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Endocrine Disruptors, Epigenetically Induced Changes, and Transgenerational Transmission of Characters and Epigenetic States

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1. INTRODUCTION TO EPIGENETICS

Developmental epigenetics is a broad phenomenon, which was initially described by Waddington as "the branch of biology which studies the causal interactions between genes and their products which bring phenotypes into being." Today, the study of epigenetic regulation of development has been sharpened because of recent work on molecular mechanisms of gene expression and developmental biology *(1)*, mainly focusing on how the environment produces alterations in gene expression patterns without changes in DNA sequences *(2)*. Epigenetics is now a well-accepted phenomenon by the scientific community, in large part because of recent discoveries in the field of the molecular biology, namely chromatin condensation, histone modifications, and DNA methylation, which are all well-identified processes.

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1.1. Mechanisms of Epigenetic Modifications

DNA methylation is by far the most widely known and most studied epigenetic modification to date. The process of DNA modification constitutes a post-replicative modification, in which a methyl group is added covalently to a DNA residue *(3)*. The reaction of DNA methylation occurs at the carbon 5 of the cytosine ring in 5′–3′-oriented CG dinucleotides (which are named as CpGs) and is catalyzed by the action of DNA methyltransferases (Dnmts) *(4)*.

Two other epigenetic mechanisms have to do with chromosome structure. Eukaryotic genomic DNA is compacted more than 10,000-fold by basic proteins named histones, which participate in the compaction of this DNA into an entity known as chromatin *(5)*. Heterochromatin is a region of the genome that is highly condensed throughout the cell cycle, in contrast to euchromatin that shows condensation exclusively during mitosis *(6)*. Because heterochromatin regions are still condensed in interphase, they are associated with repressed gene expression *(6)*. Heterochromatin is often associated with hypermethylated and hyperacetylated histones *(7,8)*. Nevertheless, there are regions (referred to as facultative chromatin) that can be transiently condensed and silenced during development *(6)*, thereby leading to variation in gene expression depending on the chromatin state (condensed or uncondensed). In turn, the chromatin state is also susceptible to modification depending on specific stimuli. For instance, there are factors responsible for the initiation of the heterochromatin formation process, such as transcriptional repressors and functional RNA, and also accessory factors that interact with many groups of proteins *(9)*. Therefore, the chromatin state may be epigenetically regulated by factors whose levels could be environmentally dependent, leading to epigenetic regulation of genes whose expression depends on chromatin state.

Histones are susceptible to a variety of post-translational modifications such as phosphorylation, acetylation, methylation, ubiquitination, sumoylation, ADP ribosylation, glycosylation biotinylation, and carbonylation *(5)*. Nevertheless, among these, methyl marks provide an epigenetic mechanism that favors the stable transfer of gene expression profiles to progeny cells *(5)*. It has been suggested that an "epigenetic conversation" exists between histones and DNA that involves cytosine methylation, histone deacetylation and methylation, all acting in synergy to generate a self-reinforcing epigenetic cycle that maintains and perpetuates a repressed chromatin state *(10)*.

Despite evidence that the aforementioned epigenetic modifications can act autonomously *(10)*, RNA factors, histone methylation, and chromatin-remodeling enzymes appear to all act together with Dnmts, resulting in the establishment and maintenance of methylation patterns and the generation of site-specific methylation and tissue-specific differences *(11)*. Among RNA factors, small RNAs (siRNA and miRNA) have recently been shown to have the ability to direct DNA methylation, a mechanism called RNA-directed DNA methylation (RdDM) that is carried out by dsRNA, which may be produced by transcription through inverted repeats *(12)*. As a whole, these data indicate that epigenetic mechanisms are not acting alone but rather are integrated such that they may be affected by stimuli, and also produce phenotypic consequences. Hence, epigenetic regulation of gene expression deals with integrating intrinsic and environmental signals *(13,14)*.

1.2. Relationship Between Epigenetics and Transgenerational Effects

In integrating the concepts of epigenetics and mechanisms of epigenetic regulation with the concept of transgenerational transmission of characters, it should be emphasized that *not every epigenetic effect can be considered as transgenerational*. This latter phenomenon depends on the susceptibility of the organism to undergo epigenetic changes and also on the capacity of the organism or environment to make those changes persist across generations. An individual's susceptibility to epigenetic changes will depend on the stages of the ontogeny when the external effect took place. Exposure to chemicals or other environmental agents may induce epigenetic changes in the genome, but only when they act during critical periods of the ontogeny *(15,16)*. In turn, persistence of the stimuli through generations will depend on the nature of the epigenetic system affected. For example, epigenetic alterations on chromatin condensation leading to the changed expression of particular genes could regulate gene expression in a variable manner during a single ontogenic event but may have a reduced capacity to transmit those changes in comparison with imprinted genes. Changing methylation in imprinted genes could have the same evolutionary value of a mutation *(15)* and may, moreover, lead to biased mutations *(17)*.

1.3. Role of Developmental Stage in Susceptibility to Epigenetic Transgenerational Effects

The timing during ontogeny when an organism is exposed to stimuli has implications on the organism's susceptibility to be affected by such stimuli. For instance, development is characterized by a high sensitivity to environmental stimuli, either external or generated by cellular productions *(13)*. Disruptions produced by a stimulus during early stages of development have more systemic consequences assessed in adulthood compared with the effect of such exposures in adulthood, which has more local and limited consequences. The reason for this is that interfering with an embryonic cell during development will produce changes in the derived cell lineage, which involves a higher number and broader types of cells than when a cell lineage is derived from an adult somatic perturbed cell. Thus, Danzo *(18)* has stated that "the greatest risks to reproductive health posed by xenobiotics would be during the embryonic, neonatal and pubertal periods, when the reproductive systems are undergoing finely tuned modulation by steroid hormones."

Any environmental effect produced in early life stages will also be of profound importance from a transgenerational perspective. Organisms in early stages of development are more susceptible to heritable structural changes that can be transmitted through the germ line, such as reprogramming of methylation patterns *(2)* or through overt mutations *(19)*, even though the effects of external agents on the early embryo may be hidden until much later in life *(20,21)*. In mammals, however, external effects in early development are strongly buffered by the uterus and placenta. Therefore, any compound interfering with mammalian early stages of development must first circumvent those barriers.

1.4. Transgenerational Effects of Endocrine-Disrupting Chemicals

Other possible transgenerational effects are those that are more related to the presence of a compound itself in the environment than with its effects on structural

features of organisms. If a given environmental compound is persistently present generation after generation in a population of individuals, such a presence may lead to altered parameters consistently and transgenerationally in this population. With regard to this point, it must be emphasized that an organism is not only transmitting to the offspring the structure that permits the realization of the phenotype. The organism also transmits to the offspring the environmental conditions allowing the realization of such a phenotype *(22)*. In this sense, if environmental conditions trigger the same phenotypic response every generation, it could be considered a transgenerational transmission of characteristics. Endocrine-disrupting chemicals (EDC) achieve that condition given that it is well known that they can be persistently present in the environment and food chain *(18,23,24)*.

In this chapter, we will focus the discussion on describing situations in which EDC exposure affects epigenetic mechanisms in mammals and which possess the structural features that enable them to be passed transgenerationally to future generations. Then, the epigenetic system for which EDC effects have been more extensively studied, that is, DNA methylation, will be discussed under two conditions: first, when such changes are triggered during early stages of development and, second, during differentiation of the germ line. Both cases represent events when possibilities for the organisms to be affected by EDC are increased. Moreover, in these models, epigenetic changes have more possibilities of being transgenerationally transmitted if such changes are induced either before or during the differentiation of the germ line, which contains intrinsically transmissible structural features of organisms.

2. EDCS AND THEIR MECHANISMS OF ACTION

Nearly 14 years ago, the scientific community acknowledged the existence of chemicals capable of interfering with or mimicking endogenous hormones and other signaling molecules of the endocrine system. Moreover, these substances have the ability to cross placental and brain barriers and to interfere with development and function *(25)*. Since then, as attention to this area of investigation has grown, these compounds have been referred to by a variety of names, such as EDCs *(25)*, xenoestrogens *(26)*, environmental hormones *(18,27)*, hormonally actives agents *(28)*, and environmental agents *(29)*. These chemicals included many chemical classes and comprise an integral part of the world economy and commerce. The United States Environmental Protection Agency (USEPA) developed a screening and testing program to detect EDCs, and the Organization for Economic Cooperation and Development (OECD) has set up a task force to identify, prioritize, and validate test methods for the detection of endocrine disruptors *(30)*. Nevertheless, few of the thousands of chemicals used today have been tested systematically for endocrine-disrupting effects in organisms.

The endocrine system of vertebrates consists of an intricate web of stimulatory and inhibitory hormone signals that control basic body functions such as metabolism, growth, digestion, and cardiovascular function, as well as more specialized traits and processes such as behavior, sexual differentiation (during embryogenesis), sexual maturation (during puberty), and adult reproduction *(23)*. For example, the circulating hormone 17β-estradiol (E2) controls a variety of cellular mechanisms, including development processes and differentiation events, as well as growth in organs such as breast,

ovary, and uterus. The timing and concentration of bioactive estrogen signals determine sexual maturity, ovulation, and pregnancy.

2.1. Nuclear Receptors and Endocrine Disruption

Families of nuclear receptors (NRs) are defined by both structural and functional homologies. The NR superfamily contains ligand-activated transcription factors that exert a wide variety of different cellular responses by positively and/or negatively regulating target gene expression *(31,32)*. Apart from receptors that bind steroid hormones, retinoic acid or thyroid hormone, the NR superfamily contains so-called orphan receptors for which no ligand is known *(33,34)*. Steroid/xenobiotic receptors (SXR, also know as pregnane X receptor [PXR]), which belong to the family of orphan receptors, recognize many classes of EDC and may activate responses resulting in the expression of EDC metabolizing enzymes, thereby providing a link between the internal and external environment *(35)*. Estrogen receptor-related receptors (ERRs) are a subfamily of orphan NRs that are closely related to the estrogen receptor family *(36,37)*. Research on ERRs has shown that this family shares target genes, co-regulators, and promoters with the estrogen receptors family *(38,39)*. On the contrary, ERRs seem to interfere with the classic ER-mediated estrogen-responsive signal in a variety of ways *(40,41)*. Interestingly, ERRs have been reported to be prognostic biomarkers in different types of cancer *(42,43)*. In addition to SXR and ERRs, other NRs have been shown to bind EDC. For example, chlorinated hydrocarbons such as some polychlorinated biphenyl (PCB) compounds, in theory, have the ability to bind to and activate the ligand-activated transcription factor, the aryl hydrocarbon receptor (AhR), to bind the thyroid hormone receptor *(44)*. Some studies also demonstrate binding activity of environmental agents to a thyroid hormone-binding protein similar to T4 but not to a thyroid hormone receptor *(45,46)*. A further discussion of these issues is provided in Chapter 6 (Adler).

To date, no synthetic environmental chemical has been reported to function as androgen. However, a growing number of pesticides have been recognized as androgen antagonist or anti-androgen. The anti-androgenicity of dichlorodiphenyltrichloroethane (DDT) and its metabolites and other insecticides *(47,48)* also highlights the diversity of structures underlying the hormonal antagonist activities of environmental compounds. The herbicide linuron, for example, has been shown to compete with ligand for binding with the androgen receptor in human, thereby altering androgen-dependent gene expression *(49,50)*.

In addition to those synthetic EDC previously mentioned, natural chemicals have also been shown to disrupt endocrine function. Plants produce versatile chemicals, called phytochemicals or phytoestrogens, which serve both as endogenous signals triggering color and scent production within the plant and as exogenous signals secreted for communications with other organisms, for example, to inhibit sexual reproduction of predatory herbivores *(51)*. Leguminous plants (soybeans, clover, and alfalfa) secrete phytoestrogens into the soil as a recruitment signal enabling symbiotic interactions with mycorrhizal fungi and *Rhizobium* soil-bacteria, which in turn, provide growth advantages to the host plant by increasing water/phosphate availability and fertilizing with nitrogen, respectively *(52)*. Structurally, phytoestrogens are isoflavones capable of binding to estrogen receptors alpha and beta (ERα and ERβ) and acting as a weak agonist *(53,54)*, competing with endogenous E2 for ER binding and activation

of estrogen-responsive genes *(55,56)*. Despite their ability to bind these receptors, phytoestrogens exhibit only a fraction ($10^{-2} - 10^{-3}$) of the estrogenic activity of estradiol *(57,58)*. In vitro binding affinities per se do not distinguish between ER agonist and antagonist, nor do they predict tissue-specific estrogen or anti-estrogenic activity. Therefore, it may be inappropriate to perform risk assessment of estrogenic compounds by estimating their potencies solely through reporter gene or binding assays. A thorough description of the mechanisms of action of phytoestrogens appears in Chapter 6 (Adler), and effects of phytoestrogens as a central nervous system EDC is provided in Chapter 4 (Walker and Gore).

Selective ER modulators (SERMs) represent another class of synthetic estrogens being developed for treatment and prevention of hormone-dependent diseases *(59)*. In human HepG2 hepatoma cells transfected with an estrogen-responsive complement C3 promoter-luciferase construct, SERMs differentially activate wild-type ERα and its variant forms expressing activation function, namely ER-AF1 and ER-AF2; these are in vitro differences that reflect SERMs' unique in vivo biologies *(60,61)*. The HepG2 cell assay has also been used to investigate the estrogenic activities of phytoestrogens and synthetic/industrial estrogenic compounds *(58,62)*. These results show that despite evidence that phyto- and synthetic estrogens have weak estrogenic activity, they induce distinct patterns of ER agonist/antagonist activities that are cell context-specific and promoter-dependent, suggesting that these compounds will induce tissue-specific in vivo ER agonist or antagonist activities. These studies suggest that other receptors such as the AhR, which also binds structurally diverse ligands, may exhibit unique responses in vivo that are not predicted in in vitro assays.

3. GENE EXPRESSION REGULATION BY EDCS THROUGH EPIGENETIC MECHANISMS

There are many ways by which EDCs could regulate gene expression *(63,64)*. Transcriptional regulation by EDC has been described, for example, in several *Hox* genes in which distinct retinoic acid-responsive elements mediate direct transcriptional regulation by retinoic acid, resulting in teratogenesis after altered transcription induction of these genes *(21)*. Nevertheless, here we will focus on available data concerning the epigenetic mechanisms for regulating gene expression by EDC. The finding that some compounds have the ability to induce alterations in DNA methylation patterns is not new [see Wachsman *(65)*]. Exposure to EDCs may result in transcriptional changes resulting from altered DNA methylation in key genes *(16)*, and this appears to be the most common mechanism for effects of EDCs. To our knowledge, the first group to report such an effect of EDC were Barrett et al. *(66)* who proposed that diethylstilbestrol (DES) could transform cells by a mechanism other than point mutations, frameshift mutations, or small deletions. By applying the current knowledge of epigenetic mechanisms, we can speculate now that such transformations reported by Barrett et al. *(66)* could have been the product of an epigenetic process. EDCs are capable of triggering impairments during the development of organs, as proposed by Li et al. *(67)*, who showed that neonatal exposure to DES produced abnormalities in the demethylation of the lactoferrin promoter. It has also been shown that the administration of the phytoestrogens coumestrol and equol to newborn mice can enhance methylation and produce inactivation of the proto-oncogene *H-ras (68)*. Later, Day

et al. *(69)* demonstrated that DNA methylation patterns can be altered in 8-week-old mice that consumed high doses of genistein. All this evidence is supported by the new finding that exposure of early embryos to TCDD, DES, or PCB153 alters Dnmt activity, which has the potential to induce a change in methylation status of genes and affect further developmental processes *(70)*. Thus, the link relating EDC and DNA methylation is becoming strongly supported by scientific evidence.

With regard to EDC effects on another epigenetic system, Hong et al. *(71)* reported that genistein and equol produce effects on histone acetylation mediated by either ERα or ERβ, which takes place through stimulation of the histone acetyltransferase activity. Singleton et al. (2006) showed that treating ERα-HA breast cancer cells (which overexpress HA-tagged ERα) with bisphenol A (BPA) or estradiol leads to differential expression of a set of genes. BPA upregulated histone H2B and downregulated histone H1, on which estradiol had no effect; moreover, BPA had no effect on histone deacetylase, which also differs from the downregulating effect of estradiol in this regard *(72)*. Interestingly, from an epigenetic perspective, these histones have implications for chromatin condensation, as previously described. Histone H2B belongs to the dimer H2A/H2B that assembles with the $(H3/H4)_2$ tetramer, forming a histone octamer wrapped in the nucleosome core particle, the fundamental unit of chromatin *(73)*. Histone H1 binds to the nucleosome surface and interacts with nucleosomal DNA at the entry and exit points, determining the higher-order folding states of chromatin *(74)*.

There are not many publications examining EDC effects on chromatin condensation. Nevertheless, it has been shown that treating in vitro oocytes that have already undergone germinal vesicle breakdown with genistein (but not daidzein) produces several consequences at the chromosomal level such as retention of metaphase configuration or prevention of the spindle translocation toward the cortex *(75)*. A more recent study has shown that chronic oral treatment with the fungicide vinclozolin (30 mg/kg per day) from conception to adulthood disrupted both the sperm nuclear morphology and the chromatin texture, having a deleterious impact on chromatin condensation homogeneity *(76)*.

4. EDC EFFECTS ON DNA METHYLATION DURING DEVELOPMENT

As previously mentioned, any compound interfering with mammalian early stages of development must first circumvent the barrier represented by the uterus or placenta. Endocrine disruptors are known to act through maternal–fetal transfer, thereby having consequences on both gene expression and embryonic phenotype. With regard to the former, Nishizawa et al. *(77)* reported that mid to late embryonic exposure (organogenesis period) to BPA changes the expression levels of NRs such as AhR, RARa, and RXRa mRNAs in adult tissues such as brain (cerebrum and cerebellum) and gonads (testes and ovaries). Nielsen et al. *(78)* and Newbold *(79)* have reported that expression of ERα mRNA and protein is induced in the uterine epithelium after prenatal DES exposure. Naciff et al. *(80)* showed that early prenatal exposure to endocrine disruptors 17α-ethinyl estradiol, BPA, or genistein lead to an altered gene expression response in several genes (this latter study will be more extensively discussed below).

In section 4.2 phenotypic changes induced in embryos by EDCs have been reported by Takai et al. *(81)*, who showed that blastocysts exposed to BPA produce adult mice that are heavier at weaning than controls, despite having similar weight at birth. Other studies show

the same effect by EDCs mediated by maternal transfer. Adeeko et al. *(82)* showed that pregnant maternal treatment with daily doses of tributyltin chloride (20 mg/kg) between gestational days 0 and 15, which includes the preimplantation period (until gestational day 5), leads to reduced weight gain in fetuses. In addition, maternal treatment with daily doses of 10 mg/kg produced the same effect in fetuses when treatment occurred between gestational days 8 and 19, that is, starting after implantation *(82)*. Another study showing maternal treatment with a variety of endocrine disruptors, such as genistein, resveratrol, zearalenone, BPA and DES, reported transient effects on the reproductive tract and mammary glands in offspring with maternal high doses of genistein and resveratrol; in turn, low and high doses of BPA and DES had transient effects on the reproductive tract and mammary glands, whereas high doses of zearalenone induced prolonged effects *(83)*. Markey et al. *(84)* reported a decrease in the absolute and relative weight of the vagina and also mammary gland dysgenesis due to fetal developmental exposure to BPA at doses 4,000-fold lower than those capable of inducing an uterotropic response. Another feature reported to be altered because of prenatal exposure to BPA is the number of days between vaginal opening and first vaginal estrus, which is reduced in mice *(84,85)*. We have found the same pattern for vaginal opening in the model of feeding mice mothers with a natural isoflavones concentrate containing genistein and daidzein (unpublished data, manuscript in preparation).

4.1. How do EDCs Reach the Fetus?

There are two possible ways in which EDC may undergo maternal transfer to reach the developing embryo and produce epigenetic changes; one is through oviductal and/or uterine endometrial secretions *(86)*. The hormonal environment within the uterus is of critical importance to fetal development, and thus, the way in which preexisting maternal hormones that are present in the fetus interact with added chemicals will determine how that uterine environment changes *(84)*. With regard to this, we have hypothesized that endocrine disruptors could be acting on embryos in the uterus even before implantation takes place, through altering maternal secretion of epithelial uterine steroids such as catecholestrogens, which in turn could lead to changes in the establishment of methylation patterns in the embryo *(15)*. In an in vitro study, Wu et al. *(87)* showed that exposure of preimplantation embryos to the contaminant 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin can indeed alter DNA methylation in *H*19 and *IGF-2*, both imprinted genes. Thus, altering the preimplantation intrauterine environment could lead to alterations in methylation patterns not only in non-imprinted but also in imprinted genes, which are known to have relatively unchanged methylation patterns throughout generations.

The other way in which EDC acts maternally on the developing embryo is transplacentally. It has been shown that EDCs such as p,p′-Dichlorodiphenyldi chloroethylene (DDE) and α-Hexachlorobenzene (HCH) can be detected in the amniotic fluid in women between 15 and 23 weeks of gestation, leading to embryonic exposure to EDCs during organogenesis *(88)*. In a very complete study in this field, Naciff et al. *(80)* showed gene expression response to transplacental exposure to endocrine disruptors, either 17-α-ethinyl estradiol, BPA, or genistein, from gestation day 11 to 20 in rat. Of 8740 genes analyzed, they detected changes in expression in 366 genes for 17-α-ethinyl estradiol, 398 genes for BPA, and 344 genes for genistein. Moreover, among those, expression of 66 genes was consistently and significantly regulated in the same direction *(80)*. Altered expression in those genes may be related to one

of the mechanisms involved in epigenetic regulation of gene expression. Newbold et al. *(89)* have shown that, after administrating DES to pregnant rats during early post-implantation development and the neonatal period, a greater susceptibility for specific tumor formation in rete testis and reproductive tract tissues occurred in F1 and reappeared in the non-exposed F2 offspring. The authors suggested that such a transgenerational phenomenon could be due to epigenetic alterations transmitted through the germ line, including changes in DNA methylation. Recent reports by Anway et al. *(90)*, Skinner and Anway *(91)*, and Anway and Skinner *(92)*, which will be discussed next, reinforce such a postulate. Moreover, this maternally mediated epigenetic effect is not limited to synthetic EDCs. We have found that feeding mice mothers with an isoflavones concentrate containing genistein and daidzein alters gene-specific methylation patterns in the offspring (unpublished data, manuscript in preparation).

5. EDC EFFECTS ON DNA METHYLATION DURING GERM LINE DIFFERENTIATION

The process of germ line segregation from somatic cells in organisms may occur through pre-formation or epigenesis; however, in metazoans, the latter is probably the main mechanism of germ cell specification *(93)*. The initiation of the functional activation of the male and female reproductive systems represents an occasion during which environmental endocrine disruptors could act to alter normal physiology *(18)*. Physiological effects due to EDC exposure have been reported to occur in germ line in both males and females during critical stages of development such as sex determination. For example, embryo exposure to methoxychlor during sex determination period affects embryonic testis cellular composition and germ cell number and survival *(94)*. Embryonic testicular cord formation is affected when embryos are exposed in vitro to vinclozolin, and transient *in utero* exposure to vinclozolin increases apoptotic germ cell numbers in the testis of pubertal and adult animals, which correlates with reduced sperm motility in the adult *(95)*. During the critical period of sexual differentiation, it is expected that exposure of a chromosomal male to antiandrogenic xenobiotics would interfere with the androgen-dependent differentiation of the Wolffian-derived structures and/or with the normal development of the male genitalia *(18)*. On the contrary, in females, it is well known that genistein has an inhibitory effect on maturation of mammalian oocytes *(96)*. Markey et al. *(84)* showed an increase in the percentage of ovarian tissue occupied by antral follicles in 3-month-old mice exposed *in utero* to 250 μg/kg BPA.

However, heritable damage can also occur in the zygote at the beginning of the embryonic development of a new individual and be transmitted to the next generation through altering features during germ line development *(19)*. Moreover, such heritable damage can be induced while germ line is developing. For example, it has been shown that either chlorambucil or melphalan is capable of inducing a high frequency of heritable deletions and other mutations in mouse germ cells *(97,98)*, thereby producing a transgenerational change because of mutations. Nevertheless, although some endogenous and exogenous agents are frequently associated with DNA mutations and transgenerational transmission, chemically induced epigenetic modifications of DNA may well have the same net effect on the phenotype of newly altered cells and on their progeny *(99)*. Regarding this, Holliday *(100)* reported that teratogens

could target mechanisms that control patterns of DNA methylation in particular regions of the genome of developing embryos modifying methylation patterns of the same DNA sequence in somatic cells, leading to a developmental alteration and subsequently to changes in germ line cells. Modifications capable of being transmitted are those (i) occurring in somatic cells before germ line segregation or (ii) in germ line cells while they are in the process of differentiation. EDC effects regarding the former have been previously described in this section. With regard to the latter, Anway et al. *(90)* have shown that exposing a mother rat to either vinclozolin or methoxychlor during embryonic days 8–15 produced transgenerational defects in the spermatogenic capacity, which was transmitted throughout four generations (F1–F4). Furthermore, the authors also detected 25 different polymerase chain reaction (PCR) products that had altered methylation patterns in the F1 born to mothers subjected to the vinclozolin administration *(90)*. Therefore, exposure of a gestating mother to EDC during critical periods of sex differentiation and testis morphogenesis, which is when cord formation takes place in the embryo, triggers the germ line effect of decreased spermatogenic capacity and sperm viability; this phenotype is transgenerationally transmitted in the male and appears to be associated with altered DNA methylation of the germ line *(91)*. This interpretation is in concordance with that suggested by Newbold et al. *(89)* for the transgenerational transmission of the increased susceptibility for tumor formation because of early exposure to DES that was in section 4.1.

6. FINAL CONSIDERATIONS

There is a variable amount of evidence describing the effects of EDC on each distinct epigenetic mechanism known to date. Nevertheless, there is existing evidence of epigenetic EDC effects in histone modification, chromatin condensation and especially in DNA methylation. In any case, each epigenetic modification may or may not have transgenerational consequences, which will depend on the epigenetic system affected, on the stage during the ontogeny when this occurs and on the persistency of the stimuli throughout generations. Epigenetics integrates intrinsic and environmental factors *(14)*. Modifications of intrinsic factors are capable of being transmitted when triggered in somatic cells before germ line segregation or in germ line cells while they are in the process of differentiation. Experiments such as those performed by Newbold et al. *(89)* and Wu et al. *(87)* strongly support the possibility that EDC action occurs through mothers to the embryo, produces epigenetic changes on them, and moreover leads to the transgenerational transmission of those changes, mechanism previously described in Guerrero-Bosagna et al. *(15)*. In parallel, experiments by Anway et al. *(90)* show that EDC are also able to induce epigenetic modification during the differentiation of germ line, also with the possibility of transgenerational transmission of those changes. Moreover, such evidence suggests that EDCs are able to reach the embryo (i) during the pre-implantation period, through uterine or oviductal secretions, (ii) while implantation is taking place, through direct contact with uterine epithelia, or (iii) after implantation occurs, through the placenta.

Although, to date, there is not considerable evidence available reporting epigenetic and transgenerational effects of EDC, the consistency of the recent aforementioned findings supports the feasibility of this postulate. The implications of this may be many, ranging from public health to ecological or evolutionary issues.

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