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Feature Articles

Diethylstilboestrol: II, Pharmacology, Toxicology and Carcinogenicity in Experimental Animals

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Diethylstilboestrol (DES) exerts several toxic effects in experimental animals, by mechanisms which are still unclear. The genotoxicity of the drug has been attributed to a quinone metabolite and is mainly clastogenic, including sister chromatid exchange, unscheduled DNA synthesis, chromosomal aberrations, disruption of mitotic spindle and aneuploidy. There is evidence that genotoxic effects may occur also transplacentally. Intrauterine and early postnatal exposure to DES can cause a variety of dysplasias. In the offspring of female mice exposed to DES during pregnancy, histological changes are observed in the vaginal and cervical epithelium, the endometrium, the ovary, the testis and the epididymis. Prenatal exposure of rats to DES led to decreased litter size and to urethrovaginal cloaca, penile and testicular hypoplasia, and cryptorchidism. Vaginal ridging, vaginal adenosis, testicular hypoplasia and cryptorchidism have been observed in rhesus monkeys following prenatal exposure. There is sufficient evidence that diethylstilboestrol is carcinogenic in experimental animals, after either prenatal or postnatal exposure. Mice show a similar type of carcinogenicity to that observed in humans, target organs being vagina, cervix, uterus, ovary, mammary gland and testis. In rats, prenatal exposure to DES produces mostly mammary and pituitary tumours, but also some tumours of the vagina. Hamsters develop tumours of vagina, cervix, endometrium, epididymis, testis, liver and kidney. DES induces ovarian papillary carcinomas in dogs, and malignant uterine mesotheliomas in squirrel monkeys. Some experimental evidence points to the possibility of a transgenerational carcinogenic effect, since prenatal treatment of mice with DES is followed by an increased incidence of uterine and ovarian carcinomas in the second-generation descendants. Experimental results could have been used to predict the adverse effects of DES observed in humans in the early 1970s: DES had been reported to be carcinogenic in mice in the 1930s, while experiments in the 1960s had provided evidence that exposure during pregnancy could result in an increased cancer risk in the progeny.
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INTRODUCTION

THE EXISTENCE of biologically active substances in the ovarian follicles, such as "folliculin" and other "oestrus producing hormones", was already known in the early years of the 20th century [1–3]. After the chemical, physiological and pharmacological characterisation of these substances, the first attempts were made to produce them synthetically [4, 5].

As a result, it was established that the oestrogenic properties may be retained even with molecular structures quite dissimilar to those of the endogenous phenanthrenic hormones, such as dihydroxy-diphenylmethane and dihydroxy-stilbene (stilboestrol) [5–7]. The diphenyl compounds had several toxic effects, but they were also powerful oestrogens. When the chain between the phenol groups was lengthened, the effect was increased, provided that the hydroxyl groups were retained [7], and by 1938, the diethyl-substituted stilbenes had been identified as the most active oestrogenic compound [8]. Given orally, diethylstilboestrol (DES) proved to be five times more potent than oestradiol, while its physiological and pharmacological profiles were closely comparable with those of the natural oestrogens [9–11].

The esters of DES exhibit a sustained action, with the dipropionate derivative having the longest activity [12]. Dienoestrol and hexoestrol are other alkylated derivatives of stilboestrol, with only minor structural and pharmacological differences from DES [13].

Very soon after its synthesis, DES was approved in the USA for therapeutic use in humans [14]. Thereafter, it was applied in many countries in cases of threatened abortion and whenever oestrogen replacement therapy was indicated. Nowadays, the use of the drug has been restricted to certain cases of prostatic and breast cancer, and it is locally applied for postmenopausal vaginal disorders. In the early 1950s, DES was approved in the USA as a growth promoter for livestock, given either as a feed additive or as an implant [15].

The medicinal and veterinary uses of DES have had to be re-evaluated several times, due to great concern over the toxicity and possible carcinogenicity of the drug in humans. The carcinogenic potential of DES has now been established by many epidemiological studies [16–19], and it is the only drug with formally proven transplacental carcinogenic action in humans [20]. Furthermore the hypothesis has been advanced that increased concentrations of oestrogens during pregnancy may

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increase the risk of occurrence of breast cancer in daughters [21].

The epidemiological evidence for the carcinogenicity of DES has been substantiated by several experimental studies, which have provided further information on the toxicity of the drug and its possible mechanism of action. In this article, the relevant experimental evidence is reviewed and evaluated.

PHARMACOKINETICS

DES is readily absorbed after oral administration, although the exact percentage of its intestinal absorption is difficult to calculate due to extensive enterohepatic circulation [15, 22].

DES is a lipid-soluble substance which is readily distributed in the whole organism. Studies on its transfer across the placenta in mice have shown that it accumulates in the fetal genital tract, where it reaches levels three times higher than in the foetal plasma [23].

In the rat, it was demonstrated that after intestinal intubation of DES or DES-glucuronide, free DES is readily absorbed through the epithelium, whereas the conjugated form requires prior hydrolysis by the intestinal microflora. It is further established that DES conjugates cannot cross any biological membrane, including the placental barrier, without previous hydrolysis [24, 25]. Whole animal autoradiography experiments showed that radioactive DES injected intravenously into rats is accumulated in the liver and small intestine within 4 h, and radioactivity was still detected in these organs after 4 days [26].

Peak plasma levels of radioactivity were found within 16 h in sheep given radiolabelled DES at single oral doses of 100, 4 or 3 mg. Radioactivity disappeared almost completely after 120 h [27]. Sheep treated daily with 4 or 3 mg of DES were killed on day 14 and tissue levels of radioactivity corresponded with less than 1 μg/kg of DES in all organs [27].

Ten days after a single oral dose of radioactive DES to steers, residues could be detected only in the small intestine, the faeces and the urine. The intestinal radioactivity, being less than 1% of the dose used, corresponded mainly to free DES, while the bile and the gall bladder contained only the DES–glucuronide [28].

In cattle given a single oral dose of radioactive DES (10 mg), the kinetics followed a biphasic depletion curve attributed to hepatic clearance. An initial steeper slope represented a biological half-life of 17 h, while the half-life for the later phase was 5.5 days [29]. Pellets of 24–36 mg DES implanted subcutaneously in cattle or steers liberated about 56–74 μg of DES per day into the circulation; the half-life was 80–90 days [22, 30]. The distribution of DES has been studied in the tissues of steers also after application of ear implants with radioactive drug [30]. Between 30 and 120 days, the concentrations of radio-activity in the bile were 30–60 times greater than in the blood. In animal models used for the pharmacokinetics of DES (with the exception of primates), it is apparent that the drug is almost exclusively eliminated through biliary excretion into the intestine, where it undergoes extensive enterohepatic circulation before being excreted in the faeces. Only traces of DES can be detected in urine [15].

METABOLISM

The metabolism of DES has attracted much interest, especially because of suggestions that toxic intermediate products may be generated during DES degradation [31, 32].

A possible pathway of DES metabolism in rats is *ortho*-hydroxylation to the catechol metabolite, followed by methylation by catechol-0-methyltransferase [33]. Major metabolites of DES in several species (rat, mouse, hamster, primates) are dienoestrol and omega-hydroxydienoestrol [31, 32, 34, 35], which are thought to originate from epoxide and quinone intermediates [32, 36]. An epoxide may also be formed at the double bond of the stilbene, to give 4′-hydroxypropiophenone, which is detected in rat urine after DES administration [32]. Both the quinone and epoxide intermediates have been suggested as the reactive metabolites of DES [31, 35]. The microsomal oxidation of DES can be enhanced by pretreatment with phenobarbital [37]. It is known that metabolites of DES, formed by rat liver microsomes or by mouse fetal cells in primary cultures, can bind covalently to DNA [38].

In mice [23, 35, 39], rats [40] and cattle [41], DES and its metabolites are extensively conjugated with glucuronic acid and then excreted into the intestine with the bile. In contrast, sulphate conjugation seems to be the most important synthetic route for the DES metabolism in the liver of the guinea pig [42].

In rhesus monkeys (*Macaca mulatta*) and chimpanzees, the urine is the main route of excretion [34, 43].

TOXICOLOGY IN ANIMALS

General toxicity

The acute toxicity potential of DES is rather high in rodents, LD_{50}s being about 35 and 70 mg/kg bw for intraperitoneal doses in mice and rats, respectively [44, 45].

In mice, DES exerts a definite effect on the thymus and the immune system as a whole. There is a decrease in delayed hypersensitivity response and increased phagocytosis of foreign material by the reticuloendothelial system [17, 46, 47]. Also, reduced haematopoiesis has been found, with diminished peripheral blood-cell counts [48]. In two different strains of mice, chronic administration of DES in the diet resulted in a markedly reduced ovarian weight when the drug exceeded the level of 25 μg/kg diet [49].

The female newborn mouse is considered to be a suitable model for investigating the effect of oestrogens on the development of the genital epithelium, a process that in humans occurs during the first trimester of pregnancy. In female mice given daily subcutaneous injections of 5 μg DES for the first 5 days after birth and killed 13 months later, extensive adenosis was observed, comprising most of the cervical wall. The vaginal epithelium showed hyperplasia and squamous metaplasia, with some glandular ducts penetrating far into the subepithelial tissue [50]. These and other similar results have generated the hypothesis that DES allows columnar epithelia of Müllerian duct origin to persist and invade or overgrow into areas which should be of a squamous cell type. The inhibition of squamous epithelial cell proliferation leads to adenosis and perhaps later to neoplastic changes in the tissue [50, 51].

Teratogenicity

In pregnant mice, DES was given on days 9–16 of gestation, at dose levels of 0.01–100 μg/kg bw, in order to study the long-term effect on the offspring. In the mature female offspring, the reproductive capacity was gradually diminished in a dose-dependent way, and was completely lost for doses over 10 μg/kg bw. There were abnormalities of the genital tract, including cystic endometrial hyperplasia, persistent cornification of the vaginal epithelium, oedema and stromal hyperplasia of the cervix and ovarian cysts. Among the mature male offspring, exposed prenatally at a dose of 100 μg/kg bw, 80% were sterile, and they had a high incidence of alterations in the reproductive tract [52].

When female mice of the NMRI strain were treated postnatally with DES (5 μg/day/animal, for the first 5 days of life), an inhibitory effect on the biochemical pathways of ovarian steroidogenesis was seen. These changes may be responsible for the disruption of the normal FSH–LH stimulation of the maturation of the ovaries during adulthood, and may also lead to dysplasia [53].

Other effects in the progeny of mice include undescended testes and hypogonadism [54] and cleft palate in a dose-dependent way [55]. In male newborn mice, very large doses of DES (1 g/kg bw) could produce histopathological changes of a benign nature, like epididymal cysts and fibrotic testes [56].

Several dysplasias were also noted in the offspring of rats given subcutaneous injections of DES (0.015–0.6 mg/kg bw) on days 13, 16, 18 and 20 of pregnancy. In female offspring, hypospadia and urethrovaginal cloaca were seen. In male offspring, there were hypospadia, phallic hypoplasia and inhibition of the growth and descent of testes [57,58].

A dose-related decrease in number of live offspring was found in female rats treated subcutaneously with DES (0.01–0.1 mg/kg bw/day) between days 10 to 18 of gestation. Above a dose of 0.02 mg/kg bw/day, there were no live offspring. The fertility of the female offspring was severely decreased (70–100% sterility), whereas that of the male offspring was affected to a much lower extent (11–18% sterility) [59].

In female rabbits, injection of DES before days 12–14 of gestation caused termination of pregnancy [60].

In pregnant rhesus monkeys (*Macaca mulatta*), administration of DES in three different time-protocols (day 21 to delivery, day 100 to delivery and day 130 to delivery) resulted in vaginal ridging and/or cervical hooding in seven out of eight female offspring. At the age of 5.5 years, vaginal adenosis was found in three of these female offspring [61]. In the same experiments, three out of five male offspring exhibited one or more abnormalities in the genitalia, including testicular hypoplasia, preputial adhesions and undescended testes [62].

Carcinogenicity studies in animals

Exposure during adult life. Lacassagne was the first to demonstrate the carcinogenicity of DES by the induction of mammary tumours in male mice [63], and his findings were confirmed by Shimkin and Andervont [64].

Newborn male and female mice were injected subcutaneously with 2 mg DES within the first 24 h of life. In female BALB/c mice, cancers of the cervix and/or vagina were found after 13–26 months in 6/17 animals, while in female C3Hf mice, the incidence was 3/10 at 24–26 months. Male mice did not show any unusual tumour but 5/10 BALB/c and 7/10 C3Hf mice had single or multiple, often bilateral, epididymal cysts [51].

In male mice of two strains (ZD_8F and AZF_1), feeding of a semisynthetic diet containing DES (average intake, 0.5 μg/animal per day) resulted in the appearance of mammary tumours. The incidences of tumours were lower (11/30 and 12/37) and the average latency times longer (14.6 months and 18.8 months) in intact males than in castrates (incidences, 33/34 and 19/20; latency times, 10.7 months and 14.3 months) [65].

The incidence of mammary carcinomas in female C3H and A mice fed DES was proportional to the dose for a range between 25 and 1000 μg/kg diet. An inverse relation was noticed for the latency times. In male C3H mice, mammary tumours were observed only at concentrations of DES as high as 500 μg/kg diet or more [55].

Male mice with a high rate of spontaneous leukaemia had a further enhanced incidence of leukaemia after weekly subcutaneous injections of 50 μg/kg DES in oil for 7 months [66].

Male and female ICR Jcl mice were injected subcutaneously with a single dose of DES (10 or 100 mg/kg) at the age of 21 days, and were killed after 12 months. The incidence of ovarian cystadenoma was increased in the female mice treated with the higher dose [52].

Pellets containing 0.2–2 mg of DES were implanted subcutaneously in C mice, and the development of tumours was followed up to the 16th month of age. Interstitial-cell tumours of the testis were detected between 6 and 11 months with a dose-dependent frequency. Lymphoid tumours were found in both male and female mice between 8 and 14 months [67]. Lymphoid tumours could be induced by DES implants also in mice of the C3H strain [68].

Pellets of DES (5 mg, subcutaneous) induced interstitial-cell carcinomas of the testis also in BALB/c mice, but several other strains were found to be resistant, probably due to genetic factors [69].

Epidermoid carcinomas of the vagina and/or the cervix developed in mice treated intravaginally with an oil solution or a pellet of DES [70].

In rats, DES given in diet, at doses of 0.02–0.2 mg/kg bw per day, resulted in an increased incidence of fibroadenomas of the mammary gland in females and in a slight increase of pituitary tumours, in both sexes after 18–24 months [71]. Also after a single subcutaneous administration of 20 mg DES to rats, gross and microscopic hyperplasia of the pituitaries was observed [72]. The considerable interstrain variation in the induction of pituitary and mammary tumours by DES in rats is probably attributable to genetic factors [73, 74].

In castrated rats, DES induced hepatic tumours when given alone or in coadministration with *N*-nitrosobutylurea [75].

In male Syrian golden hamsters (*Cricetus auratus*), subcutaneous implantation of DES (20 mg) induced malignant adenomatous renal tumours [76]. These tumours develop easily in normal or castrated hamsters, but mature female animals appear to be unaffected. The tumours of the male animals give metastases in the abdominal cavity [77, 78], and they could be transplanted in animals under DES treatment, but regressed rapidly on removal of the oestrogen pellet [79–81].

Injections of DES (thrice per week; subcutaneously dose 6 mg/animal) in male Syrian golden hamsters produced renal tumours between 6 and 9 months. When DES injections were alternated with nafoxidine (a non-steroidal anti-oestrogen) the tumorigenic effect was reportedly abolished [82].

In male and female Syrian golden hamsters treated subcutaneously with DES (1.5 mg/kg bw/week), undifferentiated sarcomas were seen around the interscapular injection site. As tumours were observed both in male and female animals, it was suggested that the local carcinogenicity of DES might not be related to its hormonal effects [83].

In Armenian hamsters (*Cricetulus migratorius*), DES implanted subcutaneously as a 15 mg pellet led to the development of hepatic tumours, as early as 45 days after the implantation. Histopathologically, the tumours appeared as multicentric adenocarcinomas of varied degrees of differentiation, and frequently contained Mallory bodies (43%). Preneoplastic nodules could not be detected [84].

In dogs, ovarian lesions (six papillary carcinomas, one papillary adenoma and one hyperplasia) were found in all eight female dogs given subcutaneous injections of 15–60 mg DES in paraffin oil at 7–8 week intervals over 19 months (total dose, 90–495 mg)

[85]. In a further study, 10 female dogs received total subcutaneous doses of 60–495 mg/animal DES for up to 455 days: nine developed ovarian tumours described as papillary carcinomas, and one had "papillary dysplasia"; three carcinomas (diagnosed by biopsy) were found at the time of DES withdrawal [86].

In squirrel monkeys, DES pellets (60 mg) were implanted subcutaneously, and the animals were killed and examined histologically between 5 and 14 months after the beginning of the experiment. Malignant uterine mesotheliomas were detected in 70% of the animals [87].

There is evidence that DES affects the hepatocytes in a similar way to phenobarbital, increasing the liver weight and enhancing the activity of enzymes involved in drug metabolism [88, 89]. It has been used successfully as a promoter in studies of hepatocarcinogenesis, after an established liver carcinogen was given for initiation [90–92].

Prenatal exposure. DES has been shown to have a transplacental carcinogenic effect in several animal species similar to that observed in humans [19].

In mice, DES was given subcutaneously on days 9–16 of pregnancy at a range of doses (0.01–100 μg/kg bw). Survival of the offspring was inversely proportional to the dose level. Adenocarcinomas of the uterine endometrium and epidermoid tumours of the cervix and vagina were observed at 6–15 months in 10% or less of the surviving female offspring in all treated groups [62].

Pregnant ICR/Jc1 mice were given single subcutaneous injections of DES (10 mg/kg bw) on days 7–19 of pregnancy. The incidence of lung papillary adenomas was significantly increased in mice of both sexes whose mothers had been treated on day 15 of pregnancy (7/29 v. 0/14 in controls). Cystadenomas and granulosa-cell tumours of the ovary were significantly increased in female offspring of mice treated on day 15, 17 or 19 of pregnancy (3/17, 6/15, 4/33 v. 0/8 controls). No tumour of the genital tract was observed [52].

In several other experiments in which mice were prenatally exposed to DES, adenocarcinomas of the uterus, cervix and vagina, epidermoid carcinomas of the uterine cervix and vagina and ovarian and mammary tumours developed [93–97].

Pregnant ACI rats were given subcutaneous injections of DES at total doses of 0.8 or 8.0 μg/animal. Over the next 10 months, the female offspring developed mammary tumours with a significantly higher incidence than in controls. The incidence was even higher in animals exposed to DES both prenatally and postnatally. Tumour multiplicity at time of killing was significantly increased in the group given the higher prenatal dose of DES [98].

In pregnant Wistar rats, different levels of DES were administered on days 18, 19 and 20 of the gestation, and the female progeny was followed until death or 2 years of age. Epithelial tumours of the vagina (adenocarcinoma, squamous cell carcinoma and mixed carcinoma) were seen, with dose-dependent incidence. Control animals had spontaneous tumours at a rate of 0.6%, whereas the incidence was 4.3% for the maternal dose of 0.5 mg/kg, and 11.5% for the maternal dose of 50 mg/kg. There was no discernible dose–response relationship for tumours of other tissues of the reproductive tract (mammary gland, ovary, oviduct, uterine cervix or uterus) [99].

Syrian golden hamsters were given DES with a gastric tube, either as single dose (20 or 40 mg/kg bw) on day 15 of gestation, or as two consecutive doses of 20 or 40 mg/kg bw on days 14 and 15 of gestation. Reproductive tract neoplasms were seen in 28% (4/14) of female progeny when a single 40 mg/kg dose was used, and in 50% (4/8) when two 40 mg/kg doses were given. The male progeny had granulomas of the epididymis (70%) and testis (40%) and epididymal cysts (20%) [100].

In other studies with hamsters treated prenatally with DES, female animals developed endometrial adenocarcinoma and squamous-cell papillomas of the cervix and vagina [101].

In female rhesus monkeys (*Macaca mulatta*) prenatally exposed to DES, despite some dysplasias in the genital tract (see section on teratogenicity above), no vaginal or cervical adenocarcinoma was detected even after a follow-up of 7 years [61].

Multigenerational carcinogenicity. There is experimental evidence that prenatal treatment with DES increases the incidence of uterine and ovarian carcinomas in the second-generation descendants, which were not exposed directly to the drug [102]. These results, and the overall complexity of DES carcinogenicity, have led to a theoretical model of a possibly multigenerational "transmission" of cancer risks [103], a hypothesis advanced on the basis of several experimental and epidemiological observations [104]. 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 unexposed females [105].

Genotoxicity

In bacterial short-term mutagenicity tests, DES has not shown any mutagenic activity. It does not induce reverse mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA100 and TA98. Negative results in *S. typhimurium* were also obtained with 11 metabolites and other derivatives of DES (106–108), although at least two of them (diethylstilboestrol-3, 4-oxide and beta-dienoestrol) are very effective in inducing sister chromatid exchanges in cultured human fibroblasts [109].

Despite the negative results in the Ames test, DES was found to lead to chromosomal aberrations in tests carried out with Chinese hamster fibroblasts *in vitro* [110]. Chromosomal damage was also seen in cultured white cells of human peripheral blood, when DES was applied at concentrations of 5, 15 and 20 μg/ml [111]. The genotoxic effect of DES on epithelial cells from the neonatal mouse uterine cervix is evident either as chromosomal aberrations after *in vivo* administration [49], or as increased frequency of sister chromatid exchanges in cell cultures [112].

DES enhanced the transformation rate of BALB/c 3T3 cells, especially when hepatocytes were added to the culture as an exogenous metabolic activation system [113].

Male mice exposed to a single intraperitoneal injection of 1, 10 or 100 mg/kg bw DES diphosphate had increased numbers of aneuploid cells in their bone marrow after 6, 24 and 48 h, but the effect was not dose-dependent [114].

Single doses of 1.0, 0.1 or 0.01 μg diphosphate dissolved in 0.85% sodium chloride were injected intraperitoneally 6, 24 or 48 h before day 9 of gestation into 45 mice of four different strains. The embryos exposed (N = 307) were collected for chromosome preparation and examination. A significant increase of aneuploidy was detected, depending on dose and time of exposure [115].

Experiments with embryonic hamster cells have verified the formation of abnormal or arrested mitotic spindles due to the disruption of microtubules, and have led to the hypothesis

that this is the mechanism by which DES causes aneuploidy [116–119].

A DES concentration of 10^{-6} mol/l induced unscheduled DNA synthesis in HeLa cells in the presence of a liver activation system [120]. It has been shown that DES can form DNA adducts in the liver, kidneys and uterus of hamsters injected with a single large dose of radiolabelled drug (220 mg/kg) [121]. This type of adduction also occurs under *in vitro* conditions, and the metabolite diethylstilboestrol-4',4h'-quinone may be the key feature in the genotoxicity of DES [122]. In the kidneys of male Syrian hamsters, it was possible to detect the formation of 8-hydroxydeoxyguanosine due to the generation of free radicals in the process of redox cycling between DES and its quinone [123].

In pregnant hamsters, DES was given on the 10th day of gestation at a single dose of 200 mg/kg and the animals were killed 5 and 24 h after treatment. The genotoxic quinone metabolite of stilbene was found to form DNA adducts in several maternal tissues, and also in the fetal heart and kidney, indicating that DES is a genotoxic agent in both adult and fetal tissues [124].

CONCLUSIONS

Diethylstilboestrol (DES) has been shown to be carcinogenic when given by several routes of administration to adult mice, rats, hamsters and monkeys. It is also mutagenic and teratogenic. Tumours of the reproductive system have been induced in mice, rats and hamsters following prenatal exposure.

The mechanism(s) through which DES exerts its carcinogenic effect remain unclear. Despite the absence of a mutagenic effect in the Ames tests, DES reduces unscheduled DNA synthesis, induces chromosomal aberrations, disruption of mitotic spindle, aneuploidy, and increases the frequency of sister chromatid exchanges. Moreover, its quinone metabolite is able to form DNA adducts in various organs, including liver, kidney and uterus. DES also produces a variety of effects which may be involved in the carcinogenic process, such as changes in the plasma levels of hormones, in the hypothalamus–pituitary–gonadal axis, in the hormonal receptors of specific target organs, and in the immune system.

DES is also a well documented human carcinogen (see ref. 19 for a review). The link between prenatal exposure to DES and the occurrence of a rare tumour in young girls was established in 1970 [16] by an alert clinician. The experimental data that already existed proving that DES had a carcinogenic activity and that carcinogens could act transplacentally were unfortunately not used to prevent adverse effects in humans. The first evidence that oestrogens, including DES, had a carcinogenic activity had been obtained with mice in the 1930s [63]. The results of Lacassagne, perhaps because they involved a few animals and were published in French, did not attract much attention outside a limited circle of oncologists. Similarly, the experimental results of the 1960s showing that the uterine environment was not totally safe, and that exposure of pregnant animals to a carcinogen could result in an increased incidence of tumours in the progeny [22], were noted by only a limited number of scientists.

In the early part of this century, the observations by Yamagiwa and Ichikawa of chemical carcinogenicity in rabbits [125], and a few years later by Tsutsui in mice [126], were acclaimed as the confirmation of the observation made by Percivall Pott on humans over a century before. The case of DES and of transplacental carcinogenesis should be taken as a reminder that experimental results can indeed be used to predict similar effects in humans.

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