Eur J Cancer, Vol. 28A, No. 6/7, pp. 1182–1189, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Feature Articles

Diethylstilboestrol: I, Pharmacology, Toxicology and Carcinogenicity in Humans

M. Marselos and L. Tomatis

Diethylstilboestrol is still used as an adjunct palliative treatment in certain patients with breast and prostate cancer. Its pharmacological, toxicological and carcinogenic properties are reviewed. In addition to the usual untoward effects following subacute or chronic administration of oestrogens, treatment with diethylstilboestrol has been associated with serious cardiovascular sequelae. Most characteristic are, however, the carcinogenic properties of this drug. Many epidemiological data provide evidence that prenatal exposure to diethylstilboestrol is causally associated with vaginal and cervical clear-cell adenocarcinomas, a very rare type of cancer in the unexposed female population. The intrauterine exposure of males leads to an increased risk of testicular cancer, although the data are less conclusive in this respect. There is some evidence that administration of diethylstilboestrol in large doses to adult women during pregnancy increases the risk of subsequent breast cancer and it probably increases the incidence of endometrial carcinoma, as has been shown with other similar oestrogens given chronically for menopausal symptoms.
Eur J Cancer, Vol. 28A, No. 6/7, pp. 1182–1189, 1992.

INTRODUCTION

IN THE early 20th century, biologically active substances were shown to be produced by the ovaries and especially the follicle. The name "oestrogens" was coined for these substances, because of their ability to induce oestrus in animals [1, 2].

The chemical, physiological and pharmacological characterisation of these hormones was accomplished by Dodds and his collaborators, in the Department of Biochemistry of the Middlesex Hospital of London [3–5]. In 1934, it was shown that the oestrogenic properties may be retained with molecular structures fairly dissimilar to those of the endogenous hormones [6], such as dihydroxy-diphenylmethane and dihydroxy-stilbene (stilboestrol) [7, 8]. The diethyl dihydroxy-stilbene derivative (diethylstilboestrol, DES) was found to be five times more potent as an oestrogen than oestradiol, after oral administration [9, 10]. The use of DES was advocated in the 1940s for many pregnancy complications, particularly in diabetic women, in the belief that the drug would help by regulating the secretion of endogenous hormones, or by substituting for them [11–15]. Only 3 years after its synthesis, DES was approved in the USA for therapeutic use in man [16], and was applied in clinical practice with a wide range of indications, such as threatened abortion, habitual abortion, prior stillborn, prior premature labour, prior pre-eclampsia or eclampsia, essential hypertension and pregnancy of diabetic women. Few clinical reports have supported such applications of the drug, however, while serious objections were raised by means of retrospective studies. For example, the results of the administration of DES to 840 patients, according to the conventional schedule proposed by Smith [15], were compared with the results of an identical placebo treatment given to 806 patients. The frequency of abortion, prematurity, postmaturity and eclampsia, and perinatal mortality, with or without diabetes, were not affected by DES [16].

In the early 1950s, DES was approved by the FDA in the USA as a growth promoter in livestock either as a feed additive or as an implant, a practice which was eventually banned there in 1979 [17].

Between 1940 and 1970, DES was given to a large number of pregnant women, but estimates of the proportion exposed as a percentage of the adult female population vary from 0 to 10% [18–20].

Several products containing DES and its esters, alone or in combination with other ingredients have been marketed under different proprietary names and in various formulations. Nowadays, most of the preparations available contain the dipropionate derivative. DES has been used in humans in the form of injectable solution, suppositories, pessaries (vaginal suppositories) and tablets [21], and in animals in the form of pellet implants [16].

DES is the only drug for which transplacental carcinogenic action in man has been formally proven [22]. The epidemiological data on its long-term toxic effects have helped the scientific community to realise the possible dangers of new drugs in general.

Regulatory authorities have accepted the necessity for testing for possible teratogenic effects, since the thalidomide tragedy. The case of DES has raised the question of whether perinatal carcinogenesis testing should be adopted routinely for new drugs.

Correspondence to L. Tomatis.
L. Tomatis is at the International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon, Cedex 08, France; and M. Marselos is at the Department of Pharmacology, Medical School, University of Ioannina, 45110 Ioannina, Greece.
Received 25 Oct. 1991; accepted 24 Dec. 1991.

EXPOSURE CONDITIONS

Clinical applications

As mentioned above, the first applications of DES were restricted to cases of various pregnancy complications, especially in diabetic women. While such early uses were still awaiting justification (which in fact was never obtained) DES was recommended for a wide variety of clinical conditions in the fields of endocrinology, gynaecology and obstetrics [21–26].

The indications for which DES has been prescribed systematically can be classified as follows: (a) conditions related to pregnancy, (b) oestrogen replacement therapy, and (c) cancer (carcinoma of the breast in postmenopausal women, carcinoma of the prostrate).

Threatened abortion is the clinical condition that was most associated with the use of DES in the past. In most European countries, DES was still used as a preventive measure for threatened abortion [27], even after its carcinogenic potential became known [18].

The current therapeutic uses of DES are in: (a) breast cancer (for palliation only) in appropriately selected women and men with metastatic disease, and (b) prostatic carcinoma, for palliative therapy of advanced disease [25–27].

When a drug is commercially available, it may be used by patients and physicians alike for situations not included in the officially accepted list of indications. Thus in The Netherlands, a preparation with DES was used until 1982 as an aphrodisiac [27]. The possibility that DES is still prescribed illegally in some countries for post-coital contraception, cannot be excluded.

Environmental exposure

In the early 1950s, many studies indicated that DES acted as an anabolic in ruminants and poultry [17]. The best response of growth in cattle was obtained with doses of 5–10 mg daily in the food, or with ear implants of 24–48 mg every 2–3 months [28, 29]. The exact mechanism of this anabolic action is not fully understood, but it seems to involve a combination of endocrine effects mostly due to an increase in the plasma levels of growth hormone and insulin [30].

In 1962, DES fell within the scope of the "Delaney clause", an amendment to the Federal Food, Drug and Cosmetic Act, stating that no residues of a substance should be allowed in food "if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal" [17]. However, the principle of zero levels was not applicable for the possible food residues of DES, due to drawbacks in the sensitivity of the available techniques. A bioassay used for its determination until 1972, the so-called "immature mouse uterine assay", could not detect DES in edible tissues below a level of 2 μg/kg [2 ppb (parts per billion)]. According to the FDA regulations, if DES was added to the diets of cattle and sheep to be slaughtered, the level could not exceed 20 mg/day for cattle and 2 mg/day for sheep. Persistent detection of DES residues in beef livers prompted the FDA in 1971 to change the required withdrawal period from 2 days to 1 week, and in 1975 from 1 week to 2 weeks [26]. It was shown in kinetic experiments using radiolabelled DES that a single oral dose of the allowed level of 10 mg could lead to residues of 0.21–0.52 μg/kg, which persisted for almost 10 days [26]. Pellets of 24–36 mg DES implanted subcutaneously in cattle or steers liberated about 56–74 μg of DES per day into the circulation and had a half-life of 80–90 days. Even after 120 days DES was present in the liver at detectable levels [28, 29].

So, between the years 1962 and 1975, the level of residues that the Delaney clause demanded be zero was in fact up to 2 ppb, and the time allowed before slaughter after the last administration was too short for the complete elimination of the drug.

It has been estimated that between 1955 and 1979 (when its use was banned), DES was administered to almost 80% of cattle and lambs in the USA [26]. Considering the methodological problems in the detection of DES residues in meat, the inadequate withdrawal time before slaughter, the difficulties in achieving a claimed daily dose by using a mixture of cattle food, and also doubts about the general compliance of animal breeders with the regulations of FDA, one concludes that it is impossible to calculate the extent of human exposure by meat consumption. As a consequence, any effort to evaluate possible public health risks associated with such exposure can only be extremely approximate.

There is evidence that people are or have been exposed to DES in the work place at concentrations high enough to produce biological effects. Cases of gynaecomastia have been reported among workers exposed to DES during the final stages of chemical synthesis [31, 32].

PHARMACOLOGICAL DATA

Bioavailability studies in animals have shown that DES is readily absorbed after oral administration, at a percentage of at least 20% of the dose [28, 33–35].

In rhesus monkeys and chimpanzees, the urine is the main route of excretion [36, 37]. Enterohepatic circulation plays an important role in the distribution of DES in rhesus monkeys, and the major metabolites found in the bile after intravenous administration of DES are the monoglucuronides of dienoestrol, ω-hydroxydienoestrol and ω-hydroxydiethyl-stilboestrol [36, 38].

In humans, orally administered DES is readily absorbed from the gastrointestinal tract and is distributed throughout the organism. The drug crosses all biological membranes with great ease and is also readily absorbed through the skin, a fact with apparent implications in occupational exposure [39]. It is slowly inactivated in the liver and excreted as a glucuronide in the urine [40]. It is also detected in the faeces, mainly as the parent compound [21, 40].

The metabolism of DES in humans is similar to that in other species, especially the non-human primates. In a study with 3 volunteers (2 males and 1 female), radioactive DES was ingested, in a gelatin capsule, at a dose of 0.5 mg/kg. The levels of radioactivity were measured in the plasma and the excreta for a week [40]. About 40% of the dose was found in the first 24 h urine, mostly as glucuronide conjugates (87%). The DES conjugate was the most important of the glucuronide fraction (68%), followed by ω-hydroxydienoestrol (20%) and dienoestrol (12%).

The plasma concentration of the drug reached a peak within 20 h after ingestion and then declined exponentially. The approximate half-life of total radioactivity was estimated as 2–3 days, without further definition of the presence or the chemical nature of any metabolites in the plasma [40].

TOXICOLOGY

Side-effects

The commonest side effect of DES is a moderate and transient nausea, which usually subsides within 1 to 2 weeks, even in cancer patients treated with large doses. Other mild side effects

include occasional vomiting, dizziness, headache, discomfort in the breasts and weight-gain, symptoms are reported to be even milder in postmenopausal women [24].

Men occupationally exposed to DES developed gynaecomastia. Similarly, exposed women had signs of hyperoestrogenism, with excessive pigmentation of the nipples and breast enlargement [31, 32]. The same signs have been found in newborns exposed to DES *in utero* [41].

Women treated with DES for suppression of lactation (120 mg for 6 days) exhibit a deterioration in their hepatic function, as reflected in a prolonged bromosulphophthalein retention time [42]. In contrast, no signs of liver damage were seen in men receiving daily doses of 500 mg DES for 3 weeks and then 60 mg oestradiol benzoate for a year, as a treatment for prostatic carcinoma [43].

The use of large doses of DES in the chemotherapy of breast and prostate cancers has led to an increased incidence of cardiovascular disease in both men and women [17, 25]. Although DES (5 mg/day) decreased the mortality due to cancer, the overall mortality increased due to cardiovascular and thromboembolic disorders [44]. These disorders can be significantly diminished by a drastic reduction of the DES dose (1 mg/day), while retaining a beneficial effect on cancer in most cases [44, 45]. The mechanism by which DES increases the incidence of cardiovascular disease may include changes in the system of coagulation, in the balance of electrolytes, in the blood volume and in the systolic blood pressure [46].

Genotoxicity

Although DES is not mutagenic in the *Salmonella* spp tests, it has given positive results in many other experimental systems, including sister chromatid exchange, unscheduled DNA synthesis, chromosomal aberration, disruption of mitotic spindle and aneuploidy. Also, its quinone metabolite is able to form DNA adducts in the liver, kidneys and uterus of the hamster [23]. In the kidneys of male Syrian hamsters, 8-hydroxydeoxyguanosine is formed due to the generation of free radicals in the process of redox cycling between DES and its quinone [47, 48].

A DES concentration of 10^{-6} mol/l induced unscheduled DNA synthesis in HeLa cells in the presence of a liver activation system [49].

In cultures of the human prostatic tumour cell line DU 145, DES was found to induce metaphase arrest which was attributed to the inhibition of microtubule assembly and consequent disturbances of the mitotic spindle [50].

Human teratogenicity and reproductive toxicity

Gross and microscopic abnormalities of the vagina and cervix have been described in females exposed to DES prenatally, and they include vaginal adenosis (columnar epithelium or its mucinous products in the vagina), cervical ectropion (eversion or erosion, columnar epithelium or its mucinous products in the cervix) and transverse fibrous ridges in the vagina or on the cervix (transverse septum, cocks-comb cervix, vaginal hood). Cervial ectropion is common among women of reproductive age even without DES exposure, but adenosis and transverse ridges occur only rarely in the general population [51–53].

Case-control studies have confirmed the association of vaginal adenosis with prenatal exposure to DES. The rate of adenosis among the exposed daughters appears to be related linearly to the time during pregnancy at which the mother began medication: it was highest in early pregnancy and was not detected if treatment was initiated in the 18th week or later [54–59].

The prevalence of adenosis has been found to vary greatly among women exposed prenatally to DES (35–90%), in many non-controlled studies [23]. In addition to methodological drawbacks, differences in the definition of observed adenosis by various investigators contribute to the discrepancies. In older patients the incidence is expected to be lower, due to the normal metaplastic replacement of the columnar epithelium by squamous epithelium [52, 53, 60, 61].

It seems that in some cases it is difficult to separate neoplastic from metaplastic squamous epithelial changes in females exposed prenatally to DES, although an increased risk of squamous-cell carcinoma of the vagina or cervix has been established among this population [62–65]. Furthermore microglandular hyperplasia of the epithelium, a benign atypical change known as "the pill lesion", can be confused with clear-cell adenocarcinoma [66].

Women exposed prenatally to DES were examined by hysterosalpingography for possible teratogenic effects in the inner genitalia. Anatomical changes, such as small endometrial cavities, "T-shaped" uteri and dilated cornual areas, were observed in 40 of the 60 women examined [67].

Males exposed prenatally to DES also had a greater frequency of abnormalities in their reproductive tracts, when compared with unexposed controls [55, 68]. The most common genital lesions were epididymal cysts, hypoplastic testes and cryptorchidism. These were observed in 30% of 289 exposed subjects, in comparison with 8% of 290 unexposed subjects. Semen analysis revealed a higher frequency of severe pathological changes (39% in 88 patients vs. 14% in 55 controls).

The effects on fertility of *in utero* exposure to DES in females and males have been evaluated by Stillman [69]. Most reports show than an overall adverse pregnancy outcome is rather frequent in DES-exposed women, corresponding with a somewhat increased tendency for spontaneous abortion, ectopic pregnancy, premature deliveries and perinatal deaths [70–73]. Some studies, however, have found no difference in fertility between exposed and unexposed women [74, 75].

Human carcinogenicity

(*a*) *Prenatal exposure.* Between 1966 and 1970, 6 cases of clear-cell adenocarcinoma of the vagina were found in teenage girls [76]. The following year Herbst *et al.* [18] reported another case and related the high incidence of this rare type of cancer to the fact that 7 of the 8 patients had been exposed to DES *in utero*. Greenwald *et al.* [77] found 4 more cases and also confirmed the use of DES by the mothers of these girls during pregnancy. Additional data suggested that prenatal exposure to DES may also cause adenocarcinoma of the cervix [78]. These initial observations were soon verified by many reports in the following years [79–83].

Studies of the clinical, pathological and epidemiological aspects of these cancers from all over the world have been filed in the "Registry for Research on Hormonal Transplacental Carcinogenesis" (University of Chicago) [84–89].

Most, but not all, data on prenatal DES exposure originate from the USA [78, 90–93]. In one of the largest epidemiological studies, it was found that in 75% of cases the mother had been treated in the relevant pregnancy with a drug, usually—but not always—as a preventive measure for threatened abortion. About 65% the reported treatment concerned either DES, or the related compounds dienoestrol and hexoestrol. Treatment was during the first half of pregnancy, and the daily doses used varied

greatly from 1.5 mg to 150 mg, with total doses consumed in a range of 135 mg to 18 g [93].

For the period 1959–1965, a total of 217 women were found to have been exposed to DES among the 57071 pregnancies included in a study of data from 12 medical centres in the USA. Reported daily DES doses varied from 2.5 to 150 mg and the duration of treatment from less than a week to 36 weeks. Total DES intake was from 175 mg to 47 g [20].

A report from the Registry included data from 519 women with clear-cell adenocarcinoma of the vagina or the cervix, 69 of whom were not from the USA (until the year 1985). About 60% of these cases were exposed to DES, 12% to another hormone or unrelated medication, 23% to non hormonal drugs, and 5% to unknown medication, if any [94].

The peak of DES exposure occurred in the 1950s, according to combined estimates of the incidence rates of clear-cell adenocarcinomas and the sales of DES tablets and other relevant pharmaceutical products [76, 94]. In the decade between 1945 and 1955, perhaps more than 1 million women received this drug during gestation just in the USA [25]. Subsequently its use began to decline, and by 1965 it was employed in less than 1% of the Boston pregnancies reviewed [20]. It has been estimated that the total number of live female offspring in the USA who were exposed to DES *in utero* during the period 1960–1970 was between 10000 and 16000 per year. Data from the UK for the period 1940–1971 also indicate that the highest exposure of women to DES was in the 1950s [95].

In Europe, a national registry has been established only in the Netherlands, where 24 cases of clear-cell adenocarcinoma, out of 39, could be ascribed to intrauterine DES exposure. Sporadic and probably incomplete data exist from other European countries, such as France (3 cases), Spain (3 cases), the UK (3 cases), Belgium (1 case), Portugal (1 case), Ireland (1 case) and Switzerland (1 case) [27, 86, 87].

By reviewing earlier epidemiological data, Herbst *et al.* [96] concluded that the risk of the exposed children of developing clear-cell adenocarcinoma of the vagina or the cervix was 0.14–1.4 per 1000, up to the age of 24.

Based on the Registry data, Melnick *et al.* [97] have estimated the risk for clear-cell adenocarcinoma of the vagina or cervix in a DES-exposed female to be about 1 in 1000, up to the age of 34. Data from the UK, where a total of at least 7500 pregnant women were treated with DES, have been used to derive a similar estimate. Assuming that half of the pregnancies are of a female child, and that fetal loss could rise to 15–20%, there would be about 3000 girls at risk. The 3 reported cases of DES-exposure-related clear-cell adenocarcinomas of the vagina or the cervix in the UK corresponding with this period of time [95, 98, 99] are consistent with the estimated level of risk.

A detailed analysis of the Registry data [100] showed that the risk for the development of DES-associated clear-cell adenocarcinoma is increased when the drug was taken before the 12th week of pregnancy (relative risk, 2.0), when there is a maternal history of at least one prior spontaneous abortion (relative risk, 2.4), or when the weight at birth was 2.5 kg or less (relative risk, 2.4).

An analysis of incidence by age of clear-cell adenocarcinoma patients whose mothers had been treated with DES showed that the neoplasm occurred primarily after menarche, although prepubertal cases have been reported [76]. The highest incidence seemed to be at the age of 19 years, with extreme cases at the ages of 7 and 29 [98, 101].

In 1987, Kramer *et al.* [102] published data from the State of New York, where about 2000 DES-exposed women had been screened since 1979. 4 women, who at their initial visit were noted to have only non-malignant DES-related changes, subsequently developed clear-cell adenocarcinoma. Similar findings have been reported by other investigators [103, 104].

Adenocarcinomas of the vagina and the cervix have been found in several instances with no apparent relation to transplacental or other DES exposure. A few cases of vaginal clear-cell adenocarcinomas were described in young women even before the introduction of DES in therapeutics [106–108]. However, the risk for the general population without DES exposure is considered to be several orders of magnitude lower, compared with the data obtained from women with a history of intrauterine exposure. Therefore, the evidence on a causal relationship between DES-exposure and clear-cell adenocarcinoma of the vagina or cervix seems conclusive [99, 109–111].

Evaluating the available scientific data, the International Agency for the Research on Cancer (IARC/WHO) has estimated that there is sufficient evidence for DES to be classified as a human carcinogen [23, 112].

Cases of other types of cancer ascribed to intrauterine DES exposure have been reported only occasionally, and they include adenocarcinoma of the endometrium in a 26-year-old woman [113], a malignant teratoma of the ovary in a 12-year-old girl [114], a pituitary adenoma in an 18-year-old woman [115] and an invasive adenosquamous cell carcinoma of the cervix in a 21-year-old woman [116].

In a follow-up study of 177 males exposed *in utero* to DES, one fatal teratoma of the testis was found [117]. Also 1 case of seminoma has been reported [118]. Exposure to exogenous oestrogenic hormones during pregnancy appears to increase the risk of testicular cancer by 3- to 5-fold [119]. Exposure specifically to DES has been investigated in five case–control studies, according to which the estimated relative risk for cancer of the testis is 2.5 ($P = 0.0014$) [120–124].

(*b*) *Postnatal exposure.* Patients with Turner's syndrome (gonadal dysgenesis and other dysplasias, due to a chromosomal anomaly), who were treated with DES for long periods of time, showed a high incidence of endometrial carcinoma [125, 126]. A total of 11 cases have been described and the average age of the patients was 31 years. Endometrial carcinoma was also found in patients treated with DES, either for gonadal deficiency secondary to hypopituitarism (Sheehan's syndrome, 1 case) [127], or for metastatic breast cancer (3 cases) [128].

There is sufficient evidence that administration of oestrogens for the control of climateric symptoms is related to an increased incidence of endometrial cancer [23] and there is no reason to believe that DES is acting in a way differing from that of other oestrogens. The development of endometrial cancer in women exposed to DES during their pregnancy has not yet been documented fully [112].

A health survey conducted by Bibbo *et al.* [129] included 693 women who had received DES as pregnancy treatment, and 668 well matched controls. A history of breast biopsy was found in 14% of the exposed and in 12% of the unexposed, and most showed fibrocystic disease. Breast cancers were 4.6% and 3.1% respectively, but this difference was not statistically significant. There was also an excess of deaths (38 exposed vs. 28 unexposed), which was accounted for almost entirely by the difference in breast cancer deaths (12 exposed vs. 4 unexposed). This excess was not statistically significant and was due to the appearance of breast cancer at a younger age among the exposed. There was

no difference in breast cancer occurrence among the women who had started DES before or after the 11th week of pregnancy.

By considering the above results together with similar follow-up studies of women exposed to DES [130–133], it has been estimated that the relative risk for the development of breast cancer is 1.4 ($P < 0.001$), with a latent period of 15–20 years after the administration of the drug [112].

In men, several cases of breast cancer thought to be due to oestrogen treatment for prostatic adenocarcinoma were eventually proven to be metastases of the primary tumour [134, 135]. In 16 out of 30 cases of males treated with DES alone, breast tumours appeared 1–57 months after the start of treatment. On the basis of reliable diagnostic techniques, 6 of the tumours were considered to be new primaries and 10 to be metastases [136].

Various other tumours of men have been sporadically reported in relation to DES administration for treatment of prostatic cancer, such as 2 cases of hepatoma [137, 138], 2 cases of hepatic angiosarcoma [139, 140], 2 cases of melanoma [141], and 3 renal carcinomas [142, 143].

CONCLUSIONS

DES has attracted the interest of many scientists for the last 50 years, first as a promising new drug for medicinal and veterinary use, and afterwards for its unusual carcinogenic properties.

Past human exposure for medicinal purposes was mainly in the context of threatened abortions and oestrogen replacement therapies. Nowadays, the use of the drug is limited to certain cases of prostatic and breast cancer, and it is also locally applied for postmenopausal vaginal disorders. However, there is still some concern that it may be misused as a post-coital contraceptive, especially in countries where it is commercially available as a proprietary product.

In meat production, DES was used for about 30 years, but exact exposure data are not available, and therefore the public health implications cannot be ascertained.

Mechanism of action

Despite its well documented carcinogenicity in humans and in experimental animals, the mechanism of this action still remains obscure. A direct genotoxicity of the drug and its metabolites has been reported, and concerns mainly clastogenic effects. In addition, it has been suggested, that DES produces many hormonal disturbances that could participate and probably interact during the process of carcinogenesis.

There is experimental evidence that prenatal treatment with DES may produce uterine and ovarian carcinomas in the second generation of offspring, which was never exposed directly to the drug [144]. These results, and the apparent overall complexity of DES carcinogenicity, have led to a theoretical model of a possibly multigenerational "transmission" of cancer [145]. Further experimental evidence for a transgeneration carcinogenic effect of DES is provided by the increased incidence of uterine and ovarian tumours in female mice obtained by mating males exposed prenatally to DES with untreated females [146].

Teratogenicity

Many studies have shown that prenatal exposure of young women to DES is associated with high incidence of vaginal adenosis and transverse fibrous ridges in the vagina. It has been estimated that among women prenatally exposed to DES, vaginal adenosis has a prevalence of 35–90%. Also gross anatomical changes, such as small endometrial cavity and dilated cornual areas, have been reported. Epididymal cysts, testicular hypoplasia and cryptorchidism have been documented in young men. Possible implications for female or male fertility have also been suggested.

Time of exposure seems to be an important factor for human dysplasias, which are always associated with DES administration before the 18th week of pregnancy.

Carcinogenicity

It has been estimated that more than 2 million pregnant women have been treated worldwide with DES for periods ranging from 1 week to several months, and with daily doses from 1.5 to 150 mg. Most of them were in the USA and Canada; few data available from the European countries concern exposure to DES.

There is sufficient evidence that intrauterine exposure of females to DES before the 12th week of pregnancy is causally associated with the appearance of vaginal and cervical clear-cell adenocarcinoma. Cancer appears usually after menarche, and at an age between 10 and 30 years. Up to the age of 24, the risk has been calculated to be in the order of 0.14–1.4/1000. Cases become very rare after the age of 25.

There are no conclusive data on the risk for other types of cancer among women prenatally exposed to DES.

Intrauterine exposure of males has been associated with testicular cancer. There are no conclusive data on possible risk from other cancers after prenatal exposure to DES.

Administration of oestrogens for the control of climacteric symptoms is known to be related to an increased incidence of endometrial cancer. DES most probably acts in a similar way, but the data are inadequate to allow any firm conclusion. Similarly, there is no documentation on the development of endometrial cancer in women treated with DES during pregnancy.

Women exposed to DES postnatally, with several therapeutic indications, have a higher risk for the development of breast cancer. The latent period after administration of the drug has been calculated to be 15–20 years.

No data are available on the possible risks from other types of human exposure, such as from DES residues in food.

1. Adler L. Zur Physiologie und Pathologie der Ovarialfunktion. *Arch Gynaekol* 1912, **95**, 349–424.
2. Frank RT. The ovary and the endocrinologist. *JAMA* 1922, **78**, 181–185.
3. Dodds EC. The bearing of recent research on the sex hormones on clinical obstetrics and gynaecology. *Proc R Soc Med* 1932, **25**, 563–569.
4. Dodds EC. Oestrus producing hormones. *Br Med J* 1934, **2**, 1187–1189.
5. Dodds EC. The practical outcome of recent research on hormones. *Lancet* 1934, **2**, 1318–1320.
6. Cook JW, Dodds EC, Hewett CL. The estrogenic activity of some condensed-ring compounds in relation to their other biological activities. *Proc R Soc Lond [Biol]* 1934, **114**, 272–286.
7. Dodds EC, Lawson W. Synthetic oestrogenic agents without the phenanthrene nucleus (letter to the editor). *Nature* 1936, **137**, 996.
8. Lawson W. Stilboestrol: biochemistry and discovery. *Middlesex Hospital J* 1941, **41**, 92–94.
9. Dodds EC, Golberg L, Lawson W. Oestrogenic activity of certain synthetic compounds (letter to the editor). *Nature* **141**, 247–248.
10. Novak E. The therapeutic use of oestrogenic substances. *JAMA* 1938, **104**, 1815–1821.
11. Smith GVS, Smith OW. Estrogen and progestin metabolism in pregnant women. With special reference to preeclamptic toxemia

and the effect of hormone administration. *Am J Obstet Gynecol* 1940, **39**, 405–410.
12. Karnaky KJ. Use of stilbestrol for treatment of threatened and habitual abortion and premature labor; preliminary report. *South Med J* 1942, **35**, 838–843.
13. Smith OW, Smith GWS, Hurwitz D. Relationship between hormonal abnormalities and accidents of late pregnancy in diabetic women. *Am J Med Sci* 1944, **208**, 25–29.
14. Smith OW, Smith GWS, Hurwitz D. Increased excretion of pregnanediol in pregnancy from diethylstilbestrol with special reference to the prevention of late pregnancy accidents. *Am J Obstet Gynecol* 1946, **51**, 411–447.
15. Smith OW. Diethylstilbestrol in the prevention and treatment of complications of pregnancy. *Am J Obstet Gynecol* 1948, **56**, 821–827.
16. Dieckmann WJ, Davis ME, Ryjenkiewicz IM, Pottinger RE. Does the administration of diethylstilbestrol during pregnancy have therapeutic value? *Am J Obstet Gynecol* 1953, **66**, 1062–1081.
17. McMartin KE, Kennedy KA, Greenspan P, Alam SN, Greiner P, Yam J. Diethylstilbestrol: A review of its toxicity and use as a growth promotant in food-producing animals. *J Environ Pathol Toxicol* 1978, **1**, 279–313.
18. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971, **284**, 878–885.
19. Noller KL, Decker DG, Lanier AP, Kurland LT. Clear cell adenocarcinoma of the cervix after maternal treatment with synthetic estrogens. *Mayo Clin. Proc* 1972, **47**, 629–633.
20. Heinonen OP. Diethylstilbestrol in pregnancy. Frequency of exposure and usage patterns. *Cancer* 1973, **31**, 573–579.
21. Reynolds EFJ, ed. *Martindale, The Extra Pharmacopoeia*, 29th Edition, London, The Pharmaceutical Press. 1989, 1413–1414.
22. Tomatis L. Overview of perinatal and multigeneration carcinogenesis. In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H, eds. *Perinatal and Multigeneration Carcinogenesis*. Lyon International Agency for Research on Cancer, Scientific Publications, No 96, 1989, 1–15.
23. International Agency for Research on Cancer *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 21, *Sex Hormones (II)*, Lyon, 1979, 173–231.
24. Murad F, Haynes R. *Estrogens and Progestins*. In: Goodman Gilman A, Goodman LS, Rall Th W, Murad F, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics* New York, Macmillans 1985, 1412–1439.
25. Noller KL, Fish CR. Diethylstilbestrol usage: Its interesting past, important present and questionable future. *Med Clin N Am* 1974, **58**, 793–810.
26. Young CL. *Diethylstilbestrol; A Cancer Control Monograph*, SRI International, Chemical Industries Center, Stanford, CA, 1978.
27. Avgerinos PM. *L'utilisation du diethylstilbestrol (DES) et ses effets sur la santé des consommateurs et leurs enfants*. Doc. B2-1242/87, *Commission de l'environnement, de la santé publique et de la protection des consommateurs*, 1989, Parlement Européen.
28. Burroughs W, Culbertson CC, Cheng E, Hale WH, Honeyer P. The influence of oral administration of diethylstilbestrol to beef cattle. *J Animal Sci* 1955, **14**, 1015–1024.
29. Trenkle A, Burroughs W. *Physiological effects of estrogens in animal feeds with emphasis on growth of ruminants*. J. paper No. J-8395 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 2027, 1976.
30. Trenkle A. The anabolic effect of estrogens in nitrogenmetabolism of growing and finishing cattle and sheep. In: Lee FC, Rendel J, eds. *Anabolic Agents in Animal Production*, Stuttgart, Georg Thieme Publishers, 1976, **5**, 79–88.
31. Fitzsimons MP. Gynaecomastia in stilboestrol workers. *Br J Ind Med* 1944, **1**, 235–236.
32. Watrous RM, Olsen RT. Diethylstilbestrol absorption in industry: a test for early detection as an aid in prevention. *Am Ind Hyg Assoc J* 1962, **20**, 469–472.
33. Mitchell GE, Neumann AL, Draper HH. Metabolism of tritium-labeled diethylstilbestrol by steers. *J Animal Sci* 1956, **15**, 1285–1287.
34. Story CD, Cheng EW, Pauls JP, Hale WH. Estrogenic activity of feces and urine following oral administration of diethylstilbestrol to lambs. *J Animal Sci* 1957, **76**, 307–311.
35. Melampy RM, Gurland J, Rakes JM. Estrogen excretion by cows after oral administration of diethylstilbestrol. *J Animal Sci* 1959, **18**, 178–186.
36. Metzler M, Muller W, Hobson WC. Biotransformation of diethylstilbestrol in the rhesus monkey and the chimpanzee. *J Toxicol Environ Health* 1977, **3**, 439–450.
37. Helton ED, Hill DE, Gough BJ, Lipe GW, King JW Jr, Horning EC, Thenot JP. Comparative metabolism of diethylstilbestrol in the mouse, rhesus monkey, and chimpanzee. *J Toxicol Environ Health* 1978, **4**, 482–483.
38. Mroszczak EJ, Riegelman S. Disposition of diethylstilboestrol in the rhesus monkey. *J Pharmacokinetics Biopharm* 1975, **3**, 303–327.
39. Palmer A, Zuckerman S. Further observations on the similarity of stilboestrol and natural oestrogenic agents. *Lancet* 1939, **1**, 933–934.
40. Metzler M. Metabolic activation of carcinogenic diethylstilbestrol in rodents and humans. *J Toxicol Environ Health* 1976, Suppl. 1, 21–35.
41. Pachyn'ski A, Budzynska A, Przytecki S, Robaczyn'ski J. Hyperestrogenism in workers in pharmaceutical establishment and their children as occupational disease. *Pol Endocrinol* 1971, **22**, 125–129.
42. Clinch J, Tindall VR. Effect of oestrogens and progestogens on liver function in the puerperium. *Br Med J* 1969, **i**, 602–605.
43. Ishibe T. Effect of long-term estrogen treatment on liver function in patients with prostatic carcinoma. *J Urol* 1975, **113**, 829–833.
44. Veterans Administration Cooperative Urological Research Group Treatment and survival of patients with cancer of the prostate. *Surg Gynecol Obstet* 1967, **124**, 1011–1017.
45. Bailar JC, Byar DP. Estrogen treatment for cancer of the prostate: early results with three doses of diethylstilbestrol and placebo. *Cancer* 1970, **26**, 257–261.
46. Kontturi MJ, Sotaniemi EA, Larmi TKI. Body blood and electrolyte balance during estrogen therapy of prostatic cancer. *J Urol* 1974, **111**, 652–655.
47. Liehr JG. Genotoxic effects of estrogens. *Mutation Res* 1990, **238**, 269–276.
48. Roy D, Floyd RA, Liehr JG. Elevated 8-hydroxydeoxyguanosine levels in DNA of diethylstilbestrol-treated Syrian hamsters: Covalent DNA damage by free radicals generated by redox cycling of diethylstilbestrol. *Cancer Res* 1991, **51**, 3882–3885.
49. Martin CN, McDermid AC, Garner RC. Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res* 1978, **38**, 2621–2627.
50. Hartley-Asp B, Deinum J, Wallin M. Diethylstilbestrol induces metaphase arrest and inhibits microtubule assembly. *Mutation Res* 1985, **143**, 231–235.
51. Pomerance W. Post-stilbestrol secondary syndrome. *Obstet Gynecol* 1973, **42**, 12–18.
52. Scully RE, Robboy SJ, Herbst AL. Vaginal and cervical abnormalities, including clear-cell adenocarcinoma, related to prenatal exposure to stilbestrol. *Ann Clin Lab Sci* 1974, **4**, 222–233.
53. Scully RE, Robboy SJ, Welch WB. Pathology and pathogenesis of diethylstilbestrol-related disorders of the female genital tract. In: Herbst AL, ed. *Intrauterine Exposure to Diethylstilbestrol in the Human*, Chicago, American College of Obstetricians and Gynecologists, 1978, 8–22.
54. Bibbo M, Al-Naqeeb M, Baccarini I, *et al.* Follow-up study of male and female offspring of DES-treated mothers. A preliminary report. *J Reprod Med* 1975, **15**, 29–32.
55. Bibbo M, Ali I, Al-Naqeeb M, *et al.* Cytologic findings in female and male offspring of DES treated mothers. *Acta Cytol* 1975, **19**, 568–572.
56. Herbst AL, Poskanzer DC, Robboy SJ, Friedlander L, Scully RE. Prenatal exposure to stilbestrol. A prospective comparison of exposed female offspring with unexposed controls. *N Engl J Med* 1975, **292**, 334–339.
57. Sestili MA. Genital tract anomalies and cancer in females exposed in utero to diethylstilbestrol. Brief report on the DESAD Project. *Publ Health Rep* 1977, **92**, 481–484.
58. O'Brien PC, Noller KL, Robboy SJ, *et al.* Vaginal epithelial changes in young women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) project. *Obstet Gynecol* 1979, **53**, 300–308.
59. Sharp GB, Cole P. Vaginal bleeding and diethylstilbestrol exposure during pregnancy: Relationship to genital tract clear cell adenocarcinoma and vaginal adenosis in daughters. *Am J Obstet Gynecol* 1990, **162**, 994–1001.

60. Ng ABP, Reagan JW, Hawliczek S, Wentz WB. Cellular detection of vaginal adenosis. *Obstet Gynecol* 1975, **46**, 323–328.
61. Ng ABP, Reagan JW, Nadji M, Greening S. Natural history of vaginal adenosis in women exposed to diethylstilbestrol *in utero*. *J Reprod Med* 1977. **18**, 1–13.
62. Fu YS, Reagan JW, Hawliczek S, Wentz WB. The use of cellular studies in the investigation of the DES-exposed woman. In: Herbst AL, ed. *Intrauterine Exposure to Diethylstilbestrol in the Human*, Chicago, American College of Obstetricians and Gynecologists, 1978, 34–44.
63. Fu YS, Robboy SJ, Prat J. Nuclear DNA study of vaginal and cervical squamous cell abnormalities in DES exposed progeny. *Obstet Gynecol* 1978, **52**, 129–137.
64. Richart RM, Fu YS, Reagan JW, Barron BA. The problem of squamous neoplasia in female DES progeny. In: Herbst AL, ed. *Intrauterine Exposure to Diethylstilbestrol in the Human*, Chicago, American College of Obstetricians and Gynecologists, 1978, 45–52.
65. Robboy SJ, Keh PC, Nickerson RJ, *et al.* Squamous cell dysplasia and carcinoma *in situ* of the cervix and vagina after prenatal exposure to diethylstilbestrol. *Obstet Gynecol* 1978, **51**, 528–535.
66. Robboy SJ, Welch WR. Microglandular hyperplasia in vaginal adenosis associated with oral contraceptives and prenatal diethylstilbestrol exposure. *Obstet Gynecol* 1977, **49**, 430–434.
67. Kaufman RH, Binder GL, Gray PM, Jr, Adam E. Upper genital tract changes associated with exposure *in utero* to diethylstilbestrol. *Am J Obstet Gynecol* 1977, **128**, 51–59.
68. Gill WB, Schumacher GFB, Bibbo M. Pathological semen and anatomical abnormalities of the genital tract in human male subjects exposed to diethylstilbestrol *in utero*. *J Urol* 1977, **117**, 477–480.
69. Stillman RJ. *In utero* exposure to diethylstilbestrol: Adverse effects on the reproductive tract and reproductive performance in male and female offspring. *Am J Obstet Gynecol* 1982, **142**, 905–921.
70. Schmidt G, Fowler WC, Talbert LM, Edelman DA. Reproductive history of women exposed to diethylstilbestrol *in utero*. *Fertil Steril* 1980, **33**, 21–27.
71. Kaufman RH, Adam E, Noller K, Irwin JF, Gray M. Upper genital tract changes and infertility in diethylstilbestrol-exposed women. *Am J Obstet Gynecol* 1986, **154**, 1312–1319.
72. Senekjian EK, Potkul RK, Frey K, Herbst AL. Infertility among daughters either exposed or not exposed to diethylstilbestrol. *Am J Obstet Gynecol* 1988, **158**, 493–498.
73. Barnes AB, Colton T, Gundersen J, *et al.* Fertility and outcome of pregnancy in women exposed *in utero* to diethylstilbestrol. *N Engl J Med* 1980, **302**, 609–614.
74. Cousins L, Karp W, Lacey C, Lucas WE. Reproductive outcome of women exposed to diethylstilbestrol *in utero*. *Obstet Gynecol* 1980, **56**, 70–76.
75. Berger MJ, Alper MM. Intractable primary infertility in women exposed to diethylstilbestrol *in utero*. *J Reprod Med* 1986, **31**, 231–237.
76. Herbst AL, Scully RE. Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). *Cancer* 1970, **25**, 745–757.
77. Greenwald P, Barlow JJ, Nasca PC, Burnett WS. Vaginal cancer after maternal treatment with synthetic estrogens. *N Engl J Med* 1971, **285**, 390–392.
78. Herbst AL, Cole P, Colton T, Robboy SJ, Scully RE. Age-incidence and risk of diethylstilbestrol-related clear cell adenocarcinoma of the vagina and cervix. *Am J Obstet Gynecol* 1977, **128**, 43–50.
79. Tsukada Y, Hewett WJ, Barlow JJ, Pickren JW. Clear-cell adenocarcinoma ("mesonephroma") of the vagina. Three cases associated with maternal synthetic nonsteroid estrogen therapy. *Cancer* 1972, **29**, 1208–1214.
80. Henderson BE, Benton BDA, Weaver PT, Linden G, Nolan JF. Stilbestrol and urogenital tract cancer in adolescents and young adults. *N Engl J Med* 1973, **288**, 354.
81. Hill EC. Clear cell carcinoma of the cervix and vagina in young women. *Am J Obstet Gynecol* 1973, **116**, 470–484.
82. Nissen EC, Goldstein AL. Stilboestrol therapy in pregnancy. Relationship to vaginal neoplasia in offspring. *Int J Gynecol Obstet* 1973, **11**, 138–142.
83. Greenwald P, Nasca PC, Burnett WS, Polan A. Prenatal stilbestrol experience in mothers of young cancer patients. *Cancer* 1973, **31**, 568–572.
84. Williams RR, Scheitzer RJ. Clear-cell adenocarcinoma of the vagina in a girl whose mother had taken diethylstilbestrol. *California Med* 1973, **118**, 53–55.
85. Alvarez Bravo AA, Andrade Sanchez A, Quiroz R. A case of primary adenocarcinoma of the vagina and pregnancy (Sp.). *Ginecol Obstet Mex* 1970, **28**, 379–386.
86. Schreiner WE, Bronz L, Wyss-Bernet M, Genton CY. Ein Fall von Adenokarzinom der Vagina nach Diaethylstilboestrol-Exposition in utero. *Schweiz Med Wochenscr* 1955, **115**, 1555–1561.
87. Iglesias GJ. Use of sex hormones during pregnancy in Spain (Sp.). *Med Clin (Barc)* 1988, **91**, 436–437.
88. Verhoeven ATM. Mesonephric carcinoma of the cervix uteri in a 8-year-old girl treated by hysterectomy. *Eur J Obstet Gynecol* 1972, **5**, 145–151.
89. Robboy SJ, Noller KL, O'Brien P, Kaufman RH, Townsend D, Barnes AB, Gundersen J, Lawrence WD, Bergstrahl E, McGorray S, Tilley BC, Anton J, Chazen G. Increased incidence of cervical and vaginal dysplasia in 3980 diethylstilbestrol-exposed young women—experience of the national collaborative diethystilbestrol adenosis project. *JAMA* 1984, **252**, 2979–2983.
90. Barrat J, Brocheriou C, Maria B, Dardois Y, Farguer C, Hervet E. Clear-cell vaginal adenocarcinoma in young girls. *A personal observation (Fr.)*. *J Gynecol Obstet Biol Reprod* 1975, **4**, 1093–1102.
91. De Graaff J, Siregar-Emck MTW, Visser G. Vaginal carcinoma in a young woman (Dutch). *Ned T Geneesk* 1976, **120**, 1569–1571.
92. Krupa J, Pelikan K. Vaginal cancer composed of clear cells found in a girl aged 18 years whose mother was treated during pregnancy by diethylstilbestrol (Agostilban) (Cz.). *Cesk Gynekol* 1977, **42**, 264–268.
93. Herbst AL, Robboy SJ, Scully RE, Poskanzer DC. Clear-cell adenocarcinoma of the vagina and cervix in girls: analysis of 170 Registry cases. *Am J Obstet Gynecol* 1974, **119**, 713–724.
94. Herbst AL, Cole P, Colton T, Robboy SJ, Scully RE. Age-incidence and risk of diethylstilbestrol-related clear cell adenocarcinoma of the vagina and cervix. *Am J Obstet Gynecol* 1977, **128**, 43–50.
95. Kinlen LJ, Badaracco MA, Moffett J, Vessey MP. A survey of the use of oestrogens during pregnancy in the United Kingdom and of the genito-urinary cancer mortality and incidence rates in young people in England and Wales. *J Obstet Gynaecol Br Commonw* 1974, **81**, 849–855.
96. Herbst AL, Cole P. Epidemiologic and clinical aspects of clear cell adenocarcinoma in young women. In: Herbst AL, ed. *Intrauterine Exposure to Diethylstilbestrol in the Human*, Chicago, American College of Obstetricians and Gynecologists, 1978, 2–7.
97. Melnick S, Cole P, Anderson D, Herbst A. Rates and risks of diethylstilbestrol-related clear-cell adenocarcinoma of the vagina and cervix. *N Engl J Med* 1987, **316**, 514–516.
98. Herbst AL, Scully RE, Robboy SJ, Welch WR, Cole P. Abnormal development of the human genital tract following prenatal exposure to diethylstilbestrol. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of Human Cancer*, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1977, 399–412.
99. Vessey MP. Epidemiological studies of the effects of diethylstilboestrol. In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H, eds. *Perinatal and Multigeneration Carcinogenesis*. Lyon International Agency for Research on Cancer. Scientific Publications, No 96, 1989, 335–348.
100. Herbst AL, Anderson S, Hubby MM, Haenszel WM, Kaufman RH, Noller KL. Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: a case control study. *Am J Obstet Gynecol* 1986, **154**, 814–822.
101. Herbst AL, Cole P, Norusis MJ, Welch WR, Scully RE. Epidemiologic aspects and factors related to survival in 384 Registry cases of clear cell adenocarcinoma of the vagina and cervix. *Am J Obstet Gynecol* 1979, **135**, 876–886.
102. Kramer MS, Seltzer V, Krumholz B, Talebian F, Tedeschi C, Chen S. Diethylstilbestrol-related clear-cell adenocarcinoma in women with initial examinations demonstrating no malignant disease. Obstet Gynecol 1987, **69**, 868–871.
103. Ghosh TK, Cera PJ. Transition of benign vaginal adenosis to clear cell carcinoma. *Obstet Gynecol* 1983, **61**, 126–130.
104. Sander R, Nuss RC, Rhatigan RM. Diethylstilbestrol-associated vaginal adenosis followed by clear cell adenocarcinoma. *Int J Gynecol Pathol* 1986, **5**, 362–370.
105. Robboy SJ, Herbst AL, Scully RE. Clear-cell adenocarcinoma of the vagina and cervix in young females: analysis of 37 tumors that

persisted or recurred after primary therapy. *Cancer* 1974, **34**, 606–614.
106. Fawcett KJ, Dockerty MB, Hunt AB. Mesonephric carcinoma of the cervix uteri: a clinical and pathologic study. *Am J Obstet Gynecol* 1966, **95**, 1068–1079.
107. Hammed K. Clear cell "mesonephric" carcinoma of uterine cervix. *Obstet Gynecol* 1966, **32**, 564–575.
108. Studdiford WE. Vaginal lesions of adenomatous origin. *Am J Obstet Gynecol* 1957, **73**, 641–656.
109. Herbst AL. Diethylstilbestrol and clear cell vaginal carcinoma. *Am J Med* 1987, **83**, 375–376.
110. Noller KL, O'Brien PC. Diethylstilbestrol and clear cell vaginal carcinoma. *Am J Med* 1987, **83**, 374.
111. Swan SH. Diethylstilbestrol and clear cell vaginal carcinoma. *Am J Med* 1987, **83**, 372–374.
112. International Agency for Research on Cancer IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Supplement 7, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs 1987, vols. 1–42, 273–278, Lyon.
113. Barter JF, Austin JM, Shingleton HM. Endometrial adenocarcinoma after *in utero* diethylstilbestrol exposure. *Obstet Gynecol* 1986, **67**, 84S–85S.
114. Lazarus KH. Maternal diethylstilboestrol and ovarian malignancy in offspring. *Lancet* 1984, **i**, 53.
115. Cunningham JR, Gidwani GP, Gupta MS, Duchesneau PM, Schumacher OP. Prolactin-secreting pituitary adenoma: occurrence following prenatal exposure to diethylstilbestrol. *Cleveland Clin Q* 1982, **49**, 249–254.
116. Vandrie DM, Puri S, Upton RT, Demeester LJ. Adenosquamous carcinoma of the cervix in a woman exposed to diethylstilbestrol *in utero*. *Obstet Gynecol* 1983, **61** (suppl.), 84S–87S.
117. Vessey MP, Fairweather DVI, Norman-Smith B, Buckley J. A randomised double-blind controlled trial of the value of stilboestrol therapy in pregnancy: long-term follow-up of mothers and their offspring. *Br J Obstet Gynaecol* 1983, **90**, 1007–1017.
118. Conley GR, Sant GR, Ucci AA, Mitcheson HD. Seminoma and epididymal cysts in a young man with known diethylstilboestrol exposure *in utero*. *J Am Med Assoc* 1983, **249**, 1325–1327.
119. Preston-Martin S. Epidemiological studies of perinatal carcinogenesis. In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H, eds. *Perinatal and Multigeneration Carcinogenesis*. International Agency for Research on Cancer, Lyon Scientific Publications, No 96, 1989, 289–314.
120. Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *Int J Cancer* 1979, **23**, 598–602.
121. Schottenfeld D, Washauer ME, Sherlock S, Zauber AG, Leder M, Payne R. The epidemiology of testicular cancer in young adults. *Am J Epidemiol* 1980, **112**, 232–246.
122. Depue RH, Pike MC, Henderson BE. Estrogen exposure during gestation and risk of testicular cancer. *J Natl Cancer Inst* 1983, **71**, 1151–1155.
123. Brown LM, Pottern LM, Hoover RN. Prenatal and perinatal risk factors for testicular cancer. *Cancer Res* 1986, **46**, 4812–4816.
124. Moss AR, Osmond D, Bachetti P, Torti FM, Gurgin V. Hormonal risk factors in testicular cancer. *Am J Epidemiol* 1986, **124**, 39–52.
125. Cutler BS, Forbes AP, Ingersoll FM, Scully RE. Endometrial carcinoma after stilbestrol therapy in gonadal dysgenesis. *N Engl J Med* 1972, **287**, 628–631.
126. Louka MH, Ross RD, Lee JH Jr, Lewis GC Jr. Endometrial carcinoma in Turner's syndrome. *Gynecol Oncol* 1978, **6**, 294–304.
127. Reid DE, Shirley RL. Endometrial carcinoma associated with Sheehan's syndrome and stilbestrol therapy. *Am J Obstet Gynecol* 1974, **119**, 264–266.
128. Khandecar JD, Victor TA, Mukhopadhyana P. Endometrial carcinoma following estrogen therapy for breast cancer. Report of three cases. *J Am Med Assoc* 1978, **138**, 539–541.
129. Bibbo M, Haenszel WM, Wied GL, Hubby M, Herbst AL. A twenty-five-year follow-up study of women exposed to diethylstilbestrol during pregnancy. *N Engl J Med* 1978, **298**, 763–767.
130. Beral V, Colwell L. Randomized trial of high doses of stilbestrol and ethisterone in pregnancy: long-term follow-up of mothers. *Br Med J* 1980, **281**, 1098–1101.
131. Greenberg ER, Barnes AB, Reseguie L, *et al.* Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med* 1984, **311**, 1393–1398.
132. Hadjimichael OC, Meigs JW, Falcier FW, Thompson WD, Flannery JT. Cancer risk among women exposed to exogenous estrogens during pregnancy. *J Natl Cancer Inst* 1984, **73**, 831–834.
133. Brian DD, Tilley BC, Labarthe DR, O'Fallon WM, Noller KL, Kurland LT. Breast cancer in DES-exposed mothers. Absence of association. *Mayo Clin Proc* 1980, **55**, 89–93.
134. Campbell JH, Cummins SD. Metastases, stimulating mammary cancer, in prostatic carcinoma under estrogenic therapy. *Cancer* 1951, **4**, 303–311.
135. Benson WR. Carcinoma of the prostate with metastases to breasts and testis. Critical review of the literature and report of a case. *Cancer* 1957, **10**, 1235–1245.
136. Bulow H, Wullstein H-K, Bottger G, Schroder FH. Carcinoma of the breast under estrogen treatment for prostatic carcinoma (Ger.). *Urologe* 1973, **A12**, 249–253.
137. Galanaud P, Chaput J-C, Buffet C, Rain B. Hepatoma after prolonged estrogen therapy. *Nouv Presse Med* 1976, **5**, 209.
138. Brooks JJ. Hepatoma associated with diethylstilbestrol therapy for prostatic carcinoma. *J Urol* 1982, **128**, 1044–1045.
139. Hoch-Ligeti C. Angiosarcoma of the liver associated with diethylstilbestrol. *J Am Med Assoc* 1978, **240**, 1510–1511.
140. Ham JM, Pirola RC, Crouch RL. Hemangioendothelial sarcoma of the liver associated with long-term estrogen therapy in a man. *Dig Dis Sci* 1980, **25**, 879–883.
141. Sadolf L, Winkley J, Tyson S. Is malignant melanoma an endocrine-dependent tumor? The possible adverse effect of estrogen. *Oncology* 1973, **27**, 244–257.
142. Bellet RE, Squitieri AP. Estrogen-induced hypernephroma. *J Urol* 1974, **112**, 160–161.
143. Nissenkorn I, Servadio C, Avidor I. Oestrogen-induced renal carcinoma. *Br J Urol* 1979, **51**, 6–9.
144. Walker BE. Tumors of female offspring of mice exposed prenatally to diethylstilbestrol. *J Natl Cancer Inst* 1984, **73**, 133–140.
145. Walker BE. *Animal models of prenatal exposure to diethylstilboestrol.* In: Napalkov NP, Rice JM, Tomatis L, Yamasaki H, eds. *Perinatal and Multigeneration Carcinogenesis*. International Agency for Research on Cancer, Lyon Scientific Publications, No 96, 1989, pp. 349–364.
146. Turusov VS, Trukhanova LS, Parfenov Yu.D, Tomatis L. Occurrence of tumours in the descendants of CBA male mice prenatally treated with diethylstilbestrol. *Int J Cancer* 1992, **50**, 131–135.

Acknowledgements—Supported by a grant from CEC (No. 90CVV01101). Dr M. Marselos spent a sabbatical year in Lyon, working as a consultant in IARC/WHO.