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TERATOGENESIS AND CARCINOGENESIS IN RAT OFFSPRING AFTER TRANSPLACENTAL AND TRANSMAMMARY EXPOSURE TO DIETHYLSTILBESTROL

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Abstract—Transplacental and transmammary exposure of rat offspring to diethylstilbestrol (DES) was studied in regard to potential teratogenesis and carcinogenesis. Pregnant and/or lactating rats received DES in oil subcutaneously. In females so exposed, abnormal development of the urogenital sinus (hypospadias and urethrovaginal cloaca formation) occurred. In exposed male offspring, hypospadias, phallic hypoplasia, inhibition of growth and descent of testes, as well as abnormalities of Wolffian derivatives, were observed. In 20–40 per cent of DES-exposed female offspring, vaginal adenosis, endometrial squamous metaplasia, and genital malignancy were encountered. Two DES-exposed offspring had a vaginal squamous carcinoma, one had an endometrial adenocarcinoma, and one had an ovarian adenocarcinoma. Vaginal squamous carcinomas may have originated in foci of squamous metaplastic epithelium of vaginal adenosis. None of the control rats developed genital malignancy.

Exposure of pregnant women to drugs and environmental chemicals may account for 2–3 per cent of congenital abnormalities [1, 2]. In the past three decades, one to two million pregnant women in the United States may have received estrogenic substances, mainly diethylstilbestrol (DES) [3, 4], for the treatment of threatened abortion. Although the direct effects of natural estrogens and DES on the reproductive tissues of man and animals are fairly well understood, the teratogenic and potential carcinogenic actions of estrogenic substances on the embryo and fetus need further study.

DES treatment during pregnancy has caused virilization of the human female fetus but has only very rarely affected the male fetus. In laboratory animals, however, a paradoxical effect of estrogenic substances has been regularly noted, i.e. natural estrogens and DES cause virilization of the female fetus and feminization of the male fetus [5–9].

In 1971, Herbst *et al.* [10] related DES, given to pregnant women for prevention of threatened abortion [11], to the occurrence of vaginal clear-cell adenocarcinoma in some female offspring 13–23 years after their mothers had received the estrogenic drug. Shortly thereafter, Greenwald *et al.* [12] reported five patients, 15–19 years of age, with vaginal clear-cell adenocarcinoma. The mothers of these young women had all received DES during the first trimester of gestation and throughout the rest of the pregnancy [12]. The association between maternal DES treatment during pregnancy and the subsequent development of vaginal clear-cell adenocarcinoma in some of their daughters was statistically highly significant [10]. This was the first time in human pregnancy that a chemical carcinogen or cocarcinogen was determined to be transported from the mother via the placenta to the fetus and to incite the development of cancer after a long latent period in the offspring.

Although the teratogenic potential of DES is known, a transplacental carcinogenic effect of DES on the female fetus of laboratory animals has not yet been observed. Therefore, it seemed of interest to determine whether genital cancer could be induced in rat offspring if pregnant and/or lactating rats were given DES. The results of our study indicate that maternal DES treatment causes teratogenic effects and probably genital cancer in exposed offspring.

MATERIAL AND METHODS

Since daily DES doses (s.c.) of 0.05 mg/kg or above in sesame oil, given during days 13–21 of pregnancy, caused intrauterine death or delivery of nonviable progeny, dosages and time intervals had to be chosen empirically in order to assure adequate exposure of the fetus to DES and survival of progeny. Accordingly, intermittent DES doses of 0.015 to 0.6 mg/kg (see Tables 1 and 2) were given at days 13, 16, 18 and 20 of pregnancy to provide DES exposure during urogenital development.

Three lactating rats were given DES in doses of 0.2 to 0.8 mg/kg during the first 3 weeks of nursing; one lactating rat received DES doses of 10.0 mg/kg at days 9 and 20 postpartum. Two of these four mother rats also received DES during pregnancy. In female and male offspring, abnormalities were grossly and histologically documented in the living or in the deceased animal at the time of surgical exploration and/or autopsy. From DES-treated mothers and their female offspring, vaginal smears were taken in 1- to 2-week intervals for evaluation of ovarian function and for possible recognition of the development of malignancy. Offspring of pregnant and/or lactating mothers who

Table 1. Teratogenesis and carcinogenesis in female rat offspring after transplacental and/or transmammary diethylstilbestrol (DES) exposure

NUMBER OF FEMALE OFFSPRING	MATERNAL TREATMENT WITH DIETHYLSTILBESTROL				GROSS FINDINGS IN OFFSPRING	HISTOLOGIC FINDINGS IN OFFSPRING
	DAYS OF GESTATION	DES DOSE: mg/kg s.c.	DAYS POST PARTUM	DES DOSE: mg/kg s.c.		
1.	13 16 18 20	0.015 0.03 0.30 0.45	2 6 10 15 20	0.8 0.8 0.6 0.8 0.6	Life span: 13 months; hypospadias; urethrovaginal cloaca with urinary stone formation; urinary incontinence	Keratinization of vaginal epithelium; endometrial squamous metaplasia
2.	–	–	9 20	10.0 10.0	Life span: 20 months; excessive tooth growth	Keratinization of vaginal epithelium; questionable endometrial squamous metaplasia
3.	–	–	6 11 15 18 20	0.4 0.4 0.4 0.4 0.4	Life span: 24 months; excessive tooth growth; left utero-ovarian cystic tumor (4 x 5 cm dia.) containing 10 ml chocolate colored fluid; tumor metastases to omentum, visceral, and parietal peritoneum, and liver	Adenocarcinoma of endometrium (origin: endometrium of left uterine horn); tumor metastases in peritoneum, omentum, liver; endometrial squamous metaplasia
4.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 24 months; hypospadias	Endometrial squamous metaplasia
5.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 16 months; intra-abdominal conglomerate tumor (4 x 4 cm dia.); hypospadias, small distal urethrovaginal cloaca; vaginal and periurethral tumor (1 x 2 cm dia.)	Vaginal adenosis; vaginal squamous carcinoma; tumor metastases in omentum and peritoneum; endometrial sarcoma
6.	13 16 18	0.015 0.03 0.60	–	–	Life span: 19 months; hypospadias, large urethrovaginal cloaca; urinary bladder distention; hydroureters; necrotizing vaginal and periurethral tumor involving the whole vagina and extending to uterine cervix and paravaginal and para-uterine tissues	Vaginal adenosis; vaginal squamous carcinoma, extending into rectum and urinary bladder; vaginal keratinization; endometrial squamous metaplasia
7.	13 16 18	0.015 0.03 0.60	–	–	Life span: 18 months; excessive tooth growth; hypospadias, urethrovaginal cloaca with urinary stone formation; paranephric abscess (5 x 5 cm dia.)	No abnormal findings
8.	–	–	6 11 15 18 20	0.4 0.4 0.4 0.4 0.4	Life span: 13 months; excessive tooth growth; no genitourinary abnormalities	Thin vaginal epithelium; questionable endometrial squamous metaplasia
9.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 24 months; hypospadias	No abnormal findings
10.	–	–	6 11 15 18 20	0.4 0.4 0.4 0.4 0.4	Life span: 26 months; excessive tooth growth; retroperitoneal abscess; tumor at end of right uterine horn (0.3 x 0.5 cm dia.)	Moderate keratinization of vagina; ovarian adenocarcinoma; endometrial squamous metaplasia

Table 2. Teratogenesis in male rat offspring after transplacental and/or transmammary diethylstilbestrol (DES) exposure

NUMBER OF MALE OFFSPRING	MATERNAL TREATMENT WITH DIETHYLSTILBESTROL				GROSS FINDINGS IN OFFSPRING
	DAYS OF GESTATION	DES DOSE: mg/kg s.c.	DAYS POST PARTUM	DES DOSE: mg/kg s.c.	
1.	13 16 18	0.015 0.03 0.60	-	-	Life span: 12 months; hypoplastic inguinal testes; hypospadias
2.	13 16 18 20	0.015 0.03 0.30 0.45	2 6 10 15 20	0.8 0.8 0.6 0.8 0.6	Life span: 17 months; hypoplastic inguinal testes; hypospadias
3.	13 16 18 20	0.015 0.03 0.30 0.45	2 6 10 15 20	0.8 0.8 0.6 0.8 0.6	Life span: 17 months; hypoplastic inguinal testes; hypospadias; excessive tooth growth
4.	13 16 18 20	0.015 0.03 0.30 0.45	2 6 10 15 20	0.8 0.8 0.6 0.8 0.6	Life span: 18 months; hypoplastic inguinal testes; hypospadias; excessive tooth growth
5.	-	-	9 20	10.0 10.0	Life span: 9 months; hypoplastic scrotal testes
6.	-	-	9 20	10.0 10.0	Life span: 18 months; hypoplastic scrotal testes; excessive tooth growth
7.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 21 months; hypoplastic, inadequately descended testes
8.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 21 months; hypoplastic, inadequately descended testes
9.	20	0.60	1 3 10 15 17 20 22	0.8 0.6 0.8 0.8 0.2 0.4 0.6	Life span: 23 months; hypoplastic, inadequately descended testes

had received injections with sesame oil served as controls.

RESULTS

Five of the DES-treated mother rats delivered a totai of thirty-one live offspring of which twenty survived. Nineteen of the twenty rats were observed and examined regularly throughout their life span, which ranged from 9 to 26 months. Out of the nineteen offspring three were exposed to DES during pregnancy, six were exposed to DES during lactation, and ten were exposed to DES both during pregnancy and lactation.

Teratogenic effects of DES

DES exposure of the female offspring during the fetal phase of gestation and/or during the first 4 days of lactation disturbed mainly the development and differentiation of the female urogenital sinus epithelium. However, urogenital sinus defects in the DES-exposed male were overshadowed by the antagonistic DES effects on testicular growth and development of Wolffian structures and gubernaculum testis. The effects of DES exposure on female offspring are presented in Table 1 and the effects on male offspring in Table 2; no genital abnormalities were observed in the controls.

Female hypospadias and a relatively large cloaca formation between the vagina and the urethra with urinary stone deposition were found in two rats (numbers 1 and 7; Table 1; Fig. 1). In these two animals intravenous urography showed normal topography and anatomy of the kidneys, ureters and urinary bladder (Figs. 2 and 3). In another female offspring (number 6; Table 1), a large urethrovaginal cloaca without stone formation was found. Hypospadias with a small cloaca was found in female offspring number 5. Vaginal smears of DES-exposed offspring consistently showed estrogenic stimulation with a predominance of intermediate and superficial cells. None of these animals had normal cyclic vaginal smears.

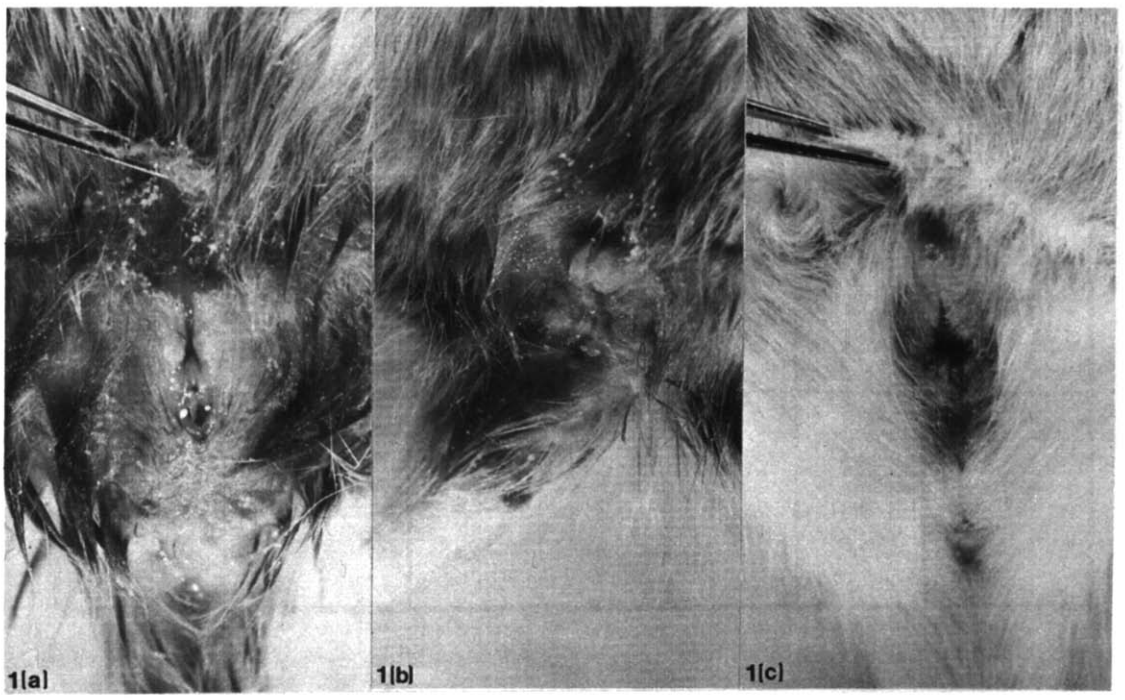
In four male offspring, hypospadias was also present (Table 2). Hypospadias is diagnosed in the male rat when a cleft phallus and urethra exist. In DES-exposed male offspring undescended and hypoplastic testes were almost always present. Teratogenic effects were most pronounced when transplacental DES exposure occurred. In such offspring, the Müllerian structures persisted in the form of rudimentary uterine horns. Wolffian structures (vas deferens, epididymis and seminal vesicles) were underdeveloped or partly absent. Also, male urogenital sinus development was disturbed, and rudimentary lower vaginal structures and a small hypospadic phallus were present. Neither female nor male offspring of DES-treated mothers could reproduce when paired with normal fertile rats.

Excessive growth of incisor teeth ("tusk" formation) occurred in five out of ten female and in three out of nine male offspring exposed to maternal DES (see Tables 1 and 2; Fig. 4). In these animals tooth clipping was necessary at 3- to 4-week intervals.

Carcinogenic effects of DES

Malignancy was not observed in DES-exposed male offspring and no genital cancer was discovered in DES-exposed mothers; one mother rat developed a mammary carcinoma. In the male and female control animals, genital malignancy did not occur.

Four out of ten exposed female offspring (one exposed *in utero* only, one *in utero* and during lactation, and two during lactation only) developed malignant tumors. Two offspring had a vaginal squamous carcinoma (Table 1; rats numbers 5 and 6) and in one of these rats (number 5) the histologic examination additionally revealed a poorly differentiated endometrial



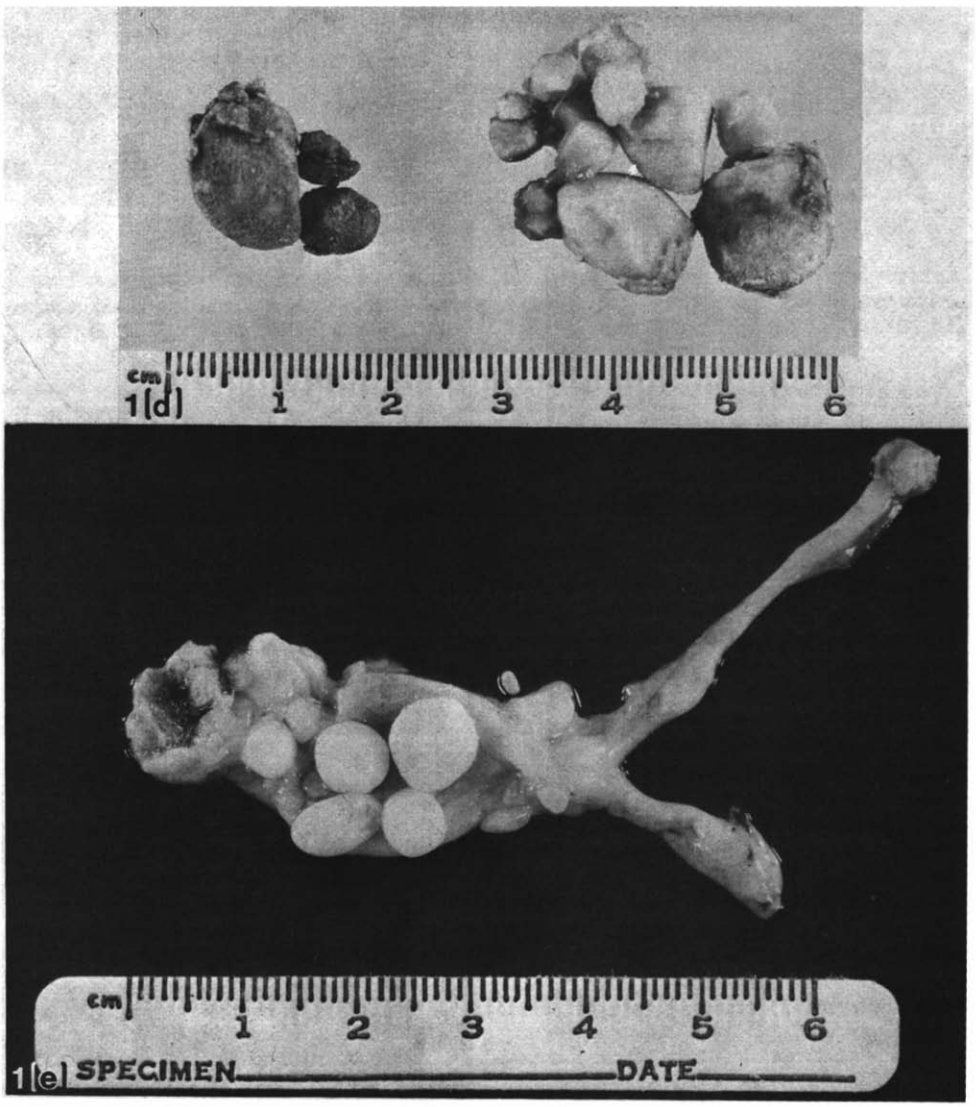


Fig. 1. Teratogenic effects of DES on the urogenital system of female rats. (a) Hypospadias in response to DES exposure (rat number 1; Table 1). Top: lower abdominal skin area partly incrusted with urinary components. Center: defective closure of vagina and urethra with incrustation of surrounding skin due to urinary incontinence. Bottom: anal area and base of rat's tail incrusted with urinary components. (b) Hypospadias in response to DES exposure (rat number 7; Table 1). Center: the opening of the urethra, located within a phallus-like structure, is split and in communication with the vagina. Incrustation of anogenital and lower abdominal area is due to urinary incontinence. (c) External genital area of a normal adult rat. Center: opening of normal vagina; a prepuce-like structure originates above the vagina and the urethra emerges into the caudal part of this structure. Bottom: anal area and base of tail. (d) Urinary stones removed from urethrovaginal cloaca of rat number 7 (left side of picture) and rat number 1 (right side). (e) Uterus, vagina and urethrovaginal cloaca with urinary stones (rat number 7; specimen at autopsy).

sarcoma. In these two animals the smear showed dysplastic, multinucleated vaginal cells with large nuclei from month 5 (rat number 5) and month 9 (rat number 6) of life on; after month 7 and 10, respectively, erythrocytes appeared in the vaginal smear. In rat number 5, at the age of 15 months, a urogenital tumor of about 1 × 2 cm in diameter was palpable and there was thin red vaginal secretion. At the time of surgical exploration and autopsy (age 16 months), a vaginal tumor 1 × 2 cm in diameter was found. The histologic diagnosis was vaginal squamous carcinoma with metastases into omentum and peritoneum; also, foci of vaginal adenosis were observed (Fig. 5). In rat number 6, at the age of 17 months, vaginal bleeding was observed and a suprapubic tumor of 1 × 1 cm in diameter was palpable. In this rat surgical exploration and autopsy at the age of 19 months revealed a vaginal squamous carcinoma of 2 × 2 cm in diameter invading the uterine cervix and the paravesicular and pararectal structures (Fig. 6); in the vagina, also foci of adenosis were observed and the endometrium displayed squamous metaplasia (Fig. 7).

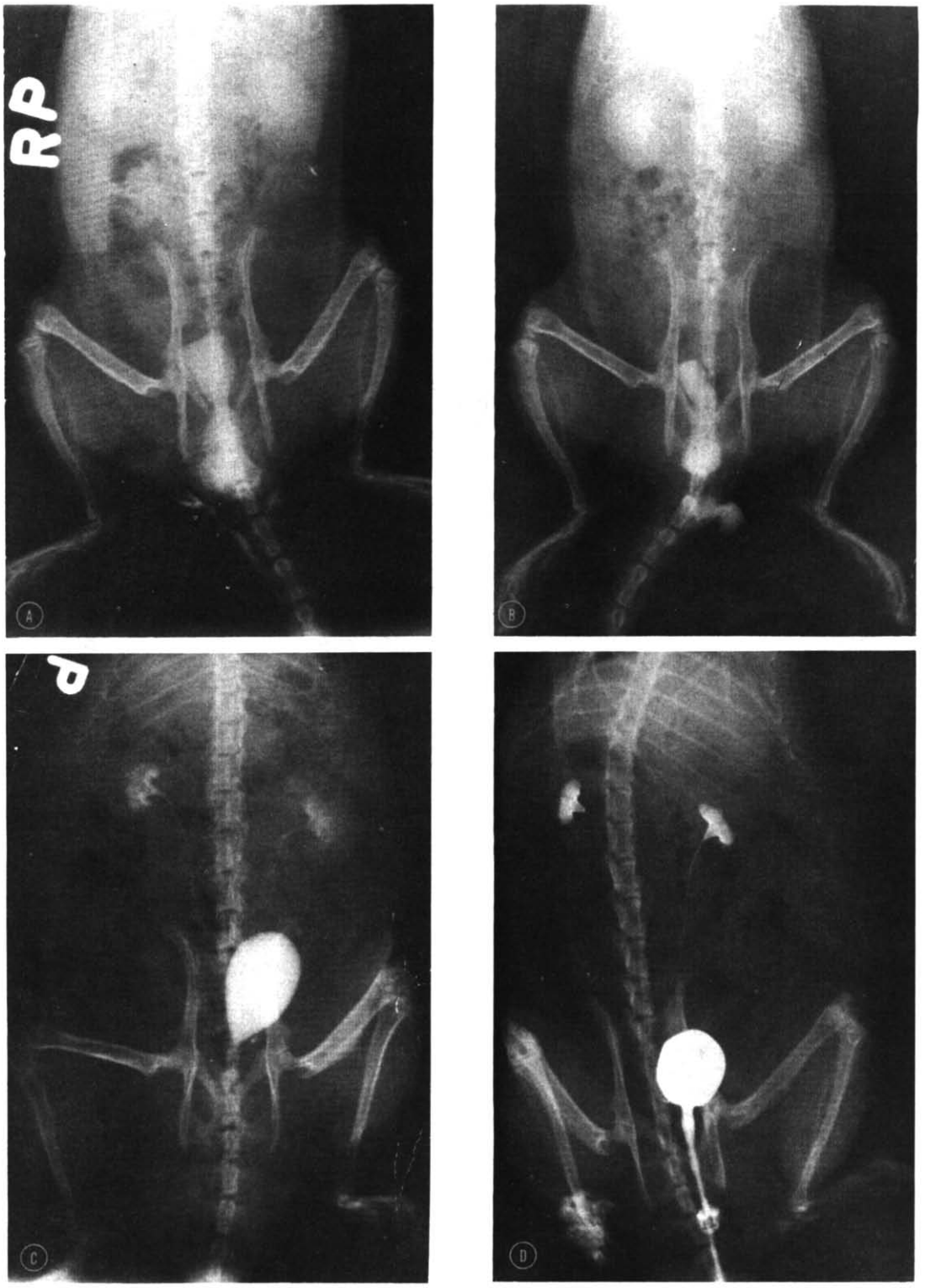


Fig. 2. Intravenous and retrograde urography of female rats. (A) Intravenous urography (rat number 1). Five min after intravenous injection of 1 ml Renografin into a tail vein, the contrast agent is excreted into the urinary bladder via the urine. Insufficient function of the urethrovesical sphincter permits the contrast agent to reach the urethrovaginal cloaca and to leak outside. (B) Intravenous urography (rat number 7). Five min after intravenous injection of 1 ml Renografin, the contrast agent is excreted into the urinary bladder via urine and leaks outside through the urethrovaginal cloaca. (C) Intravenous urography (normal rat). Five min after intravenous injection of 1 ml Renografin, the contrast agent accumulates and stays in the urinary bladder, indicating intact function of the urethrovesical sphincter. (D) Retrograde urography (rat number 5). Retrograde injection of 1 ml Renografin into the urinary bladder via a small plastic cannula inserted through the extroverted hypospadic urethra. The contrast agent reveals normal anatomy and topography of kidneys, ureters and urinary bladder; urethrovesical sphincter function appears insufficient. Note: This X-ray picture shows the rat's left-sided intra-abdominal tumor found several months later at autopsy.

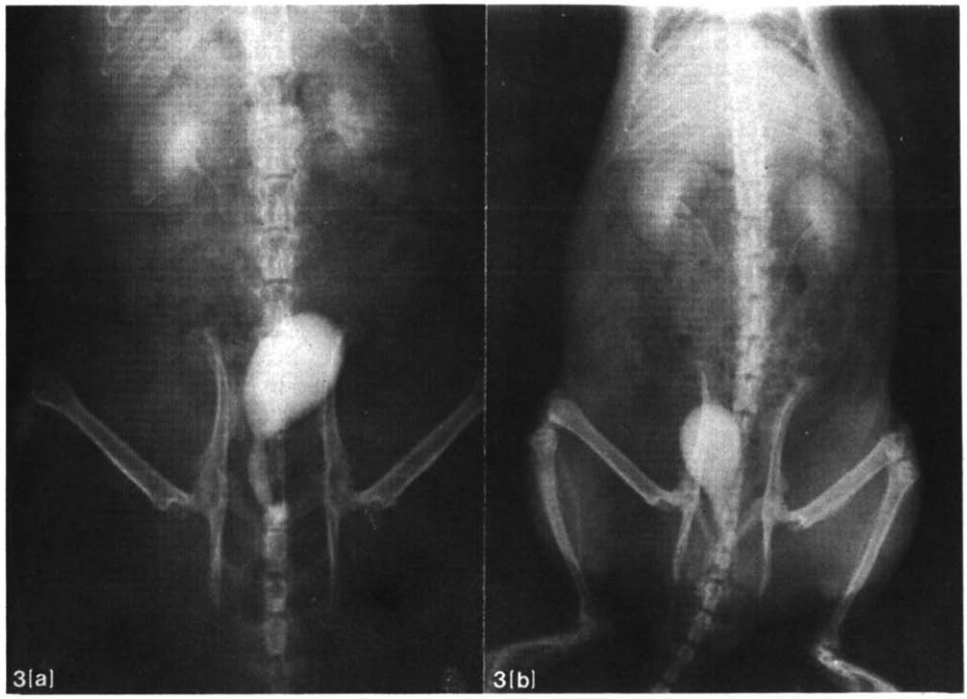


Fig. 3. Intravenous urography in male rats. (a) Five min after intravenous injection of 1 ml Renografin, the contrast agent is excreted to a large extent into the urinary bladder (rat number 1). Due to insufficient function of the urethrovesical sphincter, the contrast agent fills the urethra and the enlarged hypospadic urethral orifice. (b) Five min after intravenous injection of 1 ml Renografin, a major portion of the contrast agent is excreted into the urinary bladder and stays there (normal rat).

In rat number 3 (Table 1), exposed to DES only during lactation, a metastasizing adenocarcinoma of the endometrium was found (Fig. 8); the vaginal smear of this rat had an estrus-like picture but no suspicious cells. An ovarian adenocarcinoma developed in rat number 10, which had been exposed to DES only during lactation (Fig. 9); the possibility of the carcinoma originating from the endometrium cannot be excluded. In six rats (numbers 1, 2, 3, 4, 6 and 10; Table 1), vaginal keratinization or endometrial squamous metaplasia or both were present.

DISCUSSION

DES-induced teratogenesis of the urogenital system

Paradoxical effects of DES. In the past, teratogenic effects of natural estrogens and DES have been documented in the male and female rat fetus as well as in the human fetus (Table 3). Paradoxical estrogenic effects in male (feminization) and female (virilization) rat fetuses produced by estradiol, estrone and DES have been reported before [6–9] and in our study similar results were obtained (Tables 1 and 2). When the suckling

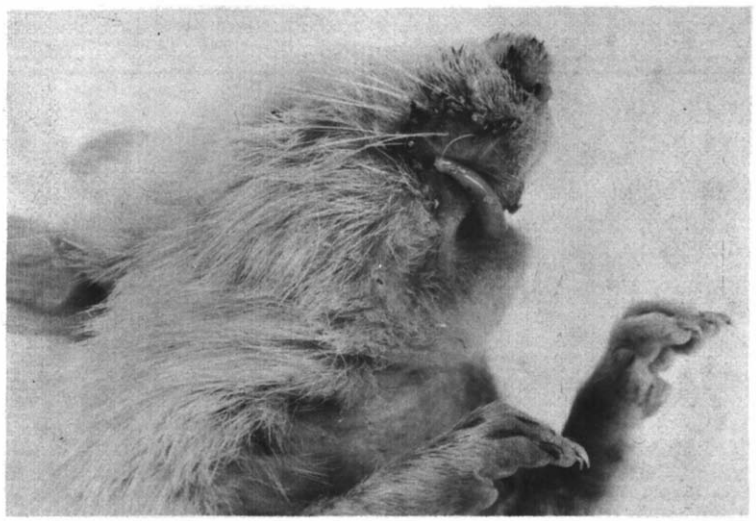


Fig. 4. Excessive tooth growth in DES-exposed rat. In this female rat (number 7), exposed to DES *in utero*, excessive tooth growth ("tusk" formation) necessitated clipping of the tooth every 3–4 weeks because of local irritation and difficulties in food intake.

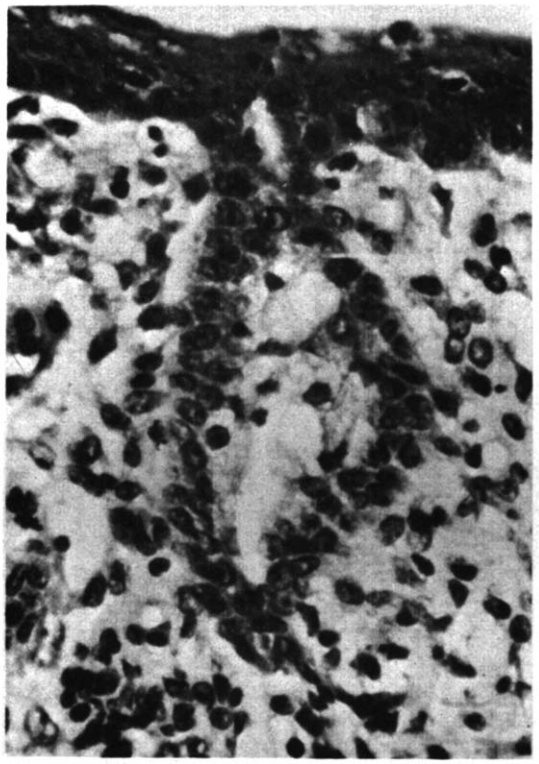
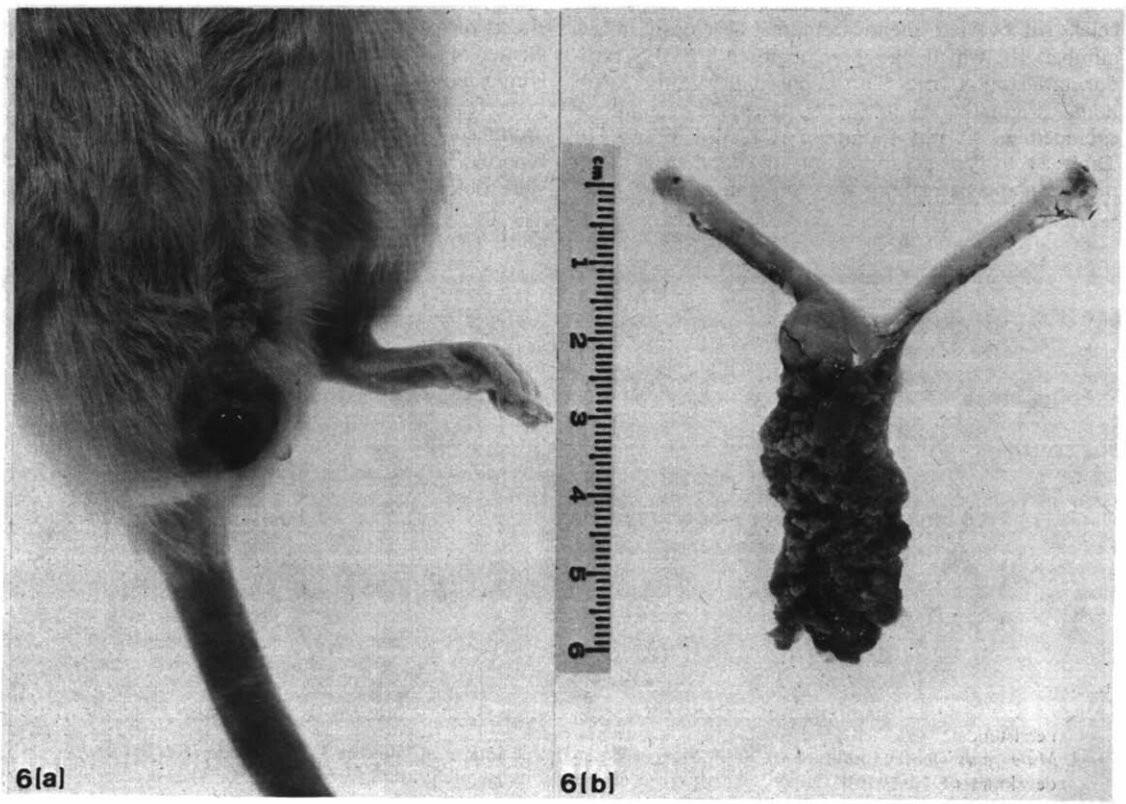


Fig. 5. Vaginal adenosis occurring in an animal with squamous vaginal carcinoma (magnification: × 700). Between areas of vaginal squamous carcinoma, foci of adenosis are present (rat number 5). From the vaginal epithelial surface (top and center of picture), a flask-like mucous gland extends into the vaginal stroma.

litter was exposed to DES excreted into the milk from day 6 of lactation, no major gross urogenital abnormalities were observed. This is in agreement with earlier findings [6] wherein no hypospadias was observed in neonatal rats treated with estrone. In a more recent study, however, estradiol and testosterone treatment of neonatal rats produced hypospadias [16]. Therefore, it appears that in DES treatment of neonatal rats, the proximity of exposure after birth and the total drug dosage applied are crucial factors in the production of urogenital abnormalities.

Hypospadias and urethrovaginal cloaca formation. Exposure of male and female offspring resulted in the development of hypospadias, i.e. a defect in urethral development affecting its ventral wall closure (Figs. 1a and 1b). In such females, the vaginal orifice was found to be common with the distal end of the urethra at the base of the clitoris. In other females (numbers 1, 6 and 7; Table 1) urethrovaginal union encompassing the lower third of the vagina, i.e. formation of a urethrovaginal cloaca, was noted (Figs. 2A and 2B). Normally the rat urethra does not open at the level of the vaginal outlet, as in women, but at the bottom of the prepuce, similar to a small penis, above the dorsal segment of the vagina. Urethrovaginal cloaca formation is the result of failure of proper differentiation and closure of the cranial lower part of the vagina and caudal wall of the urethra.

Inhibition of testicular descent, testicular hypoplasia. DES-induced testicular changes (cryptorchidism, hypoplasia) may be explained by the inhibition of fetal testicular testosterone secretion, with atrophy of both the gubernacular system and processus vaginalis; such changes have been observed in male mice exposed *in*



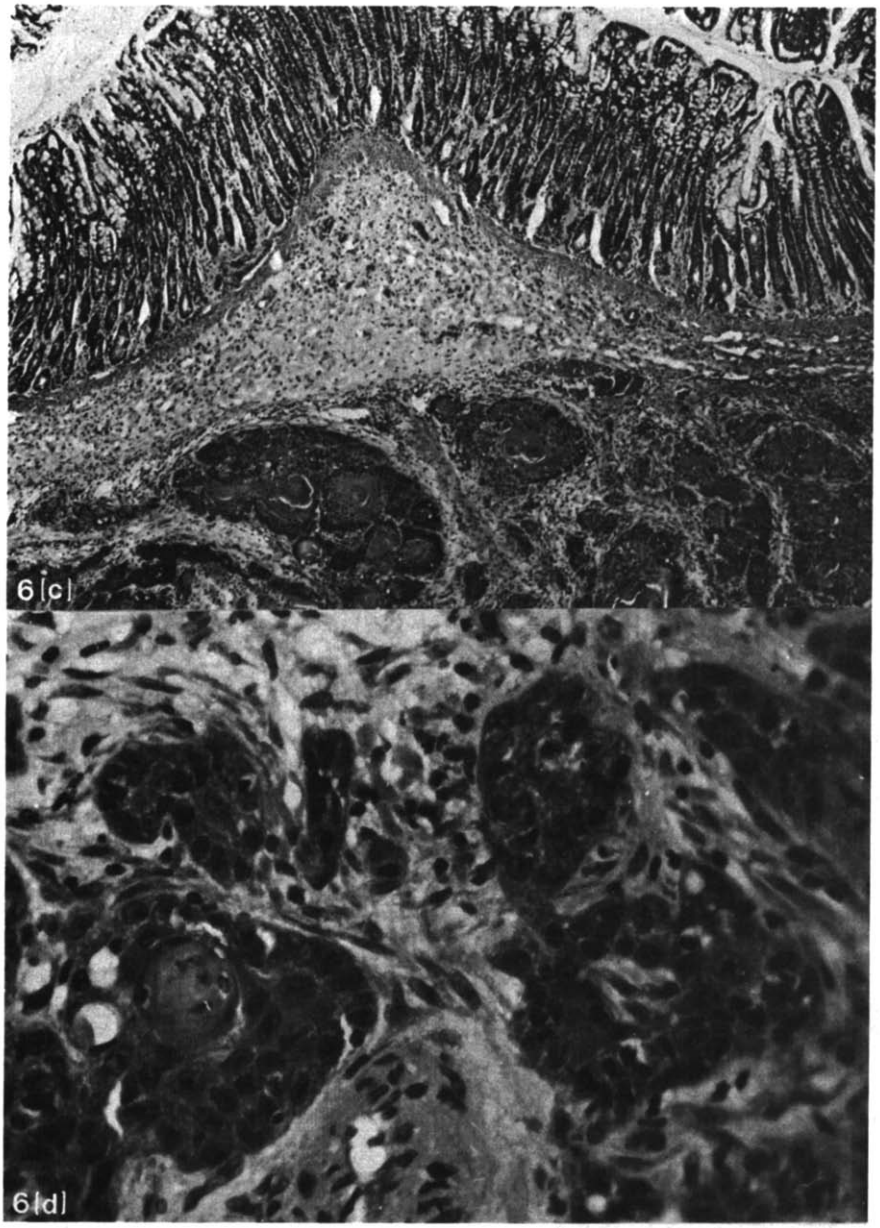


Fig. 6. Vaginal squamous carcinoma (rat number 6). (a) Tumor bulging through the urethrovaginal cloaca. (b) Gross specimen of vaginal squamous carcinoma. Malignant growth involves nearly the whole vagina with carcinomatous infiltration into paravesical, cervical, paracervical and pararectal tissues. (c) Vaginal squamous carcinoma (top) infiltrating muscularis of the rectum (magnification: × 150). (d) Higher magnification (× 700) of panel c showing invasion of the rectal wall by nests of moderately well-differentiated squamous carcinoma.

Table 3. Teratogenic effects of natural estrogens and of diethylstilbestrol on the urogenital system of rat and man*

A. RAT

1. *Female fetus:* virilization (urogenital sinus and genital tubercle develop in a masculine manner); disturbance of development of lower vagina; hypospadias; partial preservation of Wolffian structures (seminal vesicle formation); inhibition of formation of ovarian capsule.
2. *Male fetus:* feminization (urogenital sinus and genital tubercle develop in a feminine manner); inhibition of descent of testes; inhibition of development of epididymis, vas deferens, coagulation glands, seminal vesicles, and prostate; atrophy of ejaculatory ducts, underdevelopment of phallus; hypospadias (lack of closure of balanopreputial fold); epithelial proliferation of urethra; persistence of Müllerian epithelium (vagina, uterus).

B. MAN

1. *Female fetus:* virilization (clitoris enlargement, partial fusion and marked rugation of labio majora, labioscrotal fusion); persistence of Müllerian vaginal structures, normal internal female genitalia.
2. *Male fetus:* feminization in extremely rare instances; hypospadias, testicular hypoplasia; retarded psychosexual development, "feminine" behavioral pattern; decrease in athletic ability.

* Data from Refs. 5–9, 13–15.

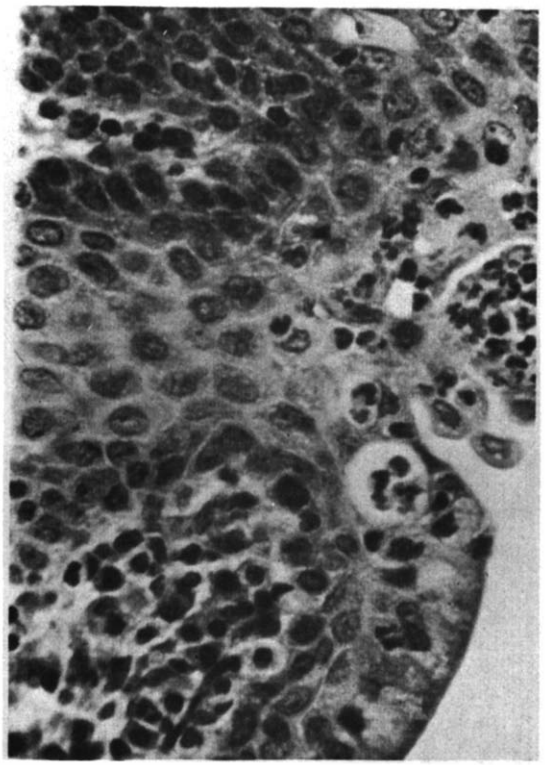


Fig. 7. Uterine horn, endometrial squamous metaplasia (rat number 6; magnification: × 700). Metaplastic squamous epithelium replaces the normal endometrial glands. This rat also had a vaginal squamous carcinoma.

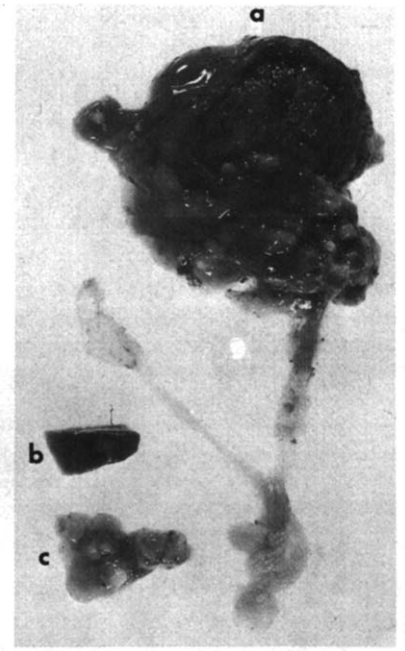


Fig. 8. Gross specimen of metastasizing endometrial adenocarcinoma (rat number 3). The endometrial adenocarcinoma originates from the upper part of the left uterine horn (a). Tumor metastasized to liver (b) and to omentum (c).

utero to estradiol [17]. The infertility in DES-exposed male rats may result from urogenital sinus defects, testicular hypoplasia, and cryptorchidism with decrease in blood supply and increase in temperature of testicular tissues resulting in impairment of spermatogenesis [17, 18]. In male mice exposed prenatally to DES, cryptorchidism (fibrotic testes) and epididymal cyst formation with ensuing sterility were observed [19].

Cloacal urinary stone formation and infertility. Urinary stone formation in the urethrovaginal cloaca of two offspring was probably the consequence of chronic local irritation and inflammation. The chemical nature of the urinary stones has not been determined yet. It is not clear to what extent DES may have contributed to the stone formation. In three out of thirty neonatal mice given a single dose of 20 mg DES subcutaneously, vaginal stone formation was observed in the absence of a urethrovaginal cloaca [20]. The infertility encountered in female rat offspring of DES-treated mothers may be explained by the urogenital abnormalities and/or disturbance of the hypothalamic–endocrine axis. In

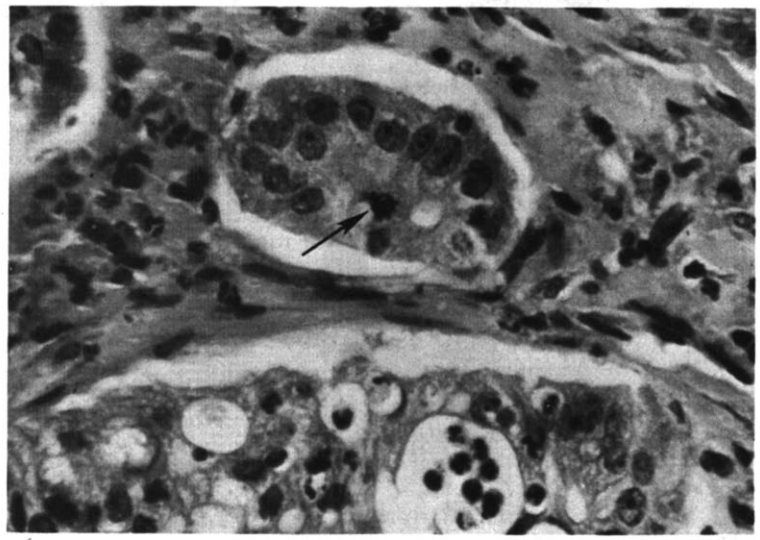


Fig. 9. Ovarian adenocarcinoma (rat number 10; magnification: × 700). Glandular nests of well-differentiated adenocarcinoma infiltrate the ovarian stroma. The malignant cells have numerous mitotic figures (arrow).

females, persistent estrus-like vaginal smears were observed, indicating dysfunction of the hypothalamo–pituitary–ovarian axis. It is well understood that early neonatal exposure of mice and rats to estrogenic and androgenic substances causes hypothalamic disturbance, leading to anovulatory cycles with a predominance of the stage of vaginal cornification (persistent estrus); these animals are usually infertile [16, 21–23].

Potential nonurogenital effects of DES: excessive tooth growth

The observation of excessive tooth growth in rats exposed to maternal DES was rather surprising. It is of interest to note that, in rats, rabbits, and dogs exposed *in utero* to antiestrogenic substances such as chlomiphene, abnormal bone development (cleft palate, malformed limbs, and missing phalanges) occurred [24–26]. In Japan, pregnant women were exposed accidentally to polychlorinated biphenyls that had leaked into cooking oil, which affected the fetus transplacentally. In such newborns, dark-brown skin staining, growth retardation, gingival hyperplasia, early tooth growth, and spotted skull calcification were observed [27]. It cannot be decided yet whether DES directly promotes accelerated tooth growth in rats or whether it brings about structural changes in the skull and jaw such that malocclusion occurs with subsequent lack of normal mechanical wear of the incisor teeth. This subject remains to be elucidated.

Mechanisms involved in DES teratogenesis

DES-induced inhibition of fetal adrenocortical and testicular 3β-hydroxysteroid dehydrogenase. Exposure of the rat fetus to DES caused pronounced urogenital abnormalities. In the rat and in the human, DES may cause adrenocortical hyperplasia, yielding increased secretion of androgenic substances [5, 28]. DES and estradiol have been described as causing inhibition of fetal adrenocortical and testicular 3β-hydroxysteroid dehydrogenase, thereby raising the production of adrenocortical 3β-hydroxysteroidal androgens (3β-ol-androgens) while decreasing the formation of testosterone in testicular interstitial Leydig cells [29–32]. In humans and in laboratory animals, DES tended to accumulate in testicular tissues, inhibiting testosterone production [5]. Estradiol and DES significantly reduced testicular testosterone secretion *in vitro* of mice and rats [33]. Although DES induces an increase in adrenal androgen production, this is not sufficient to compensate for the testicular deficiency of testosterone synthesis. As a consequence of this and of the inherent estrogenic activity of the DES molecule, feminization occurs (Table 3). Conversely, female fetuses became masculinized (clitoris hypertrophy and hypospadias) due to enhanced adrenocortical androgen production, resulting in disturbance of the development of the urogenital sinus epithelium [29].

DES-induced inhibition of fetal adrenocortical 3β-hydroxysteroid oxidoreductase. Estrogenic agents also may inhibit the activity of the adrenocortical enzyme 3β-hydroxysteroid oxidoreductase, leading to diminished secretion of cortisone in rats or cortisol in humans; through a concomitant rise in pituitary ACTH secretion, adrenocortical overproduction of 3β,5-ene androgens occurs [34]. Such a mechanism may cause virilization of the female and undervirilization (feminization) of the male fetus.

DES interference with fetal pituitary LH secretion and fetal sex steroid metabolism. Since testicular testosterone synthesis is under pituitary control [35], DES in the fetal circulation may exert a negative feedback of pituitary FSH-LH release and thus diminish testicular testosterone secretion, causing feminization.

It has also been suggested that DES may interfere with progesterone metabolism in the mother and the fetus, favoring increased formation of androgenically active compounds [32]. Also, a virilizing action of DES, disturbing the natural estrogen metabolism in the materno-fetoplacental system, and a masculinizing effect of DES metabolites have been proposed [13–15]. DES may not only compete with receptors for natural estrogens but also may stimulate microsomal liver enzymes for increased metabolism of estrogens and testosterone.

DES exposure, genital epithelial changes, and genital cancer

Vaginal keratinization and endometrial squamous metaplasia. In some of the DES-exposed rat offspring, vaginal keratinization and endometrial squamous metaplasia were observed (Table 1). Under the influence of estrogens, vaginal cells of rodents undergo keratinization [36], which is also termed cornification. Transplacental exposure of mice fetuses to ethinyl estradiol resulted in vaginal cornification, persistent estrus (absence of corpora lutea), and glandular cystic hyperplasia with squamous metaplasia of the endometrium [37]. Moreover, estradiol (50 μg/dose) injection into fetal mice (days 15 and 17 of pregnancy) produced irreversible vaginal cornification and inhibited ovarian corpora lutea formation [38]. Estradiol treatment of neonatal mice also caused vaginal parakeratosis [39] and endometrial squamous metaplasia [40]. It appears that not only exposure to exogenous estrogenic substances results in such changes, but more so, the postpuberal persistent ovarian estrogen secretion may be considered a major contributory factor in the development of vaginal keratinization and endometrial squamous metaplasia.

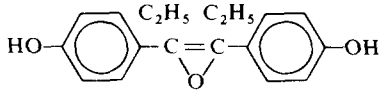
Transplacental and transmammary exposure to DES and development of cancer. To our knowledge, the development of vaginal, endometrial or ovarian cancer in rat offspring exposed to intrauterine DES or to postpartum DES via the mother's milk has not been reported before.

In animal number 6 (Table 1) intrauterine exposure to maternal DES was most likely responsible for the development of a squamous vaginal carcinoma. Another offspring with a squamous vaginal carcinoma (rat number 5; Table 1), which had been exposed to maternal DES *in utero* and during lactation, also had a poorly differentiated sarcoma of the vaginal and uterine wall. In addition to prenatal exposure, postnatal exposure to DES via milk intake may have contributed to carcinogenesis. The rate of lung carcinomas has been reported to be higher in mice exposed to maternal urethan *in utero* and during lactation, than in those exposed *in utero* only [41]. DES exposure during lactation resulted in squamous endometrial metaplasia and in the development of a metastasizing endometrial adenocarcinoma (rat number 3; Table 1).

In this small population, however, it is difficult to assess the chance of association of observed malignancies in offspring with maternal DES treatment. The belief that spontaneous vaginal and uterine carcinomas in rats are rare [42–45] is supported by our study.

Transmammary DES exposure and problems involved. Maternal DES treatment can suppress lactation by inhibiting the prolactin-induced mammary synthesis of milk. The DES dosage chosen and the time intervals of its administration allowed the suckling litter to receive enough milk for growth and survival, albeit thriving of offspring was often considerably reduced. To what extent DES, subcutaneously injected into the mother rat, entered the milk is not known to us. Because the DES solutions were injected under the abdominal skin, occasionally some of the DES may have been deposited directly into mammary tissues and in milk, possibly increasing DES uptake by the suckling offspring. It also seems possible that, after subcutaneous injection, retrograde contamination with DES of the abdominal skin and nipple area occurred; some of this DES might have been ingested by the offspring. Accordingly, in some offspring transmammary and direct postnatal DES exposure may have occurred. Postnatal DES and methylcholanthrene treatment of female rats resulted in a high incidence of endometrial squamous metaplasia and squamous carcinoma; occurrence of endometrial adenocarcinoma was rare [42]. Neonatal mice injected with DES and estradiol from birth on also developed vaginal, cervical, endometrial and ovarian cancer [46, 47].

Potential mechanisms of DES-carcinogenesis. In solution, DES exists in equilibrium with a *cis*-isomer, approximately 70:30 *trans* to *cis* form. The *cis* form is very unstable and reverts readily to the *trans* form. But the *cis* form



attracts electrons strongly and this leads to easy epoxide formation at the double bond. Like epoxide-forming chemical carcinogens, DES epoxide can attach to DNA and cause misreading or lack in enzyme formation, possibly resulting in carcinogenesis.* Whereas Miller *et al.* [48] suggested that only DES, and not its metabolites, supports development of carcinogenesis, other investigators have proposed that DES causes carcinogenesis by the interaction of its reactive metabolites with tissues [49, 50]. Accordingly, the DES metabolite, hydroxydienestrol, may via an epoxide–diol pathway develop electrophilic reactivity [50]. Fetal and early neonatal exposure to DES or to any other chemical carcinogen may result in the development of cancer due to decreased detoxification of the agent(s) and to immunological immaturity [51]. Later, the puberal ovarian estrogen secretion may induce and/or accelerate the development of malignancy; estrogens increase mitotic activity and thus may cause epithelial hyperplasia, dysplasia and neoplasia. Postpuberal ovarian estrogen secretion may stimulate columnar cellular proliferation of foci of vaginal and cervical adenosis, and thus be etiologic, in combination with the inherent carcinogenic aftereffect of DES and the possible intracellular activation of dormant type II herpes virus, for the development of malignancy in DES-exposed women [52, 53].

* J. Weissinger, personal communication (1975).

CONCLUSION

The transplacental teratogenic and carcinogenic potential of DES in humans and in rats can be recognized. However, whether natural estrogens may exert similar carcinogenic effects is not known. Also, it remains to be determined what other chemicals may cross the placenta or appear in breast milk and result in cancer of the reproductive or other organs later in life. All agents that are known to cause cancer in man also induce malignancy in animals but often respective tumor sites, histology and tumor biology are different in animals and man.

In humans, a latent period of two decades for the development of vaginal and cervical clear-cell adenocarcinoma in DES-exposed offspring has to be considered. This and the additional threat of increased development of squamous vaginal carcinoma during the fifth decade of life warrant means for recognition of potential dangers. Accordingly, an animal model which allows induction of genital or other cancer in offspring exposed to the carcinogenic agent within 1–2 years is most desirable for screening potential carcinogens. Although genital cancer developed in DES-exposed female rats, the exact condition of clear-cell adenocarcinoma of DES-exposed women could not be reproduced; perhaps this will be possible in future studies. A suitable animal model which bears close resemblance to humans in similar circumstances could contribute to a better understanding of the etiology and prevention of cancer.

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