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Estrogens and Development

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The normal development of the genital organs of mammals, including humans, is under hormonal control. A role for the female sex hormone estrogen in this process is still unclear. However, exposure of experimental animals or humans to the potent exogenous estrogen, diethylstilbestrol (DES), results in persistent differentiation effects. Since many chemicals in the environment are weakly estrogenic, the possibility of hormonally altered differentiation must be considered.

The development of the mammalian genital tract involves the remodeling of two fetal duct systems: the Müllerian or female genital precursor and the Wolffian or male counterpart. This process is apparently controlled, in the main, by two secretions from the fetal testes. In a genetic male, fetal testicular androgen is required for maintenance of the male genital anlage, while a peptide factor secreted by the testis, Müllerian inhibiting substance (MIS), determines the regression of the female duct system. In the absence of these two testicular factors, sex differentiation proceeds along female lines (*1*).

Currently it is unclear whether estrogens, either of maternal or fetal origin, play a role in sex differentiation. Although the human or rodent fetus is bathed in endogenous estrogens, these compounds are thought to be relatively inactive in the fetal genital system because they bind to extracellular carrier proteins, are conjugated, and metabolized to inactive forms.

However, it is clear that exposure to exogenous estrogens during fetal development will profoundly alter sexual differentiation. Experiments in mice that were treated with the synthetic estrogen, diethylstilbestrol (DES), demonstrated two salient features (*2*): the contralateral fetal genital duct system was retained into adulthood in both male and female offspring; in other words, DES-treated females retained both a female and male genital duct system as did treated males; and the ductal system consistent with the sex of the DES-treated mouse had numerous structural and functional defects along its length. For example, the Müllerian duct, which gives rise (starting cranially) to the oviduct, uterus, cervix, and upper vagina, had malformations of each of these organs in developmentally estrogenized females; moreover, functional defects including growth control derangements resulting in cancers are also seen in organs derived from the Müllerian duct.

Very similar defects are seen in women exposed *in utero* to DES. In fact, retention of Wolffian duct tissues as ovarian cysts (*3*), oviductal malformation (*4,5*), uterine abnormalities (*6,7*), and vaginal cancers (*8,9*) have been reported in both species. Thus, structural and functional defects are induced in the genital tracts of both species exposed to an exogenous estrogen during sex differentiation. The mechanism(s) underlying these defects are still not known. However, persistent alterations in expression of specific proteins can be demonstrated in the genital tracts of DES-treated mice (*10*). Thus, changes in the rate of cell/tissue differentiation, as well as induction of molecular defects within the target cell, are apparently involved.

These findings with the potent synthetic estrogen, DES, provide a possibly exaggerated model for what might be seen with much weaker estrogenic xenobiotics, many of which are common features in our environment. As seen in Figure 1, compounds of diverse structure all share a common biological end point—estrogenicity. Coumestrol and equol are examples of phytoestrogens, estrogenic chemicals which are made in over 300 different plants. Another naturally occurring estrogen is zearalenone, a mycotoxin produced by a fungus that infests corn. Both the polycyclic aromatic hydrocarbon, benz[a]anthracene, and the chlorinated hydrocarbon, *o,p'*-DDT, are examples of prohormones, those compounds that must first be metabolized to achieve hormonal activity. In each case, hydroxylation increases the estrogenic potency of the compound. Furthermore, in the cases of chlorinated chemicals like DDT and kepone, their biological persistence exaggerates the hormonal activity in the living organism.

The molecular basis for estrogenic activity is not readily apparent from the X-ray crystal structures of different chemicals in this class (*11*); nor are models of the estrogen receptor-ligand interaction totally satisfying (*12*). However, the structure diversity in this group of chemicals provides numerous routes for exposure to them.

Is there evidence for intoxication with environmental estrogens other than DES? Among animals this cer-

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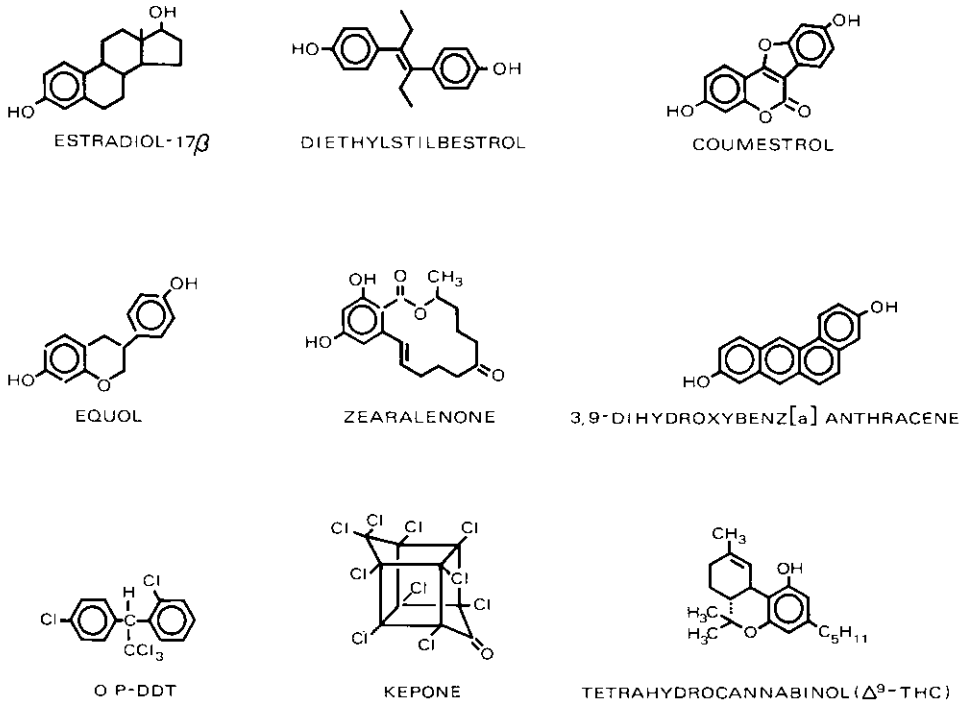

FIGURE 1. Chemicals reported to be estrogenic. Selected references include: estradiol and diethylstilbestrol (*22*); coumestrol and equol (*23*); zearalenone (*24*); 3,9-dihydroxybenz[a]anthracene (*25*); *o,p*-DDT (*26*); kepone (*27*); tetrahydrocannabinol (*28*).

tainly is the case. Sheep grazing on clover rich in phytoestrogens experience widespread infertility; this condition is associated with the naturally occurring plant hormones and is known as "clover disease" (*13*). The fertility of quail has been shown to be strictly related to the concentration of phytoestrogens in their diet (*14*). In times of drought, the effective estrogenic content of the diet is increased and reproduction markedly diminished. Likewise, a recent study with captive cheetahs has raised the possibility that the presence of phytoestrogens in their commercial diet plays a role in the infertility common to this species in zoos (*15*).

The estrogenic mycotoxin zearalenone is produced by a fungus which infests corn in silage. Pigs that eat the infested corn show signs of hyperestroginization ("the moldy corn syndrome"). This manifestation has been clearly associated with zearalenone, the fungal product (*16*).

Evidence for human intoxication is not so obvious; that which has been reported usually involves youngsters and the expression of two common parameters of estrogenization in prepubertal humans: breast growth in boys and girls and menstruation in girls. These cases appear to have another common element, i.e., inadvertent contamination with the potent synthetic estrogen, DES. The routes of exposure include, for example, the parent's clothing, contaminated vitamin pills, or meat (*17*). These examples reinforce the special sensitivity of the developing mammary gland (*18*) and reproductive system to the effects of estrogens.

Most recently, reports of premature sexual development (primarily precocious breast development, or premature thelarche) in young girls in Puerto Rico have raised the possibility of widespread exposure to, or contamination with, environmental estrogens (*19*). Since the first report of premature thelarche in 1982 (*20,21*), several studies have been directed toward understanding the nature and etiology of this condition; currently little is known and much remains to be done. However, the possibility that exogenous estrogens may modify sexual development or function presents a formidable problem for scientists and clinicians. The structural basis for the biological activity of estrogen, the special sensitivity of the immature individual to this class of chemicals, and the outcome for developmentally estrogenized animals and humans are each areas requiring investigation if we are to understand completely this new category of environmental compounds, hormonally active xenobiotics.

REFERENCES

1. Grumbach, M. M., and Conte, F. A. Disorders of sexual differentiation. In: Williams Textbook of Endocrinology, 7th ed. (J. D. Wilson and D. W. Foster, Eds.), W. B. Saunders Company, New York, 1985, pp. 312–402.
2. Newbold, R. R., and McLachlan, J. A. Diethylstilbestrol-associated defects in murine genital tract development. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 288–318.
3. Haney, A. F., Newbold, R. R., Fetter, B. F., and McLachlan, J. A. Paraovarian cysts associated with prenatal diethylstilbestrol exposure: Comparison of the human with a mouse model. Am. J. Pathol. 125: 405–411 (1986).

4. Newbold, R. R., Tyrey, S., Haney, A. F., and McLachlan, J. A. Developmentally arrested oviduct: A structural and functional defect in mice following prenatal exposure to diethylstilbestrol. Teratology 27: 417–426 (1983).
5. DeCherney, A. H., Cholst, I., and Naftolin, F. Structure and function of the fallopian tube following exposure to diethylstilbestrol (DES) during gestation. Fertil. Steril. 36: 741–745 (1981).
6. McLachlan, J. A., Newbold, R. R., and Bullock, B. C. Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. Cancer Res. 40: 3988–3999 (1980).
7. Haney, A. F., Hammond, C. B., Soules, M. R., and Creasman, W. T. Diethylstilbestrol-induced upper genital tract abnormalities. Fertil. Steril. 31: 142–147 (1979).
8. Newbold, R. R., and McLachlan, J. A. Vaginal adenosis and adenocarcinoma in mice transplacentally exposed to diethylstilbestrol. Cancer Res. 42: 2003–2011 (1982).
9. Herbst, A. L., Ulfelder, H., and Poskanzer, D. C. Adenocarcinoma of the vagina: Association of maternal stilbestrol therapy with tumor appearance in young women. N. Engl. J. Med. 292: 334–338 (1971).
10. Newbold, R. R., Carter, D. B., Harris, S. E., and McLachlan, J. A. Molecular differentiation of the mouse genital tract: Altered protein synthesis following prenatal exposure to diethylstilbestrol. Biol. Reprod. 30: 459–470 (1984).
11. Duax, W. L., and Weeks, C. M. Molecular basis of estrogenicity: X-ray crystallographic studies. In: Estrogens in the Environment (J. A. McLachlan, Ed.), Elsevier, New York, 1980, pp. 11–32.
12. Jordan, V. C., Koch, R., and Bain, R. R. Prolactin synthesis by cultured rat pituitary cells: An assay to study estrogens, antiestrogens and their metabolites *in vitro*. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 221–237.
13. Bennetts, H. W., Underwood, E. J., and Shier, F. L. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust. Vet. J. 22: 2–12 (1946).
14. Leopold, S. A., Erwin, M., Oh, J., and Browning, B. Phytoestrogens: Adverse effects on reproduction in California quail. Science 191: 98–100 (1976).
15. Setchell, K. D. R., Grosselin, S. J., Welsh, M. B., Johnston, J. O., Balistreri, W. F., Kramer, L. W., Dresser, B. L., and Tarr, M. J. Dietary estrogens—a probable cause of infertility and liver disease in captive cheetah. Gastroenterology 93: 225–233 (1987).
16. Pathre, S. V., and Mirocha, C. J. Mycotoxins as estrogens. In: Estrogens in the Environment (J. A. McLachlan, Ed.), New York, 1980, pp. 265–278.
17. New, M. I. Premature thelarche and estrogen intoxication. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 349–357.
18. Bern, H. A., Mills, K. T., and Edery, M. Estrogen-associated defects in rodent mammary gland development. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 319–326.
19. Haddock, L., Lebrón, G., Martinez, R., Cordero, J. F., Freni-Titulaer, L. W., Carrión, F., Cintron, C., and González, L. Premature sexual development in Puerto Rico: Background and current status. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 358–379.
20. Sáenz, C. A., Toro-Sola, M. A., Conde, L., and Bayonet Rivera, N. P. Premature thelarche and ovarian cysts probably secondary to estrogen contamination. Bol. Asoc. Med. P. Rico 74: 16–19 (1982).
21. Perez-Comas, A. Precocious sexual development in Puerto Rico. Lancet i: 1299–1300 (1982).
22. Korach, K. S., Metzler, M., and McLachlan, J. A. Estrogenic activity *in vivo* and *in vitro* of some metabolites and analogs of diethylstilbestrol. Proc. Natl. Acad. Sci. (U.S.) 75: 458–471 (1978).
23. Setchell, K. D. R. Naturally occurring non-steroidal estrogens of dietary origin. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 69–85.
24. Wilson, M. E., and Hagler, W. M., Jr. Metabolism of zearolenone to a more estrogenically active form. In: Estrogens in the Environment II: Influences on Development (J. A. McLachlan, Ed.), Elsevier, New York, 1985, pp. 238–250.
25. Schneider, S. L., Alks, V., Morreal, C. E., Sinha, D. K., and Dao, T. L. Estrogenic properties of 3,9-dihydroxybenz[a]anthracene, a potential metabolite of benz[a]anthracene. J. Natl. Cancer Inst. 47: 1351–1355 (1976).
26. Kupfer, D., and Bulger, W. H. Estrogenic properties of DDT and its analogs. In: Estrogens in the Environment (J. A. McLachlan, Ed.), Elsevier, New York, 1980, pp. 239–264.
27. Eroschenko, V. P., and Palmiter, R. D. Estrogenicity of kepone in birds and mammals. In: Estrogens in the Environment (J. A. McLachlan, Ed.), Elsevier, New York, 1980, pp. 305–326.
28. Rawitch, A. B., Schultz, G. S., Ebner, K. E., and Vardaris, R. M. Competition of Δ^9-tetrahydrocannabinol with estrogen in rat uterine estrogen receptor binding. Science 197: 1189–1191 (1977).