Virchows Arch. A Path. Anat. and Histol. 376, 21–28 (1977)

Virchows Archiv A

Hyperplastic and Metaplastic Lesions in the Reproductive Tract of Male Rats Induced by Neonatal Treatment with Diethylstilbestrol

Yasumasa Arai[1], Yoshihide Suzuki[1], and Yasuaki Nishizuka[2]

[1] Department of Anatomy, Juntendo University School of Medicine, Hongo, Tokyo 113 and
[2] Laboratory of Experimental Pathology Aichi Cancer Research Institute, Chikusa-ku, Nagoya 464, Japan

Summary. Newborn male Wistar rats were castrated on the day of birth (=day 1) and treated daily with diethylstilbestrol (DES) from days 1 to 30; 1 μg for the first 10 days of life, 2 μg for the next 10 days and 4 μg for the last 10 days. The animals were autopsied at 30, 90 and 270 days of age. The epithelium of the coagulating glands (CGs) and ejaculatory ducts (EDs) underwent metaplastic transformation in all DES-treated rats. These pathological changes were more marked with age. The most striking changes were found in the periurethral regions of the CGs and EDs and associated regions of the dorsal urethral wall. The normal transitional epithelial lining almost disappeared and large papillary epithelial outgrowths occurred near the opening of the EDs and CGs. This type of neoplastic change was most marked in the group of rats sacrificed at 9 months of age.

Key words: Epithelial lesions – Male reproductive tract – Neonatal diethylstilbestrol treatment.

Introduction

Accumulating evidence has shown a possible association between intrauterine exposure to diethylstilbestrol (DES) and the development of clear cell cancer of the vagina and cervix in human offspring. It is probable that non-malignant changes in the vagina such as adenosis and cervical anomalies are also an effect of DES exposure at critical fetal ages (Herbst and Scully, 1970; Herbst et al., 1971; Greenwald et al., 1971; Tsukada et al., 1972; Robby et al., 1974; Fetherston, 1975). Preneoplastic lesions are induced in the vagina and cervix of mice after neonatal exposure to exogenous estrogens including DES and probably to androgens (Dunn and Green, 1963; Takasugi and Bern, 1964;

For offprints contact: Y. Arai, Department of Anatomy, Juntendo University School of Medicine, Hongo, Tokyo 113, Japan

Kimura and Nandi, 1967; Forsberg, 1975). DES-exposed human males as yet have not shown any indication of neoplastic and preneoplastic alterations in the urogenital tract (Linden and Henderson, 1972; Greenwald et al., 1973; Henderson et al., 1973; Bibbo et al., 1975). However, McLachlan et al. (1975) reported recently that nodular lesions with squamous metaplasia of the coagulating gland (CG) and ampullae were frequently encountered in male mice exposed prenatally to DES. Similar pathological changes, notably hyperplastic downgrowth of basal cells in the area of the seminal colliculus, were reported previously in rats subjected to neonatal treatment with ovarian estrogen (Arai, 1970). The changes thus evoked in neonatal rats are unlike the histologically similar changes in adult animals treated with large amounts of estrogen, in that the later are reversible after the withdrawal of exogenous estrogen (Burrows, 1935; Zuckerman, 1940; Price, 1963). It appears that interferance with normal epithelial differentiation by exogenous exposure to sex steroids in the perinatal period results in irreversible alterations in the male reproductive tract.

In the present communication, the nature of the lesions in the reproductive tract induced by DES injections of neonatally castrated male rats will be described. A part of this study has appeared in an abstract form (Arai and Suzuki, 1975).

Materials and Methods

Wistar male rats raised in our vivarium and housed under a condition of controlled temperature ($24 \pm 1.4°$ C) and lighting (14/10 h light and dark illumination) were used in the present study. The animals were castrated within 24 h of birth under cold anesthesia, since the incidence of metaplastic changes in the male reproductive tracts induced by neonatal estrogen treatment was found to be increased markedly by the removal of neonatal testes. The development of metaplasia was also inhibited by injections of androgen along with estrogen (Arai, 1970). DES (Sigma Chemical Co., U.S.A.) was injected subcutaneously for 30 successive days from the day of birth. The daily dose of DES was increased with age: 1 μg dissolved in 0.02 ml sesame oil for the first 10 days, 2 μg in 0.04 ml oil for the next 10 days and 4 μg in 0.08 ml oil for the last 10 days. In addition, 5 neonatally castrated rats not given DES and 15 infant male rats served as controls. The animals were sacrificed at 30, 90 and 270 days of age. At autopsy genito-urinary tracts were fixed in situ in Bouin's fluid, and serial paraffin sections were stained with hematoxylin and eosin.

Results and Discussion

Histological examination showed clearly that neonatal administration of estrogen constantly resulted in metaplastic transformation of the epithelium of the coagulating glands (CGs) and ejaculatory ducts (EDs) (Table 1). Unlike the columnar epithelium in intact controls (Fig. 1) and low cuboidal epithelium in neonatally castrated controls, the epithelial lining of these organs exhibited proliferative alterations with remarkable squamous stratification and cornification. At 30 days of age metaplastic changes were limited to the ductal portion of the CGs and EDs adjacent to the urethral opening of the seminal colliculus; no epithelial downgrowths were noticed (Fig. 2). The degree of hyperplastic and metaplastic changes apparently became more intensive with age; at 90 days,

Table 1. Summary of histopathological changes in urogenital tracts in DES treated neonatally castrated male rats

Histological findings	Rats sacrificed at 30 days	Rats sacrificed at 90 days of age	Rats sacrificed at 270 days of age
Ejaculatory duct (ED)			
Metaplasia	7/7 (0/5)[a]	7/7 (0/5)	7/7 (0/5), [0/5][b]
Cornification	7/7 (0/5)	7/7 (0/5)	7/7 (0/5), [0/5]
Epithelial downgrowth	0/7 (0/5)	4/7 (0/5)	5/7 (0/5), [0/5]
Coagulating gland (CG)			
Metaplasia	7/7 (0/5)	6/7 (0/5)	5/7 (0/5), [0/5]
Cornification	7/7 (0/5)	6/7 (0/5)	5/7 (0/5), [0/5]
Epithelial downgrowth	0/7 (0/5)	5/7 (0/5)	5/7 (0/5), [0/5]
Dorsal urethral wall near opening of ED or CG			
Metaplasia	0/7 (0/5)	4/7 (0/5)	7/7 (0/5), [0/5]
Cornification	0/7 (0/5)	0/7 (0/5)	0/7 (0/5), [0/5]
Epithelial downgrowth	0/7 (0/5)	4/7 (0/5)	7/7 (0/5), [0/5]
Papillary growth	0/7 (0/5)	1/7 (0/5)	6/7 (0/5), [0/5]

[a] Numbers in parentheses indicate tthe findings in intact controls
[b] Numbers in brackets indicate the findings in neonatally castrated controls

the lumina of the CGs and EDs were distended by sloughed cornified cells, and epithelial downgrowths were observed. More advanced lesions of epidermoid metaplasia were commonly encountered in the rats killed at 270 days of age. At this age the most striking changes were found in the periurethral regions of both CGs and EDs and the associated dorsal urethral wall; the changes were of two types. The first was characterized by marked cellular downgrowth with a pattern of squamous metaplasia without cornification, but with occasional parakeratosis (Fig. 3). These downgrowths were most commonly found in the dorsal wall of the urethra between the seminal colliculus and bladder neck and were frequently accompanied by cyst formation. The second type consisted of papillary epithelial outgrowths from the urethral wall of the seminal colliculus near the openings of the CGs and EDs (Figs. 4–6), which resulted in marked disorganization of the normal transitional epithelial lining. These tumoral outgrowths consisted of stratified papillae of elongated or spindle cells. Infiltration of the stroma by lymphocytes and plasma cells was sometimes noticed.

The prostatic utricle developed to unusual size in the area of the seminal colliculus and was covered by transitional or non-karatinized stratified squamous epithelial cells; however, its distal parts which normally ended blindly showed pseudostratified or stratified columnar structure. In normal and castrated controls prostatic utricles were vestigial. As reported previously in estrogen-treated males (Arai, 1968), squamous metaplasia in the seminal vesicles and other parts of the prostate also occurred but was less prominent.

Intact and neonatally castrated controls never developed the epithelial lesions described above. In addition, estrogen injections for 30 successive days from days 21 to 50 failed to induce similar long-lasting epithelial alterations (Arai,

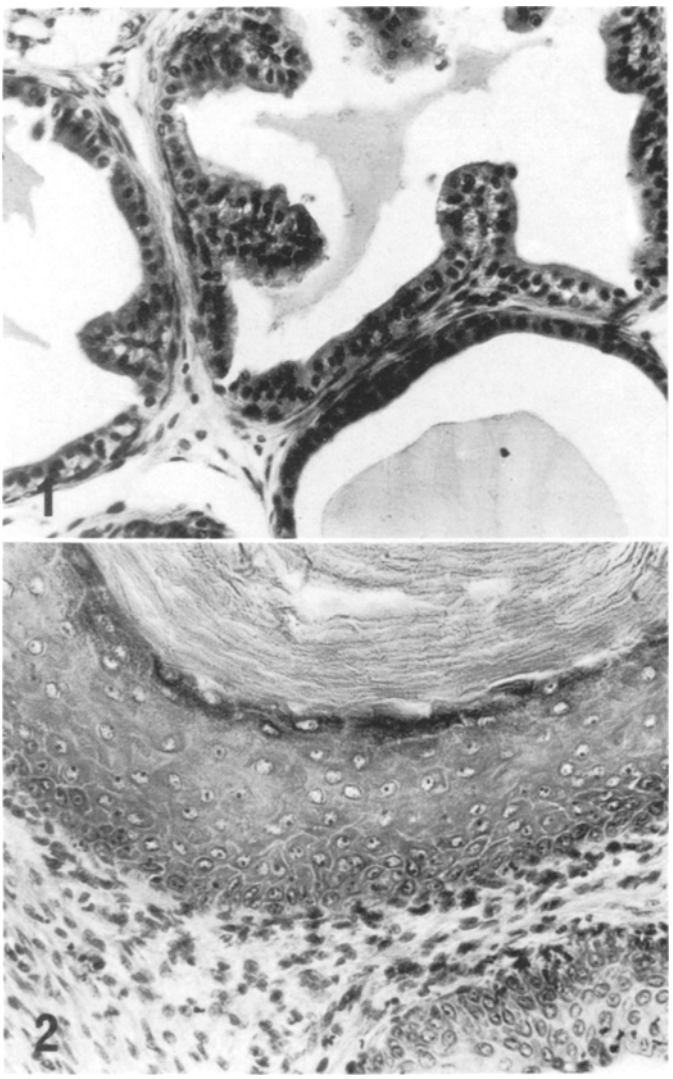
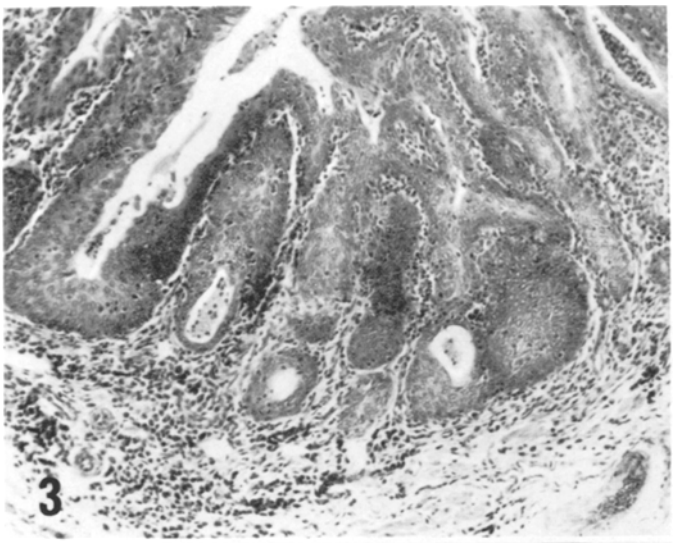


Fig. 1. Coagulating gland (*CG*) of intact control sacrificed at 270 days of age. ×250

Fig. 2. CG of DES-treated neonatally castrated rat sacrificed at 30 days of age. Note stratified squamous and cornified epithelium. ×250

1968). It seems clear that neonatal injections of DES are responsible for the induction of the permanent proliferation in the CGs, EDs and the dorsal urethral wall near the seminal colliculus. A recent study (Arai and Suzuki, unpublished) showed that squamous metaplasia first appeared on day 7 of neonatal estrogen treatment in the urethral end of the ED and prostatic utricle, and gradually extended to the whole area of the ED. Proliferative activity of the transitional epithelium in response to neonatal estrogen was also noted in the dorsal urethral



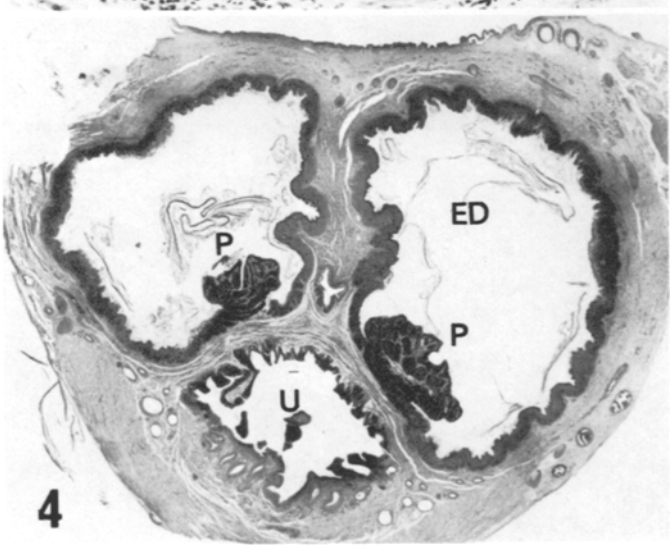
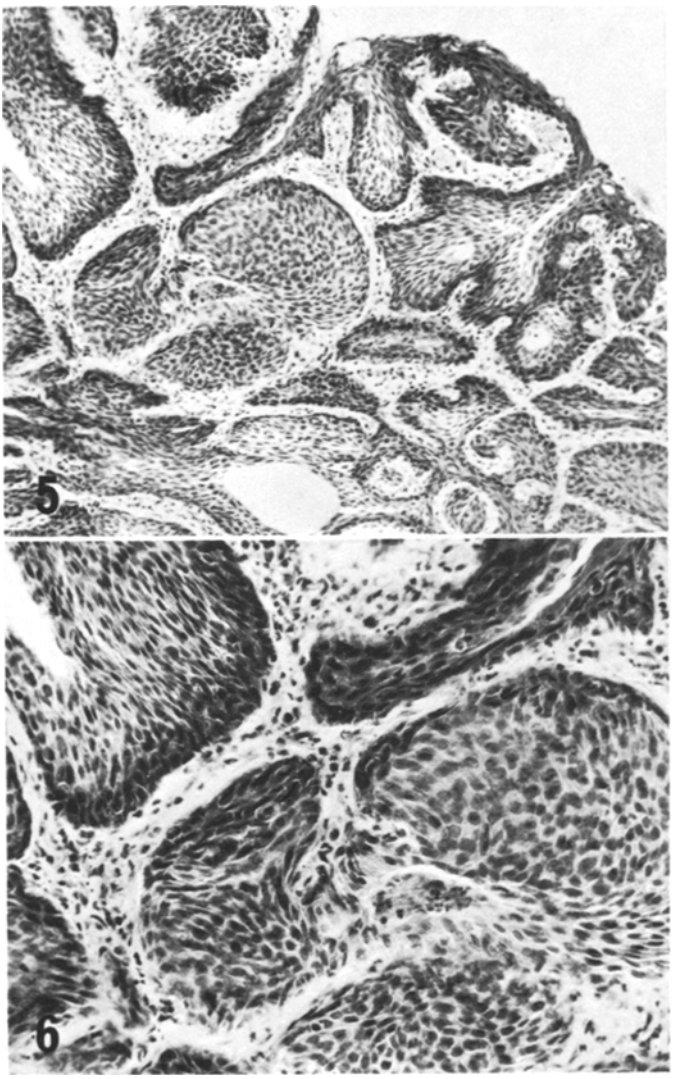


Fig. 3. Dorsal urethral wall near the opening of CG of DES-treated neonatally castrated rat killed at 270 days of age. Note marked downgrowths of the epithelium and cyst formation ×125

Fig. 4. Cross section of genito-urinary tract of DES-treated neonatally castrated rat killed at 270 days of age. Note distention of the lumina of ejaculatory ducts (*EDs*) and papillary epithelial outgrowths (*P*). *U*, urethra. ×11

wall between the seminal colliculus and bladder neck. These results suggest the possibility that disorders of proliferative activity caused by estrogen during a critical developmental period may be prerequisite for maturation of permanent cellular alterations in the male genito-urinary tracts.

It has been suggested that an increase of genito-urinary cancer may be expected among human males exposed prenatally to DES (Linden and Hender-



Figs. 5 and 6. A part of the papillary epithelial outgrowth from the same rat as in Figure 4. ×125 (**Fig. 5**), ×250 (**Fig. 6**)

son, 1972); however, there is no supportive evidence for this possibility (Carstens and Clemmesen, 1972; Henderson et al., 1973; Greenwald et al., 1973). At present, there is no evidence for or against the neoplastic nature of the epithelial lesions in the CGs and EDs in our DES-treated rats, but future fate of these persistent hyperplastic alterations into the preneoplastic state might be considered. McLachlan et al. (1975) reported that similar epithelial downgrowths occurred in one-fourth of male mice exposed to DES prenatally and that one downgrowth of them appeared to be preneoplastic. The relatively low incidence

of the pathological changes in their mice may be due to the antagonistic action of androgen during the perinatal period, because neonatal castration was found to increase the incidence of estrogen-induced metaplasia to 100% and exogenous androgen reversed it (Arai, 1970).

Possible participation of a particular cell population derived from the Müllerian duct has been suggested in vaginal tumorigenesis of the mice exposed to estrogen neonatally (Forsberg, 1974; Neumann et al., 1974; Takasugi, 1976). The origin of the estrogen-induced hyperplastic lesions in the male genito-urinary tract has not yet been investigated. Abnormal estrogenic stimulation at the stage of differentiation of the CGs and EDs may disturb the epithelial component, presumably derived from the urogenital sinus, which is thought to be very sensitive to estrogen during the perinatal development (Zuckerman, 1940; Raynaud, 1962). The high sensitivity of the urothelium on the seminal colliculus and development of metaplasia in the urethral ends of the EDs (Wolffian duct origin) and the prostatic utricles (Müllerian derivative) to neonatal estrogen treatment adds support to the possible participation of the urogenital sinus in the histogenesis of these epithelial lesions. Preliminary data indicate that papillary epithelial growths also develop frequently in the bladder of neonatally castrated male rats receiving DES or ovarian estrogen treatment at neonatal ages.

Acknowledgements. The authors wish to express their appreciation to Prof. H.A. Bern, University of California, Berkeley, for his valuable suggestions and critical reading of the manuscript. This study was supported by Research grants (701075, 801075, 901072) from the Ministry of Education of Japan and by Contract #N01-CP-55650 from National Cancer Institute, Bethesda, Md, U.S.A.

References

Arai, Y.: Metaplasia in male rat reproductive accessory glands induced by neonatal estrogen treatment. Experientia **24**, 180–181 (1968)

Arai, Y.: Nature of metaplasia in rat coagulating glands induced by neonatal treatment with estrogen. Endocrinology **86**, 918–920 (1970)

Arai, Y., Suzuki, Y.: Hyperplastic metaplastic lesions in the reproductive tracts induced by neonatal treatment with diethylstilbestrol in male rats. Proc. 10th Internat. Congr. Anat. 1975

Bibbo, M., Al-Naqeeb, M., Baccarini, I., Gill, W., Newton, M., Sleeper, K.M., Sonek, M., Wied, G.L.: Foolow-up study of male and female offspring of DES-treated mothers. J. Reprod. Med. **15**, 29–32 (1975)

Burrows, H.: Pathological conditions induced by oestrogenic compounds in the coagulating gland and prostate of the mouse. Am. J. Cancer **23**, 490–511 (1935)

Carstens, H.B., Clemmesen, J.: Genital-tract cancer in Danish adolescents. New Eng. J. Med. **287**, 172 (1972)

Dunn, T.B., Green, A.W.: Cyst of the epididymis, cancer of the cervix, granular cell myoblastoma, and other lesions after estrogen injection newborn mice. J. natn. Cancer Inst. **31**, 425–455 (1963)

Fetherston, W.C.: Squamous neoplasia of vagina related to DES syndrome. Am. J. Obstet. Gynec. **122**, 176–181 (1975)

Forsberg, J-Gunnar.: Induction of conditions leading to cancer in the genital tract by estrogen during the differentiation phase of the genital epithelium. In: Hormones and Embryonic development, Advances in the Biosciences 13, edit. by G. Rospe. Oxford, New York, Toronto, Sydney: Pergamon Press 1974

Forsberg, J-Gunnar.: Late effects in the vaginal and cervical epithelia after injections of diethylstilbestrol into neonatal mice. Am. J. Obstet. Gynec. **121**, 101–104 (1975)
Greenwald, P., Ballow, J.J., Nasca, P.C., Burnett, W.S.: Vaginal cancer after maternal treatment with synthetic estrogens. New Eng. J. Med. **285**, 390–392 (1971)
Greenwald, P., Nasca, P.C., Burnett, W.S., Polan, A.: Prenatal stilbestrol experience of mothers of young cancer patients. Cancer **31**, 568–572 (1973)
Henderson, B.E., Benton, B.D.A., Weaver, P.T., Linden, G., Nolan, J.F.: Stilbestrol and urogenital-tract cancer in adolescents and young adults. New Eng. J. Med. **288**, 354 (1973)
Herbst, A.L., Scully, R.E.: Adenocarcinoma of the vagina in adolescence: a report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). Cancer **25**, 745–757 (1970)
Herbst, A.L., Ulfelder, H., Poskanzer, D.C.: Association of meternal stilbestrol therapy with tumor appearance in young women. New Eng. J. Med. **284**, 878–881 (1971)
Kimura, T., Nandi, S.: Nature of induced persistent vaginal cornification in mice. IV. Changes in the vaginal epithelium of old mice treated neonatally with estradiol or testosterone. J. natn. Cancer Inst. **39**, 75–92 (1967)
Linden, G., Henderson, B.E.: Genital-tract cancers in adolescents and young adults. New Eng. J. Med. **286**, 760–761 (1972)
McLachlan, J.A., Newbold, R.R., Bullock, B.: Repoductive tract lesions in male mice exposed prenatally to diethylstibestrol. Science **190**, 991–992 (1975)
Neumann, F., Graf, K-J., Elger, W.: Hormone-induced disturbances in sexual differentiation. In: Hormones and Embryonic development, Advances in the Biosciences 13, edit. by G. Rospe. Oxford, New York, Toronto, Sydney: Pergamon Press 1974
Price, D.: Comparative aspects of development and structure in the prostate. In: National Cancer Institute Monograph No. 12. 1963
Raynaud, A.: The histogenesis of urogenital and mammary tissues sensitive to oestrogens. In: The Ovary II, edit. by Sir S. Zuckerman. New York, London: Academic Press 1962
Robboy, S.J., Herbst, A.L., Scully, R.E.: Clear-cell adenocarcinoma of the vagina and cervix in young females. Cancer **34**, 606–614 (1974)
Takasugi, M.: Cytological bases for permanent vaginal changes in mice treated neonatally with steroid hormones. Int. Rev. Cytol. **44**, 193–224 (1976)
Takasugi, N., Bern, H.A.: Tissue changes in mice with persistent vaginal cornification induced by early post-natal treatment with estrogen. J. natn. Cancer Inst. **33**, 855–865 (1964)
Tsukada, Y., Hewett, W.J., Barlow, J.J., Pickren, J.W.: Clear-cell adenocarcinoma ("Mesonephroma") of the vagina. Cancer **29**, 1208–1214 (1972)
Zuckerman, S.: The histogenesis of tissues sensitive to oestrogens. Biol. Rev. **15**, 231–271 (1940)

Received June 18, 1977