Transplacental Action of Diethylstilbestrol on Reproductive and Endocrine Organs, Mammary Glands, and Serum Hormone Levels in Two- and Nine-Month-Old Female Rats

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ABSTRACT

Aspects of morphology and endocrine function were studied in the reproductive tract and mammary glands of rats exposed prenatally to diethylstilbestrol (DES). Pregnant Sprague-Dawley rats received DES or vehicle on Days 15 and 18 of gestation. Female offspring exposed to DES or vehicle were assigned randomly to one of two experiments which differed in postnatal treatment. In Experiment 1, rats were intubated with either 10 mg of 7,12-dimethylbenz(a)anthracene or vehicle at 50 days of age and sacrificed 1 week later. DES-exposed animals showed significantly lower mean serum prolactin levels than did vehicle-exposed controls at this age; 7,12-dimethylbenz(a)anthracene treatment did not affect this difference associated with DES exposure. The two exposure groups did not differ significantly with respect to (a) levels of serum estrogen or progesterone; (b) wet weight of the uterus, ovaries, or pituitary; or (c) microscopic appearance of the uterus, ovaries, vagina, cervix, pituitary, and adrenal glands. In the mammary glands, ductal tissue predominated in both exposure groups, but three of 32 DES-exposed animals showed some lobuloalveolar differentiation. In Experiment 2, coincident with Experiment 1, rats from each exposure group were intubated with 10 mg of 7,12-dimethylbenz(a)anthracene at 50 days of age or given two doses of 10 mg at Days 50 and 57 and sacrificed 7 months later. The DES-exposed rats at 9 months of age had significant reductions in body weight gain compared to vehicle-exposed controls; an increased incidence of adrenal adenomata was observed among DES-exposed rats. No DES-associated differences were found with respect to serum prolactin; the wet weights of the uteri, ovaries, or pituitaries; the estrogen binding capacities of uterine cytosol; or the incidence of squamous metaplasia in uterine glands. In both exposure groups, there was variation in the morphology of the mammary gland and ovaries. Vaginal cytology at sacrifice confirmed that DES-exposed rats had similar proportions of animals in the different stages of the estrus cycle. The persistent vaginal cornification and increased incidence of other reproductive tract abnormalities (e.g., vaginal adenosis and gonadal hypogenesis) associated with neonatal estrogenization in rodents were not observed in rats exposed prenatally to this low dose of DES during the third week of gestation. Thus, the Sprague-Dawley rat is a useful model system in which to study the effects of prenatal exposure to low doses of DES on mammary gland development and carcinogenesis against a background of relatively normal reproductive tract structure and function.

INTRODUCTION

The morphogenesis and function of the rodent reproductive tract can be altered substantially by natural and synthetic estrogens administered either prenatally or neonatally. Various strains of mice have been used to determine the effects of prenatal exposure to the synthetic estrogen, DES. The results ranged from an increase of urogenital anomalies and impaired fertility to vaginal adenosis; uterine squamous metaplasia; and tumors of the ovaries, uterus, vagina, and lung (18-20, 24-26, 37). The incidences varied according to the dose and the time of exposure during gestation. When pregnant mice were given injections of estradiol (Day 15 or 17) or were given ethinyl estradiol p.o. (Days 11 to 17), their female offspring displayed persistent vaginal cornification (16, 40-42). Treatment of mouse neonates with various estrogens for the first 5 days after birth was associated with hyperplasia and cornification of the vaginal epithelium; if high doses were used, the cornification could not be altered by ovariectomy, i.e., ovarian-independent vaginal cornification (33-34, 42). Various other cervicovaginal abnormalities, including vaginal adenosis, have been found in mice treated neonatally with DES and natural estrogens (1, 10, 14, 15, 27, 28). For reviews of the effects of hormonal steroids on the newborn mouse, refer to Refs. 3, 4, and 17.

In the rat, prenatal exposure to or neonatal treatment with high doses of DES or estradiol also resulted in "permanent estrus" (23, 35, 36). In addition, such doses increased the incidence of female hypospadias, gonadal hypogenesis, and tumors of the ovaries and endometrium in adults (23, 30, 36) and caused grossly enlarged uteri in newborns (11, 12). Squamous metaplasia of the uterine epithelium (luminal and glandular) and structural anomalies of the cervix were observed in 3- to 5-month-old rats treated neonatally with DES (9). We have reported impaired fertility and increased reproductive tract abnormalities among female offspring of rats treated with 120 μg of DES during the third week of gestation (5). Lower doses (1.2 μg of DES) during the second or third weeks of gestation resulted in normal fertility and morphology of the reproductive organs in animals 4 to 5 months of age. Here, we report our continued efforts to study the effects of prenatal exposure to DES on reproductive tract structure and function in the rat. In order to correlate these data on the morphology of the reproductive organs and mam-
mary glands with other endocrine parameters, the pituitary and adrenal glands have been examined microscopically, serum hormone levels have been determined, and the estrogen binding capacities of representative uteri have been calculated. Data on the induction of mammary tumors in the DES-exposed rats used in the study reported here are presented in a companion paper (7).

MATERIALS AND METHODS

Animals and Animal Care

Noninbred rats (CD strain; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were maintained in temperature- and light-controlled animal quarters (12-hr light, 6 a.m. to 6 p.m.) and were supplied with tap water and Purina rat chow (Ralston-Purina Co., St. Louis, Mo.) ad libitum. Bedding material consisted of hard wood shavings (Beta-Chips; Northeastern Products Corp., Warrensburg, N. Y.).

Prenatal Exposure

Virgin female rats (2.5 months old) were placed with males of the same age, 3 to 4 females and one male per cage. The presence of sperm in a vaginal smear taken the following morning was used to designate Day 0 of pregnancy. Pregnant animals were assigned randomly to 2 treatment groups: 18 rats were given injections s.c. of DES (Sigma Chemical Co., St. Louis, Mo.) dissolved in sesame oil on Days 15 and 18 (2 injections of 0.6 μg in 0.3 ml of sesame oil = 1.2 μg of DES, total dose); 10 rats received 2 injections of vehicle only. Animals delivered and raised their offspring to weaning. Large litters were reduced to 10 pups by removing male offspring. Female offspring were separated from the dams 28 days following delivery and housed 5 to a cage; dams were sacrificed by decapitation to obtain serum for hormone assays. Representative offspring from each exposure group were examined daily from Day 32 to detect the time of vaginal opening; they were also used in the analysis of vaginal smears taken on Days 46 through 49 and in the determination of body weight on Day 47.

Postnatal Treatment

Female offspring from both exposure groups were ear tagged and assigned randomly to groups which differed in postnatal carcinogen treatment and the age at which animals were sacrificed. Experiment 1: 2-Month-Old Rats. Rats of both prenatal exposure groups received one gastric intubation of 10 mg of DMBA (Eastman Organic Chemicals, Rochester, N. Y.) in 1 ml of sesame oil or oil alone at 50 days of age. DMBA was prepared the afternoon before use to permit complete solubilization; the solution was kept in a foil-wrapped container to prevent photodegradation. One week later, when rats were 2 months old, they were sacrificed by decapitation; blood was collected for hormone assays; and a vaginal smear was obtained.

At autopsy, mammary gland tissue, reproductive organs, adrenal glands, and the pituitary were removed and fixed in 10% neutral buffered formalin, dehydrated through tetrahydrofuran, embedded in paraffin, sectioned at 7 to 10 μm, and stained with hematoxylin and eosin. Another sample of mammary tissue was prepared as a whole mount; this tissue was fixed between glass slides in 70% ethanol and stained in alum carmine prior to dehydration through a graded ethanol series and storage in toluene (modification of technique from Ref. 2).

Experiment 2: 9-Month-Old Rats. Other DES-exposed and vehicle-exposed rats were treated with DMBA and maintained until 9 months of age. One-half of each exposure group received one gastric intubation of DMBA (10 mg in 1 ml of sesame oil) at 50 days of age, while the other half received 2 separate 10-mg intubations of DMBA at 50 and 57 days of age. Palpation to detect mammary tumors continued weekly until animals were sacrificed at 9 months of age. Prior to decapitation, each animal was weighed. Blood was collected, and serum was prepared for hormone assays; a vaginal smear was examined. A portion of one uterine horn was frozen in liquid nitrogen for subsequent determination of estrogen binding capacity. Tissues were removed, fixed, and processed as described earlier. DES-exposed and vehicle-exposed rats receiving no postnatal treatment with DMBA were not included in this experiment, since no palpable mammary tumors were found in either exposure group at 9 months of age in an earlier experiment (6).

Serum Preparation and Assay of Serum Hormones

Blood collected immediately after decapitation was placed in plastic tubes on ice and centrifuged at 1650 × g for 10 min in an IEC (International Equipment Co., Needham Heights, Mass.) Centra 7R refrigerated centrifuge. Serum was removed and stored at −80°C until assayed. Prolactin levels were determined by competition for binding of 125I-prolactin to crude lactating rat hepatic membranes using a standard radioreceptor assay as described (31). Serum samples were compared to rat prolactin and ovine prolactin standards obtained from the hormone distribution program of the National Institute of Arthritis and Digestive Diseases. Estrogen and progesterone were determined using the New England Nuclear RIA-PAK with highly specific antibodies for the respective steroids. All aspects of the assay were according to the manufacturer's directions except that the antigen-antibody complexes were precipitated with Bio-Rad goat anti-rabbit IgG immunobeads as described (39).

Statistical Methods

In these analyses, the experimental unit was the litter. All statistical tests were calculated using transformed litter means as data. This procedure takes advantage of the central limit theorem in that sample means tend to more nearly approximate a normal distribution than do the original data. The litter means were transformed to their positive square root to compensate for skewness. All main effects were tested with a one-way analysis of variance (or analysis of covariance) for unequal sample sizes. This method was preferred to a weighted 2-way analysis of variance because it was more conservative.

RESULTS

Pregnant rats given injections of 1.2 μg of DES during the third week of gestation delivered 185 offspring in 18 litters; there were 112 offspring in 10 litters among the vehicle-treated controls. There was no significant difference between the DES-exposed and the vehicle-exposed groups in number of pups per dam, proportion of female pups per litter, or average weight of the female pups at Day 8. DES-treated and vehicle-treated dams did not differ significantly in the level of serum prolactin [336.8 ± 56.9 (S.D.)] ng/ml versus 364.4 ± 73.9 ng/ml, respectively] at sacrifice 26 days postpartum. Prior to the time when DMBA was administered, some parameters of general and sexual development were determined from 32 DES-exposed and 30 vehicle-exposed animals. Daily examination beginning at Day 32 revealed that prenatal exposure to
DES did not influence the mean time of vaginal opening (DES exposed, 36.9 ± 3.2 days, versus vehicle exposed, 36.1 ± 3.1 days). When the 2 groups were compared with respect to the number of vaginal smears judged to be in estrus over a 4-day period (Days 46 to 49), no significant difference was observed (Table 1). Also, body weights taken at 7 weeks of age did not differ between the 2 exposure groups (Table 2).

**Effect of DES Exposure on 2-Month-Old Rats.** Two-month-old rats (19 DES exposed from 6 litters and 19 control from 4 litters) were sacrificed to determine whether consequences of DES exposure could be identified at this early age when Sprague-Dawley rats are maximally sensitive to the carcinogenic action of DMBA (29). About one-half of the rats in each exposure group were treated with 10 mg of DMBA 1 week prior to sacrifice. DES-exposed rats had average serum prolactin levels which were significantly lower than the vehicle-exposed controls (Table 3); treatment with DMBA 1 week prior to sacrifice had no effect on these values. No significant differences were found in the levels of estrogen or progesterone attributable to prenatal hormone exposure (Table 3). Treatment with DMBA 1 week prior to sacrifice had no significant effect on levels of serum estrogen; however, serum progesterone was elevated significantly in DMBA-treated rats (Table 3). No significant differences were found between the 2 exposure groups for the wet weights of the uterus, ovaries, or pituitary (Table 4). In both the DES-exposed and vehicle-exposed groups, average uterine weight was reduced by approximately 100 mg as a result of DMBA treatment, a highly significant difference (Table 4).

There were no consistent differences in the degree of mammary gland development between the DES-exposed and vehicle-exposed groups; glands from rats treated 1 week earlier with DMBA were indistinguishable from those of control rats. In both exposure groups, whole mounts of most glands showed moderate ductal branching and lateral budding, with a few glands in each group characterized by marked ductal proliferation and extensive budding. Examination of the histological sections revealed marked lobuloalveolar proliferation in one DES-exposed animal (Fig. 1) with a minor degree of lobuloalveolar development seen in 2 other DES-exposed animals. Glands of the remaining animals were characterized by moderate ductal proliferation and extensive budding. Examination of representative sections of the uterine horns, vagina, cervix, and ovaries did not reveal any abnormalities associated with prenatal exposure to DES. Squamous metaplasia was seen in a uterine gland in one vehicle-exposed rat. Ovaries from rats in both exposure groups contained a combination of follicles and corpora lutea, indicative of the attainment of sexual maturity and normal cycling. Vaginal smears performed prior to autopsy revealed that all stages of the estrus cycle were represented in both exposure groups, with 50 to 60% of the animals in proestrus-estrus. Upon histological examination, no proliferative abnormalities of the adrenal cortex or medulla were found in either exposure group. Cortical necrosis appeared only in DMBA-treated animals (4 of 9 in each exposure group). All pituitary

### Table 1
Patterns of estrus determined from vaginal smears taken 4 consecutive days prior to intubation with DMBA

<table>
<thead>
<tr>
<th>Prenatal exposure</th>
<th>No. of animals in estrus</th>
<th>0/4 days</th>
<th>1/4 days</th>
<th>2/4 days</th>
<th>3/4 days</th>
<th>4/4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES (n = 32)</td>
<td>2 (30)</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 30)</td>
<td>0</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Number of animals with vaginal smears judged to be in estrus after daily observations over a 4-day period (Days 46 to 49).

### Table 2
Mean body weights of rat litters exposed to DES or vehicle

<table>
<thead>
<tr>
<th>Prenatal exposure</th>
<th>Body wt (g)</th>
<th>At 7 wk of age</th>
<th>At 9 mo of age or death</th>
<th>WT gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES (n = 31)</td>
<td>174.3 ± 10.3</td>
<td>344.9 ± 38.2</td>
<td>170.8 ± 29.6d</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 28)</td>
<td>173.9 ± 14.0</td>
<td>383.1 ± 30.7</td>
<td>219.2 ± 37.2</td>
<td></td>
</tr>
</tbody>
</table>

* Body weight taken 1 day prior to intubation with 10 mg of DMBA; rats were 7 weeks old.

### Table 3
Litter means of serum hormone levels in 2-month-old rats exposed prenatally to DES or vehicle

<table>
<thead>
<tr>
<th>Prenatal exposure</th>
<th>Serum prolactin (ng/ml)*</th>
<th>Serum estrogen (pg/ml)*</th>
<th>Serum progesterone (ng/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DMBA</td>
<td>231.8 ± 99.7f</td>
<td>463.9 ± 55.6</td>
<td>10.8 ± 3.5</td>
</tr>
<tr>
<td>10 mg DMBA</td>
<td>232.1 ± 57.8</td>
<td>492.3 ± 105.3</td>
<td>15.8 ± 4.7</td>
</tr>
</tbody>
</table>

* Overall mean of litters in each exposure group.

### Table 4
Mean organ weights of 2-month-old rat litters exposed prenatally to DES or vehicle

<table>
<thead>
<tr>
<th>Prenatal exposure, postnatal treatment</th>
<th>Organ wt (g)</th>
<th>Uterus</th>
<th>Ovaries</th>
<th>Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES, none</td>
<td>0.378 ± 0.026</td>
<td>0.108 ± 0.012</td>
<td>0.010 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>DES, DMBA</td>
<td>0.288 ± 0.053</td>
<td>0.094 ± 0.011</td>
<td>0.009 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>All DES</td>
<td>0.333 ± 0.040</td>
<td>0.101 ± 0.013</td>
<td>0.009 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Vehicle, none</td>
<td>0.383 ± 0.100</td>
<td>0.098 ± 0.020</td>
<td>0.008 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Vehicle, DMBA</td>
<td>0.295 ± 0.034</td>
<td>0.102 ± 0.008</td>
<td>0.009 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>All vehicle</td>
<td>0.339 ± 0.068</td>
<td>0.099 ± 0.014</td>
<td>0.009 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were exposed to DES or vehicle during gestation; one-half of each exposure group was then treated with 10 mg of DMBA at 7 weeks of age. All animals were sacrificed at 8 weeks of age.

16 DES-exposed rats and 19 vehicle-exposed rats examined were characterized by moderate ductal branching (Fig. 2). Examination of representative sections of the uterine horns, vagina, cervix, and ovaries did not reveal any abnormalities associated with prenatal exposure to DES. Squamous metaplasia was seen in a uterine gland in one vehicle-exposed rat. Ovaries from rats in both exposure groups contained a combination of follicles and corpora lutea, indicative of the attainment of sexual maturity and normal cycling. Vaginal smears performed prior to autopsy revealed that all stages of the estrus cycle were represented in both exposure groups, with 50 to 60% of the animals in proestrus-estrus. Upon histological examination, no proliferative abnormalities of the adrenal cortex or medulla were found in either exposure group. Cortical necrosis appeared only in DMBA-treated animals (4 of 9 in each exposure group). All pituitary
glands examined (19 DES exposed, 10 vehicle exposed) appeared normal, except for one case in the DES-exposed group, where a fluid-filled cyst covered the entire surface of the posterior lobe of the pituitary.

**Effects of DES Exposure on 9-Month-Old Rats.** All rats received one or 2 doses of DMBA beginning at 7 weeks of age. Contrasts were made between DES-exposed, DMBA-treated rats and vehicle-exposed, DMBA-treated rats. Data on mammary tumor incidence in these groups are presented in a companion paper (7).

While the DES-exposed animals and the controls did not differ in weight at birth or at 7 weeks of age, 9-month-old DES-exposed rats treated with 10 mg of DMBA weighed less than their controls, resulting in a significant reduction in weight gain (22% less) between 2 and 9 months of age compared to weight gain of controls (Table 2). Prenatal exposure to DES was not associated with a change in mean serum prolactin levels compared to controls (Table 5), in contrast to the decreased serum prolactin found in DES-exposed rats sacrificed at 2 months of age (Table 3). An effect of the dosage of DMBA was evident on the mean serum prolactin values in the 9-month-old animals with those rats treated with 2 doses of DMBA (10 mg each) having higher mean serum prolactin levels compared to those receiving a single 10-mg dose of DMBA (Table 5).

The following data on organ weights, morphology, and uterine estrogen binding capacity were obtained from 32 DES-exposed rats from 7 litters and 30 vehicle-exposed rats from 4 litters; all were treated postnatally with a single 10-mg dose of DMBA. Wet weights of various organs from DES-exposed and vehicle-exposed groups were not significantly different (uterus, 585 versus 673 mg; ovaries plus oviducts, 1.10 versus 1.13 g; pituitary, 170 versus 153 mg). Compared to mammary tissue sections of 2-month-old rats (characterized by a moderate ductal pattern), mammary tissue from these older animals was much more variable in the degree of glandular development. In both the DES-exposed and vehicle-exposed groups, approximately 60% of the mammary glands showed a moderate degree of differentiation, some with lobuloalveolar proliferation; 20 to 25% in each group were atrophic, while 15 to 20% had extensive ductal and lobuloalveolar proliferation, often with secretory activity evident. Whereas all rats examined at 2 months of age had both follicles and corpora lutea evident in representative sections of the ovaries, the 9-month-old animals displayed more variability in ovarian morphology. Among the DES-exposed rats, 50% of the ovaries contained a predominance of follicles, 19% had both follicles and corpora lutea, and 31% were composed primarily of corpora lutea; in the vehicle-exposed group, comparable figures were 47% for follicles, 36% for follicles and corpora lutea, and 17% for corpora lutea. Another reflection of the persistence of ovarian activity in these 9-month-old animals was that vaginal smears taken at necropsy were in all stages of the estrus cycle: about 60% were in proestrus-estrus, and 40% were in metestrus-diestrus in both DES-exposed and vehicle-exposed groups.

Cross-sections of the vagina and uterine horns and longitudinal sections of the cervix were studied. Of 28 slides evaluable in the DES-exposed group, one showed a large hemangioma protruding into the cervical canal near the squamocolumnar junction, and one had a patch of squamous metaplasia in the anterior cervical epithelium and also showed squamous metaplasia of uterine glands. However, such abnormalities were also seen among vehicle-exposed rats: 4 showed squamous metaplasia within the anterior cervix; and one other had squamous metaplasia of the uterus. These cases of squamous metaplasia are probably related to postnatal treatment with DMBA, since we found no instances of squamous metaplasia in the uterus of 25 untreated Sprague-Dawley rats maintained in our facility until they were 9 months old (historical controls). The mean concentration of specifically bound uterine 17β-estradiol calculated from Scatchard plots did not differ significantly between the prenatal exposure groups (271.0 ± 59.4 fmoI/mg cytosol protein in 10 uteri representing 6 DES-exposed litters and 197.1 ± 77.8 fmoI/mg cytosol protein in 9 uteri of 4 vehicle-exposed litters). Uteri from both groups showed high-affinity binding, with a range of Kd from 1.0 × 10⁻¹⁰ to 9.1 × 10⁻¹¹ M.

Adrenals in both exposure groups showed evidence of cortical necrosis, usually in the zona fasiculata; this ranged in severity from minor, focal degeneration to extensive areas of hemorrhage and necrosis. Some necrosis was observed in 16 of 29 evaluable slides in the vehicle-exposed group and in 15 of 28 slides in the DES-exposed group. The incidence of cortical adenoma, however, did differ when the prenatal exposure groups were compared, with 8 adrenals in the DES-exposed group showing at least one adenoma, while the controls contained only 4 adrenals with adenomata (p = 0.01). No pituitary lesions were observed on 24 evaluable slides in the DES-exposed group and 11 slides in the controls.

**DISCUSSION**

We demonstrated previously that the transplacental protocol under study (1.2 μg of DES in the third week of gestation) did not affect the fertility of female rat offspring (5). Results presented here show that prenatal exposure to DES did not affect (a) neonatal or peripubertal body weight of female offspring, (b) time of vaginal opening, or (c) pattern of the estrus cycle at about 7 weeks of age. Thus, we are dealing with a system which differs substantially from those where pre- or neonatal estrogen treatment results in persistent vaginal cornification and loss of ovarian cyclicity (22, 33-35).

At 2 months of age, the DES-exposed and vehicle-exposed rats were treated postnatally with a single 10-mg dose of DMBA (Table 5).
groups differed with respect to serum prolactin with significantly lower serum prolactin levels found in the DES-exposed group than in the controls. This is in contrast to neonatally estrogenized mice where 2-month-old animals in metestrus or diestrus had higher serum prolactin than did controls, while no difference was observed between groups when the mice were in proestrus–estrus (22). The levels of prolactin found in these DES-exposed rats at 2 months of age must, however, be sufficient to permit the growth of mammary tumors after DMBA treatment, since the combination of prenatal exposure to DES with postnatal DMBA treatment resulted in a higher incidence of palpable mammary tumors and decreased tumor latency compared with DMBA-treated rats lacking prior hormone exposure (6, 7). Nagasawa et al. (21) concluded that normal levels of serum prolactin must be adequate to support the growth of DMBA-induced mammary tumors, since they found that the levels of serum prolactin did not vary significantly from normal levels at various stages after DMBA treatment. Also relevant are the findings of Welsch et al. (38), demonstrating that, while hyperprolactinemia enhanced mammary carcinogenesis in DMBA-treated male rats, rats which were made hypoprolactinemic by CB-154 treatment did not have significantly fewer tumors than did controls. Adrenal cortical necrosis was observed only in animals which had been treated 1 week earlier with DMBA, in accord with the known effects of DMBA on this organ (6, 13).

Analysis of slides of mammary gland tissue from 2-month-old rats revealed that lobuloalveolar proliferation was present only in the DES-exposed group, albeit at low frequency (3 of 32). It was difficult to assign discrete categories to describe the state of mammary gland differentiation so that intergroup differences could be analyzed statistically. Those grading schemes attempted, however, did not yield statistically significant differences between exposure groups. One might have expected greater differences in the degree of mammary gland differentiation to be evident at this stage, since more mammary tumors were induced by DMBA in rats exposed prenatally to DES than in DMBA-treated animals without transplacental hormone exposure (6, 7).

Among the 9-month-old rats treated with DMBA, prenatal exposure to DES was associated with significantly reduced body weight gain compared to controls. Since differences in body weight were not observed at 2 months of age or at birth, this must reflect a failure to gain weight at a rate equivalent to controls between 2 and 9 months of age. Although there were significantly more palpable mammary tumors per rat in the DES-exposed group treated postnatally with 10 mg of DMBA, the actual weight of all palpable mammary tumors per tumor-bearing rat at sacrifice was not significantly different in the DES-exposed group (12.7 ± 17.8 g) than in controls (15.6 ± 14.7 g); also, the percentage of total body weight represented in wet weight of actual weight of all palpable mammary tumors per tumor-bearing rat was not significantly different in the DES-exposed group (12.7 ± 17.8 g) than in controls (15.6 ± 14.7 g); also, the percentage of total body weight represented in wet weight of mammary tumors, since they found that the levels of serum prolactin did not vary significantly from normal levels at various stages after DMBA treatment. Also relevant are the findings of Welsch et al. (38), demonstrating that, while hyperprolactinemia enhanced mammary carcinogenesis in DMBA-treated male rats, rats which were made hypoprolactinemic by CB-154 treatment did not have significantly fewer tumors than did controls. Adrenal cortical necrosis was observed only in animals which had been treated 1 week earlier with DMBA, in accord with the known effects of DMBA on this organ (6, 13).

Nine-month-old animals displayed more variation in mammary gland and ovarian morphology than did animals examined at 2 months of age in both prenatal hormone exposure groups. Of particular interest was the fact that the older DES-exposed animals retained significant ovulatory activity, evidenced by the presence of corpora lutea in approximately one-half of the animals and by the presence of rats with vaginal smears representing all phases of the estrus cycle in both prenatal exposure groups.

In sum, while there were some differences noted between the DES-exposed and vehicle-exposed groups in terms of serum prolactin levels, body weight gain, and incidence of adrenal adenomatous, most aspects of the development and anatomy of the reproductive tract, endocrine organs, and mammary glands analyzed here were not demonstrably affected by prenatal exposure to a low dose of DES at the 2 ages examined. Since prenatal exposure to DES is associated with significant increases in mammary tumor incidence in rats treated postnatally with DMBA (6, 7), these data demonstrate that this system provides a special opportunity to study the effects of transplacental exposure to a hormone on mammary carcinogenesis in the absence of major abnormalities in reproductive tract structure or function.

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Fig. 1. Mammary gland from a 2-month-old rat exposed prenatally to DES showing marked lobuloalveolar proliferation. Note the larger size of the adipose cells compared to those of a vehicle-exposed control (Fig. 2). H & E, × 100.

Fig. 2. Mammary gland from a 2-month-old animal exposed prenatally to vehicle; moderate ductal development is present with no evidence of alveolar differentiation or secretion. H & E, × 100.
Transplacental Action of Diethylstilbestrol on Reproductive and Endocrine Organs, Mammary Glands, and Serum Hormone Levels in Two- and Nine-Month-Old Female Rats

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