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Influence of Prenatal Maternal Stress on the Immunocompetence of the Offspring

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SOBRIAN, S. K., V. T. VAUGHN, E. F. BLOCH AND L. E. BURTON. *Influence of prenatal maternal stress on the immunocompetence of the offspring.* PHARMACOL BIOCHEM BEHAV 43(2) 537-547, 1992.—To evaluate the effects of prenatal maternal stress on the development of humoral immunocompetence in the offspring and on their hormonal and immunologic responses to postnatal stress, gravid Sprague-Dawley rats were exposed daily on gestational days 15-21 to prenatal environmental stress [(PES) 15 unsignaled, inescapable electric foot-shocks (0.05 mA for 0.5 s)] or prenatal psychological stress [(PPS) pregnant rats were placed in the nonelectrified section of the apparatus and allowed to see, hear, and smell a nonpregnant partner being environmentally stressed]. Pregnant controls (PC) were placed in the apparatus for 30 min. Serum corticosterone (CCS) and immunoglobulin G (IgG) levels were measured in the offspring every 7 days from birth to postnatal day (PND) 28. On PND 29-33, offspring were environmentally stressed; hormonal and immune status were determined on PND 34. Levels of IgG were reduced in PES and PPS offspring on PND 0 and in PES offspring on PND 7 and 28. These changes were unrelated to differences in CCS and did not reflect altered maternal-pup interactions or nutritional factors. Postnatal stress was immunosuppressive in PC pups but did not alter immune parameters in PPS offspring. In PES females, postnatal stress was also immunosuppressive. However, in PES males with already reduced IgG levels postnatal stress enhanced immune function. These data provide the first experimental evidence that prenatal maternal stress can alter immune parameters in the rat offspring.

Prenatal psychological stress Prenatal environmental stress Humoral immunity Corticosterone levels
Postnatal stress response

STATES of stress induced by a variety of environmental and psychosocial factors can modify immune processes in man and animals. Both susceptibility to and recovery from infection, allergic reactions, and autoimmune disorders have been related to life stresses (63). Direct measures of immune status and function, such as immunoglobulin levels, T-cell response to mitogens, and natural killer cell activity, have also been altered by stress. The consensus in the clinical and experimental literature [(1,2.4,77) for reviews] is that, in general, stress is immunosuppressive. Despite the fact that the response to stress is dependent upon such host factors as species, strain, sex, age, and circadian rhythms, as well as the temporal relationship between stressor and measure of immune function (2,14), it is clearly evident that psychological factors can impact immune function (4,69).

Prenatal maternal stress (PMS), that is, stress applied to or induced in the female during pregnancy, can alter the behavior of the offspring (7,38,86). Daily handling (3,5,64), anxiety (49,65), electric foot-shocks (13,73), restraint with or without bright lights and elevated temperatures (6,44,68), noise (24), and crowding (16,33,53) can affect a variety of indices in the adult offspring including sexual and reproductive behaviors (15,35,42), emotionality or reactivity (22,25), and acquisition of several avoidance tasks (31,49). Reflex and physical development, as well as the ontogeny of motor activity and alternation behavior in the preweaning offspring, are also sensitive to PMS (24,73,74). In addition, PMS appears to modify the development of serotonergic (59,60,64), noradrenergic (54,58,62), dopaminergic (26), and cholinergic (23) systems, as well as brain opiate (37,83) and benzodiazepine receptors (22).

Evidence that the brain can modulate or regulate immunologic processes has been recently reviewed (4,69). The fact that the developing CNS is particularly sensitive to insult led us to hypothesize that prenatal maternal stress, by altering the maturation of the CNS, could result in compromised immune function in the offspring. This hypothesis is supported by reports that immunocompetence of the offspring can be affected by both early life experiences and a variety of prenatal manipulations.

Immunologic responsivity of the adult rat can be modified by neonatal stimulation. The results of these studies have been

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reviewed (2) and will only be outlined briefly here. Early handling increased the survival time to and decreased the growth rate of Walker carcinoma 256 implanted in the adult and enhanced the antibody production of a bacterial antigen. In contrast, mice handled for various intervals during the preweaning period showed shorter survival times after transplantation of lymphoid leukemia as adults and increased rate of tumor development to polyoma virus. Several studies using electric shock as the neonatal stressor have reported nonsignificant protective trends with respect to mortality following viral exposure as adults.

Immunocompetence as a function of alterations in mother–young interaction has not been extensively studied. However, infant pigtail and bonnet monkeys separated from their mothers showed both impairments in cellular immunity and depressed lymphocyte response to mitogenic stimulation, which returned to normal following reunion. Maternal deprivation in rodents, accomplished by either separation or early weaning, resulted in increased mortality following exposure to encephalomyocarditis virus in rats and depressed antibody response to sheep red blood cells in mice.

Studies on "stressed" human neonates, defined as those with acute respiratory illness, bacterial infection, or delivery complication, have produced contradictory results. In an early study (90), the phagocytic and intracellular killing activity of leukocytes from stressed infants was reduced against several bacteria. Results from a second study, comparing healthy infants with a similar group of stressed neonates, found no difference in bactericidal activity of polymorphonuclear leukocytes from both groups (81).

Previous research involving prenatal maternal manipulations and immune functioning in the offspring has focused on the effects of environmental toxins, dietary alterations, and various pharmaceuticals. As the results have been reviewed (67,70), the following will provide a synopsis of the most recent findings. Cellular immunity is severely depressed in adult mice and rats exposed prenatally to pesticides (21,80), and the incidence of bacterial infection is increased as a function of age in mice exposed in utero to the environmental contaminant, methylmercury (79). Both T- and B-cell function is depressed in the offspring of rats exposed during gestation to alcohol [(20) for review], while prenatal cocaine exposure increased the rate at which viral infectivity was expressed in cultured sera from the offspring (75). Dietary deficiencies during pregnancy resulted in impaired secondary antibody responses, reduced delayed type hypersensitivity reactions, and smaller lymphatic organs in rat offspring [(70) for review], although results to the contrary have been reported (52). Prenatal irradiation reduces the primary antibody response to several antigens (36,56) and decreases the number of T-helper lymphocytes (56). In contrast, pre- and perinatal exposure to the immmunotoxicant di-*n*-octyltin dichloride have no effect on lymphoproliferative responses to B- and T-cell mitogens, natural killer cell activity, or the primary antibody response to sheep erythrocytes (72).

To investigate the relationship between brain, behavior, and immunity and the critical nature of their interaction during early life, we exposed pregnant rats to prenatal environmental stress (PES) or prenatal psychological stress (PPS) to determine if this manipulation would influence the maturation of humoral immunity in the offspring and/or modify their hormonal and immunologic responses to postnatal stress.

METHOD

Nineteen nulliparous time-pregnant Sprague-Dawley rats (Charles River Laboratories, Somerville, MA) were delivered to our laboratory on gestation day (GD) 8. Mating was confirmed by the presence of vaginal sperm and/or vaginal plug and was designated GD 1. Pregnant rats were housed individually in polyethylene maternity cages. Nineteen nonpregnant female rats of comparable age, who were to serve as partners for the pregnant rats, were delivered at the same time and housed in groups of two to three. All rats were maintained under environmentally controlled conditions (0830 h light, 1930 h dark; ad lib access to Purina Rat Chow and water; ambient temperature 23–25°C).

After random assignment to groups on GD 14, pregnant rats were exposed daily for 30 min on GD 15–21 to one of three PMS conditions:

1. *PES*: Pregnant rats were given 15 unsignaled, inescapable electric foot-shocks that were randomly programmed on a variable-interval schedule. Rats were run with a nonshocked, nonpregnant partner.
2. *PPS*: Pregnant rats were placed in the nonelectrified section of the shock apparatus and allowed to see, hear, and smell a nonpregnant partner that was being environmentally stressed. This stress paradigm has been shown to elevate plasma corticosterone and nonesterified free fatty acids in nonpregnant rats of comparable body weight (11).
3. *Prenatal Control (PC)*: Pregnant rats were placed in the shock apparatus for 30 min but were not exposed to either PES or PPS; a nonpregnant partner was placed in the adjacent compartment.

The apparatus used to administer electric foot-shock was a shuttle-box (Lafayette Instrument Co., Lafayette, IN), divided in half by a perforated Plexiglas partition. Foot-shocks (0.5 mA for 0.5 s) were delivered to only one side of the apparatus by a constant-current neon gird shocker and were randomly programmed on a variable-interval schedule (i.e., minimum interval 25 s; maximum interval 115 s). A variable-interval presentation of the unconditioned stimulus was chosen in an effort to minimize or eliminate both the development of anticipatory responses that might mitigate the animal's response and possible habituation to the stressor (87). The apparatus was cleaned with a mild liquid detergent after each individual stress session. Pregnant rats and their partners were stressed in the same order (randomly assigned on GD 14) and at approximately the same time (plus or minus 1 h) each day.

Females were allowed to deliver naturally and nurse their own offspring. At parturition [designated as postnatal day (PND) 0], dams were temporarily removed and for each litter pups were counted, sexed, weighed, and examined for external malformations. Each litter was then culled to 10 pups, balancing for sex when possible. Body weights were recorded every 7 days from birth until PND 28, at which time rats were weaned and housed in same-sex groups of three to four.

Maternal Behavior

Several indices of maternal behavior were monitored within 4 h after births were noted. Because all pups were raised by their biologic mothers, it was possible that residual stress effects could disrupt the mother–pup interactions. This disruption might subsequently alter the development of immunocompetence, which is in part accomplished through the transfer of cells and antibodies from mother to young (17,57). Evaluation of maternal behavior provided a test of this hypothesis.

A culled litter was placed at one end of a maternity cage containing clean bedding and the dam was placed at the opposite end. The latency of the female to begin nest building and

the time taken to retrieve all 10 pups was measured to the nearest tenth of a second with a stop watch. The number of pups remaining out of the nest at the end of 5 min (maximum time) was also recorded.

Thirty minutes later, females were observed for their presence in the nest and pup-directed behaviors (nursing or grooming), as well as eating, drinking, or self-grooming. Behaviors were scored as present or absent and analyzed by Fisher's exact probability test (71).

Serum Corticosterone and Immunoglobulin G Determinations

At PND 0, 7, 14, 21, and 28, one male and one female offspring from at least three litters from each of the PMS groups were killed by decapitation. Trunk blood was collected in nonheparinized tubes, centrifuged for 20 min, and the serum was removed and frozen. Corticosterone (CCS) levels were measured fluorometrically (28) and total serum immunoglobulin G (IgG) was determined by the Mancini technique [Miles Laboratories, Inc., Naperville, IL; (48)]. IgG was chosen as the primary immune measure because it is the predominant immunoglobulin transferred across the placenta. In addition, IgG is a high-affinity antibody with a long half-life, is biologically stable, and is present in milligram quantities. At killing, spleen, adrenals, thymus, and whole brain were removed and blotted to remove excess moisture and wet weights were recorded. As organ weights and body weight covary, organ/body weight ratios were also calculated and used in statistical analyses to eliminate the possibility that differences in organ weights among the groups reflected altered body weights.

Postnatal Stress

Daily from PND 29–33, male and female offspring from each of the three prenatal stress groups were exposed to postnatal environmental stress (PNES) for 15 min. Pups received eight unsignaled, inescapable foot-shocks (0.20 mA for 0.5 s), programmed to be delivered on a variable-interval schedule (minimum interval 25 s; maximum interval, 115 s). Offspring from each group, which served as controls, were placed in the apparatus for 15 min but not shocked. Twenty-four males and 24 females were used; half of each sex were stressed and the remainder served as controls. No more than two males or two females from any one litter were used in any of the 12 experimental cells.

On PND 34, pups were killed and trunk blood was used for serum CCS and total serum IgG determinations. Organ/body weight ratios were determined for spleen, adrenals, thymus, and whole brain.

Statistical Analyses

Gestational data were analyzed by one-way analysis of variance (ANOVA), as was litter size. Two-way ANOVAs were used for evaluation of pups' birth weight and the number of male and female pups. Nest-building latencies and pup retrieval times were analyzed by one-way ANOVAs. CCS and IgG levels were subjected to two-way ANOVAs. Organ weights were subjected to two types of statistical analyses. Actual organ weights, obtained on postnatal days 0–28, were initially analyzed with two-way ANOVAs. Organ/body weight ratios were then calculated and arc sine transformed prior to two-way ANOVAs. Only transformed organ/body weight ratios were analyzed for data obtained following postnatal stress. Three-way ANOVAs were used for preweaning body weight data and for analyses of all dependent variables collected following postnatal stress. Simple main effects were used to dissect significant two-way interactions and Newman-Keuls or Dunnett's test were used for significant main effects (45). The minimum α level for statistical significance was set at 0.05.

RESULTS

Gestational and Birth Statistics

Weight gain during the last third of pregnancy and the length of the gestational period were not significantly altered by either PES or PPS (Table 1). The number of females delivering viable litters, litter size, offsprings' birth weights, and the number of stillborn pups in each group were also unaffected by either PMS manipulation. Although the ratios of male to female pups were not significantly different among groups, overall female offspring outnumbered male offspring, $F(2, 26) = 21.42$, $p < 0.01$. At birth, female pups were significantly smaller than male offspring, $F(2, 26) = 11.11$, $p < 0.01$.

Maternal Behavior

When females were tested within 4 h of discovery of their litters (PND 0), there were no significant differences in the maternal behavior of PES, PPS, or PC mothers with respect to latencies to begin nest building and time to retrieve all pups (Table 2). The large variation in mean retrieval data reflected the inclusion of maximum scores of 300 s for females that did not retrieve their entire litter. Mean retrieval times, calculated only for females that successfully completed the task in each group, were 67.0 ± 34.1, 58.0 ± 8.4, and 30.0 ± 4.3 s for PES, PPS, and PC females, respectively. The remaining variability in the PES group is due to the score of one female who required 168 s to complete retrieval. Statistical analysis of these selected data revealed a significant difference between retrieval scores for PPS and PC females, $t(6) = 2.96$, $p < 0.05$. Comparison of the number of successful retrievals among the groups with Fisher's exact probability test revealed no significant differences. Despite initial differences, observations of maternal-pup interactions 30 min later revealed that all pups had been retrieved and PES and PPS females exhibited maternal behavior that was similar to that of PC mothers.

Postnatal Body Weight and Organ/Body Weight Ratios

Postnatal body weights, which served as a measure of the general health of animals, increased significantly between birth and 28 days of age in all offspring, $F(4, 91) = 14.35$, $p < 0.01$, but did not differ among the groups at PND 0, 7, 14, 21, or 28 (Fig. 1). The sexual dimorphism in body weight that existed immediately after birth was also evident at PND 28, $F(2, 25) = 8.86$, $p < 0.01$. Male PES and PC offspring pups were heavier than their female counterparts [$t(9) = 1.93$, $p < 0.05$, $t(11) = 2.12$, $p < 0.05$, respectively]; however, body weights of PPS male and female offspring did not differ.

Organ/body weight ratios were calculated for adrenals, spleen, and whole brain on PND 0, 7, 14, 21, and 28 and for thymus on PND 21 and 28; the percent values of these ratios and actual organ weights are presented in Table 3. Initial statistical analyses, computed on transformed scores (arc sine), included gender as independent variable; however, as no main effects or interactions involving this variable were significant it was not subsequently considered statistically.

Organ/body weight ratios for whole brain and thymus

TABLE 1

GESTATIONAL AND BIRTH STATISTICS (MEAN ± SEM) OF FEMALES ENVIRONMENTALLY OR PSYCHOLOGICALLY STRESSED ON GD 15-21

	Prenatal Maternal Treatment		
	Environmental Stress (PES)	Psychological Stress (PPS)	Control (PC)
Females			
GD 15-21 weight gain (g)	87.0 ± 9.2	70.4 ± 5.5	70.0 ± 5.5
Length of gestation period (days)	23.0 ± 0.0	22.4 ± 0.3	22.8 ± 0.2
Number of females delivering offspring	5/6	5/6	6/7
Litters			
Litter size	12.6 ± 1.0	13.8 ± 0.7	12.8 ± 0.8
Male/female ratio*	1 : 1.62	1 : 1.65	1 : 1.26
Birth weight (g)			
Males	6.6 ± 0.2†	6.9 ± 0.3†	6.6 ± 0.2†
Females	5.9 ± 0.3	6.0 ± 0.3	6.2 ± 0.2
Stillborn pups	1	0	1

*Male/female ratio was calculated from the total number of offspring in each treatment group.
†Significantly different from female pups, $p < 0.05$ (overall main effect).

were not significantly different among groups at any of the ages sampled. However, adrenal/body weight ratios of PES and PPS offspring at PND 0 were significantly larger than those of PC offspring [$F(2, 24) = 4.47$, $p < 0.05$; Newman-Keuls, $p < 0.05$]. This difference was also evident for adrenal organ weights at this age [$F(2, 24) = 3.83$, $p < 0.05$; Newman-Keuls, $p < 0.05$]. In contrast, 1 week later at PND 7 adrenal/body weight ratios were significantly smaller in PES and PPS offspring [$F(2, 12) = 10.49$, $p < 0.01$; Newman-Keuls: $p < 0.01$]; organ weights again reflected a similar pattern of change [$F(2, 12) = 6.74$, $p < 0.01$; Newman-Keuls: $p < 0.01$]. Further differences were not observed.

At PND 7, alterations in spleen/body weight ratios were also noted; ratios, but not organ weights, of PES offspring were significantly larger than those of PC offspring [$F(2, 12) = 4.83$, $p < 0.05$; Newman-Keuls, $p < 0.05$]. The increase in this ratio was again evident at PND 28 [$F(2, 25) = 4.31$, $p < 0.05$; Newman-Keuls, $p < 0.05$]; spleen weights of PES offspring were also significantly larger than PC offspring at this age [$F(2, 25) = 4.35$, $p < 0.05$; Newman-Keuls, $p < 0.05$].

Serum CCS. In the offspring of prenatal control dams, CCS levels increased significantly with increasing age (Fig. 2). Levels were low at birth and showed little change during the first 2 postnatal weeks; after this time, plasma CCS increased steadily and reached approximately 40-50% of adult values (55) by PND 28 [$F(4, 101) = 77.70$, $p < 0.01$; Newman-Keuls: $p < 0.01$]. Neither PES nor PPS altered this developmental pattern. Moreover, differences in CCS levels among offspring in the three groups were not evident between PND 0 and 28. Separate analysis of PND 28 CCS data, which included gender as an independent variable, did not reveal any additional group differences.

Serum IgG. The ontogenetic changes in total serum IgG were also unaffected by PES or PPS (Fig. 2). In progeny from both stressed and control dams, IgG levels increased from the first to the second postnatal week, peaked between PND 14-21, and declined to adult levels [80-160 mg/dl; (8)] at PND

TABLE 2

MATERNAL BEHAVIOR (MEAN ± SEM) OF PMS FEMALES TREATED ON GD 15-21

	Environmental Stress	Psychological Stress	Control
Initial test (PND 0)			
Latency (seconds) to begin nest building	26.0 ± 5.4	19.0 ± 4.6	20.0 ± 7.6
Time (seconds) to complete pup retrieval	113.6 ± 53.6*	91.2 ± 52.9*	114.5 ± 58.9*
Number of fully retrieved litters/total litters	4/5	4/5	4/6
30 Minutes later†			
In nest	80%	100%	66.7%
Nursing	80%	100%	50.0%
Grooming pups	20%	20%	33.3%
Self-grooming	0%	0%	33.3%
Litters with pups out of nest	0	0	0

*Includes maximum time (300 s) of animals that did not retrieve entire litter.
†Percentage of females exhibiting behavior.

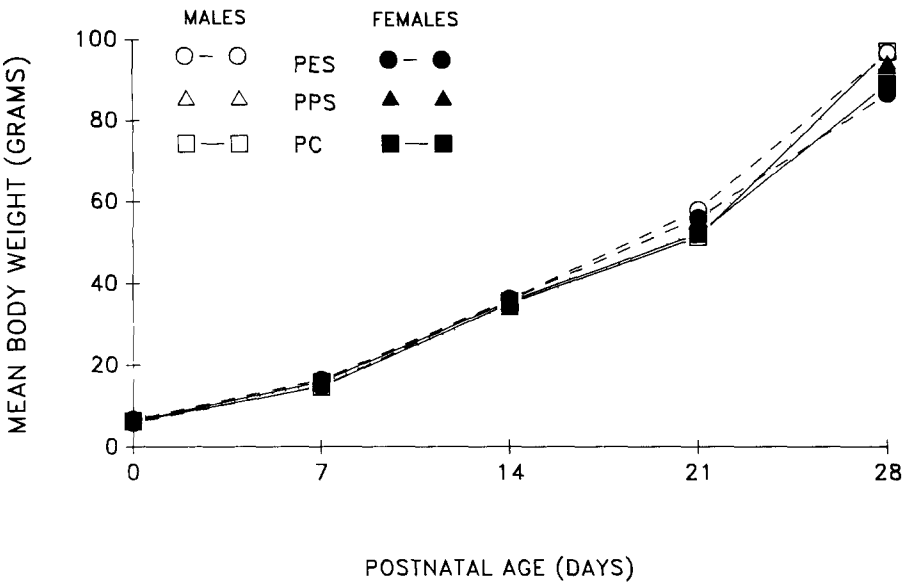

FIG. 1. Preweaning body weights of offspring of females who were exposed on GD 15-21 to either environmental of psychological stress or served as prenatal controls. Values shown are means of four to six males and four to six females at each data point.

28 [$F(4, 75) = 46.85$, $p < 0.01$; Newman-Keuls: $p < 0.01$]. However, differences in IgG levels were observed between the groups at PND 0, 7, and 28. At birth, IgG levels were significantly lower in both PES (Mean ± SEM: 19.09 ± 1.76 mg/dl) and PPS (15.02 ± 1.10 mg/dl) offspring when compared to controls (22.25 ± 2.15 mg/dl) [$F(2, 15) = 4.40$, $p < 0.05$; Dunnett's statistic: $p < 0.05$]. This difference persisted until PND 7 in PES (67.75 ± 3.80 mg/dl) but not PPS (98.91 ± 10.79 mg/dl) offspring when compared with controls (97.16 ± 11.29 mg/dl) [$F(2, 15) = 3.71$, $p < 0.05$; Dunnett's statistic: $p < 0.05$]. No further significant differences were observed until PND 28, when IgG levels were again reduced below controls (114.12 ± 11.28 mg/dl) in PES offspring (70.87 ± 7.47 md/dl) [$F(2, 18) = 3.99$, $p < 0.05$; Dunnett's statistic: $p < 0.05$]. Additional analysis of PND 28 data, including gender as an independent variable, did not reveal any further differences.

The developmental changes in IgG were not related to the changes in CCS. Correlations between IgG and CCS levels during development were $r = 0.0089$, $r = 0.113$, and $r = 0.331$ for PES, PPS, and PC offspring, respectively.

Postnatal Stress

Serum CCS. Exposure of male and female PES, PPS, and PC offspring to 5 days of PNES did not alter CCS levels when compared to their nonstressed counterparts (Table 4). However, PNES induced a sexual dimorphism in serum CCS not seen in nonstressed pups; CCS levels in females were higher than those in males, $F(1, 18) = 6.16$, $p < 0.05$. Analyses of basal CCS in nonstressed offspring revealed no significant differences.

Serum IgG. PNES significantly altered total serum IgG levels (Table 4); changes, however, reflected a three-way interaction among prenatal treatment, gender, and postnatal stress, $F(2, 30) = 19.79$, $p < 0.01$. In PES offspring, PNES significantly decreased IgG levels in females but increased IgG in males when compared to their nonpostnatally stressed counterparts. In PC offspring, both males and females had reduced serum levels of IgG following PNES. In contrast, postnatal stress did not alter IgG in either male or female PPS offspring.

Analysis of serum IgG levels among the nonpostnatally stressed offspring revealed differences in basal levels that reflected a prenatal stress × gender interaction, $F(2, 15) = 12.69$, $p < 0.01$. Among males, IgG levels in PES animals were significantly lower than those in PC and PPS offspring (Newman-Keuls: $p < 0.05$). Among females, serum IgG was reduced in PPS offspring when compared to those of PES and PC animals (Newman-Keuls: $p < 0.05$). Within-groups comparisons of males and females revealed that in PC and PES offspring levels of IgG were higher in females than in males; the reverse was found among PPS offspring (Newman-Keuls: $p < 0.05$).

Body Weights and Organ/Body Weight Ratios. Table 5 lists body weights and organ/body weight ratios for nonstressed and postnatally stressed offspring from the three prenatal groups at PND 34. Body weights of females in all groups were significantly lower than those of males, $F(1, 36) = 63.74$, $p < 0.01$. Differences due to prenatal stress condition or postnatal stress exposure were not evident. Organ/body weight ratios of the brain and adrenals were unaffected by postnatal stress exposure. However, a significant main effect with respect to gender indicated that overall the brain/body weight and adrenal/body weight ratios of females were significantly larger than in males [$F(1, 36) = 82.26$, $p < 0.01$, and $F(1, 36) = 17.65$, $p < 0.01$, respectively].

In contrast, thymus/body weight ratios were altered by postnatal exposure to environmental stress. Thymus ratios were significantly reduced in both PES and PC offspring when compared to their nonstressed counterparts, $F(1, 36) = 6.68$, $p < 0.05$. The increase in spleen/body weight ratios seen at PND 28 in PES offspring was still evident at this time; ratios in nonstressed PES animals were significantly larger than in

TABLE 3

PERCENT ORGAN/BODY WEIGHT RATIOS* OF PES, PPS, AND PC OFFSPRING FROM PND 0-28 (MEAN ± SEM)

	Postnatal Day				
	0	7	14	21	28
Brain					
PES	4.02 ± 0.35	4.72 ± 0.35	3.13 ± 0.15	2.80 ± 0.07	1.81 ± 0.06
	(259.6 ± 21.2)	(725.25 ± 14.22)	(1,137.00 ± 38.29)	(1,532.21 ± 28.09)	(1,618.23 ± 30.57)
PPS	4.16 ± 0.11	4.50 ± 0.14	3.55 ± 0.20	2.78 ± 0.05	1.66 ± 0.05
	(254.2 ± 11.0)	(692.6 ± 18.59)	(1,208.50 ± 31.90)	(1,495.75 ± 39.84)	(1,551.90 ± 38.34)
PC	3.79 ± 0.08	4.78 ± 0.42	3.48 ± 0.18	2.84 ± 0.10	1.74 ± 0.06
	(261.5 ± 8.8)	(717.87 ± 29.48)	(1,217.32 ± 20.81)	(1,466.75 ± 30.94)	(1,603.96 ± 26.15)
Adrenals					
PES	0.0428 ± 0.0040†	0.0208 ± 0.0015‡	0.0185 ± 0.0013	0.0272 ± 0.0056	0.0228 ± 0.0014
	(2.60 ± 0.40)†	(3.07 ± 0.43)†	(6.12 ± 0.42)	(15.67 ± 0.59)	(20.67 ± 0.76)
PPS	0.0413 ± 0.0059†	0.0189 ± 0.0028‡	0.0161 ± 0.0016	0.0313 ± 0.0017	0.0211 ± 0.0012
	(2.44 ± 0.30)†	(2.15 ± 0.44)†	(5.48 ± 0.42)	(16.91 ± 1.37)	(19.78 ± 1.03)
PC	0.0335 ± 0.0034	0.0223 ± 0.0012	0.0167 ± 0.0012	0.0283 ± 0.0035	0.0222 ± 0.0007
	(2.30 ± 0.22)	(3.39 ± 0.36)	(5.85 ± 0.30)	(14.67 ± 1.92)	(20.56 ± 0.64)
Spleen					
PES	0.2070 ± 0.0327	0.1831 ± 0.0227*	0.4270 ± 0.0567	0.5523 ± 0.0583	0.4112 ± 0.0263*
	(14.33 ± 2.73)	(129.67 ± 15.96)	(155.25 ± 19.0)	(314.92 ± 30.49)	(372.98 ± 40.79)*
PPS	0.2307 ± 0.0190	0.1696 ± 0.0212	0.3299 ± 0.0623	0.5715 ± 0.0385	0.3232 ± 0.0108
	(14.93 ± 1.56)	(112.98 ± 14.81)	(127.84 ± 18.73)	(307.27 ± 24.43)	(324.09 ± 32.24)
PC	0.02498 ± 0.0290	0.1535 ± 0.0027	0.3794 ± 0.0348	0.4871 ± 0.0474	0.3419 ± 0.0226
	(16.28 ± 2.90)	(90.06 ± 7.17)	(132.40 ± 13.05)	(251.83 ± 25.79)	(318.14 ± 20.71)
Thymus					
PES	ND	ND	ND	0.4299 ± 0.0161	0.3974 ± 0.0314
				(244.78 ± 16.09)	(363.73 ± 27.39)
PPS	ND	ND	ND	0.3892 ± 0.0123	0.3796 ± 0.081
				(209.21 ± 12.85)	(355.83 ± 20.19)
PC	ND	ND	ND	0.4232 ± 0.0168	0.3881 ± 0.0189
				(218.86 ± 16.28)	(359.71 ± 22.08)

Values in parentheses are actual organ weights (mg; mean ± SEM). ND,
*Percent organ/body weight ratios were calculated by multiplying ratios by 100.
†Significantly different from PC offspring, $p < 0.05$.
‡Significantly different from PC offspring, $p < 0.01$.

nonstressed prenatal controls [$F(1, 36) = 6.68$, $p < 0.05$; Newman-Keuls: $p < 0.05$].

DISCUSSION

The normal ontogeny and functioning of the immune system requires the occurrence of a number of developmentally critical events such as specific cellular migrations, cell-cell interactions, cytodifferentiations, and functional maturation (70). As some of these events occur during gestation, it is reasonable to assume that prenatal exposure to chemicals or environmental protocols might cause a temporary or permanent disruption in the genesis or functioning of the immune system. Although we are unaware of any clinical studies that have addressed this issue, there is a substantial amount of experimental evidence with respect to the expression of immunocompetence in the offspring of females exposed to a variety of prenatal manipulations. However, the results of the present experiment provide the first experimental evidence that prenatal maternal stress can affect the development of the humoral component of the immune system in the offspring and alter the postnatal response of this system to stress.

Exposure of females during the last third of pregnancy to either environmental or psychological stress produced reductions in total serum IgG levels in the offspring immediately after birth; in addition, a persistent, biphasic reduction was evident but only in offspring of environmentally stressed females. Prenatal manipulation of maternal dietary constituents have also been shown to alter serum IgG levels and influence the number of Ig-positive cells per spleen in the offspring at 2 and 6 weeks of age (19). As total Ig levels are nonspecific indicators of immunocompetence (67,72), the present findings are also in general agreement with reports of impaired humoral immune response in rats exposed prenatally to lead acetate (47), methylmercury (79), and, more recently, cocaine (75).

The altered development of the immune system reported here did not appear to reflect toxic effects of prenatal stress in the female; gestational and birth statistics were unaffected by either of the stressors. Alterations in maternal care, or the postnatal nutritional status of the pups, as indexed by postnatal body weights, do not appear to be major factors mediating the changes in immune status of the offspring.

The alterations in IgG in the offspring may have resulted from a direct effect of prenatal stress on the ontogeny of B

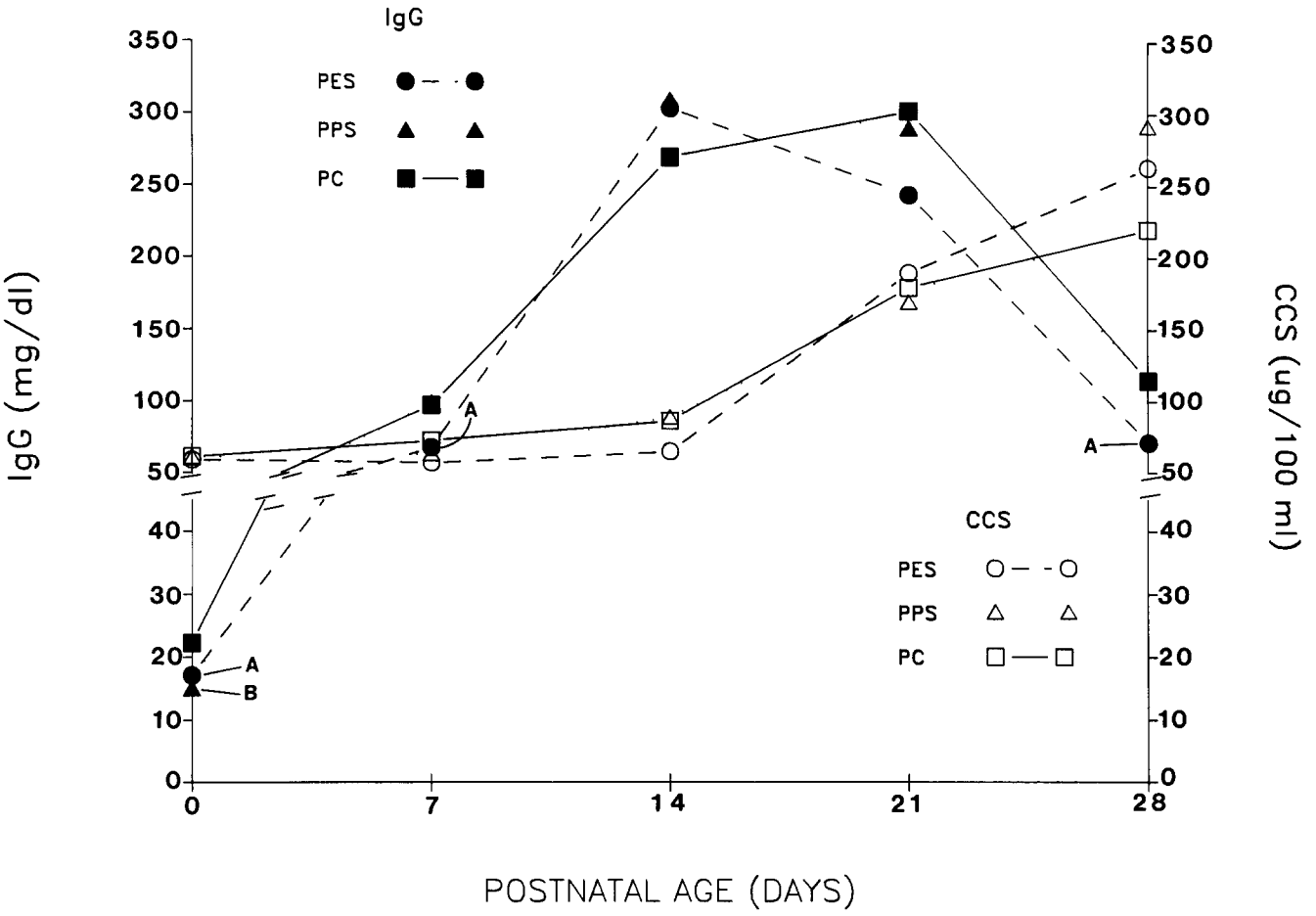

FIG. 2. Preweaning development of serum CCS and total serum IgG in offspring of PES, PPS, and PC females. Values shown are means. (A), PES offspring significantly different from PC offspring, $p < 0.05$; (B), PPS offspring significantly different from PPS offspring, $p < 0.05$.

TABLE 4

EFFECTS OF PNES ON SERUM CCS AND TOTAL SERUM IgG AT PND 34 (MEANS ± SEM)

	Serum CCS (μg/100 ml)		Serum IgG (mg/100 ml)	
	Nonstressed	PNES	Nonstressed	PNES
PES				
M	286.7 ± 17.5*	277.0 ± 17.0	79.12 ± 17.15†	202.5 ± 28.88‡
F	274.4 ± 16.6	319.9 ± 16.9§	210.83 ± 22.05§	31.50 ± 8.25‡
PPS				
M	305.9 ± 19.6	266.7 ± 20.6	150.87 ± 41.34	134.34 ± 15.88
F	319.7 ± 18.6	300.5 ± 18.6§	96.53 ± 27.42¶	84.83 ± 32.72
PC				
M	280.8 ± 18.8	296.3 ± 14.9	157.50 ± 7.63	101.72 ± 24.60‡
F	329.9 ± 40.1	346.2 ± 31.1§	228.30 ± 29.00§	161.67 ± 16.50‡

*Each value represents data from four animals.
†Significantly different from nonstressed PC males, $p < 0.05$.
‡Significantly different from nonstressed counterparts, $p < 0.05$.
§Significantly different from their male counterparts, $p < 0.05$.
¶Significantly different from nonstressed PC females, $p < 0.05$.

TABLE 5

BODY WEIGHTS (g) AND PERCENT ORGAN/BODY WEIGHT RATIOS* OF PRENATALLY STRESSED OFFSPRING EXPOSED TO PNES ON PND 29-33

	Prenatal Environmental Stress		Prenatal Psychological Stress		Prenatal Control	
	Nonstressed	PNES	Nonstressed	PNES	Nonstressed	PNES
Body Weights						
Males	137.92 ± 4.77	133.04 ± 5.11	127.95 ± 4.83	129.05 ± 5.02	132.82 ± 2.13	127.12 ± 5.66
Females	106.36 ± 3.50†	114.8 ± 6.09†	109.75 ± 2.09†	109.25 ± 3.53†	104.71 ± 4.47†	109.40 ± 2.85†
Adrenals						
Males	0.0175 ± 0.0010	0.0204 ± 0.0006	0.0206 ± 0.0002	0.0223 ± 0.0026	0.0175 ± 0.0009	0.0183 ± 0.0005
Females‡	0.0237 ± 0.0010	0.0219 ± 0.0017	0.0223 ± 0.0006	0.0206 ± 0.0009	0.0226 ± 0.0004	0.0235 ± 0.0013
Thymus						
Males	0.4110 ± 0.0227	0.3291 ± 0.0044§	0.3495 ± 0.0205	0.3240 ± 0.0158	0.3533 ± 0.0180	0.3437 ± 0.1060§
Females	0.3698 ± 0.0330	0.3550 ± 0.0232§	0.3574 ± 0.0526	0.3570 ± 0.0300	0.3984 ± 0.0232	0.3260 ± 0.0130§
Spleen						
Males	0.4220 ± 0.0355¶	0.3751 ± 0.0190	0.3590 ± 0.0213	0.4061 ± 0.0297	0.3790 ± 0.0200	0.3614 ± 0.0291
Females	0.4094 ± 0.0519¶	0.3723 ± 0.0197	0.4041 ± 0.0230	0.3594 ± 0.0230	0.3220 ± 0.0211	0.4090 ± 0.0133
Brain						
Males	1.2696 ± 0.0338	1.3425 ± 0.0447	1.3903 ± 0.0212	1.2848 ± 0.0406	1.2432 ± 0.0261	1.3644 ± 0.0308
Females‡	1.5791 ± 0.0551	1.4564 ± 0.0712	1.5564 ± 0.0430	1.5114 ± 1.028	1.5896 ± 0.0652	1.4911 ± 0.0371

*Percent organ/body weight ratios were calculated by multiplying the organ/body weight ratio by 100.
†Significantly different from their respective male counterparts, $p < 0.01$.
‡Significantly different from males, $p < 0.01$. (overall main effect).
§Significantly different from nonstressed counterparts, $p < 0.01$.
¶Significantly different from nonstressed offspring, $p < 0.05$.

lymphocytes. In mammals, immunoglobulin synthesis has been detected as early as GD 10 in the whole embryo (20,41). However, committed progenitor cells of the B lineage are not present until GD 12 and cytoplasmic Ig is first seen in large cells of the fetal liver on GD 13 (51). The number of B-cell precursors in the fetal liver increase exponentially between GDs 13 and 16, and cells with detectable membrane Ig appear in this organ between GD 16 and 17 (50,78). Prenatal stress could potentially disrupt B-cell ontogeny, preferentially, by a) interfering with the rearrangement or expression of B-cell receptor genes, b) altering the B lymphocytes' response to growth factors or inductive signals directing this differentiation, or c) interfering with the migration of mature B cells to peripheral lymphoid organs (34,55,67). Moreover, IgG may be selectively affected by prenatal insults. The latter hypothesis is further strengthened by the fact that IgG is the only Ig that crosses the placenta in both humans and laboratory rodents (78).

Alternatively, prenatal maternal stress may act indirectly through one of several mechanisms to alter immunocompetence in the offspring. As the majority of immunoglobulins present in the neonate are of maternal origin, the decrease in IgG levels evident at birth in PES and PPS offspring may reflect a stress-induced reduction in IgG of maternal origin rather than a direct effect on the developing immune system of the fetus. Although maternal IgG was not determined during the perinatal period in the present experiment because of the possibility of additional stress to the dams, previous research has shown that while the initial response to mild or moderate stress is activation of the immune system prolonged activation by PMS may deplete certain components of the system (46). This depletion, in turn, may have reduced the amount of IgG available for passive transfer from the mother to the fetus during gestation (89).

Another possibility is that the PND 0 decrease in IgG reflected altered intestinal absorption of milk-borne IgG by the newborn (84) induced by stress-related changes in corticosteroids. In the rat, corticosteroid administration has been shown to prematurely terminate the transmission of immunoglobulins and antibodies across the gut wall of the neonate (11). Transfer of IgG through the milk, which conveys passive immunity to the offspring, continues until the end of the third postnatal week (9,32). Therefore, the persistence of reduced IgG levels in PES offspring until PND 7 may again reflect either a maternal deficiency or altered transport mechanisms in the offspring. The continued appearance of a reduction in only PES progeny highlights the differential influences of various stressors on immune parameters. The fact that IgG levels are identical in PES, PPS, and PC offspring at PND 14 and 21 suggests that the decrease seen at 4 weeks of age in PES offspring represents a direct PMS-modulated effect on the developing immune system.

A fourth alternative is that prenatal stress, by altering maternal and/or fetal glucocorticoid levels (29,39,66,74), could have disrupted development of neuroendocrine tissue; a perturbation of the hypothalamic-pituitary-adrenal axis (HPAA) could affect postnatal lymphocyte function (55,82). A dynamic link exists between the immune and neuroendocrine systems (69). Hormonal changes have been shown to regulate, at least in part by a feedback mechanism, the magnitude and duration of immune responses (10). Prenatal dexamethasone has been reported to impair the antibody-forming capacity of T-cell-dependent antigens and cytolytic T-cell activity (18). However, the results of the present study do not support the

hypothesis that altered humoral immune status observed during development resulted from alterations in HPAA activity. Maturational changes in IgG did not coincide with the ontogenic changes in corticosterone levels in either stressed or nonstressed offspring.

The observed developmental changes in serum IgG provide information concerning the role of a component humoral immune system in providing protection in early life (48). Given the important role of this major class of immunoglobulins in immunocompetence, a decrease in IgG could compromise the host's defenses against environmental insult unless other humoral or cellular components of the immune response were sufficiently enhanced to counterbalance this effect (80). With respect to this hypothesis, the postnatal ages at which decreases in IgG levels were observed in PES progeny corresponded to those ages at which increases in spleen/body weight ratios were also apparent. Although splenocytes responses were not measured in the present experiment, one might speculate that these increases in spleen/body weight ratios reflect a compensatory increase in nonspecific immunity (67). However, recent evidence suggests that spleen weights are inversely correlated with functional activity; increased spleen and thymus weights were associated with decreased lymphoproliferative responses of splenocytes to the T-cell mitogens phytohemagglutinin and concanavalin A, the B-cell mitogen *Salmonella typhimurium* mitogen, and the T- and B-cell mitogen pokeweed mitogen in female rats following prenatal exposure to di-*n*-cotylyin Cl_2 on GD 10–20 (72).

The postnatal responsiveness of the HPAA to 5 days of environmental stress was assessed in prenatally stressed juvenile offspring by measuring changes in plasma CCS. Neither environmental nor psychological gestational stress altered the reactivity of the HPAA to PNES; increases in CCS were not observed in either PES or PPS postnatally stressed offspring when compared to their nonstressed counterparts. In addition, adrenal/body weight ratios were unaltered following postnatal stress exposure.

The results of previous studies are inconsistent with respect to the effects of gestational stress on HPAA reactivity. Handling (5) or conditioned avoidance training (65) of pregnant dams resulted in smaller increases in plasma corticosterone levels in the offspring than in prenatal controls when exposed to mild stress postnatally. In contrast, when saline injections were combined with handling of pregnant dams CCS levels were higher in PMS offspring after stress than in controls (61). An exaggerated HPAA response to a novel environment has also been reported for the male offspring of dams subjected to random noise stress during gestation (86).

Serum levels of CCS were not elevated in the stressed PC offspring. This suggests that hormonal adaptation may have occurred on or before the fifth exposure to the environmental stressor. Habituation of the HPAA has been reported to some forms of stress, but this adjustment is dependent upon the nature of the stressor and whether its application is given regularly or on a random basis (27,40,88). It should be noted, however, that this stress regime of a 5-day exposure to mild electric foot-shock did produce thymus atrophy in PES and PC offspring.

The immunologic consequences of exposing PMS offspring to postnatal stress was a function of both the type of prenatal stress manipulation and the sex of the offspring. In PC progeny, postnatal stress was immunosuppressive; both males and females exhibited lower IgG levels after 5 days of electric shock exposure. This immunosuppression was not evident in PPS offspring; IgG levels were the same as those observed in nonstressed PPS animals. In PES progeny, postnatal stress decreased IgG levels but only in females. It is interesting to note that this reduction occurred at a time when IgG levels in nonstressed PES female offspring were comparable to those of nonstressed PC female offspring. In contrast, postnatal stress did not further compromise the already depressed immune system status in PES males. Following electric footshock, IgG levels were elevated to levels seen in nonstressed PC males. Sexually dimorphic effects of prenatal manipulations have been reported not only for reproductive behaviors but for a variety of biochemical, morphological, and nonsexual behavior parameters (26,30,31,43,76,85). In addition, late-pregnancy stress can produce sex-specific effects on the fetal neuroendocrine system (66).

In general, prenatal environmental maternal stress was more effective than prenatal psychological maternal stress in altering immune status and reactivity to postnatal stress. However, PPS was not without effect on the offspring; it eliminated the sexual dimorphism in body weight usually seen in the third and fourth postnatal weeks. Moreover, PPS may eliminate or attenuate some aspects of the stress response. The changes in serum IgG levels and thymus/body weight ratios induced by postnatal stress in PES and PC offspring were not evident in PPS progeny. Psychological manipulations, similar to the procedure used in the present experiment, have been shown to evoke the behavioral and physiological changes characteristic of the stress response (12). However, it is possible that adaptation occurred to this mild stressor with repeated exposure, thereby eliminating the maternal hormonal response to stress (85) and its subsequent effects on the offspring.

These results provide the first experimental evidence that prenatal maternal stress can modulate immune parameters in the offspring and lend support to the CNS–immune system interaction hypothesis.

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