Journal of Steroid Biochemistry, Vol. 11, pp. 435 to 442
Pergamon Press Ltd 1979. Printed in Great Britain

THE STEROID-CANCER HYPOTHESIS AND RECENT PERTINENT EPIDEMIOLOGICAL STUDIES

ROY HERTZ
The George Washington University Medical Center, Washington, D.C., U.S.A.

SUMMARY

Extensive data concerning the tumorigenic effects of oestrogenic and progestagenic compounds in numerous species of animals indicate that dosage, genetic strain, age and duration of exposure are critical factors in eliciting such hormone-induced tumours. The occurrence of vaginal adenocarcinoma in the off-spring of women exposed to oestrogen during pregnancy extends the animal data to the human being.

Recently accumulated epidemiological studies establish a negative association between the incidence of benign breast tumours in women and exposure to oral contraceptives. However, the incidence of breast cancer is shown in a number of case-controlled retrospective studies to be unaffected by oral contraceptive use except for certain subsets consisting of unacceptably small numbers. Moreover, these studies relate to a follow-up period which is too brief for definitive evaluation since the human carcinogenic response is known to require one to two decades for full expression.

The incidence of endometrial carcinoma has been found in a number of case-controlled retrospective studies to be increased in women using oestrogens for the control of menopausal symptoms. Some of these studies indicate a greater effect with more prolonged usage. However, other studies utilizing a different basis for identification of controls fail to demonstrate an increased incidence of endometrial cancer in oestrogen-treated menopausal women.

It is clear that the currently available epidemiological data provide presumptive but not conclusive evidence of an increased incidence of endometrial cancer in oestrogen-treated menopausal women. More definitive studies are urgently needed to determine in more precise terms the relative incidence of endometrial cancer in oestrogen-treated and untreated menopausal women.

INTRODUCTION

The steroid-cancer hypothesis postulates that both endogenous and exogenous steroids and related hormonally active synthetic compounds play a significant and possibly a etiologic role in the pathogenesis of cancer in steroid responsive tissues.

The genesis of this hypothesis warrants our interest because it rests upon some of the earliest findings concerning the biologic action of the gonadal hormones. Initially, Allen and Doisy [1] in 1923 defined the essential somatic effect of the oestrogens in terms of their capacity to cornify the vagina of ovariectomized rodents. This actually led Allen *et al.* [2] to utilize colchicine to aid in the analysis of the stimulation by oestrogens of the mitotic activity in the vaginal mucosa and in the myometrium of the rat. With this technique they reported that a transverse section of the vagina at 37 h after a single injection of oestrogen contained up to 1585 mitotic figures. Comparable figures for mitotic activity were also reported for the oestrogen-stimulated uterus [3]. Hence, the experimenter had in hand a potent growth-stimulating agent which induced in the female genital tract the type of excessive mitotic activity generally associated with malignancy.

These remarkable experimental findings appeared against a background of the earlier observation of Beatson[4] in 1898 that ovariectomy ameliorated the clinical course of breast cancer in women. However, it was not until 1919 that Loeb[5] demonstrated a similar ovarian dependence in breast cancer in mice.

As the early preparations of the female sex hormone became available, their initial clinical use was attended by widespread concern as to their potential effect on the pathogenesis of cancer of the breast and the female genital tract.

This concern was increased by the finding that the potent chemical carcinogens of the methylcholanthrene type also belong to the cyclopentenophenanthrene family [6].

In many laboratories throughout the world a mass of experimental data accumulated indicating that oestrogen administration is followed by regularly reproducible tumours in five species of animals and in eight organ sites [7–9] (Table 1). These included tumours of the breast, cervix, endometrium, ovary, pituitary, testicle, kidney, and bone marrow in either mice, rats, rabbits, hamsters or dogs.

Nevertheless, the repeated failure of similar experiments to elicit neoplastic response in monkeys (*M. rhesus*) created substantial scepticism as to the potential relationship of the rodent findings to man [10–13]. However, more recent studies have demonstrated malignant uterine mesotheliomas in squirrel monkeys following oestrogen administration [14].

These animal investigations of the past 30 years have provided certain criteria as to the determinant factors involved in experimental tumour induction by exogenous oestrogens. The first of these is dosage. This has generally far exceeded estimated endogenous hormone production levels for the respective species

Table 1. Oestrogen and progestin-induced tumors in animals

Species	Site	References
Mouse	Breast	Lacassagne M. A.: Apparition de cancers de la mammelle chez la souris mal, soumise a des injections de foliculine. *C.R. Acad. Sci. (Paris)* **195** (1932) 630.
Mouse	Cervix	Gardner W. U., Allen E., Smith G. M. and Strong L. C.: Carcinoma of the cervix of mice receiving estrogens. *J.A.M.A.* **110** (1938) 1182.
Mouse	Testis	Gardner W. U.: Testicular tumors in mice of several strains receiving triphenylethylene. *Cancer Res.* **3** (1943) 92.
Mouse	Pituitary	Gardner W. U.: The effect of estrogen on the incidence of mammary and pituitary tumors of mice. *Cancer Res.* **1** (1941) 345.
Mouse	Bone marrow	Gardner W. U.: Lymphoid tumors in estrogen treated mice. *Cancer Res.* **2** (1942) 725.
Mouse	Ovary	Lipschutz A., Iglesias R., Pamosevich V. and Salinas S.: Ovarian tumors and other ovarian changes induced in mice by two 19-nor contraceptives. *Brit. J. Cancer* **21** (1967) 153.
Hamster	Kidney	Kirkman H. and Bacon R. I.: Malignant renal tumors in male hamsters treated with estrogen. *Cancer Res.* **10** (1950) 122.
Rat	Breast	Nelson W. O.: The induction of mammary carcinoma in the rat. *Yale J. Biol. Med.* **17** (1944) 217.
Rat	Pituitary	Nelson W. O.: The occurrence of hypophyseal tumors in rats under treatment with diethylstilbestrol. *Am. J. Physiol.* **133** (1941) 398.
Rabbit	Endometrium	Griffiths C. T. *et al.*: Effects of progestins, estrogens, and castration on induced endometrial cancer in the rabbit. *Surg. Forum.* **14** (1963) 399.
Dog	Ovary	Jabara A.: Induction of ovarian canine tumors by diethylstilbestrol and progesterone. *Aust. J. Exp. Biol. Med. Sci.* **40** (1962) 139–152.
Dog	Breast	Finkel M. J. V. and Berliner V. R.: The extrapolation of experimental findings (animal to man); the dilemma of systemically administered contraceptives. *Bull. Soc. Pharm. Env. Pathol.* **4** (1973) 13.
Squirrel monkey	Uterus	McClure H. M. and Graham C. E.: Malignant uterine mesotheliomas in squirrel monkeys following diethylstilbestrol administration. *Lab. Anim. Sci.* **23** (1973) 493.

utilized. However, we lack data concerning the minimal effective doses required in most instances.

A second factor is the genetic strain of the test animals. Here we have most knowledge with respect to the mouse in relation to breast cancer. Both highly responsive as well as virtually unresponsive strains have been identified. Moreover, the genetic susceptibility for the requisite interaction of hormone with the virus-like milk factor in inducing breast cancers in C_3H mice is clearly established [7].

Age of onset of exposure has proven critical in numerous experimental situations. This has led to the impression that the younger an animal is during initial exposure, the more susceptible it will be to hormonal tumour induction. This factor is further complicated by the critical role of duration of exposure, since it generally requires a substantial portion of the life span of treated rodents to effect tumour formation [7].

It would seem useful, then, to anticipate that in human exposure a significant impact might be anticipated from: (a) dosage levels; (b) age and chronicity of exposure; (c) ethnic or genetic derivation; and (d) the potential presence of predisposing virus-like factors affecting the ultimate hormone response.

Of these factors, the genetic or familial factor appears to be most patently applicable, particularly in relation to breast cancer. The increased risk of breast cancer among daughters of affected women has led to inclusion of a warning on this point in the package inserts of oestrogen preparations. Conversely, the very genetic heterogeneity of the human population may very well prove to be a basis for a very low frequency of carcinogenic response among exposed populations.

In relation to dosage, we are at a total loss to make any kind of extrapolation from the animal data. In general, it would seem that a chronic elevation above the level of endogenous oestrogen production would qualify as a potential carcinogenic dose.

This consideration is, of course, intimately related to the factor of duration of exposure. The animal data would not lead one to expect a tumour response from occasional, short-term adult exposure such as that involved in the post-coital pill or in suppression of lactation. However, such long-term exposures as are involved in chronic replacement therapy or in steroid contraception involving a substantial portion of the individual's life-span would be most suspect. In the latter instance, one would also have the additional predisposing factor of exposure of young individuals, in some cases before the completion of their general somatic development.

In addition to these considerations, the prolonged latent period known to be involved in the human response to known carcinogens must be kept in mind in the evaluation of epidemiologic data in man. A follow-up period of one or two decades is known to be required for clinical manifestations in response to such human carcinogens as aniline dyes, arsenicals, nickel, asbestos, and ionizing radiation [15, 16].

Some of these factors are put to a test in the highly conclusive reports from several sources which have recently demonstrated an association between maternal exposure to oestrogens during pregnancy and the subsequent incidence of vaginal adenocarcinoma in

the female children [17–20]. The association is statistically highly significant and a number of the epidemiologic features of the syndrome fit the expectations outlined above. First, the overall incidence among the presumably exposed and genetically heterogenous population is very small. Thus, a review of a large population of exposed mothers at the Mayo Clinic failed to reveal a single treatment-associated case [21]. Jick *et al.*[21(a)] have estimated the size of the exposed population and have deduced that a very low attack rate results. Nevertheless, the cumulative experience of an especially developed Registry has provided unequivocal evidence of an association between the disease and stilbestrol treatment and has characterized the syndrome in great detail.

As in the animal studies, the dosages employed were very high, although some cases are associated with very low dosage and some with no known exposure. The data do not provide a distinct dose–response curve, since most exposures were quite heavy.

Most interestingly, the latent period for clinical evidence of carcinogenic response can be pinpointed with unique precision. The postnatal age of the presenting patient plus the 6 months or so intervening between the known critical period for exposure provides a remarkably accurate measure of clinical latency. Accordingly, the age distribution of the presenting patients show a few cases to be younger than 10 years of age and a few older than 20 years of age [18]. The distribution is classical for human carcinogenic response [15].

However, although most of the mothers received treatment throughout pregnancy, selected cases indicate a highly specific and fairly narrow critical phase of pregnancy for effective exposure. This aspect deviates from the laboratory worker's expectation of a requisite sustained exposure. Hence, one may consider this remarkable effect to be a "terato-carcinogenic" effect rather than a usual type of postnatal carcinogenic response.

Moreover, this remarkable phenomenon jumps the species gap between animal findings and human experience. Yet it is considered that since only the "synthetic" type of oestrogen has been statistically associated here, only this class of oestrogen need be held suspect for clinical usage. This bias in the data stems from the fact that in the dosages thought to be required at the time when these mothers were exposed, the cost differential between "synthetic" and "natural steroids" was so great as to preclude the use of the more costly agents. Hence the exposure of this group of patients, as well as of most women treated for other indications, was predominantly by synthetic agents of the stilbene type. It is fallacious to infer that the observed effects are attributable to any specific type of oestrogen, since we have no experimental evidence of any such differences in effectiveness between steroidal and nonsteroidal oestrogens [7].

ENDOMETRIAL CANCER AND MENOPAUSAL OESTROGEN USE

The association of endometrical cancer with excessive endogenous oestrogen, as in the case of women with Stein–Leventhal disease and with other forms of endogenous steroid derangement, suggests a chronic stimulatory effect on this responsive tissue. Hertig and Sommers[22], Gusberg[23], Gusberg and Kaplan[24], Speert[25], and many others have emphasized the pathogenetic relationship of adenomatous hyperplasia related to excessive endogenous or exogenous oestrogen and endometrial cancer.

Until recently, the study of the association of oestrogen administration and endometrial cancer was limited to a number of epidemiologically meaningless reports. Thus by 1954 Ostergaard[26] and Freemont-Smith *et al.*[27] cited 13 prior articles bearing on this relationship and reported a frequent occurrence of endometrial cancer in oestrogen-treated patients. In contrast, other equally inadequate reports indicated an absence of such an association [28, 29]. Such studies lacked the necessary sample size and appropriate controls to warrant any conclusion.

More recently, eleven attempts to test the hypothesis of an association between oestrogen therapy for menopausal symptoms and endometrial cancer have been undertaken in epidemiological terms. Six of these studies are available in the formal literature [30–35]. Some of their salient features are partially summarized in Table 2. The remainder of these studies have been communicated but are still in process of publication [36–40].

With two exceptions, these studies have indicated an increased risk ratio for endometrial cancer among menopausal oestrogen users. In most instances this increase is shown to be greatest among long-term users and to a lesser extent also among those receiving higher dosages.

Unfortunately, such case-controlled retrospective studies are so replete with potential sources of bias and inadvertent error that the data are subject to varied interpretations. The greatest difficulty hinges upon the adequacy of the controls employed. In one instance [32] the controls are women with gynaecologic cancer other than endometrial cancer. So much is already known about the epidemiologic differences between women with, for example, cancer of the cervix and cancer of the endometrium that it seems inappropriate to make this type of comparison. The necessity for care in the matching of controls in retrospective case-controls studies cannot be overemphasized.

It should also be appreciated that the findings in any study apply to the population under investigation and may or may not be pertinent to a different population group. Thus, the selection of oestrogen-treated breast cancer patients for study yield data of significance only for women with pre-existing breast cancer [41]. Similarly, patients enrolled in a pre-paid medical service or patients consulting a single phys-

Table 2. Salient features of six epidemiological studies regarding menopausal oestrogen use and endometrical cancer

Senior Author and Reference	Source of Patient Recruitment	No. of Cases	No. of Controls	Risk Ratio	Risk Ratio by Duration of use	Method of Assessment of Drug Use
Zeil [30, 30(a)]	Prepaid medical service	94	188	7.6	5.6 (5 + years) 13.9 (7 + years)	Medical records
Mack [31]	Affluent retirement community	55	191	8.0	2.8 (1 year) 8.8 (8 + years)	Clinical records Local pharmacy interviews
Smith [32]	Local hospital admissions	317	317	4.5	Not sought	Hospital records
Dunn [33]	University hospital admissions	55	78	1.0	Not sought	Hospital records
Gray [34]	Private practice	205	205	3.1	11.5 (10 + years)	Private files
McDonald [35]	Olmsted county	145	580	1.0	7.9 (3 + years)	Clinic records

ician will present differing features which may distinguish them significantly for epidemiologic analysis [30, 34].

One of the most unacceptable features of some of these studies is the inclusion of cases arising within the first few months of oestrogen usage. This inclusion is at odds with all that we know experimentally and epidemiologically about carcinogenic response since the prolonged latent period which is so characteristically noted is lacking. This consideration raises the semantic issue of what type of response can be termed carcinogenic. It seems reasonable to consider that a carcinogenic response consists of any increment in the incidence of a proven malignancy inextricably connected with prior exposure to a given agent.

The term "proven malignancy" is used advisedly since the vagaries of histopathological diagnosis, especially in the case of hyperplastic endometrial lesions are widely appreciated. Hence, due consideration must be given to the potential impact of varying degrees of diagnostic precision in comparing cases and controls and in generalizaing from one study to another. These histopathological considerations have been appropriately stressed by Kistner[41(a)] and Richart and Ferenczy[41(b)].

It would be unproductive to belabour these considerations further except to recognize that the epidemiologic findings to date require further extensive elaboration before the apparently demonstrated association of endometrial cancer and menopausal oestrogen usage can be regarded as conclusively considered a cause and effect relationship. One can only infer that where there is such dense smoke there is quite probably also some fire.

An additional phenomenon of interest is the occurrence of endometrial carcinoma in oestrogen-treated women with gonadal dysgenesis. McCarty *et al.*[42] summarize the 13 cases thus far reported. Two such cases have also been recorded unassociated with prior oestrogen usage [42]. These rare findings are not of sufficient magnitude to permit any inferences as to a causal relationship to oestrogen. However, it is to be noted that these patients almost uniformly present an adenosquamous type of carcinoma. The remarkable resemblance between this type of lesion and the squamous metaplasia so readily produced in the rodent endometrium by chronic oestrogen administration is of interest.

In this connection, the recent observation by Reagan[43] that the proportion of adenosquamous carcinomas in proportion to adenocarcinomas of the endometrium has been increasing requires further epidemiologic study.

ENDOMETRIAL CARCINOMA AND ORAL CONTRACEPTIVES

Endometrial carcinomas in young women using the sequential form of oral contraceptives have been reported [44, 49]. In addition, two studies have clearly demonstrated a very frequent and early occurrence of endometrial hyperplasia, in some instances of the adenomatous type, in women using the sequential preparations or natural oestrogens for menopause symptoms [50, 51]. However, no properly designed epidemiological study has been developed either to test the actual pathogenetic effect of this form of oral contraceptive or to ascertain the actual role of dosage and duration of exposure. The limited observations recorded to date have, however, precipitated the withdrawal of sequential orals from use in the United States. It would seem desirable to further exploit the large-scale clinical experience with the sequentials to learn more about the nature and extent of these untoward effects in young women. Such data would aid

in delineating the active role, if any, of sequential therapy in endometrial pathogenesis.

In contrast, oral contraceptives consisting of oestrogen–progestin combinations or of progestin alone appear to induce varying degrees of endometrial atrophy. This is manifested clinically by reduction in menstrual blood loss as well as by intervals of amenorrhoea. It would seem then that this type of effect would be the opposite of what is seen with the sequentials and would therefore militate against neoplastic change. Thus far, the more comprehensive studies of the combination oral contraceptives have adduced no evidence of an increased risk of endometrial cancer [52].

CERVIX CANCER AND ORAL CONTRACEPTIVES

The monitoring of the progression of cervical changes through dysplasia, carcinoma *in situ*, to invasive carcinoma by successive Papanicoluou smears supplemented by biopsy provides a highly useful tool for the study of human carcinogenic response. A large scale study of this progression in oral contraceptive users compared with users of vaginal contraceptives indicated that the frequency of changes in smear status was essentially the same in both groups [53]. However, frequency progression from Class III smears in oral contraceptive users was double that found for those using vaginal methods [53]. Similarly, Stern[54] compared the frequency of progression either from normal smear status or from initial dysplasia to either beginning dysplasia or carcinoma *in situ* in users versus non-users over a 7-year period. The data indicated that users with initial normal smears showed the same frequency of change to dysplasia as non-users. However, prolonged oral contraceptive use was associated with a highly significant increase in rate of change [54].

The multiplicity of methodological problems in such studies frustrate any definitive conclusions. Especially noteworthy among these is the vagary of cytological and histological diagnosis. Thus, Ory *et al.* [55] indicate that among 85 cases of previously diagnosed carcinoma *in situ* presented blind to two pathologists, one pathologist confirmed the diagnosis in 65 cases and a second pathologist confirmed the diagnosis in only 6 cases.

A number of other studies on oral contraceptive use and cervical pathogenesis yeild additional data which are equally inconclusive [56–60].

On balance, however, it appears that oral contraceptive use alters the behavior of cervical epithelium in several ways, ranging from the production of cervical adenosis and adenomatous polyp formation to an as yet unquantifiable and uninterpretable effect on progression from dysplasia toward non-invasive carcinoma. Nevertheless, the role of so many potentially confounding factors in such studies require that any final conclusions must be deferred.

BENIGN AND MALIGNANT BREAST DISEASE

The complex hormonal relationships of the normal breast in such varied physiological states as adolescence, maturity, pregnancy, lactation, the menopause, and old age. This multiplicity of endocrine factors is further complicated in the presence of benign and malignant breast disease. It is therefore difficult to rationalize endocrinologically a number of known clinical and epidemiological phenomena especially when they are potentially affected by drug usage.

Nevertheless, certain positive associations with the incidence of breast cancer include multiparity, delayed first-term pregnancy, obesity, and family history. It is clear that any interpretation of data relating to a study of drug usage and breast cancer without due attention to such effects would be of limited value [61].

Twelve attempts have been made to determine the role of combined oral contraceptive use or of menopausal oestrogen use in the pathogenesis of breast disease [52, 62–72]. Except for one study on oral contraceptives [64] and two studies on menopausal oestrogens [65, 71] no positive association between hormone therapy and increased incidence of breast cancer was noted. In one of these positive studies [65] comparison was made between patients in a private practice and in the general population, hardly a valid comparison. In the other two [64, 71] positive association was observed in only certain subsets yielding very small numbers. Such observations require additional substantiation of true effect. Accordingly, one must infer for the present that no truly impressive effect of either type of hormonal therapy on breast cancer has thus far been demonstrated. However, there is general agreement that several of the above cited cancer studies not only present problems in interpretation but also fail to cover a sufficiently long period of drug use [52].

An official report of the World Health Organization concludes: "The evidence currently available only applies to relatively short periods of oral contraceptive exposure and a very limited duration of follow-up. No inference can therefore be drawn about long-term effects of oral contraceptives or the subsequent risk of breast cancer" [73].

In marked contrast, the data on the negative association between benign breast disease and oral contraceptive usage are most cogent and have been ably summarized by Ory *et al.*[55]. Moreover, the as yet unconfirmed observation of Li Volsi *et al.*[70] indicate that this negative association is limited to patients presenting lesions with limited epithelial atypia and is not demonstrable in cases with marked atypia who are considered to be cancer-prone [74, 75]. These phenomena clearly indicate that the administered hormones are significantly altering some phases of breast tissue function and this in turn is being reflected for the short term in altered pathogenetic behavior. Although this immediately favorable

effect is substantial, one has no assurance as to the ultimate end-result of these tissue effects. Moreover, it is difficult to reconcile these phenomena with Fechner's findings that the histopathology of breast biopsies from pill users is in no way distinguishable from that of non-users [76].

LIVER TUMOURS

Because of the rarity of hepatic tumours among both users and non-users of oral contraceptives, it has proven essential to develop a registry for epidemiological and histopathological analysis [77]. These data together with a host of reports of small numbers of cases combined with the information provided by a recent survey [78] serve as a basis for concluding that a causal relationship exists. It is also apparent that this tissue response increases with dosage and duration of exposure and is not limited to any specific type of oestrogen.

The morphology of these hepatic lesions ranges from focal hyperplasia to invasive carcinoma, the greatest number thus far being hepatic adenomata.

We are ill-advised to attempt at this time to finally assess the potential epidemiological impact of these presently infrequent lesions.

COMMENT

Our very valuable heritage of extensive studies of the long-term effects of steroids and related hormonally active synthetic compounds in numerous animal species provides a useful scaffold against which to project our observations in man.

The tumourigenic capacity of hormonal agents have leaped the species gap from animals to man in the case of the vaginal adenocarcinoma seen in the daughters of oestrogen-treated mothers and probably in the occurrence of liver tumours in oral contraceptive users. Both these effects exhibit a very low overall incidence as would be predicted from animal experiments with a genetically heterogenous species like man. Yet we are ill-advised to presume that in our mongrel species all such tumourigenic responses must prove to be rare. For when we are dealing with a lesion like breast cancer which has high familial occurrence and yet such wide ethnic differences in incidence we should not assume that our genetic heterogeneity will prove as protective as it has thus far.

The paucity of sound epidemiological data concerning these problems despite several decades of use of these agents by millions of women is most regretable. It is imperative that we marshal a greater effort to ascertain as completely as possible the actual result of the massive use of these agents over the years.

REFERENCES

1. Allen E. and Doisy E. A.: An ovarian hormone, a preliminary report on its localization, extraction, partial purification, and action in test animals. *J. Am. med. Ass.* **81** (1923) 819.
2. Allen E., Smith G. M. and Gardner W. U.: Accentuation of the growth effect of theelin on genital tissues of the ovariectomized mouse by arrest of mitosis with colchicine. *Am. J. Anat.* **61** (1937) 321.
3. Allen E., Smith G. M. and Reynolds S. R. M.: Hyperplasia of uterine muscle as studied by the colchicine method. *Proc. Soc. exp. Biol. Med.* **37** (1937) 257.
4. Beatson G. T.: On the treatment of inoperable cases of carcinoma of the mammae. *Lancet* **ii** (1896) 104.
5. Loeb L.: Further investigations on the origin of tumours in mice. *J. med. Res.* **40** (1919) 477.
6. Feiser L. F. and Fieser M.: *Natural Products Related to Phenathrene.* Rheinhold, New York, 3rd Edn (1949).
7. Gardner W. U., Pfeiffer C. A. and Trentin J. J.: Hormonal factors in experimental carcinogensis. In *Physiopathology of Cancer.* Harper & Row, New York, 2 Edn (1959) p. 152.
8. Jabara A.: Induction of canine ovarian tumours by diethylstilbestrol and progesterone. *Aust. J. exp. Biol. med. Sci.* **40** (1962) 139.
9. Lipschutz A.: *Steroid Hormones and Tumors.* Williams & Wilkins, Baltimore (1950).
10. Crossen R. J. and Stuzelff V.: Endometrial polyps and hyperplasia produced in an aged monkey with estrogen plus progesterone. *Archs Path.* **50** (1950) 721.
11. Engle E. T., Krakower C. and Haagensen C. D.: Estrogen administration to aged female monkeys with no resultant tumor. *Cancer Res.* **3** (1943) 858.
12. Overholser M. D. and Allen E.: A typical growth induced in cervical epithelium of monkey by prolonged injections of ovarian hormone combined with chronic trauma. *Surg. Gynec. Obstet.* **60** (1935) 129.
13. Pfeiffer C. A. and Allen E.: Attempts to produce cancer in rhesus monkeys with carcinogenic hydrocarbons and estrogens. *Cancer Res.* **8** (1948) 97.
14. McClure H. M. and Gucham C. E.: Malignant uterine mesotheliomas in squirrel monkeys following diethylstilbestrol administration. *Lab. Anim. Sci.* **23** (1973) 493.
15. Hueper W. C.: *Occupational Tumor and Allied Diseases.* C. C. Thomas, Springfield (1942).
16. Selikoff I. J., Hammond E. C. and Chung J.: Neoplasia risk associated with occupational exposure to airborne inorganic fibers. In *Proceedings of the 10th International Cancer Congress,* Year Book Medical Publishers, Chicago, Vol. 5 (1970) p. 55.
17. Greenwald P., Barlow J. J. and Nasca P. C.: Vaginal cancer after maternal treatment with synthetic estrogens. *New Engl J. Med.* **285,** (1971) 390.
18. Herbst A. L., Kurman R. J., Scully R. E. and Poskanzer D. C.: Clear cell adenocarcinoma of the genital tract in young females: Registry report. *New Engl J. Med.* **287** (1972) 1259.
19. Barber H. R. K. and Sommers S. C.: Vaginal adenosis, dysplasia and clear cell adenocarcinoma after DES treatment in pregnancy. *Obstet. Gynec.* **43** (1974) 645.
20. Herbst A. L., Kurman R. J., Scully R. E. and Poskanzer D. C.: Clear cell adenocarcinoma of the genital tract in young females—Registry report. *New Engl J. Med.* **287** (1972) 1259.
21. Lanier A. P., Noller K. L., Decker D. G., Elveback L. R. and Kurland L. T.: Cancer and stilbestrol. A follow-up of 1719 persons exposed to estrogens *in utero* and born 1943–1959. *Mayo Clin. Proc.* **48** (1973) 793.

21(a). Jick *et al.*: Boston Collaborative Drug Surveillance Programme. *Lancet* **1** (1973) 1399.

22. Hertig A. T. and Sommers S. C.: Genesis of endometrial carcinoma—I. Study of prior biopsies. *Cancer* **2** (1949) 946.
23. Gusberg S. B.: Precursors of corpus carcinoma, estrogens and adenomatous hyperplasia. *Am. J. Obstet. Gynec.* **54** (1947) 905.

24. Gusberg S. B. and Kaplan A. L.: Precursors of corpus cancer. *Am. J. Obstet. Gynec.* **86** (1963) 662.
25. Speert H.: Cancer of the endometrium in young women. *Surg. Gynec. Obstet.* **88** (1949) 332.
26. Ostergaard E.: Estrogens in the etiology of cancer of the corpus uteri. In *Proceedings of the International Congress of Gynecology and Obstetrics*, Geneva, July 26, 1954, p. 222.
27. Fremont-Smith M., Meigs J. V., Graham R. M. and Gilbert H. H.: Cancer of the endometrium and prolonged estrogen therapy. *J. Am. med. Ass.* **131** (1946) 805.
28. Geist S. H., Walter R. I. and Salmon V. J.: Are estrogens carcinogenic in the human female? *Am. J. Obstet. Gynec.* **41** (1941) 29.
29. Mustacchi P. and Gordan G. S.: Frequency of cancer in estrogen-treated osteoporotic women. In *Second Biennial Louisiana Cancer Conference*, St. Louis, Missouri. C. V. Mosby, St. Louis (1958) p. 163.
30. Zeil H. K. and Finkle W. D.: Increased risk of endometrial carcinoma among users of conjugated estrogens. *New Engl J. Med.* **293** (1975) 1167.
30(a). Gordon I., Reagen J. W., Finkle W. D. *et al.*: *New Engl J. Med.* **297** (1977) 570.
31. Mack T. M., Pike M. C., Henderson D. E., Pfeffer R. I., Gerkins V. R., Arthur M. and Brown S. E.: Estrogens and endometrial cancer in a retirement community. *New Engl J. Med.* **294** (1976) 1262.
32. Smith D. C., Prentice R., Thompson D. J. and Herrmann W. L.: Association of exogen estrogen and endometrial cancer. *New Engl J. Med.* **293** (1975) 1164.
33. Dunn L. J. and Bradbury J. T.: Endocrine factors in endometrial carcinoma. *Am. J. Obstet. Gynec.* **97** (1967) 465.
34. Gray L. A., Christopherson W. M. and Hoover R. N.: Estrogens and endometrial carcinoma. *Obstet. Gynec.* **49** (1977) 385.
35. McDonald T. W., Arnugens J. R., O'Fallon W. *et al.*: Exogenous estrogen and endometrial carcinoma: Case control and incidence study. *Am. J. Obstet. Gynec.* **127** (1977) 572.
36. Wigle D. T.: Reproductive cancer and hormone use. Read before the Society for Epidemiologic Research, Seattle, June 15, 1977.
37. Stolley B. D., Antones C. and Rosenschein R.: An investigation of the role of estrogens in endometrial hyperplasia and carcinoma. Read before the Society for Epidemiologic Research, Seattle, June 15, 1977.
38. Horwitz R. I. and Feinstein A. R.: New methods of sampling and analysis to remove bias in case-control research: No association found for estrogens and endometrial cancer. Read before the Society for Epidemiologic Research, Seattle, June 15, 1977.
39. Hoover R. and Gray L. A.: Menopausal estrogen use and the risk of non-mammary cancer. *J. natn. Cancer Inst.* (In press).
40. Prentice R.: Endometrial cancer histopathology and survival in relation to exogenous estrogen. Read before the Society for Epidemiologic Research. Seattle, June 15, 1977.
41. Hoover R., Fraumeni J. R., Everson *et al*: Cancer of the uterine corpus after hormonal treatment for breast cancer. *Lancet* **2** (1976) 885.
41(a). Kistner R. W.: Treatment of carcinoma *in situ* of the endometrium. *Clin. Obstet. Gynec.* **5** (1962) 1166. Kistner R. W.: Estrogens and endometrial cancer. Editorial. *Obstet. Gynec.* **48** (1976) 479.
41(b). Richart R. M. and Ferenczy A.: Endometrial morphologic response to hormonal environment. *Gynec. Oncol.* **2** (1974) 180.
42. McCarty K. S., Barstow T. K., Peete C. H. and Creasman W. T.: Gonadal dysgenesis with adenocarcinoma of the endometrium. *Cancer* **42** (1978) 512.
43. Reagan J. W.: The changing nature of endometrial cancer. *Gynec. Oncol.* **2** (1974) 144.
44. Lyon F. A.: The development of adenocarcinoma of the endometrium in young women receiving long-term sequential oral contraception. *Am. J. Obstet. Gynec.* **2** (1975) 299–301.
45. Silverberg S. G. and Makowski E. L.: Endometrial carcinoma in young women taking oral contraceptive agents. *Obstet. Gynec.* **46** (1975) 503–506.
46. Kaufman R. H. *et al.*: Severe atypical endometrial changes and sequential contraceptive use. *J. Am. med. Ass.* **236** (1976) 923–926.
47. Cohen C. J. and Deppe G.: Endometrial carcinoma and oral contraceptive agents. *Obstet. Gynec.* **49** (1977) 390–392.
48. Kelley H. W. *et al.*: Adenocarcinoma of the endometrium in women taking sequential oral contraceptives. *Obstet. Gynec.* **47** (1976) 200–202.
49. Silverberg S. G. *et al.*: Endometrial carcinoma in women under 40 years of age. *Cancer* **30** (1977) 592–598.
50. Vandersick G., Beernaert J., De Mylder E. and Ferin J.: Hormonal Contraception: Sequential formulations and the endometrium. *Contraception* **12** (1975) 655.
51. Whitehead M. J., McQueen J., Beard R. J., Minardi J. and Campbell S.: *Acta scand. Obstet. Gynec.* Suppl. 62 (1977) 91.
52. Royal College of General Practitioners: *Oral Contraceptives and Health*; Pitman Medical, London (1974).
53. Fuertes de la Haba *et al.*: Changing patterns in cervical cytology among oral and non-oral contraceptive users. *J. Reprod. Med.* **10** (1973) 3.
54. Stern E. *et al.*: Steroid contraceptive use and cervical dysplasia; increased risk of progression. *Science* **196** (1977) 1460.
55. Ory H. W., Conger B. S., Naib Z., Tyler C. W. and Hatcher R. A.: Preliminary analysis of oral contraceptive use and risk of developing premalignant lesions of the uterine cervix. In *Pharmacology of Steroid Contraceptive Drugs*. Raven Press, New York (1977) p. 211.
56. Peritz E. *et al.*: The incidence of cervical cancer and duration of oral contraceptive use. *Am. J. Epidem.* **106** (1977) 462.
57. Boyce J. G. *et al.*: Cervical carcinoma and oral contraception. *Obstet. Gynec.* **49** (1977) 139.
58. Worth A. J. and Boyes D. A.: A case-control study into the possible effects of birth control pills on preclinical carcinoma of the cervix. *J. Obstet. Gynaec. Br. Common.* **79** (1972) 673.
59. Thomas D. B.: Relationship of oral contraceptives to cervical carcinogenesis. *Obstet. Gynec.* **40** (1972) 508.
60. Nash H. A.: Depo-Provera, a review. *Contraception* **12** (1975) 377.
61. MacMahon B., Cole P. and Brown J.: Etiology of human breast cancer. *J. natn. Cancer Inst.* **50 (1973) 21.**
62. Vessey M. P., Doll R. and Sutton P. M.: Oral contraceptives and breast neoplasia: a retrospective study. *Br. med. J.* **3** (1972) 719.
63. Greenblatt D. J., Ory J. W. and Levy M.: Boston Collaborative Drug Surveillance Program: Oral contraceptives and venous thromboembolic disease, surgically confirmed gall-bladder disease, and breast tumors. *Lancet* **2** (1973) 1399.
64. Fasal E. and Paffenberger R. S.: Oral contraceptives as related to cancer and benign lesions of the breast. *J. natn. Cancer Inst.* **55** (1975) 767.
65. Hoover R., Gray L. A., Cole P. and MacMahon B.: Menopausal estrogens and breast cancer. *New Engl J. Med.* **295** (1976) 401.
66. Ory H. W., Cole P., MacMahon B. and Hoover R.: Oral contraceptives and reduced risk of benign breast diseases. *New Engl J. Med.* **294** (1976) 419.

67. Vessey M. P., Doll R. and Jones K.: Oral contraceptives and breast cancer. *Lancet* **2** (1975) 941.
68. Kelsey J. L., Lindfors K. K. and White C.: A case control study of the epidemiology of benign breast diseases with reference to oral contraceptive use. *Int. J. Epidem.* **3** (1974) 333.
69. Sartwell P. E., Arthes F. J. and Tonscia J. A.: Epidemiology of benign breast lesions: Lack of association with oral contraceptive use. *New Engl. J. Med.* **288** (1973) 551.
70. Li Volsi V. A., Stadel B. V., Kelsey J. L., Holford T. R. and White C.: Fibrocystic disease in oral contraceptive users: Histopathological evaluation of epithelial atypia. *New Engl. J. Med.* **299** (1978) 381.
71. Ross R., Mack T., Hill A., Gerkins V. and Menck H.: Breast cancer and post-menopausal estrogen therapy in two Los Angeles retirement communities. (1978) (in press).
72. Burch J. C., Byrd B. F. and Vaughn W. K.: The effect of long-term estrogen on hysterectomized women. *Am. J. Obstet. Gynec.* **118** (1974) 778.
73. World Health Organization: Steroid contraception and the risk of neoplasia. Report of a WHO Scientific Group; WHO Technical Report Series No. 619 (1978) Geneva.
74. Black M. M., Barclay T. H. C. and Cutler S. J.: Association of atypical characteristics of benign breast lesions with subsequent risk of breast cancer. *Cancer* **29** (1972) 338.
75. Kodlin D., Winser E. E. and Morgenstern N. L.: Chronic mastopathy and breast cancer. *Cancer* **39** (1977) 2603.
76. Fechner B.: National Breast Cancer Conference. Washington, D.C. (1976).
77. Increased risk of hepatocellular adenoma in women with long-term use of oral contraceptives. *Morbidity and Mortality Weekly Report* **26** (1977) 293.
78. Vana J.: Primary liver tumors and oral contraceptives. *J. Am. med. Ass.* **238** (1977) 2154.