

Steroid Concentrations in Serum, Glandular Breast Tissue, and Breast Cyst Fluid of Control and Progesterone-Treated Patients

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Growth and development of the human breast is dependent on several hormones among which estradiol-17 β (E₂) and progesterone (P) are of great importance (2,3). Both hormones are accumulated and retained in breast tissues (7,8), acting alternately on glandular tissue, connective tissue, and blood vessels. Estradiol and progesterone are antagonists in the regulation of cell division and capillary permeability (24). Estradiol stimulates ductal growth and hyperplasia of the connective tissue and may induce edema. Progesterone inhibits vasodilatation and edema and stimulates differentiation of galactophores and acini, thus inducing the development of secretory tissue (1,3).

Since estradiol and progesterone are partly antagonistic and partly synergistic for the normal development and function of the breast, the equilibrium between these two hormones is determinative for tissular eutrophy. During the menstrual cycle, the equilibrium between E₂ and P is changing continuously in a controlled manner. This hormonal equilibrium may be disturbed by ovarian dysfunction, leading to an inadequate luteal phase (20) characterized by an insufficient progesterone secretion and a relative or absolute excess of estradiol in the blood circulation. If repeated regularly in subsequent menstrual cycles, this hormonal imbalance may give rise to breast anomalies. In most patients with benign breast disease, plasma progesterone levels were significantly lower than reference values of normal women (22). In addition, the increased risk for breast cancer in women with benign breast disease has been emphasized by several authors (4,9,13).

Since it could be expected that insufficient blood progesterone levels were accompanied by abnormally low concentrations of progesterone in the breast tissues, the question was raised if hormonal treatment would be salutary. Subsequently, several studies have demonstrated the beneficial effect of progesterone and progestational agents in the prevention and treatment of benign breast disease (12,15).

Recently, an easy and efficient way of treating women with benign breast disease was developed through topical application of progesterone, dissolved in a hydroalcoholic gel (Progestogel®). Since the percutaneous resorption of progesterone was

found to be approximately 10% of the applied dose (14), the application of 50 mg of progesterone in the gel could result in the absorption of 5 mg of active progesterone in the breast. Consequently, the eventual deficiency of progesterone in breast tissues could be compensated and normal levels restored.

We have studied the concentrations of estradiol, progesterone, and one or more additional steroids in serum, in glandular breast tissue, and in breast cyst fluid of patients suffering from gross cystic disease (11). In a first phase, we determined if progesterone was accumulated and retained in the breast tissues; in a second phase, we determined the extent of this accumulation. In both phases, breast cyst fluid was analyzed for the following steroids: progesterone, 20 α -dihydroprogesterone, estradiol, estrone, androstenedione, testosterone, dihydrotestosterone, and dehydroepiandrosterone sulfate. Statistical differences were calculated with the Wilcoxon test for paired or unpaired series (21).

ACCUMULATION OF PROGESTERONE IN GLANDULAR BREAST TISSUE

The concentrations of progesterone, 20 α -dihydroprogesterone (DHP)¹, and estradiol were determined in breast tissue of different groups of women. DHP, the major metabolite of P in human glandular breast tissue (23), was determined in order to evaluate local metabolism of progesterone. In this way, the selective accumulation and retention of P in breast tissue could be compared with the elimination of the biologically insignificant metabolite DHP via the blood.

Tissues were obtained at the time of surgery from 34 patients with fibrocystic disease or fibroadenoma of the breast. None received hormonal treatment during the last 6 months. The patients were divided into a control group and a treated group. The control group consisted of 8 women in the luteal phase and 14 in the follicular phase, receiving no treatment. The treated group included 12 women in the follicular phase, receiving a single dose of 50 mg of progesterone in a gel, rubbed into the breast skin 24 hr before surgery.

The concentrations of E₂ and P in serum of controls were compared with reference values of normal women (Table 1). The significantly lower progesterone serum concentrations were indicative of luteal phase insufficiency.

Thirty min to 4 hr after treatment, significantly elevated serum P concentrations were observed in most patients. Twenty-four hr after treatment, i.e., at the time of surgery, the progesterone serum concentrations had returned to normal values (5). The concentrations of P and DHP in breast tissues are presented in Fig. 1. Twenty-four hr after a single percutaneous treatment of women in the follicular phase with 50 mg progesterone, the concentration of P in glandular breast tissue was comparable to the concentration observed in controls in the luteal phase, and significantly higher than in controls in the follicular phase. The concentration of the metabolite of P, i.e., DHP, was higher in the luteal phase than in the follicular phase of controls.

¹DHP, 20 α -hydroxy-4-pregnen-3-one.

TABLE 1. Concentrations (mean \pm SD) in serum of normal women in comparison with references

	N	Estradiol Follicular ^a
References	(181)	53 \pm 27
Patients	(22)	54 \pm 65

^a Follicular phase: 14 to 2 days before ovulation

^b Luteal phase: 2 to 11 days after ovulation

^c $p < 0.001$; statistically significant

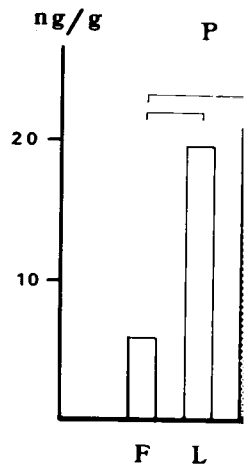


FIG. 1. Steroid concentrations in glandular breast tissue of progesterone (P) and of 20 α -dihydroprogesterone (DHP) in the follicular (F) and luteal (L) phases. Statistically significant differences between phases are indicated by P.

However, after a single dose of progesterone, the tissue concentrations of DHP were elevated. This elevation via the blood seems to have more to do with a single dose of P. Progesterone is retained in breast tissue, whereas DHP, being biologically insignificant, is eliminated via the blood. The finding of elevated serum DHP concentrations is indicative of DHP via the blood.

EFFECT OF PROLONGED TREATMENT

In order to evaluate the extent of accumulation of progesterone in breast tissue, we analyzed tissues from 30 patients with fibrocystic disease of the breast. None received hormonal treatment during the last 6 months.

TABLE 1. Concentrations of estradiol and progesterone (mean \pm SD) in serum of patients with benign breast disease: comparison with reference values obtained in normal healthy women

	N	Estradiol pg/ml		Progesterone ng/ml	
		Follicular ^a	Luteal ^b	Follicular ^a	Luteal ^b
References	(181)	53 \pm 27	160 \pm 73	0.48 \pm 0.30	13.74 \pm 5.60
Patients	(22)	54 \pm 65	156 \pm 117	0.36 \pm 0.23	4.20 \pm 0.57

^a Follicular phase: 14 to 2 days before ovulation.

^b Luteal phase: 2 to 11 days after ovulation.

^c $p < 0.001$: statistically significant difference (Wilcoxon ranking test).

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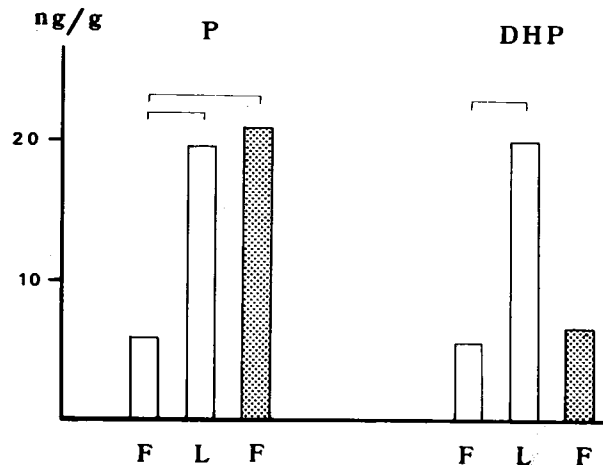


FIG. 1. Steroid concentrations in glandular breast tissue. Concentrations of progesterone (P) and of 20 α -dihydroprogesterone (DHP) in breast tissue of control \square and treated \square patients in the follicular (F) and luteal (L) phases of the cycle are expressed in ng/g fresh tissue. Statistically significant differences between two groups are given by the horizontal lines \square .

However, after a single dose of progesterone in the follicular phase, no elevated tissue concentrations of DHP were observed. A continuous supply of progesterone via the blood seems to have more influence on the DHP tissue levels than treatment with a single dose of P. Progesterone was thus accumulated and retained in glandular breast tissue, whereas DHP, being a biologically weak metabolite, was not retained. The finding of elevated serum DHP levels 24 hr after treatment suggested elimination of DHP via the blood.

EFFECT OF PROLONGED TREATMENT WITH PROGESTERONE

In order to evaluate the extent of the accumulation of progesterone in the breast, we analyzed tissues from 30 patients with fibrocystic disease or fibroadenoma of the breast. None received hormonal treatment during the last 6 months before

surgery. The control group receiving no treatment consisted of 16 patients in the luteal phase of the cycle. In the treated group, the patients received daily 50 mg progesterone percutaneously during 8 to 16 consecutive days. Treatment ended 24 hr before surgery, in the luteal phase (10 patients) or in the follicular phase (4 patients).

The mean serum progesterone concentration of the treated patients in the luteal phase was 9 ng/ml. This is not significantly different from the reference value of 13.7 ng/ml (see Table 1). The concentrations of P, DHP, and E₂ in breast tissue are shown in Fig. 2. Of these three steroids, only progesterone was present in significantly higher concentration in glandular breast tissue than in serum of the four groups of women: controls and those treated in the follicular or luteal phases of the cycle, suggesting selective accumulation and retention of progesterone in human breast tissue (6). After prolonged treatment with progesterone, the mean tissular P concentration attained 70 ng/g tissue and was significantly higher than tissular levels in controls or 1-day-treated women.

Tissular DHP concentration was elevated after prolonged treatment that ended in the luteal phase: half the P concentration 24 hr after the last percutaneous

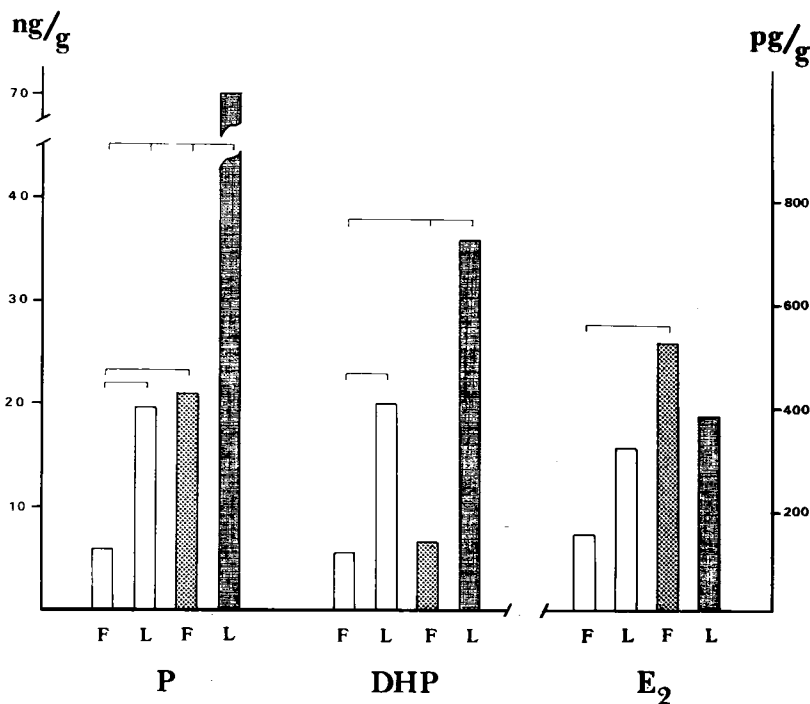


FIG. 2. Steroid concentrations in glandular breast tissue. The concentrations of progesterone (P) and 20 α -dihydroprogesterone (DHP), expressed in ng/g fresh tissue, and of estradiol (E₂) in pg/g tissue in the follicular (F) and luteal (L) phases of the menstrual cycle were compared in control patients \square and in patients treated percutaneously with progesterone in a single application ▨ , or during 8 to 16 days ▩ . Significant differences between groups are given by horizontal lines \square .

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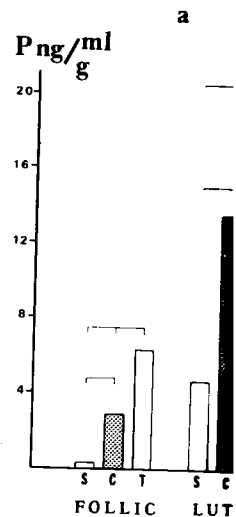
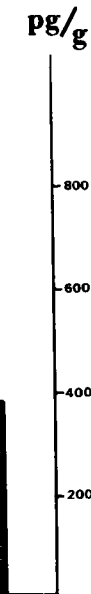


FIG. 3. Progesterone concentrations in glandular breast tissue. Progesterone concentrations are expressed in ng/g fresh tissue in glandular breast tissue. Treatment was for 1 day (1d) or 8 to 16 days (8-16d) given by horizontal lines \square .

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administration of progesterone. This elevated DHP concentration may be explained by a continuous formation of DHP from P in the glandular breast tissue (23). The elevated DHP serum level after prolonged treatment (7.5 ng/ml) was significantly higher than in controls in the luteal phase (2.8 ng/ml). This finding suggested elimination of the biologically ineffective metabolite of P via the blood.

The concentration of E₂ in glandular breast tissue was significantly elevated after a single percutaneous treatment with progesterone in the follicular phase (6), but these differences disappeared after prolonged treatment ending in the luteal phase of the cycle. There is no evidence for the origin of the elevated E₂ concentration after a single percutaneous treatment with progesterone. The normalized E₂ level after prolonged treatment with progesterone may be attributable to the enzyme 17 β -hydroxysteroid dehydrogenase present in normal human breast tissue (18). The activity of this enzyme, which converts estradiol to estrone, is highest in the luteal phase. This is probably a progesterone-dependent phenomenon similar to that observed in human endometrium (10) and myometrium (19).

STEROIDS IN BREAST CYST FLUID

The concentration of progesterone in breast cyst fluid was significantly higher than in serum (Fig. 3) and lower than in glandular breast tissue. This lower level of P in cyst fluid than in tissue was significant, except for control patients in the luteal phase. Significant differences were observed only if the two groups of controls and treated patients (a and b in Fig. 3) were subdivided into follicular and luteal

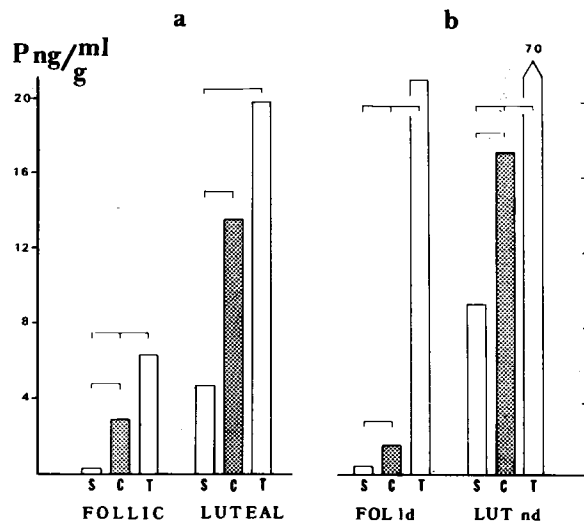


FIG. 3. Progesterone concentrations in serum, and in cyst fluid and tissue of the breast. Progesterone concentrations are expressed in ng/ml in serum (S) and breast cyst fluid (C) and in ng/g fresh tissue in glandular breast tissue (T) of untreated controls (a) and treated (b) patients. Treatment was for 1 day (1d) or 8 to 16 days (nd). Significant differences between groups are given by horizontal lines [—].

groups. The finding of specific progesterone-binding components in breast cyst fluid (16,17) may account for the specific accumulation of progesterone in the cyst fluid. Although treatment with progesterone significantly increased tissular P levels, cyst fluid P levels were not increased significantly after 1 day or 8 to 16 days of treatment.

The concentration of DHP in cyst fluid was significantly higher in the luteal phase than in the follicular phase of controls. Other differences were not observed. The concentrations of estradiol, estrone, androstenedione, and testosterone in cyst fluid were higher in the luteal than in the follicular phase of controls, but the differences were not significant. No differences were found for 5α -dihydrotestosterone and dehydroepiandrosterone sulfate (DHAS) between follicular and luteal phases of the cycle. DHAS levels in cyst fluid were significantly elevated over the serum levels.

SUMMARY

In patients with benign breast disease, we observed signs of luteal phase insufficiency and probably too-low levels of progesterone in glandular breast tissue since, after percutaneous treatment with progesterone, higher tissular progesterone levels were obtained. This progesterone was accumulated and retained in the tissue. Its metabolite 20α -dihydroprogesterone apparently was not accumulated or retained. Estradiol tissue concentrations did not change after prolonged treatment.

In breast cyst fluid, significant differences in steroid concentrations between follicular and luteal phases were observed for progesterone in controls and treated patients, and for 20α -dihydroprogesterone in controls only. No significant differences were observed for estradiol, estrone, androstenedione, testosterone, 5α -dihydrotestosterone, and dehydroepiandrosterone sulfate. Treatment with progesterone did not influence this pattern.

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