

Environmental Signaling: What Embryos and Evolution Teach Us About Endocrine Disrupting Chemicals

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ABSTRACT

The term “endocrine disrupting chemicals” is commonly used to describe environmental agents that alter the endocrine system. Laboratories working in this emerging field—environmental endocrine research—have looked at chemicals that mimic or block endogenous vertebrate steroid hormones by interacting with the hormone’s receptor.

Environmental chemicals known to do this do so most often with receptors derived from the steroid/thyroid/retinoid gene family. They include ubiquitous and persistent organochlorines, as well as plasticizers, pharmaceuticals, and natural hormones. These chemicals function as estrogens, antiestrogens, and antiandrogens but have few, if any, structural similarities. Therefore, receptor-based or functional assays have the best chance of detecting putative biological activity of environmental chemicals. Three nuclear estrogen receptor forms— α , β , and γ —as well as multiple membrane forms and a possible mitochondrial form have been reported, suggesting a previously unknown diversity of signaling pathways available to estrogenic chemicals.

Examples of environmental or ambient estrogenization occur in laboratory experiments, zoo animals, domestic animals, wildlife, and humans. Environmentally estrogenized phenotypes may differ depending upon the time of exposure—i.e., whether the exposure occurred at a developmental (organizational and irreversible) or post-developmental (activational and reversible) stage. The term “estrogen” must be defined in each case, since steroidal estrogens

differ among themselves and from synthetic or plant-derived chemicals.

An “estrogen-like function” seems to be an evolutionarily ancient signal that has been retained in a number of chemicals, some of which are vertebrate hormones. Signaling, required for symbiosis between plants and bacteria, may be viewed, therefore, as an early example of hormone cross-talk.

Developmental feminization at the structural or functional level is an emerging theme in species exposed, during embryonic or fetal life, to estrogenic compounds. Human experience as well as studies in experimental animals with the potent estrogen diethylstilbestrol provide informative models. Advances in the molecular genetics of sex differentiation in vertebrates facilitate mechanistic understanding. Experiments addressing the concept of gene imprinting or induction of epigenetic memory by estrogen or other hormones suggest a link to persistent, heritable phenotypic changes seen after developmental estrogenization, independent of mutagenesis.

Environmental endocrine science provides a new context in which to examine the informational content of ecosystem-wide communication networks. As common features come to light, this research may allow us to predict environmentally induced alterations in internal signaling systems of vertebrates and some invertebrates and eventually to explicate environmental contributions to human reproductive and developmental health. (*Endocrine Reviews* 22: 319–341, 2001)

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I. Introduction

IN 1958, Dr. Roy Hertz described the “steroid cycle,” anticipating what we now call endocrine disrupter research, as follows: “. . . we have to consider that the introduction of . . . [hormones into cattle feed lots] leads to the exposure . . . of individuals who might otherwise not ever in their lives come in contact with such materials . . . This is not a theoretical consideration because we . . . now have encountered two families, each with two children, who presented with simultaneously developing gynecomastia attributable to the accidental contamination of vitamin capsules by estrogens during manufacture. If such estrogens can, by stray handling, get into such pharmaceutical preparations, can they not very readily get where they are not wanted on the farm?

There is one additional consideration in this regard . . . The fecal excretion of these materials . . . will be dropped on the soil and . . . over generations there will be constant replenishment of the soil surface with steroidal substances of this kind. This in turn has its effect potentially on surface water-supply contamination and also potentially on the veg-

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etable content of steroids in crops raised on such soil . . . I think that we are now actually setting up a steroid cycle in our environment, and we have to give very serious consideration to its implications for our subsequent development and growth and possibly reproductive functions" (taken from the discussion following Ref. 1).

The "reproductive functions" mentioned by Hertz are, in most invertebrates and all vertebrates, under the control of an integrated network of chemical signals—the endocrine system. This finely tuned communication system relies on messenger molecules, hormones of great sensitivity and specificity, to maintain the complex information flow required for normal health. Chemicals in the environment that mimic or block endogenous hormones might upset this fine balance in ways that, while unexpected, are at least predictable based on the known biology of the endocrine system. The potential implications for human health as well as the health of numerous wildlife species are self-evident.

The emerging field of scientific inquiry commonly referred to as "endocrine disruption" is thus of growing public health and environmental concern. The concerns arise from the real and perceived deleterious effects of environmental chemicals on the development or function of the reproductive system in species as diverse as snails, alligators, and humans.

Hormonal Chaos, a recent book by Sheldon Krinsky (2), is an excellent introduction to the politics, sociology, science, history, and philosophy that pertain to endocrine disruption. Krinsky, a Professor in Urban and Environmental Policy at Tufts University, states what he terms ". . . the environmental endocrine hypothesis, [which] asserts that a diverse group of industrial and agricultural chemicals in contact with humans and wildlife have the capacity to mimic or obstruct hormone function—not simply disrupting the endocrine system like foreign matter in a watchworks, but fooling it into accepting new instructions that distort the normal development of the organism. . . . From the standpoint of human pathology, the environmental endocrine hypothesis could turn out to be the most significant environmental health hypothesis since the discovery of chemical mutagenesis." Krinsky examines the scientific roots, public response, and implications in the context of the development of ideas in science. It is all the more interesting that the book appears so early in the scientific history of the field: it was not until 1980 that the proceedings of the first meeting held on this topic were published and the compounds associated with ambient hormonal activity were termed environmental estrogens (3). Since then, as attention to this area of investigation has grown, these compounds have been variously called endocrine disrupting chemicals (4), xenoestrogens (5), environmental hormones (6, 7), hormonally active agents (8), and environmental signals (9).

The field of research dealing with the environmental endocrine hypothesis is, as with most new fields, rife with debate, inconsistencies, and controversy. This may, to some extent, be a result of the multidisciplinary nature of the topic. Meetings on endocrine disrupting chemicals often include ecologists (theoretical, field, economic), chemists (synthetic, combinatorial, analytic, modeling), endocrinologists (molecular, steroid biochemistry, clinical), toxicologists (global and organismic, mechanistic, regulatory and industrial), zoologists (representing phyla from worms to whales), policy

wonks and mavens, and often, but not always, the media. The regulatory and media interest in the topic often move at a faster pace than the science. On the other hand, the challenges posed by this important area of investigation have led to novel approaches and findings driven, or at least influenced, by the multidisciplinary nature of the work, the intensity of the debates, and the interest of the public. These concerns were given public voice with the release of the Emmy-award winning documentary, *Assault on the Male* by Deborah Cadbury (1993), and the publication of the influential book, *Our Stolen Future* in 1997 (10).

It is also the case that the environmental endocrine hypothesis resides at the boundary of endocrinology and toxicology, challenging the common wisdom of both fields. For example, Crews *et al.* (11) outlined some of the salient points that distinguish environmental endocrine disruption from other toxicological approaches. They contrast the "traditional toxicological approach," which utilizes a carcinogenic model and mortality or acute toxicity, with the "endocrine disrupter approach," which relies on a developmental model and delayed dysfunction. They also look for a common element between the effective concentration of endogenous hormones compared with exogenous xenoestrogens found in the environment singly or in mixtures.

The multidisciplinary nature of environmental endocrine research is difficult to comprehend with a single review article. Several recent reviews have looked at different aspects of this area of research. For example, reviews on the environment and male reproductive health (12), screening methods for endocrine disrupting chemicals (13), and endocrine disruption in wildlife (14, 15) summarize much of what is known in the field. LeBlanc (16) took an ecological approach based on work with invertebrates and raised the possibility that lower organisms may serve as sentinel species for human health effects if we can interpret their signals. He described the importance of ecological networks that may provide early response signs to biologically active environmental contaminants.

Also, published proceedings from some of the seminal meetings on the subject provide important sources of information as well as historical perspectives. These include the three meetings on Estrogens in the Environment, the first in 1979 (3); the second, Estrogens in the Environment II: Influences on Development in 1985 (17); and the third, Estrogens in the Environment III: Global Health Implications in 1994 (18). The Wingspread Meeting on the Human-Wildlife Connection (4), in 1992, highlighted the important associations between human and wildlife health.

This present article is a brief review of selected literature concerning primarily estrogens and estrogenic chemicals, synthesizing what is known to date and offering a central thesis for this emerging area of research. It will attempt to illuminate patterns in our environment that are relevant to the endocrine system and its function. A key pattern resides in signaling systems in developmental and evolutionary biology as well as endocrinology. Thus, this review will both return to, and refine, the concept of environmental signaling that our laboratory introduced 2 yr ago (9). This term describes what is now known about environmental endocrine science, acknowledges an informative evolutionary link, and

anticipates additional signaling pathways that may include other hormonal activities as well as activities related to the nervous and immune systems.

Science and medicine have benefited from discovering patterns in observed phenomena and refining the recognized patterns into theories or syndromes (*e.g.*, the androgen insensitivity syndrome). If chemicals from many sources are indeed adding to the hormonal burden of humankind, one may use a mechanism-based pattern-recognition approach to gain understanding. This review attempts to provide a context in which to reconcile how apparently unrelated environmental chemicals might alter reproductive function. Again, as Hertz said in 1958, in discussing the addition of hormones to our environment, "... we have to give very serious consideration to its implications for our subsequent development and growth and possibly reproductive functions."

II. Environmental Hormones

As seen in Fig. 1, the steroid/thyroid/retinoid or nuclear receptor gene family encodes for a large number of receptors. It is thought that while thyroid hormone and retinoid receptors evolved coordinately, they diverged from the steroid hormones early in evolution. Moreover, the hypothesis has

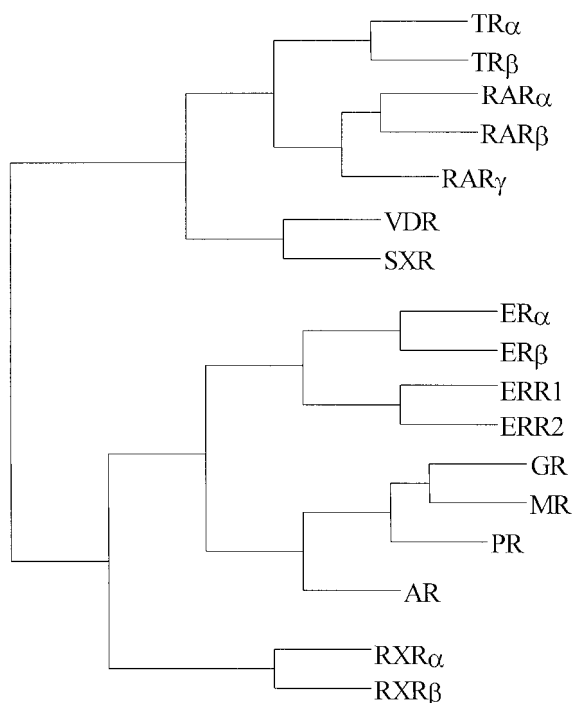


FIG. 1. Evolutionary relationships between selected members of the steroid/thyroid/retinoid gene family of nuclear receptors. Most of the known environmental chemicals with hormonal activity derive that activity through interaction with one or more of these receptors. Abbreviations used are: TR, thyroid hormone receptor; RAR, retinoid receptor; VDR, vitamin D receptor; SXR, steroid xenobiotic receptor; ER, estrogen receptor; ERR, estrogen-related receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; AR, androgen receptor; RXR, retinoid orphan receptor. Another ER form, ER γ has recently been reported (20). [Derived from (19).]

been advanced that ligand binding is an evolutionarily late event in the development of the receptors (19). Both functionally and phylogenetically, this is an important concept. The information content of chemicals destined to become ligands could be refined and diversified in concert with the gain of function in the receptor proteins that would recognize them.

The family of receptors is defined by both structural and functional homologies. While the functions and ligands of many receptors in the group are known, there are a large and growing number of receptors with no known function or ligand—the so-called orphan receptors. The orphan receptor, SXR (steroid/xenobiotic receptor), recognizes many classes of xenobiotic chemicals and activates a response that results in the expression of xenobiotic metabolizing enzymes, providing a link between the internal and external environment (17). If SXR functions as a "xenobiotic sensor" as proposed, the gradient between endogenous hormones and exogenous environmental signaling chemicals may not be as great as we thought.

In addition to SXR, other nuclear receptors have been shown to bind environmental chemicals (Table 1 and Refs. 21–23). Recent reports of synthetic environmental chemicals that activate the retinoid receptor system raise the possibility of environmental retinoids (24). Chlorinated hydrocarbons, such as some polychlorinated biphenyls (PCBs), have long been conjectured to bind the thyroid hormone receptor on theoretical grounds (25), but this has not, as yet, been demonstrated. Zoeller *et al.* (21) recently reported that some PCBs clearly activate the thyroid hormone system without a direct demonstration of thyroid hormone receptor binding. Studies also demonstrate binding activity of environmental agents to thyroid hormone binding protein similar to T₄, but not to the thyroid hormone receptor (22, 25).

As yet, no synthetic environmental chemicals have been reported that function as androgens. However, a growing number of pesticides have been recognized recently as androgen antagonists. The antiandrogenicity of dichlorodiphenyltrichloroethane (DDT) metabolites and some insecticides

TABLE 1. Environmental hormonal activities

Hormonal activity	Environmental	
	Hormone	Antihormone
Estrogen	Yes, many ^a	Yes, few ^a
Progestin	?	?
Androgen	Yes, few ^b	Yes, many ^c
Glucocorticoid	? ^d	?
Mineralocorticoid	?	?
Retinoid	Yes, one	?
Thyroid	? ^e	?

^a See representative structures in Fig. 5.

^b Androstenedione, the product of bacterial metabolism of stigmasterol; see Fig. 3.

^c See representative structures in Fig. 2.

^d Arsenic is reported to block the GR activation at the receptor binding level (23).

^e PCB congeners elicit a thyroid hormone-like response, but no binding data for the thyroid hormone receptor is available (21). One study that evaluated binding of chlorinated hydrocarbons to the thyroid hormone receptor and thyroid binding proteins did not demonstrate specific receptor binding, while binding to transthyretin was of the same affinity as T₄ (22).

(26–29) also points out the structural diversity underlying the antihormonal activities of environmental compounds. Figure 2 shows the structures of those environmental antiandrogens compared with the pharmaceutical antiandrogen, hydroxyflutamide. Very recently, the herbicide linuron was shown to be a competitive binding ligand for the androgen receptor in rats or humans, and it altered androgen-dependent gene expression in castrate rats (30). The organophosphate insecticide, fenitrothion, was also shown to be a competitive reversible inhibitor of the androgen receptor (31). The *in vitro* potency of fenitrothion as an antagonist (K_B , 2.18×10^{-8} M) was comparable to that seen for the pharmaceutical antiandrogen flutamide. This value was approximately 8- to 35-fold greater than that determined for *p,p'*-dichlorodiphenyl ethylene (DDE) (28, 32) and linuron (33).

While no synthetic environmental compounds have yet been reported to have androgenic activity, the work of Howell and colleagues has elucidated a route to significant androgenic contamination of the environment. Twenty years ago, Howell and colleagues (34) described masculinization of female mosquito fish, *Gambusia affinis holbrooki*, which were caught downstream from the effluent discharged from a paper mill in Cantonment, Escambia County, Florida. The females exhibited male secondary sex characteristics such as a male sex organ or gonopodium as well as male sexual behavior. The authors remarked that this fish species was known to be sensitive to the masculinizing effects of androgens and proposed, as one of three hypotheses, that an androgenic material was produced in the effluent. In subsequent studies (35), Howell's group determined that the masculinizing agent was indeed androgen, but androgen from a very unusual source. While steroidal androgens were not actually found in the paper waste, the plant sterol, stigmasterol, was. It was further determined that *Mycobacterium smegmatis*, which produces androgens as metabolic products, had formed extensive colonies in the effluent path. These bacterial "mats" were utilizing the plant cholesterol (stigmasterol) as a carbon source and metabolizing the plant

sterol to a potent androgen, androstenedione (Fig. 3). This is a well established pathway in microbial metabolism and is known in the laboratory. The environmental implications for the reproductive system of vertebrates of this bacterial biochemistry were not established before the work with mosquito fish. Since these initial findings, masculinization of mosquito fish downstream from pulp and paper mills has been described in many other areas of the United States (36).

The production of biologically active androgens by bacterial mats has added a new dimension to the field of environmental endocrine science. So has a recent report that nontoxic concentrations of arsenic can function as a glucocorticoid receptor (GR) modulation factor by selectively inhibiting GR-mediated transcription through altered nuclear function rather than a decrease in hormone-induced GR activation or nuclear translocation (23). This raises the possibility that metals may indeed interact at fundamental levels with the cell processes associated with hormones and their receptors. These findings suggest that the demonstration of the effect of environmental chemicals on the GR may now require consideration of other heavy metals. Previous studies have shown that arsenite, cadmium, and selenite interact with the cysteines of the ligand binding domain of the GR and inhibit binding of dexamethasone to the receptor (37).

Finally, a wholly new kind of ligand-ER complex has been reported that may change the ways we think that environmental factors mimic hormones as well as shed light on the interaction between arsenite and GR mentioned previously. The heavy metal, cadmium (Cd), has been shown to mimic the effects of estradiol in estrogen-responsive breast cancer cell lines, both in cell proliferation and regulation of gene expression (38). Cadmium has recently been demonstrated to activate ER α by interacting with the ligand-binding domain of the receptor (39). These results led the authors to assert that "the heavy metal, cadmium, is a new environmental estrogen."

Indeed, much of the focus in environmental endocrine science has been on those chemicals that mimic the female sex hormone, estradiol-17 β . The first synthetic chemical found to

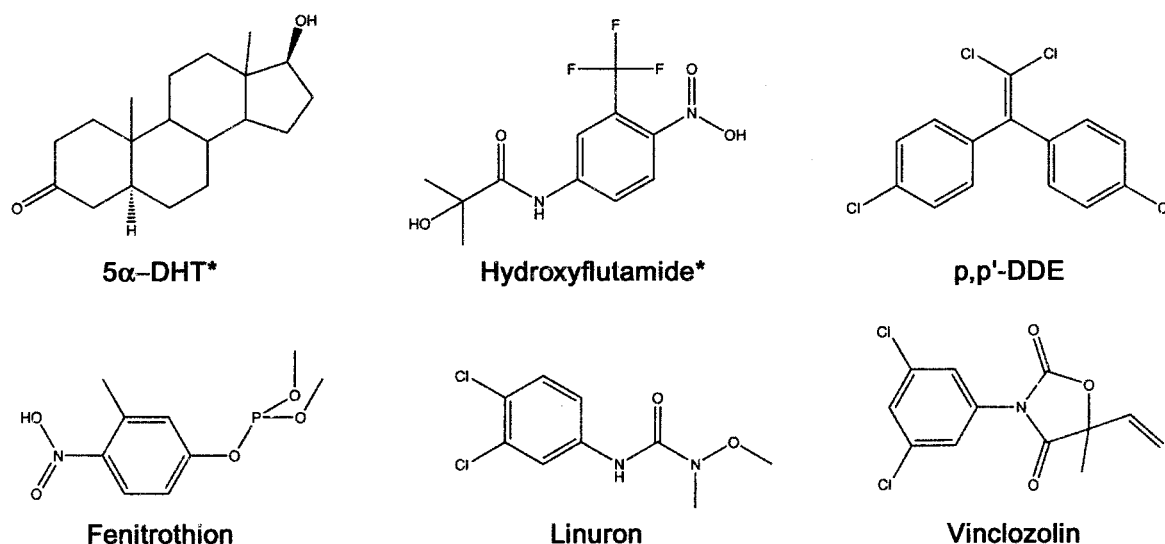


FIG. 2. Structural diversity among environmental chemicals reported to be antiandrogenic. The steroidal androgen, 5 α -dihydroxytestosterone (5 α -DHT) and its pharmaceutical antagonist, hydroxyflutamide, are shown for comparison. *p,p'*-DDE is a persistent contaminant, while the remaining are currently used pesticides: fenitrothion, an insecticide; linuron, an herbicide; and vinclozolin, a fungicide.

mimic the activity of an endogenous steroidal hormone was an estrogen. In 1933, Dodds and colleagues (40) described 1-keto-1:2:3:4 tetrahydrophenanthrene (Fig. 4) as "the first compound of known chemical constitution found to have definite oestrus-exciting activity" (40). Dodds continued to examine the structural basis for estrogenicity and, in a short landmark paper (41), described the first synthetic estrogen without the phenanthrene nucleus, the ring structure common to steroids (Fig. 4). In this paper, he evaluated a series of diphenyl compounds and concluded that only those with two hydroxyl groups in the para positions would be active as estrogens. The compound, di-(p-hydroxyphenyl) dimethylmethane, called bisphenol A, or BPA, may be the first synthetic selective estrogen receptor modulating (SERM) chemical reported. Dodds' experiments represent a pharmacological breakthrough in rational chemical synthesis, opening many routes to the same biological function exhibited by a variety of chemical structures, *e.g.*, estrogenicity, as determined using the ovariectomized rat vaginal cornification assay introduced just 8 yr earlier.

These early structural studies culminated in 1938 with the discovery of diethylstilbestrol or DES (42). DES, a derivative of the stilbene nucleus (Fig. 4), was shown to be significantly more efficacious than all the previous compounds tested by

Dodds. As it was also orally active, relatively stable, and less expensive to produce than isolated or synthesized steroidal estrogens, DES quickly became the synthetic estrogen of choice for medicine and, later, agriculture. Its medical uses included estrogen replacement therapies, lactation suppression, postcoital contraception, pregnancy maintenance, and prostate cancer therapy. In agriculture, DES was used to chemically caponize chickens and stimulate growth in cattle.

While the widespread use of DES in cattle feed lots led to the introduction of tons of potent estrogens into the ecosystem, BPA was destined for far greater use. BPA was found to be an efficient cross-linking chemical and came to be used widely in the production of plastic polymers, primarily polycarbonates. It is somewhat ironic that two synthetic chemicals, the potent estrogen, DES, and the weak-acting estrogen, BPA, which have been so important to our understanding of environmental estrogens can be traced to one laboratory, that of Sir Charles Dodds.

Many structurally diverse chemicals have been reported to function as estrogens (Fig. 5). As with all steroid hormones, 17 β -estradiol contains the three-ring phenanthrene; for estrogenicity, the first or A ring must contain a phenolic hydroxyl group. The pharmaceutical estrogens DES and ethinyl estradiol are as potent as the parent compound, estradiol. In

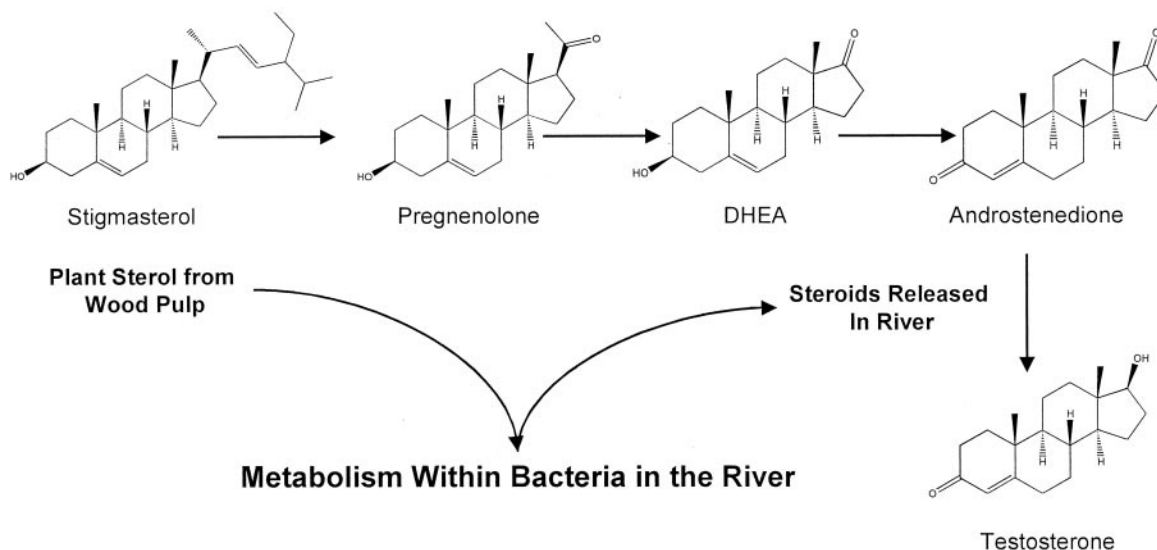


FIG. 3. The production of androgenic compounds by bacteria. Stigmasterol, a major plant sterol found in wood pulp, is efficiently metabolized to androgenic steroids such as androstenedione by the bacteria, *Mycobacterium smegmatis*. *M. smegmatis* form extensive colonies, or "bacterial mats," at the effluent site of pulp and paper mills. The natural plant sterol, stigmasterol, contained in the pulp effluent is converted by *M. smegmatis* into androstenedione, which is released into the river or stream. Female mosquito fish exposed to these androgens develop male structures. (See Refs. 34, 35, and 36 for details.)

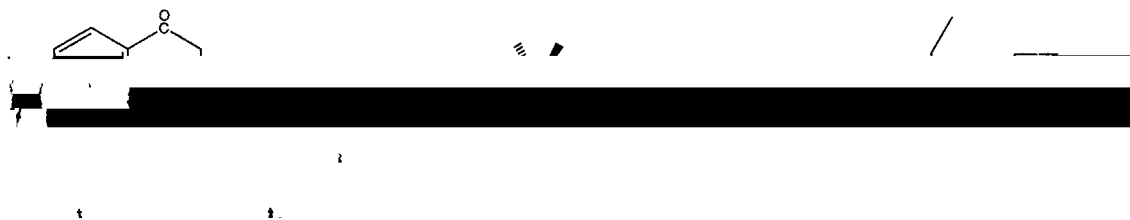


FIG. 4. First synthetic estrogenic chemicals or selective estrogen receptor modulators (SERMS). The laboratory of Dodds *et al.* reported the synthesis and bioassay of 1-keto-1:2:3:4 tetrahydrophenanthrene in 1933 (40), bisphenol A in 1936 (41), and DES in 1938 (42). Each successive compound was more estrogenic than the preceding one.

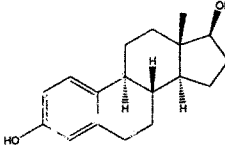
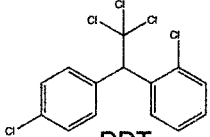
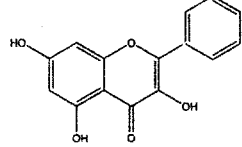
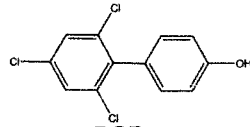
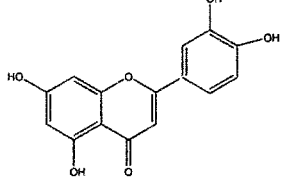
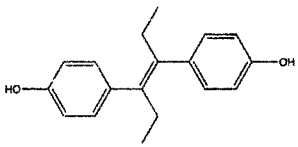
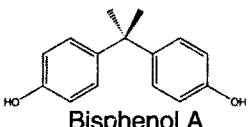
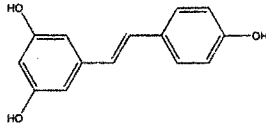
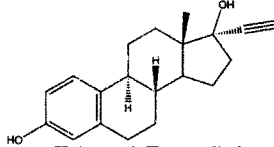
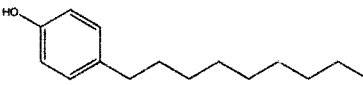
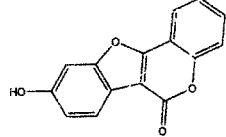
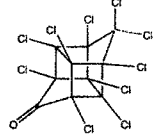
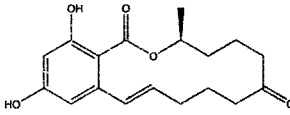
Steroids	Pollutants	Plant Products
 17β-Estradiol	 DDT	 Genistein (isoflavone)
Pharmaceuticals	 PCB	 Luteolin (flavone)
 Diethylstilbestrol	 Bisphenol A	 Resveratrol (stilbene)
 Ethynyl Estradiol	 Nonylphenol	 Coumestrol (coumarin)
Fungal Products	 Kepone	
 Zearalenone		

FIG. 5. Chemicals found in the environment reported to be estrogenic. This list is not comprehensive, but illustrates representative structures of estrogenic compounds from various sources. Information on these compounds is contained in the text.

viewing the structures of synthetic chemical contaminants, the most striking feature is, perhaps, the absence of a consistent structural motif. There is often, but not always, the presence of an aromatic ring or two. Several representative chemicals contain chlorine atoms. The role that chlorine plays in hormonal activity is still not clear. For instance Kepone, a structurally restricted, cubic molecule containing chlorine on every carbon but one, is known to be estrogenic (43).

In most cases these environmental chemicals were synthesized with no apparent hormonal intent. We can consider these chemical contaminants to be "inadvertent" estrogens or chemicals whose synthetic rationale was unrelated to their ultimately determined hormonal activity. One of the best known examples is the pesticide, DDT. While the intended function of DDT was far removed from that of a vertebrate

sex hormone, it was shown in 1950 by Burlington and Lindman (44) to estrogenize cockerels exposed to it. The estrogenic activity of DDT was rediscovered by Conney and colleagues in 1969 (45), while testing various pesticides as inducers of P_{450} enzyme activity. One of several comprehensive reviews on the estrogenicity of DDT and its congeners, written by David Kupfer (46), appeared 20 yr ago.

In addition to DDT, other chlorinated hydrocarbons, PCBs, have been shown to function as estrogens in both *in vivo* and *in vitro* assays (47). The degree of hydroxylation and the location of the chlorine and hydroxyl groups are important determinants in biological activity. In the case of substituted alkyl phenols, such as para-nonyl phenol, the potency of the compound as an estrogen has been shown to be related to side chain length and branching (48). However, all

known "inadvertent" estrogens are much less potent than the steroidal estrogen, 17 β -estradiol.

The hormonal activities of environmental chemicals apparently reside in a functional attribute rather than a structural one. To discover the intrinsic function in chemicals, one could use an approach of functional toxicology or receptor-based toxicology (49). Chemicals could be screened through various hormone receptor activation assays, and the biological activity or function would be determined along with the potency of the compound relative to the parent hormone (Fig. 6; Refs. 49–51). As seen in Fig. 6, the receptor may behave as a signal integration unit and collect information from growth factors, other nuclear receptors, and a series of chaperone proteins and coregulator proteins. All of these signal inputs are routes for environmental chemicals to mimic or block hormones.

The total amount of environmental contamination with synthetic chemicals that give an estrogen signal is not known; however, 45,000 metric tons of the weak estrogen, p-nonylphenol, were produced in 1976, and by 1982 the total annual production of all alkyl phenol polyethoxylates was estimated at 140,000 metric tons. In 1993 BPA production in the United States was 640,000,000 kg; of that, 44,000 kg (0.10%) were reported recycled, land filled, incinerated, or released in the environment.

In addition to the many synthetic chemicals resulting from industrial practices, human activities have added in other

ways to the hormonal burden on the environment. Many pharmaceutical chemicals were synthesized to function as estrogens, but their environmental impact was not considered. For example, DES, the potent synthetic estrogen, was used as a growth-promoting substance in cattle for more than 40 yr. In 1971 alone, Knight (52) estimated that 27,600 kg of DES were used for this purpose (52). While concern was expressed for the levels of synthetic hormones present in edible portions of beef, DES and its metabolites were also excreted into the ecosystem with unknown consequences. Metcalf (53) explored the fate of radiolabeled DES in a model ecosystem and reported that it was persistent and bioaccumulated. While the health outcomes are not known, studies in humans have demonstrated that after oral administration, the conjugated form of DES, DES glucuronide, is readily metabolized by intestinal bacteria and absorbed into the blood stream as the biologically active parent compound (54). These results suggest that conjugation of excreted environmental estrogens may not limit the efficacy of the hormone.

Another category of environmental contamination with synthetic estrogens is the excretion of components of pharmaceuticals used for contraception or hormone replacement in humans. There have been few studies on the levels of drugs in waste water and fewer on levels in drinking water. An early report in 1977 that 8.5 kg/day of salicylic acid, a metabolite of aspirin, was found in the waste water effluent

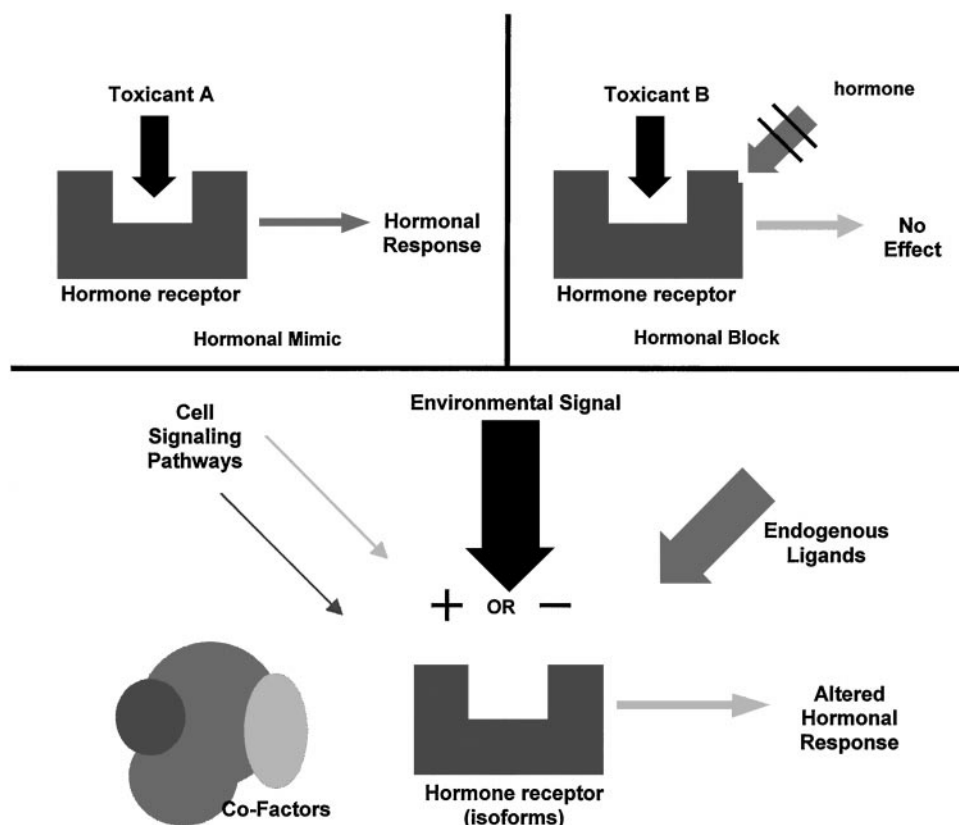


FIG. 6. Functional or receptor-based toxicology. The practice of using steroid hormone receptors to determine the hormonal or antihormonal activity of environmental chemicals is more than a decade old (49). The relatively simple concept presented then (*upper panels*), must now accommodate the advances in knowledge over the last 10 yr. These include the convergence of activating ligands and cellular signals on the ER (50), the multiple isoforms of the ER (51), contributions of coactivators and corepressors, and the gradation in response (50).

in Kansas City (55) prompted speculation that, "With millions of women taking oral contraceptives, some environmental contamination with estrogenic materials is a distinct possibility" (56). Very recently, an estrogenic component of commonly used oral contraceptives, 17 α -ethinyl estradiol, was found in trace amounts in waste water effluent (57, 58). Estrogens and their glucuronides have been found in municipal sewage in Germany, Canada, and Brazil (59) and in surface and waste waters in The Netherlands (60).

Studies on the effects on wildlife that live in ponds containing wastes from livestock are underway only now, but the total impact of natural and synthetic hormones discharged by cattle, hog, and chicken farming may be greater than previously believed. In a pilot study, Irwin and Oberdoerster (61) demonstrated feminization of turtles living in ponds that received waste runoff from cattle farms.

III. Environmental Estrogens

A. At the lab bench

While hormones in the environment seem to be a relatively new phenomenon and their biological effects uncertain, there are several well characterized examples of unexpected estrogenization of biological systems in cell culture, or, if you will, "inadvertent [estrogen] contamination in a controlled experimental micro-environment." In a laboratory setting, it was known for decades that cells derived from estrogen-responsive tissues were only weakly responsive to the mitogenic signal of estrogen in tissue culture (62, 63). In 1986, Berthois and colleagues (64) demonstrated that the commonly used pH indicator in tissue culture media, phenol red, was a weak estrogen. It was later shown that a contaminant of the phenol red preparation, bis-(4-hydroxyphenol)-[2-(phenoxy sulfonyl)phenol] methane, was the actual estrogenic chemical (65). The unexpected estrogen in tissue culture media was sufficiently biologically active to stimulate cells in culture to proliferate maximally, often negating a detectable effect of the addition of estrogen to the media. In fact, most publications after 1986 that discuss estrogen action in cell or organ culture explicitly describe using phenol red-free media.

Five years later, Soto and colleagues (66) solved another problem encountered in the culture of estrogen-responsive cells. Many laboratories conducting *in vitro* experiments noted a marked difference in response to estrogens when they switched plasticware vendors. Soto *et al.* showed that some plastic petri dishes and tubes contained a residue of p-nonyl-phenol. This alkyl phenol contaminant was shown to be estrogenic, and the finding presaged by a few years the association shown by Sumpter's group (67) between p-nonyl-phenol contamination and feminization of fish in UK streams.

Another example of an unexpected estrogen at the lab bench is that of bisphenol A, a monomeric constituent of polycarbonate plastic, which, as previously described in this review, was originally synthesized as an estrogen. Feldman *et al.* (68) described an estrogen binding protein and an endogenous ligand in the yeast *Saccharomyces cerevisiae*. After an exhaustive set of studies, Feldman and colleagues (69)

discovered that the estrogenic substance thought to be of yeast origin was actually bisphenol A, which is released from the polycarbonate flasks when they are autoclaved (69).

B. In animals other than humans

There are numerous reports of reproductive and developmental abnormalities in species ranging from snails to humans that have been associated with exposure to environmental hormones (primarily estrogens) (Table 2 and Refs. 15, 34, 70–84). With careful evaluation of the findings, it may be possible, over time, to discern an "environmental estrogen phenotype" that has two components—one developmental or "organizational" and the other, adult or "activational." This analysis will be the subject of a later review by our laboratory.

As an example of direct activational effects of environmental estrogens in wildlife, studies with cheetahs are informative. Cheetahs face extinction in the wild and exhibit reproductive failure and liver disease in captivity. Clinical symptoms such as hepatic venoocclusive disease found in 100 cheetahs in the Cincinnati Zoo suggested hyperestrogenization (85). HPLC and gas chromatography coupled with mass spectrometry revealed large amounts of the phytoestrogens, daidzein and genistein, in their diet. The authors estimated that the cheetahs consumed approximately 50 mg of the compounds per day. When four animals were given meat instead of soy-based diets, their liver function improved; thus, dietary estrogens were thought to be detrimental to this carnivorous species.

Thigpen and colleagues (86) showed that standard formula rodent diets can vary greatly in content of the phytoestrogens daidzein and genistein as well as in uterotrophic activity (86). In an earlier report (87) they show that 15-day-old weanling CD-1 mice fed an American Institute of Nutrition diet (AIN-76A) for 7 days had uterine weight gains close to that seen in mice fed a certified rodent chow containing 6 ppb of DES. The influence of differing dietary estrogen content on experimental results in rodents is an increasingly important variability factor in design of toxicology assays for environmental hormones as well as in more fundamental studies of hormone response in genetically manipulated mice. The experimental background levels of unintended estrogens are reminiscent of studies described earlier concerning estrogen-containing tissue culture media and plasticware.

Zearalenone, a fungal mycotoxin produced by *Fusarium*, binds the estrogen receptor (ER) (88) and is uterotrophic in the newborn rat (89). Consumption of corn contaminated with *Fusarium sp* has been associated with estrogenic effects in poultry and livestock such as cloacae prolapse in turkeys (90), impaired fertility in cattle (91), and hyperestrogenicity in swine (92). The last disorder has been termed the "moldy corn syndrome." The exact extent of estrogenic mycotoxin contamination of human foodstuffs is not known, but is estimated to be 3 μ g/person/day in North America.

C. In humans

There have been case studies in the clinical literature that illustrate the acute, reversible (activational) effects of exog-

TABLE 2. Examples of reproductive and developmental abnormalities attributed to endocrine disruption

Species	Observation	Contaminant	References
Mammals			
Humans	Gynecomastia, oligospermia, impotence, hypogonadism, decreased libido, reduced sperm counts and motility, menstrual cycle irregularities	DDT, kepone, oral contraceptive exposure, stilbene derivatives	(70)
Cattle	Infertility	Coumestrol	(71)
Sheep	Infertility, dystocia	Isoflavonoids, coumestans	(71)
Seals	Impaired reproductive functions	PCBs	(15)
Mink	Population decline, developmental toxicity, hormonal alterations	PCBs, dioxins	(72)
Rabbits	Infertility, failure of ovulation, failure of implantation	Isoflavonoids	(71)
Guinea pigs	Infertility	Isoflavonoids, coumestans	(71)
Mice	Proliferative lesions, reproductive tract tumors, infertility, inhibition of estrus, inhibition of ovulation	DES, isoflavonoids	(71,73)
Birds			
Japanese quail	Abnormal reproductive behavior, hematology, and feather morphology	<i>o,p'</i> -DDT	(74)
Gulls	Abnormal development of ovarian tissue and oviducts in male embryos	<i>o,p'</i> -DDT	(75)
Waterbirds	Egg shell thinning, mortality, developmental abnormalities, growth retardation	DDE, PCBs, AhR agonists	(72)
Reptiles			
Alligators	Abnormal gonads, decreased phallus size, altered sex hormone levels	<i>o,p'</i> -DDT, <i>p,p'</i> -DDE, dicofol	(76,77)
Red-eared slider turtle	Anomalous reproductive development	<i>trans</i> -Nonachlor, <i>cis</i> -Nonachlor, arochlor 1242, <i>p,p'</i> -DDE, chlordane	(78)
Fish			
Mosquito fish	Abnormal expression of secondary sex characters, masculinization	Androstenedione	(34)
Roach	Hermaphroditism, vitellogenin in males, altered testes development	Sewage effluent mixture	(72)
Lake trout	Early mortality, deformities, blue sac disease	Dioxin, related AhR agonists	(72)
White sucker	Reduced sex steroid levels, delayed sexual maturity, reduced gonad size	Bleached kraft plup, mill effluent mixtures	(72)
Flatfish	Decreased hormone levels, reduced ovarian development, reduced egg/larvae viability	PAHs	(72)
Invertebrates			
Snails	Masculinization, imposex, formation of additional female organs, malformed oviducts, increased oocyte production	Tributyltin, bisphenol A, octylphenol	(79–82)
Marine copepods	Stimulate sexual maturation and egg production	Bisphenol A	(83)
<i>Daphnia magna</i>	Delayed molting time	PCB29, arochlor 1242, diethyl phthalate	(84)

Abbreviations: AhR, aryl hydrocarbon receptor; DES, diethylstilbestrol; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyl-trichloroethane; PAH, polyaromatic hydrocarbons; PCB, polychlorinated biphenyl.

enous estrogen on the human male. The most informative is a case entitled, "The mortician's mystery: gynecomastia and reversible hypogonadotropic hypogonadism in an embalmer" (93), in which a 50-yr-old man presented with a progressive loss of libido, a decrease in testicular size and beard growth, and marked breast development. As these symptoms are associated with excess estrogen in a male, the patient, a mortician, was examined carefully for an estrogen producing tumor and excess serum steroidal estrogens. Failure to find a clinical answer suggested that there might be an exogenous or environmental source of estrogen exposure. Organic chemical extraction of the patient's serum revealed an unknown substance that effectively displaced radiolabeled estradiol from its receptor. A similar activity was discovered in the embalming cream used by the patient. When the source of environmental estrogen was removed, the patient experienced a significant restoration of libido, testes

size, and sperm count, as well as reduction in breast size. The authors remarked upon the reversibility of the clinical symptoms and reached the following conclusion: "some principles observed in our patient may be generalizable to groups . . . although he presented with striking clinical findings, it is possible that lesser degrees of exposure to estrogen in . . . industrial exposures are more common and induce less profound disturbances of reproductive function, such as oligospermia in men and menstrual irregularities in women."

IV. Estrogens and Estrogenic Signaling

In 1979, the first Symposium on Estrogens in the Environment was held, ". . . to determine what an estrogen is and how it works, and what effect estrogenic substances might have on human health . . . since many chemicals with diverse

chemical structures, some of which are environmental contaminants, have been endowed with 'estrogenic' properties" (3). This seemed a fairly straightforward goal at the time; however, 20 yr later the attainment of that goal remains elusive.

One of the difficulties in the field of environmental endocrine research is semantic. What is an environmental estrogen? According to The American Heritage Dictionary of the English Language, ed 4 (2000), an estrogen is "any of several steroid hormones produced chiefly by the ovaries and responsible for promoting estrus and the development and maintenance of female secondary sex characteristics." The word, which first appeared in 1927, is comprised of the following components, "estr(us)" (again, from the same dictionary, "estrus [is] the periodic state of sexual excitement in the female of most mammals, excluding humans, that immediately precedes ovulation and during which the female is most receptive to mating; heat.") plus "o" (the combining form) and "-gen" ("producer; one that is produced."). Thus, estrogen is a word of recent origin with a functional definition, *i.e.*, something that produces a period of heat in a female—a signal.

To "induce estrus" is a behavioral and physiological process involving many organ systems and a commitment of time. As scientific knowledge of estrogen action has evolved, so has the functional definition. Over time, an estrogen has been defined in the scientific literature as a chemical capable of inducing vaginal cornification in an immature mouse; a chemical that increases uterine weight in an ovariectomized mouse; chemicals associated with proliferation of the uterine epithelium in castrate female mice; chemicals capable of stimulating an increased number of cells from estrogen target organs grown in tissue culture; chemicals that form ligands for the ER and displace radiolabeled estradiol from its binding; chemicals that regulate the expression of estrogen target genes; and, chemicals that transactivate ER-driven reporter genes in cells in culture. While one would think that any or all of these functional definitions would apply, the use of one or another has led to controversy. If a chemical binds the ER with a high affinity and specificity, is it an estrogen? Or must it also activate ER-regulated genes? Must it lead to a functional response? Hertz said, "Notwithstanding this complex array of variably associated effects of estrogens, the *sine qua non* of estrogenic activity remains the mitotic stimulation of the tissues of the female genital tract. A substance which can elicit this response is an estrogen; one that cannot do this is not an estrogen" (94).

This semantic problem is not unique to environmental estrogens. Semour Lieberman (95) recently posed the questions, "When is an estrogen an estrogen? When is it not?", whereby he revisited the concept of estrogenicity and the precision by which it should be defined. He was considering the use of the term estrogen to describe pharmaceutical and environmental compounds as well as natural hormones. In fact, Lieberman points out, we still do not know whether estradiol and estriol, two natural steroidal compounds, are really both estrogens, even though they have been called such for 60 yr, since behavioral estrus is induced by estradiol, not estriol. He raises the deliciously provocative possibility that estriol, the estrogen of pregnancy in humans, may ac-

tually have a different role than one might surmise from its classification as estrogen.

Therefore, when we say, for example, that plants make estrogen, precision requires us to say that plants make compounds that induce some responses traditionally associated with the steroid hormone, estradiol, either *in vivo* or *in vitro*. The language used to describe compounds that may alter the endocrine system presents a challenge in linguistic research as intriguing as much of the laboratory research in this area.

The signaling molecule, estradiol, regulates reproduction in many invertebrates and all vertebrates. Of invertebrates, Cnidarians (coral) (96, 97), crustaceans [water fleas (98, 99) and lobsters (100)], mollusks (snails) (101), and echinoderms (starfish) (102) are reported to produce estradiol. The phylogenetic distribution of estradiol production in the animal kingdom suggests that estrogenically active chemicals may be evolutionarily conserved signals. It also suggests the possibility that all animals are sensitive to estrogens, whether endogenous or environmental. In addition to the ligand signal, it appears that the signal recognition system is also widely distributed phylogenetically. As seen in Table 3 (20, 50, 51, 84, 103–116), ERs have been found in many vertebrate species. In those species in which it has been studied—including mammals, birds, and fish—both ER α and - β subtypes have been found. Very recently, a third distinct form of ER, ER γ , has been cloned from a teleost fish, the Atlantic croaker, *Micropogonias undulatus* (20). This represents the first identification of a third classical ER in vertebrates. Phylogenetic analysis suggests that ER γ evolved through gene duplication from ER β early in teleost lineage. As both ER β and - γ bind 17 β -estradiol with high affinity, the presence of three subtypes of ER in teleost fish suggests that the estrogen signal

TABLE 3. Selected examples of species containing ER or related receptors

Species	ER or ER-related molecules	References
Mammals		
Humans	ER α , ER β	(50)
Cattle	ER α	(103)
Sheep	ER α , ER β	(104)
Dogs	ER	(105)
Cats	ER	(106)
Rats	ER α , ER β	(51)
Mice	ER α , ER β	(50)
Birds		
European starlings	ER α , ER β	(107)
Japanese quail	ER α , ER β	(108)
Reptiles		
Alligator	aER	(109,110)
Red-eared slider turtle	ER	(111)
Fish		
Rainbow trout	rtER α , rtER β	(112)
Tilapia	OaER	(113)
Atlantic croaker	ER α , ER β , ER γ	(20)
Invertebrates		
<i>Daphnia magna</i>	EcR	(84)
Crayfish	EcR	(114)
<i>Drosophila</i>	EcR	(115)
Mosquito	EcR	(116)

Abbreviations: aER, alligator estrogen receptor; EcR, ecdysone receptor; ER, estrogen receptor; OaER, *Oreochromis aureus* estrogen receptor; rtER, rainbow trout estrogen receptor.

may be distributed in networks that we have not yet even considered. It remains to be demonstrated that ER γ exists in other vertebrate species, although in a study using the ER α knock out (ERKO) mouse, investigators explained their results in which catechol estrogens and methoxychlor, but not 17 β -estradiol, stimulated uterine cell proliferation and lactotransferrin induction in the ER α minus mouse by raising the possibility of a third, or γ , form of the ER (117).

In studies reported so far, ER has not been found in invertebrate species. Other members of the nuclear receptor super gene family have been shown including the ecdysone receptor (Table 3) and several vertebrate "orphan receptors" such as COUP II (chicken ovalbumin upstream promoter II). This is an important area for further investigation and will help evaluate and refine the concepts of environmental signaling contained in this review.

For many years the characterization of an ER associated with the cell membrane has been sought as a means to explain the rapid responses seen after estrogen stimulation (118). There is now a growing body of literature describing the localization of ER molecules in the plasma membrane of estrogen target cells (119, 120). The membranes of endothelial cells are reported to contain ER α coupled to nitric oxide synthase as a functioning signaling module (121, 122). ER has also been shown in perimembrane activity in neural cells (123) and is thought to mediate rapid nongenomic estrogen signaling. The activation of coupled membrane receptors for estrogens and dopamine by environmental estrogens opens an exciting new dimension in environmental signaling (124). Finally, to explain the induction of mitochondrial gene expression with ethinyl estradiol (125), Chen and Yager (126) recently localized ERs - α and - β to the mitochondria of estradiol-treated cells (126). This provides yet another area of control for estrogenic signaling and locates a response module in the oxidatively active cellular organelle that may utilize the electron-donating capacity of catechol estrogens.

V. Environmental Signaling

We begin to understand the broad environmental signaling aspects of estrogenic chemicals when we consider chemicals produced by plants that evolved long before vertebrates and yet have been found to be hormonally active in numerous mammalian species (71). Subsequently, some of these chemicals have been termed estrogens, or phytoestrogens (literally, "plant estrogens"), as a result of their hormone signaling effects in animals. While plant estrogens probably did not originate as what we now think of as "estrogens," later vertebrate dietary exposure and response to these compounds led to the concept of phytoestrogens or plant chemicals with estrogenic activities (71). In a now classic report by Bennets *et al.* published in 1946 (127), compounds derived from plants such as subterranean clover were found to be capable of compromising the fertility of grazing sheep by overestrogenizing them.

Since the corollary evolution of animals has required the formation of internal signaling molecules of reproductive importance, it is tempting to speculate that estrogenic plant signals may have been internalized, or rather may have

evolved, into the endocrine system, playing a crucial role in the coevolution of both major phyla. This also raises the question of interphyla cross-talk, *i.e.*, since animals recognize plant hormones, do plants recognize animal hormones? This question may be of environmental importance given the increasing burden of synthetic and other estrogenic compounds released into the environment.

Phytochemical signals, some of which are estrogenic, have evolved to benefit the plants that produce them. The most commonly studied phytochemicals are the flavonoids, including isoflavones and flavones, represented by genistein and luteolin, respectively. In fact, it has been shown that the isoflavones, like genistein, bind vertebrate forms of ER α or ER β and alter the transcription of estrogen-responsive genes (128, 129). The report that isoflavones are better ligands for ER β than ER α raises the possibility that environmental estrogens may exert greater effects on tissues or species with higher ER β /ER α content (129).

The flavonoids represent a family of phytochemicals that have been thought to function to deter herbivores from eating the plant containing them, protect the plant from fungal and bacterial pathogens, and initiate symbiosis with nitrogen-fixing bacteria (130). It is this last function that is the defining signaling role for flavonoids.

Leguminous plants, such as soybean and alfalfa, produce such flavonoids. Symbiosis occurs as a result of complex signaling between host plant and bacteria, which is initiated by plant recruitment of bacteria to root hairs through the release of these small molecule polyphenolic compounds (131). *Rhizobium* bacteria exist as free-living organisms in the soil or as nitrogen-fixing symbionts of leguminous plants. In response to phytochemical signals (release of flavonoids), *rhizobia* infect the roots of host plants and induce the formation of specialized organs called root nodules (132). *Rhizobia* then colonize the root nodules and, in exchange for carbon nutrients from the host plant, provide the plant with a nitrogen source by changing atmospheric nitrogen into a nitrogen fertilizer (133). This is called "symbiotic nitrogen fixation."

For example, alfalfa, *Medicago sativa*, secretes the flavonoid luteolin, or 3',4',5,7-tetrahydroxyflavone, from its root hairs into the surrounding soil where the soil bacterium *Sinorhizobium meliloti* is located (134). Luteolin interacts with constitutively produced rhizobial NodD proteins, and this interaction activates transcription of a cassette of nodulation (*nod*) genes necessary for symbiosis (135). Host and bacteria specificity is necessary to maintain symbiotic partners, *e.g.*, alfalfa and *S. meliloti*, or soybeans and *Bradyrhizobium japonicum*. Each plant producing a unique profile of phytochemical signals achieves this specificity. For example, upon recognition of alfalfa's primary phytochemicals, luteolin and apigenin, *S. meliloti* NodD proteins activate *nod* gene transcription, but other flavonoids, such as chrysin and coumestrol, inhibit NodD-induced gene activation (141).

The ability of flavonoids to initiate, maintain, and regulate symbiosis requires unique signal recognition by, and activation of, the bacterial transcription regulator NodD. The ability of flavonoids to activate symbiosis is in part determined by the specific type of NodD protein within a *Rhizobium* species and, as illustrated above, is also regulated by the

specific phytochemical signaling molecules (136, 137). This suggests a complex interaction between flavonoids exuded by legume roots to recruit *Rhizobium* and those flavonoids found within the root that might function to negatively regulate this interaction. This balance of positive and negative inducing flavonoids serves a crucial function for the maintenance of symbiosis.

Some pollutants and organochlorine pesticides affect endocrine signaling in animals and human cell culture systems by weakly binding to ERs and modulating their ability to turn on transcription of estrogen-responsive genes (63, 138). These same environmental contaminants may interfere with plant-rhizobial signaling. This hypothesis is based on studies showing that endocrine disrupting chemicals, as well as phytochemicals produced by leguminous plants as a signal to *Rhizobium*, are both able to bind ERs in animals and affect the transcriptional activation of responsive genes (15, 129, 139). Some of the same phytochemicals that are able to bind the ERs and activate transcription are also able to cause transcription of responsive nodulation genes by interacting with the *Sinorhizobium meliloti* NodD protein. The NodD protein and ER α share not only similarities in compounds that they are able to bind or respond to, but may also share a degree of sequence homology in their ligand-binding regions. One study has reported that two regions of NodD1 share 45% and 35% amino acid homology with two regions in the hormone-binding domain of the mammalian ER α (140). Therefore,

these two distinct proteins may share an evolutionary connection in sequence as well as the ability to bind estrogen-like compounds. Functional similarities and transcriptional effects are also shared by ER α and NodD; each protein binds a specific phenolic hormonal ligand, and this binding alters expression of key target genes leading to morphogenetic and metabolic responses.

The tightly controlled expression of these target nodulation genes allows *Rhizobium* to respond to the specific phytoestrogen signal emitted by the host plant and begin the process of symbiosis (130, 141). In light of the genetic and functional similarities between ER α and NodD, endocrine disrupting chemicals that are able to bind ERs and modulate signaling may employ the same mechanism to modulate the ability of *S. Meliloti* NodD to respond to the phytoestrogen signal, luteolin. Therefore, our laboratory used a construct containing key nodulation genes linked to a reporter gene (135) to study the effect of endocrine disrupting chemicals on signaling between the NodD protein, a proposed evolutionary relative of the ERs, and its natural phytoestrogen ligand. Fox *et al.* (Fox, J. E., M. Starcevic, K. Y. Kow, M. E. Burow, and J. A. McLachlan, submitted) show that under these conditions, DES, but not 17 β -estradiol, inhibits luteolin-NodD-induced gene activation. Similar levels of inhibition have been seen with known endocrine disrupting chemicals. These results raise the possibility that endocrine disruption may be seen in symbiotic environmental signaling systems

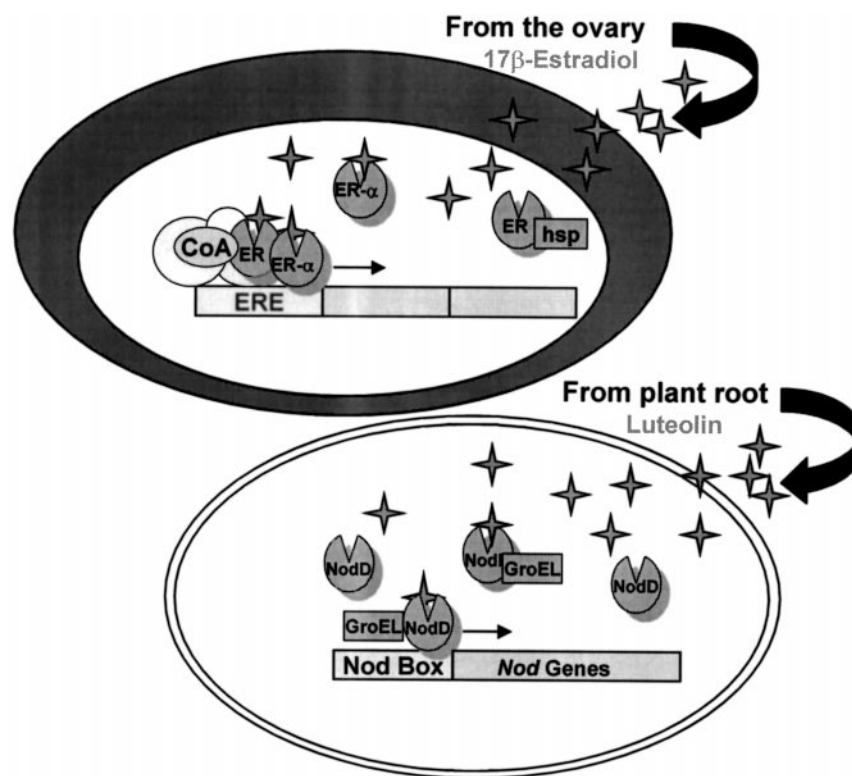


FIG. 7. Functional analogy between estradiol endocrine signaling and luteolin symbiotic signaling. The *upper panel* depicts the ovarian secretion of 17 β -estradiol, the signaling molecule that activates the transcriptional apparatus controlled by the ER in association with a specific DNA recognition motif, estrogen response element (ERE), in the estrogen target cell (*e.g.*, uterine cell). The *lower panel* depicts the secretion by the plant (alfalfa) root of luteolin into the surrounding soil. This provides the signal that activates the transcriptional apparatus controlled by the interaction of the NodD binding protein and the DNA recognition motif called a Nod Box contained in the promoter region of target genes. Luteolin-NodD association activates the transcription of multiple bacterial nodulation genes.

that exist between organisms rather than within them. Figure 7 shows a stylized model of the functional analogy that may be derived from endocrine signaling by estradiol and symbiotic signaling by luteolin.

Another symbiotic relationship between plants and microbes involves flavonoid signaling and arbuscular mycorrhizal fungi (142). This important mutual interaction in the rhizosphere is ancient (>400 million years old) and universal (representing fungal colonies in the roots of most vascular plants) (143). Flavonoids such as quercetin and kaempferol are known to stimulate the hyphal growth of the fungus, *Gigaspora margarita* (144). Thus, as with rhizobial bacteria, root phenolics are secreted secondary plant metabolites that function as growth or differentiation signals for mutualistic fungi.

Since many phytochemicals that participate in plant-microbe symbiosis are also estrogenic in vertebrate systems (145), one might ask what the effect of "vertebrate estrogens" and antiestrogens are in the root-mycorrhizal signaling system. Using a transformed organ culture of carrot roots, Poulin *et al.* (144a) were able to demonstrate hyphal growth in *G. margarita* and *Globus intraradices* induced by the flavonoids, quercetin, or biochanin A, respectively. Using a highly specific antiestrogen, EM-652 [Dovalla-Bell *et al.* (145a)], they were further able to demonstrate a dose-related reduction in the biochanin A signal. 17 β -Estradiol produced a 2.4-fold increase in hyphal growth, but was effective only at the highest concentration (5 μ M).

Thus, as pointed out by Baker (145), consideration of the evolutionary role of signaling molecules used by plants to alter the behavior of microbes can provide insights into the mechanism of action of estrogenic compounds at many phylogenetic levels. This approach should be informative for elucidating the chemical structures and functions inherent to environmental agents that behave in a hormonally active fashion.

Since several hormonally active xenobiotics are chlorinated hydrocarbons, the principle of environmental signaling suggests a search for naturally occurring signaling molecules that contain chlorine. In fact, a potent developmental signal, differentiation-inducing factor-1 (DIF-1) is a chlorinated alkyl phenone produced by the slime mold, *Dictyostelium*. This chlorinated signaling molecule is released by the amoeba and induces it to differentiate into stalk cells (146). DIF-1 regulates the central cell fate decision during *Dictyostelium* development (147). With DIF-1 the cells differentiate into stalk cells, and without it, they become spores (148). The DIF-1 levels rise during cellular differentiation (149). As the cells differentiate they produce an inactivating enzyme, DIF-1 dechlorinase, which prevents further increases in the signal (150).

As in the legume-rhizobial bacteria system, the DIF-1 system provides a signal-dependent mechanism for cellular aggregation and differentiation. In the case of *Rhizobium* the process results in a root nodule, while in *Dictyostelium*, it results in a fruiting body. It is interesting to speculate that the signaling properties seen in vertebrate estrogenic signaling may be related to evolutionarily ancient systems developed in soil bacteria and slime molds.

Similarly, for chemicals such as PCBs or other chlorinated

hydrocarbons, environmental bacteria can play a role in bioactivating hormonally inert compounds into more estrogenic forms by first dechlorinating and then hydroxylating the parent compound (151). The extent of such conversion is not known, but it provides another avenue for the production of hormonally active environmental compounds.

The conversion, then, of nonhormonally active compounds in the environment to active "hormones" is an important issue to consider both in the context of environmental endocrine disrupting chemicals as well as in naturally occurring hormonally active environmental signaling systems. Are there adaptive benefits to *Gambusia* exposed through generations to testosterone or, as is more likely, are there more proximate targets for utilization of the androgen produced by the bacterial mats? While plant sterols serve as a mere carbon source for organisms that do not respond to the androgenic metabolic products, organisms sharing the same environment, such as mosquito fish, interpret these metabolic products as "masculinizing signals" that alter their body plans and endocrine systems.

From a toxicological view, the insights into the structure-function relationships of modern day contaminants may be significantly advanced through evolutionary considerations of the ancient signaling molecules known to elicit differentiation responses. Likewise, we must add to our calculations of distribution of natural and synthetic chemicals the role played by microbial conversion.

VI. Estrogens and Fetal Development

A. Effects of estrogens and estrogenic chemicals on development of males

Many of the reports regarding effects of environmental estrogens on wildlife have dealt with altered sexual development. Hydroxylated PCBs have been shown to sex reverse male-determined turtle embryos in much the same way estradiol does (152). Fry and Toone (75) have shown that DDT will feminize gulls in ovo. The reduced phallus size and altered estrogen-to-testosterone levels seen in alligators in Lake Apopka, Florida, suggest a developmental exposure to environmental estrogen or antiandrogens (76).

These results in wildlife raise the possibility that prenatal estrogen levels in humans might be associated with later genital anomalies in the male offspring. One way to explore that issue is to ask what the estrogen status was during the fetal development of men with retained or cryptorchid testes. When maternal pregnancy conditions were noted for men with cryptorchidism it was found that the clinical conditions reported for the mother during the fetal life of the men—obesity, hyperemesis, first pregnancy, or hypertension—were each associated with elevated estrogen levels in their mothers (153).

The term "organizational effects" describes the persistent developmental effects of hormones while "activational effects" describes the acute, reversible effects of hormones. Studies on developmental exposure to the potent estrogenic chemical DES may shed light on the mechanisms underlying the apparent "organizational" effects of estrogens.

1. *DES as a model for developmental estrogenization.* Studies in our laboratory and others have helped to define a phenotype typical of male mice exposed *in utero* to DES and other estrogens. The structural or functional changes associated with the phenotype include undescended testes, cysts of the epididymis, prostatic lesions, distended seminal vesicles, retained Müllerian ducts, reduced fertility, and abnormal spermatogenesis (even in a scrotal testis). In a smaller number of cases, the occurrence of testicular cancers was noted (154–157). The severity of these changes was dose-dependent as were the appearance of all the lesions in the suite. It was subsequently shown that the epididymal cysts were of Müllerian duct origin (158); it was apparent that the enlarged prostatic utricle was also the Müllerian contribution to the prostate gland.

The results of Sharpe *et al.* (159) and vom Saal and associates (160) and others who have studied the effects of steroidal and environmental estrogens on the genital tract of the fetal male rodent have provided further confirmation that estrogen can induce long-term functional changes. More recently, studies with the ER α null mouse (161) have added strong support to the concept that male genital tract development may have an estrogen component.

The similarity in the morphogenesis of the reproductive system in mammals as diverse as the mouse and human provides comparative insights into the spectrum of effects associated with *in utero* estrogen exposure. Mice and human fetuses progress from an “indifferent” stage of internal genitalia in which both the presumptive male (Wolffian duct) and female (Müllerian duct) reproductive organs coexist regardless of the genetic sex of the fetus to the definitive structure of the appropriate gender. This process is under the control of hormones from the fetal testes after differentiation of the fetal gonad. The configuration will be female unless the fetal testis intervenes by secreting Müllerian Inhibiting Substance (MIS) to induce regression of the Müllerian duct and testosterone to maintain the Wolffian duct (162). In mice, DES exposure *in utero* results in the retention of both male and female genital ducts, thus forming a male pseudohermaphrodite or a genetic male with functioning testes and a male genital tract as well as a female genital tract. Failure of testicular descent is also commonly observed.

Studies in organ culture confirm the retention of female genital anlage in the DES-exposed tissues. They also extend the *in vivo* observations to demonstrate that the DES effect is not on the synthesis or secretion of MIS from the fetal testes, but in the Müllerian duct resistance to the apoptotic signal of MIS (163).

2. *Features in the human male.* Genital tract defects similar to those seen in DES-treated mice were also observed in men whose mothers had taken DES (164, 165). A group at the University of Chicago reported that DES-exposed men had a higher incidence of undescended (cryptorchid) testes and epididymal cysts than comparable unexposed men. Gill and colleagues (166, 167) went on to confirm and extend these studies and showed, in addition, a higher incidence of hypoplastic testes and abnormal sperm. In one study reporting testicular cancer in one DES-exposed man, the possibility of cancer of the testis as a result of prenatal exposure to DES was

raised by Gill *et al.* (167). A few other case reports of testicular cancer (seminoma) and epididymal cysts in prenatally DES-exposed men have been reported (168).

Comparison of mouse and human data demonstrates the importance of understanding the timing of biological events involved in the development of the reproductive tract of each. For example, when comparing the total dose of DES administered during pregnancy to the mouse, as compared with the human, to produce retained testes, the Relative Potency Index (RPI) was more than 80. However, when the dose comparison was made during the biologically relevant period for testicular descent in both species (days 14–16 of gestation in the mouse and weeks 7–27 in the human), the RPI was between 1 and 2 (169).

Thus, the male offspring of DES-exposed pregnancies of both mice and humans share some defects in common, including undescended testes, epididymal cysts, and sperm abnormalities. A recent study by the Wilcox group confirmed the occurrence of structural abnormalities in DES-exposed men but found that there was not a significant difference in fertility between the study participants and control subjects (170). While retention of the female genital anlage, the Müllerian duct, was a prominent feature in DES-exposed male mice, no report from similarly exposed men has addressed this issue. One might expect some element of Müllerian duct retention in the human male, since the hormone responsible for regression of the female duct is also thought to play a role in testicular descent, a defect common to both species.

One of the earliest reports of adverse effects of prenatal exposure to DES on male progeny was a single case of pseudohermaphroditism in a male infant. The child's mother had been given high doses of DES during pregnancy (50 mg/day commencing in the sixth week of gestation; 200 mg/day by the eighth week and for the duration of the pregnancy) (171). The genital lesions in the boy included hypospadias and testes apparently devoid of germ cells. The period of gestation during which exposure occurred appears important since Davis and Potter (172) observed no abnormalities in the external genitalia of four male infants whose mothers were treated after the first trimester with high doses of DES. Finally, in parallel with the studies on the prenatally DES-exposed mouse, the Müllerian duct derivative in the prostate, the prostatic utricle, was hypertrophic and contained areas of squamous metaplasia, suggesting that the fetal Müllerian derivatives responded the same in both species to the estrogenizing effect of DES *in utero* (173).

B. Molecular mechanisms for the developmental actions of estrogen

The developmentally estrogenized male phenotype—retained or cryptorchid testes, decrease in sperm number, increase in abnormal sperm, retained Müllerian ducts, epididymal cysts, hypospadias, and prostatic disease—has been seen, in whole or in part, in mice, rats, hamsters, and humans exposed to estrogens *in utero*. The genes involved in the process of male genital tract morphogenesis are only now being identified. The acute or persistent modulation of the expression of developmentally critical or hormone-respon-

sive gene in the male genital tract by estrogenic compounds is currently ongoing in numerous laboratories.

1. *Cryptorchidism*. Emmen *et al.* (174) have recently shown that the cryptorchidism associated with prenatal treatment with various estrogens in mice may be the result of estrogen-related inhibition of insulin-like factor 3 (Insl3), produced by the fetal testes. Insl3 had been shown earlier to affect testicular descent through signaling from the fetal testes to the gubernaculum. Insl3 mutant mice exhibit bilateral cryptorchidism; this is thought to occur as a result of altered development of a component of the genital mesentery, the gubernaculum, which retains an elongated "female" structure (175, 176).

2. *Hypospadias*. Hypospadias, a defect of the external male genitalia associated with prenatal estrogen treatment, has also recently gained molecular dimensions. In this case, the Yamada group (177) has reported that the development of the external genitalia of the mouse involves signaling by fibroblast growth factor (FGF) during formation of the genital tubercle. FGF 10 knock-out mice show abnormal development of the glans penis, suggesting an important role for that signaling molecule in the induction of hypospadias.

3. *Müllerian duct retention*. The molecular mechanism for Müllerian duct retention associated with DES is becoming clearer. While it had been shown that the effect of DES on Müllerian duct retention resides at the level of the duct rather than the fetal testis (163), the molecular alteration in the duct has recently been shown to be a failure of the MIS receptor in the fetal duct to respond to the peptide (178). The molecular mechanisms associated with Müllerian pathogenesis after prenatal exposure to DES is starting to be understood. Ma *et al.* (179) studied the localization of the Hox genes related to morphogenesis of the fetal Müllerian duct in the mouse. By concentrating on Hoxa, they determined the longitudinal distribution of these genes along the developing genital tract and were able to relate changes seen in Hoxa-10 gene disruption to that seen in prenatal exposure to DES.

4. *Molecular feminization of the developmentally estrogenized male tissues*. Androgen-dependent secretory proteins, SVS-IV, V, and VI, have been characterized from the rat (180, 181) and mouse (182) seminal vesicle. In both the rat and mouse, SVS-IV was under control of androgen and, therefore, not expressed in female or castrate male tissues. An estrogen-dependent uterine secretory protein (183) and the gene encoding it were identified as lactotransferrin, a member of the transferrin gene family (184). Lactotransferrin is under powerful control by estrogen, is located in the uterine epithelium, and varies with estrogen levels during the estrous cycle (185). Lactotransferrin was expressed in mammary gland and leukocytes but was not regulated by estrogen in these tissues (185). Lactotransferrin was not expressed in ovariectomized female mice or in male control mice. Male mice that were castrated and treated with estrogen did not express lactotransferrin in their seminal vesicles (186).

When the seminal vesicles of prenatally DES-exposed males were analyzed, lactotransferrin mRNA was detected. The level of expressed lactotransferrin message increased

after castration of the male and exceeded that of the uterus when each was estrogen stimulated. These results comprise the first demonstration of hormonally altered sexual development at the gene level. Further analysis of the seminal vesicles of DES-treated mice demonstrated that while they retained the cytoarchitecture associated with the male organ, they expressed epithelial gene products associated with the uterus, *i.e.*, lactotransferrin and ER α (187). In fact, virtually all the cells of the DES-exposed mouse seminal vesicle epithelium express antigens recognized by antisera to lactotransferrin and ER α .

Additional experiments were conducted to assess whether prenatal exposure to DES had feminized the seminal vesicle cells or, alternatively, blocked the masculinization of the organ at the molecular level. It was shown that DES-exposed mouse seminal vesicles were competent to express the seminal vesicle specific protein SVS-IV under androgen control as well as lactotransferrin. In fact, the same cell was often able to make both products (188).

VII. Mechanisms in Altered Fetal Development

Estrogens are considered reversible cellular signals. Administration of estrogen to an ovariectomized mouse results in organ growth, cell proliferation, and target gene expression in the uterus (189). When estrogen is withdrawn, uterine size and weight as well as expression of estrogen-regulated genes return close to the unstimulated state.

On the other hand, when estrogens are given to newborn mice, at least one gene under estrogen control is expressed persistently, even in the absence of estrogen (190). The estrogen treatment that results in persistent expression of lactotransferrin also results in epithelial cancers of the uterus in a majority of the mice (191). This leads to the question, how does a reversible signal become irreversible in the absence of detectable gene mutation? As described earlier, during the process of cell differentiation, genes are differentially turned on or off. For the most part, a key event in establishing the pattern of gene expression in a cell is the methylation or demethylation of regulatory elements of the gene.

Five CpG sites available for methylation occur in a region upstream from the ERE in the mouse lactotransferrin promoter. In the developmentally estrogenized mouse, two sites remain unmethylated, while in the corresponding control, only one CpG site remains unmethylated (192). It has been shown previously that a 1-bp change in methylation pattern can have a strong effect on expression of the gene (193). Adult mice treated with the same dose of DES for the same period of time did not have a change in the DNA methylation pattern of the lactotransferrin gene. This is consistent with the inability of such treatment in the adult to persistently change expression of the gene. The actual mechanism underlying the molecular feminization of genes by estrogen has still not been elucidated. However, other studies in our laboratory on the control of lactotransferrin expression may prove informative. We have shown that developmental exposure to estrogens resulted in the persistent overexpression of lactotransferrin in the uterus of females (190). The cellular secretory protein is expressed at the mRNA and protein

levels as if the mouse is receiving injections of estrogen, even 3 weeks after ovariectomy. More recently, we have shown that the persistent expression of a normally hormone-regulated gene may be the result of developmental imprinting (192). A fundamental event in cell differentiation involves programming genes to be differentially regulated later in life. One mechanism for developmental gene programming is selective DNA methylation or demethylation of its promoter.

The altered methylation pattern associated with estrogen treatment during differentiation of uterine epithelial cells provides a mechanism for irreversible expression of a normally reversible signal or provides one possible route for the change from epigenetic to genetic in hormonal carcinogenesis. The generalization of this mechanism to hormonal carcinogenesis requires studies on methylation and expression of different genes that are important to cell differentiation or proliferation. It has been shown that breast cancer cells have silenced genes that otherwise would function as tumor suppressor genes and that the silencing is a function of altered methylation (194). It is not known whether estrogens play any role in the methylation or demethylation of these genes.

Genomic imprinting refers to a very specific phenomenon: "the non-Mendelian inherited epigenetic form of gene regulation that results in monoallelic expression" or, "genomic imprinting is an epigenetic form of gene regulation that results in the expression of only one parental allele" (195). The elements of genomic imprinting are the persistent change in gene expression that is accomplished in an epigenetic fashion, and the silencing of a gene from one parent, but not the other. The process of cell differentiation is based on differential gene expression or the sequential turning on and off of different genes to establish a stable phenotype. While this process may not be monoallelic, it is both epigenetic and inherited. In developmental toxicology, the term "gene imprinting" refers to persistent changes in gene expression that occur through nonmutagenic mechanisms.

The same conundrum has appeared in the molecular biology of cancer, in which "silencing" of tumor suppressor genes has been associated with the cancer process. Since tumor suppressor genes are in many cases genomically imprinted monoallelic genes, the process fits the specific definition. However, in cases where the neoplastic transformation process involves persistent activation or repression of nongenomically imprinted monoallelic genes, the term gene imprinting is useful.

The terminology is especially useful when considering mechanisms of hormonal carcinogenesis. It is well known that treatment of various species, including humans, with exogenous estrogen is associated with tumors in different organs (196). Estrogens have been largely assumed to function in the tumorigenic process as a secondary stimulus or promoter, due to the paucity of evidence that estrogens or estrogenic chemicals are point mutagens (197). In spite of the failure to conclusively demonstrate that estrogenic chemicals, unlike other carcinogenic chemicals, do not form covalent adducts to DNA, nor induce structural DNA mutations, it has been shown that estrogens of various structures and biological potencies can function as carcinogens. This evidence includes neoplastic transformation of cells in culture in the absence of enhanced cell proliferation (198–200), car-

cinogenic effects in the adult hamster kidney (201), or neonatal mouse (191), and hamster (202) uteri.

In fact, very little is known regarding the role of estrogens or other hormones in gene imprinting. A case can be made, however, that estrogen-associated signaling pathways may contribute to DNA methylation or demethylation. For example, our laboratory showed earlier that estrogen-like effects were induced in uterine cells in culture (203) or in the mouse uterus *in vivo* (189) by epidermal growth factor (EGF). Subsequently, we demonstrated that the EGF signal required the presence of a functioning ER in cell culture for the expression of the growth factor effect (204). This study was subsequently confirmed in mice lacking the ER α gene (205). The demonstration of cross-talk between membrane receptors and steroid hormone receptors has also been shown for IGF-I and ER, dopamine and PR, and both IGF-I and EGF and ER in cell culture. The studies of receptor cross-talk have established the ER as an important signal transduction molecule in peptide hormone signaling systems. We have recently demonstrated additional evidence of this signaling convergence by showing that the cell survival pathway associated with phosphatidylinositol-3 kinase and AKT also requires a functioning ER for its action (Burow, M. E., B. N. Duong, D. E. Frigo, S. Elliott, C. B. Weldon, B. M. Collins-Burow, J. Alam, B. S. Beckman, and J. A. McLachlan, submitted). Thus, pathways involved in cell proliferation, differentiation, and survival apparently converge on the ER.

Finally, it has been shown previously that both EGF and estrogen increase the levels of *c-fos* or *c-jun* in target cells or tissues (206). The response of the immediate early genes to estrogen also proceeds, apparently, via an ER. In the case of developmental estrogenization, *c-fos* and *c-jun* were persistently expressed. Recently, a demonstration that cells constitutively overexpressing *c-fos* up-regulated cytosine methyltransferase, the enzyme involved in DNA methylation, has suggested to the authors that *fos* may be one regulator of the process (207).

In summary, we suggest a mechanism whereby estrogen either directly or through related signaling pathways plays a role in programming or imprinting genes involved in cell proliferation, differentiation, or survival. This happens in such a way that the genes are either persistently over- or underexpressed or will respond to a later cue, such as another hormone leading to altered cell function and ultimately disease. Given that the process of gene imprinting is a normal one seen in cell differentiation and genomic allelic silencing, one may speculate that for estrogens or estrogenic chemicals, the distinction between physiology and pathology is blurred. The case for estrogen-associated gene imprinting is strengthened by two recent papers in which it was shown that specific genital tract neoplasms associated with developmental exposure to DES can be generationally transmitted to both female (208) and male (73) progeny.

Cellular imprinting by estrogenic compounds may arise through at least two mechanisms. In one case, the estrogenic chemical may directly imprint the gene through a process leading to persistent genetic change, probably at the level of DNA methylation. On the other hand, altering components of signaling pathways at key points in cell differentiation such that altered gene expression would ensue could form a

biochemical memory. The most likely genes to be important to this process would be those involved in response to secondary hormonal cues. Thus, when a gene programmed to respond to estradiol at puberty is misprogrammed or reprinted by developmental exposure to a hormonally active chemical, it will respond abnormally to the secondary cue, resulting in a functional cellular abnormality. It is this concept of epigenetic memory that is most compelling in dissecting the mechanism of action of hormones during development. Vitellogenin, or egg yolk protein, has been well studied in this way (209–216). Prior estrogen stimulation in chickens or frogs results in an accelerated induction of vitellogenin following a second stimulus. The molecular “memory” apparently is coded in the conformation of the chromatin site associated with vitellogenin. While studies of this type have not been thoroughly done with mammals, it provides an additional model with which to view the possible imprinting effects of estrogen during development.

VIII. Lessons Learned

In environmental endocrine science, we have made a series of observations that, at first, seemed unconnected. However, now, as the observations start to establish a pattern, we can begin to discern the linkages between them. In the last 20 yr we have discovered the intrinsic biological signaling properties of numerous synthetic environmental chemicals. We are also beginning to learn about the complex network of signaling molecules that facilitate information flow in the communication system of ecological life. In the same time period, cell and molecular biology has elucidated many of the signaling molecules necessary for intra- and intercellular communications. The similarities between the signaling strategies adopted by the internal and external world are probably more than coincidental if the evolution of the signaling systems followed, in any way, the convergent pathways suggested in this review.

Environmental signals are chemical messenger molecules functioning in a communication network linking numerous species. One may speculate that the functional aspects of this more globally distributed network might have provided a framework or blueprint to build the internal communication networks of animals, which we call their endocrine systems. As such, similarities in response to such signals in some cases should not be unexpected. Indeed, a central strategy for all life forms is the transmission of important characteristics to their offspring. This also is a form of information transfer in which the signal is embedded in the physical entity being transmitted. Thus, the transmission of genetic information and the utilization of signaling molecules and pathways are intrinsic to life from bacteria to humans. As de Loof (217) states, “communication is the one essential property for life.” Chemicals that alter either or both levels of information flow can have consequences that may be deleterious to the individual or population.

From the study of embryos and evolution the following three patterns emerge:

1. The structural diversity of environmental hormones may reflect the evolutionary background of these chemicals

as plant signaling molecules or differentiation specific signals for organisms that do not require an endocrine system. Moreover, signals developed for one communication system may be functionally misinterpreted by another system.

2. Evolutionarily important signals are likely to be those related to reproduction or differentiation of the species and its cells. This information is most crucial to the survival of that species and, likely conserved as pathways, most often misinterpreted. Chemicals synthesized to disrupt the reproductive capacity of insect pests stand a good chance of affecting cell differentiation in unintended species and in unintended ways.

3. Molecules with high informational content can induce long-term changes in a communication system if the information is disseminated at inappropriate times. Hormones that may alter the processing of information by imprinting a response pathway or imparting memory functions in a cell can be expected to have long-term effects on developing organisms. The effect will be related to the number and types of programming mistakes induced.

The patterns underlying evolution and embryogenesis are being uncovered through systematic inquiry using the techniques of molecular and cell biology. As patterns begin to emerge in environmental endocrine science, recognition of similarities to those associated with evolution and development should provide insights to mechanisms and outcomes. Without pattern recognition, there is not the ability to predict, and without prediction there is not the possibility to prevent. If male fruit bats are lactating in Malaysia (218), look for the environmental hormone. If there is a dramatic increase in the cases of premature breast development in Puerto Rico (219), look for the environmental hormone. And, if a 50-yr-old mortician presents with gynecomastia and hypogonadotrophic-hypogonadism with no estrogen-producing tumor (93), look for the environmental hormone.

It has not always been recognized as such, nor has it been applied to environmental endocrine science, but making public health predictions based on environmental chemical confusion or environmental signal misinterpretation has a long and mostly successful history in environmental health sciences and toxicology. One informative example will suffice. From the Middle Ages until as recently as 1959, an acute behavior disorder could result from eating moldy rye bread; the fungal product associated with this condition is a neuroactive compound in humans called ergot and the condition is known as ergotism (220). In the Middle Ages and later, ergotism was known as St. Anthony's fire since humans consuming infested rye flour behaved as if they were on fire and were thought to be possessed by the devil. This level of knowledge was consistent with the unpleasant consequences usually visited on such individuals. As the association between mold in bread and disease in humans was made, the scientific explanation, the human body's misreading of the fungal signal, provided a course of public health action—prevent mold from developing in rye flour or if it does, don't make bread from it.

As the principles underlying environmental endocrine science are developed or discovered, the opportunity to apply them to understand and anticipate the environmental component of human reproductive diseases and developmental

disorders should excite endocrinologists. From an environmental stewardship perspective, the evolving concept of environmental signals can provide insights with which to address the impact of hormonally active chemicals on humans and the ecosystems that they share with other species. Disruption of this apparently broad communication system has the potential for global change that transcends the endocrine system.

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Note Added in Proof

Embryos. A very recent study on sex reversal in humans describes a plausible molecular mechanism for a female phenotype in genetic males. Overexpression of WNT-4, a putative sex-determining gene, due to a duplication of 1P31-P35 led to female development in an XY individual. The authors propose that this genetic imbalance is associated with conditions including ambiguous genitalia and failure of testicular descent (221).

Evolution. An elegant case is made for the elaboration of the steroid receptor family through gene duplication and ligand exploitation in which the metabolic intermediates of hormones become ligands. Analysis of the evolution of steroid receptors and endocrine complexity concludes that the estrogen receptor was the first steroid hormone receptor and, if so, may explain why so many different taxa may be sensitive to environmental pollutants that interact with the estrogen receptor (222).

References

- Gassner FX, Reifstein Jr EC, Algeo JW, Mattox WE 1958 Effects of hormones on growth, fattening, and meat production potential of livestock. *Recent Prog Horm Res* 14:183-217
- Krimsky S 2000 Hormonal Chaos: The Scientific and Social Origins of the Environmental Endocrine Hypothesis. Johns Hopkins University Press, Baltimore, MD
- McLachlan JA (ed) 1980 Estrogens in the Environment. Elsevier, New York
- Colborn T, Clements C (eds) 1992 Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing, Princeton, NJ
- Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H 1993 Medical hypothesis: xenoestrogens as preventable causes of breast cancer. *Environ Health Perspect* 101:372-377
- Danzo BJ 1998 The effects of environmental hormones on reproduction. *Cell Mol Life Sci* 54:1249-1264
- Cheek AO, McLachlan JA 1998 Environmental hormones and the male reproductive system. *J Androl* 19:5-10
- Knobil E, Chair, Committee on Hormonally Active Agents in the Environment 1999 Hormonally Active Agents in the Environment. National Research Council, Washington, DC
- Cheek AO, Vonier PM, Oberdorster E, Burow BC, McLachlan JA 1998 Environmental signaling: a biological context for endocrine disruption. *Environ Health Perspect* 106 [Suppl 1]:5-10
- Colborn T, Myers JP, Dubanowski D 1996 Our Stolen Future. Dutton, New York
- Crews D, Willingham E, Skipper JK 2000 Endocrine disruptors: present issues, future directions. *Q Rev Biol* 75:243-260
- Toppari J, Larsen JC, Christiansen P, Giwerzman A, Grandjean P, Guillelte Jr LJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkebaek NE 1996 Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 104[Suppl 4]:741-803
- Gray Jr LE, Kelce WR, Wiese T, Tyl R, Gaido K, Cook J, Klinefelter G, Desaulniers D, Wilson E, Zacharewski T, Waller C, Foster P, Laskey J, Reel J, Giesy J, Laws S, McLachlan J, Breslin W, Cooper R, Di Giulio R, Johnson R, Purdy R, Mihaich E, Safe S, Colborn T 1997 Endocrine Screening Methods Workshop report: detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. *Reprod Toxicol* 11:719-750
- Guillelte Jr LJ 2000 Contaminant-induced endocrine disruption in wildlife. *Growth Horm IGF Res* 10[Suppl B]:S45-50
- Tyler CR, Jobling S, Sumpter JP 1998 Endocrine disruption in wildlife: a critical review of the evidence. *Crit Rev Toxicol* 28:319-361
- LeBlanc GA 1995 Are environmental sentinels signaling? *Environ Health Perspect* 103:888-890
- McLachlan JA (ed) 1985 Estrogens in the Environment: Influences on development. Elsevier, New York
- McLachlan JA, Korach KS 1995 Symposium on estrogens in the environment. III. *Environ Health Perspect* 103[Suppl 7]:3-4
- Escriva H, Safi R, Hannon C, Langlois MC, Saumitou-Laprade P, Stehelin D, Capron A, Pierce R, Laudet V 1997 Ligand binding was acquired during evolution of nuclear receptors. *Proc Natl Acad Sci USA* 94:6803-6808
- Hawkins MB, Thornton JW, Crews D, Skipper JK, Dotte A, Thomas P 2000 Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleosts. *Proc Natl Acad Sci USA* 97:10751-10756
- Zoeller RT, Dowling ALS, Vas AA 2000 Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology* 141:181-189
- Cheek AO, Kow K, Chen J, McLachlan JA 1999 Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environ Health Perspect* 107:273-278
- Kaltreider RC, Davis AM, Lariviere JP, Hamilton JW 2001 Arsenic alters the function of the glucocorticoid receptor as a transcription factor. *Environ Health Perspect* 109:245-251
- Harmon MA, Boehm MF, Heyman RA, Mangelsdorf DJ 1995 Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. *Proc Natl Acad Sci USA* 92:6157-6160
- Rickenbacher U, McKinney JD, Oatley SJ, Blake CC 1986 Structurally specific binding of halogenated biphenyls to thyroxine transport protein. *J Med Chem* 29:641-648
- Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray Jr LE 1994 Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol Appl Pharmacol* 126:276-285
- Gray Jr LE, Ostby JS, Kelce WR 1994 Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. *Toxicol Appl Pharmacol* 129:46-52
- Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM 1995 Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* 375:581-585
- Wong C, Kelce WR, Sar M, Wilson EM 1995 Androgen receptor antagonist vs. agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. *J Biol Chem* 270:19998-20003

30. **Lambright C, Ostby J, Bobseine K, Wilson V, Hotchkiss AK, Mann PC, Gray Jr LE** 2000 Cellular and molecular mechanisms of action of linuron: an antiandrogenic herbicide that produces reproductive malformations in male rats. *Toxicol Sci* 56:389–399
31. **Tamura H, Maness SC, Reischmann K, Dorman DC, Gray LE, Gaido KW** 2001 Androgen receptor antagonism by the organophosphate insecticide fenitrothion. *Toxicol Sci* 60:56–62
32. **Maness SC, McDonnell DP, Gaido KW** 1998 Inhibition of androgen receptor-dependent transcriptional activity by DDT isomers and methoxychlor in HepG2 human hepatoma cells. *Toxicol Appl Pharmacol* 151:135–142
33. **McIntyre BS, Barlow NJ, Wallace DG, Maness SC, Gaido KW, Foster PM** 2000 Effects of *in utero* exposure to linuron on androgen-dependent reproductive development in the male Crl:CD(SD)BR rat. *Toxicol Appl Pharmacol* 167:87–99
34. **Howell WM, Black DA, Bortone SA** 1980 Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally-induced masculinization. *Copeia* 4:676–681
35. **Denton TE, Howell WM, Allison JJ, McCollum J, Marks B** 1985 Masculinization of female mosquitofish by exposure to plant sterols and *Mycobacterium smegmatis*. *Bull Environ Contam Toxicol* 35:627–632
36. **Davis WP, Bortone SA** 1992 Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning? In: Colborn T, Clements C (eds) *Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton Scientific Publishing Co, Inc, Princeton, NJ, pp 113–127
37. **Simons SS, Chakraborti PK, Cavanaugh AH** 1990 Arsenite and cadmium(II) as probes of glucocorticoid receptor structure and function. *J Biol Chem* 265:1938–1945
38. **Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB** 1994 Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *J Biol Chem* 269:16896–16901
39. **Stoica A, Katzenellenbogen BS, Martin MB** 2000 Activation of estrogen receptor- α by the heavy metal cadmium. *Mol Endocrinol* 14:545–553
40. **Cook JW, Dodds EC, Hewett CL** 1933 A synthetic oestrus-exciting compound. *Nature* 131:56–57
41. **Dodds EC, Lawson W** 1936 Synthetic oestrogenic agents without the phenanthrene nucleus. *Nature* 137:996
42. **Dodds EC, Goldberg L, Lawson W, Robinson R** 1938 Oestrogenic activity of certain synthetic compounds. *Nature* 141:247–249
43. **Eroschenko VP, Palmiter RD** 1980 Estrogenicity of kepone in birds and mammals. In: McLachlan JA (ed) *Estrogens in the Environment*. Elsevier/North Holland, New York, pp 305–326
44. **Burlington HA, Lindman VF** 1950 Effect of DDT on testes and secondary sex characters of white Leghorn cockerels. *Proc Soc Exp Biol Med* 74:48–51
45. **Welch RM, Levin W, Conney AH** 1969 Estrogenic action of DDT and its analogs. *Toxicol Appl Pharmacol* 14:358–367
46. **Kupfer D, Bulger WH** 1980 Estrogenic properties of DDT and its analogs. In: McLachlan JA (ed) *Estrogens in the Environment*. Elsevier/North Holland, New York, pp 239–264
47. **Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD** 1988 Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol Pharmacol* 33:120–126
48. **Mueller GC, Kim UH** 1978 Displacement of estradiol from estrogen receptors by simple alkyl phenols. *Endocrinology* 102:1429–1435
49. **McLachlan JA** 1993 Functional toxicology: a new approach to detect biologically active xenobiotics. *Environ Health Perspect* 101:386–387
50. **Muramatsu M, Inoue S** 2000 Estrogen receptors: how do they control reproductive and nonreproductive functions? *Biochem Biophys Res Commun* 270:1–10
51. **Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA** 1996 Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 93:5925–5930
52. **Knight WM** 1980 Estrogens administered to food producing animals: environmental considerations. In: McLachlan JA (ed) *Estrogens in the Environment*. Elsevier/North-Holland, New York, pp 391–402
53. **Metcalf R** 1980 Model ecosystems for the environmental studies of estrogens. In: McLachlan JA (ed) *Estrogens in the Environment*. Elsevier/North-Holland, New York, pp 203–212
54. **Metzler M** 1976 Metabolic activation of carcinogenic diethylstilbestrol in rodents and humans. *J Toxicol Environ Health [Suppl 1]*:21–35
55. **Hignite C, Azarnoff DL** 1977 Drugs and drug metabolites as environmental contaminants: chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. *Life Sci* 20:337–341
56. **Rall DP, McLachlan JA** 1980 Potential for exposure to estrogens in the environment. In: McLachlan JA (ed) *Estrogens in the Environment*. Elsevier/North Holland, New York, pp 199–202
57. **Lagana A, Bacaloni A, Fago G, Marino A** 2000 Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry. *Rapid Commun Mass Spectrom* 14:401–407
58. **Harries JE, Sheehan DA, Jobling S, Matthiessen P, Neall P, Sumpter JP, Tylor T, Zaman N** 1997 Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environ Toxicol Chem* 16:534–542
59. **Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, Servos M** 1999 Behavior and occurrence of estrogens in municipal sewage treatment plants. I. Investigations in Germany, Canada and Brazil. *Sci Total Environ* 225:81–90
60. **Belfroid AC, Van der Horst A, Vethaak AD, Schafer AJ, Rijs GB, Wegener J, Cofino WP** 1999 Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Sci Total Environ* 225:101–108
61. **Irwin LK, Oberdoerster E** 2001 Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol. *Aquatic Toxicol*, in press
62. **Sonnenschein C, Weiller S, Farookhi R, Soto AM** 1974 Characterization of an estrogen-sensitive cell line established from normal rat endometrium. *Cancer Res* 34:3147–3154
63. **Sonnenschein C, Soto AM** 1980 But . . . are estrogens *per se* growth-promoting hormones? *J Natl Cancer Inst* 64:211–215
64. **Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS** 1986 Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci USA* 83:2496–2500
65. **Bindal RD, Katzenellenbogen JA** 1988 Bis(4-hydroxyphenyl)[2-(phenoxy)sulfonyl]phenylmethane: isolation and structure elucidation of a novel estrogen from commercial preparations of phenol red (phenolsulfonphthalein) [published erratum appears in *J Med Chem* 1989 Dec;32(12):2582]. *J Med Chem* 31:1978–1983
66. **Soto AM, Justicia H, Wray JW, Sonnenschein C** 1991 p-Nonylphenol: an estrogenic xenobiotic released from “modified” polystyrene. *Environ Health Perspect* 92:167–173
67. **Sumpter JP, Jobling S** 1993 Male sexual development in “a sea of estrogen.” *Lancet* 342:124–125
68. **Feldman D, Do Y, Burshell A, Stathis P, Loose DS** 1982 An estrogen-binding protein and endogenous ligand in *Saccharomyces cerevisiae*: possible hormone receptor system. *Science* 218:297–298
69. **Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D** 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* 132:2279–2286
70. **Degen GH, Bolt HM** 2000 Endocrine disruptors: update on xenoestrogens. *Int Arch Occup Environ Health* 73:433–441
71. **Hughes Jr CL** 1988 Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environ Health Perspect* 78:171–174
72. **Safe SH, Foster WG, Lamb JC, Newbold RR, Van der Kraak G** 2000 Estrogenicity and Endocrine Disruption. Council for Agricultural Science and Technology, Ames, IA, vol 16:1–16
73. **Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA** 2000 Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 21:1355–1363
74. **Bryan TE, Gildersleeve RP, Wiard RP** 1989 Exposure of Japanese quail embryos to o,p'-DDT has long-term effects on reproductive

- behaviors, hematology, and feather morphology. *Teratology* 39: 525–535
75. Fry DM, Toone CK 1981 DDT-induced feminization of gull embryos. *Science* 213:922–924
 76. Guillette Jr LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR 1994 Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* 102:680–688
 77. Guillette Jr LJ, Woodward AR, Crain DA, Pickford DB, Rooney AA, Percival HF 1999 Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. *Gen Comp Endocrinol* 116:356–372
 78. Willingham E, Crews D 1999 Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. *Gen Comp Endocrinol* 113:429–435
 79. Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, Brandt I, Vethaak AD 2000 Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Crit Rev Toxicol* 30:71–133
 80. Mizunashi S, Ikegaya Y, Matsuki N 2000 Pharmacological property of tributyltin *in vivo* and *in vitro*. *Environ Toxicol Pharmacol* 8:205–212
 81. Oehlmann J, Schulte-Oehlmann U, Tillmann M, Markert B 2000 Effects of endocrine disruptors on prosobranch snails (*Mollusca: Gastropoda*) in the laboratory. I. Bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology* 9:383–397
 82. Schulte-Oehlmann U, Tillmann M, Markert B, Oehlmann J, Watermann B, Scherf S 2000 Effects of endocrine disruptors on prosobranch snails (*Mollusca: Gastropoda*) in the laboratory. II. Triphenyltin as a xeno-androgen. *Ecotoxicology* 9:399–412
 83. Andersen HR, Halling-Sorensen B, Kusk KO 1999 A parameter for detecting estrogenic exposure in the copepod *Acartia tonsa*. *Ecotoxicol Environ Safety* 44:56–61
 84. Zou E, Fingerman M 1997 Effects of estrogenic xenobiotics on molting of the water flea, *Daphnia magna*. *Ecotoxicol Environ Safety* 38:281–285
 85. Setchell KD, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, Kramer LW, Dresser BL, Tarr MJ 1987 Dietary estrogens—a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology* 93:225–233
 86. Thigpen JE, Setchell KD, Ahlmark KB, Locklear J, Spahr T, Caviness GF, Goelz MF, Haseman JK, Newbold RR, Forsythe DB 1999 Phytoestrogen content of purified, open- and closed-formula laboratory animal diets. *Lab Anim Sci* 49:530–536
 87. Thigpen JE, Li LA, Richter CB, Lebetkin EH, Jameson CW 1987 The mouse bioassay for the detection of estrogenic activity in rodent diets. II. Comparative estrogenic activity of purified, certified and standard open and closed formula rodent diets. *Lab Anim Sci* 37:602–605
 88. Powell-Jones W, Raeford S, Lucier GW 1981 Binding properties of zearalenone mycotoxins to hepatic estrogen receptors. *Mol Pharmacol* 20:35–42
 89. Sheehan DM, Branham WS, Medlock KL, Shanmugasundaram ER 1984 Estrogenic activity of zearalenone and zearalanol in the neonatal rat uterus. *Teratology* 29:383–392
 90. Meronuck RA, Garren KH, Christensen CM, Nelson GH, Bates F 1970 Effects on turkey poults and chicks of rations containing corn invaded by *Penicillium* and *Fusarium* species. *Am J Vet Res* 31:551–555
 91. Roine K, Korpinen EL, Kallela K 1971 Mycotoxicosis as a probable cause of infertility in dairy cows. *Nord Vet Med* 23:628–633
 92. Bristol FM, Djurickovic S 1971 Hyperestrogenism in female swine as the result of feeding mouldy corn. *Can Vet J* 12:132–135
 93. Finkelstein JS, McCully WF, MacLaughlin DT, Godine JE, Crowley Jr WF 1988 The mortician's mystery. Gynecomastia and reversible hypogonadotropic hypogonadism in an embalmer. *N Engl J Med* 318:961–965
 94. Hertz R 1985 The estrogen problem-retrospect and prospect. In: McLachlan JA (ed) *Estrogens in the Environment. II. Influences on Development*. Elsevier Science Publishing Co, Inc, New York, pp 1–11
 95. Lieberman S 1996 Are the differences between estradiol and other estrogens, naturally occurring or synthetic, merely semantical? *J Clin Endocrinol Metab* 81:850–851
 96. Tarrant AM, Atkinson S, Atkinson MJ 1999 Estrone and estradiol-17 β concentration in tissue of the scleractinian coral, *Montipora verrucosa*. *Comp Biochem Physiol A Mol Integr Physiol* 122:85–92
 97. Atkinson S, Atkinson MA 1992 Detection of estradiol-17 β during a mass coral spawn. *Coral Reefs* 11:33–35
 98. Baldwin WS, LeBlanc G 1994 *In vivo* biotransformation of testosterone by phase I and II detoxication enzymes and their modulation of 20-hydroxyecdysone in *Daphnia magna*. *Aquatic Toxicol* 29:103–117
 99. LeBlanc GA, McLachlan JB 2000 Changes in the metabolic elimination profile of testosterone following exposure of the crustacean *Daphnia magna* to tributyltin. *Ecotoxicol Environ Safety* 45:296–303
 100. Waddy S, Aiken D, deKleijn D 1995 Control of growth and reproduction. In: Factor JR (ed) *Biology of the Lobster Homarus americanus*. Academic Press, New York
 101. Feral C, LeGall S 1983 The influence of a pollutant factor (tributyltin) on the neuroendocrine mechanism responsible for the occurrence of a penis in the females of *Ocenebra erinacea*. In: Lever JB, Boer HH (eds) *Molluscan Neuroendocrinology*. Elsevier/North Holland, Amsterdam
 102. LeBlanc G 1999 Susceptibility of invertebrates to endocrine disrupting environmental chemicals. In: Guillette LJ (ed) *Environmental Endocrine Disruptors: An Evolutionary Perspective*. Taylor and Francis, London
 103. van Eerdenburg FJ, Daemen IA, van der Beek EM, van Leeuwen FW 2000 Changes in estrogen- α receptor immunoreactivity during the estrous cycle in lactating dairy cattle. *Brain Res* 880:219–223
 104. Zieba D, Bilinska B, Schmalz-Fraczek B, Murawski M 2000 Immunohistochemical localization of estrogen receptors in the ovine corpus luteum throughout the estrous cycle. *Folia Histochem Cytophiol* 38:111–117
 105. Vermeirsch H, Simoens P, Lauwers H, Coryn M 1999 Immunohistochemical detection of estrogen receptors in the canine uterus and their relation to sex steroid hormone levels. *Theriogenology* 51:729–743
 106. Martin De Las Mulas J, Millan Y, Bautista MJ, Perez J, Carrasco L 2000 Estrogen and progesterone receptors in feline fibroadenomatous change: an immunohistochemical study. *Res Vet Sci* 68: 15–21
 107. Bernard DJ, Bentley GE, Balthazart J, Turek FW, Ball GF 1999 Androgen receptor, estrogen receptor α , and estrogen receptor β show distinct patterns of expression in forebrain song control nuclei of European starlings. *Endocrinology* 140:4633–4643
 108. Foidart A, Lakaye B, Grisar T, Ball GF, Balthazart J 1999 Estrogen receptor- β in quail: cloning, tissue expression and neuroanatomical distribution. *J Neurobiol* 40:327–342
 109. Vonier PM, Crain DA, McLachlan JA, Guillette Jr LJ, Arnold SF 1996 Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. *Environ Health Perspect* 104:1318–1322
 110. Vonier PM, Guillette LJ, McLachlan Jr JA, Arnold SF 1997 Identification and characterization of estrogen and progesterone receptors from the oviduct of the American alligator (*Alligator mississippiensis*). *Biochem Biophys Res Commun* 232:308–312
 111. Bergeron JM, Gahr M, Horan K, Wibbels T, Crews D 1998 Cloning and *in situ* hybridization analysis of estrogen receptor in the developing gonad of the red-eared slider turtle, a species with temperature-dependent sex determination. *Dev Growth Differ* 40:243–254
 112. Pakdel F, Le Guellec C, Vaillant C, Le Roux MG, Valotaire Y 1989 Identification and estrogen induction of two estrogen receptors (ER) messenger ribonucleic acids in the rainbow trout liver: sequence homology with other ERs. *Mol Endocrinol* 3:44–51
 113. Tan NS, Freceer V, Lam TJ, Ding JL 1999 Temperature dependence of estrogen binding: importance of a subzone in the ligand binding domain of a novel piscine estrogen receptor. *Biochim Biophys Acta* 1452:103–120
 114. Willig A, Keller R 1976 Biosynthesis of α - and β -ecdysone by the crayfish *Orconectes limosus* *in vivo* and by its Y-organs *in vitro*. *Experientia* 32:936–937

115. Li T, Bender M 2000 A conditional rescue system reveals essential functions for the ecdysone receptor (EcR) gene during molting and metamorphosis in *Drosophila*. *Development* 127:2897–2905
116. Miura K, Wang SF, Raikhel AS 1999 Two distinct subpopulations of ecdysone receptor complex in the female mosquito during vitellogenesis. *Mol Cell Endocrinol* 156:111–120
117. Das SK, Taylor JA, Korach KS, Paria BC, Dey SK, Lubahn DB 1997 Estrogenic responses in estrogen receptor- α deficient mice reveal a distinct estrogen signaling pathway. *Proc Natl Acad Sci USA* 94:12786–12791
118. Horton MJ, Szego CM 1984 Chromatin proteins of rat preputial gland: acute changes in response to estrogen. *Int J Biochem* 16:447–460
119. Watson CS, Pappas TC, Gametchu B 1995 The other estrogen receptor in the plasma membrane: implications for the actions of environmental estrogens. *Environ Health Perspect* 103[Suppl 7]:41–50
120. Watson CS, Campbell CH, Gametchu B 1999 Membrane oestrogen receptors on rat pituitary tumour cells: immuno-identification and responses to oestradiol and xenoestrogens. *Exp Physiol* 84:1013–1022
121. Haynes MP, Sinha D, Russell KS, Collinge M, Fulton D, Morales-Ruiz M, Sessa WC, Bender JR 2000 Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. *Circ Res* 87:677–682
122. Chambliss KL, Yuhanna IS, Mineo C, Liu P, German Z, Sherman TS, Mendelsohn ME, Anderson RG, Shaul PW 2000 Estrogen receptor α and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ Res* 87:E44–52
123. Clarke CH, Norfleet AM, Clarke MS, Watson CS, Cunningham KA, Thomas ML 2000 Perimembrane localization of the estrogen receptor α protein in neuronal processes of cultured hippocampal neurons. *Neuroendocrinology* 71:34–42
124. Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B 2000 Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β . *Proc Natl Acad Sci USA* 97:11603–11608
125. Chen J, Li Y, Lavigne JA, Trush MA, Yager JD 1999 Increased mitochondrial superoxide production in rat liver mitochondria, rat hepatocytes, and HepG2 cells following ethinyl estradiol treatment. *Toxicol Sci* 51:224–235
126. Chen J, Yager JD 2001 Mitochondrial localization of estrogen receptor (ER)s α and β . *Proc Am Assoc Cancer Res* 42:882
127. Bennets HW, Underwood EJ, Shier FL 1946 A specific breeding problem of sheep on subtropical clover pastures in Western Australia. *Aust Vet J* 22:2–12
128. Makela S, Davis VL, Tally WC, Korkman J, Salo L, Vihko R, Santti R, Korach KS 1994 Dietary estrogens act through estrogen receptor-mediated processes and show no antiestrogenicity in cultured breast cancer cells. *Environ Health Perspect* 102:572–578
129. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology* 139:4252–4263
130. Koes RE, Quattrocchio F, Mol JN 1994 The flavonoid biosynthetic pathway in plants: function and evolution. *Bioessays* 16:123–132
131. Baker ME 1998 Flavonoids as hormones. A perspective from an analysis of molecular fossils. *Adv Exp Med Biol* 439:249–267
132. Bladergroen MR, Spalink HP 1998 Genes and signal molecules involved in the rhizobia-leguminosae symbiosis. *Curr Opin Plant Biol* 1:353–359
133. Schultze M, Kondorosi A 1998 Regulation of symbiotic root nodule development. *Annu Rev Genet* 32:33–57
134. Peters NK, Frost JW, Long SR 1986 A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. *Science* 233:977–980
135. Mulligan JT, Long SR 1985 Induction of *Rhizobium meliloti* nodC expression by plant exudate requires nodD. *Proc Natl Acad Sci USA* 82:6609–6613
136. Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, Prome JC, Denarie J 1990 Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature* 344:781–784
137. Broughton WJ, Perret X 1999 Genealogy of legume-Rhizobium symbioses. *Curr Opin Plant Biol* 2:305–311
138. Burow ME, Tang Y, Collins-Burow BM, Krajewski S, Reed JC, McLachlan JA, Beckman BS 1999 Effects of environmental estrogens on tumor necrosis factor α -mediated apoptosis in MCF-7 cells. *Carcinogenesis* 20:2057–2061
139. Baker ME 1992 Similarities between legume-rhizobium communication and steroid-mediated intercellular communication in vertebrates. *Can J Microbiol* 38:541–547
140. Gyorgypal Z, Kondorosi A 1991 Homology of the ligand-binding regions of *Rhizobium* symbiotic regulatory protein NodD and vertebrate nuclear receptors. *Mol Gen Genet* 226:337–340
141. Peters NK, Long SR 1988 Alfalfa root exudates and compounds which promote or inhibit induction of *Rhizobium meliloti* nodulation genes. *Plant Physiol* 88:396–400
142. Simon L, Levesque RC, Lalonde M 1993 Identification of endomycorrhizal fungi colonizing roots by fluorescent single-strand conformation polymorphism-polymerase chain reaction. *Appl Environ Microbiol* 59:4211–4215
143. Remy W, Taylor TN, Hass H 1994 Four hundred-million-year-old vesicular arbuscular. *Proc Natl Acad Sci USA* 91:11841–11843
144. Becard G, Douds DD, Pfeffer PE 1992 Extensive *in vitro* hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO₂ and flavonols. *Appl Environ Microbiol* 58:821–825
- 144a. Poulin M-J, Simard J, Catford J-C, Labrie F, Piché Y 1997 Response of symbiotic endomycorrhizal fungi to estrogens and antiestrogens. *MPMI* 10:481–487
145. Baker ME 1992 Evolution of regulation of steroid-mediated intercellular communication in vertebrates: insights from flavonoids, signals that mediate plant-rhizobia symbiosis. *J Steroid Biochem Mol Biol* 41:301–308
- 145a. Doualla-Bell F, Bonneau MJ, Labrie F, Fortier MA 1995 Differential effects of a steroidal antiestrogen, EM-139, on prostaglandin and cyclic adenosine 3',5'-monophosphate production in the circular and longitudinal layers of bovine myometrium. *Biol Reprod* 52:1358–1363
146. Town CD, Gross JD, Kay RR 1976 Cell differentiation without morphogenesis in *Dictyostelium discoideum*. *Nature* 262:717–719
147. Kay RR 1998 The biosynthesis of differentiation-inducing factor, a chlorinated signal molecule regulating *Dictyostelium* development. *J Biol Chem* 273:2669–2675
148. Kay RR, Jermyn KA 1983 A possible morphogen controlling differentiation in *Dictyostelium*. *Nature* 303:242–244
149. Brookman JJ, Town CD, Jermyn KA, Kay RR 1982 Developmental regulation of a stalk cell differentiation-inducing factor in *Dictyostelium discoideum*. *Dev Biol* 91:191–196
150. Insall R, Nayler O, Kay RR 1992 DIF-1 induces its own breakdown in *Dictyostelium*. *EMBO J* 11:2849–2854
151. McKinney JD, Korach KS, McLachlan JA 1990 Detoxification of polychlorinated biphenyls [letter; comment]. *Lancet* 335:222–223
152. Bergeron JM, Crews D, McLachlan JA 1994 PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:780–781
153. Santti R, Newbold RR, Makela S, Pylikkanen L, McLachlan JA 1994 Developmental estrogenization and prostatic neoplasia. *Prostate* 24:67–78
154. McLachlan JA, Newbold RR, Bullock B 1975 Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. *Science* 190:991–992
155. McLachlan JA 1979 Transplacental effects of diethylstilbestrol in mice. *Natl Cancer Inst Monogr* 51:67–72
156. Newbold RR, Bullock BC, McLachlan JA 1985 Lesions of the rete testis in mice exposed prenatally to diethylstilbestrol. *Cancer Res* 45:5145–5150
157. Newbold RR, Bullock BC, McLachlan JA 1986 Adenocarcinoma of the rete testis. Diethylstilbestrol-induced lesions of the mouse rete testis. *Am J Pathol* 125:625–628
158. Newbold RR, Bullock BC, McLachlan JA 1987 Mullerian remnants of male mice exposed prenatally to diethylstilbestrol. *Teratog Carcinog Mutagen* 7:377–389
159. Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP 1995

- Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. *Environ Health Perspect* 103:1136–1143
160. Palanza P, Parmigiani S, Liu H, vom Saal FS 1999 Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. *Pharmacol Biochem Behav* 64:665–672
 161. Eddy EM, Washburn TE, Bunch DO, Goulding EH, Gladen BC, Lubahn DB, Korach KS 1996 Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. *Endocrinology* 137:4796–4805
 162. Jones Jr HW, Scott WW 1971 Hermaphroditism, Genital Anomalies and Related Endocrine Disorders, ed 2. Williams & Wilkins, Baltimore, MD
 163. Newbold RR, Suzuki Y, McLachlan JA 1984 Müllerian duct maintenance in heterotypic organ culture after *in vivo* exposure to diethylstilbestrol. *Endocrinology* 115:1863–1868
 164. Bibbo M, Al-Naqeeb M, Baccarini I, Gill W, Newton M, Sleeper KM, Sonek RN, Wied GL 1975 Follow-up study of male and female offspring of DES-treated mothers a preliminary report. *J Reprod Med* 15:29–32
 165. Bibbo M, Ali I, Al-Naqeeb M, Baccarini I, Climaco LA, Gill W, Sonek M, Wied GL 1975 Cytologic findings in female and male offspring of DES treated mothers. *Acta Cytol* 19:568–572
 166. Gill WB, Schumacher GF, Bibbo M 1977 Pathological semen and anatomical abnormalities of the genital tract in human male subjects exposed to diethylstilbestrol *in utero*. *J Urol* 117:477–480
 167. Gill WB, Schumacher GF, Bibbo M, Straus II FH, Schoenberg HW 1979 Association of diethylstilbestrol exposure *in utero* with cryptorchidism, testicular hypoplasia and semen abnormalities. *J Urol* 122:36–39
 168. Conley GR, Sant GR, Ucci AA, Mitcheson HD 1983 Seminoma and epididymal cysts in a young man with known diethylstilbestrol exposure *in utero*. *JAMA* 249:1325–1326
 169. Hogan MD, Newbold RR, McLachlan JA 1987 Extrapolation of teratogenic responses observed in laboratory animals to humans: DES as an illustrative example. In: Banbury Report 26: Developmental Toxicology: Mechanisms and Risk. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 257–269
 170. Wilcox AJ, Baird DD, Weinberg CR, Hornsby PP, Herbst AL 1995 Fertility in men exposed prenatally to diethylstilbestrol. *N Engl J Med* 332:1411–1416
 171. Kaplan NM 1959 Male pseudohermaphroditism. *N Engl J Med* 261:641–644
 172. Davis ME, Potter EL 1948 The response of the human fetal reproductive system to the administration of diethylstilbestrol and testosterone propionate during early pregnancy. *Endocrinology* 42:370–378
 173. Driscoll SG, Taylor SH 1980 Effects of prenatal maternal estrogen on the male urogenital system. *Obstet Gynecol* 56:537–542
 174. Emmen JM, McLuskey A, Adham IM, Engel W, Verhoef-Post M, Themmen AP, Grootegoed JA, Brinkmann AO 2000 Involvement of insulin-like factor 3 (Insl3) in diethylstilbestrol-induced cryptorchidism. *Endocrinology* 141:846–849
 175. Nef S, Parada LF 1999 Cryptorchidism in mice mutant for Insl3. *Nat Genet* 22:295–299
 176. Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W, Adham IM 1999 Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. *Mol Endocrinol* 13:681–691
 177. Haraguchi R, Suzuki K, Murakami R, Sakai M, Kamikawa M, Kengaku M, Sekine K, Kawano H, Kato S, Ueno N, Yamada G 2000 Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation. *Development* 127:2471–2479
 178. Visser JA, McLuskey A, Verhoef-Post M, Kramer P, Grootegoed JA, Themmen AP 1998 Effect of prenatal exposure to diethylstilbestrol on Müllerian duct development in fetal male mice. *Endocrinology* 139:4244–4251
 179. Ma L, Benson GV, Lim H, Dey SK, Maas RL 1998 Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in Müllerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 197:141–154
 180. Ostrowski MC, Kistler MK, Kistler WS 1979 Purification and cell-free synthesis of a major protein from rat seminal vesicle secretion. A potential marker for androgen action. *J Biol Chem* 254:383–390
 181. Harris SE, Mansson PE, Tully DB, Burkhart B 1983 Seminal vesicle secretion IV gene: allelic difference due to a series of 20-base-pair direct tandem repeats within an intron. *Proc Natl Acad Sci USA* 80:6460–6464
 182. Chen YH, Pentecost BT, McLachlan JA, Teng CT 1987 The androgen-dependent mouse seminal vesicle secretory protein IV: characterization and complementary deoxyribonucleic acid cloning. *Mol Endocrinol* 1:707–716
 183. Teng CT, Walker MP, Bhattacharyya SN, Klapper DG, DiAugustine RP, McLachlan JA 1986 Purification and properties of an oestrogen-stimulated mouse uterine glycoprotein (approx. 70 kDa). *Biochem J* 240:413–422
 184. Pentecost BT, Teng CT 1987 Lactotransferrin is the major estrogen inducible protein of mouse uterine secretions. *J Biol Chem* 262:10134–10139
 185. Teng CT, Pentecost BT, Chen YH, Newbold RR, Eddy EM, McLachlan JA 1989 Lactotransferrin gene expression in the mouse uterus and mammary gland. *Endocrinology* 124:992–999
 186. Pentecost BT, Newbold RR, Teng CT, McLachlan JA 1988 Prenatal exposure of male mice to diethylstilbestrol alter the expression of the lactotransferrin gene in seminal vesicles. *Mol Endocrinol* 2:1243–1248
 187. Newbold RR, Pentecost BT, Yamashita S, Lum K, Miller JV, Nelson P, Blair J, Kong H, Teng C, McLachlan JA 1989 Female gene expression in the seminal vesicle of mice after prenatal exposure to diethylstilbestrol. *Endocrinology* 124:2568–2576
 188. Beckman Jr WC, Newbold RR, Teng CT, McLachlan JA 1994 Molecular feminization of mouse seminal vesicle by prenatal exposure to diethylstilbestrol: altered expression of messenger RNA. *J Urol* 151:1370–1378
 189. Nelson KG, Takahashi T, Bossert NL, Walmer DK, McLachlan JA 1991 Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. *Proc Natl Acad Sci USA* 88:21–25
 190. Nelson KG, Sakai Y, Eitzman B, Steed T, McLachlan J 1994 Exposure to diethylstilbestrol during a critical developmental period of the mouse reproductive tract leads to persistent induction of two estrogen-regulated genes. *Cell Growth Differ* 5:595–606
 191. Newbold RR, Bullock BC, McLachlan JA 1990 Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res* 50:7677–7681
 192. Li S, Washburn KA, Moore R, Uno T, Teng C, Newbold RR, McLachlan JA, Negishi M 1997 Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res* 57:4356–4359
 193. Yokomori N, Moore R, Negishi M 1995 Sexually dimorphic DNA demethylation in the promoter of the Slp (sex-limited protein) gene in mouse liver. *Proc Natl Acad Sci USA* 92:1302–1306
 194. Ferguson AT, Evron E, Umbricht CB, Pandita TK, Chan TA, Hermeking H, Marks JR, Lambers AR, Futreal PA, Stampfer MR, Sukumar S 2000 High frequency of hypermethylation at the 14–3-3 sigma locus leads to gene silencing in breast cancer. *Proc Natl Acad Sci USA* 97:6049–6054
 195. Jirtle RL 1999 Genomic imprinting and cancer. *Exp Cell Res* 248:18–24
 196. Hertz R 1976 The estrogen-cancer hypothesis. *Cancer* 38:534–540
 197. Degen GH, Metzler M 1987 Sex hormones and neoplasia: genotoxic effects in short term assays. *Arch Toxicol Suppl* 10:264–278
 198. Barrett JC, Wong A, McLachlan JA 1981 Diethylstilbestrol induces neoplastic transformation without measurable gene mutation at two loci. *Science* 212:1402–1404
 199. McLachlan JA, Wong A, Degen GH, Barrett JC 1982 Morphologic and neoplastic transformation of Syrian hamster embryo fibroblasts by diethylstilbestrol and its analogs. *Cancer Res* 42:3040–3045
 200. Tsutsui T, Maizumi H, McLachlan JA, Barrett JC 1983 Aneuploidy induction and cell transformation by diethylstilbestrol: a possible chromosomal mechanism in carcinogenesis. *Cancer Res* 43:3814–3821

201. Li JJ, Li SA 1990 Estrogen carcinogenesis in hamster tissues: a critical review. *Endocr Rev* 11:524–531
202. Leavitt WW, Evans RW, Hendry III WJ 1981 Etiology of DES-induced uterine tumors in the Syrian hamster. *Adv Exp Med Biol* 138:63–86
203. Tomooka Y, DiAugustine RP, McLachlan JA 1986 Proliferation of mouse uterine epithelial cells *in vitro*. *Endocrinology* 118:1011–1018
204. Ignar-Trowbridge DM, Nelson KG, Bidwell MC, Curtis SW, Washburn TF, McLachlan JA, Korach KS 1992 Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. *Proc Natl Acad Sci USA* 89:4658–4662
205. Curtis SW, Washburn T, Sewall C, DiAugustine R, Lindzey J, Couse JF, Korach KS 1996 Physiological coupling of growth factor and steroid receptor signaling pathways: estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor. *Proc Natl Acad Sci USA* 93:12626–12630
206. Kamiya K, Sato T, Nishimura N, Goto Y, Kano K, Iguchi T 1996 Expression of estrogen receptor and proto-oncogene messenger ribonucleic acids in reproductive tracts of neonatally diethylstilbestrol-exposed female mice with or without post-pubertal estrogen administration. *Exp Clin Endocrinol Diabetes* 104:111–122
207. Bakin AV, Curran T 1999 Role of DNA 5-methylcytosine transferase in cell transformation by fos. *Science* 283:387–390
208. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA 1998 Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 19:1655–1663
209. Andres AC, Muellener DB, Ryffel GU 1984 Persistence, methylation and expression of vitellogenin gene derivatives after injection into fertilized eggs of *Xenopus laevis*. *Nucleic Acids Res* 12:2283–2302
210. Baker HJ, Shapiro DJ 1978 Rapid accumulation of vitellogenin messenger RNA during secondary estrogen stimulation of *Xenopus laevis*. *J Biol Chem* 253:4521–4524
211. Corthesy B, Leonnard P, Wahli W 1990 Transcriptional potentiation of the vitellogenin B1 promoter by a combination of both nucleosome assembly and transcription factors: an *in vitro* dissection. *Mol Cell Biol* 10:3926–3933
212. Edinger RS, Mambo E, Evans MI 1997 Estrogen-dependent transcriptional activation and vitellogenin gene memory. *Mol Endocrinol* 11:1985–1993
213. Farmer SR, Henshaw EC, Berridge MV, Tata JR 1978 Translation of *Xenopus* vitellogenin mRNA during primary and secondary induction. *Nature* 273:401–403
214. Folger K, Anderson JN, Hayward MA, Shapiro DJ 1983 Nuclease sensitivity and DNA methylation in estrogen regulation of *Xenopus laevis* vitellogenin gene expression. *J Biol Chem* 258:8908–8914
215. Gerber-Huber S, Felber BK, Weber R, Ryffel GU 1981 Estrogen induces tissue specific changes in the chromatin conformation of the vitellogenin genes in *Xenopus*. *Nucleic Acids Res* 9:2475–2494
216. Gschwendt M, Rincke G, Schuster T 1982 The estrogen-induced vitellogenin synthesis in chicken liver after estrogen withdrawal or antiestrogen treatment. *Mol Cell Endocrinol* 26:231–242
217. de Loof A 1993 Schroedinger 50 years ago: “What is life?” “The ability to communicate”, a plausible reply? *Int J Biochem* 25:1715–1721
218. Francis C, Anthony ELP, Brunton JA, Kunz TH 1994 Lactation in male fruit bats. *Nature* 367:691–692
219. Colon I, Caro D, Bourdony CJ, Rosario O 2000 Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environ Health Perspect* 108:895–900
220. Tanner JR 1987 St. Anthony’s fire, then and now: a case report and historical review. *Can J Surg* 30:291–293
221. Jordan BK, Mohammed M, Chiung ST, Delot E, Chen X-N, Dewing P, Swain A, Rao PN, Elejalde BR, Vilain E 2001 Upregulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. *Am J Hum Genet* 68:1102–1109
222. Thornton JW 2001 Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. *Proc Natl Acad Sci USA* 98:5671–5676