Progesterone Receptors in Normal Mammary Gland: Receptor Modulations in Relation to Differentiation

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ABSTRACT The biological basis for the observed modulation in cytoplasmic progesterone receptors (PgR) of normal mammary gland occurring during mammary development was investigated. Specifically, the relative roles of hormones vs. differentiation on (*a*) the decrease in PgR concentration during pregnancy and lactation and (*b*) the loss of mammary responsiveness to estrogen during lactation were examined. PgR were measured using the synthetic progestin, R5020, as the ligand. The hormones estrogen and progesterone were tested in vivo for their effect on PgR concentration. Mammary gland differentiation was assessed morphologically and by measuring enzymatically active α-lactalbumin.

These studies show that there is a stepwise decrease in PgR that occurs in two stages. The first decrease is completed by day 12 of pregnancy and the second decrease occurs only after parturition. There appears to be a hormonal basis for the first decrease and it appears to be caused by the negative effect of progesterone on estrogen-mediated increase in PgR. In direct contrast, the absence of PgR during lactation and the mammary tissue insensitivity to estrogenic stimulation of PgR were not related to the hormonal milieu of lactation but were directly related to the secretory state of the mammary gland and lactation per se.

In rodents the two ovarian hormones, estrogen and progesterone, are both critically required for mammary cell proliferation and lobuloalveolar differentiation (LAD) during pregnancy. Estrogen stimulates cell proliferation (2, 23, 29), whereas progesterone in concert with estrogen causes morphogenesis of mammary alveoli (2, 11, 23, 29). Progesterone also prevents the initiation of lactation before parturition (8–10, 19, 39). Also, once lactation is established, it can proceed normally in the absence of ovaries (7).

For steroid hormones to produce a biological response, it is believed that they must initially interact with their macromolecular cytoplasmic receptors (17). In target tissues for estrogen and progesterone such as the uterus and certain normal and neoplastic mammary tissues, cytoplasmic estrogen receptors (ER) and progesterone receptors (PgR) are present. Furthermore, PgR synthesis in these tissues appears to be under estrogenic control and thus PgR can also serve importantly as markers of estrogen action (13–15, 20, 26, 28, 37, 38, 41, 43). We have previously reported that PgR concentration per cell varies with the developmental state of the mammary gland; PgR are present in virgin gland, decrease during pregnancy, are undetectable during lactation, and reappear during lactational involution (14). Most striking is the observation that not only are PgR absent during lactation but also that estrogen administration to lactators fails to result in the increased concentration of mammary PgR. This inability to respond to estrogen is specific to lactating mammary tissue because (*a*) uteri of lactating mice respond to exogenous estrogen with increased PgR levels and (*b*) in virgin mammary gland the level of PgR is augmented by estrogen administration in a manner similar to that for the uterus (13). The present studies were undertaken to determine the basis for (*a*) the observed modulation in PgR concentration during mammary development and (*b*) the loss of mammary responsiveness to estrogen during lactation. We found that there was a hormonal basis for the decrease in PgR concentration that occurs during pregnancy and that this decrease was most likely attributable to progesterone. In contrast, the absence of PgR during lactation was not related to the hormonal milieu of lactation but was directly related to the secretory state of the gland and lactation per se.[1]

[1] A preliminary report of this work has been published: Haslam, S. Z., and G. Shyamala. 1979. Modulation of progesterone receptors in normal mammary tissue. *J. Cell Biol.* 83(2, Pt. 2):238*a* (Abstr.).

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MATERIALS AND METHODS

Animals

Female BALB/c mice were used at 2–5 mo of age and were from our own colony. Intact or ovariectomized virgin, pregnant, or lactating mice were obtained and used as described previously; ovariectomized virgin mice were used 14 d after ovariectomy (13, 14). In certain experiments, animals were ovariectomized and hysterectomized on day 14–16 of pregnancy, and hormone injections were initiated 24 h later. Unilateral thelectomy (nipple removal) was accomplished by cauterization before mating; litter sizes were adjusted to six pups.

Isotope

The synthetic progestin [^{3}H]R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) (sp act, 86.0 Ci/mmol) was purchased from New England Nuclear, Boston, Mass.

Hormones

All hormones used for injections were purchased from Sigma Chemical Co., St. Louis, Mo. Unlabeled R5020 was purchased from New England Nuclear. The hormones were administered by subcutaneous injection as a solution in 1% ethanol in saline or in sesame oil.

Steroid Receptor Assay

Tissue homogenates were prepared in a phosphate-glycerol buffer (5 mM sodium phosphate, 10 mM thioglycerol, 10% glycerol, pH 7.4) and centrifuged at 12,350 *g* for 1 h. Mammary glands were homogenized at a concentration of 1 gm/ml. Unless otherwise specified, the supernates designated as cytoplasmic extracts were used for steroid receptor assays. For measurements of PgR, aliquots of cytoplasmic extracts were incubated with 20 nM of [^{3}H]R5020 alone or in the presence of a 100-fold excess of unlabeled R5020. All cytoplasmic extracts were incubated with hormones at 4°C for 4 h before assay. The bound readioactive steroid in all the incubations was estimated using the dextran-coated charcoal assay of Korenman (18) as described previously (42) for mammary glands.

Some further comments on the estimation of PgR concentration in the present studies using R5020 as the ligand are necessary. Previously, we have reported that dexamethasone can significantly compete for certain specific R5020-binding sites; however, these binding sites did not appear to be high-affinity PgR (42). Subsequent studies have revealed that inclusion of dithiothreitol (DTT) in the buffer augments the degree of competition by dexamethasone to specific R5020-binding sites, and this is compatible with recent observations in our laboratory that the glucocorticoid-binding sites in mammary tissues are stabilized by DTT (24). Therefore, in the present studies, DTT was not included in the homogenizing buffer; however, it should be mentioned that even when DTT was excluded, some dexamethasone competition was still observed. In all cases, even if the binding data are corrected for dexamethasone competition, the relative results remain unchanged. For these reasons and because the precise identity of these dexamethasone-competible sites is as yet unclear, the specific binding data reported in these studies do not include any correction for competition by dexamethasone to specific R5020-binding sites. We have also determined that endogenously bound progesterone would be exchanged 100% by R5020 under the present assay conditions[2] in a manner similar to that reported for mouse uterus (36); thus, values for specific R5020 binding represent the total number of cytoplasmic PgR.

α-Lactalbumin Measurement

α-Lactalbumin activity was assayed by the method of Ip and Dao (16), based on that of Ebner et al. (10), with modifications as follows. Mammary tissue was homogenized with one 15-s burst of a Polytron PT10-ST (Brinkmann Instruments, Inc., Westbury, N. Y.). Homogenates were not centrifuged but used after filtration through organza. The reaction mixture contained either 20 or 50 μl of homogenate, 2 μmol Tris-HCl buffer (pH 7.4), 1 μmol $MnCl_2$, 60 nmol UDP-galactose (supplemented with ~15,000 cpm UDP-[^{14}C]galactose [New England Nuclear]). The total volume of the reaction mixture was 100 μl. α-Lactalbumin activity was estimated in the presence of excess bovine milk galactosyl-transferase (5 mU; Sigma Chemical Co.) and 2 μmol of D-glucose acceptor to form [^{14}C]lactose. A standard curve was generated with increasing amounts of bovine α-lactalbumin to ensure that the reaction was in the linear portion of the curve with respect to α-lactalbumin concentration. To correct for the nonspecific production of [^{14}C]galactose resulting from endogenous hydrolysis of UDP-[^{14}C]galactose, a control reaction was included for each sample, using distilled water in place of glucose as the acceptor. The incubations of the reaction mixture were carried out at 37°C for 30 min in a shaking water bath; the reaction was stopped by cooling in ice and the addition of 100 μl of cold water. The content of each tube was passed through a column (0.5 × 4 cm) of Bio-Rad AG 1-X2 anion exchange resin (Bio-Rad Laboratories, Richmond, Calif.) in the chloride cycle. Reaction tubes were washed with 0.5 ml of water that was then transferred to the columns. Neutral sugars on the column were eluted with another 1 ml of water directly into scintillation vials. Radioactivity in the eluate was measured with 10 ml of formula 950A (New England Nuclear) by liquid scintillation counting. Recovery of neutral sugars from the column was >80% with no elution of UDP-galactose. The product of the enzymatic reaction was identified as lactose by paper chromatography (34).

To assess mammary gland morphology and extent of LAD, mammary tissue samples were prepared for histological examination as described previously (14). The extent of LAD was evaluated by estimating the percentage of mammary tissue that was represented by epithelial cells in lobuloalveolar organization. Sections of mammary tissue in which ~50% of the epithelial cells were lobuloalveolar were scored as ++, whereas sections in which close to 100% of the epithelial cells were lobuloalveolar were assigned a score of ++++. An average of 10 pieces of tissue were examined microscopically for each experimental condition that was evaluated for LAD.

DNA content of the tissues was estimated according to Ceriotti (3), and protein concentration was assayed according to Lowry et al. (22).

RESULTS

The Pattern of PgR Modulation in Relation to Mammary Gland Differentiation in Intact Mice

Our earlier studies indicated that mammary PgR concentration was modulated as a function of mammary gland development. Namely, we found that PgR were abundant in virgin gland, were decreased during pregnancy, and were totally undetectable during lactation (14). To identify the physiological basis for these observed modulations in mammary PgR, in the following studies we examined the relationship between mammary PgR and mammary differentiation. There are a number of morphological and biochemical criteria that can be used to measure the progression of mammary gland differentiation from the undifferentiated ductal epithelium of virgin mice to the fully differentiated secretory lobuloalveolar epithelium of lactating mice. In the present studies one morphological criterion and one biochemical criterion were used, namely degree of lobuloalveolar differentiation (LAD) and amount of α-lactalbumin activity. In mice, LAD occurs mainly during

TABLE I

Relationship between Mammary PgR Concentration and Mammary Gland Differentiation

Developmental state	Specific [^{3}H]R5020 binding	α-Lactalbumin activity	Mammary gland morphology
	fmol/ mg DNA	*pmol lactose formed/ mg tissue/ 30 min*	*degree of LAD**
Virgin	1,610 ± 193	Not detectable	Ductal: none
Pregnant, *d*			Lobuloalveolar:
12	350 ± 32	—	+
14	349 ± 63	5 ± 1	++
16	315 ± 26	13 ± 1	+++
19	342 ± 88	82 ± 37	++++
Lactating, *d*			Lobuloalveolar/ secretory:
2	0	189 ± 7	++++
11–15	0	277 ± 50	++++

* For method of estimating degree of LAD, see Materials and Methods. All values represent mean ± SEM of two to four experiments.

[2] S. Z. Haslam and G. Shyamala, unpublished observations.

pregnancy, whereas α-lactalbumin activity, which is barely detectable during pregnancy, increases dramatically around parturition and reaches peak levels during lactation (25).

The results of the studies on the relationship between mammary gland differentiation and PgR levels during normal mammary gland development in intact untreated mice as summarized in Table I reveal two stages of PgR modulation. The first 80% reduction in mammary PgR is completed by day 12 of pregnancy and the second reduction, resulting in a total loss of PgR, occurs after parturition. It is of interest that after day 12 of pregnancy, although there is no further decrease in mammary PgR, the gland continues to become progressively more differentiated as indicated by the increases in α-lactalbumin activity and LAD (Fig. 1 *a–f*). Thus these data suggested that there is not a causal relationship between mammary PgR and differentiation. This raised the possibility that the factors responsible for the decrease in PgR were also simultaneously leading to mammary differentiation.

The Effects of Estrogen and Progesterone on Mammary PgR and Differentiation

The two major hormones of pregnancy, estrogen (E) and progesterone (P), are known to influence that PgR level in various target tissues (20, 25, 38, 43) and are also known to cause epithelial cell proliferation and LAD in mammary glands of ovariectomized virgin mice (2, 29). For these reasons, in the following experiments the simultaneous effect of E and P on mammary PgR levels and differentiation was examined. To distinguish the direct effects of the hormones on PgR levels

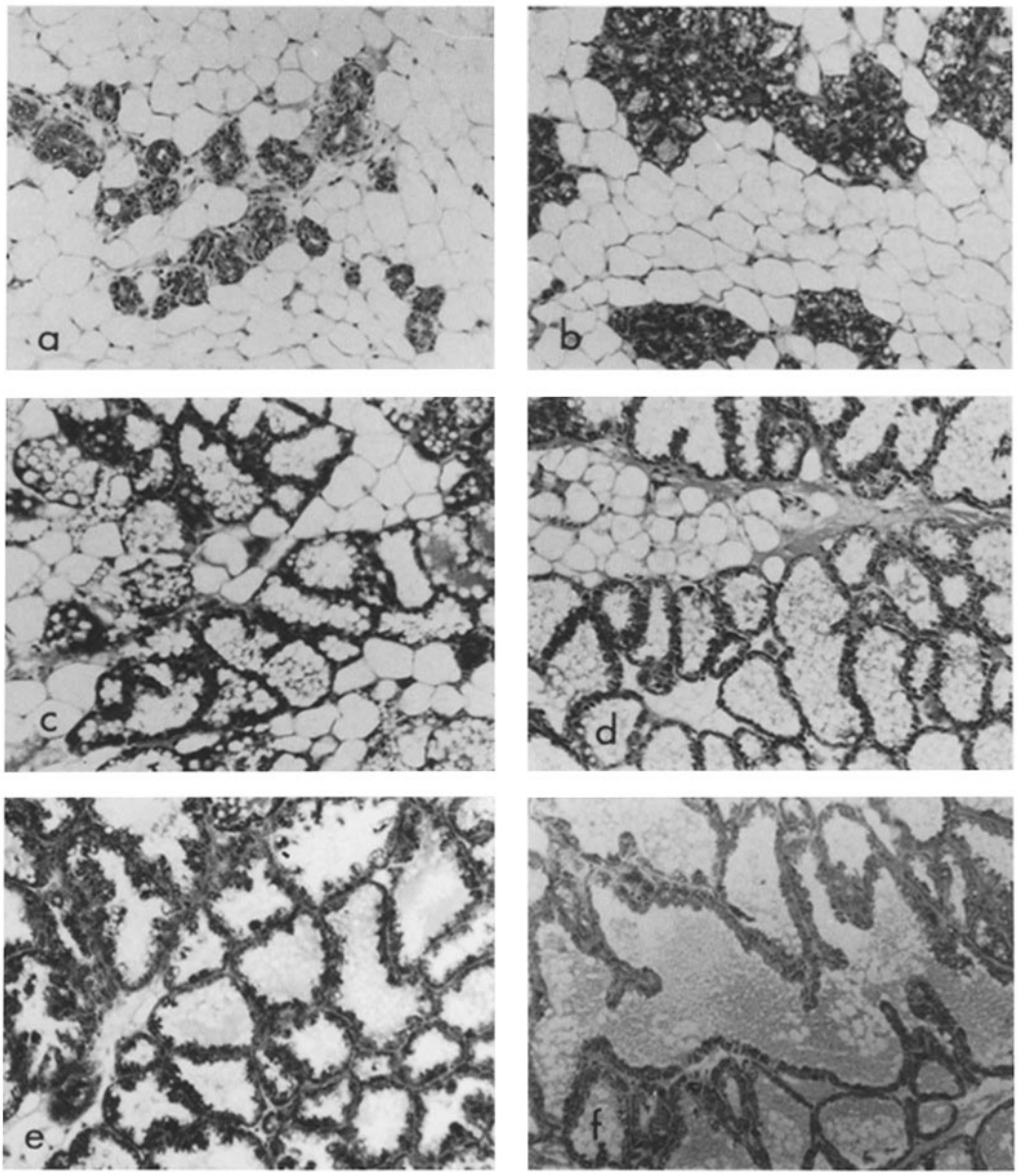

FIGURE 1 Mammary gland morphology during pregnancy and lactation. (*a*). Day 12 of pregnancy. Adipose cells are predominant; epithelial cells starting to form small lobules of alveoli. (*b*). Day 14 of pregnancy. An increased number of epithelial cells are present and the alveolar lobules are larger than in *a*. (*c*) Day 16 of pregnancy. The amount of adipose tissue has been reduced and alveolar lumina are dilated and appear to be filled with fat droplets. (*d*) Day 19 of pregnancy. There has been a further decrease in adipose cells. (*e* and *f*) Days 2 and 7 of lactation. No adipose cells are visible. Alveolar lumina are extensively dilated and secretion filled, indicating an actively secretory state of the gland. Hematoxylin and eosin. × 125.

from their possible indirect effect resulting from mammary gland differentiation, we quantitated PgR after different lengths of time of hormone treatment and correspondingly different degrees of LAD.

The morphology of mammary glands of ovariectomized virgin mice after estrogen and progesterone treatment is shown in Fig. 2 *a–e*. After treatment with oil, E or P alone for 7 or 14 d or E and P for 7 d, mammary glands were predominantly ductal and thus undifferentiated. In contrast, the mammary glands of mice treated with E and P for 14 d had begun differentiation as indicated by the presence of alveoli.

The effect of E and P on mammary PgR concentration in ovariectomized virgin mice is shown in Fig. 3. Administration of E alone for either 7 or 14 d resulted in a significant increase in mammary PgR. Administration of P alone for either 7 or 14 d resulted in PgR levels similar to those of vehicle control, whereas P in combination with E for 7 d resulted in PgR values not significantly different from those obtained with E alone. However, administration of P in combination with E for 14 d resulted in a 20% decrease of mammary PgR as compared to treatment with E alone. Although it was clear that E could cause an increase in mammary PgR and that P can cause a decrease in PgR, the 20% reduction in PgR caused by progesterone under the above experimental conditions could not account for the 80% reduction in PgR that is observed during pregnancy. This raised the possibility that during pregnancy the loss of PgR might have resulted from either a decreased sensitivity of mammary tissue to estrogen or an increased ability of progesterone to decrease PgR. The following experiments were carried out to distinguish between these two possibilities.

In these studies, 24 h before the initiation of hormone treatment, pregnant mice (14–16 d) were ovariectomized and hysterectomized to remove the major sources of endogenous hormones, and then the effects of E and P on mammary PgR were tested. The results of these experiments are presented in Fig. 4. Withdrawal of hormones for 24 h (time zero control) or 5 d (vehicle group) resulted in a decrease in PgR, whereas administration of E alone produced a significant increase in PgR. In contrast to its effect in mammary glands of ovariectomized virgins, P in combination with E produced a greater decrease in PgR (60% vs. 20%) and the PgR concentration was similar to that observed in intact pregnant mice. Thus the data in Fig. 4 clearly indicated that the decrease in mammary PgR during pregnancy was not the result of a decrease in estrogenic sensitivity of the tissue with respect to PgR increase but rather was the result of an enhanced ability of progesterone to decrease PgR. Furthermore, it also demonstrated that the differentiation of mammary gland in itself did not alter the mammary tissue responsiveness to estrogen. Consequently, it was also clear that factors other than mammary gland differentiation that occurs during pregnancy must have been responsible for the total loss of PgR and the loss of responsiveness to estrogen that is observed after parturition. This led us to consider that lactation and secretion per se might have an independent effect on PgR concentration and responsiveness to estrogen.

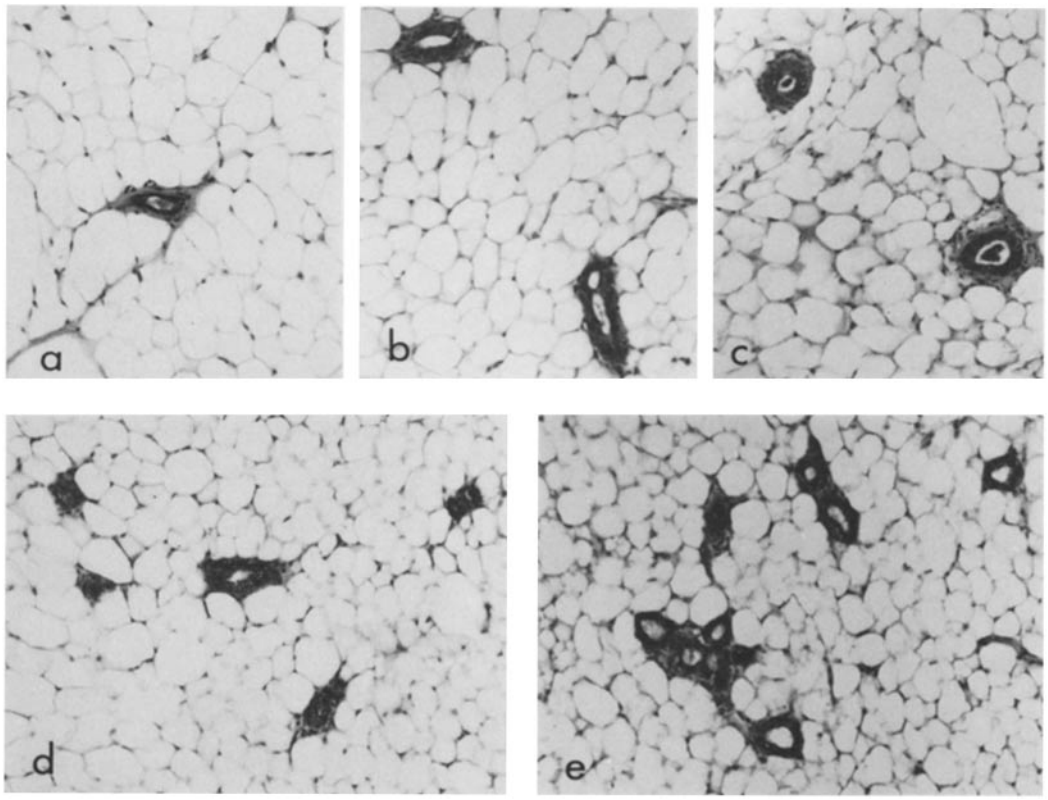

FIGURE 2 Mammary gland morphology of ovariectomized virgins after administration of estrogen and progesterone. (*a*) Control (14 d oil). (*b*) Progesterone treatment (14 d). (*c*) Estrogen treatment (14 d). In all three treatment groups, there is a predominance of adipose cells and sparse ducts composed of epithelial cells. (*d*). Estrogen and progesterone treatment (7 d). The amount of epithelial cells and number of ducts are increased. (*e*) Estrogen and progesterone treatment (14 d). The amount of epithelial cells is further increased and a small lobule of alveoli is present. Hematoxylin and eosin. × 125.

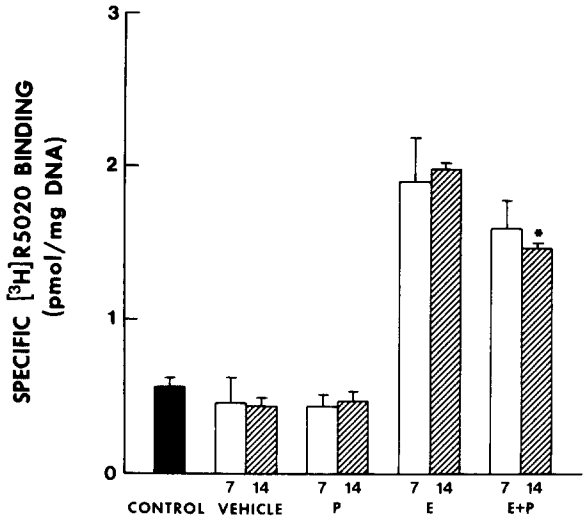

FIGURE 3 The effects of estrogen and progesterone on mammary PgR concentration. Virgin mice ovariectomized 14 d before the onset of hormone treatment were injected daily for 7 (□) or 14 (▨) d with either *vehicle*, progesterone (*P*, 1 mg), estrogen (*E*, 1 μg), or estrogen plus progesterone (*E* + *P*, 1 μg + 1 mg, respectively). Control (■) mice were assayed 14 d after ovariectomy alone; i.e., this represents the concentration of PgR at the initiation of hormone treatment. Cytoplasmic extracts of mammary glands were assayed for specific [^{3}H]R5020 binding 24 h after the last injection. Each value represents the mean ± SEM of three to four experiments; tissues from two animals were pooled for each experiment. Asterisk, P = .05 14-d E + P < 14-d E (Student's *t* test).

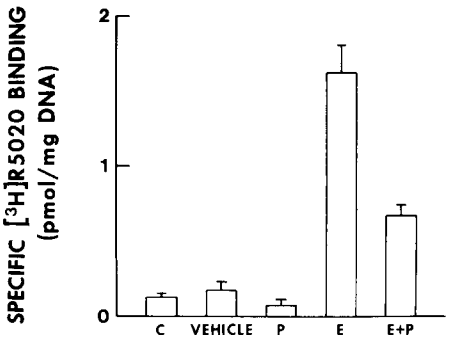

FIGURE 4 Effects of estrogen and progesterone on PgR concentration in mammary gland after 14–16 d of pregnancy. Pregnancies were terminated by ovariectomy and hysterectomy on day 14–16; starting 24 h later, the animals received a further five daily injections of vehicle, or estrogen (*E*, 1 μg) or progesterone (*P*, 1 mg) alone or in combinations, as indicated. Controls (*c*) were assayed at 24 h after ovariectomy and received no other treatment. 24 h after the last injection, cytoplasmic extracts were assayed for specific [^{3}H]-R5020 binding. Each value represents the mean ± SEM of three to four experiments.

Relationship of Lactation to PgR Modulation and Estrogen Responsiveness of Mammary Gland

To identify the precise effect of lactation on PgR, we felt that it was necessary to dissociate the hormonal milieu of lactation from the secretory state of the tissue. It is well known (7) that, in addition to the appropriate hormonal milieu, initiation and maintenance of copious milk secretion requires the physical removal of milk; this is accomplished in nature by suckling. By preventing suckling on one side of a lactating mouse, using the technique of unilateral thelectomy (nipple removal), we were able to obtain fully lactating mammary tissue from nipple-intact glands and nonlactating mammary tissue from the thelectomized glands from the same postpartum mouse (40). As can be seen from Table II, thelectomy in itself had no effect on mammary PgR in virgin and pregnant animals and it also did not prevent the decrease in PgR observed during pregnancy. A significant difference between PgR levels of intact and thelectomized glands was detectable only in postpartum mice; in this case, PgR were always detectable in the thelectomized, nonlactating glands but were absent in the contralateral intact lactating glands. Table II also shows that although thelectomy had no effect on mammary differentiation during pregnancy as assessed by α-lactalbumin activity, in postpartum mice the thelectomized glands had less α-lactalbumin activity compared to the intact lactating glands. The low α-lactalbumin activity of thelectomized glands was attributable to the non-lactational state of the gland and was corroborated histologically (Fig. 5 *a–d*).

We next examined the effect of estradiol on PgR of mammary glands in unilaterally thelectomized mice. These results are presented in Table III. We were consistently able to increase PgR concentration by exogenous administration of E in thelectomized, nonlactating mammary tissue, whereas PgR failed to be augmented by E in lactating nonthelectomized mammary tissue; the uteri of these animals also responded to E with increased level of PgR.

DISCUSSION

The results of our present studies indicate that as mammary glands of virgin mice differentiate there is a stepwise loss of PgR that occurs in two stages. The first decrease in PgR is completed by day 12 of pregnancy, whereas the second decrease occurs after parturition and results in a total loss of PgR in mammary tissue. Estrogen and progesterone appear to be the principal regulators of PgR concentration in mammary tissue; estrogen increases PgR concentration, whereas progesterone reduces the concentration of PgR. In view of these hormonal effects on PgR concentration, and because estrogen and pro-

TABLE II

Effect of Thelectomy on PgR Concentration and α-Lactalbumin Activity in Mammary Gland

	Specific [^{3}H]R5020 binding		α-Lactalbumin activity	
Developmental state	Thelectomized* mammary gland	Intact mammary gland	Thelectomized mammary gland	Intact mammary gland
	fmol/ mg DNA		*pmol lactose formed/ mg tissue/ 30 min*	
Virgin	1,884 ± 308	1,972 ± 234	—	—
Pregnant, *d*				
14	486 ± 33	507 ± 86	5 ± 1	6 ± 1
Lactating, *d*				
2	338 ± 33	0	55 ± 5	197 ± 8
7	182 ± 72	0	—	—

All values represent the mean ± SEM of two to five experiments.
* Mice were thelectomized before mating or, in case of virgins, 2–4 wk before tissue was assayed.

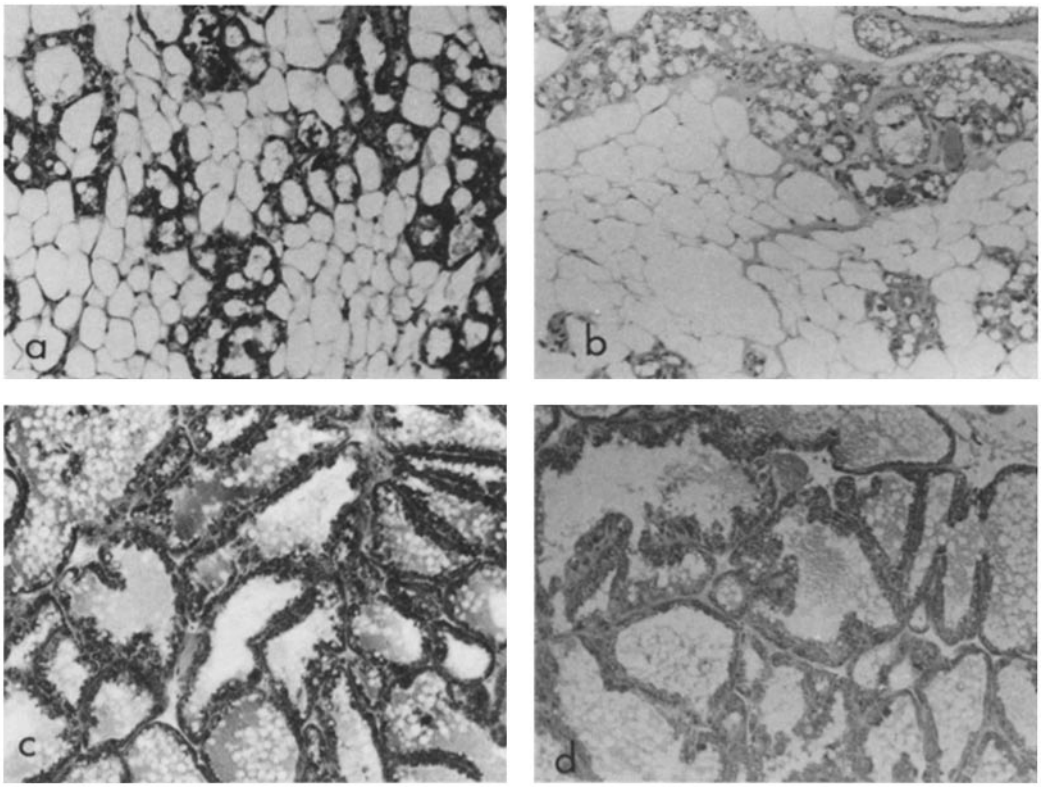

FIGURE 5 Effect of thelectomy on mammary glands of postpartum mice. Thelectomized mammary glands (*a*). 2 d postpartum and (*b*) 7 d postpartum. Mammary gland morphology is similar to that at 16 d of pregnancy (Fig. 1 *c*); the glands are lobuloalveolar, but alveoli have small lumina indicative of a nonsecretory condition. Contralateral nipple-intact mammary glands (*c*) 2 d postpartum and (*d*) 7 d postpartum. In contrast to Fig. 5 *a* and *b*, alveolar lumina are extensively dilated and secretion filled, thus indicating an actively secretory condition. Hematoxylin and eosin. × 125.

TABLE III

Estrogen Stimulation of PgR in Thelectomized Mice

Treatment*	Specific [^{3}H]R5020 binding	
	Thelectomized	Intact
	fmol/ mg DNA	*fmol/ mg DNA*
Mammary Gland		
NaCl	175 ± 22	0
E	325 ± 39	0
Uterus		
NaCl	5,860 ± 1,370	
E	11,263 ± 1,355	

* Mice were unilaterally thelectomized before mating and, 11 d postpartum, were injected subcutaneously with 3 μg of either E or NaCl. Tissues were assayed for PgR 24 h later. Each value represents the mean ± SEM of three to five experiments.

gesterone are also major hormones of pregnancy, we believe that the decrease in PgR occurring during pregnancy is attributable to the negative effect of progesterone on PgR. Because estrogen and progesterone also cause differentiation of the mammary gland, the net effect of these hormones on both processes leads to the previously observed apparent inverse relationship between mammary PgR and differentiation (14). However, it does not appear that the decrease in PgR and differentiation are causally related, because during pregnancy the progression of differentiation as determined by LAD and α-lactalbumin activity did not result in a progressive loss of PgR. The second decrease in PgR, which occurs at postpartum, appears to be specifically related to the secretory state of the gland rather than to a negative effect of the hormonal milieu of lactation on PgR.

The mechanisms by which cytoplasmic mammary PgR are decreased either by hormones or during lactation are not known. As mentioned earlier, under our present assay conditions we are measuring total cytoplasmic PgR and thus it is not likely that the reduction of or lack of PgR is attributable to endogenously filled sites. But because we only measured cytoplasmic PgR, it is conceivable that reduction of PgR could be the result of a high concentration of nuclear bound PgR. However, studies on the nuclear translocation and retention of PgR in uteri of a number of mammalian species indicate that a relatively small fraction of total cytoplasmic PgR are ever translocated to the nucleus (4, 45). An alternative explanation for reduced level of PgR may be the degradation/deactivation of existing PgR and the failure of new PgR to be synthesized. In view of a number of reports on the ability of molybdate to stabilize glucocorticoid and progesterone receptors (26, 30–32) in various target tissues, we tested its effect on mammary PgR. We found that 20 mM molybdate added to homogenization buffer did not enhance PgR binding in virgin mammary tissues and, most importantly, it did not reveal masked or unapparent PgR in lactating mammary tissue.[3] However, we cannot rule out the possibility that PgR are indeed resynthesized but remain sequestered in the nucleus in a form not bound to hormone. Although examples of this latter phenomenon are lacking in normal target tissues, certain human mammary

[3] S. Z. Haslam and G. Shyamala, unpublished observations.

carcinoma cell culture lines have been demonstrated to possess unoccupied nuclear receptor sites for estrogen (12, 47). Although further studies are obviously required to determine what mechanisms are operating to decrease PgR during lactation in normal mammary tissues, it should be emphasized that whatever mechanisms are operative they are specific to the secretory state of the gland.

The present studies have demonstrated for the first time that progesterone can decrease the concentration of its own receptor in mammary tissues, which agrees with similar findings in uterine tissue (4, 21, 27, 44, 45); this lends further credence to the concept that progesterone's effects may also be receptor-mediated in mammary tissue. The present studies also revealed some important information about the ability of progesterone to modulate estrogen action in mammary tissue. Progesterone when administered in combination with estrogen for 7 d failed to affect the estrogen-mediated increase in mammary PgR and, during this period, the mammary glands were predominantly ductal and thus undifferentiated. In contrast, when progesterone was administered with estrogen to pregnant animals whose mammary glands were extensively lobuloalveolar and thus differentiated, it significantly decreased mammary PgR when administered with estrogen. Thus, it is tempting to speculate that the ability of progesterone to decrease PgR may depend on the state of mammary differentiation and also that this ability may be acquired during lobuloalveolar differentiation. This speculation might explain the results of recent studies on the transplantable urethan-induced mouse mammary carcinoma MXT-3590, which is of ductal origin. In this tumor, estrogen can augment both tumor growth and PgR concentration but progesterone fails to antagonize tumor growth (46). It is possible that the inability of progesterone to antagonize estrogen-mediated tumor growth is a reflection of the ductal origin of the MXT-3590 tumor. The effects of estrogen and progesterone on PgR have also been examined in dimethylbenzanthracene-induced primary rat mammary tumors, and from this study it appears that there can be a dissociation between estrogenic regulation of mammary growth and PgR (15). However, that PgR, in both hormone-dependent mouse and rat mammary tumors, are under acute estrogenic regulation is most comparable to the situation present in mammary tissue of virgin and pregnant mice, but distinct from the estrogen-insensitive state of lactating mammary tissue.

The effect of estrogen to increase and progesterone to decrease PgR has also been reported for uterine tissue (1, 6, 20, 28, 38, 43). However, it is not known whether uterine cytodifferentiation acts to modify the response of the uterus to hormones, as occurs in the mammary gland. In this regard, differential responsiveness of cells to P or E and P have been described in oviduct development and function, and such differences appear to be determined by the types of cells present and by the stage of oviduct development (32, 33, 35). Also, in recent studies of estrogen action and estrogen antagonists in the rat uterus, it has been proposed that the cell type (endometrial vs. myometrial cells) may determine that nature of the biological response to the hormone antagonists (5).

The mechanisms by which lactation results in mammary gland estrogen insensitivity, and how this might be reversed, are currently being investigated. Understanding how cells modify their requirement for, or response to, growth regulatory molecules such as hormones is critical to our understanding of the basis of the loss of regulation that occurs in certain disease states such as neoplasia.

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