Environmental oestrogens have been implicated in the pathogenesis of hormonally treated cancers (such as breast and prostate cancer), male infertility, and abnormalities of the male and female reproductive tracts. They may be derived from plants (phytoestrogens), pharmaceuticals, or other synthetic compounds not originally intended to have oestrogenic activity (including soy based infant formulas). This review will discuss the evidence from both animal studies and humans for an effect of these ubiquitous compounds on the development of the human female genital tract, in addition to prolonging the menstrual cycle, alleviating symptoms of the menopause, and protecting against the development of endometrial carcinoma.

N
atural oestrogens are involved in the development and function of the male and female genital tract, neuroendocrine tissues, bone, and breast. At the cellular level, they promote cellular proliferation and hypertrophy of the female secondary sexual organs and induce the synthesis and secretion of cell type specific proteins. It has been widely established that exposure to natural (endogenous) oestrogens is the principle risk factor for the development of endometrial cancer. However, over the past 50 years an increasing body of evidence has accumulated indicating that several environmental chemicals exist that also have oestrogenic and/or potentially antioestrogenic effects both on aquatic and land based wildlife, and on humans. Although the effect of environmental oestrogens on the breast has been considered elsewhere, there has been little attention paid to their effects on the human female reproductive tract. Therefore, we discuss the origin and nature of oestrogenic compounds within our environment, and review the evidence that such agents might affect the pathophysiology of the female genital tract.

THE NATURE OF ENVIRONMENTAL OESTROGENS
Environmental oestrogens derive from two main sources: phytoestrogens and synthetic (pharmaceutical and non-pharmaceutical) oestrogens.

Phytoestrogens
Phytoestrogens are naturally occurring phytochemicals found in plants and plant products, which are structurally and functionally similar to 17β-oestradiol (isoflavones) or synthetic oestrogens such as diethylstilboestrol (lignins). They form a diverse group with a variety of biochemical and biological properties. The principle phytoestrogens are the isoflavones (for example, coumestrol, genistein, daidzein, and equol) and lignins (for example, enterolactone and enterodiol), derived from precursors in the diet by the gut microflora, and mycotoxins derived from fungal moulds or grain (for example, zearalenone). The isoflavones have received the greatest attention with regard to female genital tract pathology and are low molecular weight diphenolic antimicrobial compounds (phytoalexins), which are synthesised de novo in plants in response to exposure to bacterial pathogens. Boué and colleagues—for example, have shown that Aspergillus sojae induces the production of the coumestan phytoalexin, coumestrol, and the pterocarpan phytoalexins, glycollins I-III in soybean cotyledons. Glycollin prevents the accumulation of alfatoxin B₁, in cultures of Aspergillus flavus.

Coumestrol is the most potent of the phytoestrogens, but is some 100–200 times less potent than 17β-oestradiol, and almost 3000 times less potent than diethylstilboestrol. Using cytosolic preparations of rat endometrial adenocarcinoma cells, Hopert et al have shown that the affinity ranking to the oestrogen receptor (ER) is 17β-oestradiol >>> coumestrol > genistein > daidzein >> mangostin, such that the affinity for the ER of coumestrol is approximately 2660 times that of mangostin. Given that coumestrol is a fluorescent compound, it is possible to demonstrate directly a binding affinity with the ER that is approximately 20% of that seen for 17β-oestradiol.

Phytoestrogens are present as glycosides in the diet because it is rich in the glycoside of daidzein, which is metabolised in the gut to equol.
such that circulating concentrations of isoflavones in human infants fed these formulas may be 13 000–22 000 times higher than plasma oestriadiol concentrations in early life.43 Lactating females may pass dietary oestrogens to their offspring: animal studies have shown that milk from lactating females fed on a low dose coumestrol diet may also be a biologically important source of phytooestrogens.44 Coumestrol is also present in certain food supplements, such as alfalfa tablets.45

The metabolism of phytoestrogens has been reviewed in detail elsewhere21 and a detailed discussion is beyond the scope of this review. However, Sonnenschein and Soto1 and Whitten and colleagues1 have suggested that phytooestrogens may exert their biological activity by: (1) mimicking the action of endogenous oestrogens; (2) acting as oestrogen antagonists; (3) altering the pattern of synthesis and metabolism of endogenous hormones; and (4) modifying hormone receptor values. In addition, Aldercreutz et al1 have shown that there is a positive correlation between fibre intake, urinary excretion of enterolactone, and serum steroid hormone binding globulin (SHBG), and a negative correlation between urinary excretion of enterolactone and serum oestradiol.15 Thus, the ingestion of phytooestrogens stimulates the hepatic synthesis of SHBG and indirectly reduces the amount of free (biologically active) oestradiol in the serum. Therefore, in the absence of oestrogen, isoflavones have a weakly oestrogenic effect, but may exhibit an antioestrogenic effect when oestrogen is present.24

However, it must be remembered that phytooestrogens may exert biological activity by other mechanisms. For example, the isoflavone genistein is a potent selective inhibitor of tyrosine kinase in both human26 and rat myometrial cells.27 28 A similar effect is also seen in human ovarian carcinoma cells29 and in adenovirus infected cell lines.30 It is of note that daidzein, which has a similar structure to genistein, has no effect on tyrosine kinase activity. However, in human leiomyosarcoma cells, both genistein and daidzein inhibit fast Na+ current by directly blocking fast Na+ channels.31 Kellis and Vickery32 have shown that flavonoids inhibit the aromatisation of androstenedione and testosterone to oestrogens catalysed by human placental and ovarian microsomes.

Given that phytoestrogens are readily identified within the human diet, the lack of associated deleterious effects is at first glance surprising. However, this might be explained by adaptive phenomena during the coevolution of animals and plants. Thus, it is likely that sudden exposure to a phytoestrogen not normally present in an animal's ecosystem (for example, the recent widespread introduction of soymeal into the Western diet) is more likely to have deleterious effects on health than a phytoestrogen that is normally present and ingested over a long time course.1 23 However, no studies have yet explored this in humans.

Xenoestrogens

A wide variety of synthetic chemicals present within the environment have oestrogenic activity. These may be subdivided into pharmaceutical oestrogens and other synthetic compounds that have been produced for other purposes, but have subsequently been found to have oestrogenic activity. Pharmaceutical oestrogens including diethylstilboestrol33 and oral contraceptives can be found both in sewage and river water.

Following the widespread use of hormonal contraception there has been increasing concern that contraceptive steroids may enter the water supply. Both natural and synthetic oestrogenic chemicals are present in raw domestic sewage.34 Despite treatment in a modern sewage disposal plant, oestrogens can be detected, albeit at low concentrations, in the effluent from such plants.35 Purdom et al36 have shown that both male and female trout exposed to the effluent from sewage treatment works have evidence of exposure to exogenous oestrogens, as determined by the measurement of plasma vitello-genin values, an oestrogen regulated egg yolk protein synthesised in the liver.36 The oral contraceptive steroid 17α-ethinyl oestradiol is a more potent inducer of vitellogenesis than 17β-oestradiol, primarily because it shows little binding to oestrogen binding plasma proteins.37 In addition, they determined that the oral contraceptive steroid 17α-ethinyl oestradiol is a more potent inducer of vitellogenesis than 17β-oestradiol. In The Netherlands, Belfroid et al38 have demonstrated the presence of oestrone and 17β-oestradiol (hormones that are naturally excreted in human and animal urine) in treated wastewater at concentrations of 47 ng/litre (oestrone) and 1–12 ng/litre (17β-oestradiol).39 The contraceptive steroid 17α-ethinyl oestradiol has also been detected in treated waste water. The concentrations of such steroids are greater in domestic than in industrial effluent. In general, the concentrations of oestrogenic hormones within surface water in The Netherlands were lower (1–4 ng/litre), and of these oestrone was the most prevalent.

Despite the evidence above that sewage plant effluent contains oestrogenic compounds that exert an effect on aquatic life, human health is only likely to be affected if such compounds re-enter the drinking water supply or the food chain from these sources. In Korea, where standards of water treatment are insufficient, upstream and downstream samples of river water have been shown to be contaminated with oestrogenic compounds.30 In Israel, where water recycling is far more pronounced than in the UK, oestrogen has been detected in lake water used as a source of drinking water, possibly as a result of contamination by sewage water.40 Studies in the UK have repeatedly failed to detect oestrogenic compounds in drinking water supplies.41 42

A detailed discussion of the nature and mechanism of action of the vast range of chemicals that have been found to have weak oestrogenic activity is beyond the scope of this review, and has been covered in detail elsewhere.40 It is sufficient to note that such chemicals include pesticides, such as dichlorodiphenyltrichloroethane (DDT),41 methoxychlor,42 and kepone;43 polycyclic aromatic hydrocarbons, such as 3,9-dihydroxybenz[a]anthracene; drugs, such as digoxin and cimetidine; antioxidants in foods, such as butylated hydroxyanisole;44 resin based composites and sealants used in dentistry;45 and some polychlorinated industrial byproducts, such as polychlorinated biphenyls and chlorofluorocarbon.46

It is interesting to note that oestrogen-like substances may be released from plastic centrifuge tubes into plasma stored within them. Soto et al47 have shown that nonylphenol, an antioxidant used in the manufacture of plastics, is responsible for this.48 In addition, Krishnan et al49 have shown that the oestrogenic compound bisphenol-A is released into cultures during the autoclaving of the polycarbonate plastic ware used to sterilise the culture medium.49 Bisphenol-A is also released from the lacquer coatings of food cans50 and certain sealants and resins used in dentistry.51 Jobling et al52 have shown that phthalate esters (used in the production of various plastics) isolated from liquid sewage have oestrogenic activity and are able to combine with both piscine and mammalian ERs.53 That such compounds may be released from plastics into specimens must be remembered when considering any study of environmental oestrogens where samples are collected into or stored in plastic containers, rather than into glass, and subsequently used in bioassays to determine their oestrogenicity. Nonylphenol is also known to leach from the PVC tubing used in milk processing, and from food packaging.1

THE EFFECT OF ENVIRONMENTAL OESTROGENS ON THE FEMALE GENITAL TRACTS OF ANIMALS

Most of what is known of the effects of environmental oestrogens on the female genital tract comes from iatrogenic human exposure to diethylstilboestrol and studies on laboratory animals and domestic livestock. In broad terms, these effects can
be divided into maturational and morphological abnormalities, and disruption of the oestrous cycle and infertility.

**Maturational and morphological effects**

It has now been well established that a low dose phytoestrogen can induce developmental and maturational abnormalities in both laboratory animals and domestic livestock. In rats, Whitten *et al* have shown that a low dose (0.01%) coumestrol diet (a concentration of coumestrol found naturally in human food) fed to lactating dams induces vaginal opening within female pups at a lower body weight than in pups from dams fed with a control diet, although the age at vaginal opening did not differ across treatment groups. Although coumestrol treated animals were significantly lighter than the controls, coumestrol was shown to exert a significant effect on weight at vaginal opening, independent of its effects on growth rate. Murthy *et al* found that a benzene extract from the flowers *Hibiscus rosa-sinensis* (a plant purported to act as an antifertility agent in rural folk practice in India) induces precocious vaginal opening in mice. Similarly, Burroughs *et al* found that the administration of coumestrol subcutaneously to newborn mice in the first 5 days of life induced eye opening and vaginal opening at an earlier age than was seen in untreated mice, and that these effects were similar to those seen in mice given diethylstilboestrol, although vaginal opening was more advanced in the group treated with diethylstilboestrol. In contrast, they observed no difference in body weights between the treated and untreated groups. However, Awoniyi *et al* were unable to demonstrate precocious vaginal opening in rats born to dams fed genistein on day 17 of gestation, although these animals did show prominent ovarian follicular atresia.

Human concentration doses of coumestrol are uterotrophic in rats. Uterine wet and dry weights are increased above controls in neonatal rats receiving either 10 µg or 100 µg coumestrol for the first 5 days of postnatal life. This effect is confined to the first 10 days of postnatal life. After 10 days, the uterine weight is significantly lower than in untreated animals. Genistein has a similar uterotrophic effect. In addition, after 5 days of 10 µg/day coumestrol treatment ER values are lowered to 60% of controls by 5 days, and to 15–20% of controls in ovariectomised rats, but that low dose genistein (0.1 mg/kg) administered intravenously suppresses LH luteinising hormone (LH) surge at the expected time, suggesting a role for hypothalamic or pituitary responses in the acyclic condition. A similar pattern of acyclicity with vaginal cornification is seen in mice treated with coumestrol in the neonatal period. Burroughs *et al* have repeatedly shown that this state persists even after bilateral oophorectomy, further evidence that exposure to phytoestrogens in the neonatal period has lasting effects on the hypothalamo–hypophysial–ovarian axis.

**Effects on the oestrous cycle and fertility**

In addition to producing the maturational and morphological abnormalities described above, environmental oestrogens can affect the oestrous cycle and cause infertility. Neonatal rats exposed to a low dose phytoestrogen in the first 5 days of postnatal life exhibit persistent cornification of vaginal epithelial cells on cytological examination by 4 months of age; evidence of a persistent oestrous state. Such animals fail to respond to oestrogen priming followed by progesterone with a luteinising hormone (LH) surge at the expected time, suggesting a role for hypothalamic or pituitary responses in the acyclic condition. A similar pattern of acyclicity with vaginal cornification is seen in mice treated with coumestrol in the neonatal period. Burroughs *et al* have repeatedly shown that this state persists even after bilateral oophorectomy, further evidence that exposure to phytoestrogens in the neonatal period has lasting effects on the hypothalamo–hypophysial–ovarian axis. Similar effects are seen when neonatal mice receive natural or synthetic oestrogens. Hughes *et al* found that orally administered genistein had no effect on tonic LH values in ovariectomised rats, but that low dose genistein (0.1 mg/kg) administered intravenously suppresses LH concentrations. Genistein blocked the post gonadotrophin releasing hormone LH surge in such animals. In a follow up study, Hughes *et al* showed that neither genistein nor the mycoestrogens zearealenone or zearelalol provided oestrogenic priming for progesterone induced LH secretion, even though both genistein and zearelalol blocked gonadotrophin releasing hormone induced LH secretion.

More recently, Murthy *et al* showed that a benzene extract from flowers of *H rosa-sinensis* administered intraperitoneally to adult albino mice induced an irregular oestrous cycle, with a dose dependent prolonged oestrous and metestrus. The ovariates of affected mice show a decrease in the number of Graafian follicles, an absence of corpora lutea, and an increased number of atretic follicles. In addition, the ovaries show a significant increase in cholesterol and ascorbic acid.
indicating the non-availability of pituitary gonadotrophins for steroidogenesis.

The effect of phytoestrogens on fertility in sheep (so called oestrous state, as demonstrated by vaginal cornification. In neonatal exposure results in acyclicity and a persistent exposure. From the discussion above it is evident that the development of this axis in humans occurs in evolutionary terms the chemical content of our diet has resulted in the permanent redifferentiation of the cervix to resemble uterus, both microscopically and functionally. This is associated with loss of sexual characteristics, which is possible in sheep because the genes that control sexual differentiation are not fully deactivated at birth.5

The effect of phytoestrogens on the female genital tract is dependent upon the age at exposure and the duration of exposure. From the discussion above it is evident that neonatal exposure results in acyclicity and a persistent oestrous state, as demonstrated by vaginal cornification. In contrast, Cline and colleagues72 have shown that although an oestrogenic response can be produced in postmenopausal macaques by conjugated equine oestrogens and by tamoxifen, the vaginal cytological pattern in animals treated with soybean phytoestrogens (genistein) at doses comparable to a human diet showed no difference to control animals. Similarly, Anthony et al have shown that a soybean isoflavone rich diet fed to peripubertal rhesus monkeys lowers their cardiovascular risk factors but does not affect the reproductive tract.73 Interestingly, the serum SHBG concentration was also unaffected.

THE EFFECT OF ENVIRONMENTAL OESTROGENS ON THE HUMAN FEMALE GENITAL TRACT

As we have seen, there is considerable evidence to indicate that several naturally occurring and synthetic chemicals have weak oestrogenic activity that can affect the female reproductive tract of fish, rodents, and livestock. Despite the large body of literature describing the occurrence of such compounds within the human diet, there have been surprisingly few studies exploring the effects of phytoestrogens and xenoestrogens on the human female genital tract. However, there are some indications that xenoestrogens may play a role in the pathogenesis of breast cancer86-88 and male genital tract abnormalities.71-73 It is tempting to draw inferences from the animal work above, but it must be remembered that there are pronounced interspecies variations in the metabolism of dietary oestrogens, notably the effects of ruminant digestion on phytoestrogens, which leads to their pronounced effects on fish, rodents, and livestock. Besides, phytoestrogens and synthetic oestrogens, and bioassays have been developed to determine the oestrogenicity of such agents isolated from human serum.89-91 In one such analysis of human serum, Soto et al have recently shown that cumulative exposure to DDT metabolites has a synergistic oestrogenic effect.92 Furthermore, Markiewicz et al have shown that isoflavonoids enhance alkaline phosphatase activity in a human endometrial adenocarcinoma cell line (Ishikawa-Var 1) and that the complexes formed between the human ER, oestradiol, and the isoflavonoids are functionally equivalent.93 Those studies that have been undertaken to explore the effect of exposure to environmental oestrogens in humans can be divided into two categories. The first body of evidence deals with the association between environmental oestrogen exposure and the risk of neoplasia in the genital tract. Of these, the most prominent is the now well established role of exposure to diethylstilboestrol in utero in the subsequent development of adenocarcinoma of the vagina and cervix. One in 1000 women exposed to diethylstilboestrol in utero will develop the disease.94 Such exposure is iatrogenic, has been discussed elsewhere,95-97 and given that it is not our aim in this review to discuss the effects of iatrogenically administered oestrogens, falls outside the scope of this review. The remainder of the literature explores the effects of environmental oestrogens on fertility and the existing literature is confined to the effect of phytoestrogens on the female genital tract. To the best of our knowledge, the role of xenoestrogens in human female infertility and gynaecological neoplasia has not been studied.

Effects of environmental oestrogens on the menstrual cycle

Evidence is emerging that phytoestrogens may possibly exert an effect on the human menstrual cycle, although the findings are inconsistent.3 In a study of six premenopausal women evaluated in a metabolic suite, Cassidy and colleagues98 showed that a diet containing 60 mg soy protein/day (equivalent to approx 25 mg daidzein and 20 mg genistein/day) delays menstruation by one to five days and prolongs the follicular phase of the menstrual cycle by 2.5 ± 1.6 days. Such a prolongation of the menstrual cycle was significant when compared with the cycle length in the same women on a diet containing no soy protein. There was no change in the duration of the luteal phase. In addition, soy protein ingestion results in a significant suppression of the midcycle LH and follicle stimulating hormone (FSH) peak plasma concentrations, whereas the plasma concentration of oestradiol is increased and the concentration of cholesterol is reduced in the follicular phase. Thus, in premenopausal women, phytoestrogens apparently exert an antioestrogenic effect. However, the phytoestrogen diet did not affect the plasma SHBG concentration.99 The addition of flax seeds (which contain the lignins enterodiol and enterolactone) to an omnivorous diet similarly lengthens the luteal phase of the menstrual cycle, although the overall cycle length remained unchanged.3 This matter is far from settled, however. In a study of 14 women, Duncan et al found that a diet rich in soy isoflavones (128 mg ± 16mg/day) resulted in a modest reduction in plasma oestrone concentrations, but had no impact on the length of the menstrual cycle, follicular phase, or luteal phase.90 Other groups have suggested that soy isoflavone ingestion in premenopausal women leads to unchanged follicular phase length, midcycle LH, and FSH91; increased,100 or unchanged101 oestradiol; decreased100 dehydroepiandrosterone sulfate; and decreased102 or unchanged103 luteal phase progesterone (reviewed in Cassidy and colleagues104).

It has been suggested that phytoestrogens may also modify the symptoms of the menopause. Lock87 reported that in comparison with Canadian women, Japanese women are less likely to suffer from hot flushes during the menopause. Alder-creutz et al have hypothesised that the high isoflavonoid phytoestrogen content of the Japanese diet might be responsible
for this, noting that the urinary excretion of genistein, daidzein, and equol is much greater in Japanese than in North American women. Albertazzi et al found that menopausal women given a daily dietary supplement of 60 g soy protein had a significant reduction (p = 0.01) in the mean number of hot flushes compared with a placebo group. Dietary supplementation with wheat flour (which contains zearalenone) similarly reduces the frequency of hot flushes. However, the findings are mixed and a series of studies has failed to demonstrate any effect of phytoestrogens on menopausal symptoms.

In postmenopausal hysterectomised women receiving transdermal oestrogen, Nicholls et al have shown that dietary soy phytoestrogens mildly suppress the LH surge following a gonadotrophin releasing hormone challenge, and thus have a mild antioestrogenic effect. The results failed to achieve significance however (possibly a function of the small sample size), and must be interpreted with caution. Baird and colleagues noted that a diet rich in soy was associated with minor oestrogenic changes in vaginal cytology, but observed no alteration in LH or FSH. Duncan et al have shown that postmenopausal women receiving a high isolavone diet (2.00 mg/kg/day) had modestly reduced serum oestradiol and oestrone values, although there was no significant effect on vaginal cytology or endometrial biopsy. In contrast, Wilcox et al found that dietary supplementation with phytoestrogens led to increased vaginal cytological maturation.

Environmental oestrogens and the risk of gynaecological malignancy

It is generally agreed that the high fat, low fibre diet of the industrialised world is associated with an increased risk of cancer, whereas a diet rich in fruit, vegetables, whole grains, and legumes is protective. In a study comparing women with endometrial cancer with women admitted with non-gynaecological disorders, Levi et al have shown that a diet rich in vegetables, fresh fruit, whole grain bread, and pasta, independent of total energy intake, reduces the risk of endometrial carcinoma by 40–60%.

We cannot immediately assume that the antioestrogenic effect of a diet rich in fruit, vegetables, and legumes (and hence fibre) is a result of the presence of phytoestrogens. Gordin and colleagues and Rose and colleagues have shown that a high fibre diet itself reduces the serum oestrogen concentration in premenopausal women. Supplementation of the diet with wheat bran significantly reduces the serum oestrone and oestriol concentrations without altering the SHBG concentration. The presence of fibre within the gut alters the enterohepatic circulation of oestrogens. Oestrogens conjugated in the liver to form biologically inactive glucuronides and sulphoglucuronides are excreted in the bile. These are metabolised by bacterial glucuronidase and sulfatase to active metabolites, which are reabsorbed. In vegetarian women, who consume less fat and more fibre than omnivorous women, there is a positive correlation between faecal weight and faecal excretion of oestrogens. Vegetarian women have significantly lower faecal β-glucuronidase activity, and a higher faecal weight. Thus, a greater proportion of the oestrogen excreted in the bile is lost in the faeces, and such women have a lower plasma oestrone and oestriol concentration. Postmenopausal women have a lower urinary excretion of oestriol and a higher plasma SHBG concentration.

Nonetheless, there is evidence to suggest that phytoestrogens themselves are cancer preventative agents. Circumstantial evidence is available in the lower prevalence of endometrial cancer in Japanese women (who eat a diet rich in phytoestrogens) compared with American women. Furthermore, irrespective of the amount of fibre consumed, women with endometrial cancer eat less vegetables, fruit, and dairy products than controls. Goodman et al found that although there was a negative correlation between dietary fibre intake and the risk of endometrial cancer, there was also an inverse correlation between soy protein consumption and the risk of endometrial cancer. This also held true for other sources of phytoestrogens such as whole grains, vegetables, fruits, and seaweeds.

CONCLUSION

There is considerable evidence that environmental oestrogens impact upon the development of the female genital tract and subsequent fertility in fish, laboratory rodents, and livestock. Such oestrogenic compounds may be (1) naturally occurring compounds synthesised by plants to resist pathogens, (2) natural steroidal oestrogens or synthetic oestrogens excreted into the environment, or (3) chemicals synthesised for another purpose and found subsequently to have oestrogenic activity. In humans, there is growing evidence that such compounds may have an impact on male fertility and on the risk of developing breast cancer. The literature on the effect of environmental oestrogens on the human genital tract is surprising by its absence. It is evident that such compounds are in our environment, and that we ingest them. Furthermore, it is clear that they may affect the length of the human menstrual cycle. The true effect of such compounds on the pathophysiology of the female genital tract remains unknown. The animal data suggest that the timing of exposure to such compounds is crucial, with neonatal exposure having the most pronounced effects. Given the exposure of neonates to phytoestrogens, this should be a cause for concern. The impact of exposure to phytoestrogens in adulthood is also worthy of further study. Because there is clear evidence that natural and synthetic steroid oestrogens excreted into the environment may affect wildlife and also enter the human water supply, the impact of such agents deserves attention. Clearly, however, large scale longitudinal studies will be needed to separate the effects of environmental oestrogens from those of the abundant confounding factors.

REFERENCES


