

## CHARACTERIZATION OF PROGESTERONE RECEPTORS AND METABOLISM OF PROGESTERONE IN THE NORMAL AND CANCEROUS HUMAN MAMMARY GLAND

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### SUMMARY

The molecular interaction of progesterone was investigated in 18 cancerous and 12 normal human mammary gland tissues. The time course analysis of progesterone localization showed that it was initially concentrated in the cytosol and subsequently transferred to the nucleus. In the cancerous tissue the nuclear fraction had significantly higher amount of the steroid than in the cytosol fraction while in the normal mammary gland tissue localization of progesterone in the nuclear fraction was only slightly higher compared with the cytosolic fraction. Both in the normal and cancerous mammary tissue, progesterone was bound to a 4.3S receptor but a characteristic 6.7S binding component was noted in the normal mammary tissue. The number of specific binding sites in the normal and cancerous cytosol was of the order of 2.1 and 1.5 pmol/mg protein and the dissociation constant was  $0.42 \times 10^{-9} \text{M}^{-1}$  and  $0.98 \times 10^{-9} \text{M}^{-1}$  respectively. The binding component was a thermolabile protein but different than plasma proteins. Ligand specificity studies revealed that along with progesterone, other progestins compete for the binding sites. In the mammary gland, progesterone was metabolised mainly to 20-hydroxy-4-pregnen-3-one, 5 $\alpha$ -pregnane-3,20-dione and polar compounds. The breast carcinoma tissue showed higher metabolism than the normal breast tissue. In the breast carcinoma 20 $\beta$ -hydroxy-4-pregnen-3-one was the major metabolite whereas in the normal mammary gland 20 $\alpha$ -hydroxy-4-pregnen-3-one was the major metabolite.

### INTRODUCTION

Growth and development of mammary gland are hormone dependent phenomena. The binding of hormone to receptor proteins has been suggested to be one of the initial steps in the mechanism of action of a hormone in the target tissue for its physiological response [1-4]. Initial studies on the binding of oestradiol in rat uterus [5-6] paved the way for the extrapolation of such studies on rat and human mammary tumours [7-13]. The value of the binding of oestradiol to specific receptors in the cancerous cytosol for differentiating hormone dependent and independent tumours and the clinical usefulness of the observations is being explored [10, 14-19]. The binding of progesterone to the uterine receptors and its physico-chemical nature has been shown [20-25]. However, only limited biochemical studies have been done on the progesterone interaction with normal mammary gland and breast carcinoma tissue [26-32]. The present study is a detailed investigation on the metabolic fate of progesterone and its interaction with the receptor proteins in the human normal and cancerous breast tissue.

### EXPERIMENTAL

**Reagents.** [1,2- $^3\text{H}$ ]-progesterone (S.A. 47.8 Ci/mmol) and [1,2,6,7- $^3\text{H}$ ]-progesterone (S.A. 98 Ci/mmol) were obtained from New England Nuclear Corp. Boston

MA, U.S.A. The purity was checked every two months by t.l.c. using chloroform-acetone (9:1, v/v).

**Tissue preparation and cell fractionation.** Normal and cancerous mammary tissue specimens obtained at mastectomy operations were transported from the hospital to the laboratory under ice cold conditions within a few minutes of operation. The normal and cancerous tissues for the experiments were identified histopathologically. The age of the patients ranged between 25-54 years. The tissues were teased off fat, minced and washed thoroughly 4-5 times with cold buffer. The minces were homogenized in Tris-HCl buffer pH 7.4 containing 10% glycerol using a Polytron-PT 10 homogenizer and fractionated into different subcellular preparation by differential ultracentrifugation as described earlier [22]. Protein was estimated by the method of Lowry *et al.* [33].

**Characterization of progesterone receptors in the normal and cancerous mammary gland cytosol**

**Sephadex G-200 column chromatography.** Sephadex G-200 columns (2.5 cm  $\times$  50 cm) were prepared and equilibrated with 0.01 M Tris-HCl buffer pH 7.4. Normal cytosol (15 mg protein) and cancerous cytosol (25 mg protein) were incubated with [1,2- $^3\text{H}$ ]-progesterone (20.9 pmol) for 90 min at 0°C and the steroid-cytosol complex was layered on the top of the Sephadex G-200 column and eluted with Tris-HCl

buffer. Fractions of 4 ml were collected and the protein was measured at 280 nm. Aliquots were taken for [ $^3\text{H}$ ]-radioactivity counting.

**Sucrose density gradient.** Linear gradients of 5–20% sucrose in 0.01 M Tris-HCl 10% glycerol buffer were prepared using a Beckman density gradient former and kept at 4°C. Samples (0.2 ml) incubated with [ $^3\text{H}$ ]-progesterone were layered on the cold gradients and centrifuged at 48000 rev/min for 15 h using SW-50 rotor. Fractions (2 drops/fraction) were collected by piercing the bottom of the tube and tritium in each fraction was determined. Human serum albumin (HSA) was run along with the samples. The sedimentation coefficient and the molecular weight of the steroid binding components were determined according to the method of Martin and Ames [34].

**Charcoal-Dextran treatment.** 0.5 ml charcoal suspension (0.5% Norit A and 0.05% Dextran T70 in buffer tris-HCl 10% glycerol, pH 7.4) was added to each assay tube, vortexed for 5 s, allowed to stand for 15 min and centrifuged at 2500 rev/min for 15 min. Supernatant (bound fraction) was taken into vials and counted for radioactivity.

**Equilibrium dialysis.** The normal and cancerous cytosols (1 ml each) in dialysis tubings were dialysed against 10 ml of Tris-HCl-10% glycerol buffer containing [ $^3\text{H}$ ]-progesterone (10,000 c.p.m.) at 4°C for 20 h with gentle shaking. At the end of dialysis, aliquots in duplicate were taken from inside and outside the dialysis bag for counting. The per cent binding of progesterone to the cytosol protein was calculated [20].

**Immunoabsorption of the plasma protein.** Anti-normal human serum was polymerized using glutaraldehyde according to Avrameas and Ternynck [35]. The cytosols were mixed with polymerized anti-normal human serum at 4°C for 10 min and centrifuged at 1500 g for 10 min. This step was repeated thrice to remove possible plasma proteins. Protein content of the supernatant was measured. The binding capacity of the immunoabsorbed cytosol was then studied by equilibrium dialysis.

**Polyacrylamide gel electrophoresis.** Polyacrylamide gel electrophoresis of the cytosol was performed essentially as described earlier [22]. Progesterone-mammary gland cytosol protein binding was achieved by incubating the normal or cancerous cytosol with [ $^3\text{H}$ ]-progesterone at 0°C for 1 h by which time the equilibrium was attained. The cytosol was layered on the 7.5% polymerized acrylamide gel (pH 9) and electrophoresed at 4°C with a constant current of 2.5 mA/gel. Cytosol alone and human serum albumin were also electrophoresed simultaneously along with samples. After electrophoresis the gels were cut transversely into 2 mm slices, dissolved in 30% hydrogen peroxide and counted for radioactivity. Gel columns containing cytosol and HSA were stained with amido black. The electrophoretic mobility of the binding protein was calculated in relation to bromophenol blue.

**Ligand specificity.** The competition of various steroids for progesterone receptors was investigated with the cancerous cytosol prepared in Tris-HCl-glycerol buffer (pH 7.4) containing  $1 \times 10^{-6}$  M cortisol to eliminate the interference of plasma cortico-steroid binding globulin (CBG) and 0.1% monothioglycerol, 20% glycerol, 5 mM diisopropylfluoro-phosphate and 5 mM mercaptoethanol for receptor stabilization. Cytosol (1.5 mg protein/100  $\mu\text{l}$ ) was added to tubes containing  $3.2 \times 10^{-14}$  mol of [ $^3\text{H}$ ]-progesterone and different concentrations of progesterone and other competing steroids. Steroid protein complex was incubated at 4°C for 12–16 h and [ $^3\text{H}$ ]-progesterone binding was measured by dextran-charcoal adsorption method. The concentrations of unlabelled progesterone and of steroid competitors that reduced [ $^3\text{H}$ ]-progesterone binding by 50% were determined from the semilog plot drawn as per cent bound vs steroid concentrations. The effectiveness of a competitor was established using the ratio of unlabelled progesterone concentration for 50% competition to competitor concentration for 50% competition. For the relative binding affinity, the ratio was multiplied by 100. The binding affinity of progesterone was taken as 100 [37].

**Metabolism of progesterone in the mammary gland tissue:** Normal and cancerous mammary gland tissue (500 mg) were minced and incubated at 37°C for 2 h with [ $^3\text{H}$ ]-progesterone (41.8 pmol) in Krebs-Ringer phosphate buffer [38] pH 7.4 containing 1 mg of glucose/ml. The reaction was terminated by adding acetone to the incubation flasks. The steroid was extracted thrice with acetone and once with methanol. The extract was pooled and the organic solvent was evaporated till the buffer remained. The aqueous portion was re-extracted with diethyl ether. The ether extract was dried and redissolved in 70% aqueous methanol and delipidized at -20°C [38]. The methanolic extract was dried and analysed by thin layer chromatography (t.l.c.) using following solvent systems [40]: I, hexane: ethyl acetate (5:2, v/v); II, chloroform-acetone (9:1, v/v); III, chloroform-methanol (9:1, v/v); IV, hexane-diethyl ether (4:1, v/v); V, cyclohexene-cyclohexanone (9:1, v/v). Identification of the metabolites was carried out as described earlier [41].

**Radioactivity counting.** Radioactivity was measured in a liquid scintillation spectrometer (Packard Tricarb Model 3314) as described earlier [22].

## RESULTS

### *Localization of progesterone in the human mammary gland*

Table 1 shows the distribution of [ $^3\text{H}$ ]-progesterone in cancerous and normal breast tissue cell fractions, 90 min after *in vitro* incubations. On an average 1.9 pmol and 4.7 pmol of the incubated [ $^3\text{H}$ ]-progesterone (41.8 pmol/500 mg tissue) was concentrated by the normal and cancerous mammary gland tissue re-

Table 1. Distribution of progesterone and its metabolites in the normal and cancerous breast tissue

Patients	Normal breast				Carcinoma breast			
	Nuclear	Mitochondrial	Microsomal	Cytosol	Nuclear	Mitochondrial	Microsomal	Cytosol
S.F. (25)					67.0	17.9	8.4	5.1
S.W. (27)	49.1	18.0	11.7	21.1	70.4	9.0	10.2	10.2
S.R. (30)	47.6	4.7	2.4	45.1	69.0	8.1	6.9	16.4
P.K. (32)	51.0	11.3	3.9	33.6	71.1	9.0	1.7	18.0
U.D. (35)					60.4	3.4	2.8	30.7
S.K. (35)					81.0	11.0	1.8	6.1
L.W. (42)	44.1	8.2	4.8	42.8	50.8	12.8	7.5	29.1
S.D. (42)	47.0	5.7	11.4	33.7				
R.C. (45)					61.2	9.5	2.7	26.1
C.M. (48)	23.1	10.4	3.5	57.6				
G.D. (50)	43.9	8.2	2.6	45.1	69.9	6.6	1.8	21.5
K.K. (50)	47.3	22.1	16.4	24.0	66.2	10.7	15.9	7.0
Mean $\pm$ S.E.	44.1 $\pm$ 3.1	11.1 $\pm$ 2.1	7.1 $\pm$ 1.9	38.1 $\pm$ 4.2	66.7 $\pm$ 2.5	9.8 $\pm$ 1.2	6.0 $\pm$ 1.5	17.0 $\pm$ 3.1

The normal and cancerous mammary gland tissues (500 mg) were minced and incubated with [ $1,2\text{-}^3\text{H}$ ]-progesterone (41.8 pmol) in Krebs-Ringer phosphate buffer (pH 7.4) at 37°C for 90 min. At the end of incubation, the tissues were washed with cold buffer thrice to remove the adhering radioactive steroid. The tissues were homogenized, fractionated and the radioactive steroid from each preparation was estimated as previously described [40]. The results are expressed as per cent of the radioactivity in each unit of tissue (radioactivity in nuclear, mitochondrial, microsomal and cytosol preparation). The figures in parentheses represent the age of the patients.

spectively. Subcellular fractionation of the tissue into nuclear, mitochondrial, microsomal and cytosol preparations revealed that the cancerous breast tissue 800 g nuclear preparation localized considerably higher amounts of progesterone than the 105,000 g cytosol preparation. The mitochondrial and microsomal preparations contained relatively low amounts of progesterone. In the normal tissue the localization of progesterone in the 800 g nuclear preparation was in general only slightly higher than cytosol preparation. The mitochondrial and microsomal preparations contained considerably smaller amount of progesterone. The age of the women did not make any significant difference in the subcellular distribution pattern of progesterone in these tissues.

#### Time course study of progesterone localization in the nuclear and cytosol preparations

Figure 1 shows the per cent distribution of [ $^3\text{H}$ ]-progesterone in the nuclear and cytosol preparations at different times of incubation. In the normal mammary gland at 1 and 5 min of incubation more of progesterone was concentrated in the cytosol fraction. At 15 min of incubation the amount of progesterone in the cytosol and nuclear preparations was almost equal. At 30 min and 60 min the nuclear uptake was higher than in the cytosol fraction. The uptake in the nuclear preparation was maximum at 30 min and it showed a plateau thereafter. In the case of cancerous mammary gland, progesterone localization even as early as 1 min was slightly higher in the nuclear fraction than in the cytosol fraction. The amount of progesterone in the nuclear preparation increased gradually up to 30 min and it remained almost constant upto 60 min whereas in the cytosol progesterone decreased gradually with time up to 30 min.

**Binding of [ $^3\text{H}$ ]-progesterone to breast tissue cytosol**  
*Sephadex gel filtration chromatography.* On gel chromatography the mammary gland cytosol progesterone binding macromolecules resolved into three

major 280 nm absorbing peaks (Fig. 2A and B). The first two peaks were proteinous in nature as determined by 280/260 nm absorption ratio. [ $^3\text{H}$ ]-Progesterone was bound to both the protein peaks of the Sephadex column chromatography. The elution pattern of the binding protein of both the cancerous and normal cytosols were comparable.

*Sucrose gradient sedimentation analysis.* Progesterone was bound to two macromolecules in the normal cytosol fraction (Fig. 3A). The heavier macromolecular receptor had a sedimentation value of about 6.7S with an approximate molecular weight of 132,000.

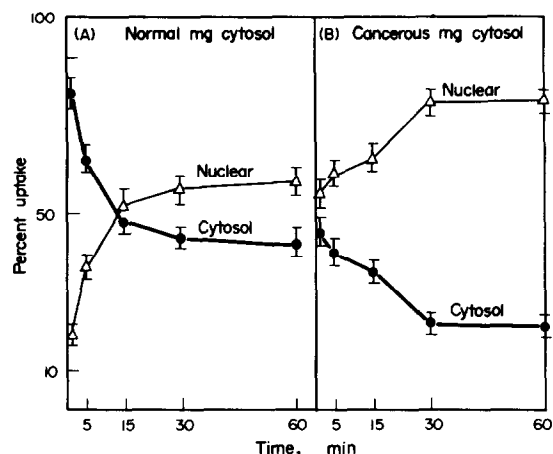


Fig. 1. Distribution of [ $1,2\text{-}^3\text{H}$ ] progesterone in the nuclear and cytosolic preparation of normal and cancerous breast tissues: Normal (A) and cancerous (B) breast tissue 500 mg each were incubated with [ $1,2\text{-}^3\text{H}$ ]-progesterone (20.9 pmol) at 0°C for 5 min to allow diffusion of the steroid into the cell and then at 37°C for 1, 5, 15, 30 and 60 min. The tissues were homogenized and fractionated into nuclei (800 g) and (105,000 g) cytosolic fractions by ultracentrifugation. The results are expressed as per cent progesterone and its metabolites in the nuclear and cytosol fractions when the steroid concentrated in these two fractions was taken as 100 per cent. The results represent the mean  $\pm$  S.E. values from three individual cases (T.B., R.P. and S.L.).

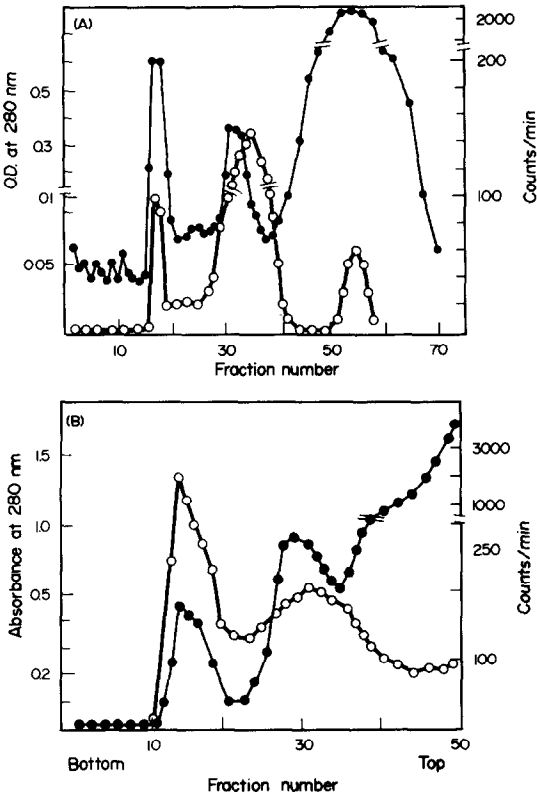


Fig. 2. Sephadex G-200 column chromatographic pattern of the human normal (A) and cancerous (B) mammary gland cytosol progesterone binding protein.

The 6.7S binding component was observed in four of the seven cases studied (Table 2). Progesterone was bound to another lighter macromolecular receptor which had a sedimentation value of about 4.3S, with an approximate molecular weight of 56,000. This peak was relatively more constant being present in all the cases studied. The concentration of the 6.7S binding component varied in different cases. In some cases total [<sup>3</sup>H]-progesterone in the 6.7S peak was more than in the 4.3S peak while in others the 4.3S peak contained more radioactivity than the 6.7S peak. The cancerous mammary gland cytosol sucrose gradient sedimentation pattern (Fig. 3B) showed only 4.3S peak in all the six cases studied with an approximate

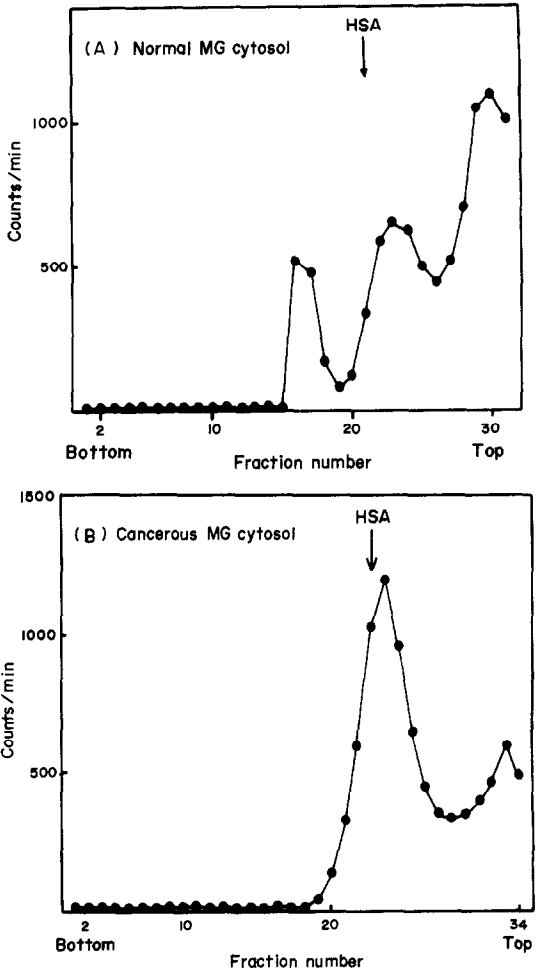


Fig. 3. Sucrose density gradient sedimentation analysis of cytosols: Normal (A) and cancerous (B) cytosols (0.3 ml each) were incubated with [<sup>1,2-3</sup>H]-progesterone (10.5 pmol) at 0°C for 90 min. 0.2 ml of the cytosol was layered on 4.8 ml of 5–20% sucrose–10% glycerol gradient and centrifuged at 48,000 rev/min for 15 h. HSA was used as the molecular marker protein. At the end of centrifugation equal number of drops were collected in vials and counted for radioactivity.

molecular weight of 60,000 daltons. The binding of [<sup>3</sup>H]-progesterone to 6.7S in the normal and 4.3S in the cancerous as well as normal cytosol was greatly affected when studied in the presence of 100 fold

Table 2. Sucrose gradient sedimentation analysis of the normal and cancerous mammary gland cytosol progesterone binding proteins

Patients	Normal cytosol S value of:		Cancerous cytosol S value of:	
	Peak I	Peak II	Peak I	Peak II
R.C.	—	4.4	*	*
S.F.	—	4.2		4.4
K.K.	6.9	4.8	*	*
T.B.	6.7	3.8		4.2
A.D.	—	4.2		4.5
P.D.	6.3	4.3		4.4
R.K.	*	*		4.2
H.F.	*	*		4.2
V.S.	7.0	4.4	*	*
Mean ± S.E.	6.7 ± 0.16	4.3 ± 0.11		4.32 ± 0.06

\* Tissues not studied.

excess of non labelled progesterone thereby showing the specificity of the progesterone binding peaks for progesterone (Figs not shown).

#### *Polyacrylamide gel electrophoresis of the normal and cancerous mammary gland cytosol*

The polyacrylamide gel electrophoretic pattern of the normal and cancerous cytosol progesterone binding proteins and the effect of cortisol and progesterone on the binding are shown in Fig. 4. No characteristic progesterone binding was observed up to one third of the gel length from the top. In all the gels, scanned for [ $^3\text{H}$ ]-progesterone binding, five definite peaks were observed with  $R_F$  values of 0.52, 0.59, 0.7, 0.83 and 0.9. The peaks having  $R_F$  0.52 and 0.59 appeared to be specific progesterone binding proteins of the mammary gland tissue. These peaks were affected by cold progesterone and not by cortisol. The protein peak having  $R_F$  0.52 showed more binding to the cancerous mammary gland cytosol and the other having  $R_F$  0.59 appeared more specific to normal cytosol progesterone binding protein. The peak having  $R_F$  0.7 was affected by cortisol and not by progesterone. The protein peaks having  $R_F$  0.83 and 0.9 were affected neither by cortisol nor by progesterone indicating their high capacity nature. The later peak  $R_F$  0.9 had identical mobility with human serum albumin.

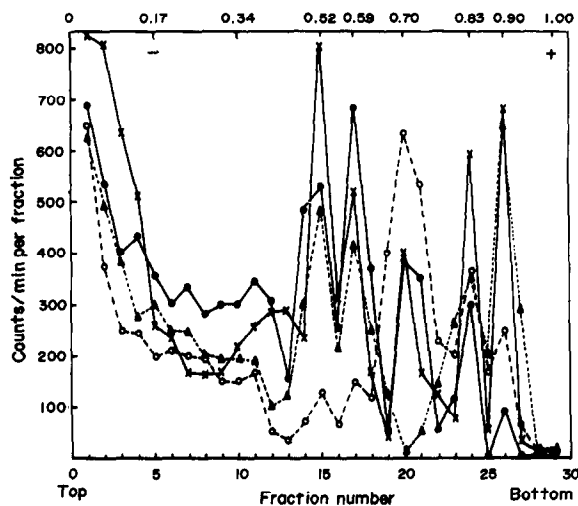


Fig. 4. Polyacrylamide gel electrophoretic pattern of normal and cancerous mammary gland cytosol progesterone binding protein: The normal and cancerous cytosols (200  $\mu\text{l}$ ) were incubated with 1. [ $1,2\text{-}^3\text{H}$ ]-progesterone (0.5  $\mu\text{Ci}$ ); 2. [ $1,2\text{-}^3\text{H}$ ]-progesterone (0.5  $\mu\text{Ci}$ ) and unlabelled progesterone (1  $\mu\text{g}$ ) and 3. [ $1,2\text{-}^3\text{H}$ ]-progesterone (0.5  $\mu\text{Ci}$ ) and unlabelled cortisol (1  $\mu\text{g}$ ) at  $0^\circ\text{C}$  for 1 h and 20  $\mu\text{l}$  samples were electrophoresed on polyacrylamide (7.5%) gels. The radioactivity in each gel fraction ( $\sim 2\text{ mm}$ ) was determined and plotted.  $\bullet\text{---}\bullet$  normal cytosol,  $\times\text{---}\times$  cancerous cytosol,  $\blacktriangle\text{---}\blacktriangle$  effect of cortisol on cancerous cytosol progesterone binding protein,  $\circ\text{---}\circ$  effect of progesterone on cancerous cytosol progesterone binding protein. Similar effects (not shown in the Fig.) were observed with normal cytosol.

#### *Nature of progesterone receptors*

Progesterone binding to normal and cancerous cytosol was not affected by RNase but trypsin abolished this binding, thereby showing the protein nature of the receptor. Progesterone receptors were thermolabile as heat treatment of the cytosol at  $60^\circ\text{C}$  for 20 min completely denatured progesterone receptor. As an effect of 2-mercaptoethanol, a sulphhydryl group protecting agent, progesterone binding to cytosol was facilitated. The ratio of progesterone binding in the 2-mercaptoethanol treated to untreated cytosol was 1.4 in the normal and 1.25 in the cancerous cytosol. The effect of high salt concentrations (0.4 M KCl) on the progesterone receptor in the normal cytosol showed that the binding in the 6.7S region was dissociated and a single binding peak was observed in the 4.3S region (Figs not shown).

#### *Effect of immunoabsorbant on progesterone binding*

The binding of progesterone to the immunoabsorbed cytosol and plasma was studied by equilibrium dialysis. The binding in the immunoabsorbed normal and cancerous cytosols decreased only a little when compared with unabsorbed cytosols, while it decreased considerably in the immunoabsorbed plasma. When 0.5 mg protein/dialysis was used, the binding of progesterone to the unabsorbed normal cytosol was 74% and in immunoabsorbed, it was 68%. Similarly in the unabsorbed cancerous cytosol, the binding of progesterone was 78% and in the immunoabsorbed 71% while in plasma, the binding decreased from 87% in the unabsorbed to 47% after immunoabsorption. The binding of progesterone to the immunoabsorbed cytosols was further confirmed by sucrose gradient sedimentation analysis.

#### *Binding affinity of progesterone to normal and cancerous cytosols*

Scatchard analysis of the progesterone binding protein showed two classes of binding both in the normal and cancerous cytosols (Figs 5A and 5B). Progesterone at lower concentrations was bound to a high affinity low capacity protein with a high specificity. At higher concentrations of the steroid another class of binding was observed which was not easily saturable. The number of specific binding sites in the normal cytosol was of the order of  $2.1 \times 10^{-12}$  mol/mg protein and in the cancerous cytosol it was  $1.5 \times 10^{-12}$  mol/mg protein. The dissociation constant of progesterone binding protein in the normal cytosol was  $0.42 \times 10^{-9} \text{ M}^{-1}$  and in the cancerous cytosol  $0.98 \times 10^{-9} \text{ M}^{-1}$ .

#### *Ligand specificity of the progesterone binding proteins*

Progesterone binding peaks in the normal and cancerous cytosols were abolished by 100 fold excess of cold progesterone. The ligand specificity of the progesterone binding protein in the cancerous cytosol to

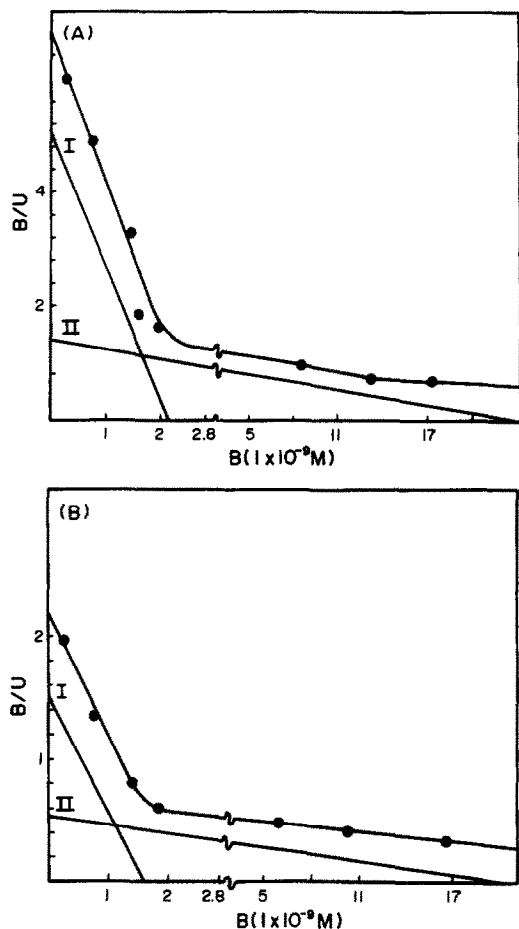


Fig. 5. Scatchard plot of the normal and the cancerous mammary gland cytosol progesterone binding proteins: Human normal (A) and cancerous (B) mammary gland cytosols (1 mg protein/dialysis) were dialysed against different concentrations of unlabelled progesterone (0.3–30 nM) and [ $1,2\text{-}^3\text{H}$ ]-progesterone (10,000 c.p.m.) in Tris-HCl (0.01 M) glycerol (10%) buffer (pH 7.4) for 20 h at 4°C. Scatchard plot was drawn from the concentration of the bound steroid (B) on X axis against bound/unbound ratio (B/U) on Y axis. The straight lines I and II were obtained from the experimental curve according to Rosenthal [36].

other natural and synthetic steroids is shown in Fig. 6. Synthetic progestins competed efficiently for the progesterone receptor. The relative binding affinities were 77, 65, 41, 29 and 11 for norgestrel, norethisterone, chlormadinone acetate, dihydroprogesterone and norethisterone acetate respectively, when the affinity for progesterone was considered 100. Of the natural steroids tested  $5\alpha$ -pregnane-3, 20-dione showed maximum competition for progesterone binding sites, while cortisol, testosterone and oestradiol showed little or no competition.

#### Metabolism of progesterone by the mammary gland

Table 3 shows the metabolism of progesterone by the normal and cancerous mammary gland tissues. The mean values of the different experiments showed that the metabolism of progesterone in the cancerous mammary tissue was more than that in the normal

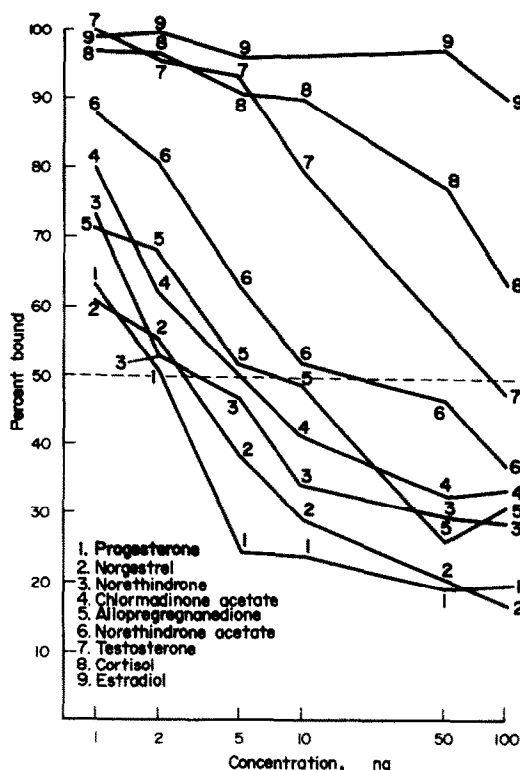


Fig. 6. Ligand specificity of the progesterone binding receptor: Competition of progesterone, progestins and other steroids for the progesterone receptor in the cancerous cytosol was carried out by the dextran coated charcoal method using 1–100 ng of the test compounds. Results are expressed as the per cent inhibition of the binding ability by different steroids of 50% competition level as described under Experimental.

mammary tissue. Formation of  $5\alpha$ -pregnane-3,20-dione in the cancerous tissue was 2.6 times more than in the normal. The amount of pregnanolone isomer, 20 $\alpha$ -hydroxy- $5\alpha$ -pregnan-3-one was formed little. 20-Hydroxy-4-pregnen-3-one region of the chromatogram had considerable radioactivity in the normal and cancerous tissues. In three cases (S.R., L.W., R.C.) 20-hydroxy-4-pregnen-3-one region when analysed for 20 $\alpha$  and 20 $\beta$ -hydroxy-4-pregnen-3-one showed that the hydroxylation of progesterone was facilitated in the 20 $\alpha$ -position in the normal tissues and 20 $\beta$ -position in the cancerous mammary tissue. Polar compounds like  $5\alpha$ -pregnane-3,20-diol and those which did not move from the origin were formed more in the cancerous than in the normal tissue.

#### DISCUSSION

The uptake, binding and biotransformation of progesterone with the cancerous and normal mammary gland revealed a specific interaction of progesterone with the target organ. Significantly higher amounts of progesterone localization in the nuclei of cancerous tissue differed from normal tissue. Further, the time-course studies suggested that the uptake pattern of

Table 3. Metabolism of [1,2-<sup>3</sup>H]progesterone in the normal and cancerous mammary tissue

Steroids	Normal breast tissue						Cancerous breast tissue					
	S.W.	S.R.	L.W.	R.C.	Mean $\pm$ S.E.	S.K.	S.W.	S.R.	L.W.	R.C.	K.K.	Mean $\pm$ S.E.
5 $\alpha$ -pregnane-3,20-dione	1.6	1.2	1.5	1.0	1.3 $\pm$ 0.14	4.4	1.9	3.6	4.9	2.2	3.8	3.5 $\pm$ 0.49
Progesterone converted	10.6	7.9	12.2	10.9	10.3 $\pm$ 0.9	20.0	23.9	21.8	18.9	21.9	25.9	22.1 $\pm$ 1.04
5 $\alpha$ -pregnan-20 $\gamma$ -ol-3-one	0.2	0.3	0.2	0.4	0.3 $\pm$ 0.05	1.0	1.1	1.2	1.2	1.4	1.0	1.2 $\pm$ 0.06
20 $\gamma$ -hydroxy-4-pregnen-3-one	—	2.8	1.6	3.5	2.6 $\pm$ 0.55	—	—	1.6	0.7	1.8	—	1.4 $\pm$ 0.33
20 $\beta$ -hydroxy-4-pregnen-3-one	3.9*	0.4	1.0	0.4	0.6 $\pm$ 0.2†	5.0*	10.6*	5.0	1.2	5.4*	8.8	3.9 $\pm$ 1.32†
Unidentified	—	0.3	1.2	0.2	0.5 $\pm$ 0.3	—	—	2.5	3.2	1.4	—	2.4 $\pm$ 0.53
6 $\beta$ -hydroxy-progesterone	0.8	0.5	0.8	0.7	0.7 $\pm$ 0.07	1.1	0.7	1.1	1.6	1.5	1.8	1.3 $\pm$ 0.16
5 $\alpha$ -pregnan-3,20-diol	1.0	0.8	2.5	1.9	1.6 $\pm$ 0.4	3.7	4.5	2.9	3.2	3.4	4.7	3.7 $\pm$ 0.31
Origin	1.5	1.2	3.1	2.5	2.1 $\pm$ 0.4	4.5	4.3	3.1	2.6	4.1	5.4	4.0 $\pm$ 0.41

500 mg of tissue was incubated with [1,2-<sup>3</sup>H]-progesterone (41.8 pmol) at 37°C for 2 h as described under 'Experimental'. The results are expressed as per cent metabolite formed of the substrate and as percent progesterone converted. \*Radioactive steroid which move in 20-hydroxy-4-pregnen-3-one region in hexane:ethyl acetate (5:2, v/v) and not resolved further. †Mean value of 20 $\beta$ -hydroxy-4-pregnen-3-one of patients S.R., L.W., R.C.

progesterone in the normal mammary gland nuclei and cytosol was different from cancerous tissue. The faster transport of progesterone to the cancerous cell nuclei suggested that the transport mechanism of progesterone in this tissue was altered. However, the transport of progesterone into the nuclei from the cytosol was similar to the two-step hypothesis suggested by Jensen *et al.* [42] for oestradiol in the rat uterus.

Binding of progesterone to the immunoabsorbed cytosol suggested the presence of specific progesterone receptors in the mammary gland and was different than that of plasma progesterone binding proteins. The electrophoretic pattern of progesterone binding components in the cytosols studied did not vary qualitatively. Two progesterone specific binding peaks were observed in the normal and the breast carcinoma tissues. However, the quantity of these two binding components varied under normal and cancerous conditions. The presence of a complex mixture of proteins of widely different electrophoretic mobility was earlier reported in breast cyst fluid [43] and in the human endometrial carcinoma [44]. On the other hand, the progesterone binding components on sucrose gradient sedimentation showed qualitative difference such as easily detectable two peaks in the normal cytosol while one in the cancerous cytosol.

The ligand specificity of the specific proteins was proved by the addition of excess of progesterone and cortisol and other natural and synthetic steroids. The compounds with progestational properties competed for the progesterone binding sites, while cortisol, oestradiol and testosterone showed little or no competition for progesterone binding sites. The binding of progestogens to uterine progesterone receptor has partially been correlated with their biological potency for the endometrial growth [37]. Such a biological correlation may also hold true for progesterone action on the mammary gland.

The differences in the biotransformation of progesterone in the normal and cancerous mammary gland suggested a subtle change in the biochemical constitution of the cancerous tissue. Metabolism of progesterone by the cancerous tissue was about two times more than by the normal tissue. Significantly

higher amounts of 20 $\beta$ -hydroxy-4-pregnen-3-one was formed with the cancerous mammary gland which is not normally observed with the normal mammary gland of human [45], rat [46] and rabbit [47]. This suggested that in the cancerous mammary gland 20 $\beta$ -hydroxy steroid dehydrogenase is activated more than in the normal. 20 $\beta$ -hydroxy-4-pregnen-3-one by Hooker-Forbes and Clauberg assays was reported to be two times more potent than progesterone and 20 $\alpha$ -hydroxy-4-pregnen-3-one was only 2/5 times as potent as progesterone [48]. Since these assays are based on growth of the endometrium and mitosis of cells, it may be suggested that the hyperplastic activity of the mammary tissue may be due to the increased formation of 20 $\beta$ -hydroxy-4-pregnen-3-one. The formation of 5 $\alpha$ -pregnane-3,20-dione in higher quantity in the cancerous tissue as compared with the normal, suggested the enhanced 5 $\alpha$ -reductase activity in this tissue. The presence of this enzyme has been indirectly shown by the conversion of testosterone to dihydro-testosterone in the mammary carcinoma [49, 50].

In conclusion, a few significant observations made from this study were: 1. the nuclear transport of progesterone from the cytosol in the cancerous tissue was faster and more than in the normal tissue, 2. the progesterone binding component is a progestogen specific, thermolabile, macromolecular protein, 3. the nature of the progesterone binding protein was altered due to tumourigenesis, 4. progesterone metabolism in the cancerous tissue was two times more than in the normal tissue, 5. significantly higher amounts of 20 $\beta$ -hydroxy-4-pregnen-3-one was formed in the cancerous tissue, while 20 $\alpha$ -hydroxy 4-pregnen-3-one was formed more in the normal tissue. It is suggested that during the process of tumourigenesis these changes in the breast tissue might have taken place or these biochemical alterations may be some of the factors adding to the development of mammary gland tumour.

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