Reproductive Tract Lesions in Male Mice Exposed Prenatally to Diethylstilbestrol

Abstract. *Sixty percent of the male offspring from pregnant mice treated with diethylstilbestrol during gestation were sterile. The affected animals had gonadal changes which included intra-abdominal or fibrotic testes, or both. Additionally, nodular masses in the ampullary region of the reproductive tract were observed in 6 of 24 animals; one of these appeared to be preneoplastic.*

The synthetic estrogen, diethylstilbestrol (DES), is currently used as a food additive for cattle and a postcoital contraceptive in women. For 25 years preceding 1971, DES was used as an obstetrical medication to treat threatened abortion. Recent reports have described the latent appearance of a previously rare genital tract tumor, vaginal adenocarcinoma, in young women whose mothers had been given DES during their gestations (*1*). The only reported adverse effects of prenatal DES exposure on the male offspring is the pseudohermaphroditic neonate described by Kaplan (*2*).

In the present study, 20 timed pregnant CD-1 mice (Charles River Breeding Laboratories) were treated subcutaneously with DES (Sigma) in corn oil on days 9 through 16 of gestation. The daily dose was 100 μg per kilogram of maternal body weight. The mice usually delivered their young 3 to 4 days after the last DES injection; no gross malformations were observed in the offspring. The average number of male offspring born to control and DES-treated mothers was 4.6 and 2.6, respectively. The external appearance and rate of weight gain were the same in young from corn oil–treated and DES-treated mothers.

At 7 months of age, representative males (each from different litters) were housed with untreated females of known fertility (each male with two females). The reproductive capacities of ten male mice exposed to DES prenatally differed from those ten derived from corn oil–treated mothers. All the control males were able to impregnate one or both females during the 17-day breeding time. On the other hand, six of the DES-exposed males were sterile, producing no pregnant females during the entire breeding period.

When the prenatally drug-treated males were killed and examined at 9 to 10 months of age, testicular changes were found in 15 of 24 animals. Six of the affected mice had at least one intra-abdominal testis, firmly attached to the posterior pole of the kidney (Fig. 1a). The retained testes were hypoplastic, fibrotic, and usually contained foci of calcification (Fig. 1b). Moreover, there were small nodules and sheets of interstitial cells in these intra-abdominal gonads. Eight animals had epididymal cysts; six of these also had testicular lesions. As seen in Fig. 1b, epididymal cysts were usually associated with a fibromuscular outgrowth from the testis. It

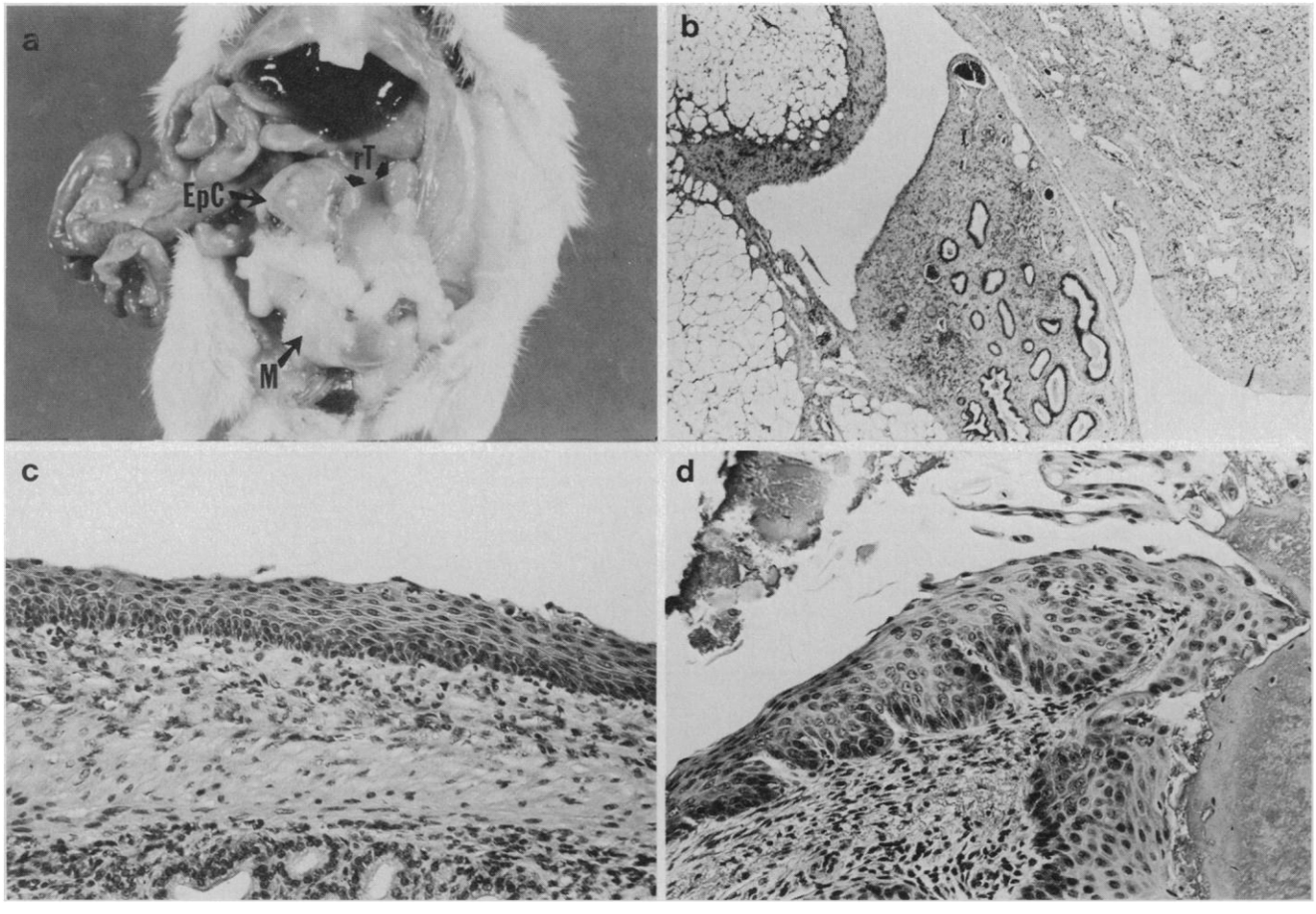

Fig. 1. (a) Reproductive tract lesions in a 9-month-old male mouse exposed prenatally to DES. Lesions included retained testes (*rT*), epididymal cyst (*EpC*), and nodular enlargement (*M*) in the region of the seminal colliculus. (b to d) Photomicrographs of selected reproductive organs from 9- to 10-month-old male offspring exposed prenatally to DES; hematoxylin and eosin staining (× 54). (b) A fibrotic testis (right) is accompanied by an attenuated epididymis (center). The epididymal fat pad (left) contains a thick connecting fibromuscular band. (c) Squamous metaplasia is seen in the epithelial lining of the coagulating gland. The basal layer is relatively smooth. (d) Irregular downgrowths and cellular pleomorphism are seen in the epithelial lining of the coagulating gland. This section is taken from the area adjacent to the seminal colliculus. The natural history of the lesion is unknown but its appearance is similar to early neoplasia.

Table 1. Lesions in the reproductive tract of male mice exposed prenatally to DES. Males were the 9- to 10-month-old offspring of CD-1 mice treated with DES (100 μg/kg, subcutaneously) on days 9 to 16 of gestation.

Prenatal treatment	Incidence of lesions*	Location of lesions		
		Testis†	Epididymis‡	Accessory sex glands§
Corn oil	0/14	0	0	0
DES	18/24	15	10	6

*Expressed as the ratio: (number of male offspring with one or more reproductive tract lesions)/(number of male offspring examined). †Lesions included (i) intra-abdominal retention and/or fibrosis and calcification of testes, and (ii) reduction in number of spermatogonia with multinucleate cells in lumina of testes. ‡Epididymal cysts. §Lesions seen in the area of the seminal colliculus; the nodular masses of five animals were associated with squamous metaplasia; the cellular atypia in one animal resembled early neoplasia.

should be noted that testicular lesions may be secondary to cryptorchidism. However, the appearance of multinucleate giant cells within the seminiferous tubules of scrotal testes indicates an additional effect of prenatal DES treatment on the gonad.

Significant alterations were seen in the accessory sex glands of male mice exposed prenatally to DES (Table 1). These glands were usually distended with hard secretory material and spermatozoa. Nodular enlargements of the seminal vesicles or coagulating glands, or both, were found adjacent to the seminal colliculus of eight animals. Inflammation, including erosions or ulcers and escape of secretions into the surrounding tissues, was associated with enlargements of the seminal vesicle.

Nodular enlargements of the coagulating glands and ampullae found in five mice were associated with squamous metaplasia (Fig. 1c). Whether these metaplastic changes are preneoplastic or constitute benign lesions is unclear; squamous metaplasia of the accessory sex glands of male rodents given high doses of estrogen has been described (*3, 4*). However, adjacent to and in the duct of the coagulating gland of the prostate of one animal, there were downgrowths and cellular pleomorphism (Fig. 1d) suggesting a more serious, and possibly preneoplastic, growth disturbance.

Carcinoma of the male rodent accessory sex glands is very rare (*5*). Indeed, treatment of mice with large doses of DES as adults results in mammary (*6*) or testicular tumors (*7*), but no other genital tract neoplasms were noted. Similarly, neonatal administration of DES at a dose of approximately 1 g/kg failed to produce lesions of the male accessory sex glands although epididymal cysts and fibrotic testes were observed (*8*).

Another group of nine mothers was treated with DES in the present study. All of their 1- to 4-day-old male offspring had testes anterior to the urinary bladder or, in some cases, firmly fixed to the posterior pole of the kidney. In the young from a corresponding number of control mice, the testes were always at the level of the bladder. Similarly, Greene *et al.* (*4*) found intra-abdominal testes in newborn rats exposed prenatally to high doses of estradiol diproprionate.

In light of these results in rodents, the incidence of cryptorchidism in young boys whose mothers had been treated with DES during gestation may be of clinical importance. Obviously, these offspring should be further evaluated for latent alterations of the genital tract, since changes in the adult male human reproductive tract similar to those we observed in the mouse might be dismissed as secondary to inflammation. Some of these lesions could be important causes of infertility even when viable sperm are produced.

The observed effects of DES on the reproductive tract may be explained, in part, by considering the development of this organ system. In the female mouse (*9*) and human (*10*), the Müllerian ducts are the fetal precursors of the definitive oviduct, uterus, and anterior portion of the vagina. It has been suggested that the embryonic origin of the DES-induced vaginal adenocarcinoma in women is Müllerian duct tissue (*11*); these suggestions are reinforced by studies with DES on the vaginal epithelium of neonatal (*9, 12*) and fetal mice (*13*). Although the Müllerian ducts regress during the normal differentiation of the genital tract in the male, vestiges of these embryonic tissues do persist as the appendix testis and prostatic utricle (*10, 14*). The positions of the fibromuscular growths and nodular masses in the male offspring of DES-treated mice are consistent with the anatomical locations of the appendix testis and prostatic utricle, respectively. Although an action of DES on the fetal urogenital sinus must be considered, these findings raise the possibility that the disturbance of genital tract development following prenatal exposure to DES may be mediated, in part, through Müllerian tissue in the male, as well as the female, fetus.

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References

1. A. L. Herbst, H. Ulfelder, D. C. Poskanzer, *N. Engl. J. Med.* **284**, 878 (1971); P. Greenwald, J. J. Barlow, P. C. Nasca, W. S. Burnett, *ibid.* **285**, 390 (1971).
2. N. M. Kaplan, *ibid.* **261**, 641 (1959).
3. H. Burrows, *Am. J. Cancer* **23**, 490 (1935); Y. Arai, *Experientia* **24**, 180 (1968); T. J. Triche and J. C. Harkin, *Lab. Invest.* **25**, 596 (1971).
4. R. R. Greene, M. W. Burrill, A. C. Ivy, *Am. J. Anat.* **67**, 305 (1940).
5. J. D. Prejean, J. C. Peckam, A. E. Casey, D. P. Griswold, E. K. Weisburger, J. H. Weisburger, *Cancer Res.* **33**, 2768 (1973).
6. A. B. Okey and G. H. Gass, *J. Nat. Cancer Inst.* **40**, 225 (1968).
7. H. B. Andervont, M. B. Shimkin, H. Y. Canter, *ibid.* **18**, 1 (1957).
8. T. B. Dunn and A. W. Green, *ibid.* **31**, 425 (1963).
9. J.-G. Forsberg, *Am. J. Obstet. Gynecol.* **115**, 1025 (1973).
10. H. Tuchmann-Duplessis and P. Haegel, *Illustrated Human Embryology* (Springer-Verlag, New York, 1972), vol. 2, pp. 78–98.
11. A. L. Herbst, R. J. Kurman, R. E. Scully, D. C. Poskanzer, *N. Engl. J. Med.* **287**, 1259 (1972); S. G. Silverberg and L. S. DeGiorgi, *Cancer* **29**, 1680 (1972); A. L. Herbst, D. C. Poskanzer, S. J. Robboy, L. Friedland, R. E. Scully, *N. Engl. J. Med.* **292**, 334 (1975).
12. J.-G. Forsberg, *Am. J. Obstet. Gynecol.* **113**, 83 (1972).
13. J. A. McLachlan, R. Newbold, T. Hanner, in preparation.
14. L. J. Wells, M. W. Cavanaugh, E. L. Maxwell, *Anat. Rec.* **118**, 109 (1954); A. Raynaud, in *The Ovary*, S. Zuckerman, Ed. (Academic Press, New York, 1962), vol. 2, p. 179.

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Lysinoalanine: Presence in Foods and Food Ingredients

Abstract. *Lysinoalanine, N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine, an unusual amino acid implicated as a renal toxic factor in rats, has been found in proteins of home-cooked and commercial foods and ingredients. Although it has been reported to occur in both edible and nonfood proteins only after alkali treatment, it has now been identified in food proteins that had not been subjected to alkali. Lysinoalanine is generated in a variety of proteins when heated under nonalkaline conditions.*

The preference of affluent industrialized societies for manufactured foods as opposed to home cooking has prompted consideration of the need to determine the effect of processing procedures on the wholesomeness and nutritive value of various foods. Heating and alkali treatment have been reported to adversely affect the nutritional value of proteins (*1, 2*). Heating may abolish the nutritive value of essential amino acids originally present in a protein by means of racemization (*3*) and chemical