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A DISCUSSION OF THE ROLES OF OESTROGEN AND PROGESTIN IN HUMAN MAMMARY CARCINOGENESIS

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Summary—Oestrogens and progestins are important for both the genesis of human breast cancer and growth of those tumours once formed. Their role at different stages of the neoplastic process are reviewed and discussed within the context of a change in sensitivity of epithelial cells during either initiation or promotion stages. Evidence favours, but does not conclusively prove, the view that progestins are the predominant mitogen for normal breast epithelium whilst oestrogen assumes that function in neoplastic epithelium. Alterations in oestrogen receptor levels could provide the key for such a change. There are insufficient data on physiological progestin concentrations to judge their effect on established cancer. Models for steroidal effects on cell proliferation and oestrogen and progestin receptor regulation that are based on endometrial data are not appropriate for breast.

INTRODUCTION

Professor James' contributions to endocrinology have been numerous and many are recognized in other papers in this issue. In this article, I wish to combine two different features of his scientific life, his interest in breast cancer and his commendable caution. The role of steroids and their receptors in cancer development will be used to illustrate the need for caution in making simple generalizations about endocrine-sensitive cancers.

The concept that oestrogens are the major adverse factor in human breast cancer has dominated thinking in this area [1, 2]. This opinion is based on three main lines of evidence: (a) the ability of oestrogens to generate mammary tumours in rodents [3, 4]; (b) epidemiologically-derived risk factors such as the protective effect of ovariectomy and increased risk of breast cancer in young women given diethylstilboestrol to prevent abortion [1, 2]; and (c) the mitogenic effects of oestrogens on established breast cancer cell lines [5, 6] and efficacy of antioestrogens in treating established breast cancer [7].

Conversely, the other ovarian steroid progesterone and its synthetic derivatives (progestins) are thought to be protective, a view largely based on their antioestrogenic and therefore antiproliferative effects on endometrium [8]. Supportive evidence for beneficial effects of progestins comes from their clinical use in advanced breast cancer [9] and their ability to decrease tumour yield under certain conditions in rodents [3, 4].

The "oestrogens bad, progestins good" model adequately explains the endometrial cancer data but should be questioned when applied to both the genesis and behaviour of human breast cancer [10, 11].

A central feature of general carcinogenesis is that it is a multistage process with cell sensitivities changing during progression from normal→hyperplastic→hormone-sensitive cancer→hormone-insensitive state (Fig. 1). Histologically, this continuum of change in response to environment is seen in an increased epithelium:stroma ratio and breakdown of epithelial cell–epithelial cell regulation, resulting in carcinoma *in situ* in which cell clumps are still confined within a basement membrane. Subsequent changes enable the cancer cells to overcome the limiting influence of the basement membrane and metastasis ensues. Well-differentiated metastatic breast cancers retain some of the regulatory influences of the environment in which they are growing but additional changes lead to complete autonomy. With all these regulatory changes occurring, it would be surprising if hormone sensitivity remained constant. Indeed, we know that changes do occur in the terminal stages of progression to autonomy but little attention has been given to the possibility of earlier changes. Evidence for such

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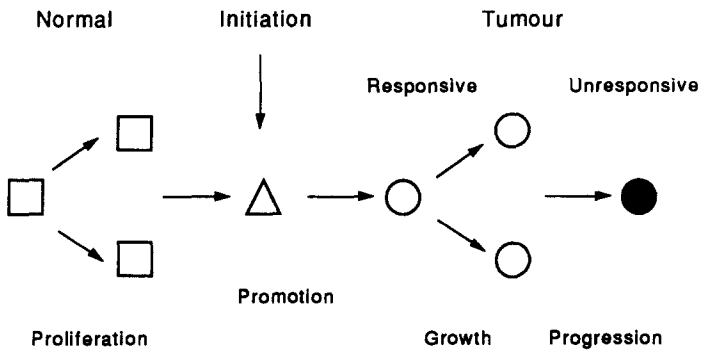

Fig. 1. Stages of carcinogenesis.

changes does exist and it is these data that lead me to question the oestrogen bad, progestin good model for human breast cancer.

As depicted in Fig. 1, neoplastic development is a multifactorial process but one feature, proliferation, is common throughout. The effects on this function are relevant at any stage, regardless of whether it increases: (a) the number of normal cells as potential targets for initiating agents; (b) the proliferation of initiated but preneoplastic cells; or (c) the growth of established cancer cells. Indeed, models of hormonal involvement in breast [12, 13] and endometrial [14] cancer have been published that are based on the proliferative effects of steroids. However, proliferation alone does not generate a cancer, abnormal regulation of that growth and the ability to invade other tissues are also crucial. Steroids are said to be promotional agents [12, 15], which is correct as long as that definition includes enlargement of the pool of target cells for the initiating agent(s). There is little evidence to indicate that any common steroidal agent can act as an initiating agent although the non-steroidal oestrogen, diethylstilboestrol may be exceptional [16].

Given the importance of cell proliferation, it is not surprising that this feature has received much attention.

STEROID EFFECTS ON THE PROLIFERATION OF HUMAN BREAST EPITHELIA

Normal epithelium

Breast cancers are thought to arise in the epithelial cells of the terminal ductal lobular unit (TDLU) and therefore the behaviour of these cells is important [17]. Histologically normal breast adjacent to fibroadenomas can be used to assess its proliferative activity at the time of surgery. Our data so obtained [18, 19] indicate optimal proliferation during the luteal (progestagenic) phase of the menstrual cycle (Fig. 2); similar work by other groups agree with this observation [20–22]. Furthermore, both combination oestrogen + progestin and progestin-only oral contraceptives increase epithelial proliferation *in vivo* over that seen in the natural cycle [19]. Interestingly, the activity of a proven progestin-inducible enzyme, fatty acyl synthetase, increases in proportion to proliferative activity in human breast epithelia [23]—again hinting at a progestin influence on proliferation. These indications of progestin-related proliferation are reinforced when normal breast is compared with normal endometrium analysed by the same method (Fig. 3). Endometrial epithelium shows the expected, oestradiol-induced proliferation which is antagonized by progesterone in the luteal phase [24]. This clear-cut difference between normal breast and endometrial epithelia indicates important differences in response pathways and is most easily explained for breast by a progestin-related mitogenic effect as is known to exist in rodents [25]. Such an explanation

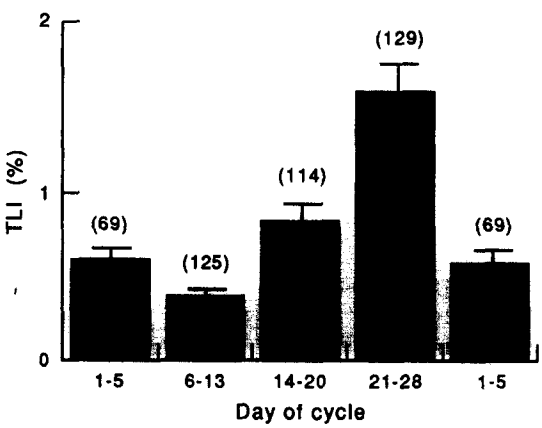

Fig. 2. [^{3}H]Thymidine labelling index (TLI) of normal lobular mammary epithelium from young, premenopausal women. Data are presented as mean ± SEM (No. observations) and are taken from Ref. [19].

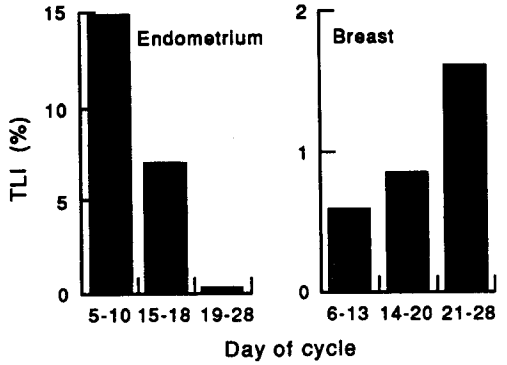

Fig. 3. Comparison of [^{3}H]thymidine labelling index (TLI) of normal endometrial [left hand panel] and breast [right hand panel] epithelia at different stages of the menstrual cycle. The endometrial data are taken from Ref. [24] and the breast from Ref. [19].

does not rule out a contributory influence of oestrogen in the breast; oestrogens are known to sensitize target cells to progestin effects [26].

All data discussed thus far were obtained directly with histologically normal breast TDLUs adjacent to benign fibroadenoma from clinically characterized young women. If such tissue is excised and grown in hormonally treated, immunodeficient mice a different picture emerges; oestrogen stimulates growth of the TDLU and progestins have no effect [27]. Epithelial cells cultured from normal human breast are also stimulated by oestrogen and not progestin [28] although the exact cell type was not characterized.

Developmental changes in the mammary gland provide additional clues about the roles of oestrogen and progestin. In girls, differential development of the cellular components occurs at about the time of menarche. Ductal growth is stimulated by oestrogen, whereas additional progestin is required for lobular development [17, 29]. A similar pattern occurs in rodents [17, 25]. Full proliferative development of the terminal regions of the mammary tree requires the endocrine environment of pregnancy which includes both oestrogen and progestin. Oestrogens without progestin will induce breast development in girls with Turner's Syndrome [30, 31] but it is not clear if this includes TDLU formation as no histological data on such breasts are available.

A requirement for both oestrogen and progestin (ovular as opposed to anovular cycles) has also been advanced to explain the adverse influence of early menarche on breast cancer development [32].

Although the conflicting data obtained by different techniques do not allow a firm conclusion as to whether progestins, either alone or in combination with oestrogen, are breast epithelial mitogens, there is certainly no evidence that progestins counteract the mitogenic effects of oestrogens.

Neoplastic epithelium

Oestrogens clearly stimulate the proliferation of established breast cancer cells, as determined both by clinical [7] and laboratory studies [33, 34]. An example of the large proliferative response elicited by oestradiol on human breast cancer cell lines is shown in Fig. 4. According to which cell line is analysed, either complete [ZR-75 or MCF-7 (McGrath)] or partial [T-47-D or MCF-7 (KO)] dependence on oestradiol can be demonstrated.

This big proliferative effect of oestrogens on established breast cancer contrasts with that seen *in vivo* with normal epithelial cells (see above) and indicates either an increased or changed cell sensitivity to oestrogen.

Data on progestin effects on neoplastic human breast epithelium are both sparse and confusing. Clinically, high doses of progestins induce regression of some breast cancers [7, 9] but interpretation of such data in the context of physiological concentrations is difficult. It is well-established that high doses of oestrogen can induce regressions [7] despite their mitogenic effect at lower concentrations; such a

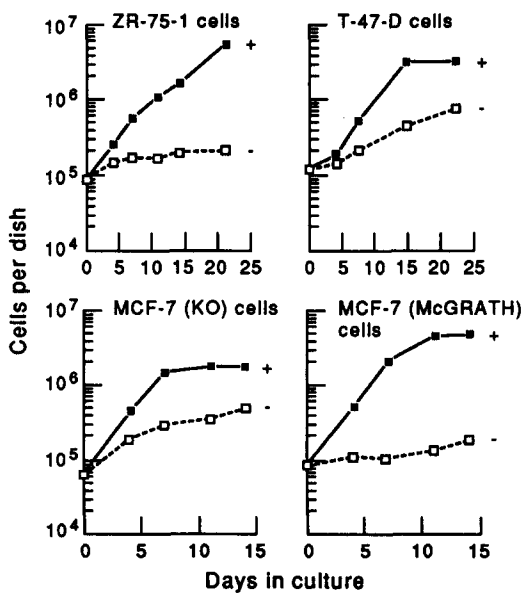

Fig. 4. Proliferation of four human breast cancer cell lines in the presence (+) and absence (−) of 10^{-8} M oestradiol. Data taken from Ref. [34].

biphasic effect might also occur with progestins. With human breast cancer cell lines, progestins do inhibit proliferation but the effect is small and dependent on culture conditions. The pH indicator, phenol red, normally added to culture media, is oestrogenic for human breast cancer lines [35] and, in this environment progestins are weakly growth inhibitory [36, 37]. In the absence of phenol red progestins induce a proliferative response although again, the effect is small [38, 39]. This is illustrated in Fig. 5 with the T47D cell line. Note the small proliferative response to progestins as compared with the large oestrogenic effect with the same cells (Fig. 4). There are two conflicting reports on the actions of the antiprogestin, RU486 in cell culture [36, 38].

The safest general comment that one can make about progestin effects on established breast cancer is that more data are required; no conclusions are possible as to whether physiological levels of progestins are good or bad for breast cancer growth.

STEROID RECEPTOR REGULATION IN HUMAN BREAST EPITHELIUM

As most steroid effects are mediated by specific intracellular receptors, analysis of these proteins might provide clues about oestrogen and progestin actions. Also, as we have speculated about a change of steroid sensitivity during carcinogenesis (see above) receptors could be involved. When oestradiol receptors (ER) were first being quantitated they were difficult to detect in normal human breast, whilst breast tumours often had very high ER levels. The problem with normal breast was a methodological one, due in part to low cellularity but also to low levels of ER. For example, ER positive breast tumours frequently contain

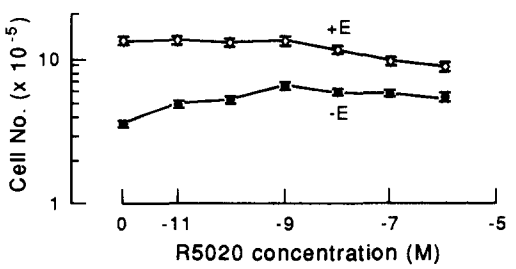

Fig. 5. Influence of different concentrations of a synthetic progestin, R5020 on the proliferation of T-47-D human breast cancer cell line in the absence (−E) and presence (+E) of 10^{-8} M oestradiol. Cells were grown in the absence of phenol red. Data from P. Darbre (personal communication).

more than 100 fmol ER/mg protein, whereas peak levels in normal breast are about one-tenth of that value [40]. Thus, ER is present in normal epithelium, albeit at low concentrations. Although inadequately analysed at the present time, an upregulation of ER content seems probable—which could be the driving force for changed sensitivity as it is now clear that receptor numbers can influence magnitude of response [41, 42]. A clue as to the stage of progression at which changes in ER might occur comes from the observation that, after correction for cellularity, invasive tumours have appreciably higher levels of ER than intraduct counterparts [43]. Whether this reflects changes in the ER gene itself or to altered regulatory influences due to the different cellular environment remains to be established. Another indication that important changes in oestrogen sensitivity occur at about this stage of progression comes from the histochemical analysis of an ER-related protein, p29 which is clearly associated with oestrogen action, albeit is an unknown way [44]. This protein is low in normal breast, is high in hormone-sensitive, invasive tumours and exhibits an intermediate, heterogeneous staining pattern in carcinoma—*in situ* [44, 45].

As well as differences in amounts of receptor between normal and cancer cells, their hormonal regulation could be important. We have used an histochemical approach with normal premenopausal breast [46] that indicates ER regulation to be analogous to that in endometrium, whereas progesterone receptor (PR) may differ (Fig. 6). The percentage of ER positive samples is decreased both in the luteal (progestagenic) phase of the cycle and by use of oral contraceptive pills (mainly combined oestrogen + progestin types). Interestingly, PR exhibits a high positivity rate throughout the cycle and is little affected by the pill. These data contrast with the endometrial picture, in which staining decreases under the influence of progesterone [48, 49], and thereby provide another example of different regulatory patterns in breast and endometrium. However, a major defect in these histochemical studies is the lack of quantitation which will be essential before full interpretation can be achieved. This might explain some of the conflicting data in the literature with some reports indicating more ER [40, 50] and PR [40] in normal breast epithelium during the first half of the menstrual cycle, whilst others suggest little change [51].

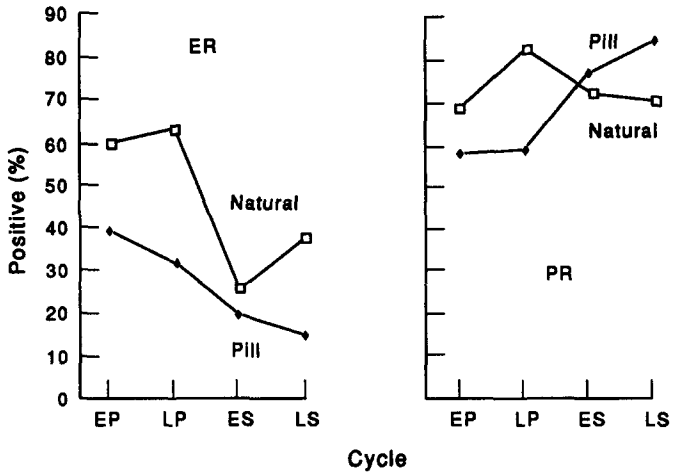

Fig. 6. Immunohistochemical analysis of ER (left hand panel) and PR (right hand panel) in normal human breast epithelium from young women who were either taking oral contraceptives (Pill) or no steroids (Natural) at the time of biopsy. EP, LP, ES & LS refer to early and late proliferative and early and late secretary phases of the menstrual cycle, respectively. % Positive refers to the number of samples in the stated group showing positive staining. Data are taken from Ref. [46].

In the light of the preceding discussions on changed hormone sensitivity of proliferation, it would be interesting to know if receptor regulation differed between normal and cancer but the data are too fragmentary to allow conclusions. Some [52–54] but not all [50, 51, 55, 56] publications report higher cancer ER during the first half of the menstrual cycle than in the second half. Most authors [54–56] found little change in cancer PR throughout the cycle.

TUMOUR PROGRESSION

Steroid sensitivity is known to change after establishment of breast cancer and it is this progression to hormone insensitivity that generates major clinical problems. The heterogeneous nature of this progression has been continually emphasized [57, 58] but good experimental models have been sparse. Animal models have been informative but their complexity limits their utilization, particularly for molecular studies. The essential requirement for a model system with which to study progression is a known precursor–product relationship. Breast culture methods have now been described which fulfil that requirement and indicate three very different routes to the same objective namely autonomous growth. Amplification of the multidrug-resistance gene in MCF-7 cells by exposure to increasing concentrations of adriamycin leads to loss of both ER and oestrogen-sensitive growth, whereas EGF receptor numbers are increased [59]. A second experimental method follows the spontaneous development of oestrogen-resistant T47D cells accompanied by changes in ploidy and ER gene structure [60, 61]. We, and others have utilized a third approach based on long-term steroid deprivation of cloned cultures [34, 57, 62]. Unresponsive mammary cultures generated by this technique have two important common features. Firstly, the cells that are steroid insensitive for proliferation have functional receptors and, secondly, basal proliferation in the absence of steroid increases. These changes have been fully discussed elsewhere [5, 34, 57] but our general conclusion is that this type of insensitivity results from upregulation of steroid-independent pathways of growth and that steroid receptors loss is a consequence and not cause of that change.

CHANGES IN STEROID SENSITIVITY OF HUMAN BREAST EPITHELIUM DURING CARCINOGENESIS AND PROGRESSION: A WORKING MODEL

The events represented in Fig. 7 could explain the data outlined in this article. In normal breast epithelium, progestins are mitogenic although we cannot exclude the possibility of an additional small contribution from oestrogen. Although PR levels have a large constitutive component in breast epithelium, the existing data do not preclude an additional oestrogen effect on proliferation via induction of PR. Increased levels of ER in neoplastic epithelium sensitize those cells to oestrogen which becomes the major steroidal mitogen. The data are too incomplete to decide whether or not progestin sensitivity changes. Progression from steroid sensitive to insensitive state involves increased activity of steroid independent pathways of

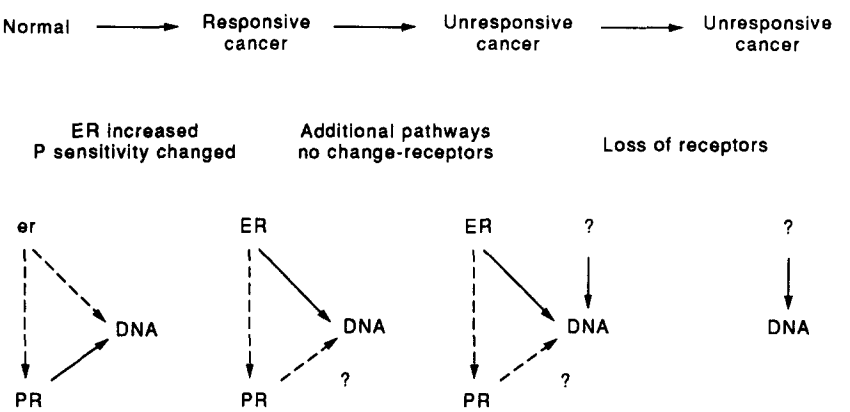

Fig. 7. Model of steroid sensitivity changes during human mammary carcinogenesis and progression. er, ER—low and high levels, respectively of oestradiol receptor; PR—progesterone receptor; DNA—DNA synthesis; solid arrows—established effects; dashed arrows—possible effects.

proliferation without changes in ER, although subsequent loss of this entity could occur.

CONCLUSION

This article has highlighted aspects of progestin effects on human breast epithelium that question the common assumption that this class of female sex hormones has beneficial effects on neoplasia. The data are still too sparse to warrant firm conclusions but, given the importance of this class of compounds, additional information is urgently required. I have also suggested that during the complex process of carcinogenesis, alterations in steroid sensitivity accompany the other changes in regulation of cell behaviour that occur. In the context of the "oestrogen bad, progestin good" model that is appropriate for endometrial cancer, a more correct statement for breast might be that carcinogenesis results in the transition from "progestin bad, oestrogen?" to "oestrogen bad, progestin?"

REFERENCES

1. Henderson B. E., Ross R. and Bernstein L.: Estrogens as a cause of human cancer. *Cancer Res.* **48** (1988) 246–253.
2. Vessey M. P.: The involvement of oestrogen in the development and progression of breast disease: epidemiological evidence. *Proc. R. Soc. Edinb.* **95B** (1989) 35–48.
3. Fowler E. H.: Progesterone and synthetic progestins: their biological activity and role in neoplasia. In *Influences of Hormones in Tumor Development* (Edited by J. A. Kellen and R. Hilf). CRC Press, Boca Raton, FL (1979) pp. 45–84.
4. Welsch C. W.: Hormones and murine mammary tumorigenesis: an historical view. In *Hormonal Regulation of Mammary Tumors* (Edited by B. S. Leung). Eden Press, Montreal (1982) pp. 1–29.
5. Darbre P. D. and King R. J. B.: Steroid hormone regulation of cultured breast cancer cells. In *Breast Cancer: Cellular and Molecular Biology* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Academic, Boston, MA (1988) pp. 307–341.
6. Dickson R. B. and Lippman M. E.: Breast Cancer: Cellular and Molecular Biology. Kluwer Academic, Boston, MA (1988) pp. 119–165.
7. Santen R. J., Manni A., Harvey H. and Redmond C.: Endocrine treatment of breast cancer in women. *Endocrine Rev.* **11** (1990) 221–265.
8. King R. J. B. and Whitehead M. I.: Assessment of the potency of orally administered progestins in women. *Fert. Steril.* **46** (1986) 1062–1066.
9. Horwitz K. B., Wei L. L., Sedlacek S. M. and D'Arville C. N.: Progestin action and progesterone receptor structure in human breast cancer: a review. *Recent Prog. Horm. Res.* **41** (1985) 249–316.
10. King R. J. B.: Role of oestrogen and progestin in human mammary carcinogenesis (1990) In *Endocrine Therapy of Breast Cancer V* (Edited by A. Goldhirsch). *European School of Oncology Monographs*. Springer, Berlin (1990) pp. 3–8.
11. King R. J. B.: Biology of female sex hormone action in relation to contraceptive agents and neoplasia. *Contraception* **43** (1991) 527–542.
12. Moolgavkar S.: Hormones and multistage carcinogenesis. *Cancer Surv.* **5** (1986) 635–648.
13. Key T. J. and Pike M. C.: The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur. J. Cancer Clin. Oncol.* **24** (1988) 29–43.
14. Key T. J. and Pike M. C.: The dose–effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br. J. Cancer* **57** (1988) 205–212.
15. King R. J. B.: Experimental strategies for studying the development of breast cancer with special reference to steroid hormones, growth factors and oncogenes. In *Endocrine Therapy of Breast Cancer* (Edited by F. Cavalli). *European School of Oncology Monographs*. Springer, Berlin (1986) pp. 5–26.
16. World Health Organization/International Agency for Research on Cancer Genetic and Related Effects: An updating of selected IARC Monographs from Vols 1–42. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Supplement (1987) pp. 250–256, 293–295, 369–371, 426–433, 437–443.
17. Russo J., Gusterson B. A., Rogers A. E., Russo I. H., Wellings S. R. and van Zwieten M. J.: Biology of

disease: comparative study of human and rat mammary tumorigenesis. *Lab. Invest.* **62** (1990) 244–278.
18. Going J. J., Anderson T. J., Battersby S. and MacIntyre C. C. A.: Proliferative and secretory activity in human breast during natural and artificial menstrual cycles. *Am. J. Path.* **130** (1988) 193–203.
19. Anderson T. J., Battersby S., King R. J. B., McPherson K. and Going J. J.: Oral contraceptive use influences resting breast proliferation. *Hum. Path.* **20** (1989) 1139–1144.
20. Meyer J. S.: Cell proliferation in normal human breast ducts, fibroadenomas and other duct hyperplasias, measured by nuclear labelling with tritiated thymidine: effects of menstrual phase, age, and oral contraceptive hormones. *Hum. Path.* **8** (1977) 67–81.
21. Longacre T. A. and Bartow S. A.: A correlation morphologic study of human breast and endometrium in the menstrual cycle. *Am. J. Surg. Path.* **10** (1986) 382–393.
22. Potten C. S., Watson R. J., Williams G. T., Tickle S., Roberts S. A., Harris M. and Howell A.: The effect of age and menstrual cycle upon proliferative activity of the normal human breast. *Br. J. Cancer* **58** (1988) 163–170.
23. Chalbos D., Escot C., Joyeux C., Tissot-Carayon M.-J., Pages A. and Rochefort H.: Expression of the progestin-induced fatty acid synthetase in benign mastopathies and breast cancer as measured by RNA *in situ* hybridization. *J. Natn. Cancer Inst.* **82** (1990) 602–606.
24. Ferenczy A., Bertrand G. and Gelfand M. M.: Proliferation kinetics of human endometrium during the normal menstrual cycle. *Am. J. Obstet. Gynec.* **133** (1979) 859–867.
25. Russo J., Tay L. K. and Russo I. H.: Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res. Treat.* **2** (1981) 5–73.
26. Clark J. H. and Peck E. J. Jr: *Female Sex Steroids. Receptors and Function.* Springer, Berlin (1979) pp. 99–113.
27. McManus M. J. and Welsch C. W.: The effect of estrogen, progesterone, thyroxine and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. *Cancer* **54** (1984) 1920–1927.
28. Mauvais-Jarvis P., Kuttenn F. and Gompel A.: Antiestrogen action of progesterone in breast cancer. *Breast Cancer Res. Treat.* **8** (1986) 179–188.
29. Vorherr H.: Development of the female breast. In *The Breast: Morphology, Physiology and Lactation* (Edited by H. Vorherr). Academic Press, New York (1974) pp. 1–19.
30. Pertzelan A., Yalon L., Kauli R. and Laron Z.: A comparative study of the effect of oestrogen substitution therapy on breast development in girls with hypo- and hypergonadotrophic hypogonadism. *Clin. Endocr.* **16** (1982) 359–368.
31. Vanderschueren-Lodeweyckx M., Massa G., Maes M., Craen M., van Vliet G., Heinrichs C. and Malaux P.: Growth-promoting effect of growth hormone and low dose ethinyl estradiol in girls with Turner's Syndrome. *J. Clin. Endocr. Metab.* **70** (1990) 122–126.
32. Vihko R. and Apter D.: Endogenous steroids in the pathophysiology of breast cancer. *CRC Crit. Rev. Oncol. Hemat.* **9** (1989) 1–16.
33. Lippman M. E. and Dickson R. B.: Growth control of normal and malignant breast epithelium. *Proc. R. Soc. Edinb.* **95B** (1989) 89–106.
34. Darbre P. D. and Daly R. J.: Effects of oestrogen on human breast cancer cells in culture. *Proc. R. Soc. Edinb.* **95B** (1989) 1–16.
35. Berthois Y., Katzenellenbogen J. A. and Katzenellenbogen B. S.: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 2496–2500.
36. Gill P. G., Vignon F., Bardon S., Derocq D. and Rochefort H.: Difference between R5020 and the antiprogestin RU 486 in antiproliferative effects on human breast cancer cells. *Breast Cancer Res. Treat.* **10** (1987) 37–45.
37. Sutherland R. I., Hall R. E., Pang Y. N., Musgrove E. A. and Clarke C. L.: Effect of medroxyprogesterone acetate on proliferation and cell cycle kinetics of human mammary carcinoma cells. *Cancer Res.* **48** (1988) 5084–5091.
38. Hissom J. R., Bowden R. T. and Moore M. R.: Effects of progestins, estrogens, and antihormones on growth and lactate dehydrogenase in the human breast cancer cell line T47D. *Endocrinology* **125** (1989) 418–423.
39. Braunsberg H., Coldham N. G., Leake R. E., Cowan S. K. and Wong W.: Actions of the progestagen on human breast cancer cells: mechanisms of growth stimulation and inhibition. *Eur. J. Clin. Oncol.* **23** (1987) 563–571.
40. Carpenter S., Georgiade G., McCarty Sr. K. S. and McCarty K. S. Jr: Immunohistochemical expression of oestrogen receptor in normal breast tissue. *Proc. R. Soc. Edinb.* **95B** (1989) 59–66.
41. Vanderbilt J. N., Miesfeld R., Maler B. A. and Yamamoto K. R.: Intercellular receptor concentration limits glucocorticoid-dependent enhancer activity. *Molec. Endocr.* **1** (1987) 68–74.
42. Rabindran S. K., Danielsen M. and Stallcup M. R.: Glucocorticoid-resistant lymphoma cell variants that contain functional glucocorticoid receptors. *Molec. Cell. Biol.* **7** (1987) 4211–4217.
43. Hawkins R. A., Tesdale A. L., Ferguson W. A. and Going J. J.: Oestrogen receptor activity in intraduct and invasive breast carcinomas. *Breast Cancer Res. Treat.* **9** (1987) 129–133.
44. King R. J. B., Finley J. R., Coffer A. E., Millis R. R. and Rubens R. D.: Characterization and biological relevance of a 29-kDa, oestrogen receptor-related protein. *J. Steroid Biochem.* **27** (1987) 471–475.
45. Cano A., Coffer A. I., Adatia R., Millis R. R., Rubens R. D. and King R. J. B.: Histochemical studies with an estrogen receptor-related protein in human breast tumors. *Cancer Res.* **46** (1986) 6475–6480.
46. Battersby S., Robertson B. J., Anderson T. J., King R. J. B. and McPherson K.: Influence of menstrual cycle, parity and oral contraceptive use on steroid hormone receptors in normal breast. Submitted for publication.
47. Garcia E., Bouchard P., De Brux J., Berdah J., Frydman R., Schaison G., Milgrom E. and Perrot-Applanat M.: Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. *J. Clin. Endocr. Metab.* **67** (1988) 80–87.
48. Press M. F. and Greene G. L.: Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. *Endocrinology* **122** (1988) 1165–1175.
49. Press M. F., Nousek-Goebl N., King W. J., Herbst A. L. and Greene G. L.: Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle. *Lab. Invest.* **51** (1984) 495–503.
50. Markopoulos C., Berder U., Wilson P., Gazet J. C. and Coombes R. C.: Oestrogen receptor content of normal breast cells and breast carcinoma throughout the menstrual cycle. *Br. Med. J.* **296** (1988) 1349–1351.
51. Jacquemier J. D., Hassoun J., Torrente M. and Martin P. M.: Distribution of estrogen and progesterone receptors in healthy tissue adjacent to breast lesions at various stages—immunohistochemical study of 107 cases. *Breast Cancer Res. Treat.* **15** (1990) 109–117.

52. Saez S., Martin P. M. and Chouvet C. D.: Estradiol and progesterone receptor levels in human breast adenocarcinoma in relation to plasma estrogen and progesterone levels. *Cancer Res.* **38** (1978) 3468–3473.
53. Heise E. and Görlich M.: Estradiol receptor in human breast cancers throughout the menstrual cycle. *Oncology* **39** (1982) 340–344.
54. Smyth C. M., Benn D. E. and Reeve T. S.: Influence of the menstrual cycle on the concentrations of estrogen and progesterone receptors in primary breast cancer biopsies. *Breast Cancer Res. Treat.* **11** (1988) 45–50.
55. Weimer D. A. and Donegan W. L.: Changes in estrogen and progesterone receptor content of primary breast carcinoma during the menstrual cycle. *Breast Cancer Res. Treat.* **10** (1987) 273–278.
56. Coradini D., Cappelletti V., Miodini P., Ronchi E., Scarone G. and Di Fonzo G.: *Tumori* **70** (1984) 339–344.
57. King R. J. B. and Darbre P. D.: Progression from steroid responsive to unresponsive state in breast cancer. In *Endocrine Therapy of Breast Cancer III* (Edited by F. Cavalli). *European School of Oncology Monographs*. Springer, Berlin (1989) pp. 3–15.
58. King R. J. B.: Heterogeneity of endocrine response within tissues. *Ann. N.Y. Acad. Sci.* **595** (1990) 242–250.
59. Vickers P. J., Dickson R. B., Shoemaker R. and Cowan K. H.: A multidrug-resistant MCF-7 human breast cancer cell line which exhibits cross-resistance to antiestrogens and hormone-independent tumor growth *in vivo*. *Molec. Endocr.* **2** (1988) 886–892.
60. Reddel R. R., Alexander I. E., Koga M., Shine J. and Sutherland R. L.: Genetic instability and the development of steroid hormone insensitivity in cultured T 47D human breast cancer cells. *Cancer Res.* **48** (1988) 4340–4347.
61. Mark L., Graham I. I., Krett N. L., Miller L. A., Leslie K. K., Gordon D. F., Wei L. L. and Horwitz K. B.: $T47D_{CO}$ cells, genetically unstable and containing estrogen receptor mutation are a model for the progression of breast cancers to hormone resistance. *Cancer Res.* **50** (1990) 6208–6217.
62. Katzenellenbogen B. S., Kandra K. L., Norman M. T. and Berthois Y.: Proliferation, hormonal responsiveness and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. *Cancer Res.* **47** (1987) 4355–4360.