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*Review*

# Antiestrogen action of progesterone in breast tissue

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**Keywords:** antiestrogen, antiprogestin, estrogen action, luteal phase defect, mammary differentiation, progesterone, tumor promotion

## Abstract

In normal breast, estrogen stimulates growth of the ductal system, while lobular development depends on progesterone. Thus, estrogen and progesterone, when secreted in an adequate balance, permit the complete and proper development of the mammary gland. Progesterone may also have an antagonistic activity against estradiol, mediated through a decrease in the replenishment of the estrogen receptor, and also through increased 17β-hydroxysteroid dehydrogenase which leads to accelerated metabolism of estradiol to estrone in the target organ. Thus, it can be inferred that long periods of luteal phase defect leading to an unopposed estrogen effect on the breast might promote breast carcinogenesis.

## Introduction

Many controversies have arisen recently in the literature about progesterone action in the breast [23, 31, 37, 38]. These controversies are related to the possibility that an unopposed estrogen effect due to a defect in progesterone secretion during the luteal phase might be a promoting factor in human breast cancer genesis [23, 31]. The fact that this hypothesis remains controversial is due to insufficient knowledge on the antiestrogenic activity of progesterone and progestins in human breast cells. Whereas the information has been well documented for the endometrium [5, 45], only few data are available regarding breast cells. These recent data, obtained either in animal or in human breast target cells in culture, demonstrated that breast target cells respond to the synergistic and antagonistic action of estradiol and progesterone, in the same way as the endometrium.

## Experimental data in animals

Experimentally there are many indirect data to suggest an antagonism between estradiol and progesterone at the level of breast tissue. Many investigators have shown that the mammary gland of different species responds differently to estrogens if the hormone is administered in physiological or surpraphysiological doses or in combination with progesterone or alone [1, 4, 8, 10, 28] (Table 1).

High doses of estrogen administered for a prolonged time to castrated female rats induce proliferation and dilation of the lobules in the glandular tissue with formation of cysts and overgrowth of the epithelium [4]. In addition, estrogen provokes an increase of circumcanalicular and intralobular connective tissue [1] (Table 2). The successive sequence of mammary alterations following the administration of estradiol to female rats is [10] the proliferation of tubular system secretion, the dilatation of ducts, formation of cysts, and fibrosis. These changes observed with supraphysiologic doses of estrogen seem to be comparable to human

fibrocystic disease [1, 4]. In contrast, when estradiol is administered in combination with progesterone, complete and proper development of the mammary gland is observed when the ratio between estrogen and progesterone is adequate [10, 28]. Cowie et al. [8] found that a combination of estrogen and progesterone in castrated goats resulted in uniform development and secretion when the dose of estrogen remained low (0.25 mg/day). An increase in the estrogen dose to 1.0 mg/day resulted in cysts and epithelial proliferation.

## Biochemical data

Biochemically, the antiestrogen activity of progesterone is well documented. The mechanisms by which progesterone and progestins exert their antiestrogenic action in women include a reduction of estrogen secretion in the systemic circulation, an inactivation of estradiol by its metabolism at the target tissues, a reduction of estrogen receptor levels in these tissues, [27] and a direct effect on cell multiplication.

### *Effects on blood estrogen levels*

Progestins in pharmacological doses may lower the circulating levels of estrogens by suppressing gonadotropins and ovarian function [29]. In particular, androstane derivatives are strong antigonadotropic agents [32]. In a large investigation performed in women with benign breast disease, we were able to confirm that lynestrenol administered from day 10 to day 25 of the menstrual cycle not only suppressed the ovulation peak of LH but also resulted in plasma estradiol levels less than or equal to 50 pg/ml [33].

### *Effects on estrogen receptor levels (Fig. 1)*

Another mechanism by which progestins can be antiestrogenic is their ability to lower estrogen receptor levels in the endometrium [5, 6, 27]. How this effect is mediated has not been biochemically elucidated. It has been suggested that the decrease in intracellular concentrations of estradiol, brought about by the increase in 17β-hydroxysteroid dehydrogenase (see further), may be responsible for the decline in estradiol binding sites. Experiments in rats have shown, however, that progestins affect estrogen receptor levels, even though they do not influence the activity of the enzyme [15]. Progestins do not alter estradiol affinity for binding to its receptor, and it is likely that their effect involves a reduction of estrogen receptor synthesis. In the hamster uterus, progestins decrease the levels of nuclear estrogen receptors through processes inhibited by actinomycin or cycloheximide [27].

Uncertainties concerning the heterogeneity of the estradiol receptor complicate the definition of the problem. In particular, Clark et al. [7] found two distinct types of cytosol receptors: one (type I) is translocated by estrogens into the nucleus, while

*Table 1.* Optimal ratio for obtaining harmonious development of the mammary gland (from Bassler, 1970: Ref. 4).

| Species | Ratio of estrogen: progesterone | Authors |
|---|---|---|
| Rat | 1:1,000–5,000 | Kirkham and Turner (1954) |
| | 1:3,000–5,000 | Smith (1955) |
| | 1:4,000–5,000 | McDonald and Reece (1962) |
| Mouse | 1:75–250 | Mixner and Turner (1943) |
| Rabbit | 1:10–40 | Lyons and McGinty (1944) |
| | | Scharf and Lyons (1944) |
| | 1:67 | Yamamoto and Turner (1956) |
| Guinea Pig | 1:20–100 | Benson, Cowie, Cox, Goldzweig (1957) |
| Goat | 1:140 | Benson, Cowie, Cox, Flux, and Folley (1955) |

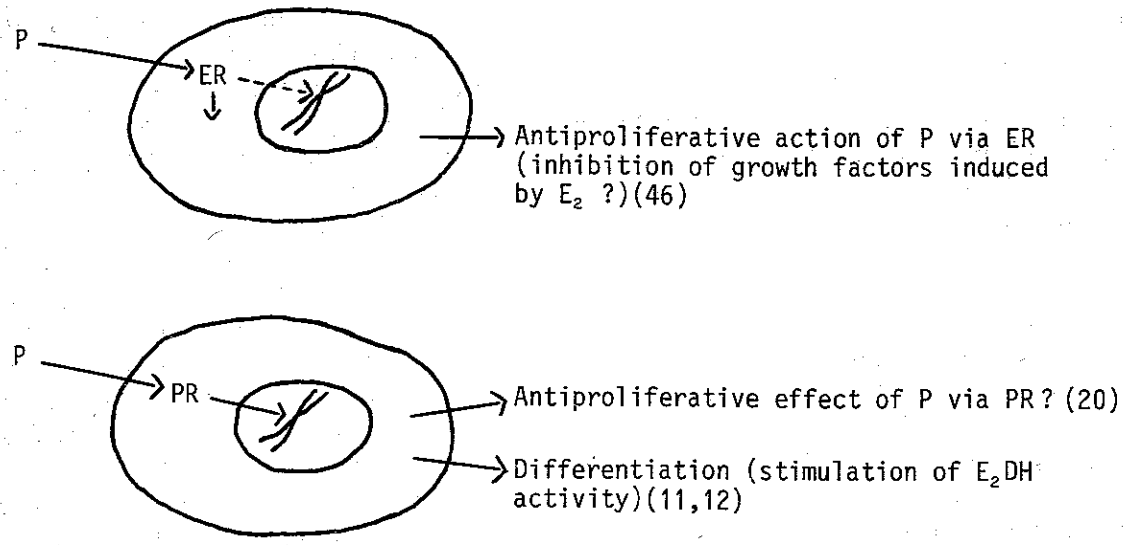


*Fig. 1.* A model describing the possible sites of action of progesterone and progestins (P) on breast cell. ER: decrease in estradiol receptor; PR: progesterone receptor; $E_2$DH: 17β-hydroxysteroid dehydrogenase activity.

the other (type II) is not. These authors also described the presence of two types of nuclear receptors. Progesterone blocks the estrogen stimulation of nuclear type II sites and inhibits uterine growth.

In human noncancerous tissue there is no direct proof that progesterone antiestrogenic activity is mediated through its action on the receptor. Only indirect information is available. First, in fibroadenomas removed at different times of the menstrual cycle, as reported previously [26] there was a continuous decrease in the level of both cytosolic and nuclear estradiol receptors (ERc and ERn) throughout the luteal phase. In addition, when patients with fibroadenomas were treated 30 days before surgery with progestins (10 mg lynestrenol daily + percutaneous progesterone) their fibroadenomas did not contain any trace of estradiol receptor (unpublished data).

*Table 2.* Modifications in breast morphology provoked by administration of estrogen to castrated mice (from Eisen, 1942).

| Lesions | Length of treatment |
|---|---|
| Proliferation of ductal system | 24 days |
| Dilatation of ducts | 60 to 90 days |
| Formation of cysts | 90 to 150 days |
| Fibrosis | 150 to 180 days |

*Effects on breast cell multiplication (Table 3)*

The opposite action of estradiol and progesterone on cell multiplication has been extensively studied in the endometrium, in which estradiol has a strong proliferative effect, whereas progesterone inhibits this effect and is actually involved in cell differentiation [5, 7, 20]. The first *in vitro* data were obtained on breast cancer cells by Vignon et al. [46] who observed that estradiol stimulated the growth of T47D breast cancer cells in culture whereas the progestin promegestone (R5020) inhibited the cell growth induced by estradiol. In a recent study Horwitz and Freidenberg [18] emphasized the role of the progesterone receptor (PR) in the control of breast cancer cell growth by using the breast cancer cell line T47Dco, which is lacking ER and is antiestrogen resistant. In this model R5020 clearly suppresses cell growth with a parallel translocation of PR. This group made the same observation on the T47Dco cell line with RU 486 [19, 20], which like

other antihormones, seems to have both agonist and antagonist action on PR.

To the best of our knowledge, the only report on the respective effects of estradiol and progesterone on normal breast cell division is that of Welsh and McManus [34]. The studies were carried out on normal human breast tissue transplanted in athymic castrated nude mice, and confirmed that estradiol stimulates normal breast cell growth whereas progesterone has no action.

In our laboratory, normal human epithelial cell cultures are currently obtained from reductive mammoplasties. These cells have been shown to be hormone-dependent [40]. They provide a useful model for the study of the action of estradiol and progestins on breast cell division. Cell growth was estimated by daily cell counts using a histometric method providing histometric growth index and DNA assay [12]. Estradiol stimulation of cell growth obtained on secondary cultures could not be observed when the cells were cultured in the usual medium. This may be due to the presence of small amounts of free or conjugated estrogens in the 5% human serum added to the medium. Indeed, under minimal conditions of supplementation (i.e., 1% serum instead of 5%) and with only low amounts of insulin and EGF, a significant stimulation of cell growth by estradiol was apparent and was dose dependent for estradiol concentrations ranging from $10^{-10}$ to $10^{-8}$ M.

Under optimal culture conditions, R5020 slowed down cell proliferation, and this inhibition was dose-dependent. In addition, the inhibition occurred in the absence as well as in the presence of estradiol. This result confirms data obtained by other groups on breast cancer cell lines in culture [3, 18, 20, 46], especially T47Dco in which, despite the absence of ER, physiologic concentrations of progestins directly inhibit cell proliferation. However, it is interesting to note that, when estradiol was added to R5020, cell growth inhibition was less effective than with R5020 alone. This suggests that the specific proliferative effect of estradiol is preeminent over a presumed estrogen-priming effect on PR levels [17].

Interestingly, the antiprogestin RU 486, when added to the medium with or without estradiol, was also capable of inhibiting normal breast cell growth, but to a lesser extent than R5020 (since $10^{-7}$ M RU 486 is required to achieve the same effect as that observed with $10^{-9}$ M R5020) [12]. This antiproliferative effect of RU 486 has already been observed in breast cancer cell lines [3, 19, 20].

The mechanism by which RU 486 inhibits breast cell growth remains unclear since this compound was first described as a progesterone antagonist in clinical trials and, as such, was proposed as a contragestive or chemical precocious abortifacient ]36]. However the dual properties of antihormones and especially antiestrogens are well-known [16, 35], and it seems probable that, like tamoxifen, RU 486 will have biphasic (agonistic and antagonistic) properties, depending on the presence of a pure progestin such as R5020 in the medium. Indeed, when the culture medium is supplemented with an equimolar concentration of R5020 and RU 486, it can be noted that cell growth inhibition decreases.

*Table 3.* Decrease in breast cell proliferation provoked by progesterone, progestins, and antiprogestin RU 486

| Material | Compound used | Reference |
|---|---|---|
| Nude mice + graft of normal human breast tissue | Progesterone | McManus and Welsch (34) |
| Human breast cancer cell line T47D (culture) | R 5020 (Progestin) | Vignon et al (48) |
| Human breast cancer cell line (T47Dco) (without ER) | R 5020 (Progestin) | Horwitz and Freidenberg (18) |
| Normal human breast cells in culture | R 5020 (Progestin) | Gompel et al (12) |
| Human breast cancer cell line (T47D) (culture) | RU 486 (Antiprogestin) | Bardon et al (3) |
| Human breast cancer cell line (T47Dco) (without ER) | RU 486 (Antiprogestin) | Horwitz (19) |
| Normal human breast cells in culture | RU 486 (Antiprogestin) | Gompel et al (12) |

### Estrogen metabolism at target level: the importance of 17β-hydroxysteroid dehydrogenase (Fig. 2)

The high affinity of estradiol for its receptor suggests that the receptor level is the main factor in determining the amount of available hormone bound under physiologic conditions. However, other considerations suggest that hormone-metabolizing enzymes in the cell may interfere with the binding of the hormone by the receptor (15). The main enzyme involved in the antiestrogenic activity is the progesterone-dependent 17β-hydroxysteroid dehydrogenase [43, 45]. The activity of the NAD-dependent 17β-hydroxysteroid dehydrogenase ($E_2DH$) plays, indeed, an important antiestrogenic role since it converts a potent estrogen, estradiol, into a less active one, estrone. Indeed, the estrogen receptor has a lower affinity for estrone and the complex dissociates more rapidly [7]. Previous studies have demonstrated that $E_2DH$ activity is present in human endometrium and other tissues [11, 22, 24, 25, 39, 45]. Extensive investigations were therefore carried out in our laboratory in order to study the activity of this enzyme in breast cells. Two types of investigations were performed: 1) in fibroadenomas; 2) in normal breast cells in culture.

*Fibroadenomas*

This benign tumor was chosen for receptor studies because it offers a relatively homogenous epithelial concentration that is very close to that of normal breast tissue. Breast fibroadenomas were surgically removed from 54 patients [11]. In 28 of those patients, surgery was performed during the follicular phase, in 18 others, it was performed during the luteal phase. Eight other patients were under hormonal treatment: 5 were under progestin therapy (10 mg lynestrenol/day for 10 days) and 3 were treated with progesterone percutaneously applied upon the breast (50 mg/day in an alcoholic gel allowing 10–15% local absorption of the total dose of progesterone) [32].

When $E_2DH$ activity in fibroadenomas was considered with respect to the day of the menstrual

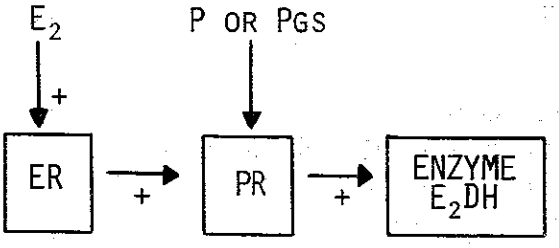


*Fig. 2.* Hormone and receptor dependence of 17β-hydroxysteroid dehydrogenase enzyme activity ($E_2DH$). $E_2$ = estradiol; P = progesterone; Pgs = progestins; ER = estradiol receptor; PR = progesterone receptor.

cycle of the patients, it was low during the follicular phase without significant variations. After ovulation, $E_2DH$ activity progressively increased to reach its maximal level at the end of the luteal phase. This delay in $E_2DH$ activity increase during the luteal phase might be due to an inadequate corpus luteum with low progesterone secretion in most patients with fibroadenomas [11, 29, 30]. Under such conditions, $E_2DH$ stimulation might occur more slowly and later than during a normal luteal phase. However, the variations in $E_2DH$ activity observed at different phases of the menstrual cycle in the patients studied are in agreement with *in vitro* results obtained by different investigators studying human and monkey endometrium [24, 27, 39, 43, 44].

It is interesting to note that the increase in $E_2DH$ activity observed during the luteal phase varies according to the level of epithelial cellularity of the fibroadenoma studied. $E_2DH$ activity is relatively high in tumors with high epithelial cellularity, but is low in tumors with significant fibrosis and no epithelial cells. These results may be correlated with studies on estradiol and progesterone receptors in fibroadenomas [26, 29, 30]. Progesterone receptors disappear rapidly during the course of the disease, and since $E_2DH$ seems to be dependent on the presence and efficiency of the progesterone receptor, it is tempting to consider $E_2DH$ activity as a particularly fine index of cellular differentiation, at least in benign breast lesions. It would, therefore, be interesting to test this enzyme activity in breast cancer in order to determine whether it is a more sensitive marker of hormone

dependency than the progesterone receptor.

The marked increase in $E_2DH$ activity noted in fibroadenomas treated with either oral progestin or topically applied progesterone is yet further proof that this enzyme is progesterone dependent. It also provides an additional basis for the treatment of benign breast disease with locally or systemically administered progesterone or progestins. In addition to the progesterone and progestin antiestrogenic effect due to the decrease in ER synthesis, progesterone-dependent $E_2DH$ activity effectively opposes the elevated concentration of estradiol inside breast tissue, particularly in the case of insufficient production of progesterone by the corpus luteum [29, 42].

These results confirm our previous hypothesis on the hormonal background and substantiate our proposal for the treatment of benign breast disease with progesterone and progestins as a potential prevention against the co-carcinogenic role of estrogen in the development of breast cancer [32].

*Primary cultures of normal breast cells*

Primary cultures of normal breast cells prepared from surgical specimens of reduction mammoplasty were used to study the activity of $E_2DH$ [40].

Epithelial cells and fibroblasts have completely different morphologies and growth rates. Epithelial cells are small, round, and slightly pear-shaped, with a massive nucleus which is round and very refringent. Fibroblasts are characteristically spindle shaped with a small nucleus and filaments extending in several directions. The kinetics of estrone formation after incubation of epithelial cells with $^3$H-estradiol for periods of time ranging from 15 to 40 minutes showed that the reaction is linear for the first 30 minutes. In fibroblast culture, estrone formation was much slower, and a 24 h incubation was necessary to obtain the same amount of estrone as found after 1 h in epithelial cells.

When estradiol was added to the culture medium, it had no effect on $E_2DH$ activity in either cell type, though the affinity and capacity of $E_2DH$ were greater in epithelial cells than in fibroblasts. In the absence of progestin treatment, $E_2DH$ activity increased slowly over the course of cell culture whether estradiol was added or not [12]. However, the stimulation of $E_2DH$ activity by the progestins MPA and R5020 was only observed in a medium containing estradiol [12, 40]. An estrogen priming effect is therefore necessary to the action of progestin on $E_2DH$ stimulation. This contrasts with the action of the progestin R5020 on cell proliferation, which can be observed even in the absence of estrogen supplementation to the medium. In addition, as already observed in human breast cancer cell lines [3, 19, 20], RU 486 results in different effects depending on the biological marker considered. It has progesterone-agonist properties and a partial antagonist effect on cell growth. Moreover, this RU 486 effect does not require the presence of estradiol. In contrast, if $E_2DH$ activity is used as a biological marker, RU 486 displays only an agonistic property and, like R5020, requires the presence of estradiol.

In addition to their intrinsic importance, the present results may have several implications concerning the genesis of breast cancer. They are in agreement with the hypothesis of a protective effect of progesterone or progestins against the mitogenic activity of estrogens in breast target tissue [23, 31] and the fact that the progestin R5020 inhibits breast cell multiplication may be opposed to the recent speculations of Pike et al. [37, 38] on the role of progestins contained in oral contraceptive preparations as factors increasing breast cell division. While progestins directly inhibit breast cell division, the efficiency with which physiologic concentrations of progesterone counteract the mitogenic effect of estrogens is not as well documented. Progesterone, indeed, is much less effective than R5020 in culture [46]. This is only due to the fact that progesterone is markedly metabolized in cell cultures [46]. However, like R5020, this natural steroid suppresses the replenishment of PR in T47Dco cells in culture [18]. In addition, progesterone, when topically applied over the breast of patients with fibroadenomas, significantly increases $E_2DH$ activity measured *in vitro* in the epithelial cells of the tumors [11].

Several conclusions can be drawn from this study: 1) estradiol and the progestin R5020 have an opposite effect on proliferation of normal human breast epithelial cells in culture – estradiol stimulates whereas R5020 inhibits cell multiplication; 2) the antiproliferative action of R5020 is effective even in the absence of estradiol; 3) apart from its antiproliferative effect, R5020 appears to favor cell differentiation, as the stimulation of $E_2DH$ enzymatic activity by progesterone or progestins is considered an index of such a differentiation; and 4) the antiprogestin RU 486 has a dual progestin agonist/antagonist action, depending on the physiological response considered, and perhaps also on the presence on the DNA progesterone acceptor of another compound, binding more strongly or more specifically the site of the chromatin involved in transcriptional progesterone activity (Fig. 2).

**Preventive action of progesterone against experimental animal mammary carcinogenesis (Table 4)**

The effects of progestins on established mammary tumors are well known. Progesterone alone cannot maintain tumor growth. In ovariectomized and adrenalectomized rats with growing DMBA tumors, estradiol alone or in combination with progesterone can sustain continued tumor growth, but progesterone alone cannot and the tumor regresses (2). Estradiol alone, but not progesterone, restores tumor growth.

More interesting is the fact that early treatment with progesterone protects the mammary gland against the tumor-promoting effect of estrogens and various carcinogens [20]. The protective action of progesterone against the carcinogenic induction of mammary tumors was first shown by Segaloff [41] in an interesting experiment on estrogen and Xray-induced estrogen-induced mammary carcinogenesis in female A X C rats. The continuous administration of progesterone protected these animals from the synergism between estrogen and radiation. In addition, it also appeared that progesterone had an overall protective effect against the individual carcinogenic agents, since fewer tumors occurred on the opposite side as well as on the radiated side.

In other experiments [22, 47] it has been demonstrated that tumor development induced by DMBA in Sprague Dawley rats might be inhibited when progesterone was administered 7 to 25 days before DMBA administration. The latency period was prolonged and fewer rats developed tumors, with fewer tumors per rat [47]. Recently it has been observed that N-nitrosomethyl urea (NMU) provokes the mutagenesis of the H-ras-1 oncogene in rats and subsequent carcinogenesis [48]. More-

*Table 4.* Decrease in total number of mammary tumors in rats treated with progesterone (P) before carcinogen exposure.

| Animals | Protocol | Results | Reference |
|---|---|---|---|
| 48 A × C rat with intact ovaries | P pellets before irradiation + DES pellets | no tumors in P treated animals on radiated side | Segaloff [41] (1973) |
| 61 Sprague Dawley rats with intact ovaries | P treatment (4 mg/day) × 7 days before NMU exposure (4 mg/100 gm body wt) | 35% tumors in P treated animals + NMU instead of 80% without P + NMU | Gottardis et al. [13] (1983) |
| 140 Sprague Dawley rats with intact ovaries | P treatment (4 mg/day) or P + $E_2$ 20 mg × 10 days before NMU 50 mg/kg body wt × 3 weeks | 42% tumors in P + $E_2$ treated animals + NMU instead of 80% without P + NMU | Grubbs et al. [14] (1985) |
| Sprague Dawley rats castrated | P treatment (4 mg/day) × 20 days prior DMBA | 70% inhibition of tumor incidence in treated group compared to control | Kledzik et al. [22] (1974) |
| Sprague Dawley rats castrated | P treatment (4 mg/day) × 10 days before DMBA (× 40 days) | 80% inhibition of tumor incidence in treated group compared to control | Welsch et al. [47] (1968) |
| 50 WFU rats castrated | P treatment (2 mg/days) × 2 weeks before administration of $E_2$ or DES | 23% tumors in P treated group instead of 94% in control + $E_2$ or DES | Inoh et al. [21] (1985) |

over, in an elegant experiment it was reported that chronic treatment of Sprague Dawley rats with progesterone 7 days before NMU administration significantly decreased the cumulative incidence of NMU-induced mammary tumors as well as their total number (13). A similar observation was made by another group in female Wistar-Furth rats [21]. These rats were ovariectomized and treated with DES or estradiol. When progesterone was administered simultaneously with the estrogens the mammary tumor incidence decreased from 96% to 23%.

More recently, Grubbs et al. [14] have clearly demonstrated that stimulation of the mammary gland to a highly differentiated state early in life can provide protection against future carcinogen exposure. In the Sprague-Dawley rat model the authors administered to the animal immediately after puberty either NMU alone, or estradiol or progesterone or both followed by NMU exposure, in order to determine whether prevention of NMU-induced tumors was possible. They showed that pretreatment of rats with 17β-estradiol and progesterone resulted in a 88% reduction in cancer incidence.

In these different experiments the protective effect of progesterone against various carcinogens was generally related to the stimulation by progesterone of terminal duct differentiation, thus protecting lobulo-alveolar glands from carcinogens. However, it is interesting to note that in the NMU model, the mutagenesis of an oncogene is postulated. Thus, the antiestrogen effect of progesterone which inhibits the estrogen-dependent proliferation required for tumor induction might provide conditions that are adequate for suppressing the phenotypic expression of the NMU-activated ras-1 oncogene – at least in rats (48). The fact that tamoxifen, like progesterone, has a protective effect against mammary carcinogenesis initiated by estrogens in ovariectomized rats [21] may be considered to be in favor of this last hypothesis.

## Summary

In most target cells of the female genital tract, adequate cell differentiation is obtained via the successive and synergistic actions of estradiol ($E_2$) and progesterone (P). This is primarily due to the fact that P receptor (PR) synthesis involves the prior action of estradiol via its receptor (ER). In normal breast, $E_2$ stimulates the growth of the ductal system, whereas lobular development depends on P secretion. In other words $E_2$ +P, when secreted in an adequate balance, permit the complete and proper development of the mammary gland. On the other hand, P may also have an antagonistic action against $E_2$. The antiestrogen activity of P is mediated through a decrease in the replenishment of $E_2$ receptor along with the synthesis of 17β-hydroxysteroid dehydrogenase which leads to accelerated metabolism of $E_2$ to $E_1$ in the target organ itself. These biochemical events, well documented in the endometrium, have been shown in cultures of normal breast epithelial cells as well as in differentiated fibroadenomas with high cellular density. In addition, data from the literature show that $E_2$ added to human breast cells increases cell multiplication, possibly because of the synthesis of growth factors. P and progestins have opposite effects to estrogen. Data from our laboratory indicate that in normal cultured cells, $E_2$ and P are likewise antagonistic with regard to cell multiplication. Based on these different data, it is inferred that in human beings, long periods of luteal phase defect leading to an unopposed estrogen effect might be a promoter agent involved in breast carcinogenesis.

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