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Distinction between Progestin- and Glucocorticoid-Binding Sites in Mammary Glands

APPARENT LACK OF CYTOPLASMIC PROGESTERONE RECEPTORS IN LACTATING MAMMARY GLANDS

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The cytosol fraction of the lactating mammary glands of mice does not appear to contain detectable amounts of progesterone receptors. Mixing experiments indicate that the absence of receptors is not due to interference by other factors in the cytosol. However, in the cytosol of mammary glands, there is specific binding of progestins to certain low-affinity sites which have characteristics of specific glucocorticoid-binding sites.

The presence of specific progesterone receptors in the uterine cytosol of various species including mice has been documented by using either radioactive progesterone (Baulieu *et al.*, 1970; Milgrom *et al.*, 1970; Feil *et al.*, 1972; McGuire & Bariso, 1972; Leavitt *et al.*, 1974) or R5020 ([17-*methyl*-^{3}H]-17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione) as the ligand (Philibert & Raynaud, 1973; Philibert *et al.*, 1975; McGuire *et al.*, 1977). The synthesis of uterine progesterone receptors is under the acute control of oestrogens (Toft & O'Malley, 1972; Rao *et al.*, 1973; Milgrom *et al.*, 1973), and subsequently it has been shown that the amounts of progesterone receptors in certain experimental mammary tumours are also modulated by oestrogens (Horwitz & McGuire, 1977; Koenders *et al.*, 1977). Although progesterone is involved in the growth and differentiation of both normal and neoplastic mammary tissues in rodents (Lyons *et al.*, 1958; Nandi, 1958; Bresciani, 1965*a*,*b*, 1968), very little is known about progesterone receptors in the normal mammary glands of these animals. A single report on the presence of progesterone receptors in normal mammary glands of the goat has been published (Markland & Hutchens, 1977), and a previous report about progesterone receptors in lactating rat mammary glands was inconclusive (McGuire *et al.*, 1977). We have initiated a series of detailed studies on progesterone receptors in normal mammary tissues, since such studies are fundamental to our understanding of the molecular basis for the direct role of progesterone in the growth, development and function of normal mammary glands. Furthermore, progesterone receptors may be useful as markers of oestrogen action and may provide an indication of the functional integrity of oestrogen receptors in both normal and neoplastic mammary tissues. In the present studies, we have shown that the cytosol of lactating mammary glands does not contain significant amounts of progesterone receptors and have concluded that any binding of progestins observed in this tissue may be due to their binding to sites that are more characteristic of glucocorticoid-binding sites. A preliminary report of this work has been published (McBlain *et al.*, 1978).

Materials and Methods

Steroids

All radioactive steroids were purchased from New England Nuclear Corp., Boston, MA, U.S.A., with specifications as follows: R5020 (86.0Ci/mmol), dexamethasone (37.6Ci/mmol), oestradiol (98.5Ci/mmol), medroxyprogesterone acetate (58Ci/mmol) and dihydroprogesterone (5α-pregnane-3,20-dione) (55.7Ci/mmol). Radioinert R5020 was also purchased from New England Nuclear Corp. Other unlabelled steroids were purchased from Sigma, St. Louis, MO, U.S.A., Steraloids, Wilton, NH, U.S.A., or Mann Research, New York, NY, U.S.A.

Animals

Female Balb/c mice were from our own colony and had been lactating for 7–10 days at the time of death.

Tissue preparation

All procedures were performed at 0–4°C. After excision, mammary tissues were weighed, rinsed twice in buffer, minced and homogenized in 1 vol. of buffer with two 15s bursts of a Polytron PT 10-ST (Brinkmann Instruments, Westbury, NY, U.S.A.). The tissues were homogenized in either Tris/glycerol buffer [50mM-Tris/HCl, 1.5mM-EDTA, 10mM-thioglycerol, 10% (v/v) glycerol, pH7.4] or phos-

phate/glycerol buffer (5mM-sodium phosphate, 10mM-thioglycerol, 10% glycerol, pH7.4) containing 10mM-dithiothreitol. The homogenates were centrifuged for 60min at either 12350*g* or 105000*g*. The resulting supernatant was referred to as the cytosol. The choice of centrifuge depended on the yield of homogenate and the experimental protocol required. Steroid binding did not differ for the two preparative centrifugation procedures.

Samples of the cytoplasmic extracts were incubated at 0–4°C with predetermined concentrations of ^{3}H-labelled steroids with or without an excess of non-radioactive steroid for defined periods of time. All steroids were added to the incubations as ethanol solutions in concentrations which limited the ethanol volume in the incubation to a maximum of 5%. The amount of steroid bound was determined by either a dextran-coated-charcoal assay procedure based on that of Korenman (1970), by Sephadex G-25 filtration (Puca & Bresciani, 1968) or by sucrose-density-gradient analysis as described previously (Toft *et al.*, 1967).

Protein concentrations in the cytoplasmic extracts were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Results

Attempts to demonstrate progesterone receptors in cytosol of lactating mammary glands

(*a*) *Concentration-dependent binding of ^{3}H-labelled R5020 in cytosol of mammary glands and uteri.* Initially experiments were carried out to ascertain whether saturable and specific binding of ^{3}H-labelled R5020 could be demonstrated in the cytosol of lactating mammary glands. As a control, the cytosol of the uteri of the same lactating animals was also studied, because these uteri are known to contain progestin receptors (Philibert & Raynaud, 1977; Gomez *et al.*, 1977). Since mammary glands of lactating animals have oestrogen receptors (Shyamala & Nandi, 1972; Gardner & Wittliff, 1973*b*), as another control the binding of [^{3}H]oestradiol in the cytosol of lactating mammary glands was also examined simultaneously. In approximately one-third of all lactating-mammary-gland cytosol preparations, there was no detectable specific binding of R5020, even though high concentrations of ^{3}H-labelled R5020 (up to 50nM) were used in the incubation medium; this lack of specific binding was not related to the choice of buffers, as no difference was observed between cytosols in the Tris/glycerol buffer or phosphate/glycerol buffer. Different times of incubation with steroid (1, 4 and 24h) or the choice of binding assay also did not elicit specific binding in these cases. In instances where specific binding was present, it exhibited a non-saturable type of kinetics, as illustrated in Fig. 1(*a*). In contrast, at the same range of ligand concentrations,

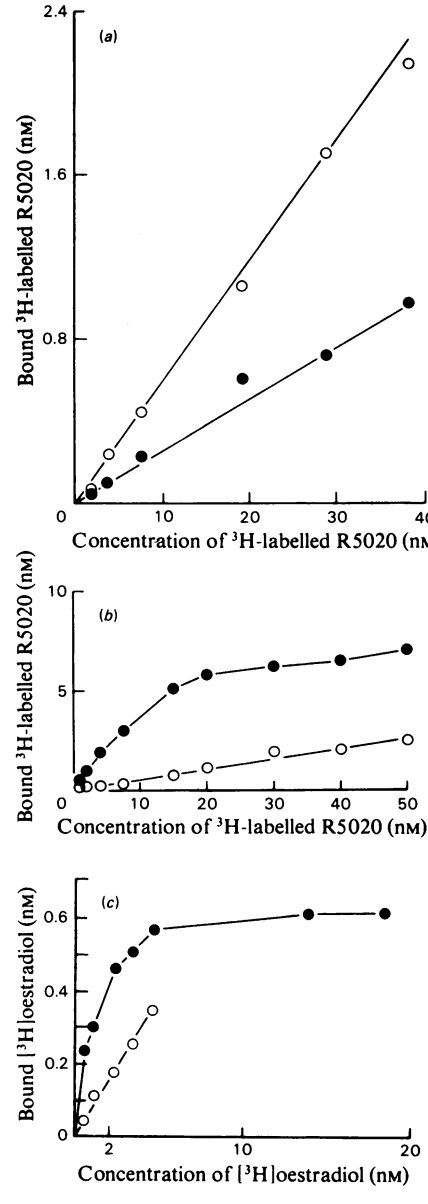

Fig. 1. *Binding of ^{3}H-labelled R5020 and [^{3}H]oestradiol in cytosol of mammary glands and uteri of lactating mice*
In all experiments, samples of cytosol were incubated with various concentrations of [^{3}H]steroid with or without 100-fold excess of unlabelled steroid for 4h at 0–4°C. (*a*) Binding of ^{3}H-labelled R5020 in the cytosol of mammary glands, (*b*) binding of ^{3}H-labelled R5020 in uterine cytosol and (*c*) binding of [^{3}H]-oestradiol in cytosol of mammary glands. The amount of radioactivity remaining bound in incubations containing an excess of competing unlabelled steroid is referred to as the non-specific binding (○). Specific binding (●) is the difference between total and non-specific binding.

specific and saturable binding of ^{3}H-labelled R5020 in the cytoplasmic extract of uteri (Fig. 1*b*) and specific and saturable binding of [^{3}H]oestradiol in the cytoplasmic extract of mammary glands (Fig. 1*c*) were observed. The affinity constants for the binding of ligands in control experiments as estimated by Scatchard analysis were similar to those previously reported (the K_d for binding of oestradiol in mammary cytosol was 0.7 nM and the K_d for binding of R5020 in uterine cytosol was 6 nM). In all experiments the control mammary-gland and uterine samples had specific oestrogen and progesterone binding respectively.

(*b*) *Gel-filtration and sucrose-density-gradient analyses.* The nature of ^{3}H-labelled R5020 interaction with the mammary cytosol was also studied by gel-filtration and sucrose-density-gradient analyses. As before, samples of mammary cytosol incubated with [^{3}H]-oestradiol and uterine cytosol incubated with ^{3}H-labelled R5020 were analysed in an identical manner to serve as controls. The results of the gel-filtration experiments are shown in Fig. 2 and indicate that most of the specific binding of ^{3}H-labelled R5020 in mammary cytosol dissociated during gel filtration (Fig. 2*a*), whereas the specific binding in the above-specified control samples was intact (Figs. 2*b* and 2*c*). Sucrose-density-gradient centrifugation of mammary cytosol incubated with ^{3}H-labelled R5020 resulted in the appearance of non-specifically bound and free ^{3}H-labelled R5020 only (results not shown). These results were not surprising when it became apparent that the specific binding of ^{3}H-labelled R5020 in mammary cytosol that was observed at 4h was completely lost by 24h, whereas the specifically bound steroid in the control samples was quite stable at 24h (specific uterine binding of ^{3}H-labelled R5020 at 24h was 92% of that observed at 4h and specific

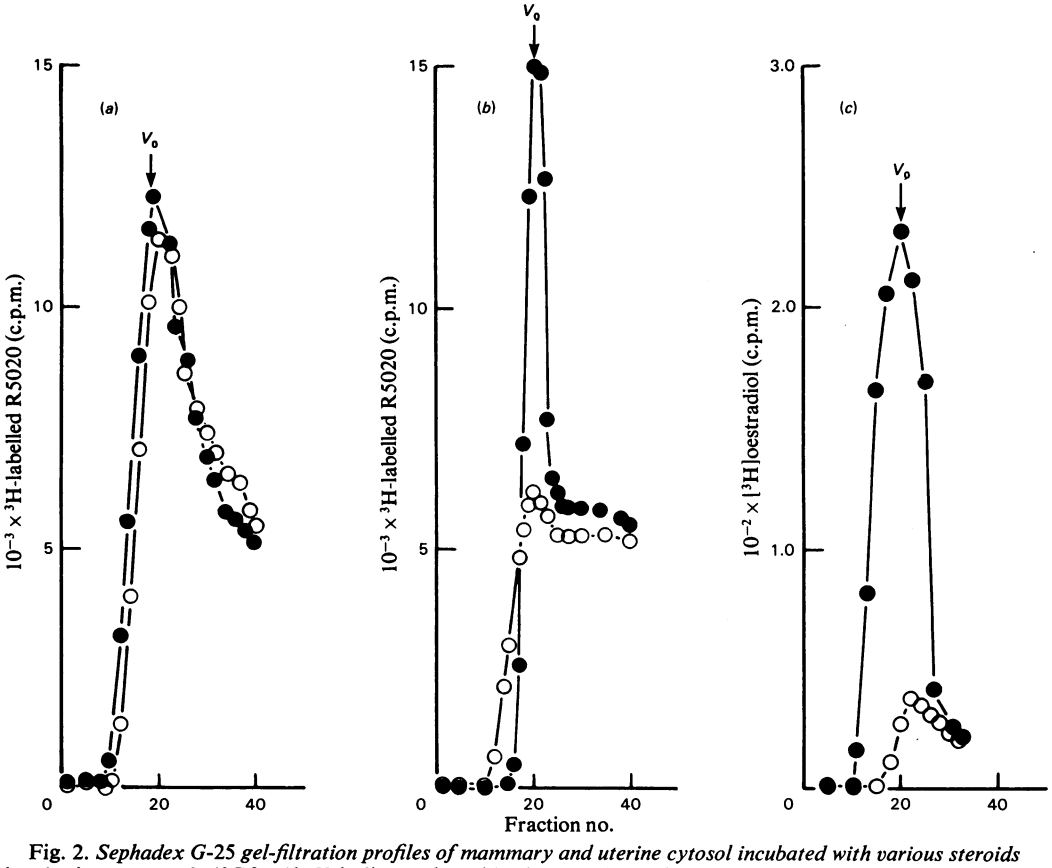

Fig. 2. *Sephadex G-25 gel-filtration profiles of mammary and uterine cytosol incubated with various steroids*
All incubations were at 0–4°C for 4h. V_0 indicates the void volume as determined by the elution of Blue Dextran. Volumes of fractions were 0.2ml. (*a*) Mammary cytosol incubated with 50nM-^{3}H-labelled R5020 only (●) or in combination with 100-fold excess of unlabelled R5020 (○). (*b*) Uterine cytosol incubated with 50nM-^{3}H-labelled R5020 only (●) or in combination with 100-fold excess of unlabelled R5020 (○). (*c*) Mammary cytosol incubated with 10nM-[^{3}H]oestradiol only (●) or in combination with 100-fold excess of unlabelled oestradiol (○).

oestradiol binding in mammary cytosol at 24h was 99% of that at 4h).

These data on ^{3}H-labelled R5020 binding as analysed by gel filtration, sucrose-density-gradient centrifugation and stability of the binding suggested that any specific binding of ^{3}H-labelled R5020 observed in mammary cytosol was perhaps due to its binding to low-affinity sites and not to progesterone receptors.

Distinction between ^{3}H-labelled R5020 binding to progestin receptors and glucocorticoid receptors

(*a*) *Steroid specificity of the binding of ^{3}H-labelled R5020 in mammary cytosol.* The experiments described thus far had failed to detect saturable and high-affinity binding of ^{3}H-labelled R5020 in the cytosol of lactating mammary glands. Since at high concentrations of ^{3}H-labelled R5020 specific binding had been observed in approximately two-thirds of all samples tested, it was important to establish the nature of this binding. To this end, the steroid-binding specificity for the binding of ^{3}H-labelled R5020 in mammary cytosol was established by competition analysis and the data are summarized in Table 1. The most striking observation was the high affinity of dexamethasone and cortisol for ^{3}H-labelled R5020-binding sites in mammary cytosol; these findings were not typical of those obtained for R5020 binding to progesterone receptors in uteri of various animals (Philibert & Raynaud, 1973, 1977; Philibert *et al.*, 1975; McGuire *et al.*, 1977; Gomez *et al.*, 1977; Walters & Clark, 1977) or for those for certain mammary cells (Asselin *et al.*, 1976; Horwitz & McGuire, 1977; Koenders *et al.*, 1977; Markland & Hutchens, 1977; Lippman *et al.*, 1977).

Table 1. *Relative binding affinities of various steroids to ^{3}H-labelled R5020-binding sites in lactating-mammary-gland cytosol*

Samples of cytosol of lactating mammary glands were incubated with 20nM- or 50nM-^{3}H-labelled R5020 alone or in the presence of 100-fold excess of competing steroids. Non-specific binding has been subtracted. The results are averages of three to five experiments.

Competing steroid	Specific binding of ^{3}H-labelled R5020 (% of control)
None	100
Oestradiol-17β	100
5α-Dihydrotestosterone	100
Norgestrel	56.8
Deoxycorticosterone	19.7
Cortisol	17.2
Progesterone	13.8
Corticosterone	10.3
Dexamethasone	0

(*b*) *Relative binding of dexamethasone and R5020.* Previous studies from our laboratory and others have shown that progesterone can bind to glucocorticoid receptors present in the cytoplasmic fraction of lactating mammary glands (Shyamala, 1973; Gardner & Wittliff, 1973*a*). Since R5020 is a potent progestin, we tested the possibility that the observed binding of ^{3}H-labelled R5020 might represent its interaction with the glucocorticoid receptors. The results of these experiments are shown in Fig. 3. R5020 can compete better than progesterone for glucocorticoid receptors (as measured by saturable and specific binding of dexamethasone), but with a lower affinity than dexamethasone, and this competition is due to its binding at the binding site for the glucocorticoid (Fig. 3*a*). The relative binding affinity of R5020 compared with dexamethasone to glucocorticoid-receptor sites is better illustrated in Figs. 3(*b*) and 3(*c*), which show that R5020 is much less effective than dexamethasone in competing for either R5020- or dexamethasone-binding sites in mammary cytosol. It is also clear from Figs. 3(*b*) and 3(*c*) that at higher concentrations both R5020 and dexamethasone have an equal affinity for R5020- or dexamethasone-binding sites; however, such competition by dexamethasone to R5020-binding sites in mammary cytosol is in sharp contrast with that which occurs in the control uterine cytosol (Fig. 3*d*).

Binding of ^{3}H-labelled R5020 in mixed cytoplasmic extracts from uteri and mammary glands

Studies were carried out to determine whether mammary cytosol might have contained factors that, although having no effect on oestrogen and glucocorticoid receptors, might have prevented the detection of progestin receptors. To this end, combined cytoplasmic extracts of mammary glands and uteri were assayed for specific ^{3}H-labelled R5020 binding. The mixed cytoplasmic extracts were obtained either by combining the individual cytosols or by co-homogenizing the uteri with mammary glands and then centrifuging the co-homogenate. To ensure that the estimated specific binding of ^{3}H-labelled R5020 was due only to its binding to progesterone receptors and not to any sites also bound by glucocorticoids, unlabelled dexamethasone was also used as a competing steroid. As shown in Table 2, mixing the uterine cytosol with mammary-gland cytosol either directly (*a*) or by co-homogenization (*b*) failed materially to decrease the expected number of ^{3}H-labelled-R5020-binding sites contributed by the uterine cytosol.

Studies were also carried out to determine if the dilution of the mammary cytosol or addition of phenylmethanesulphonyl fluoride, which inhibits proteinase activities, could result in the detection of saturable specific binding of ^{3}H-labelled R5020 in

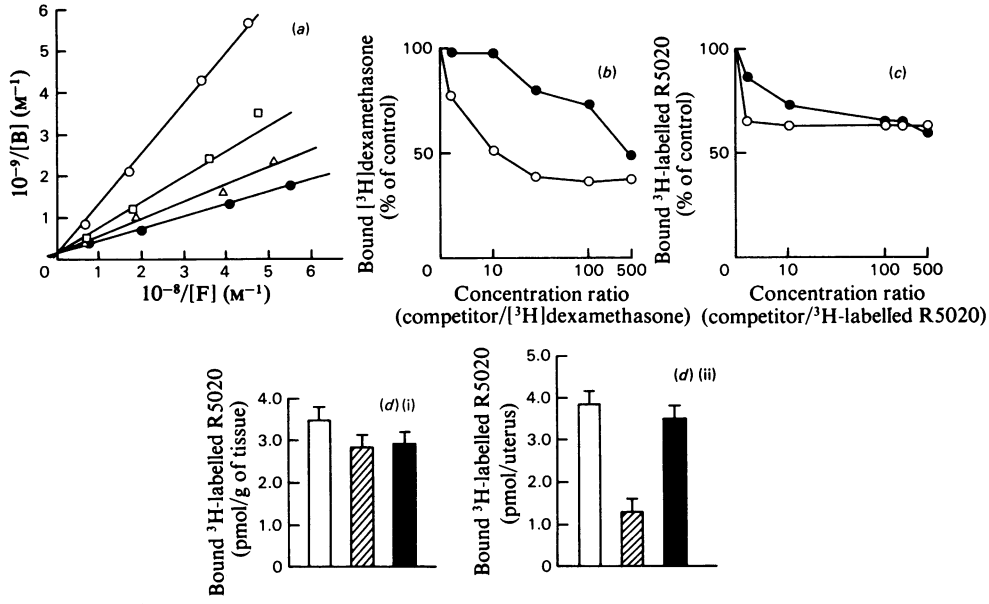

Fig. 3. *Characteristics of binding of R5020 and dexamethasone in mammary and uterine cytosols*

(*a*) Competitive inhibition of [^{3}H]dexamethasone binding (●) by dexamethasone (○), progesterone (△) and R5020 (□). Binding of [^{3}H]dexamethasone in lactating-mammary-gland cytosol was determined at various concentrations of [^{3}H]dexamethasone and competing steroids. The co-ordinates refer to the reciprocals of the molar concentrations of bound [^{3}H]dexamethasone (1/[B]) or free [^{3}H]dexamethasone (1/[F]). (*b*) Comparison of the relative affinity of dexamethasone and R5020 for [^{3}H]dexamethasone-binding sites. Samples of cytosol from lactating mammary glands were incubated with 20nM-[^{3}H]dexamethasone with or without competing steroids (●, R5020; ○, dexamethasone) for 4h at 0°C. Binding values obtained in the absence of competitor are taken as 100%. (*c*) Comparison of the competitive affinity of dexamethasone and R5020 to ^{3}H-labelled R5020 binding sites. Samples of cytosol from lactating mammary glands were incubated with 20nM-^{3}H-labelled R5020 with or without competing steroids (●, R5020; ○, dexamethasone) for 4h at 0°C. Binding was determined by charcoal-adsorption assays; values in the absence of competitor are taken as 100%. (*d*) Binding affinity of dexamethasone to ^{3}H-labelled R5020 binding sites in mammary (i) and in uterine (ii) cytosol. Samples of mammary or uterine cytosol were incubated with 50nM-^{3}H-labelled R5020 alone (□) or with 100-fold excess of unlabelled R5020 (■) or with 100-fold excess of unlabelled dexamethasone (■) for 4h at 0–4°C. Data are expressed as the mean ±S.E.M. (bars) of results of five experiments.

mammary cytosol. Neither the dilution nor the addition of phenylmethanesulphonyl fluoride to mammary cytosol had an effect on the binding data presented above (results not shown).

Interaction of medroxyprogesterone acetate and dihydroprogesterone with mammary cytosol

It is known that R5020 has several distinct advantages over other progestins, including progesterone, for its use as a ligand for studies on progesterone receptors, such as its lack of affinity for corticosteroid-binding globulin and a lower rate of dissociation than progesterone from the progestin receptor (Philibert & Raynaud, 1974; Philibert *et al.*, 1977). Similarly, medroxyprogesterone acetate, another synthetic progestin, also does not bind to corticosteroid-binding globulin (Feil *et al.*, 1978); although medroxyprogesterone acetate has a lower dissociation rate from the progestin receptor compared with progesterone in the human endometrium (Feil *et al.*, 1978), it has a higher dissociation rate than progesterone in the uterine cytosol of the guinea pig (Feil *et al.*, 1976). To verify that our failure to detect specific progesterone receptor in mammary cytosol was not due to the choice of ligand, we also studied the interaction of medroxyprogesterone acetate with the mammary cytosol. The results of a typical experiment are presented in Table 3. In all experiments, specific binding of medroxyprogesterone acetate was readily observed in mammary cytosol, and both R5020 and progesterone had a high affinity for these sites, as demonstrated by their degree of competition. Although this progestin competition could be interpreted initially as an indication that the binding sites were those belonging to progesterone receptors, it was observed that these sites also had a high affinity for dexamethasone; furthermore, all the specific binding was lost when

Table 2. *Binding of ^{3}H-labelled R5020 in uterine and mammary cytosol*

In all experiments 1.0ml of mammary cytosol was equivalent to 1 g of tissue and 1.0ml of uterine cytosol was equivalent to five uteri. (*a*) Mixed cytosols were incubated at 0°C for 4h with 50nM-^{3}H-labelled R5020 either alone or in the presence of 100-fold excess of unlabelled R5020 or dexamethasone. (*b*) In Expt. 1, 7.6nM-^{3}H-labelled R5020 was used with 100-fold excess of unlabelled R5020 or dexamethasone. In Expt. 2, 50nM-^{3}H-labelled R5020 was used with 100-fold excess of unlabelled R5020 or dexamethasone.

(*a*) Incubation	Specifically bound ^{3}H-labelled R5020* (fmol/ml)	Non-specifically bound ^{3}H-labelled R5020 (fmol/ml)	Protein concn. (mg/ml)
Mammary cytosol + buffer (1:1, v/v)	None	2828	20.1
Uterine cytosol + buffer (1:1, v/v)	5473	2608	4.1
Mammary cytosol + uterine cytosol (1:1, v/v)	5533	2992	—

(*b*) Incubation	Specific binding of ^{3}H-labelled R5020 (fmol/ml)	
	Expt. 1	Expt. 2
Uterine cytosol	3945	17001
Mammary cytosol	None	None
Mixed cytosol from co-homogenates	1844	9548

* Represents specific binding of ^{3}H-labelled R5020 which was not competed for by unlabelled dexamethasone.

Table 3. *Relative affinity of various steroids to specific binding of ^{3}H-labelled medroxyprogesterone acetate in cytosol of mammary glands and uteri of lactating mice*

Samples of cytosol were incubated with 20nM-^{3}H-labelled medroxyprogesterone acetate with or without 100-fold excess of unlabelled steroids for 4h at 0–4°C. The results are averages of three separate experiments with duplicate determinations. The 100% values at 4h for mammary-gland cytosol were 732 ± 96fmol/ml and in uterine cytosol it was 2840fmol/uterus. At 24h there was no specific binding in mammary cytosol, whereas uterine cytosol had the same binding as at 4h.

Competing steroid	Specific binding of ^{3}H-labelled medroxyprogesterone acetate (% of control)	
	Mammary glands	Uterus
None	100	100
Progesterone	0	3.7
Dexamethasone	12.8	58.0
R5020	7.0	24.4

assayed at 24h. This behaviour of medroxyprogesterone acetate with mammary cytosol was once again in sharp contrast with that observed with uterine cytosol (also shown in Table 3). The affinity of dexamethasone for medroxyprogesterone acetate-binding sites in mammary cytosol can be readily explained if the medroxyprogesterone acetate binding was in fact to glucocorticoid-receptor sites; such a possibility is likely, since medroxyprogesterone acetate has been shown to bind to glucocorticoid receptors in liver and thymus cytosol (DiSorbo *et al.*, 1977).

5α-Pregnane-3,20-dione, a progesterone metabolite known to bind to progesterone receptors in uterine cytosol of, guinea pig (Saffran *et al.*, 1978) failed to reveal any specific binding sites in the mammary cytosol (results not shown).

Discussion

The results of the studies described in this paper have led us to conclude that there are no specific progesterone receptors in the cytosol of lactating mammary glands; our conclusion was derived from extensive studies on the nature of binding of ^{3}H-labelled R5020 in the mammary cytosol of lactating mice and comparison of the results obtained with that obtained for the uteri of these animals. There were two critical differences between the behaviour of R5020 binding in mammary cytosol and in uterine cytosol. The binding in mammary cytosol was such that the specific binding was not consistently observed, and when observed it was indicative of binding to low-affinity sites, since it was never stable for a period of 24h. The steroid specificity of the specific binding sites for R5020 was unequivocally not characteristic of progesterone receptors, but rather was indicative of glucocorticoid-binding sites. We verified that the observed binding pattern was not the result of our choice of ligand by using another synthetic progestin, medroxyprogesterone acetate. We also verified that there were no specific inhibitors that might have interfered with the detection of

progesterone receptors, by performing experiments with mixed cytosol. Although there exists the possibility that the progesterone receptors in mammary cytosol might have been bound by endogenous progesterone and hence were not detectable under our assay conditions, this appears unlikely, because it is known that under the conditions of the assay used in the present studies, ^{3}H-labelled R5020 can easily exchange with endogenously bound progesterone (Philibert *et al.*, 1977). Furthermore, mammary cytosol of lactating mice ovariectomized on either day 2 or 7 of lactation still did not contain detectable amounts of progesterone receptors S. Haslam & G. Shyamala, unpublished work).

The interpretation of the binding data obtained with ^{3}H-labelled R5020 in the lactating mammary-gland cytosol was complicated by the presence of relatively high concentrations of glucocorticoid receptors which appear to bind R5020. It is known that progesterone has an affinity for glucocorticoid receptors in several other target tissues (Cake & Litwack, 1975; Munck & Leung, 1977), and since both R5020 and medroxyprogesterone acetate are potent progestins, the affinity of these steroids to glucocorticoid receptors need not be surprising. However, since progestins bind to glucocorticoid receptors with low affinities, saturation of glucocorticoid sites with progestins will require a much higher concentration of ligand than that used in the present studies, and thus may explain the non-saturable kinetics shown in Fig. 1. These studies do not, however, preclude the possibility that the observed binding of R5020 was due to some hitherto unidentified binding sites and not to the glucocorticoid receptors previously described for this tissue (Shyamala, 1973).

The data in the present paper indicate an apparent lack of progesterone receptors in lactating mammary tissue. This finding raises an important question with respect to the mechanism of progesterone action in mammary tissues. The lack of progesterone receptors in mammary tissues appears to be peculiar to the lactational phase of development only, since we have successfully identified and characterized the progesterone receptors that are present in non-lactating mammary tissues (S. Haslam & G. Shyamala, unpublished work). It is known that progesterone is an antagonist of lactogenesis (Wilkman & Davis, 1968; Kuhn, 1969; Davis *et al.*, 1972; Denamur & DeLouis, 1972) and can inhibit molecular events leading to casein synthesis (Rosen *et al.*, 1978; Matusik & Rosen, 1978). Thus it is possible that the lack of progesterone receptors in lactating mammary tissues is related to the prevention of this influence of progesterone during lactation. It is also known that mammary tissues undergo cell proliferation under the influence of ovarian hormones, principally oestrogens (Bresciani, 1965*a*,*b*, 1968); although such cell proliferation is enhanced during pregnancy and early lactation, there is no further increase in DNA synthesis during established lactation (Munford, 1963). Since progesterone receptors are believed to be synthesized under the influence of oestrogens in target tissues (Toft & O'Malley, 1972; Rao *et al.*, 1973; Milgrom *et al.*, 1973), a lack of progesterone receptors during lactation may indicate a lack of an oestrogenic effect. Lactating mouse mammary glands do contain relatively large amounts of oestrogen receptors (Shyamala & Nandi, 1972; Gardner & Wittliff, 1973*b*), and in this regard it is necessary to note that although the uteri of lactating mice respond to injected oestradiol with an increase in amounts of progesterone receptor, the mammary glands of the same animals still do not contain detectable amounts of progesterone receptors (McBlain *et al.*, 1978). Studies are now required to elucidate the molecular basis for the absence of progesterone receptors from lactating mammary tissues.

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