are generally greater with more severe restriction (12,16,19,20). In the pig, maternal protein reduction from the normal 14 to 0.5 to 1.0% resulted in a 10- to 25-mmHg increase in mean arterial pressure of the adult offspring (8). Furthermore, in most low-protein rat models, a normal protein diet has been provided after birth. This design may model the situation of a suboptimal fetal environment followed by an adequate postnatal environment. It may also model the phenomenon of catch-up growth (accelerated increase in body mass index in childhood), which in humans is predictive of adult hypertension independent of a reduced birth weight effect (24).

**Manipulation of Other Maternal Dietary Factors.** Only a few studies have considered the effects of other nutrients. In one study, a low-protein diet that contained more fat and starch led to increased systolic BP in offspring compared with its own control diet, whereas a low-protein diet that contained more sugar failed to program for hypertension compared with its own control diet (25). Thus, differences in the nutrient(s) (carbohydrate and/or fat) used to make up the protein deficit in the low-protein diet in different rat models can also confound interpretation of experimental findings. Recently, increased maternal dietary fat intake (26), high (27) or low sodium (28) intake, iron deficiency (29), and water deprivation (30) during pregnancy have been reported to program offspring hypertension. Thus, the picture is emerging that the hypertension-inducing capability is not unique to any specific maternal dietary manipulation. This is perhaps not surprising considering that a specific maternal nutrient deficiency may not result in a similar deficiency in the fetus, which is well equipped to “extract” nutrients even from a deprived mother.

**Maternal Glucocorticoid Treatment.** Because fetal programming associated with maternal undernutrition and intrauterine growth restriction (IUGR) has been proposed to involve fetal overexposure to maternal glucocorticoids, the effects of exogenous glucocorticoid administration to pregnant mothers have been studied by several investigators. Dodic and co-workers (11,31–33) reported extensively on a sheep model. Remarkably, in their hands, a dexamethasone or cortisol infusion for only 48 h between 22 and 29 d of gestation (term approximately 146 d) programs the offspring to become hypertensive. In the rat, we have shown that maternal dietary protein or calorie restriction programs the male and female offspring for hypertension when imposed either throughout or only during the last half of gestation but has no effect on offspring adult BP when given only during the first half of pregnancy (16,20,21). In contrast, Langley-Evans et al. (37) reported that a 9% casein diet programmed hypertension in male offspring whether given to pregnant rats during the first, second, or third trimester, although the largest effect was seen in offspring that were exposed throughout pregnancy. One study reported that feeding a 9% casein diet to pregnant rats for only the first 4.25 d of pregnancy (preimplantation period) was sufficient to program the male but not the female offspring for increased systolic BP (38). The reasons for the discrepancies between the studies are not clear. In any case, in our hands, exposure to maternal protein restriction must occur during the latter half of rat pregnancy to program the offspring for hypertension.

Relative to the length of gestation, the critical period in sheep seems to differ from that in the rat. A mild (15%) reduction in maternal food intake in the first half of sheep pregnancy leads to an elevated mean arterial pressure (by approximately 10 mmHg) in postnatal life (23). The experiments of Dodic et al. (11) using maternal glucocorticoid treatments have further narrowed the window of susceptibility in sheep. Administration of dexamethasone to pregnant ewes for 2 d during the first but not the second trimester causes hypertension in the juvenile and adult offspring.

Thus, the critical period for programming of hypertension seems to be earlier in gestation in the sheep than in the rat. Sheep are more mature at birth than rats, and thus the critical periods in the two species may represent comparable stages of development of a specific organ system. In particular, the critical period for programming of hypertension overlaps with the window of nephrogenesis. Recognizing the species-specific windows of sensitivity to programming is important for design and interpretation of mechanistic studies as discussed further below.

**Gender Differences**

In the rat, a 70% reduction of food intake throughout pregnancy (18) or to 6% protein diet (12,16) induced a similar degree of hypertension in male and female offspring. Other
studies, however, have described a gender difference. A 30% reduction in food intake from 0 to 18 d of rat gestation produced hypertension in the offspring that appeared earlier and was more pronounced in male than in female rats (39). In our studies, female rat offspring of mothers that were fed an 8.5% protein diet throughout pregnancy were not hypertensive compared with offspring of mothers on the normal-protein diet, but their male littermates had mean arterial BP that were significantly higher than in male controls (by approximately 10 mmHg) (19). At least two factors could account for the apparent discrepancies among these studies. First, different methods were used to measure BP: Most studies have used the indirect tail-cuff method, whereas the studies that reported gender differences used more direct determination via an indwelling catheter. Second and more important, food or protein restriction was more modest in the studies that reported a gender difference. Indeed, our recent findings in chronically instrumented conscious animals showed that a modest maternal protein restriction throughout pregnancy causes increased mean arterial pressure in male but not in female offspring, whereas a more severe maternal protein restriction results in hypertension in both male and female offspring (19,20,40). We have also found that a moderately high maternal sodium intake in pregnancy and lactation leads to hypertension in male but not in female offspring, whereas a very high maternal sodium intake programs both male and female offspring for increased BP (27).

Thus, female rat offspring may be relatively protected from the hypertensive effect of maternal dietary manipulations, but if the maternal insult is severe enough, then both genders are susceptible. However, whether this concept applies to all species and other types of prenatal perturbations is not yet clear (3,4,28,31,32,34).

**Characteristics of Disease in Offspring**

**Time Course and Survival.** Because the measurement of BP very early in life is often difficult, the exact age of onset of hypertension in many experimental models is not well defined. Although the mechanisms that lead to hypertension must have their origins in prenatal life, the offspring may not be born hypertensive. In the rat low-protein models, hypertension has been noted at or around the time of weaning and is well established in young adolescents, by 8 wk of age (12,16). Fetal BP has been measured in the sheep models and found to be unchanged (41) or even decreased (42) in late pregnancy despite that the offspring are hypertensive by 4 mo of age (23,43). It is interesting that some human studies have shown a positive, not negative, correlation between neonatal BP and birth weight (44), whereas contradictory results have been obtained in adolescents (45,46).

Once developed, prenatally programmed hypertension seems to persist indefinitely (14,16,20,47) and to get progressively worse with age (Figure 1) (14,16,20), a finding that has also been described in humans with low birth weight (5). In general, both rat and sheep models exhibit only mild to moderate hypertension, approximately 5- to 40-mmHg increase in systolic pressure in young adults (16,20,31,33,48), compared with the much more severe hypertension in genetic models such as the spontaneously hypertensive rat (49). Telemetry monitoring was used by Tonkiss et al. (50) to characterize BP variations in rat offspring of protein-restricted dams. Hypertension was seen only during the physically active period, at night, and the authors suggested that high pressures that were measured during daytime by others using the more common tail-cuff method may be due to exaggerated stress response during the handling of the animal. However, we have measured mean intra-arterial pressure in animals that are chronically instrumented, trained, and acclimatized to the restrainer, so stress is minimized if not entirely avoided, yet sustained hypertension is still present (19,20).

In the rat, an interesting finding has been the susceptibility of prenatally programmed hypertension to postnatal modulation. Both early postnatal angiotensin-converting enzyme (ACE) inhibition and low-Na diet after weaning, even when given only for a 2- to 3-wk period, seem to have a pronounced and sustained ameliorating effect on the development of hypertension (51,52); conversely, high-Na diet seems to accelerate the hypertension (52). The mechanisms remain to be elucidated. The timing of these treatments was after completion of nephrogenesis, indicating that the postnatal modulatory effect is not through a change in the final number of nephrons. Because newborn rats are very immature compared with, for instance, sheep and human newborns, the postnatal window may be species specific.

Very few studies have followed the experimental animals to determine the long-term survival. In the rat with hypertension induced by maternal low-protein diet, we observed a markedly reduced 18-mo survival, 44% compared with 93% in control rats (14,16). The cause of death in these animals was undetermined. However, it did not seem to be renal failure or hyperkalemia (14), despite that renal function may deteriorate with age (53,54). It is interesting that life-long normalization of BP by ACE inhibitor treatment improved but did not totally abolish the extra mortality (14), suggesting that factors unrelated to BP participate in decreased survival.

**Associated Morbidity.**

IUGR. The initial human observations linked low birth weight to later hypertension (1,2), and IUGR has been de-
scribed in many of the experimental models, whether induced by maternal dietary changes (12,16,19), maternal glucocorticoid treatment (55), or surgical impairment of blood flow to the fetus (15,34). However, some maternal glucocorticoid treatment regimens in the rat and sheep seem to program hypertension without fetal growth impairment (11,33,48,56,57), and IUGR does not invariably result in adult hypertension (36,58,59). Thus, IUGR and later hypertension can be dissociated, suggesting that the programming is not mediated by IUGR per se.

**Metabolic Syndrome.** The metabolic syndrome (syndrome X), including obesity, insulin resistance, and dyslipidemia in addition to hypertension, has been linked to low birth weight in humans (1), and it therefore is of interest to examine its presence in animal models. Hyperglycemia, impaired insulin secretion, insulin resistance, and even frank diabetes in the offspring have been induced by some prenatal maneuvers with or without IUGR (55,60–66). Underlying mechanisms may include decreased number of pancreatic β cells (67–69), upregulation of hepatic enzymes that may convey insulin resistance (55,60), and upregulation of the glucocorticoid receptor (60,70,71). In contrast, Gatford et al. (72) reported that 5-yr-old sheep made that were hypertensive by prenatal administration of dexamethasone exhibited unchanged insulin sensitivity. The relationship among IUGR, hypertension, and impaired insulin secretion or insulin resistance is unclear at the time of the writing of this review. However, it is unlikely that hypertension is the result of insulin resistance.

Adult obesity develops in some rat models of perinatally programmed hypertension that is induced by maternal calorie restriction (17) but not in those in which hypertension is induced by protein restriction (14,16) or in rats (55) and sheep (11) after prenatal dexamethasone. Alterations in cholesterol metabolism have been described in some models, but the direction of change has varied between studies (73,74). Qualitative or quantitative changes in body fat, without increased body weight, have also been reported (75). In summary, IUGR and most aspects of the metabolic syndrome can be induced by prenatal manipulations in experimental animals, but these findings can also be dissociated from the development of hypertension.

### Mechanisms of BP Programming

#### Maternal and Placental Factors

The nature of the signal from mother to fetus that is responsible for initiating fetal programming has not been delineated unequivocally. Some investigators have proposed that the key is increased fetal exposure to glucocorticoids (9,76), caused by elevated maternal glucocorticoid levels (as a result of stress), by increased passage of glucocorticoids to the fetus, or by activation of the fetal hypothalamic-pituitary-adrenal axis (HPA). Fetal corticosterone levels have been reported to be increased by maternal food restriction (77,78) but not by maternal protein restriction (79). Administration of dexamethasone, a synthetic glucocorticoid that is not inactivated by the placenta (see below), to pregnant rats or sheep on normal diet leads to hypertension in the offspring (9,11,21,48), supporting the hypothesis. However, in the rat studies, reduced maternal food intake may have played a role. In our hands, dexamethasone treatment decreased food intake; when control pregnant animals were pair-fed with dexamethasone-treated rat dams, all offspring became equally hypertensive, suggesting that the hypertensive effect of dexamethasone in the rat may be an indirect one (21).

The studies by Dodic et al. (11,33) in the sheep, however, are unlikely to involve significant nutritional deprivation because of the short duration of the treatment and that the offspring were not growth-restricted at birth.

The fetus is normally protected from exposure to excess maternal glucocorticoids by the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βH2), which metabolizes the active glucocorticoid (cortisol in humans and sheep, corticosterone in rats) to an inactive form. Activity of the enzyme may be reduced in placentas from rats that are on protein-restricted diets, possibly allowing increased transplacental passage of glucocorticoids (80,81). Also, administration of carbenoxolone, an inhibitor of 11βH2, to pregnant rats was reported to program for hypertension in their offspring (61,82), but these results could not be duplicated in a study that used a slightly lower but still biologically active dose of carbenoxolone (83); the discrepancy may be due to other nonspecific effects of high doses of the drug (84).

Virtually no information is available on other potential mediators, such as other maternal-to-fetal hormones and growth factors that may participate in the programming of the fetus.

### Targets of Programming in the Fetus

#### Extrarenal Mechanisms.

Structural, functional, and biochemical abnormalities have been described in various organ systems in many experimental models of prenatal programming. Only those findings that have been proposed to be involved in BP programming are discussed here.

**HPA and Glucocorticoid Receptor.** There is evidence that the prenatal environment may program the HPA, resulting in changes in circulating glucocorticoids, glucocorticoid receptor expression, other endocrine functions, and behavior in later life (85). The role of the HPA in perinatally programmed hypertension, however, is less obvious. In the sheep, both maternal calorie restriction and steroid administration may induce demonstrable changes in the offspring HPA. Calorie restriction in early pregnancy has been reported to suppress the HPA during fetal life (23,86,87), to increase glucocorticoid receptor expression in several tissues of the newborn (70), and to result in augmented cortisol response in later life (23). Dodic and coworkers (32,33) examined the HPA in the sheep-steroid model. Although they found alterations in the hippocampal glucocorticoid receptor expression in the fetus, the changes did not persist in the postnatal life, and the offspring exhibited no alterations in either basal or stimulated cortisol levels (11,32,33), leading the authors to conclude that steroid-induced programming of hypertension is not mediated by the HPA (32).

Inconsistent findings have also been reported in rat models of hypertension programmed by prenatal protein restriction or steroid administration. Both unchanged (88) and increased (13,89) plasma corticosterone (the major rodent glucocorticoid) levels, as well as normal corticosterone response to stress (13,55,90), have been described. Upregulation of the glucocor-
ticoid receptor has been reported in kidney, liver, lung, and brain in both fetal and adult life in the rat with prenatally programmed hypertension (81), whereas decreased expression in the hippocampus was observed in one study (13).

In summary, changes in HPA can be demonstrated in some models of prenatally programmed hypertension, but they do not seem to correlate consistently with the presence of hypertension, casting doubt on a direct role of the HPA in the development of hypertension. This conclusion is further strengthened by the fact that in some models, the prenatal manipulation has not induced hypertension despite similar changes in the HPA in the offspring (91). Possible contribution of generalized overexpression of the glucocorticoid receptor to the development of hypertension has not been ruled out definitively.

**Vascular and Sympathetic Function.** Vascular reactivity has been examined either in vivo as the BP response to vasoconstrictors or vasodilators or *in vitro* using vessel segments to measure vasoconstriction, vasorelaxation, or nitric oxide (NO) production in response to a variety of stimuli. In the rat hypertension models induced by various maternal dietary manipulations, impaired vascular relaxation seems to be present, whereas variable responses to vasoconstrictors have been reported (10,26,92,93). Sheep offspring that are made hypertensive by prenatal food restriction or glucocorticoid administration seem to have a normal response to vasoconstrictors (11,43,56). Because functional vascular alterations accompany most types of hypertension and may be consequences of hypertension, these findings cannot be taken as proof of primary vascular pathophysiology in prenatally programmed hypertension. Moreover, some prenatal manipulations have resulted in similar vascular or baroreceptor changes without resulting in hypertension (22,43).

Increased plasma noradrenaline level in response to stress has been described in rat offspring at 4 mo of age after perinatal malnutrition (90), suggesting dysfunction of the sympathetic system. Altered fetal sympathetic activity, assessed by the response in heart rate to BP variation, has been reported in rats and sheep (42,43,56,57), and the suggestion has been made that low BP during prenatal life may result in resetting of baroreflex control and thereby contribute to the later development of hypertension (42). However, at least in sheep, the baroreflex changes can be dissociated from hypertension; early-gestation dexamethasone treatment programs hypertension (11), whereas later exposure results in altered baroreflex without hypertension (43).

**Mineralocorticoid Effect.** Limited information is available on mineralocorticoid activity in prenatally programmed hypertension. Both suppressed (13) and increased (90) hippocampal mineralocorticoid receptor expression has been reported in the rat, whereas no long-term changes were seen in sheep (32,33). Expression of the mineralocorticoid receptor in the kidney does not seem to be increased in either species (81,94). We observed an increase in plasma aldosterone in a rat low-protein–induced model (16), but the pathophysiologic significance of this finding is not yet defined. Mineralocorticoid action in target organs could be enhanced by deficiency in the 11HD2 enzyme; this possibility is discussed further below.

**Systemic and Brain Renin-Angiotensin System.** The role of the renin-angiotensin system (RAS) in the genesis of hypertension is still unclear, despite a large body of experimental data. In human IUGR, neonatal plasma renin activity, measured in cord blood, has been reported to be increased (95). Several studies have examined the state of systemic RAS in experimental prenatally programmed hypertension, with mixed results (16,19,37,96). Differences among prenatal manipulations, gender, and timing of the measurements may explain some of the differences. Circulating plasma renin activity during early life seems to be suppressed (16). In later life, both unchanged (11,96) and increased (37) plasma renin activity has been reported. In our longitudinal study, we observed a switch from low to high plasma renin activity in the rat, but the switch occurred only after hypertension was established (14). Plasma angiotensin I and II concentrations were unchanged during the prehypertensive stage (97). These results cast doubt on the role of circulating hyperreninemia in the pathogenesis of the hypertension. Similarly, Peers et al. (96) concluded on the basis of their studies in sheep that the peripheral RAS does not play a role in the hypertension induced by prenatal steroids. Increased pulmonary ACE activity has been described in the rat (12), but its pathophysiologic importance remains unknown.

The brain RAS has received considerable attention. In their model of prenatal dexamethasone administration in sheep, Dodic et al. (32) reported increased expression of angiotensinogen in hypothalamus and angiotensin II type 1 receptor (AT1R) in medulla oblongata in fetuses, and some of the changes seemed to persist in mature adult offspring; in another study by the same group, prenatal cortisol treatment resulted in upregulated hippocampal AT1R expression in the fetus, but the change did not persist after birth (33). In rat hypertension programmed by a prenatal low-protein diet, increased AT1R expression in the subfornical organ and lamina terminalis was seen, and the hypertension was ameliorated by direct infusion of ACE inhibitor or AT1R blocker intracerebrally (57). This intriguing observation requires further study before a role of the central nervous system RAS can be assigned. The role of the intrarenal RAS is discussed below.

**Renal Mechanisms.** Comparison of the two most extensively studied species, rat and sheep, reveals that the window of susceptibility to BP programming seems to coincide with the early stages of kidney development in each case, during the second half of pregnancy in the rat (16,20) and during the first trimester in the sheep (11,33), suggesting that abnormal renal ontogeny may be involved. Guyton et al. (98) were the first to advance the hypothesis that any sustained hypertension requires the participation of the kidney in the pathogenesis via the pressure-natriuresis mechanism. A right shift of the pressure-natriuresis curve may be a general feature of all hypertension (22,43). However, as the BP response to vasoconstric-
low, there is additional strong information to support a critical role of the kidney in prenatally programmed hypertension.

**Number of Nephrons.** In 1968, Zeman (100) first reported that rat offspring of severely protein-restricted mothers had kidneys that contained fewer glomeruli than normal. This finding has been confirmed more recently by several laboratories, and a nephron deficit has been proposed to play a causal role in prenatally programmed hypertension (101). The methods for estimating the total number of glomeruli (and hence nephrons) have included maceration-digestion of whole kidney as well as counting of glomeruli captured in random tissue sections. The gold standard, used less frequently because of the time and effort involved, is determination of glomerular number by unbiased stereologic techniques such as the disector-fractionator method.

Using stereologic methods and inulin clearance, the laboratory of one of the authors has investigated the effect of different degrees of protein restriction on the number of glomeruli and on GFR in the Sprague-Dawley rat (19,20,40). The findings are summarized in Figure 2. It was confirmed that the relatively severe degree of maternal protein restriction (5% protein) used by Zeman causes a reduction of 30 to 45% in the number of nephrons in the offspring (20). This effect is present in both male and female rats but tends to be greater in male rats. More modest protein restriction also results in a decrease in nephron number in male rats, although to a lesser degree: An 8.5% protein maternal diet decreased the total nephron number by 25% in male offspring (19). The average volume of individual glomeruli was increased, so the total volume of all glomeruli was not different from that in normal animals, consistent with glomerular hypertrophy in response to a reduction in nephron number (102). In contrast, female offspring of these modestly protein-restricted mothers had a normal number of glomeruli that were of normal size (40). Thus, use of stereologic techniques and only a modest protein restriction revealed a gender difference in programming of nephron number. Importantly, the changes in nephron number paralleled changes in BP: Hypertension was present in the groups with decreased nephron number and not in those with a normal number of nephrons. This supports a role for reduced nephron number in causing hypertension. The results of a stereologic study by Zimanyi et al. (103), using modest protein restriction in the Wistar-Kyoto rat, are in general agreement with our experience. Less information is available in other models and species. Decreased nephron number has been described in rats that were made hypertensive by prenatal dexamethasone administration (48), in pigs after prenatal protein restriction (8), and in sheep after prenatal dexamethasone (47). In the last study, however, the offspring were studied at 7 yr of age, making the distinction between primary congenital deficit and secondary nephron loss difficult.

There is additional experimental evidence to support the hypothesis that reduced nephron number is responsible for later hypertension. In normal rats, uninephrectomy on the first day of postnatal life causes salt-sensitive hypertension as early as 8 wk of age, and this precedes the onset of renal disease, which did eventually occur at least in male rats (104,105). A recent study in sheep confirmed this finding; uninephrectomy during the period of nephrogenesis in the ovine fetus leads to increased mean arterial pressures at 6 and 12 mo of age (106).

**GFR.** Our studies have used inulin clearance for GFR determination in the rat low-protein model (19,20). Absolute GFR was reduced by 15 to 25% in both male and female offspring of severely protein-restricted mothers; GFR normalized to body weight was significantly reduced in male offspring and also tended to be reduced in female rats. With less severe protein restriction, GFR was unchanged in female offspring (which did not develop hypertension) but was reduced by approximately 10% in male protein-restricted offspring compared with controls (Figure 2). This did not reach statistical significance, but this difference is at the limit of detection of the technique, and a decrease of this magnitude would be physiologically significant. In addition, a decrease of only 10% in whole-kidney GFR coupled with a decrease of 25% in nephron number suggests that single-nephron filtration is increased in these animals compared with controls, a finding that may have important implications for the progression of renal disease. Decreased GFR in the rat model of prenatally programmed hypertension has been reported by others as well (53). However, in some models, hypertension seems to develop without a discernible decrease in GFR. Rat offspring that were made hypertensive by prenatal dexamethasone had unchanged GFR despite having a reduced number of nephrons (48). Moreover, hypertension induced by decreased placental perfusion in the rat did not result in decreased GFR at 8 wk of age (34), and studies by one of the

![Figure 2. Arterial pressure, renal function, and nephron (glomerular) number in adult male offspring of rats that were fed normal protein (NP, 19%) or low protein (8.5% [LP] or 5% [LLP]) diets throughout pregnancy or during the last half of pregnancy (NP/LLP). Mean ± SEM. *P < 0.05 versus the values in NP offspring. Redrawn from references 19 and 20.](image-url)
authors have shown normal nephron number and GFR in rats that were made hypertensive by high maternal dietary sodium (27). Thus, although the hypertension has been suggested to result directly from impaired Na filtration as a result of reduced nephron count and GFR, the mechanism may be more complex and involve other renal mechanisms (107). Furthermore, the mechanisms of programming for hypertension may not be identical in different models.

Na Reabsorption. We have examined the possibility that a decrease in the number of nephrons is accompanied by resetting of Na reabsorption by the tubules. Direct experimental evidence for increased Na reabsorption is lacking in that Na transport has not been measured in any nephron segment in prenatally programmed hypertension. Instead, we have examined the expression of the apical Na transporters along the rat nephron segments and shown that the expression of two transporter proteins, the thick ascending limb Na-K-2Cl co-transporter and the distal convoluted tubule Na-Cl co-transporter are increased to 302 and 157% of control levels, respectively, during the prehypertensive stage (Figure 3), accompanied by an increase in their mRNA levels (108). No changes were seen in the expression of NHE3 or ENaC subunits; however, our studies cannot rule out altered activity of these transporters by other regulatory mechanisms, for instance, by trafficking of the transporters between the apical membrane and the intracellular compartment. Bertram et al. (81) reported increased Na-K-ATPase expression in whole rat kidney at 12 wk of age, also consistent with increased Na transport.

We have described increased plasma aldosterone concentration in the rat in the prehypertensive stage (16), and decreased expression of the 11HD2 enzyme, which normally “protects” the mineralocorticoid receptor from stimulation by glucocorticoids, has been reported in the rat (81) and ovine (70) kidney. These findings may indicate upregulated Na transport in the distal convoluted tubule or the collecting duct via inappropriate mineralocorticoid receptor activation, but more direct confirmation is required.

Intrarenal RAS. The role of the RAS in altered renal development has received considerable attention. Angiotensin II is known to be important in normal renal development (109,110), and variations in dietary protein can alter the RAS (111). In the rat low-protein model, we have shown that renal tissue renin protein and mRNA, AT1R protein and mRNA, and angiotensin II levels are reduced in the newborn and young offspring compared with controls (16,19,97). Furthermore, littermates of these same offspring had fewer than the normal number of nephrons and were hypertensive in adulthood. We also showed that pharmacologic blockade of the RAS with losartan in normal rats for the first 12 d after birth (the latter portion of nephrogenesis in the rat) led to a reduced number of nephrons and hypertension in adulthood (112). Thus, there seems to be a cause-and-effect relationship among the RAS during development, nephron number, and adult hypertension. These findings support the hypothesis that maternal protein restriction programs the offspring for hypertension by suppressing the intrarenal RAS during development, leading to a reduced nephron endowment, renal dysfunction, and consequent hypertension later in life.

The intrarenal RAS may also participate directly in the genesis of the offspring hypertension in postnatal life. The importance of the intrarenal RAS in BP control is gaining increasing experimental support. Accumulating evidence suggests that activation of the RAS within the kidney, independent of the state of the systemic RAS, may induce Na retention and sustained hypertension (113). Despite that the intrarenal RAS is suppressed in the neonatal period in prenatally programmed hypertension in the rat, AT1R expression increases above control levels during the prehypertensive stage (97,114), without feedback suppression in kidney angiotensin I or II contents (97). In sheep that have been programmed to become hypertensive via maternal glucocorticoid treatment (115) or by maternal food restriction (70), upregulation of renal AT1R has been documented already in late gestation and at birth, respectively, perhaps reflecting the much more advanced maturation state of the ovine kidney. These findings are consistent with activation of RAS via upregulated AT1R and offer a potential role for the intrarenal RAS in the pathogenesis of prenatally programmed hypertension. The effect of upregulated AT1R may be accentuated by a downregulation of the counterregulatory angiotensin II type 2 receptor (116). However, most of the described changes in the intrarenal RAS were quantitatively modest, and more work is needed to establish unequivocally a role for the intrarenal RAS in the genesis of the hypertension. Delineating

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**Figure 3.** Apical Na transporters quantified by immunoblotting in kidneys of offspring from maternal low-protein (6% protein) pregnancies at 4 wk of age. Values expressed as percentage of those of control kidneys (horizontal line). NHE3, proximal tubule Na-H exchanger; BSC1, thick ascending limb Na-K-2Cl co-transporter; TSC, distal convoluted tubule Na-Cl co-transporter; ENaC-α, ENaC-β, and ENaC-γ, subunits of the collecting duct epithelial Na channel. Mean ± SEM. *P < 0.05, ***P < 0.001 versus control values.

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the expression of RAS components along the nephron is an important next step to clarify their role further.

**Oxidative Stress and Inflammation.** Oxidative stress has long been associated with hypertension, and recent experimental studies strongly support the hypothesis that accumulation of reactive oxygen species in the kidney is, in fact, a cause of hypertension (117). Oxidative stress may be increased in prenatally programmed hypertension (118), but its pathogenetic role is largely unexplored. Our recent results (119) have demonstrated increased nitrotyrosine content (“footprint” of oxidative stress) in kidneys preceding the onset of hypertension. More important, we were able to prevent the development of hypertension and nitrotyrosine accumulation by administration of the reactive oxygen species scavenger tempol to the rats (Figure 4). As a potential source of oxidative stress, we observed an increased presence of immune cells in the kidneys, and pharmacologic blockade of the cell infiltration has effects similar to those of tempol (Figure 4). These results mimic those obtained in other types of experimental hypertension and suggest that oxidative stress in the kidney may be part of a common pathogenetic pathway.

**Summary and Future Perspectives**

There is a wealth of data to show convincingly that adult hypertension is programmable by the prenatal environment in a variety of species. It is also evident from the above discussion that the picture of prenatal programming is far from complete. In comparing studies, it is critical to consider the details of methodologic differences, such as specific dietary components and BP measurement techniques, as well as gender differences, all of which may affect the conclusions drawn. Nevertheless, the current available mechanistic information is difficult to fit neatly into a single pathogenetic scheme, and it may become necessary, in contrast to previous expectations, to postulate different mechanisms for hypertension programmed by different prenatal maneuvers. However, another possibility is that investigators so far are feeling different parts of the elephant without having been able to get their arms around the whole yet.

The “thrifty phenotype” hypothesis, stating that the “starving” fetus is programmed to conserve nutrients in later life (120), may apply to Na homeostasis as well. Because dietary salt has been scarce throughout the human history except for the last two millennia, we (and most experimental animals) may be well equipped to conserve Na but poorly adapted to defend ourselves against Na overload and hypertension. Prenatal conditions that endanger the fetus, for instance, ischemia or hypotension, may permanently program a resetting of the Na-conserving mechanisms as a defense against volume depletion, perhaps utilizing the orchestrated efforts of several organ systems. This programming of Na homeostasis would be inappropriate in postnatal life with plentiful dietary Na, resulting in hypertension.

Because the role of the kidney seems important in the programming, one of the areas that require further research is renal ontogeny to explain what causes abnormal renal development (decreased number of nephrons). Apart from a study revealing increased apoptosis in the developing kidney (121) and the changes in RAS, very little is known of the perturbations during renal organogenesis. Undoubtedly, some of the array of growth factors that participate in normal kidney ontogeny are also responsible for the abnormal kidney development.

The emerging field of epigenetics is also opening up new horizons. Whatever function is programmed in the fetus, it is likely the result of altered gene expression. There is evidence that epigenetic regulation of gene expression, through methylation-demethylation of DNA, could be the mechanism of prenatal programming (122). A disconcerting aspect of the concept is the potential for transgenerational passage; in other words, prenatally programmed disease could be passed on to the next generation as a result of covalent methylation of the responsible gene(s) (122–124).

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