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Antiestrogen action of progestogens on human breast cells

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INTRODUCTION

Many controversial studies have been published on the increased risk of breast cancer with the pill or with estrogen replacement therapy for menopause. It is unanimously accepted that estradiol stimulates breast cell multiplication; it can therefore increase the risk of errors arising at the time of cell replication and act as a 'promoter' of carcinogenesis. The role of progesterone is more disputed. Data obtained in experimental animals, and in *in vitro* and *in vivo* studies of breast cell growth and differentiation, and epidemiological data on hormonal risk factors in breast cancer, have caused most authors to conclude that progesterone slows down estradiol-induced cell multiplication and to stimulate functional maturation of cells.

However, the publication of discrepant results has thrown the medical community into confusion. It is therefore essential to gather as much information as possible on the hormone dependence of breast tissue and on the interactions of estrogens and progestogens. The stakes are high, since the conclusions have relevance for the estrogen/progestogen balance in contraceptive pills, the choices of treatment of menopause, and strategies for preventing breast cancer.

What is the role of estradiol and its interaction with progesterone, as deduced from physiological and experimental data on animals, *in vitro* and *in vivo* studies on human breast tissue and epidemiological studies of breast cancer?

EXPERIMENTAL STUDIES IN ANIMALS

Numerous animal experiments have clearly demonstrated the mitogenic effect of estradiol on the mammary gland[1–5]. Eisen[5] showed that administration of high doses of estrogen to mice successively leads to proliferation of the tubular system after 26 days, with dilatation of ducts, formation of cysts and fibrosis. After longer periods of estrogen administration, malignant tumors develop, in all stages from benign to malignant. Progesterone inhibits the development of these estradiol-induced tumors.

Many studies of mammary carcinogenesis in animals have used physical carcinogens (such as irradiation) or chemical carcinogens (dimethylbenzanthracene or *N*-nitrosomethylurea known to raise the incidence of mutations in the Ha-*ras*-I oncogene) which have a role as initiators of carcinogenesis. Administration of estradiol to animals exposed to carcinogens increases the number, size and aggressiveness of the induced tumors, and shortens the time before tumor appearance. Estradiol has a role as a promoter of carcinogenesis. In contrast, pretreatment with progesterone prevents or decreases these effects of estradiol[6–9].

INTERACTION BETWEEN ESTRADIOL AND PROGESTERONE IN HUMAN BREAST TISSUE. PHYSIOLOGICAL AND EXPERIMENTAL DATA

The pubertal period

During the pubertal period, development of the breast tissue is estradiol-dependent: estradiol is secreted alone for several years. After the beginning of the ovulatory cycles, the breast is subjected to the alternate secretion of estradiol and progesterone.

During the menstrual cycle

In breast biopsies obtained at various times in the menstrual cycle, Vogel and colleagues[10] observed a proliferative aspect and numerous mitoses of the duct epithelium during the follicular phase; this aspect is similar to that observed in estrogen-treated animals[1]. During the luteal phase, there were very few mitoses; a secretory aspect was observed similar to that described after progestogen treatment of estradiol-pretreated animals[2,3].

Not only the epithelial cells are regulated by sex steroids. Under the influence of estrogen, the mesenchymatous component of the gland is transformed into intralobular connective tissue. The estrogens stimulate the production of an edematous substance, rich in mucopolysaccharides,

which precedes hyalinization[11] and has an important role in the genesis of breast fibrocystic disease. Progesterone counteracts this effect of estrogen on the mesenchyma[12].

In breast cancer cell lines

Most studies of the hormone dependence of the human breast have been performed using cancer cell lines. Vignon and colleagues[13] and Horwitz and co-workers[14] have shown that the progestin R5020 clearly inhibits the growth of T47D cells.

In a recent paper, Musgrove and colleagues[15] showed that the progestin medroxyprogesterone acetate (MPA) slows the growth of breast cancer cell lines positive for estrogen and progesterone receptors. However, MPA has a biphasic effect: it causes a transient acceleration on cell cycles that have already started; it then blocks cells in the G_0/G_1 phase and prevents them from entering further cycles.

In normal breast cells

The stimulatory effects of estradiol and the inhibitory effects of progesterone on mitosis in human breast tissue have been demonstrated by the incorporation of [^{3}H]thymidine in explants from normal or adenomatous breast tissue, maintained in culture from 2–8 days[16].

Longman and Buehring[17] showed that progesterone or progestins, when added alone, did not stimulate cell growth in explants of normal mammary tissue. When added to ethinylestradiol, which has the greatest stimulatory effect, progestins slow down cell growth.

It is interesting to note that the inhibitory effect of progestins is less marked in cancer cells than in normal cells. Cancer cells may escape from normal regulatory cell mechanisms and no longer respond to hormone action.

In our laboratory, we routinely obtain cultures of normal human breast cells established from samples obtained during reductive mammoplasty. In these cultures, it is possible to conduct separate studies of epithelial cells and fibroblasts[18]. We have focused on the interactions of estradiol and progestogens, especially on the epithelial cells. Ultrastructural characteristics of normal human breast epithelial (HBE) cells were examined by both scanning (SEM) and transmission (TEM) electron microscopy[19]. Under SEM, the cells exhibit a homogeneous pattern: they are large, polygonal and flattened. Microvilli are short and rare. Following estradiol treatment, the cells appear young and show extensive protrusions, with

numerous bunches and blebs. Microvilli increase markedly in number and density, indicating intense replicative activity. However, the addition of the progestin promegestone (R5020) causes the cells to appear flattened without blebs; they are similar to control cells, with sparse microvilli. Parallel transmission electron microscopy reveals extensive Golgi apparatus and secretory activity under R5020 treatment.

Growth of these normal epithelial cells in culture was evaluated using daily cell counting and DNA assay. Growth was stimulated by estradiol in a dose-dependent manner and inhibited by the progestin R5020, also in a dose-dependent manner (Figures 1 and 2)[20–22]. Progesterone itself inhibits cell growth. [^{3}H]thymidine incorporation after 4 days of treatment (i.e. during the exponential phase of cell growth) was higher following estradiol treatment than in control cells. Incorporation decreased when either progesterone or R5020 was added to estradiol, in a dose-dependent manner (Figure 3).

Interesting and comparable results on the interaction and effects of estradiol and progesterone on breast cell multiplication have recently been obtained *in vivo*[23]. A total of 32 patients was treated with either estradiol, progesterone or a placebo, applied locally to the breast during the follicular phase prior to surgery for benign breast diseases. The number of mitoses in the epithelial cells of the normal part of the breast was counted. After estradiol administration, both estradiol concentration in breast tissue and the number of mitoses were high. After progesterone administration, progesterone concentration in breast tissue was high, but the number of mitoses was low. These results support the conclusion that progesterone has an antimitotic effect.

Mechanisms of estradiol and progesterone antagonism in normal human breast cells

Two mechanisms underlying the progesterone regulation of estradiol action – also observed on breast fibroadenomas and cancer – have been characterized in normal human breast cells:

(1) Progesterone stimulates the enzyme 17β-dehydrogenase (E2DH) which converts estradiol, the active estrogen into estrone, its less active metabolite[18,20]; and

(2) Progesterone decreases the estradiol receptor (ER) content[24].

We had first studied these mechanisms in breast fibroadenomas, which are considered to be a good model since they offer a rich epithelial cell

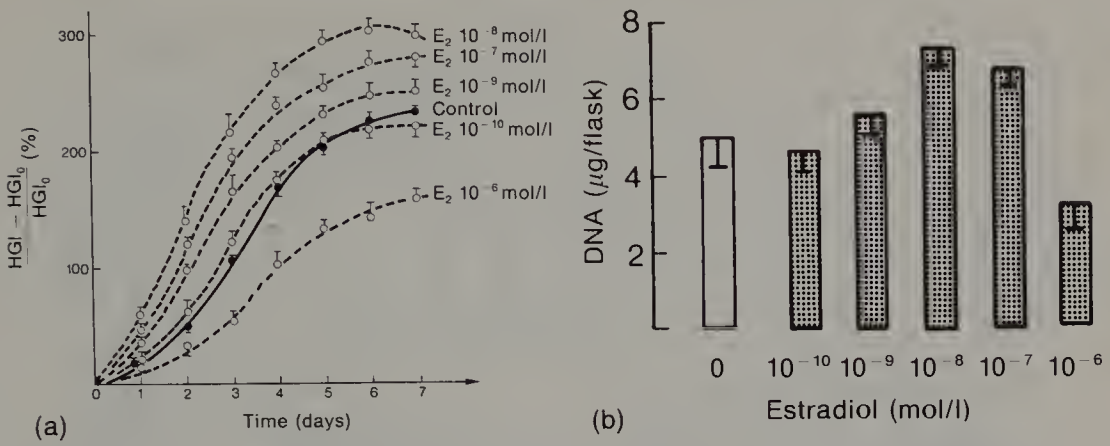

Figure 1 (a) Stimulatory dose-dependent effect of estradiol (E_2) on the growth of normal human breast epithelial cells in secondary culture. The study of cell growth was based on daily cell counting and determination of a histometric growth index (HGI). The results are expressed as percentage increase in HGI compared to the value on day 0 (HGI_0). (b) DNA values on day 7 (mean of triplicate flasks; bars, SD) (Reproduced from reference 22, with permission)

concentration, that still closely resembles normal tissue[25,26]. We then demonstrated that these mechanisms also operate in human breast cells in culture.

E2DH activity

This was measured as the production of estrone by cells incubated with [^{3}H]estradiol. E2DH activity was high and stimulated by progestins in epithelial cells, but low and not stimulated by progesterone in fibroblasts[18]. Consequently, E2DH was proposed as a marker of epithelial cells, of progesterone dependency and also of progesterone receptor (PR) operativity[20,27]. It therefore seemed interesting to compare the action of progestin on E2DH activity and on the DNA content of HBE cells. Progestin treatment lowered DNA content and stimulated E2DH activity in these cells. It thus slows down cell multiplication while favoring cell differentiation[20].

Steroid receptors

These are difficult to study in cultured normal breast cells due to their lower levels compared with cancer cells. Large numbers of cells are therefore required when using the classical biochemical methods for receptor assay. However, immunocytochemical studies using monoclonal

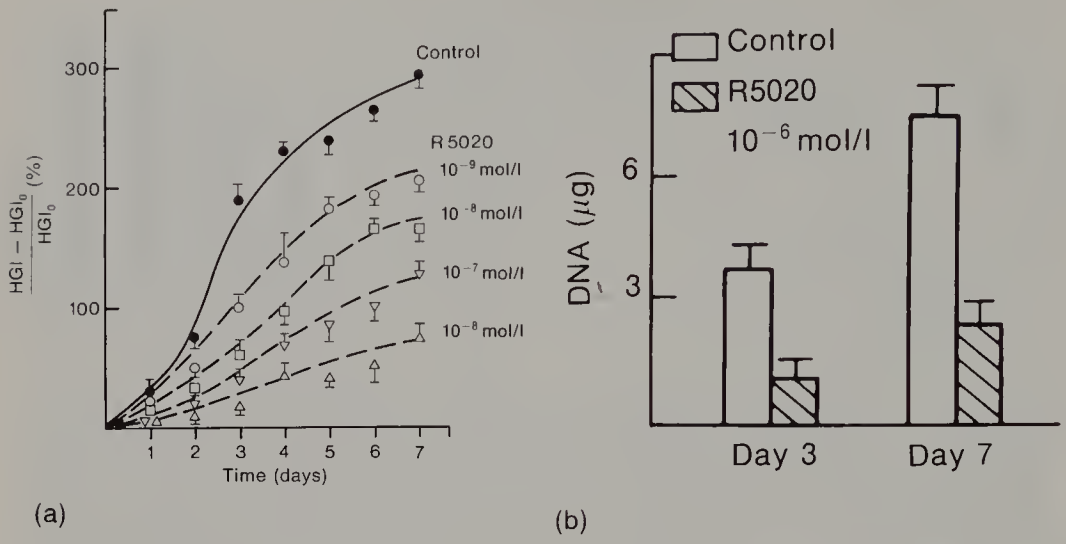

Figure 2 (a) Inhibitory effect of the progestin promegestone (R5020) on the growth of normal HBE cells in culture. The study of cell growth was based on daily cell counting and determination of a histometric growth index (HGI). The results are expressed as percentage HGI compared to the value on day 0 (HGI_0). (b) DNA values on day 3 and 7 in control cell cultures (no hormone addition) and cells cultured in the presence of R5020 (10^{-6} mol/l) (mean ± SD of determinations in triplicate flasks). (Reproduced from reference 20, with permission)

antibodies have enabled the characterization of ER and PR in these cells[24]. Immunostaining specific for ER has been observed in epithelial cells: it is nuclear, and varies from cell to cell in positivity and intensity. Moreover, it is enhanced in estradiol-treated cells and decreases after addition of the progestin R5020. PR was also detected with antibodies provided by Greene[24]. It is also hormone-modulated, increased by estradiol exposure and decreased by R5020.

In normal breast epithelial cells, therefore, estradiol stimulates both its own receptor and the progesterone receptor, whereas the progestin R5020 lowers the number of both ER and PR (Table 1). ER and PR are also present in fibroblasts, but at a lower level than in epithelial cells, and they are only weakly hormone-dependent, if at all[24].

C-myc *proto-oncogene expression HBE cells*

In breast cancer cell lines, the stimulatory effect of estradiol on cell growth can partly operate through the stimulation of proto-oncogene expression[28,29] and growth factor production.

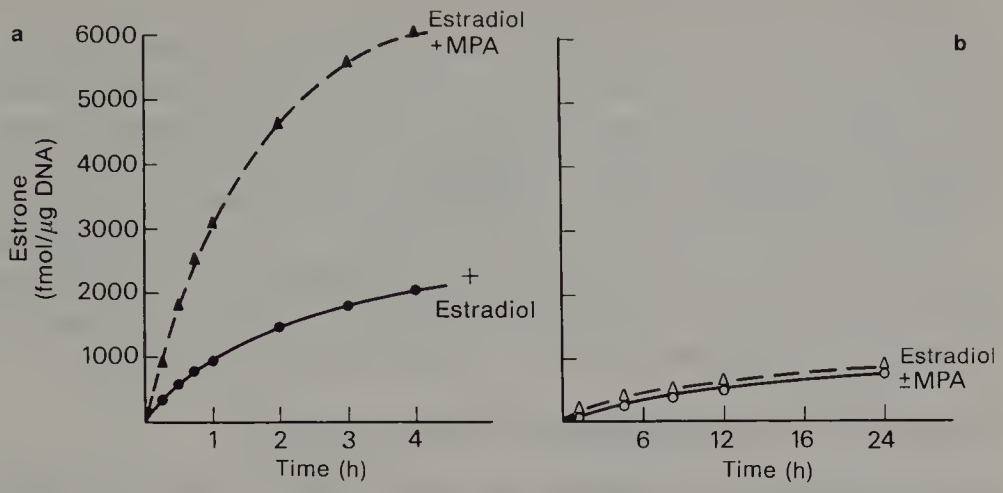

Figure 3 Time course of estrone formation after incubation with [^{3}H]estradiol (incubation medium 5 ml, final concentration = 2 nmol/l) in (a) normal human breast epithelial cells or (b) fibroblasts cultured in the presence of either estradiol (10^{-8} mol/l) alone (no difference from control), or estradiol + medroxyprogesterone acetate (MPA) (10^{-8} mol/l). (Reproduced from reference 18, with permission)

Table 1 Variations of estrogen receptor immunostaining in human breast epithelial cells cultured for 8 days in the absence of steroids (control) or in the presence of estradiol (10^{-8} mol/l), or estradiol (10^{-8} mol/l) + R5020 (10^{-7} mol/l) or R5020 (10^{-7} mol/l) alone. Reproduced from reference 24, with permission

Estrogen receptor immunostaining (% of stained cells)	*Positive* (%)	*Negative* (%)	*Intensity of staining*
Control	55	45	+ +
Estradiol	73	27	+ + +
Estradiol + R5020	44	56	+ +
R5020	35	65	+

The proto-oncogene c-*myc* is involved in the stimulation of cell replication and can itself be stimulated by numerous mitogens, including estradiol. Estradiol stimulation of c-*myc* was demonstrated in human breast cancer cell lines[28,29] and also in non-cancerous estradiol-dependent target tissues (uterus, chick oviduct)[30,31]. In contrast, progestins[32] and triphenylethylenic antiestrogens[29] inhibit c-*myc* expression.

We are investigating whether the foregoing mechanisms operate in

normal breast cells. We have immunocytochemically demonstrated the presence of the c-*myc* protein[33]. The expression of this protein is exclusively nuclear, heterogeneous, and the intensity of staining varies from cell to cell. In addition, c-*myc* expression is hormone-modulated: the number of positive cells and intensity of staining increase following estradiol treatment and decrease after the addition of R5020 to estradiol.

Preliminary results from Northern blot studies confirm the interaction of estradiol with c-*myc* expression: early stimulation of mRNA occurred 30 min after estradiol treatment followed by later stimulation after 2 h. Interactions of progestins and c-*myc* are now being investigated.

HORMONAL RISK FACTORS FOR BREAST CANCER

As well as genetic susceptible factors in breast cancer, hormonal factors are most frequently mentioned, especially the effects of progesterone deficiency with unopposed estrogen administration. Considering these hormonal risk factors[34–38], nulliparity and late first pregnancy may be related to hypofertility and ovulatory disorders, early menarche and late menopause are responsible for prolonged anovulatory periods at each end of the reproductive life; periods when estradiol but no progesterone is secreted, and irregular menstrual cycles, as well as benign breast disease, are the consequences of ovulatory disorders with progesterone deficiency.

Interaction of estradiol and progesterone has also been observed in models of breast carcinogenesis in humans. The women of Hiroshima and Nagasaki who survived the 1945 atomic bomb[39] constitute a model for the study of cooperation between an initiator of carcinogenesis (irradiation) and a promoter (estradiol). Analysing the incidence of breast cancer among irradiated women by age at the time of the explosion, the risk was increased four or five times in women 11–14 years old at the time of irradiation, and doubled in women who were aged 15–19. The risk does not seem to be increased in women who were > 19 years old at the time of irradiation[39]. A similar observation was made among adolescent girls who were subjected to repeated radiological examination as part of antituberculosis treatment[40]. Breast cells therefore seem to be most susceptible to radiation-induced mutations in those young women who were irradiated during the para- or immediate post-pubertal period. This period is characterized by relative hyperestrogenism, anovulatory cycles, no progesterone secretion, and the greatest breast sensitivity to estradiol, consequently inducing rapid proliferation and development.

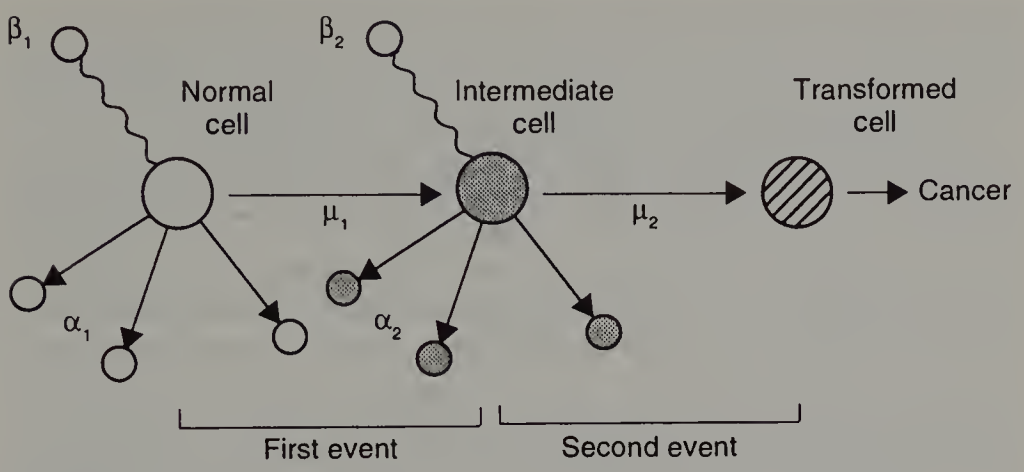

Figure 4 Scheme for two-step carcinogenesis according to Moolgavkar. (Reproduced from reference 41, with permission)

ROLE OF ESTRADIOL AND PROGESTINS IN THE TWO-STEP SCHEME FOR CARCINOGENESIS ACCORDING TO MOOLGAVKAR[41]

Considering the effects of estradiol and progestins on cell growth and differentiation, we can try to understand how they are involved in the process of carcinogenesis.

Moolgavkar has proposed a two-step scheme for carcinogenesis (Figure 4)[41]. This proposes that a normal cell has two possibilities: it either divides into new cells ($\alpha 1$) or it matures, differentiates ($\beta 1$ cell) and finally dies. However, if the normal cell is subjected to an initiator of carcinogenesis, it can evolve ($\mu 1$) into an intermediate (precancerous) cell. Intermediate cells can also either divide ($\alpha 2$) or differentiate ($\beta 2$) and die. But, if a second process of initiation intervenes ($\mu 2$), the cell definitely evolves into a cancer cell.

Cell division is a vulnerable phase for the cells with risks of errors of replication, oncogene activation, or exogenous carcinogen intervention. By stimulating cell multiplication, estrogens increase the risk of error at the time of replication. In this way, they can act as promoters of carcinogenesis. By orienting cells toward maturation, progesterone and progestins exclude them from the pool of vulnerable dividing cells and could be protective. Any situation characterized by an imbalance of endogenous or exogenous progesterone and estradiol with an unopposed estrogen effect should be avoided or corrected.

MIGHT PROGESTERONE BE A CARCINOGENIC FACTOR?

Progesterone itself and progestins have been presented as possible factors of carcinogenesis in a small number of controversial articles[42–44].

Ferguson and Anderson[42] claimed to have observed the greatest number of mitoses in the epithelial cells of normal breast tissue on days 24–25 of the cycle. They concluded that progesterone could have a mitogenic effect. However, their tissue samples came from women who had undergone surgery for benign breast diseases; these patients often suffer from anovulation or dysovulation. The occurrence of ovulation and the existence of a luteal phase were not confirmed by basal body temperature or by plasma progesterone assay. Although biopsies were carried out in the second part of the cycle, therefore, there was no guarantee of a luteal phase. It also seems that the mitotic index had been calculated by adding mitosis and apoptosis (i.e. dead cells).

In a further study of patients taking oral contraceptives, Anderson and co-workers[45] observed a correlation between the number of mitoses in breast tissue and the estrogen potency of the contraceptive (low, medium or high), but no correlation with the progestogen content of pills. They observed only a noticeable number of mitoses with progestogen micropills. However, in these contraceptives the progestogen dose is very low, and is frequently associated with endogenous hyperestrogenism.

Potten and colleagues[46] observed the highest rate of mitoses on day 21 of the cycle, which is too early in the luteal phase to be attributed to the cumulative effect of secreted progesterone. This high rate of mitoses could possibly reflect the cumulative effect of estradiol since the beginning of the cycle.

In an article published in 1983, Pike[43] suggested that oral contraceptives containing the highest doses of progestogen increased the risk of breast cancer. However, the Swyer test used to evaluate the progestogen potency of these pills has been criticized as non-specific. In particular, the pills that he claimed to contain the highest progestogen content were, in fact, the highest in estrogen.

The most recent and controversial article is by Bergkvist and co-workers[44], who evaluated breast cancer risk in postmenopausal women receiving estrogen replacement therapy. Whereas the global risk was 1.1, they found a relative risk of 4.4 when a progestogen was combined with estrogen in the treatment. However, this evaluation was based on only ten patients. The authors themselves declared that these results were not statistically significant and could be due to chance. The lay press echoed Bergkvist's findings but missed the last point, while his article was criticized

in scientific journals. A detailed epidemiological review examining the questionable relationship between progestin exposure in contraceptive and hormone replacement therapies and breast cancer risk failed to find evidence of an association between progestins and breast cancer[47,48].

It is therefore essential to keep in mind the numerous experiments and data on the role of progesterone in controlling the action of estradiol:

(1) Extensive experimental studies on animals have demonstrated a proliferative effect of estradiol on breast tissue, whereas progesterone inhibits estradiol-induced proliferation and induces differentiation;

(2) Numerous studies have confirmed the respective effects of estradiol and progesterone on human breast tissue in cell cultures, explants and biopsies obtained at various times of the menstrual cycle or during estradiol or progesterone treatment;

(3) Clinical and epidemiological observations suggest that the hormonal profile in progesterone insufficiency and unopposed estrogenism seems to favor benign breast disease in the short term and increases the risk of breast cancer in the long term[34–37,49–52]; and

(4) There is an unequivocal beneficial effect of progestins in benign breast disease[53].

All of these data contradict the hypothesis of a carcinogenic effect of progesterone on breast tissue.

CONCLUSION

Normal human breast epithelial cells, which remain hormone-dependent in culture, constitute a useful tool for investigating the hormone-dependence of the normal human mammary gland. In this culture system, estradiol stimulates and progestins inhibit cell growth. In addition, progestins favor cell differentiation.

From these observations, as well as from the data obtained by many other authors, it appears that by stimulating cell proliferation, estradiol can act as a promoter of carcinogenesis. Any condition characterized by estrogen–progesterone imbalance with an unopposed estrogen effect should therefore be avoided or corrected. This should be taken into account in spontaneous anovulatory or dysovulatory menstrual cycles (puberty, perimenopause, hyperprolactinemia, polycystic ovarics, weight changes, stress: i.e., the 'estrogen-windows' of Korenman), the treatment

of benign breast diseases and strategies for the prevention of breast cancer, and therapeutic choices for contraception and the menopause.

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REFERENCES

1. Bassler, R. (1970). Morphology of hormone induced structural changes in the female breast. *Curr. Top.*, **53**, 1–89
2. Benson, G. K., Cowie, A. T., Cox, C. P. and Goldzveig, S. A. (1957). Effects of oestrone and progesterone on mammary development in the guinea-pig. *J. Endocrinol.*, **15**, 126–44
3. Benson, G. K., Cowie, A. T., Cox, C. P., Folley, S. J. and Hosking, S. D. (1965). Relative efficiency of hoexestrol and progesterone as oily solution and a crystalline suspension in inducing mammary growth and lactation in early and late ovariectomized goats. *J. Endocrinol.*, **31**, 157–64
4. Lyons, W. R. and McGinty, D. R. (1941). Effects of estrone and progesterone on male rabbit mammary glands. I. Varying doses of progesterone. *Proc. Soc. Exp. Biol. Med.*, **48**, 83–9
5. Eisen, M. J. (1942). The occurrence of benign and malignant mammary lesions in rats treated with crystalline estrogen. *Cancer Res.*, **2**, 632–44
6. Segaloff, A. (1973). Inhibition by progesterone of radiation–estrogen-induced mammary cancer in the rat. *Cancer Res.*, **33**, 1136–8
7. Inoh, A., Kamiya, K., Fujii, Y. and Yokoro, K. (1985). Protective effect of progesterone and tamoxifen in estrogen-induced mammary carcinogenesis in ovariectomized w/Fu rats. *Jpn. J. Cancer Res. (Gann)*, **76**, 699–704
8. Gottardis, M., Erturk, E. and Rose, D. P. (1985). Effects of progesterone administration on *N*-nitrosomethylurea-induced rat mammary carcinogenesis. *Eur. J. Clin. Oncol.*, **19**, 1479–84
9. Grubbs, C. J., Farnell, D. R., Hill, D. L. and McDonough, K. C. (1985). Chemoprevention of *N*-nitro-*N*-methylurea-induced mammary cancers by pretreatment with 17β-estradiol and progesterone. *J. Natl. Cancer Inst.*, **74**, 927–30
10. Vogel, P. M., Georgiade, N. G., Setter, B. F., Vogel, S. and McCarty, K. (1981). The correlation of histologic changes in the human breast with the menstrual cycle. *Am. J. Pathol.*, **104**, 23–34
11. Ozello, L. and Speer, F. D. (1958). The mucopolysaccharides in the normal and diseased breast. Their distribution and significance. *Am. J. Pathol.*, **34**, 993–1009
12. Asboe-Hansen, G. (1958). Hormonal effects on connective tissue. *Physiol. Rev.*, **38**, 446–62
13. Vignon, D., Bardon, S., Chalbos, D. and Rochefort, H. (1983). Antiestrogenic effect of R5020, a synthetic progestin in human breast cancer cells in culture. *J. Clin. Endocrinol. Metab.*, **56**, 1124–30
14. Horwitz, K. B. and Freibenberg, G. R. (1985). Growth inhibition and increase

of insulin receptors in antiestrogen-resistant $T47D_{co}$ human breast cancer cells by progestins – implications for endocrine therapies. *Cancer Res.*, **45**, 167–73

15. Musgrove, E. A., Lee, C. S. L. and Sutherland, R. L. (1991). Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor α1, epidermal growth factor receptor, c-*fos* and c-*myc* genes. *Mol. Cell. Biol.*, **11**, 5032–43
16. Welsch, C. W., McManus, M. J., Haviland, Th. J., Dombroske, S. E., Swim, E. L., Sharpe, S. and Conley, E. (1983). Hormone regulation of DNA synthesis of normal and hyperplastic human breast tissues *in vitro* and *in vivo*. In: Angeli, A. (ed.) *Endocrinology of Cystic Breast Diseases*, pp. 47–58. (New York: Raven Press)
17. Longman, S. M. and Buehring, G. C. (1987). Oral contraceptives and breast cancer. *In vitro* effect of contraceptive steroids on human mammary cell growth. *Cancer*, **59**, 281–7
18. Prudhomme, J. F., Malet, C., Gompel, A., Lalardrie, J. P., Ochoa, C., Boué, A., Mauvais-Jarvis, P. and Kuttenn, F. (1984). 17β-Hydroxysteroid dehydrogenase activity in human breast epithelial cell and fibroblast cultures. *Endocrinology*, **114**, 1483–9
19. Gompel, A., Chomette, G., Malet, C., Spritzer, P., Pavy, B., Kuttenn, F. and Mauvais-Jarvis, P. (1985). Estradiol-progestin interaction in normal human breast cells in culture. Ultrastructural studies. *Breast Disease*, **1**, 149–56
20. Gompel, A., Malet, C., Spritzer, P., Lalardrie, J. P., Kuttenn, F. and Mauvais-Jarvis, P. (1986). Progestin effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. *J. Clin. Endocrinol. Metab.*, **63**, 1174–80
21. Mauvais-Jarvis, P., Kuttenn, F. and Gompel, A. (1986). Estradiol–progesterone interaction in normal and pathological breast cells. *Ann. N.Y. Acad. Sci.*, **464**, 152–67
22. Malet, C., Gompel, A., Spritzer, P., Bricout, N., Kuttenn, F. and Mauvais-Jarvis, P. (1988). Tamoxifen and hydroxytamoxifen isomers *versus* estradiol effects on normal human breast cells in culture. *Cancer Res.*, **48**, 7193–9
23. Barrat, J., de Lignières, B., Marpeau, L., Larue, L., Fournier, S., Nahoul, K., Linares, G., Giorgi, H. and Contesso, G. (1990). Effet *in vivo* de l'administration locale de progestérone sur l'activité mitotique des galactophores humains. *J. Gynecol. Obstet. Biol. Reprod.*, **19**, 269–74
24. Malet, C., Gompel, A., Yaneva, H., Cren, H., Fidji, N., Mowszowicz, I., Kuttenn, F. and Mauvais-Jarvis, P. (1991). Estradiol and progesterone receptors in cultured normal human breast epithelial cells and fibroblasts – immunocytochemical studies. *J. Clin. Endocrinol. Metab.*, **73**, 8–17
25. Kuttenn, F., Fournier, S., Durand, J. C. and Mauvais-Jarvis, P. (1981). Estradiol and progesterone receptors in human breast fibroadenomas. *J. Clin. Endocrinol. Metab.*, **52**, 1225–9
26. Fournier, S., Kuttenn, F., De Cicco, N., Baudot, N., Malet, C. and Mauvais-Jarvis, P. (1982). Estradiol 17β-hydroxysteroid dehydrogenase activity in human breast fibroadenomas. *J. Clin. Endocrinol. Metab.*, **55**, 428–33
27. Fournier, S., Brihmat, F., Durand, J. C., Sterkers, N., Martin, P. M., Kuttenn, F. and Mauvais-Jarvis, P. (1985). Estradiol 17β-hydroxysteroid dehydrogenase, a marker of breast cancer hormone dependency. *Cancer Res.*, **45**, 2895–9

28. Dubik, D. and Shiu, R. P. C. (1988). Transcriptional regulation of c-*myc* oncogene expression by estrogen in hormone responsive human breast cancer cells. *J. Biol. Chem.,* **253**, 12705–8
29. Santos, G. F., Scott, G. K., Lee, W. M. F., Liu, E. and Benz, C. (1988). Estrogen-induced post-transcriptional modulation of c-*myc* protooncogene expression in human breast cancer cells. *J. Biol. Chem.,* **263**, 9565–8
30. Murphy, L. J., Murphy, L. C. and Friesen, H. G. (1987). Estrogen induction of N-*myc* and c-*myc* protooncogene expression in rat uterus. *Endocrinology,* **120**, 1882–8
31. Weisz, A. and Bresciani, F. (1988). Estrogen induces expression of c-*fos* and c-*myc* protooncogenes in rat uterus. *Mol. Endocrinol.,* **2**, 816–24
32. Fink, K. L., Wieben, E. D., Woloschak, G. E. and Spelberg, T. C. (1988). Rapid regulation of c-*myc* protooncogene expression by progesterone in the avian oviduct. *Proc. Natl. Acad. Sci. USA,* **85**, 1796–800
33. Leygue, E., Louis-Sylvestre, C., Gol, R., Malet, C., Baudot, N., Cumins, C., Mowszowicz, I., Kuttenn, F. and Mauvais-Jarvis, P. (1993). Estrogen regulation of c-*myc* in normal human breast epithelial (HBE) cells in culture. Presented at *75th Annual Meeting American Endocrinology Society,* Las Vegas, June, Abstr. 1914
34. MacMahon, B., Cole P. and Brown, J. (1973). Etiology of human breast cancer: a review. *J. Natl. Cancer Inst.,* **50**, 21–42
35. Cowan, L. D., Gordis, L., Tonascia, J. A. and Jones, G. S. (1981). Breast cancer incidence in women with a history of progesterone deficiency. *Am. J. Epidemiol.,* **114**, 209–17
36. Korenman, S. G. (1980). The endocrinology of breast cancer. *Cancer,* **46**, 874–8
37. Sherman, B. M. and Korenman, S. G. (1974). Inadequate corpus luteum function: a pathophysiological interpretation of human breast cancer epidemiology. *Cancer,* **33**, 1306–12
38. Dupont, W. D. and Page, D. L. (1985). Risk factors for breast cancer in women with proliferative breast disease. *N. Engl. J. Med.,* **312**, 146–51
39. Tokunaga, M., Norman, J. E. and Agano, M. (1979). Malignant breast tumours among atomic bomb survivors Hiroshima and Nagasaki 1959–1974. *J. Natl. Cancer Inst.,* **62**, 1347–52
40. Miller, A. B., Home, G. R., Sherman, G. J., Lindsay, J. P., Yaffé, M. J., Dinner, P. J., Risch, H. A. and Preston, D. L. (1989). Mortality from breast cancer after irradiations during fluoroscopic examination in patients being treated for tuberculosis. *N. Engl. J. Med.,* **321**, 1285–9
41. Moolgavkar, S. H., Day, N. E. and Stevens, R. C. (1980). Two-stage model for carcinogenesis: epidemiology of breast cancer in females. *J. Natl. Cancer Inst.,* **65**, 559–69
42. Ferguson, D. J. P. and Anderson, T. J. (1981). Morphological evaluation of cell turnover in relation to the menstrual cycle in the resting human breast. *Br. J. Cancer,* **44**, 177–81
43. Pike, M., Henderson, B. and Krailo, M. (1983). Breast cancer in young women and use of oral contraceptives: possible modifying effect of formulation and age at use. *Lancet,* **2**, 926–30
44. Bergkvist, L., Adami, M., Persson, I., Hoover, R. and Schairer, C. (1989). The risk of breast cancer after estrogen and estrogen–progestin replacement.

N. Engl. J. Med., **321**, 293–7
45. Anderson, T. J., Battersby, S., King, R. J. B., McPherson, K. and Going, J. J. (1989). Oral contraceptive use influences resting breast proliferation. *Hum. Pathol.*, **20**, 1139–44
46. Potten, C. S., Watson, R. J., Williams, G. T., Tickle, S., Roberts, S. A., Harris, M. and Howell, A. (1988). The effect of age and menstrual cycle upon proliferative activity on the normal human breast. *Br. J. Cancer*, **258**, 163–70
47. Staffa, J. A., Newschaffer, C. J., Jones, J. K. and Miller, V. (1992). Progestins and breast cancer: an epidemiologic review. *Fertil. Steril.*, **57**, 473–91
48. Clarke, C. L. and Sutherland, R. L. (1993). Progestin regulation of cellular proliferation: update 1993. *Endocrine Rev.*, **1**, 132–5
49. Mauvais-Jarvis, P., Sitruk-Ware, R., Kuttenn, F. and Sterkers, N. (1979). Luteal phase insufficiency: a common pathophysiological factor in development of benign and malignant breast diseases. In Bulbrook, R. D. and Taylor, D. J. (eds.) *Commentaries on Research in Breast Disease*, pp. 25–29. (New York: Alan R. Liss Inc.)
50. Vorherr, H. (1986). Fibrocystic breast disease: pathophysiology, pathomorphology, clinical picture and management. *Am. J. Obstet. Gynecol.*, **154**, 161–79
51. Sitruk-Ware, R., Thalabard, J. C., Benotmane, A. and Mauvais-Jarvis, P. (1989). Risk factors for breast fibroadenoma in young women. *Contraception*, **40**, 251–68
52. Plu-Bureau, G., Thalabard, J. C., Sitruk-Ware, R., Asselain, B. and Mauvais-Jarvis, P. (1992). Cyclical mastalgia as a marker of breast cancer susceptibility: results of a case–control study among French women. *Br. J. Cancer*, **65**, 945–9
53. Mauvais-Jarvis, P. and Kuttenn, F. (1993). Benign breast disease. In Bardin, C. W.(ed.) *Current Therapy in Endocrinology and Metabolism*, Vth edn. (Philadelphia: B.C. Decker Inc.), in press