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# PROSTATIC BINDING OF ESTRADIOL-17$\beta$ IN THE BABOON

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SUMMARY

A specific receptor for estradiol-17$\beta$ ($E_2$) has been identified in the caudal and cranial lobes of the baboon prostate. Following *in vivo* infusion of [$^3$H]-$E_2$ into the hypogastric arteries of castrated and intact baboons, the prostate, particularly the cranial lobe, concentrated more radioactivity than any other tissue or fluid not involved in the metabolism or excretion of $E_2$. Sucrose density gradient centrifugation of *in vivo* and *in vitro* labeled cytosols showed that the bound $E_2$ complex had a sedimentation coefficient of $\simeq 4S$. Sephadex G-25 filtration of cytosols and 0.4 M KCl nuclear extracts of caudal and cranial lobe preparations from intact and 40 h castrates infused with [$^3$H]-$E_2$ showed bound radioactivity in all cases. Analysis of nuclear residues resistant to 0.4 M KCl extraction revealed significant concentrations of radioactivity. Testosterone (T) and dihydrotestosterone (DHT) did not compete *in vitro* with [$^3$H]-$E_2$ for the estrogen receptors, but $E_2$ and diethylstilbestrol (DES) were effective competitors. Nafoxidine, and to some extent estracyt, displaced labeled $E_2$ from cytosol binding sites. Analysis of cytosols and nuclear preparations following *in vitro* incubations of prostatic tissues revealed further evidence of specific cytosol binding and demonstrated nuclear translocation of a [$^3$H]-$E_2$ bound complex. Scatchard and double reciprocal plots indicated $K_a$ and $K_D$ values for the $E_2$ binding to be about $1.2 \times 10^9$ M and $8.3 \times 10^{-10}$ M for the caudal lobe and $7.7 \times 10^9$ M and $1.3 \times 10^{-10}$ M for the cranial lobe, respectively.

## INTRODUCTION

The use of estrogens in the treatment of cancer of the prostate [1] followed the demonstration of their effectiveness in reducing the size of the gland [2]. Regression of the prostate following estrogen administration has generally been assumed to be mediated indirectly via suppression of the hypothalamic-pituitary-gonadal axis [3], though some direct effects of estrogens on the prostate have been reported [4].

The present work was initiated following the observation [5] that radioactivity associated with injected estrogens is deposited in prostatic tissues of dogs and baboons. Since then, the binding of estradiol-17$\beta$ ($E_2$) to specific cytoplasmic macromolecules has been identified in the rat ventral prostate [6–8] and in normal [9], benign hyperplastic [10] and malignant conditions of the human prostate [9].

Recently, this laboratory reported the existence of a binding protein for $E_2$ in the baboon caudal prostate [11]. Since cytoplasmic receptors appear to be essential to the action of steroids in some target tissues, we have further investigated the properties of estrogen binding in the baboon prostate, which, as a primate model, may provide information of potential value for direct application to the human gland.

## MATERIALS AND METHODS

### *Baboons*

Adult male baboons (*Papio anubis*) weighing 18–25 kg were caged individually and, along with a daily supplement of fresh fruits and vegetables, were fed Teklad Primate Diet and water *ad libitum*.

For all surgical procedures, anesthesia was induced with Sernylan (2.5 mg/kg, i.m.) followed by Valium (2.5 mg/kg, i.v.). Where indicated, bilateral orchiectomy was performed via a scrotal incision; arterial infusions and prostatectomies were performed after appropriate exposure through a vertical midline abdominal incision. The caudal and cranial lobes of the excised prostates, which weigh between 4–7 g and 1.5–3 g, respectively, were separated by sharp dissection through their natural planes.

### *Materials*

[6,7-$^3$H]-$E_2$ (46.7 Ci/mmol), and [2,4,6,7-$^3$H]-$E_2$ (100 Ci/mmol) were purchased from the New England Nuclear Corporation. These compounds were checked periodically for purity by paper chromatography in the system benzene–methanol–water (10:5.5:4.5, by vol.). Estracyt (estramustine phosphate), radioinert or tritiated at positions 6 and 7 of the $E_2$ moiety (3.65 $\mu$Ci/$\mu$mol), was obtained from AB Leo, Helsingborg, Sweden. Radioinert $E_2$, testosterone (T), dihydrotestosterone (DHT) and DES were purchased from the Sigma Company. Nafoxidine hydrochloride (Upjohn 11,100A) was a gift from Upjohn Company. Tris and EDTA were obtained from the Eastman Kodak Company, sucrose (Ultra Pure) from Schwartz/Mann, Sephadex G-25 from Pharmacia Fine Chemicals and medium 199-1X from Grand Island Biological Company. All other chemi-

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cals used were reagent grade (Fisher Scientific Company).

*Sucrose density gradient centrifugation (SDGC)*

Samples of cytosol (0.5 ml) were layered on the top of 5–20% (w/vol) sucrose gradients [12] prepared in 0.02 M Tris–HCl buffer, pH 7.5, at 20°C, containing 1.5 mM EDTA (TE). Using bovine serum albumin (BSA) as a reference standard, the gradients were centrifuged at 4°C for 20 h at 41,000 rev./min (average force 201,000 *g*). Forty fractions of 10 drops each were collected from the bottom of the tubes into counting vials. The migration of BSA (S = 4.6, mol wt = 68,000) was assayed by measuring the absorbance at 280 nm after dilution with water.

*Sephadex G 25 filtration (SG25)*

Bound radioactivity in samples (0.5 ml) of cytosol and 0.4 M KCl nuclear extracts was determined by using gel filtration on SG25 columns (24 cm × 0.8 cm), thus removing free hormone from the void vol. [13, 14]. The columns were equilibrated with the same buffer in which the samples were dissolved. Fifty-four fractions of 5 drops each were collected into counting vials.

*Determination of radioactivity*

Tissues and nuclear pellets were oxidized using a Packard Instrument Company model 306 sample oxidizer [5] and Packard model 3375 or 2450 spectrometers were used for determination of radioactivity. At least 16,000 c.p.m. were accumulated (corresponding to ±2.5% S.D.). Determination of radioactivity in aqueous samples was performed in 10 ml pre-mixed scintillation fluid (ACS from Amersham, Searle Company). Counting efficiency was 45% for tritiated samples.

*Protein determinations*

The protein concentrations of the various samples were determined [15] using BSA as standard, and radioactivity determinations were normalized to sample protein content.

In vivo *infusions*

Ia. *SDGC analysis.* The hypogastric arteries of intact ($n = 4$) and 40 h castrate ($n = 1$) baboons were cannulated with a 25 gauge needle and 1.0 mCi of [6,7-$^3$H]-$E_2$ dissolved in 30 ml of 0.9% saline was infused into each artery over a 1 h period, using a Harvard Infusion/Withdrawal Pump (model 931). Following prostatectomy, the caudal and cranial lobes were rinsed in ice-cold TE and homogenized in an equal vol. of TE with a Potter–Elvehjem glass homogenizer. Radioactivity bound in the 4.2 *S* peak of the caudal lobe cytosol from one of the intact baboons was isolated by combining gradient fractions 28–31 and treating them as a single sample. The sample was extracted with chloroform:methylene chloride (1:1, v/v) and dried at 50°C under nitrogen and subjected to paper chromatography.

Ib. *SG25 analysis.* One intact and one 40 h castrate baboon were infused for 1 and 2 h, respectively. 1 mCi of [2,4,6,7-$^3$H]-$E_2$ dissolved in 45 ml of 0.9% saline was administered through tygon cannulas (0.51 mm i.d. × 1.5 mm o.d.) into each common iliac artery (the external iliac arteries were tied off) of the intact baboon and into each hypogastric artery of the castrated baboon.

The excised prostates were rinsed in ice-cold 0.5 M STKM buffer [0.5 M sucrose in TKM buffer (0.05 M Tris–HCl, 0.025 M KCl, 0.005 M $MgCl_2$, pH 7.5, at 20°C)], and put into a vol. of 0.5 M STKM equal to 3 times the tissue weight. The tissues were homogenized with a Polytron (PT10-35, Brinkman Instruments, Inc.) by three 10-s bursts (setting 5.5) interrupted with 2 min cooling intervals. The homogenate was filtered through cheese cloth and nylon mesh. The filtrate was centrifuged at 760 *g* for 10 min to produce a crude nuclear pellet. The supernatant was centrifuged at 103,000 *g* for 1 h to obtain cytosol. The crude nuclear pellet was washed gently in 0.5 M STKM, centrifuged at 500 *g* for 10 min, suspended in 0.5 M TKMS, layered over a cushion of 2.0 M STKM and centrifuged at 103,000 *g* for 1 h. The resulting nuclear pellet was washed 2 more times in 0.5 M STKM. Using phase contrast microscopy, the nuclei appeared spherical and free of extraneous cellular debris. The pellet of "clean" nuclei was then incubated (0–2°C) for 1 h in 0.4 M KCl-TE with periodic vortexing, then centrifuged at 10,000 *g* for 20 min to produce a nuclear extract and a nuclear residue.

In vitro *studies*

IIa. *SDGC analysis.* Prostate cytosols (prepared as described in Ia, above) of one intact, one 24 h castrate and five 40 h castrate baboons were used for the identification of $E_2$ binding components. In other *in vitro* studies, 0.5 ml samples of prostate cytosol were incubated (90 min, 4°C) with 1.0 μCi of [6,7-$^3$H]-$E_2$ and with 20 μg of radio-inert estracyt and with 0.06 μg, 1.2 μg, 3.0 μg or 20 μg nafoxidine.

IIb. *SG25 analysis.* Alternate caudal prostate slices (0.5 mm, prepared on a Stadie-Riggs microtome) of 40 h castrates ($n = 3$) were incubated for 1 h in air at 4 and 37°C with Medium 199 (1:10, w/vol) and 50 μCi of [6,7-$^3$H]-$E_2$. The tissues were rinsed and homogenized with a Teflon homogenizer in a vol. of 0.5 M STKM equal to 3 times the tissue weight. Cytosols, 0.4 M KCl nuclear extracts and residues were prepared as outlined above (Ib). Identical procedures were followed using the cranial lobes from the same prostates.

In another experiment, caudal and cranial lobe cytosols were each combined with 1 μCi of [6,7-$^3$H]-$E_2$/ml cytosol, divided equally, and incubated for 1 h at 0° and 37°C. Equal portions of "clean" nuclei from the same homogenate were suspended in the heated and unheated cytosol-estradiol mixtures and the incu-

bations continued for 1 h, after which the nuclei were separated by centrifugation at 0–2°C for 10 min at 760 *g*.

Determination of total, nonspecific and specific $E_2$ binding sites in the 40 h castrate ($n = 2$) was accomplished by incubation (90 min, 4°C) of ten identical aliquots of caudal and cranial lobe cytosols with decreasing concentrations of [6,7-$^3$H]-$E_2$ (20 nM to 0.2 nM) in the presence of a 100-fold molar excess of DES. Separation of bound and free steroid was accomplished by SG25. Double reciprocal plots of bound and free steroid and Scatchard analyses (16) were used to determine association constants ($K_a$), the number of high affinity binding sites (N) and the dissociation constants ($K_D$).

*Specificity studies.* Hormone specificity was studied in fresh cytosols from a 40 h castrate. Binding of [2,4,6,7-$^3$H]-$E_2$ was measured in the presence of various unlabeled steroids which were added in 1, 10 and 100-fold molar excess to the labeled $E_2$ ($1 \times 10^{-9}$ M in 0.5 ml cytosol). Incubations were carried out at 30°C for 2 h and then subjected to SG25 analysis.

## RESULTS

### I. In vivo *infusion of* [6,7-$^3$*H*]-$E_2$; *SDGC*

The distribution and deposition of the radioactivity (d.p.m. $\times 10^3$/gm of whole tissue) of [6,7-$^3$H]-$E_2$ in the prostate and other organs of the baboon following infusion into the hypogastric arteries were measured. Significant amounts of radioactivity were concentrated into the caudal lobe ($\simeq$400 d.p.m.'s) and, particularly, in the cranial lobe ($\simeq$850 d.p.m.'s), as compared to that found in the bladder ($\simeq$250 d.p.m.'s), seminal vesicles ($\simeq$200 d.p.m.'s), plasma ($\simeq$200 d.p.m.'s) and muscle ($\simeq$500 d.p.m.'s). The uptake of radioactivity in the liver and kidneys, organs primarily involved in the metabolism and excretion of $E_2$, was 600 and 1000 d.p.m.'s per gm of tissue, respectively.

The SDGC patterns of radioactivity obtained from the caudal and cranial lobe cytosols (Fig. 1) revealed bound radioactivity having sedimentation coefficients of 4.2 *S*. A definite peak is seen in the caudal lobe, whereas in the profile obtained with the cytosol prepared from the cranial lobe of the same prostate, only a shoulder of bound radioactivity is identified. The difference between the caudal and cranial peaks under the conditions of this particular experiment is difficult to explain, though we think it may be due to methodological effects and/or some blunting of the cranial peak by the significant amount of non-specific binding (see above paragraph) in this particular preparation. As has been shown previously for other steroids [17–19], the demonstration of such peaks of radioactivity in cytosol is important in the identification of a steroid receptor. In order to precisely identify the labeled ligand, the caudal lobe cytoplasmic fraction containing the peak was extracted and the steroids in it identified using paper chromatography and isotope dilution techniques. The radioactive peak was found to have the same mobility as co-chromatographed $E_2$. An important indication of the specificity of the $E_2$ binding component was the observation that large daily doses (2 mg/kg, s.c.) of T administered for 3 days to a 40 h castrate did not affect significantly the $E_2$ receptor interaction in the cytosol of the caudal lobe. Castration 40 h prior to the infusion of [6,7-$^3$H]-$E_2$ enhanced the resolution (4.2 *S*) and magnitude of the peaks of bound $E_2$ in the cytosols of both prostatic lobes.

### II. In vitro *cytosol competitive binding studies*; *SDGC*

When caudal lobe cytosols from an intact and a 24 h castrate baboon were incubated with [6,7-$^3$H]-$E_2$, we were unable to demonstrate specific binding using SDGC (Fig. 2). However, when cytosol obtained from a 40 h castrate was incubated with [6,7-$^3$H]-$E_2$ and then subjected to SDGC, a peak of bound radioactivity having a sedimentation coefficient of 4.2 *S* was clearly demonstrated (Fig. 2).

Cytosol obtained from the caudal prostate of 40 h castrates was incubated with [6,7-$^3$H]-$E_2$ and various amounts of nafoxidine. The SDGC pattern in Fig. 3 demonstrates the ability of nafoxidine to compete with $E_2$. Nafoxidine competition is dose related and highly effective at the 3.0 $\mu$g level. Estracyt, an agent combining $E_2$ and an alkylating moiety, competed slightly for $E_2$ binding sites and reduced the peak of radioactivity in the caudal lobe cytosol of the baboon castrate by 12%.

### III. In vitro *tissue incubations*; *SG*25

As shown in Table 1, mean uptake of radioactivity by slices of caudal and cranial prostate incubated with [6,7-$^3$H]-$E_2$ was greater at 37°C than at 4°C.

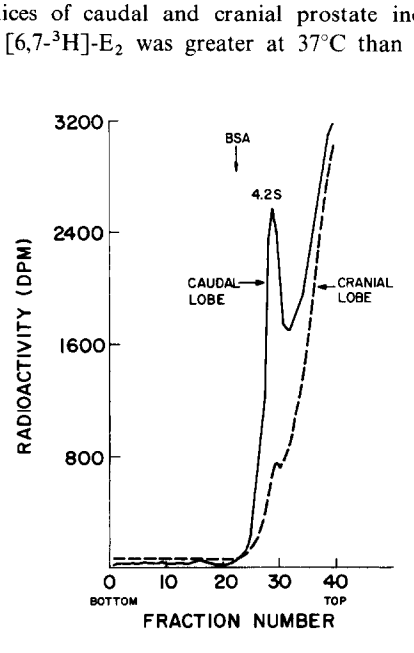


Fig. 1. SDGC of cytosols prepared from the caudal and cranial lobes of the baboon prostate following *in vivo* infusion of [6,7-$^3$H]-$E_2$ into the hypogastric arteries.

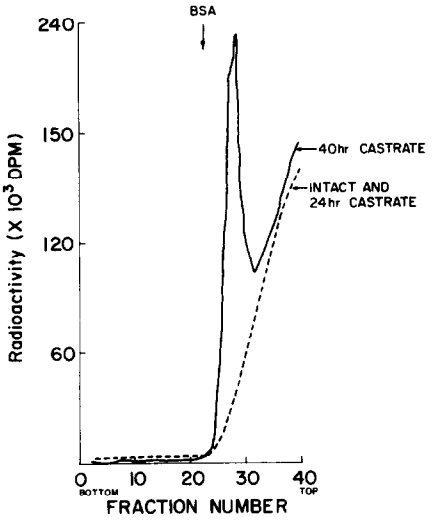


Fig. 2. SDGC of cytosols from the caudal lobes of intact, 24 and 40 h castrated baboons incubated with [6,7-$^3$H]-$E_2$ *in vitro*. A definite peak of bound label is evident with the cytosol from the 40 h castrate, whereas such a peak could not be demonstrated with cytosol of the 24 h castrate or intact baboon. The latter may be due to the inability of [6,7-$^3$H]-$E_2$ to displace endogenous steroid bound to receptor sites, which would be vacant in the cytosol of a 40 h castrate.

There was also a trend toward greater cytosol incorporation of label (d.p.m./mg protein) in the 37°C vs. 4°C incubations in both the caudal ($160 \times 10^3$ vs. $140 \times 10^3$) and cranial ($340 \times 10^3$ vs. $270 \times 10^3$) lobes. SG25 analysis of these cytosols revealed that the bound steroid values of the 4°C and 37°C incubations were similar within separate lobes, but were two times greater in the cranial than in the caudal lobe. These differences carried through to the total radioactivity measured in the 0.4 M KCl nuclear extract, in which bound label was readily identified. The SG25 analyses of the 0.4 M KCl nuclear extract, however, did not reveal any striking differences in the amount of bound radioactivity transferred to the nuclei at 4°C and 37°C in the prostate lobes. In general, however, the various cranial lobe preparations were found to incorporate greater amounts of radioactivity than the caudal lobe. Also, the nuclear extract data given in Table 1 indicate that the concentrations of bound radioactivity in the caudal lobe are similar to those of its cytosol, whereas twice as much bound radioactivity was measured in the cytosol of the cranial lobe than in its nuclear extract. Since these data do not appear to show a temperature dependent nuclear uptake of the bound radioactivity in the 0.4 M KCl extract, subsequent nuclear uptake studies also included analysis of the nuclear residue.

### IV. In vivo *infusion of* [2,4,6,7-$^3H$]-$E_2$; *SG25*

Distribution of radioactivity following arterial infusion of [2,4,6,7-$^3$H]-$E_2$ into intact and 40 h castrate baboons is given in Table 2. The amounts of total and bound radioactivity were greater in the cranial vs. caudal lobe cytosol of the castrated baboon, with a similar trend suggested by the data for the intact baboon prostate cytosol. These data also show that significant amounts of label were concentrated as bound complexes in the 0.4 M KCl nuclear extracts

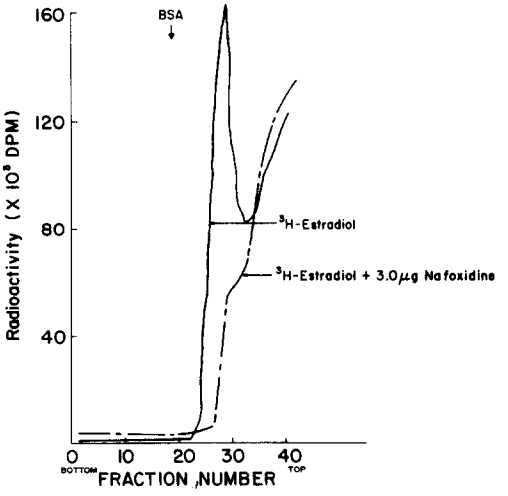


Fig. 3. SDGC of the cytosol from the caudal lobe of a castrated baboon incubated *in vitro* with [6,7-$^3$H]-$E_2$ in the absence and presence of nafoxidine. The curves show definite competition by nafoxidine with [6,7-$^3$H]-$E_2$ for the binding sites of the cytosol.

Table 1. Mean (±S.D.) distribution of radioactivity in the baboon ($n = 3$) prostate following *in vitro* incubations of whole tissue with [6,7-$^3$H]-estradiol-17$\beta$

| | Caudal | | Cranial | |
|---|---|---|---|---|
| | 4°C | 37°C | 4°C | 37°C |
| Tissue uptake d.p.m. ($\times 10^6$)/g wet tissue | 11 ± 0.6 | 15 ± 2.8 | 21 ± 1.6 | 30 + 2.0 |
| Total cytosol radioactivity d.p.m. ($\times 10^3$)/mg protein | 140 ± 7.6 | 160 ± 8.2 | 270 ± 17.3 | 340 ± 19.7 |
| Cytosol bound radioactivity d.p.m./mg protein | 2600 ± 161 | 2700 ± 496 | 7000 ± 1924 | 6200 ± 1189 |
| 0.4 M KCl Nuclear extract total d.p.m. ($\times 10^3$)/mg protein | 61 ± 18.5 | 35 ± 7.1 | 136 ± 33.6 | 135 ± 29.8 |
| 0.4 M KCl Nuclear extract bound d.p.m./mg protein | 3300 ± 1940 | 2700 ± 1788 | 2800 ± 1512 | 3500 ± 644 |

Table 2. Distribution of radioactivity in caudal and cranial lobes of the baboon prostate following arterial infusion with [2,4,6,7-$^3$H]-estradiol-17$\beta$

| | Intact* | | Castrated† | |
|---|---|---|---|---|
| | Caudal | Cranial | Caudal | Cranial |
| Tissue weight—g | 4.5 | 2.7 | 7.4 | 4.1 |
| Tissue uptake d.p.m. ($\times 10^3$)/g wet tissue | 620 | 480 | 140 | 395 |
| Total tissue uptake d.p.m. ($\times 10^3$) | 2,790 | 1,296 | 1,036 | 1,619 |
| Total cytosol radioactivity d.p.m./mg protein | 1,670 | 2,040 | 1,430 | 2,590 |
| Cytosol bound radioactivity d.p.m./mg protein | 680 | 910 | 465 | 1,940 |
| 0.4 M KCl Nuclear extract total d.p.m./mg protein | 2,334 | 1,649 | 3,980 | 3,410 |
| 0.4 M KCl Nuclear extract bound d.p.m./mg protein | 1,650 | 1,080 | 470 | 1,750 |
| 0.4 M KCl Nuclear residue d.p.m./g wet weight | 7,156 | 8,378 | 2,092 | 4,802 |

* One h infusion ($n = 1$).
† Two h infusion ($n = 1$).

Table 3. Radioactivity associated with baboon ($n = 1$) prostatic cytosol and nuclear preparations after incubating cytosol with [$^3$H]-$E_2$ and then with nuclei

| | Caudal | | Cranial | |
|---|---|---|---|---|
| | 0°C | 37°C | 0°C | 37°C |
| Cytosol volume (ml) | 4.7 | 4.7 | 6.3 | 6.3 |
| d.p.m. ($\times 10^6$) Incubated | 10.0 | 10.0 | 12.0 | 12.0 |
| Cytosol—pre-incubation with nuclei d.p.m. ($\times 10^5$)/mg protein | 3.1 | 3.4 | 2.3 | 2.0 |
| Cytosol—post-incubation with nuclei d.p.m. ($\times 10^5$)/mg protein | 2.7 | 2.8 | 2.0 | 1.7 |
| Cytosol bound radioactivity post-incubation d.p.m. ($\times 10^3$)/mg protein | 4.9 | 4.6 | 3.3 | 3.7 |
| 0.4 M KCl nuclear extract total d.p.m. ($\times 10^5$)/mg protein | 2.0 | 4.0 | 4.8 | 4.1 |
| 0.4 M KCl Nuclear extract—(SG25) bound d.p.m. ($\times 10^3$)/mg protein | 2.4 | 1.3 | 3.0 | 2.8 |
| Nuclear residue d.p.m. ($\times 10^5$)/mg protein | 2.0 | 3.1 | 3.6 | 7.7 |

of both the caudal and cranial lobes of castrate and intact baboons. The amounts of bound radioactivity concentrated in the nuclear extracts and cytosols were similar, except in the case of the caudal lobe of the intact baboon, where over twice as much was concentrated in the nuclear extract. Moreover, significantly greater amounts of total radioactivity were concentrated in the nuclear residues. These data indicate that bound radioactivity associated with $E_2$ had been transferred from the cytosol to the nucleus in both lobes of the prostate.

V. In Vitro *nuclear uptake studies*; *SG25*

Results of the cytosol-nuclei recombination experiments are presented in Table 3. Samples (0.1 ml aliquots) of the cytosols were analysed before and after incubation with the nuclei. A decrease of approximately 15% in cytosol radioactivity was measured in the 0°C and 37°C incubations for both lobes, indicating nuclear uptake of label at both temperatures. SG25 analyses showed that bound radioactivity was present in the cytosols after incubation with nuclei. 0.4 M KCl nuclear extract contained, in all cases, bound radioactivity, and the nuclear residue contained radioactivity which was resistant to 0.4 M KCl extraction. Oxidation of the residues from the 37°C incubations produced tritiated water in which greater amounts of radioactivity were concentrated than in the residues from the 0°C incubations, suggesting that a temperature dependence of translocation of steroid–receptor complex is more readily ascertained with analysis of nuclear residue than with 0.4 M KCl nuclear extract preparations. Again, as was shown with the *in vivo* infusions (Table 2), significantly greater amounts of radioactivity were associated with the nuclear residue than with the nuclear extract.

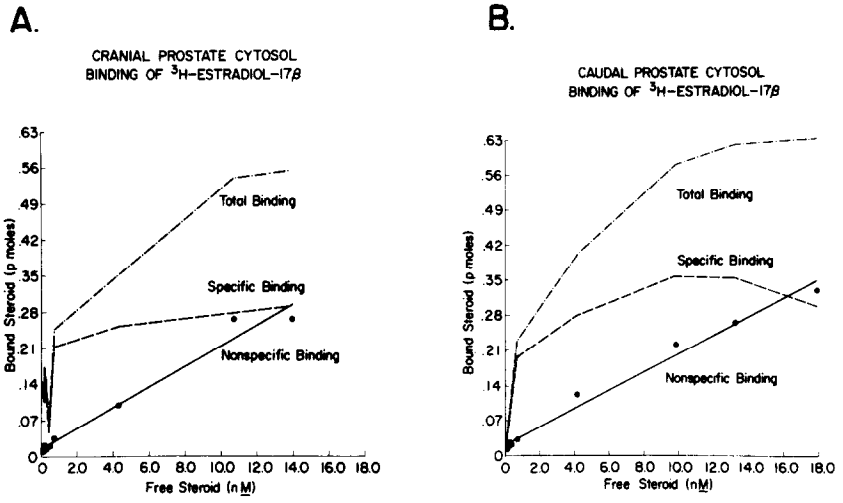


Fig. 4. Estradiol binding to specific and non-specific sites in the cranial (A) and caudal (B) lobe cytosols of the baboon prostate.

VI. In vitro *specificity and affinity studies; SG25 analysis*

Differentiation of [$^3$H]-$E_2$ binding to specific and non-specific prostatic cytoplasmic sites in the caudal and cranial prostate is shown in Fig. 4, where the quantity of specifically bound steroid equals the difference between non-specific and total binding. These data clearly show the linearity of the non-specific binding and the saturability of total and specific binding of $E_2$ in both lobes of the prostate. These same data were used to produce the Scatchard plots shown in Figs 5 and 6. Caudal and cranial lobe dissociation constants were calculated to be $8.3 \times 10^{-10}$ M and $1.3 \times 10^{-10}$ M, respectively. The number of high affinity $E_2$ binding sites for the caudal and cranial lobes, as determined by the X intercept of the Scatchard plots were 265 fmol and 168 fmol per mg of cytosol protein, respectively. Double reciprocal plots of the binding data (ratio of 1/bound labeled $E_2$ as a function of 1/free labeled $E_2$) for the caudal and cranial lobes had high correlation coefficients (0.99 and 0.97) and from these plots the association constants were calculated to be $1.2 \times 10^9$ M and $7.7 \times 10^9$ M, respectively.

The specificity of the prostate cytosol receptor labeled with [$^3$H]-$E_2$ was demonstrated by the addition of a 100-fold molar excess of unlabeled $E_2$ or DES, which reduced the amount of labeled complexes to 56% and 55% in the caudal lobe, respectively; corresponding values for the cranial lobe of 44 and 46% indicated even greater competition (Table 4). The

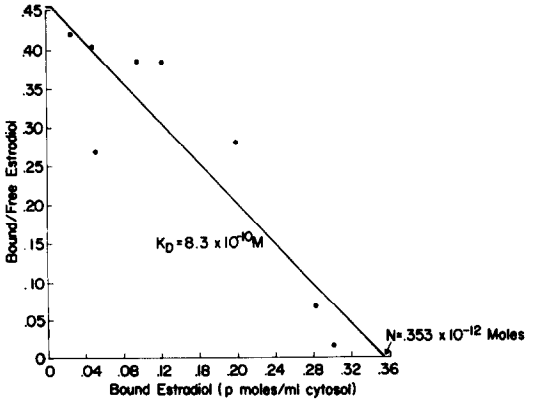


Fig. 5. Scatchard plot of estradiol binding data from baboon caudal prostate cytosol showing the ratio bound/free labeled $E_2$ as a function of the amount of bound labeled $E_2$. The straight line ($r = 0.90$) indicates a single class of binding sites ($N = 265$ fmol per mg protein) and an apparent $K_D$ value of $8.3 \times 10^{-10}$ M. Best fit of the line through the points was determined by linear regression analysis.

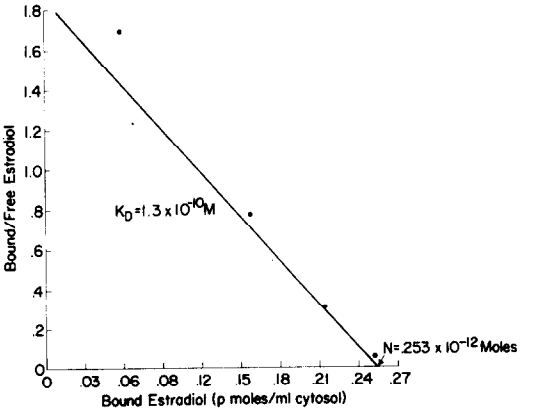


Fig. 6. Scatchard plot of $E_2$ binding data from baboon cranial prostate cytosol showing the ratio bound/free labeled $E_2$ as a function of the amount of bound labeled $E_2$. The straight line ($r = 0.92$) indicates a single class of binding sites ($N = 168$ fmol per mg protein) and an apparent $K_D$ value of $1.3 \times 10^{-10}$ M. Best fit of the line through the points was determined by linear regression analysis.

Table 4. *In vitro* competition of various unlabeled steroids with [$^3$H]-estradiol for cytosol estrogen receptors in the baboon ($n$ = 1) prostate

| Steroid added | Molar ratio to estradiol | Caudal | | Cranial | |
|---|---|---|---|---|---|
| | | $^3$H-estradiol (bound d.p.m./mg protein) | % of control | $^3$H-estradiol (bound d.p.m./mg protein) | % of control |
| [$^3$H]-estradiol—control | — | 3036 | 100 | 2695 | 100 |
| 17$\beta$-estradiol ($E_2$) | 100 | 1705 | 56 | 1177 | 44 |
| | 10 | 2295 | 76 | 1567 | 58 |
| | 1 | 2465 | 81 | 2141 | 79 |
| Diethylstilbestrol | 100 | 1678 | 55 | 1245 | 46 |
| (DES) | 10 | 2443 | 80 | 1628 | 60 |
| | 1 | 2624 | 86 | 2254 | 84 |
| Testosterone (T) | 100 | 3168 | 100 | 2715 | 100 |
| | 10 | 2934 | 97 | 2692 | 100 |
| | 1 | 2957 | 97 | 2610 | 97 |
| Dihydrotestosterone | 100 | 3081 | 100 | 2702 | 100 |
| (DHT) | 10 | 3168 | 100 | 2775 | 100 |
| | 1 | 3063 | 100 | 2704 | 100 |

specificity of the receptor was further demonstrated by competitive experiments in which T and DHT did not affect labeled $E_2$ binding.

## DISCUSSION

The present study demonstrates cytosol $E_2$-binding molecules in the caudal and cranial lobes of the baboon prostate and their interaction with the nuclei of the gland. The specificity of the $E_2$ binding component is indicated by the inability of T and DHT to compete with labeled $E_2$ for specific binding sites in the cytosol. On the other hand, the labeled $E_2$ was readily displaced by carrier $E_2$ and DES, thus indicating the affinity of the $E_2$ binding molecules for those substances which are thought to compete with labeled $E_2$ on specific binding sites. Furthermore, nafoxidine, which is a nonsteroidal antiestrogen that has been shown to compete with $E_2$ for binding sites on specific receptors [17, 18], also displaced labeled $E_2$.

Since $E_2$ undergoes extensive *in vivo* metabolism, primarily by the liver [20], it was possible that the radioactivity in the 4.2 *S* peak of the prostatic cytosol represented a receptor complex associated not necessarily with the infused labeled $E_2$, but with any of its metabolites. However, we were able to show that the steroid associated with the binding protein *in vivo* was almost exclusively $E_2$ rather than any labeled metabolite such as estrone, estriol or one of their conjugates.

Since neither excess T nor DHT was able to significantly displace bound $E_2$ would suggest that $E_2$ was not binding to an androgen receptor. Indeed, it has been recently reported that $E_2$ and androgen binding do not occur at the same receptor site in the rat ventral prostate [21]. In addition, if such binding were due to testosterone-estradiol binding globulin (TeBG), one would predict some displacement by the androgens tested. In contrast, both $E_2$ and DES were successful in displacing bound labeled $E_2$ in both caudal and cranial lobe cytosols. It has also been shown that DES does not displace $E_2$ bound to TeBG [22]. In recent studies in which we have demonstrated TeBG in serum of male baboons, DES did not compete with $E_2$ or DHT binding to TeBG (Karr, J. P. *et al.*, unpublished data). Similarly, $E_2$ binds to a plasma protein in the pregnant rat uterus, whereas DES does not [23]. This is not the case with $E_2$ bound to cellular receptors, e.g. in breast tumors or to the binding sites in the present studies, in which DES readily displaced $E_2$.

The results of the present study point to the presence of specific and saturable estrogen binders in the caudal and cranial lobe cytosols. Moreover, the physical constants presented, which to our knowledge are the first to be reported for the baboon prostate, reflect the high affinity nature of these binders. The $K_D$'s we determined for the caudal and cranial lobes are similar to those reported for other $E_2$ receptors [24, 25].

Nuclear translocation of $E_2$–receptor complex has been shown to occur in $E_2$ sensitive tissues [17, 18, 24]. We also measured bound radioactivity in the 0.4 M KCl nuclear extracts. Even though quantitative differences in the translocation of bound 0.4 M KCl extractable radioactivity at 0–4°C and 37°C were not significant, it is important to note that the nuclear fraction resistant to 0.4 M KCl extraction concentrated greater amounts of radioactivity at 37°C. Other investigators have also described insoluble, specific nuclear binding components of high affinity for estrogen and other steroids [25–29]. The possibility that the non-extractable radioactivity represents more tightly bound $E_2$ receptors, which may actually regulate gene activity, has yet to be determined. Furthermore, it is not known whether, in the human, the $E_2$–receptor complex produces its effects through translational [30, 31] or transcriptional effects [17, 18]. To serve this end, the baboon will continue to be an important model.

In conclusion, we have demonstrated estrogen binding molecules in the caudal and cranial lobes of the baboon prostate which satisfy several essential characteristics of a true receptor. These criteria include hormone specificity, high affinity and saturability at low steroid concentrations. In addition, both lobes of the prostate concentrate large amounts of the $E_2$ *in vivo*. Radioactivity associated with labeled $E_2$ was translocated from the cytosol to nucleus, where it was partly identified in a bound state in the 0.4 M KCl extractable fraction of the nuclei. However, labeled $E_2$ was primarily transferred to insoluble nuclear residue and showed some temperature-dependent nuclear uptake.

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