[19] Cox DR, Oakes D. Analysis of survival data. London, UK: Chapman Hall, 1984.

[20] Newbold RR. Lessons learned from perinatal exposure to diethylstilbestrol. *Toxicol Appl Pharmacol* 2004;**199:**42–150.

[21] Walker BE, Uterine tumors in old female mice exposed prenatally to diethylstilbestrol. *J Natl Cancer Inst* 1983;**70:**177–81.

[22] Newbold RR, McLachlan JA. Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. *Cancer Res* 1982;**42:**2003–11.

[23] McLachlan JA, Newbold RR, Bullock BC. Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res* 1980;**40:**3988–99.

[24] McLachlan JA, Newbold RR, Shah HC, Hogan M, Doxon RL. Reduced fertility in female mice exposed transplacentally to diethylstilbestrol (DES). *Fertil Steril* 1982;**38:**364–71.

[25] McLachlan JA, Burow M, Chiang T-C, Li SF. Gene imprinting in developmental toxicology: a possible interface between physiology and pathology. *Toxicol Lett* 2001;**120:**161–64.

[26] Li S, Hursting SD, Dzvis BJ, McLachlan JA, Barrett JC. Environmental exposure, DNA methylation, and gene regulation. Lessons from diethylstilbesterol-induced cancers. *Ann N Y Acad Sci* 2003;**983:**161–69.

[27] Li JJ, Hansman R, Newbold R, Davis B, McLachlan JA, Barrett JC. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res* 1997;**57:**4356–59.

[28] Couse JF, Dixon D, Yates M *et al*. Estrogen receptor-α knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. *Dev Biol* 2001;**238:**1–15.

[29] Mericskay M, Carta L, Sassoon D. Diethylstilbestrol exposure *in utero*: A paradigm for mechanisms leading to adult disease. *Birth Defects Res A Clin Mol Teratol* 2005;**73:**133–35.

[30] Kamiya K, Sato T, Nishimura N, Goto Y, Kano K, Iguchi T *et al*. Expression of estrogen receptor and proto-oncogene messenger ribonucleic aids in reproductive tracts of neonatally diethylstilbestrol-exposed female mice with or without post-pubertal estrogen administration. *Exp Clin Endocrinol Diabetes* 1996;**104:**111–12.

[31] Yamashita S, Takayanagi A, Shimizu N. Effects of neonatal diethylstilbestrol exposure on c-fos and c-jun protooncogene expression in the mouse uterus. *Histol Histopathol* 2001;**16:**131–40.

[32] Falck L, Forsberg J-G. Immunohistochemical studies on the expression and estrogen dependency of EGF and its receptor and C-fos proto-oncogene in the uterus and vagina of normal and neonatally estrogen-treated mice. *Anat Rec* 1996;**245:**459–71.

[33] Ma L, Benson GV, Lim H, Dey SK, Maas RL. Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in mullerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 1998;**197:**141–54.

[34] Block K, Kardana A, Igarashi P, Taylor HS. *In utero* diethylstilbestrol (DES) exposure alters Hox gene expression in the developing mullerian system. *FASEB J* 2000;**4**:1101–08.

[35] Sato T, Ohta Y, Okamura H, Hayashi S, Iguchi T. Estrogen receptor (ER) and its messenger ribonucleic acid expression in the genital tract of female mice exposed neonatally to tamoxifen and diethylstilbestrol. *Anat Rec* 1996;**44:**74–385.

[36] Okada A, Sato T, Ohta Y, Buchanan DL, Iguchi T. Effect of diethylstilbestrol on cell proliferation and expression of epidermal growth factor in the developing female rat reproductive tract. *J Endocrinol* 2001;**170:**539–54.

[37] Wilcox AJ, Umbach DM, Hornsby PP, Herbst AL. Age at menarche among diethylstilbestrol granddaughters. *Am J Obstet Gynecol* 1995;**73:**835–36.

[38] Perez K, Titus-Ernstoff L, Hatch EE *et al*. Reproductive outcomes in men with prenatal exposure to DES. *Fertil Steril* 2005;**84:**1649–56.

[39] Kaufman RH, Adam E. Findings in female offspring of women exposed *in-utero* to diethylstilbestrol. *Obstet Gynecol* 2002;**99:**197–200.

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Commentary: Prenatal exposure to diethylstilbestrol (DES): a continuing story

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When Arthur L Herbst and his colleagues first reported, in 1971,[1] an association between the administration of diethylstilbestrol (DES) to pregnant women and cervico-vaginal adenocarcinoma found in their daughters, it was called a 'biological time bomb'. This was the first documented example of transplacental carcinogenesis in humans—the carcinogen (DES) was taken by the mother, but the cancer appeared in the offspring, and then, only after the onset of puberty.

In addition to these rare genital tract cancers, other alterations were subsequently observed in much higher prevalence in the daughters of DES-exposed mothers—in both humans and mice. These included structural (or cellular) anomalies of the vagina, uterus, and fallopian tubes, subfertility and infertility, and menstrual/oestrous cycle irregularities.[2] This is significant because until the early 1970s, between

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2 and 4 million women were administered DES while pregnant.[3]

The DES-exposed population of men and women became the model through which understanding of delayed effects of gestational exposures to hormones or other compounds could be gained. The earliest exposed people are now in their fifth decade and other reproductive tract effects are only now being recorded, such as a higher prevalence of uterine fibroids,[4] suggesting a continued cellular or molecular lesion in the genital tract of women exposed to DES *in utero*.

Over the course of the last 35 years, work from our laboratory and others using mice, rats, hamsters, or non-human primates has shown that most of the changes seen in the DES-exposed man or woman could be replicated in laboratory animals including the signal lesion of cervico-vaginal adenocarcinoma.[5] In fact, recent publications citing increases in uterine fibroids in DES-exposed women had already been shown in exposed mice[5] and rats.[6]

From the beginning, the scientific and clinical communities have sought to discover what the mechanism might be whereby an oestrogenic chemical like DES could cause an effect that persisted into daughter cells only to show up after puberty. Many laboratories, including my own, investigated the mutagenic potential of DES. Most now conclude that mutagenicity or the ability to alter the base structure of DNA is not the only or, indeed, most prominent mechanism for the persistent effects of DES.

We now believe that the very mechanisms underlying cell differentiation and organogenesis may play a major role in the expression of DES effects during development. In the normal establishment of cell differentiation pathways and the developmental fate of cells themselves, changes in the pattern of gene expression occur; indeed, the underlying strategy of development is differential gene expression. In the very simplest terms, skin expresses skin genes, while bones express bone genes; just as importantly, bone-specific genes are turned off in skin and vice versa. The persistent expression or suppression of genes is accomplished through a process called epigenetic change in which the DNA sequence is not altered. It involves the addition of methyl groups to specific bases in specific parts of genes and has been shown to regulate gene expression; thus, it underlies cell differentiation. For more details about epigenetics, evolution, endocrine disruption, health, and disease, see the recent paper by Crews and McLachlan.[7]

Work from our laboratory[8] and others raised the possibility that early exposure to DES may cause persistent epigenetic changes in some genes and not others such that the fate of tissues or organs is altered. For example, the promoter region of a persistently expressed oestrogen-responsive gene displayed an altered pattern of DNA methylation following developmental exposure to DES. The epigenetic change can actually persist through generations of cells in one organism or, if the change occurs in the germ cell line, could even persist into the next generation of an organism. Thus, my colleague Retha Newbold and I showed that rare cancers seen in female mice exposed to DES prenatally were seen again in the next generation of mice.[9] Recently, a report of a transgenerational effect of hormonally active chemicals in rats associated with altered patterns of methylation also points to epigenetics as a mechanism.[10]

In their paper in this issue of the Journal,[11] Titus-Ernstoff and colleagues conclude that women who were not themselves exposed to DES *in utero* may have altered reproductive tract function if their mothers had been exposed *in utero*. This is a remarkable finding, if replicated, since it would mean that in humans, maternal ingestion of DES during pregnancy can not only alter the reproductive capacity of the woman exposed directly while a fetus, but that the alteration may be passed on to another generation (the so-called DES granddaughter effect). The effect described in the current paper, later attainment of menstrual regularization and more irregular periods, is small, but biologically consistent for an oestrogenic effect.

So, many decades after the first pregnant woman was exposed to DES, we now have preliminary evidence that this drug can alter the genes of the target cells in a way that persist into her daughter's daughter's generation. More than half a century of DES experience has shown us that numerous defects can be encoded in the genome of the exposed fetus to be expressed later in life. This paper and the supporting animal research data suggest that these encoded or imprinted defects may, in some cases, persist into the next generation. This is a small study with many questions, but the questions are profound enough that they merit an enhanced and continued follow-up of DES-exposed offspring and their offspring.

It is notable, and somehow fitting, that Dr Herbst, author of the first paper describing human transplacental carcinogenesis may, 35 years later, be illuminating the first example of transgenerational effects of DES in humans as one of the authors of the current Titus-Ernstoff paper. In both cases, animal studies as early as 1962[12] were key to understanding the human findings.

References

[1] Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilboestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;**284:** 878–81.

[2] Newbold RR, McLachlan JA. Transplacental hormonal carcinogenesis: diethylstilbestrol as an example. In: Huff J, Boyd J, Barrett JC. (eds). *Cellular and Molecular Mechanisms of Hormonal Carcinogenesis: Environmental Influences*. New York: Wiley Liss & Sons, 1996, pp. 131–47.

[3] Schrager S, Potter BE. Diethylstilbestrol exposure. *Am Fam Phys* 2004;**69:**2395–400.

[4] Baird DD, Newbold RR. Prenatal diethylstilbestrol (DES) exposure is associated with uterine leiomyoma development. *Reprod Toxicol* 2005:**20:**81–84.

[5] McLachlan JA, Newbold RR, Bullock BC. Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res* 1980:**40:**3988–99.

[6] Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci USA* 2005:**102:**8644–49.

[7] Crews D, McLachlan JA. Epigenetics, evolution, endocrine disruption, health and disease. *Endocrinology* 2006:**147:** S4–S10.

[8] Li S, Washburn KA, Moore R *et al*. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in the mouse uterus. *Cancer Res* 1997:**57:** 4356–59.

[9] Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 1998:**19:**1655–63.

[10] Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005:**308:**1466–69.

[11] Titus-Ernstoff L, Troisi R, Hatch EE *et al*. Menstrual and reproductive characteristics of women whose mothers were exposed *in utero* to diethylstilbestrol (DES). *Int J Epidemiol* 2006;**35:**862–68.

[12] Takasugi N, Bern HA, DeOme KB. Persistent vaginal cornification in mice. *Science* 1962:**138:**438–39.