Effects of Transplacental Exposure to Diethylstilbestrol on Carcinogenic Susceptibility during Postnatal Life in Hamster Progeny

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ABSTRACT

Prenatal exposure to a single dose of diethylstilbestrol (DES) produced a significant increase in carcinogenic response of hamster progeny that were subsequently subjected to the carcinogenic stimulus of 7,12-dimethylbenz(a)anthracene (DMBA) during postnatal life. The compounds were administered according to the following schedules. The pregnant animals (second group) received a single dose of DES, 10 mg/kg, on Day 14 of gestation. Postnatally, at 6 weeks of age, the progeny were given DMBA, 25 mg/kg p.o., twice weekly for 8 weeks. The first group received DMBA at 6 weeks of age, 30 mg/kg p.o., twice weekly for 18 weeks.

The progeny exposed to DES prenatally and DMBA postnatally (DES-DMBA-exposed progeny) developed a greater multiplicity of tumors per tumor-bearing animal (p < 0.001) and higher rates of neoplasms of the reproductive tract, e.g., ovarian and uterine tumors, mammary gland and forestomach tumors, and dermal melanomas. The prenatally DES-exposed progeny also had significantly higher incidences of malignant tumors, e.g., carcinomas of the mammary gland (p < 0.001) and carcinomas of the forestomach (p < 0.001), than did the hamsters given DMBA alone during postnatal life.

Endocrine imbalance produced by exposure in utero may heighten the sensitivity of the progeny to development of neoplasms after a challenge with carcinogenic stimuli in adult life. The significance of these experimental data to the human situation is discussed.

INTRODUCTION

The profound effects of an induced hormonal imbalance on certain experimental and human neoplasms have been well established (3, 15, 17–19, 34, 35). These effects are observed particularly in tissues and organs that are physiologically under the direct influence of sex hormones, i.e., target tissues such as the reproductive organs and the breast. A number of publications have reported a relationship between sex hormones and chemical carcinogenesis in several animal species (12, 14, 16, 18, 19, 41) with varying results according to the experimental design, species of animal, type of carcinogen, hormonal environment of the host, and sites of tumor formation.

For example, rats subjected to a single dose of DMBA developed a high incidence of mammary carcinomas (16).

These tumors regressed in females following ovariectomy but persisted in males after orchidectomy. The reciprocal application of sex hormones to gonadectomized mouse progeny subjected prenatally to ethynitrosourea resulted in a significant increase in hepatocellular neoplasms in male offspring, indicating a definite influence of sex hormones on development of hepatic tumors (29). Our transplacental studies in hamsters indicated that gonadectomy increased the incidence of neoplastic tumors and broadened the profile of other tumor types in hamster progeny that were exposed prenatally to ethynitrosourea precursors (34, 35), indicating also that carcinogenesis in this animal species is responsive to the induction of hormonal imbalance (16).

The transplacental exposure of hamsters and mice to the synthetic estrogen DES resulted, however, in the induction of neoplasms and a variety of potentially malignant epithelial lesions in different segments of the genital tract of the progeny (21, 35, 37, 38).

The mechanism of action of DES appears to be mediated by a disturbance induced in the hypothalamic-pituitary axis (6, 9). This produces an endocrine imbalance and alters the secretion pattern of gonadotrophins, which in turn results in gonadal dysfunction and, in female progeny, persistent estrogenic stimulation (8, 9, 37). The direct or indirect action of DES on fetal target organs, however, should also be considered (37, 40).

The effects of an altered endocrine balance on carcinogenesis in rats exposed neonatally to estradiol and subsequently during adulthood to N-hydroxy-N-2-fluorenylacetamide demonstrated that the altered endocrine status and metabolic activation of the carcinogen by the host affected the outcome of liver carcinogenesis (47). An increased susceptibility to carcinogen and, consequently, a greater proclivity of the mammary gland to undergo dysplastic alteration have been recorded in mice treated perinatally with estradiol and subsequently in adult life with DMBA (45, 46).

The present study explored the neoplastic response in hamster progeny that were exposed in utero to DES and subsequently in postnatal life to the carcinogenic stimuli of DMBA.

MATERIALS AND METHODS

Animals

A group of 9 randomly bred female Syrian golden hamsters from the Eppley Institute colony were mated individually for transplacental exposure and housed with their litters until 5 weeks after delivery. The progeny were separated by sex and kept in groups of 3 to 5 on San-i-cel bedding (Anderson Laboratory, Maumee, Ohio) in clear plastic cages. Another group of 100 hamsters of both sexes that were not treated...
prenatally were obtained from the same source and cohort. All animals were fed Wayne pellet diet (Allied Mills, Inc., Chicago, Ill.) and given tap water *ad libitum*.

**Compounds**

DES (Eli Lilly and Company, Indianapolis, Ind.) was suspended in 0.2% gelatin solution at a concentration of 0.2%. DMBA (Eastman Kodak, Rochester, N. Y.) was also suspended in 0.2% gelatin solution at a concentration of 0.2% and delivered by i.g. intubation immediately after preparation.

**Treatment Procedure**

The administration of chemicals consisted of 2 separate experimental sets: the first set received DMBA postnatally only; and the second set received prenatal exposure to DES and subsequently in postnatal life DMBA (DES-DMBA-exposed progeny).

The animals in the first experimental setting were subjected to i.g. intubation of DMBA, 30 mg/kg, delivered at a rate of 0.01 ml/2g of body weight administered over a period of 17 weeks, for a total amount of 510 mg/kg beginning at 6 weeks of age. This high dosage of carcinogen was administered in an attempt to induce mammary tumors, since it is known that this animal species is very resistant to the development of mammary tumors even after treatment with powerful carcinogens (6, 7, 13). The technical details of i.g. application were described previously (36).

In the second experimental setting, the pregnant animals were given on the 14th day of gestation a single dose of DES, 10 mg/kg, delivered at a rate of 0.01 ml/2g of body weight by i.g. tube. Treatment with DMBA began at the sixth week of age. The progeny were given 16 i.g. doses of DMBA, 10 and/or 25 mg/kg, delivered at a rate of 0.01 ml/2 g of body weight twice weekly over a period of 8 weeks for a total amount of 190 mg/kg. This reduction in the amount of DMBA administered to DES-exposed progeny was due to an apparently greater sensitivity of these animals to postnatal treatment with DMBA since they showed clinical signs of excessive toxic effects at the early stages of treatment.

**Experimental Groups**

**First Experimental Setting.** This consisted of 2 groups of 50 females (Group 3) and 50 males (Group 4) 6 weeks of age. Due to an excessive toxicity of DMBA for males, 14 animals died within the first 10 weeks of age, i.e., after 4 weeks of DMBA treatment, and were excluded from statistical evaluation.

**Second Experimental Setting.** Of the 70 siblings that originated from 9 litters exposed prenatally to DES, 40 progeny (57%) were weaned and separated by sex into 2 groups consisting of 20 females and 20 males (Groups 1 and 2, respectively) (Table 1). Thirty siblings were cannibalized or missing within the first few days postdelivery. The lower than normal (80% in unexposed hamsters) weaned rate in DES-exposed hamsters was probably due to the toxic effects of the drug, although other ancillary factors influencing survival of the newborns cannot be excluded. Two animals died within the first week after inception of DMBA treatment at 6 weeks of age.

All the animals, including the DES-treated mothers, were weighed and checked regularly at weekly intervals throughout their life span. The grossly observed tumors and pathological abnormalities were routinely recorded. The animals were left to die spontaneously, and only a few were sacrificed when found moribund. Complete autopsies were performed on all animals, and sections were taken from all organs including the different regions of the genital tract. The tissues were fixed in 10% buffered formalin solution, embedded in paraffin, processed, and stained conventionally with hematoxylin and eosin. Selective stainings were used when needed.

Microscopic examinations were done routinely on all tumors, livers, kidneys, lungs, spleen, pancreas, stomachs, selected segments of intestines, bone marrow, lymphocytic tissues, central nervous system, hypophysis, thyroids, and adrenal glands and selected segments of the entire genital tract, including ovaries, oviduct, uterus, cervix, vagina, testes, epididymis, seminal vesicles, Cowper's glands, and prostate. Microscopic evaluation was also performed on any other organ or tissue that showed gross pathological alterations.

The data were analyzed statistically with consideration of tumor and death rates (2, 26).

**RESULTS**

Table 1 summarizes the effective number of animals, overall tumor incidences, and the average latency and distribution patterns of neoplasms developing in the forestomach, skin, and other tissues.

The survival of hamsters exposed prenatally to DES and postnatally to DMBA (Groups 1 and 2) ranged from 11 to 41 weeks, and survival of those exposed only to DMBA (Groups 3 and 4) ranged from 12 to 29 weeks. The average life span was 23.5 weeks for Group 1, 24.8 weeks for Group 2, and 20.3 weeks for both females and males of Groups 3 and 4. The life expectancies of animals that did not die prematurely (until weaning) were not significantly different among the groups.

The majority of animals died from rapidly developing mammary gland malignant tumors and occasionally from squamous cell carcinomas of the forestomach which extensively invaded the surrounding viscera of the abdominal cavity.

Females of both experimental sets developed a multiplicity of tumor types, while males had somewhat lower, but not significantly different, rates of neoplasm formation. The total number of tumors per tumor-bearing animal (tumors originating from various tissues or organs), however, was significantly greater in female progeny exposed prenatally to DES and postnatally to DMBA (Group 1) than in those that received postnatal treatment with DMBA only (Group 3) (4.2 versus 1.7; *p* = 0.001). The male progeny in Group 2 DES-DMBA-exposed progeny also developed a significantly greater number of tumors per tumor-bearing animal (*P* = 0.050) than did the males in Group 4 that were treated with DMBA only during adulthood. The average latent periods at which various neoplasms developed in both males and females of all experimental groups, however, were not statistically different.

The overall incidences of tumors, average latency periods, occurrences of tumors of the ovaries and reproductive tract, and frequencies of benign and/or malignant neoplasms of the mammary gland are shown in Table 2. Since Table 1 presents the distribution pattern and the basic data for the neoplasms,
the narrative will consider only the nature of the tumors and their statistical significance.

Mammary Tumors

The DES-DMBA-exposed female progeny developed a greater incidence of mammary tumors than did females treated with DMBA alone during postnatal life (68.4 versus 42.0% for Groups 1 and 3, respectively) (Table 2). However, the incidence of malignant mammary tumors, i.e., carcinomas, was statistically significantly greater in the DES-DMBA-exposed female progeny than in those not exposed (68.4 versus 42.0% in Groups 1 and 3, respectively; p ≤ 0.001). All progeny of Group 1 bore multiple carcinomas with 2.2 tumors/tumor-bearing animal. Four animals also had benign adenomas. The average latency period was 24 weeks. In Group 3, 12 females (24%) had carcinomas with 1.5 tumors/tumor-bearing animal. Six females bore both malignant and benign tumors, and 12 had benign tumors (adenomas) only. The average latency period was 22 weeks. The average latency period for mammary tumors did not differ significantly between Groups 1 and 3.

Macroscopically, the mammary tumors appeared as solitary or conglomerated soft nodular masses in the breast gland areas. They grew from a few mm to several cm, usually within 2 to 4 weeks. Their consistency varied greatly. They underwent rapid necrotic and regressive alterations and showed cystic cavitations containing proteinaceous serous mucus or hemorrhagic material and extensive necrosis. Malignant and benign mammary tumors were observed. The general criteria for characterization of malignant mammary tumors were morphological, such as histological architecture and cytological features (anaplasia) of the tissues, and biological, such as invasion, metastases, and transplantability. The benign mammary tumors were intraductal in origin and papillary in type. The papillary stroma and glandular epithelium lining the cystic dilations and/or forming the acini were composed of uniform cellular elements. Both types of mammary tumors are briefly described as follows. Microscopically, the malignant mammary tumors were predominantly papillary carcinomas originating intraductally. The papillary projections that protruded into cystic spaces were composed of varying amounts of stroma. The epithelial cells covering the papillae were cuboidal or flattened, similar to epithelial elements lining the cystic spaces (Fig. 1). Frequently, these cellular elements formed papillary projections, appearing as masses of sheets of neoplastic cells or areas of acinar formations within the papillary stroma. Metastasis to the lungs occurred in a few instances (Fig. 2). Squamous cell metaplasia sometimes occurred in the acini or glandular structures and in the epithelium lining the cystic spaces, which occasionally contained cellular debris. There were extensive areas of hemorrhage and necrosis. Adenocarcinomas appeared as gland-like structures in which the epithelial cells formed regular or irregular acinar spaces. The neoplastic epithelium lining the acini was cuboidal or flattened and varied from one to several layers in thickness. The neoplastic tissue was commonly intersected by sparse fibrovascular stroma (Fig. 3). Occasionally, a cribriform pattern was seen. The arrangement of neoplastic cells within this tumor type resembled lobular hyperplasia. Around the sheets of tumor cells, this stroma was scanty. The size and shape of cellular elements that formed the acinar spaces varied. This tumor pattern was usually associated with other tumoral types. A few neoplasms exhibited features of anaplastic carcinoma characterized by poor differentiation. The neoplastic cells composing this tumor type tended to be large and varied in shape and size. The nuclei were prominent, cellular pleomorphism, and mitotic figures were also quite common.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective no. of animals</th>
<th>Sex</th>
<th>% T/TBA</th>
<th>Latent period (wk)</th>
<th>No.</th>
<th>%</th>
<th>Latent period (wk)</th>
<th>No.</th>
<th>%</th>
<th>Latent period (wk)</th>
<th>Other neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DES, 10 mg/kg prenatally + DMBA, 190 mg/kg postnatally</td>
<td>19</td>
<td>F</td>
<td>100.0</td>
<td>4.2</td>
<td>23.0</td>
<td>19</td>
<td>100.0</td>
<td>23.0</td>
<td>9</td>
<td>47.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>DES, 10 mg/kg prenatally + DMBA, 190 mg/kg postnatally</td>
<td>19</td>
<td>M</td>
<td>95.0</td>
<td>2.2</td>
<td>25.0</td>
<td>18</td>
<td>95.0</td>
<td>25.0</td>
<td>6</td>
<td>32.0</td>
<td>27.0</td>
</tr>
<tr>
<td>3</td>
<td>DMBA, 510 mg/kg postnatally</td>
<td>50</td>
<td>F</td>
<td>100.0</td>
<td>1.7</td>
<td>20.0</td>
<td>50</td>
<td>100.0</td>
<td>20.0</td>
<td>5</td>
<td>10.0</td>
<td>21.0</td>
</tr>
<tr>
<td>4</td>
<td>DMBA, 510 mg/kg postnatally</td>
<td>36</td>
<td>M</td>
<td>88.9</td>
<td>1.2</td>
<td>20.0</td>
<td>32</td>
<td>89.0</td>
<td>22.0</td>
<td>3</td>
<td>8.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

* T/TBA, tumor-bearing animals with any type of neoplasms.

b Average age at which tumors were found at autopsy and recorded.

c DES given by i.g. intubation once, 10 mg/kg, on Day 14 of gestation.

d DMBA given by i.g. intubation at 25 or 10 mg/kg twice weekly for a total of 190 mg/kg postnatally, beginning at 6 weeks of age, for a period of 8 weeks.

A, 2 squamous cell papillomas of esophagus (26, 30); 1 malignant lymphoma, histiocytic type (23); 1 hemangiom of liver (25); 1 adenoma of pituitary gland (38); 1 hemangiom of spleen (26); 1 adenocarcinoma of small intestine (22); 1 hemangiom of uterus (20); 1 neurinoma of lumbosacral plexus (22); B, 3 malignant lymphomas, 1 stem cell type (38), 1 histiocytic type (22), 1 lymphocytic type (38); 3 hemangiom of spleen (22, 38, 42); 2 squamous cell papillomas of skin (30, 42); 2 adenomas of adrenal cortex (39, 42); 2 squamous cell papillomas of esophagus (24, 39); 1 leiomyosarcoma of prostate gland (38); 1 sarcoma of skin (30); 1 adenoma of pituitary gland (38); C, 1 malignant lymphoma, histiocytic type (21), 1 adenocarcinoma of small intestine (22); D, 1 adenocarcinoma of small intestine (23); 1 cholangioadenoma of liver (23); 1 squamous cell papilloma of esophagus (21).
Several randomly selected mammary tumors were transplanted into homologous animals and grew successfully in subsequent generations. The histological examination of the initial crop of neoplasms showed an increase in cellularity and marked differentiation of the structure over the original tumors.

The majority of benign mammary tumors originated from intraductal epithelium and formed dilations and cystic spaces. The papillary formations which protruded into dilated ductal luminary and cystic spaces were characterized by an abundance of fibrous stroma and were lined by a single layer of cuboidal cells. Acinar structures formed by a single layer of uniform epithelial cells were common (Fig. 4).

**Tumors of the Reproductive Tract**

**Tumors of the Ovary.** Ovarian tumors developed only in the DES-DMBA-exposed progeny. There were 8 neoplasms (42.1%) in Group 1, and none occurred in Group 3. The difference in incidence between the groups is statistically highly significant (42.1% versus 0 in Groups 1 and 3, respectively; \( p < 0.001 \)). The tumors were of the granulosa theca cell type (Fig. 5). Their morphological distinctions were described in detail previously (37). The average latency was 24 weeks.

**Tumors of the Uterus.** The DES-DMBA-exposed female progeny developed a significantly greater proportion of uterine tumors than did females subjected to DMBA postnatally only (68.4 versus 23.3% for Groups 1 and 3, respectively; \( p < 0.001 \)). Grossly, the tumors appeared as polypoid growths within the uterine lumen and often were multiple. Histologically, most of the tumors appeared to be benign polyps covered by columnar, tall, frequently pseudostratified, endometrial epithelium with areas of ulcerations.

The epithelial cells were usually tall and not atypical; mitoses were common. In many instances, the main mass of the tumor consisted of hyperplastic glands that were surrounded by lamina propria in an adenomatous pattern. In most of the tumors, the stroma was composed of various amounts and densities of connective tissue deriving from the endometrium. Occasionally, the stroma exhibited areas of hyperplasia and sarcomatous alteration of the tissue, but these were small and fairly well circumscribed. Two animals had mixed homologous tumors (carcinosarcomas) that were composed of myosarcomatous (sarcomatous) and carcinomatous elements. These developed from the uterine wall close to the cervix at 22 and 27 weeks and were characterized as homologous mixed Müllerian tumors, since both tissue elements composing the neoplasm were thought to derive from multipotential elements of the Müllerian system.

**Tumors of the Cervix.** Three female progeny exposed to DES-DMBA had polyps of the cervix, and one had a squamous cell papilloma. These developed from the endocervix as pedunculated excrescences that protruded into the cervical canal. Microscopically, the stroma of the tumor stalk contained vascular tissue and was covered by tall columnar epithelium with occasional areas of denudation. Stratified keratinized squamous cells covered the stalk of the papilloma. None of these tumors occurred in animals of Group 3 exposed only to DMBA.

**Tumors of the Vagina.** Tumors of the vagina were benign squamous cell papillomas. Five females of the DES-DMBA-exposed progeny and 6 animals treated with DMBA alone

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mammary gland (malignant)</th>
<th>Mammary gland (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>DES-DMBA</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>DES-10 mg/kg, DMBA 100 mg/kg</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>DMBA 100 mg/kg</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>DMBA 510 mg/kg</td>
<td>36</td>
<td>12</td>
</tr>
</tbody>
</table>

* Average age at which tumors were found at autopsy and necropsy.
* DES given by i.g. intubation once, 10 mg/kg, on Day 14 of gestation.
* DMBA given by gavage, once, 510 mg/kg, on Day 14 of gestation.

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developed these neoplasms. The difference in incidences of squamous cell papillomas was not significant (26 versus 12% for Groups 1 and 3, respectively). The morphology of these tumors was described in detail previously (33).

**Foreostomach Tumors**

All the females in both groups had foreostomach tumors which were squamous cell carcinomas and squamous cell papillomas (Table 1). The females of Group 1 exposed to DES-DMBA had significantly greater incidences of squamous cell carcinomas than did females treated solely with DMBA in adulthood [16, (84.2%) for Group 1 versus 20 (44.4%) for Group 3; \( p \leq 0.002 \)].

The males of Group 2 (DES-DMBA exposed) also had a significantly higher incidence of squamous cell carcinomas, i.e., malignant tumors, than did males of Group 4 (16 of 18 or 84.2% for Group 2 versus 16 of 36 or 44.4% for Group 4; \( p \leq 0.006 \)). In contrast, the number of squamous cell papillomas was significantly higher in both males and females (\( p \leq 0.002 \) and \( \leq 0.025 \), respectively) in Groups 3 and 4, i.e., in animals receiving DMBA alone during postnatal life. The morphological features of squamous cell carcinomas in this species were described in detail previously (33).

**Melanomas of the Skin**

Dermal melanomas developed at a significantly higher rate in DES-DMBA-exposed female and male progeny of Groups 1 and 2 than in animals of Groups 3 and 4. The difference in the incidence of melanomas in females (47.0% in Group 1 versus 10.0% in Group 3) is statistically significant (\( p \leq 0.005 \)), as is that in males (31.0% in Group 2 versus 8.0% in Group 4; \( p \leq 0.050 \)).

The morphology, pathogenesis, and influence of sex hormones on the development of these tumors were elaborated on in previous studies (25, 28, 33).

**Other Tumors**

In addition to the tumors described, several other tumor types were observed in both DES-DMBA-exposed progeny and animals treated postnataIly with DMBA (see the footnotes to Table 1). The occurrence of malignant lymphomas, squamous cell papillomas of the skin, squamous cell papillomas of the esophagus, pituitary adenomas (chromophobe), and a leiomyosarcoma of the prostate are worth noting since these tumor types are extremely rare in hamsters. The development of a somewhat greater rate of malignant lymphoma in DES-DMBA-exposed male progeny (2.7% versus 15.6%) over that in males treated with DMBA alone is of particular interest. The pathohistological and morphological features of malignant lymphomas (43) and squamous cell tumors (33) were described. The leiomyosarcoma observed in a DES-DMBA-exposed male progeny was composed of densely pocketed spindle or fusiform cellular elements which frequently formed a blurring interlacing pattern. The cells had an elevated rate of mitotic activity and commonly showed nuclear hyperchromatism (Fig. 6). The incidence and tumor types occurring spontaneously in the control animals were reported in previous communications (33, 35, 36). In a recent communication, we described the spectrum of neoplasms and the distribution pattern of tumors occurring in hamster progeny that were transplacentally exposed to DES alone (37).

**Miscellaneous Lesions**

Microscopic examination of reproductive organs of female progeny revealed hyperplasia of the ovaries, cystic glandular hyperplasia with polypoid projections of the endometrium, squamous cell metaplasia with keratinization and parakeratosis of uterine epithelium, glandular hyperplasia with polypoid projections and keratinization with marked parakeratosis of the endocervix, and squamous cell hyperplasia with keratinization of the epithelium in the upper vagina. These lesions occurred in greater proportions in progeny of Group 1, i.e., in DES-DMBA-exposed progeny, than in animals of Group 3 that received DMBA alone.

The nonneoplastic lesions of the reproductive tract of the male progeny consisted of spermatic granulomas of the epididymis, smooth muscle hyperplasia, and tubular (sometimes cystic) dilation of the epididymis. Atrophy of the seminal tubular epithelium in the testes could also be observed. The few granulomas appeared only in DES-DMBA-exposed male progeny. These lesions consisted of histiocytic cell elements that contained eosinophilic cytoplasm and brown pigment. Occasionally, blue-gray spherules reminiscent of Michaelis-Gutmann bodies were present in these cells. Giant cells of foreign body type were seen, particularly at the sites of contact with spermatocytes. An increase of fibromuscular stroma, commonly infiltrated by inflammatory cell infiltrate, was also found in the epididymis. In general, all types of lesions were more frequently observed in the DES-DMBA-exposed male progeny.

**DISCUSSION**

The presented findings indicate that prenatal exposure to a single dose of DES significantly increases the carcinogenic response of the progeny if they are subjected to DMBA during postnatal life. This increase in carcinogenic sensitivity of progeny exposed transplacentally to DES was evident in spite of the fact that animals treated with DMBA alone after birth received substantially more carcinogen (total of 190 mg/kg versus 510 mg/kg; \( p \leq 0.001 \)).

Thus, the DES-DMBA-exposed progeny developed not only a greater multiplicity of tumors per tumor-bearing animal (\( p = 0.001 \)) but also a broader spectrum of neoplasms and significantly higher rates of several tumor types. These tumors occurred primarily in the endocrine-responsive organs, i.e., in the reproductive tract (e.g., ovarian tumors, uterine tumors) and mammary gland. In addition, forestomach and skin (melanosomas) tumors were observed. The DES-DMBA-exposed progeny had also significantly higher rates of malignant tumors than did hamsters given DMBA alone, i.e., carcinomas of the mammary gland (\( p \leq 0.001 \)) and carcinomas of the forestomach (\( p \leq 0.001 \)).

The spontaneous incidence of mammary tumors in hamsters is extremely low (6, 10, 13, 42), and life-long repeated exposure to massive doses of DES and other estrogens does not appear to affect the occurrence of these tumors in the hamster (18, 30). No mammary gland tumors were observed in hamster progeny transplacentally exposed to DES that were subjected to one or multiple repeated doses of this drug as already.
reported in a separate communication (37). The experimental induction of this type of neoplasm in hamsters has been reported following administration of various carcinogens including 3-methylcholanthrene (6), DMBA (7), o-aminazotoluene (42), urethan (30, 44), and 1-acetoxypyroplpyrpylnitrosamine (1). In most instances, mammary tumors were accompanied by the neoplasms and/or proliferative changes of the genital tract (1, 6, 7, 30–32) regardless of the type of carcinogen used. This association of mammary tumors with those of the genital tract may suggest that polycyclic hydrocarbons may act through a hormonal mechanism (6, 7).

In the current study, the mammary tumors were associated with tumors of the ovary, vagina, cervix, and uterus, as well as with squamous cell metaplasia, cystic hyperplasia, and polyposis of all segments of the reproductive tract; and stromal hyperplasia of the ovaries and the pituitary gland. These lesions were more frequent (100%) and more extensive in DES-DMBA-exposed female progeny than in DMBA-treated females; in fact, the former also had significantly greater proportions of ovarian, uterine, and cervical neoplasms. It has been suggested that the estrogenic hormones are of essential importance in the initiating process of mammary carcinogenesis (5, 22–24) and that the carcinogenic action of the polycyclic hydrocarbon can be achieved effectively only when the epithelial cells exhibit an increased rate of mitotic activity stimulated by estrogens (5). The enhanced carcinogenic activity in the mammary gland that resulted in a significant ($p \leq 0.001$) increase of mammary carcinomas in the DES-DMBA-exposed progeny over the DMBA-treated females appears to be due to an altered pattern of gonadotrophin secretion and a consequent state of continuous estrogenic stimulation from the altered ovaries which was mediated through the endocrine disbalance in the hypothalamic-pituitary axis induced by DES during the prenatal life.

Accordingly, it can be speculated that the persistent estrogenic stimulation was responsible for the initiation phase and contributed to the promotion phase after the exposure to carcinogenic stimuli of DMBA had been already accomplished. The predominance of malignant mammary tumors (carcinomas) is thought to be the consequence of an interaction between estrogen and DMBA which resulted in a synergistic (not additive) carcinogenic effect. The influence of other factors involved in the mammary carcinogenesis, e.g., the altered secretion of prolactin, the quantitative relationship of estrogen in the progyn, and the dose of a given carcinogen (DMBA), should be considered, since estrogens do not exert a complete carcinogenic effect on the mammary gland in this animal species (7, 11, 30). The polycyclic hydrocarbons, including DMBA, per se also seem ineffective in inducing neoplastic transformation (5).

In this context, it should be emphasized that DES-DMBA-exposed progeny received less than one-half of the total dose of the carcinogenic polycyclic hydrocarbon, DMBA, given to hamsters receiving only DMBA (190 versus 510 mg/kg; $p \leq 0.001$). The prenatal exposure to DES (single dose, 10 mg/kg) might have increased the carcinogenic effect of DMBA, either by indirectly interacting with the estrogen produced by a continuous state of estrogenic stimulation or by increasing the sensitivity of the biological substrate, i.e., the differentiating cellular elements of the mammary gland and other target organs. It is of interest that over 90% of the ovarian tumors were associated with mammary neoplasms and 100% occurred with tumors of the uterus. It can be speculated that ovarian tumors were hormonally active and may have influenced the development of both mammary and uterine neoplasms, since it is known that these organs are under the direct influence of sex hormones. The development of ovarian tumors and neoplasms of the uterus, cervix, and vagina, as well as other hyperplastic and metaplastic lesions in the reproductive tract, again at greater rates in the DES-DMBA-exposed female progeny, suggests that persistent estrogenic stimulation and the carcinogenic hydrocarbon, DMBA, were involved in the carcinogenic process. It is important to note that all the segments of the reproductive tract are target tissues and as such are under the direct or indirect influence of the ovarian and/or pituitary hormones.

The significantly greater incidences of squamous cell carcinomas, i.e., malignant neoplasms, in DES-DMBA-exposed progeny over non-DES-treated animals are of particular interest since the forestomach is not usually considered an estrogen target organ. It is possible, however, that a change occurred in the hypothalamic-pituitary-adrenal axis similar to that in gonads, which in turn resulted in altered levels of glucocorticoids, which are known to affect the development and function of the gastrointestinal tract.

The role of sex hormones in the carcinogenic processes of dermal melanomas has been established in several animal species (14, 20), and the influence of estrogens on the development of these neoplasms in prenatal carcinogenesis in hamsters has been described in a previous report (34).

The information obtained from this experiment is of considerable relevance to carcinogenesis from 2 aspects. First, the induction of mammary tumors represents a useful experimental model for study of the genesis of these neoplasms at various levels of development, involving both hormonal effects and carcinogenic stimuli of a chemical acting in succession during prenatal and postnatal life. The biological substrate of origin of these tumors in hamster is embryogenetically identical to those occurring in humans, since over 80% of neoplasms derive from the ductal epithelium of the mammary gland.

Second, the implications of these findings to the human situation are obvious. There are between 500,000 and 2,000,000 young women in the United States alone who were exposed to DES in utero for therapeutic reasons (37). The possibility that the proliferative and metaplastic lesions and abnormalities in certain segments of the genital tract in these subjects may have resulted through the endocrine imbalance induced by the action of prenatal DES exposure on the hypothalamic-pituitary pathway has been suggested (4, 8, 9, 35), and the probability that these alterations will undergo malignant transformation following the introduction of a carcinogenic stimulus later in life should be considered (27, 39). Consideration should be given to the surveillance of people exposed in utero to DES as a potentially high-risk group for estrogen-mediated tumorigenesis.

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REFERENCES

1. Althoff, J., Grandjean, C., Pour, P., and Gold, B. Local and systemic effects...
Effects of Transplacental Exposure to Diethylstilbestrol on Carcinogenic Susceptibility during Postnatal Life in Hamster Progeny

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