Vaginal Adenosis and Adenocarcinoma in Mice Exposed Prenatally or Neonatally to Diethylstilbestrol

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ABSTRACT

The association of intrauterine exposure to diethylstilbestrol (DES) and the subsequent development of reproductive tract abnormalities in young women has been well documented. Although the incidence of vaginal adenocarcinoma was low in the exposed population, vaginal adenosis, a nonmalignant abnormality, was quite common. In order to study the pathogenesis of adenocarcinoma and to determine the frequency of adenosis following prenatal exposure to DES, timed pregnant CD-1 mice were treated s.c. with DES (dose range, 5 to 100 \( \mu g/kg/day \)) on Days 9 through 16 of gestation. This period corresponds to major organogenesis of the reproductive tract in the mouse. Female offspring were sacrificed between 1 and 18 months of age. In addition to nonmalignant abnormalities, some of which have been described in women exposed prenatally to DES, two cases of vaginal adenocarcinoma (2%) were observed in 91 prenatally DES-treated animals. No comparable epithelial lesions were seen in 158 control female mice. One other case of adenocarcinoma of the vagina was reported previously by this laboratory using the prenatally exposed animal model.

In another series of mice treated prenatally with DES, 100 \( \mu g/kg/day \), 3 of 20 (15%) 1-month-old animals and one of 10 (10%) 18-month-old treated offspring had glandular epithelium abnormally located in the vaginal fornices (adenosis). Other cervicovaginal abnormalities observed after prenatal DES exposure included structural alterations, cervical enlargement, squamous metaplasia in the endocervical canal, excess keratinization of the ectocervix and vagina, transverse folds and basal cell hyperplasia in the upper vagina, and prominent Wolffian duct remnants. Thus, vaginal adenosis in the mouse does not appear to be a common abnormality following treatment with DES in utero. Neonatal exposure to DES on Days 1 to 5, on the other hand, resulted in six of eight (75%) animals with adenosis at 35 days of age. Since perinatal mouse studies have reported high incidences of vaginal adenosis but, to our knowledge, no cases of vaginal adenocarcinoma, the results presented in this report suggest that the stage of cellular differentiation at the time of DES exposure may be critical in the final expression of these abnormalities.

INTRODUCTION

The long-term consequences of transplacental exposure to DES\(^2\) on the developing female fetus were originally described by Herbst et al. in their report of clear cell adenocarcinoma in adolescent women (17, 19). Although the incidence of adenocarcinoma is low in these DES-exposed females, other genital abnormalities are common. These include the presence of glandular epithelium or its mucinous products in the vagina (vaginal adenosis) or in the portio vaginalis of the cervix (cervical ectropion). Vaginal adenosis and cervical ectropion are abnormalities observed in unexposed females, including fetuses and neonates (21); however, they are encountered more often and to much greater extent in prenatally DES-exposed women (34).

The frequency of adenosis in women according to several studies (2, 18, 36) ranged from 35 to almost 90% of the exposed population. In addition to this reported high incidence, interest in this lesion lies in the hypothesis several investigators have developed that vaginal adenosis is a premalignant stage of adenocarcinoma (3, 14, 20, 34). In a recent report, Antonioli et al. (1) suggested a possible relationship between the 2 lesions by showing transitional states between adenosis and adenocarcinoma in the upper portion of the vagina of prenatally DES-exposed human females.

Reports from this laboratory have described an animal model using the CD-1 mouse to evaluate the transplacental effects of DES (23–26) including the long-term effects on the female mouse genital tract such as vaginal adenocarcinoma; only one case of vaginal adenosis was demonstrated in these prenatally DES-exposed mice (26). Until Plapinger and Bern (30) reported adenosis-like lesions in the cervicovaginal region of several strains of mice, DES- or estradiol-associated vaginal adenosis was thought to be confined to the NMRI mouse (11).

The present study was undertaken to provide information on the incidence of adenosis and the relationship of this lesion to adenocarcinoma in the transplacentally DES-exposed mouse as well as to compare the incidence of neonatally induced adenosis in the CD-1 mouse strain used in this study with that already published (26).

MATERIALS AND METHODS

Outbred CD-1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and were bred to male mice of the same strain in the animal facility at the National Institute of Environmental Health Sciences. Vaginal plug detection was considered Day 0 of pregnancy. Pregnant mice were housed separately in a room with controlled temperature (21–22\(^\circ\)) and lighting (14-hr light and 10-hr dark periods) and were provided with synthetic bedding, fresh water, and NIH 31 laboratory mouse chow \textit{ad libitum}.

Prenatally DES-treated Animal Experiments. Pregnant female mice were given s.c. injections of DES (Sigma Chemical Co., St. Louis, Mo.) dissolved in corn oil on Days 9 through 16 of gestation. The daily dose of DES was 100 \( \mu g/kg \) body weight; the offspring of these animals are referred to in this report as DES-100. The total volume of corn oil administered was 0.01 ml/g maternal body weight. Purity of the DES was checked by thin-layer chromatography, high-pressure liquid chro-
matography, and gas chromatography-mass spectrometry and exceeded 99% (27).

Pregnant mice delivered their young, and all litter sizes were randomly reduced to 8. At 25 days of age, the mice were weaned and housed in groups of 5/cage. At 1 month (20 control and 20 DES-100) or 18 months of age (10 control and 10 DES-100), female offspring were sacrificed by cervical dislocation. Only one to 2 mice per litter were included in each group. Reproductive tract tissues were removed, fixed, embedded, and serially sectioned according to the method described by Plapinger and Bern (30). Briefly, a transverse cut was made through the cranial cervix approximately 0.5 mm caudal to the junction of the uterine horns. The portion of the reproductive tract caudal to that cut was fixed in Bouin’s solution. The fixed tissue was embedded in paraffin and 6-μm serial transverse sections were made, starting at the cranial end and stopping after approximately three-fourths of the tissue was sectioned. This procedure provided serial sections through the cervical canal, vaginal fornices, and upper portions of the vaginal canal. A light microscope was used to evaluate hematoxylin and eosin-stained sections of reproductive tract as well as representative sections of liver, lung, kidney, adrenal, and spleen. Cervical measurements were made as described previously (26).

An additional series of pregnant animals was similarly exposed to daily s.c. DES doses of either 5, 10, or 100 μg/kg of body weight on Days 9 to 16 of gestation. Their offspring are referred to as DES-5, DES-10, and DES-100, respectively. Animals sacrificed included 158 controls (72 six- to 11-month-old mice, 53 twelve to 18 months old, 33 older than 18 months), 29 DES-5 (zero to 11 months old, 26 twelve to 18 months old, 3 older than 18 months), 16 DES-10 (zero 6 to 11 months old, three 12 to 18 months old, 13 older than 18 months), and 46 DES-100 (19 six to 11 months old, 17 twelve to 18 months old, 10 older than 18 months).

The reproductive tract tissues of these animals were fixed in 10% neutral buffered formalin, embedded in paraffin, and serially sectioned at 6 μm as described previously (26). In this study, the vagina and cervix were cut in the midsagittal plane. Both halves were embedded in the same block and were serially sectioned sagittally for 10 sections starting at the sagittal midline. If a microscopic lesion was observed, additional serial sections were made to include the entire area of pathological change. In addition to reproductive tract, representative sections of liver, lung, kidney, adrenal, and spleen were stained with hematoxylin and eosin.

Neonatally DES-treated Animal Experiments. Eighteen untreated pregnant female mice, housed individually, delivered their young on Day 19 of gestation. All litter sizes were adjusted to 8 at birth. Ten litters were untreated (control), and 8 litters were given DES dissolved in corn oil as a s.c. injection on Days 1 through 5 of neonatal life (2 μg/pup/day in 20 μl corn oil). These animals were maintained and weaned by the same schedule described for the prenatally exposed females. The offspring of these treated mice are designated DES-50, DES-100, and DES-10, respectively. Animals sacrificed included 158 DES-50 (19 six to 11 months old, 17 twelve to 18 months old, 10 older than 18 months), 16 DES-10 (zero 6 to 11 months old, three 12 to 18 months old, 13 older than 18 months), and 46 DES-100 (19 six to 11 months old, 17 twelve to 18 months old, 10 older than 18 months).

RESULTS

Cervicovaginal Changes in Female Mice Exposed Prenatally to DES. The genital tract of prenatally DES exposed females had numerous cervicovaginal abnormalities in 1- and 18-month-old animals. The prevalence of abnormalities of the vagina ranged from 0 among control mice at 1 month and 20% at 18 months to 85% at 1 month and 90% at 18 months of age in the prenatally DES-100 exposed females (Table 1). The abnormalities in control animals at 18 months were consistent with usual age-related changes (26) whereas the changes in the DES-100 animals at 1 and 18 months were not. These DES-related changes included prominent Wolffian duct remnants (Fig. 1) observed in serial cross-sections of the cervicovaginal region. The incidence of these remnants in 1-month-old animals was 11 in 20 and in 18-month-old animals was 3 in 10; the ductal remnant in one of the 18-month-old animals was cystic. In contrast, no prominent Wolffian remnants were observed in this series of control females.

Another common feature observed in these mice after in utero exposure to DES was excessive vaginal keratinization. The squamous epithelium throughout the vagina of most DES-100 mice was composed of many layers of immature and mature cells covered by sheets of keratin. Leukocytes were commonly seen in the superficial layers. In 4 of 10 (40%) 18-month-old animals, the increase in keratinization combined with basal cell hyperplasia resulted in irregular rete pegs of epithelium which extended into the subjacent stroma (Fig. 2). In another case, the vaginal epithelium formed papillary projections into the lumen (Fig. 3).

Urethral openings, abnormally located anterior to the vulva (persistent urogenital sinus), were found in 20% of the 1-month-old DES-100 animals. ‘Gland-like structures’ associated with this abnormality were of urothelial origin.

All of the prenatally DES-100 mice showed hypertrophy in the structure of the portio vaginalis (cervix) (Chart 1). The outside diameter of the cervical area in 1-month-old animals averaged 3.6 ± 0.5 (S.D.) mm for DES-100 compared to 2.85 ± 0.7 mm for controls and at 18 months averaged 4.37 ± 0.45 mm for DES-100 and 2.8 ± 0.4 mm for controls. Histological evaluation of tissues from this area showed that both the stroma and epithelium were affected by DES and contributed to the enlargement seen macroscopically. Stromal edema in this area was seen in 75% of the 1-month-old and 80% of the 18-month-old animals. In cross-section, the epithelial mucosa of the DES animals had fewer folds, and those that were present were less convoluted and shallower than were those in control tissues. In some animals, the uterine and cervical lumen appeared as a simple tube. Squamous metaplasia in the endocervical canal in conjunction with excessive keratinization in some cases was observed in 45% of the 1-month-old and 80% of the older animals.

Table 1

<table>
<thead>
<tr>
<th>Abnormalities of cervicovaginal area in female mice exposed prenatally to DES</th>
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</thead>
<tbody>
<tr>
<td>No. of mice with abnormalities</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Abnormality</td>
</tr>
<tr>
<td>Structural alterations b</td>
</tr>
<tr>
<td>Cervical enlargement</td>
</tr>
<tr>
<td>Squamous metaplasia in endocervical canal</td>
</tr>
<tr>
<td>Excessive keratinization in ectocervix and upper vagina</td>
</tr>
<tr>
<td>Basal cell hyperplasia in vagina with irregular rete pegs of epithelium</td>
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<tr>
<td>Prominent Wolffian remnants</td>
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<tr>
<td>Leukocytic infiltration</td>
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</table>

* Females were the offspring of CD1 mice treated on Days 9 through 16 of gestation with DES, 100 μg/kg/day. Tissues were processed by the method of Plapinger and Bern (30).

b Defects in Mullerian duct fusion and vaginal fornix formation. Statistical significance of DES-exposed animals to corresponding age-matched controls by Fisher exact test. p < 0.0001.

a p < 0.001.

b Not significant.

p = 0.04.
Vaginal Abnormalities in DES-exposed Mice

Fig. 1. a, cross-section through the common cervical canal of a 1-month-old mouse exposed prenatally to DES. Lining epithelium is cornified and stroma is mildly hyperplastic. WD, the lumen of prominent remnants of the Wolffian duct. DES-100. H & E, x 15. b, higher magnification of the field shown in a. WD, Wolffian duct remnant. DES-100. H & E. x 75.

CONTROL PRENATAL DES NEONATAL DES

Chart 1. Schematic drawing of reproductive tracts from 1-month-old female CD-1 mice. Control, untreated females. Prenatal DES, females which were offspring derived from CD-1 mice treated with DES, 100 μg/kg/day, on Days 9 to 16 of gestation. Treatment resulted in general enlargement in the area of the cervix. Oviductal malformations and abnormal urethral openings in the vagina were other abnormalities commonly observed. Neonatal DES, females which were offspring from control CD-1 mice treated neonatally on Days 1 to 5 with DES, 2 μg/kg/day/pup. Treatment resulted in a hypotrophic uterus which was much smaller than were any unstimulated control tissues. The vagina was enlarged, but there were no cervicovaginal or oviductal malformations or abnormal urethral openings.

A more striking structural abnormality was observed in serial cross-sections through the cervicovaginal region of female mice exposed prenatally to DES. In the most severely affected cases, the Müllerian ducts did not fuse to form a common cervical canal. In other less severe cases, the cervix was short craniocaudally but still had a relatively large diameter, both internal and external, with increased stromal elements. In addition, the vaginal fornix was always shallower than were controls and, in one 35-day-old animal, it was not present (Chart 2).

It was extremely difficult to distinguish changes in the location of the squamocolumnar junction between control and prenatally DES-exposed animals. Normally in the cycling control female, the squamocolumnar junction varied between animals, and the border was more or less distinct depending on the stage of the estrous cycle (22).

The columnar epithelium described as adenosin in this report did not refer to tall mucus-producing cells present in the superficial layer of stratified epithelium in the normal cycling mouse vagina but rather to columnar cells arranged either in a single layer in the lining epithelium or forming a gland-like structure within the vaginal stroma. Columnar epithelium within the vaginal fornices (adenosis) was observed in the post-pubertal (1 month) as well as the older prenatally exposed animals (18 months), but in no controls. The number of animals involved, however, was low, and the region of involvement in each animal extended through only a few serial sections. Three of 20 (15%) 1-month-old and one of 10 (10%) 18-month-old exposed animals had columnar cells abnormally located in the vaginal fornices (Table 2). In one of the 1-month-old mice, a layer of high columnar cells formed a cyst-like structure immediately basal to the lining epithelium (Fig. 4). The opening of this gland into the vaginal lumen was observed in another section. The second case of adenosin similarly demonstrated columnar cells lining a subepithelial gland but with no demonstrable opening into the vaginal lumen. In the third 1-month-old DES female, a patch of columnar cells was found in the region of the vaginal fornix that is normally lined by squamous epithelium (Fig. 5). There appeared to be early gland formation, but the abnormality was only observed in two 6-μm serial sections. Vaginal adenosin that was observed in the one 18-month-old female resembled those described in the younger animals. Again, columnar cells formed a cyst in the stroma under the epithelial layers. The lumen of this "gland-like" cystic structure...
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Table 2
Incidence of adenosis in female mice exposed to DES

<table>
<thead>
<tr>
<th>Prenatal treatment</th>
<th>No. of animals</th>
<th>Mice with adenosis</th>
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<tbody>
<tr>
<td>1-mo.-old offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0/20 (0)</td>
</tr>
<tr>
<td>DES-100</td>
<td>20</td>
<td>3/20 (15)%</td>
</tr>
<tr>
<td>16-mo.-old offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>DES-100</td>
<td>10</td>
<td>1/10 (10)%</td>
</tr>
<tr>
<td>Neonatal treatment</td>
<td></td>
<td></td>
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<tr>
<td>1-mo.-old offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>DES</td>
<td>8</td>
<td>6/8 (75)%</td>
</tr>
</tbody>
</table>

a Females were offspring derived from CD-1 mice treated with DES, 100 μg/kg/day, on Days 9 through 16 of gestation.

b Numbers in parentheses, percentage.

c Not significant.

d Females were offspring from control CD-1 mice treated neonatally on Days 1 through 5 with DES, 2 μg/day/pup, or averaging approximately 1 mg/kg/day. Tissues were embedded and processed by the method of Plapinger and Bern (30).

p < 0.01.

was more dilated than were those seen in younger animals (Fig. 6). The cases of adenosis that were described occurred in animals with only slight structural abnormalities. In an attempt to study the relationship between adenosis and adenocarcinoma in the vagina of mice, the second study included a larger group of animals treated in utero with various doses of DES. In this series of animals, the incidence of vaginal adenocarcinoma was as follows: controls, 0 of 158; DES-5, 1 of 29; DES-10, 0 of 16; and DES-100, 1 of 46. One DES-5 female became moribund and therefore was sacrificed at 17 months of age. Observations at the time of necropsy included ovarian cysts and mild cervical enlargement, but no other readily apparent abnormalities. Microscopic evaluation of the genital tract showed gland-like structures in the submucosal area of the vaginal fornix. These glands were lined by well-differentiated mucus-secreting columnar cells with a low degree of nuclear pleomorphism. Invasion into surrounding tissues resulted in this lesion being diagnosed as vaginal adenocarcinoma (Fig. 7).

Another 17-month-old animal exposed to DES-100 had a large stone in the vagina. Serial longitudinal sections through the entire reproductive tract of this mouse showed an abnormal urethral opening with urethral glands in the lower vagina. The surface epithelium of the vagina was heavily keratinized, and in some areas there were small squamous papillomas. These alterations and focal inflammatory changes were probably related to the presence of the stone. In addition to urethral glands, a gland-like structure lined by tall mucus-secreting cells (adenosis) opened into the lumen of the upper vagina. In the area of the opening of this gland, mild focal atypia in the luminal epithelium was observed. An area directly adjacent to this showed changes consistent with well-differentiated squamous cell carcinoma (Fig. 8). Additional submucosal glands in the vaginal fornix, not associated with the abnormal urethral opening, contained areas of squamous metaplasia (Fig. 9) and adenocarcinoma (Fig. 10).

Cervicovaginal Changes in Female Mice Exposed Neonatally to DES. At 35 days of age, the uteri and cervix of neonatally DES-exposed mice were hypotrophic (Charts 1 and 2). There was little gland formation in the uteri resulting in slender tubular structures. The cervical mucosa had fewer folds than did controls but more than did the prenatally DES-exposed animals. In addition, vaginal keratinization was not seen.

Adenosis was observed in the neonatally exposed animals in 6 of 8 DES-treated animals while it was not apparent in any of the controls. Adenosis occurred as columnar cells lining the vaginal fornix and in some cases formed folds toward the underlying stroma resembling glands (Fig. 11).

DISCUSSION

Previous reports from this laboratory described a mouse model for the transplacental effects of DES on the developing fetal female reproductive tract and the subsequent associated abnormalities (21, 23, 25, 26). Vaginal adenocarcinoma was observed in low incidence (1 of 140 DES-treated mice, all doses) in these experiments (26); likewise, vaginal adenosis was a rare histological observation (1 of 140 DES-treated mice). This latter observation was surprising since Forsberg demonstrated a high incidence of vaginal adenosis in NMRI mice after neonatal treatment with estrogen (11); more recently, Plapinger and Bern (30) reported adenosis after perinatal estrogenization.

The number of cases of adenosis observed in this prenatally DES-exposed animal model may have been conservative because excessive cornification in the cervix and vagina, inter-animal variability in the squamocolumnar junction in the control as well as in the treated animals, and other structural abnormalities in the DES-exposed animals made further determinations inconclusive. Considering these problems in identifying adenosis in the mouse, the differences in incidence between prenatal and neonatal exposure are still apparent. Some possibilities which may explain the lower incidence of adenosis in the CD-1 mouse following prenatal DES treatment rather than neonatal DES exposure were: (a) route of exposure and dose of DES; (b) differences in sectioning techniques; (c) age of mouse; (d) strain of mouse; (e) stage of genital tract development during DES exposure. In order to determine the actual occurrence of adenosis in the prenatal mouse model and its relationship to the pathogenesis of adenocarcinoma, this report investigated each of these possibilities.

The route of exposure to DES in studies which reported vaginal adenosis (11, 30) was direct s.c. administration to the neonatal mouse whereas the prenatal reports (26) described s.c. injections to the mother. Thus, the direct neonatal dose of DES, averaging approximately 1 mg/kg/day, was higher than the indirect doses (5 to 100 μg/kg/day) used in the prenatal studies. Higher prenatal doses were severely fetotoxic, making investigation of the relationship of adenosis to increasing amounts of prenatally administered DES impossible. It is interesting to note, however, that 2 of 3 cases of vaginal adenocarcinoma reported in the prenatal mouse model [one in the present report and one reported previously (26)] occurred in offspring exposed to lower doses, not higher doses, of DES.

The progression to neoplasia, or at least its expression, may be more obvious in the absence of the extensive tissue changes associated with the higher doses of DES. For example, in an animal with adenosis, columnar cells in this lesion were partially replaced by squamous cells (squamous metaplasia). Clinical reports have suggested that squamous metaplasia is a healing process of adenosis, and in some cases adenosis was replaced completely by squamous cells (36). Differentiation of squamous...
metaplasia into mature, stratified squamous epithelium occurred; this epithelium became impossible to distinguish from the normal squamous epithelium of the vagina resulting in a complete involution of adenosis (38). It is possible that high prenatal doses may amplify the process of squamous metaplasia and therefore result in lower incidences of observable adenosis. The development of squamous metaplasia in adenosis has raised the possibility of increased incidence of dysplasia and squamous carcinoma to several investigators (15, 35). This was not the finding, however, of clinicians following women at Massachusetts General Hospital (33) or patients enrolled in the National Cooperative Diethylstilbestrol Adenosis project (32). Their data suggest that rates of dysplasia are quite low in the DES-exposed population. Although squamous cell carcinoma is a lesion reported in the present and other studies (5, 6, 14, 37), more evidence is needed to prove it to be a significant health problem in women exposed prenatally to DES. This lesion, however, does suggest additional reason for continued careful follow up of the exposed population.

The second possibility for the lower incidence of adenosis in the prenatally DES-exposed mouse was the difference in sectioning techniques. Data from reproductive tracts of the animals that were reported in the previous long-term prenatal study (26) were cut in the sagittal midline with the cut surface embedded down in the paraffin block, and 10 serial sections were made. If an abnormality was observed, additional sections were made. The possibility existed that the vaginal fornix may not have been reached in all of the animals; therefore, in the present study, serial sections through the entire block were done to ensure the inclusion of the vaginal fornix. In addition, serial cross-sections obtained by the method described by Plapinger and Bern (30) were done in another group of prenatally DES-exposed animals. The incidence of vaginal adenosis in transplacentally treated mice determined by both techniques was still much lower than were the reported cases of adenosis observed after neonatal DES exposure and therefore does not appear to be a possible explanation.

The age and strain of mice used were also considered as possibilities attributing to the lower incidence of adenosis in the prenatal mouse model compared to the neonatal mouse model. The initial findings of Forsberg suggested that adenosis occurred only in NMRI mice (10, 11, 13). However, Plapinger and Bern (30) have demonstrated vaginal adenosis in perinatally estrogenized mice in 4 strains (NCS, BALB/cCrl, C3H/Crl, C57/Crl) and at ages reported in this study. In addition, adenosis was recently described for CD-1 mice exposed late in gestation to DES (38). Therefore, strain differences are unlikely to account for our observations of lower incidences of adenosis.

The last possibility, stage of genital tract development during DES exposure, seems to be the most likely explanation for the differences observed. For example, Plapinger and Bern (30) reported that the NCS mouse, with only neonatal exposure to estradiol benzoate, had an incidence of 68% adenosis in comparison to 60% with neonatal exposure plus 1 day prenatal exposure, 38% with neonatal plus 2 days prenatal exposure, and 0 with neonatal plus 4 days prenatal exposure. Likewise, neonatal treatment with DES resulted in 100% adenosis (30). On the other hand, prenatal treatment with ethinyl estradiol resulted in only 3% of adenosis in offspring (39); moreover, the later in gestation DES was given in the small series reported by Walker (38), the higher was the incidence of adenosis. Although the numbers of animals in these reports were small and 2 of the strains studied by Plapinger and Bern (30) did not have the same temporal relationship for adenosis, these data, plus observations in this current study, suggest that adenosis can be demonstrated after neonatal exposure to DES but prenatal exposure results in a lower incidence of the lesion.

The exact effect of DES on genital tract development remains to be determined; however, studies on differentiation of the vagina may be informative. Forsberg (12) has suggested that exposure to DES during development probably interferes in some manner with the replacement of the Müllerian columnar epithelium by squamous epithelium (vaginal transformation) resulting in the persistence of Müllerian cells similar to those of the endocervix, endometrium, and fallopian tube in an abnormal area. Using the neonatal animal model, Forsberg (11) has produced adenosis in a system where vaginal transformation takes place after birth. Recently, Forsberg and Kalland (14) have reported development of squamous cell carcinomas and adenosis with squamous metaplasia in the vaginas of mice treated neonatally with DES; in addition, these investigators demonstrated apparent adenocarcinomas in regions of adenosis in the cervix, not the vagina, following such early postnatal exposure to DES. In our laboratory prenatal exposure, before vaginal epithelial transformation has occurred, did not produce a high incidence of adenosis. In the 225 mice exposed to DES-5, -10, or -100 in this and a previous report (26), a total of 3 cases of vaginal adenocarcinoma were obtained.

A recent report from this laboratory showed that DES causes transformation in a culture system using Syrian hamster embryo cells (4). It was known that DES induced genetic damage in cells by chromosome loss since Rao and Engleberg (31) have shown chromosome nondisjunction in HeLa cells and Chrisman (7) has shown aneuploidy in 8-day-old mouse embryos after DES exposure. An important correlation can be made with these studies and a recent report which shows an association of aneuploidy with vaginal cellular dysplasia in young women exposed prenatally to DES (15).

There is little information about the ultimate fate of columnar epithelium in the mouse vagina other than the possibility of a replacement of the abnormality with upgrowing squamous epithelium (14, 25). An alternative fate of adenosis in the mouse might be the rare development of vaginal adenocarcinoma. Although various squamous epithelial changes have been observed, vaginal adenocarcinoma has not been reported following neonatal or late prenatal treatments with DES (5, 9, 13, 14, 28, 30, 37, 38). Using this strain of mouse, if there is a correlation between adenosis and adenocarcinoma, it would be reasonable to expect a higher incidence of adenocarcinoma at later ages in the neonatal DES-exposed animals. This merits further investigation.

However, the present results suggest that adenosis is a lesion that may be inducible during the process of transformation; adenocarcinoma may require an earlier time for induction. Support for this theory can also be found in clinical studies because there are several reports which suggest that adenosis is not seen in women exposed to DES after the 20th week of gestation, a time when vaginal transformation has already occurred in humans (18). Also, recent data from the National Cooperative Diethylstilbestrol Adenosis project indicate that vaginal epithelial changes (adenosis and squamous metaplasia)
rarely develop if the first exposure occurred well after the 20th week (29).

Although the time for induction may be different for these lesions, it is apparent that the basic biological relationship between adenosin and adenocarcinoma of the vagina is not completely understood.

This could account for the epithelial abnormalities (basal cell hyperplasia with irregular rete pegs) as well as other anatomical abnormalities of connective tissue observed in this study (papillary structures resembling transverse ridges in exposed women). With abnormalities of the stroma, including stromal hyperplasia and edema (cervical enlargement) and neoplasia in mice exposed prenatally to DES (26), the possibility of a primary stromal action of DES must be considered.

The role of Wolffian duct-derived tissues in the abnormal development of typically Müllerian structures, such as cervicovaginal tissues, is presently unclear. As long ago as 1959, Gruenwald (16) suggested that Wolffian duct mesenchyme contributed to the definitive stroma of the cervix and uterus (Müllerian-derived structures). The association of the hyperplastic, retained Wolffian structures observed in the present study with the abnormally developing Müllerian tissue is an area of current investigation. In fact, we reported previously (26) that cystic Wolffian duct remnants were associated with vaginal sections in 63% of the 12-month-old DES-100-treated mice, while the corresponding control value was 9% (26).

A striking change in the cervicovaginal area of DES-treated mice involved structural defects which included malformation of the Müllerian ducts as well as structural alterations in the vaginal fornices (Chart 2). Whether these malformations play a role in the expression of epithelial lesions seen in the cervicovaginal tissue of DES-exposed mice is currently being investigated.

Although a recent study suggests that DES-related abnormalities diminish with age (1), the potential for in utero transformation of actively differentiated cells as well as environmental factors later in life affecting normal cells in an abnormal location (adenosis) resulting in adenocarcinoma are possibilities that must be considered. Forsberg (10) observed that ovarioectomy at puberty prevented development of adenosis in the cervicovaginal region of female mice treated neonatally with estrogen or DES. These observations, in conjunction with the fact that adenosis and adenocarcinoma were observed in prenatally exposed women more often at puberty, suggest a possible role of estrogen as a promoter in the pathogenesis of these lesions at puberty.

In summary, the results presented in this report combined with additional knowledge of the effects of DES in other systems suggest that the stage of cellular differentiation at the time of DES exposure may be critical in the final expression of a particular abnormality.

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14. Forsberg, J.-G., and Sellâ, T. Neonatal estrogen treatment and epithelial abnormalities in the cervicovaginal region of female mice treated neonatally with estrogen or DES. These observations, in conjunction with the fact that adenosis and adenocarcinoma were observed in prenatally exposed women more often at puberty, suggest a possible role of estrogen as a promoter in the pathogenesis of these lesions at puberty.

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Fig. 2. Basal cell hyperplasia in the vagina of an 18-month-old female exposed prenatally to DES; irregular extension of epithelium into the underlying stromal tissue. DES-100. H & E, x 75.

Fig. 3. Vaginal epithelium forming papillary projections into the lumen of an 18-month-old animal. DES-100. H & E, x 75.

Fig. 4. a, adenosis (Ad) in a cross-section through the vaginal fornix of a 1-month-old mouse exposed prenatally to DES. Gland-like structure in stroma of fornix opens into the vaginal lumen in another section. Lining epithelium is keratinized, and there is stromal edema. DES-100. H & E, x 15. b, higher magnification of a. A layer of tall columnar cell lines gland-like structure. DES-100, H & E, x 75.
Vaginal Abnormalities in DES-exposed Mice

Fig. 5. a, portion of cross-section through vaginal fornix of a 1-month-old mouse exposed prenatally to DES. Immature squamous epithelium is interrupted by an area of columnar cells. Ad, Adenosis. DES-100. H & E, × 75. b, higher magnification of adenosis in a. Columnar cells approximate a gland-like structure in the stroma of the vaginal fornix. This abnormality was observed in only two 6-μm sections. DES-100. H & E, × 120.

Fig. 6. Adenosis in the vaginal fornix of an 18-month-old animal prenatally exposed to DES. Columnar cells form a cyst in the stroma under the epithelial layers. The lumen of this gland-like structure is more dilated compared to the younger animals. DES-100. H & E, × 75.

Fig. 7. Vaginal adenocarcinoma in a 17-month-old female exposed prenatally to DES. Gland-like structures in the submucosal area of the vaginal fornix are lined by mucus-secreting columnar cells with a low degree of nuclear pleomorphism. Invasion into the underlying tissue is consistent with well-differentiated adenocarcinoma of the vagina. DES-100. H & E, × 75.

Fig. 8. Squamous cell carcinoma in the vagina of a 17-month-old prenatal DES-treated mouse. There is cellular pleomorphism and a lack of orderly progression from basal to squamous cells. DES-100. H & E, × 75.

Fig. 9. Submucosal glands in the vaginal fornix of the same 17-month-old DES-exposed female in Fig. 8, containing areas of squamous metaplasia (arrow). DES-100. H & E, × 75.

Fig. 10. Adenocarcinoma of the vagina lying adjacent to the area shown in Fig. 9. Cellular pleomorphism and invasion are characteristics of this tumor. DES-100. H & E, × 100.

Fig. 11. Adenosis (arrow) in the vaginal fornix of a 35-day-old female exposed neonatally to DES. Columnar epithelium forms fold toward the underlying stroma. Neonatal DES. H & E, × 75.
Vaginal Adenosis and Adenocarcinoma in Mice Exposed Prenatally or Neonatally to Diethylstilbestrol

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