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# High-Affinity Binding of Oestradiol-17β by Cytosols from Testis Interstitial Tissue, Pituitary, Adrenal, Liver and Accessory Sex Glands of the Male Rat

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The specificity of the binding of oestradiol-17β by cytoplasmic fractions of several tissues of the male rat was investigated. 1. Agar-gel electrophoresis, Sephadex chromatography, adsorption by dextran-coated charcoal and sucrose-gradient centrifugation were used to estimate the binding capacity and specificity. The four different methods all gave similar results for the capacity of the specific oestradiol-17β-binding macromolecules in the testis. 2. The presence of a specific saturable binding protein with a sedimentation coefficient of 8S was demonstrated in liver, adrenal, pituitary, prostate, epididymis and testis interstitial tissue. The highest concentration of oestradiol-17β-binding macromolecules was found in testis interstitial tissue (0.12pmol/mg of protein) and in the pituitary (0.075pmol/mg of protein). 3. The oestradiol-17β receptor in the testis cytosol showed the characteristics of a protein with respect to Pronase treatment and temperature sensitivity. In competition experiments with different steroids the receptor showed a high affinity for oestradiol-17β, a moderate affinity for diethylstilboestrol and oestradiol-17α and a low affinity for oestrone, oestriol, testosterone and 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one). 4. The wide distribution of oestradiol-17β receptors in the male rat is in apparent contradiction to the current concept of the specificity of steroid-hormone action. Further research is required to investigate a possible physiological meaning of the presence of specific receptors in the different tissues.

The action of sex steroid hormones on target tissues may be mediated through specific receptors. The presence of an oestradiol-17β receptor has previously been demonstrated in the interstitial tissue of the rat testis (Brinkmann *et al.*, 1972). A nuclear form of receptor has also been found and it appears that the cytoplasmic receptor can be transferred to the nuclei of testis interstitial tissue (Mulder *et al.*, 1973).

Baulieu *et al.* (1971) formulated the criteria for a true steroid receptor, i.e. a receptor should show high affinity, saturability, steroid specificity and tissue specificity. It was previously shown that the cytoplasmic oestradiol-17β receptor in testis interstitial tissue has a high affinity for oestradiol-17β ($K_a$ is $10^{10}$ M$^{-1}$) and that only a limited number of binding sites are present (Brinkmann *et al.*, 1972). In the present paper we present the results of an investigation on tissue specificity and steroid specificity of cytoplasmic oestradiol-17β binding.

## Materials and Methods

### Materials

[2,4,6,7-$^3$H]Oestradiol-17β (specific radioactivity 105Ci/mmol) was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.). The radiochemical purity was verified by paper chromatography and t.l.c.

Deoxyribonuclease, ribonuclease and Pronase were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

### Preparation of subcellular fraction and incubation procedures

Male Wistar rats (200–250g) were killed by decapitation. The isolated tissues were homogenized in 1vol. of 10mM-Tris–HCl buffer, pH7.4, at 0°C with three strokes of a Potter–Elvehjem homogenizer at 1100rev./min. The homogenate was centrifuged at 105000*g* for 60min at 0°C. The 105000*g* supernatant (cytosol) was incubated with steroid for 2h at 0°C.

Isolated interstitial tissue and seminiferous tubules were obtained by wet dissection of decapsulated whole testis tissue (Christensen & Mason, 1965).

### Measurement of steroid binding

When cytosol is incubated with oestradiol-17β the steroid may bind to specific and non-specific

binding proteins. The cytosol was incubated with [$^3$H]oestradiol-17$\beta$ to determine total binding and with [$^3$H]oestradiol-17$\beta$ plus a 100-fold excess of unlabelled oestradiol-17$\beta$ to determine non-specific binding (Williams & Gorski, 1973). The quantity of specifically bound hormone was calculated by subtracting the value for [$^3$H]oestradiol-17$\beta$ bound in the presence of a 100-fold excess of unlabelled oestradiol-17$\beta$ (non-specifically bound oestradiol-17$\beta$) from the value for total [$^3$H]oestradiol-17$\beta$ binding.

After incubation of cytosol with steroids bound and unbound steroid were separated by one of the following techniques. (In control experiments buffer with labelled steroid was used instead of cytosol.)

(*a*) *Gradient centrifugation.* After incubation with steroid 200$\mu$l of cytosol was layered on 5ml of a 5–20% (w/v) sucrose gradient prepared in 10mM-Tris–HCl buffer, pH7.4. After centrifugation in a Beckman L2-65B centrifuge at 0°C for 16h at 150000$g_{av.}$ in a SW65 rotor the bottom of the tube was pierced and 30 fractions were collected. Radioactivity was measured in each fraction.

(*b*) *Dextran-coated charcoal adsorption method.* A 0.25% charcoal suspension (200$\mu$l), containing 0.025% dextran, was added to 100$\mu$l of cytosol after incubation with steroid. After mixing the suspension was kept at 0°C for 15min. The samples were then centrifuged for 10min at 1200$g$ to separate bound from free steroid. A 200$\mu$l portion of the supernatant was taken for measurement of radioactivity in the bound-steroid fraction.

(*c*) *Agar-gel electrophoresis.* Agar-gel electrophoresis was performed essentially as described by Wagner 1972). A 50$\mu$l portion of incubated cytosol was layered on an agar plate (100mm×85mm×5mm thick) kept at 0°C (agar Noble; Difco, Detroit, Mich., U.S.A.). It was possible to apply ten samples on one plate. After electrophoresis for 90min at 130mA per plate (200–250V) at 0°C, the plate was cut into ten strips, each containing one sample, and each strip was divided in 20 fractions of 4mm. For counting of radioactivity, steroid from the individual agar fractions was dissolved by shaking for 12h at room temperature in 10ml of Triton-containing scintillation fluid (see under 'Measurement of radioactivity').

(*d*) *Sephadex chromatography.* Sephadex chromatography was performed as described by Williams & Gorski (1973). A 50$\mu$l portion of incubated cytosol was layered on a column (8cm×0.6cm) of Sephadex G-25 (superfine grade). The column was eluted with 10mM-Tris–HCl buffer, pH7.4, at 0°C and the excluded volume (bound radioactivity) was collected in a vial and radioactivity was measured.

### *Pretreatment with charcoal*

When indicated excess of unbound steroid was removed by adding 0.5mg of dextran-coated charcoal to 200$\mu$l of incubated cytosol. After mixing, the suspensions were incubated for 15min at 0°C. Charcoal was removed by centrifugation for 10min at 1200$g$.

### *Protein determination*

The protein content of the isolated cytosols was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Generally the cytosols contained 20–25mg of protein/ml. Cytosols of interstitial tissue, pituitary, hypothalamus and uterus, however, contained 4–10mg/ml of cytosol.

### *DNA determination*

DNA content was measured as described by Giles & Myers (1965).

### *Measurement of radioactivity*

Radioactivity was measured in a Packard model 3375 liquid-scintillation spectrometer. The scintillation fluid consisted of a mixture of Triton X-100 (Rohm and Haas, Philadelphia, Pa., U.S.A.) and toluene (1:2, v/v) containing 0.1g of POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene]/l and 4.8g of PPO (2,5-diphenyloxazole)/l (Packard Instrument S.A. Benelux, Brussels, Belgium).

## Results

### *Properties of the oestradiol-17$\beta$ receptor in the testis cytosol*

*Steroid specificity of the oestradiol-17$\beta$ receptor in the testis.* Relative affinities of a number of steroids for the oestradiol-17$\beta$ receptor in the testis are presented in Fig. 1. These experiments were carried out by

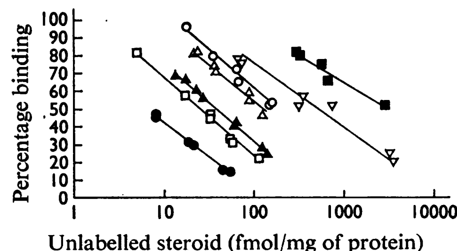


Fig. 1. *Binding affinity of various steroids for the testis cytosol oestradiol-17$\beta$ receptor*

Testis cytosol was incubated with 0.07nM-[$^3$H]oestradiol-17$\beta$ and increasing amounts of unlabelled steroids. After incubation (2h) the percentage binding was measured by the charcoal technique. ●, Oestradiol-17$\beta$; □, diethylstilboestrol; ▲, oestradiol-17$\alpha$; △, oestrone; ○, oestriol; ▽, 5$\alpha$-dihydrotestosterone; ■, testosterone.

Table 1. *Comparative binding affinity of various steroids for the testis cytosol oestradiol-17β receptor*

Testis cytosol was incubated with 0.07 nM-[$^3$H]oestradiol-17β and increasing amounts of unlabelled steroids. Percentage binding was measured by the charcoal technique. Comparative binding affinity was calculated as the amount of steroid that will decrease the initial percentage binding of the labelled oestradiol-17β to 50%.

| Steroid | Comparative binding affinities (fmol/mg of protein) |
|---|---|
| Oestradiol-17β | 6.3 |
| Diethylstilboestrol | 25 |
| Oestradiol-17α | 38 |
| Oestrone | 127 |
| Oestriol | 190 |
| Dihydrotestosterone | 500 |
| Testosterone | 3000 |

incubating 200 μl of cytosol with 3500 d.p.m. (0.07 nM of [$^3$H]oestradiol-17β and increasing amounts of the different unlabelled steroids. The percentage binding was measured by using the charcoal technique. In the competition experiments with testosterone and dihydrotestosterone it was necessary to decrease the concentration of endogenous steroids by hypophysectomy of the animals 8 days before the experiment. Comparative binding affinities were determined by the method of Korenman (1969). The results are given in Table 1. The highest competition for the binding sites was achieved with oestradiol-17β, diethylstilboestrol and oestradiol-17α. The decrease in percentage binding after incubation with increasing amounts of unlabelled oestradiol-17β was the same whether the testis cytosol was prepared from normal or hypophysectomized animals.

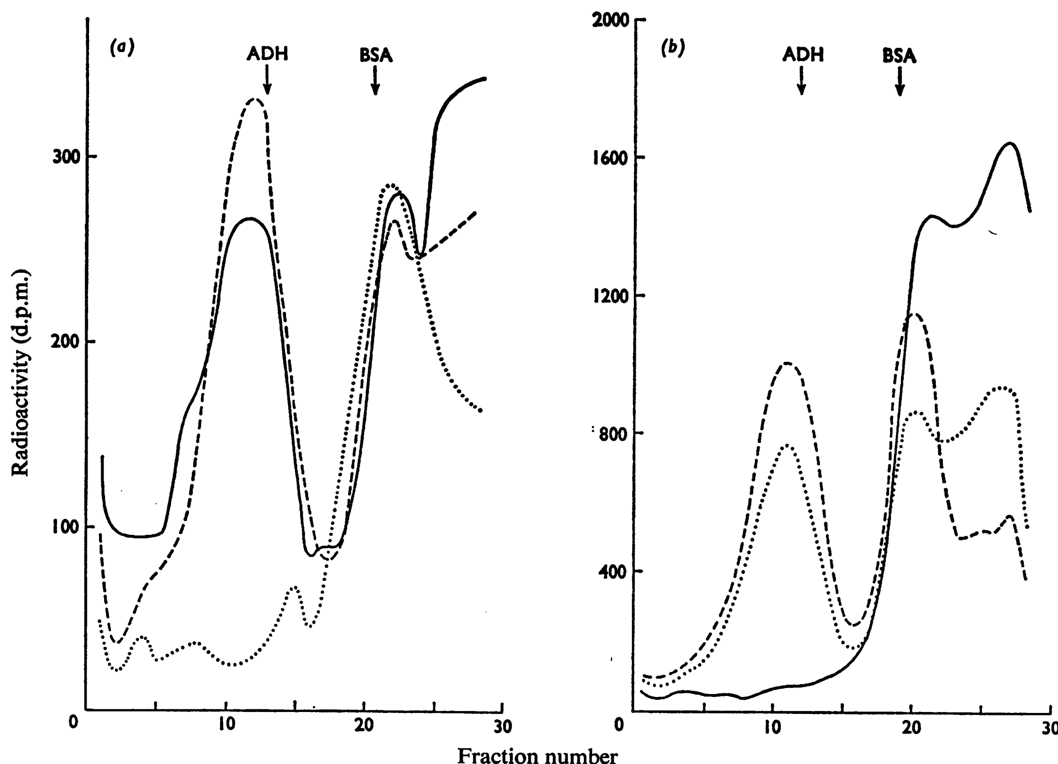


Fig. 2. *Effect of enzyme digestion and preincubation at different temperatures on oestradiol-17β binding by cytosol of rat testis*

(*a*) Testis cytosol was preincubated with 2 μg of Pronase, 1 mg of deoxyribonuclease or ribonuclease for 30 min at 12°C. After 2 h incubation with 0.08 nM-[$^3$H]oestradiol-17β a 200 μl portion was layered on a sucrose gradient. Alcohol dehydrogenase (ADH, 7.4 S) and bovine serum albumin (BSA, 4.6 S) were used as sedimentation markers. —— Ribonuclease treatment; ----, deoxyribonuclease treatment; ····, Pronase treatment. (*b*) Sucrose-gradient analysis was performed after labelling *in vitro* of testicular cytosols preincubated at 0°C, 30°C and 37°C for 30 min. ——, Preincubation at 37°C; ····, preincubation at 30°C; ----, preincubation at 0°C.

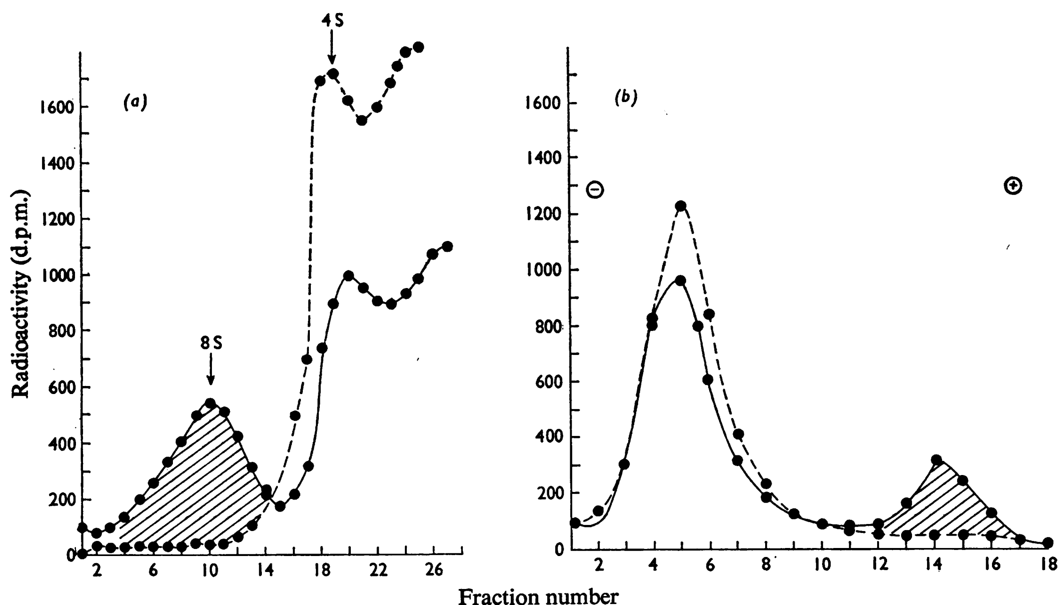


Fig. 3. *Gradient centrifugation and agar-gel electrophoresis of testis cytosol labelled with [$^3$H]oestradiol-17β*

Testicular cytosol was incubated with 0.4nM-[$^3$H]oestradiol-17β (●——●) or with 0.4nM-[$^3$H]oestradiol-17β plus 0.04μM-unlabelled oestradiol-17β (●----●). After incubation binding was determined by gradient centrifugation (*a*) or agar-gel electrophoresis (*b*). Shading indicates specific binding. Alcohol dehydrogenase and bovine serum albumin were run in a parallel gradient to determine the sedimentation values.

*Protein character of the oestradiol-17β receptor in the testis.* Testis cytosol (1.0ml) was preincubated with Pronase, deoxyribonuclease or ribonuclease. It was shown by gradient centrifugation that the 8S receptor was absent only after Pronase treatment, as shown in Fig. 2(*a*). Preincubation of cytosols at 30° and 37°C affected the amount of receptor-bound steroid. After preincubation at 30°C a decrease in steroid bound to 8S receptor was observed and after preincubation at 37°C no 8S steroid–receptor complex was found (Fig. 2*b*).

*Comparison of different techniques for quantitative analysis of hormone binding*

In four series of experiments cytosol of total testis tissue was incubated with different amounts of labelled oestradiol-17β ranging from 0.3 to 3.9nM. Analysis of oestradiol-17β binding by agar-gel electrophoresis and gradient centrifugation is illustrated in Fig. 3. Non-specific and specific binding were calculated as described in the Materials and Methods section.

The results are presented in Table 2. When the results for each concentration were compared by the rank-correlation test of Wilcoxon (1945) it appeared that the values for non-specific binding calculated from the gradient-centrifugation curves were significantly lower ($P = 0.05$) than the values obtained with the other techniques. Highest values for non-specific binding were calculated from the results obtained with the charcoal techniques ($P < 0.05$).

The apparent increase in non-specific binding with increasing amounts of oestradiol-17β was not caused by an incomplete separation of free and bound oestradiol-17β. When 4nM-[$^3$H]oestradiol-17β was incubated with buffer only, none of the four techniques showed the presence of radioactivity in the fractions which normally contain the macromolecular bound oestradiol-17β. A comparison by using Wilcoxon's (1945) test for the values of the amount of specifically bound oestradiol-17β showed no significant difference between the results obtained with the four different techniques. This may reflect that after sucrose-gradient centrifugation the specifically bound oestradiol-17β is completely present in the 8S area, which was used for estimation of specific binding. In all further experiments agar-gel electrophoresis and Sephadex chromatography were used for quantitative analysis of specific binding. Sucrose-gradient analysis was not used because in tissues other than testis, specific binding proteins might

Table 2. *Analysis by different techniques of oestradiol-17β binding by testis cytosol*

Testis cytosols from different rats were incubated in four series of experiments with increasing amounts of labelled oestradiol-17β (0.3–3.9nM). Non-specific binding and specific binding were determined by using agar-gel electrophoresis, the charcoal technique, Sephadex chromatography and sucrose-gradient centrifugation. Determinations of non-specific and specific binding after incubation with [$^3$H]oestradiol-17β are presented.

| Expt. no. | Concn. of [$^3$H]-oestradiol (nM) | Non-specific binding (fmol/mg of protein) | | | | Specific binding (fmol/mg of protein) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Agar | Charcoal | Sephadex | Gradient | Agar | Charcoal | Sephadex | Gradient |
| I | 1.4 | 1.8 | 5.0 | 1.9 | — | 6.4 | 8.0 | 6.0 | — |
| | 3.8 | 5.0 | 11.0 | 2.9 | — | 7.7 | 7.8 | 9.0 | — |
| II | 0.3 | 1.2 | 0.9 | 0.7 | 0.6 | 2.9 | 2.9 | 2.9 | 4.8 |
| | 1.6 | 3.5 | 3.7 | 2.1 | 1.4 | 6.2 | 5.4 | 5.6 | 6.3 |
| | 3.9 | 4.5 | 10.0 | 3.0 | 2.1 | 8.4 | 7.0 | 7.4 | 5.7 |
| III | 0.4 | 1.7 | 1.6 | 0.8 | 1.3 | 6.1 | 6.9 | 5.7 | 5.8 |
| | 1.5 | 3.7 | 4.7 | 3.5 | 1.5 | 12.2 | 14.3 | 15.2 | 14.2 |
| | 3.0 | 6.6 | 10.0 | 9.0 | — | 12.4 | 13.7 | 11.0 | — |
| IV | 2.2 | 4.3 | 15.1 | — | 3.9 | 9.3 | 10.2 | — | 8.3 |

Table 3. *Specific binding of oestradiol-17β by different tissues of the male rat*

Cytosols of different tissues of the male rat were incubated with 4nM-[$^3$H]oestradiol-17β and with 4nM-labelled plus 0.4μM-unlabelled oestradiol-17β. Specific binding was determined by both agar-gel electrophoresis and Sephadex chromatography. Specific binding is expressed as fmol/mg of protein of the cytosol and as fmol/mg of DNA of the homogenate. Each value is the mean of at least two determinations. The presence or absence of a binding protein with a sedimentation value of 8S is indicated with a plus or minus mark. For the sake of comparison the value of the specific binding in female rat uterus is also included.

| Tissue | Specific binding (fmol/mg of protein) | | | Specific binding (fmol/mg of DNA) | | | 8S |
|---|---|---|---|---|---|---|---|
| | Agar gel | | Sephadex | Agar gel | | Sephadex | |
| Liver | 2.3 | – | 2.1 | 32.2 | – | 29.4 | + |
| Kidney | 1.4 | – | 20.0 | 6.6 | – | 94.0 | – |
| Adrenal | 22.2 | – | — | 121.3 | – | — | + |
| Plasma | <0.5 | – | <0.5 | <2.5 | – | <2.5 | – |
| Skeletal muscle | <0.5 | – | <0.5 | <2.5 | – | <2.5 | – |
| Pituitary | 75.6 | – | 75.6 | 68.7 | – | 68.7 | + |
| Hypothalamus | 2.6 | – | 1.5 | 13.8 | – | 8.0 | – |
| Prostate* | 11.4 | – | 8.5 | 69.1 | – | 51.5 | + |
| Epididymis | 8.7 | – | 7.9 | 46.1 | – | 41.9 | + |
| Seminal vesicle | 0.1 | – | 0.7 | 0.7 | – | 4.9 | – |
| Total testis tissue | 9.8 | – | 9.6 | 35.2 | – | 34.6 | + |
| Seminiferous tubules | <0.5 | – | <0.5 | <2.5 | – | <2.5 | – |
| Testis interstitial tissue | 140 | – | 100 | 518 | – | 370 | + |
| Uterus | 240 | – | — | — | – | — | + |

* The 4S peak also contained specifically bound oestradiol-17β.

occur in the 4S area of the gradients and the separation between bound steroid in the 4S area and free steroid on top of the gradient is less accurate. Also the gradient centrifugation is more time-consuming. With the charcoal technique the high percentage of non-specific binding makes the estimation of specific binding less accurate, particularly in tissues containing a low amount of specific binding proteins.

*Occurrence of oestradiol-17β receptors in different tissues of the male rat*

Cytosols of 13 different tissues of the male rat were incubated with 4nM-[$^3$H]oestradiol-17β. Specific binding was determined by using both agar-gel electrophoresis and Sephadex chromatography. The presence of an 8S receptor in each tissue was deter-

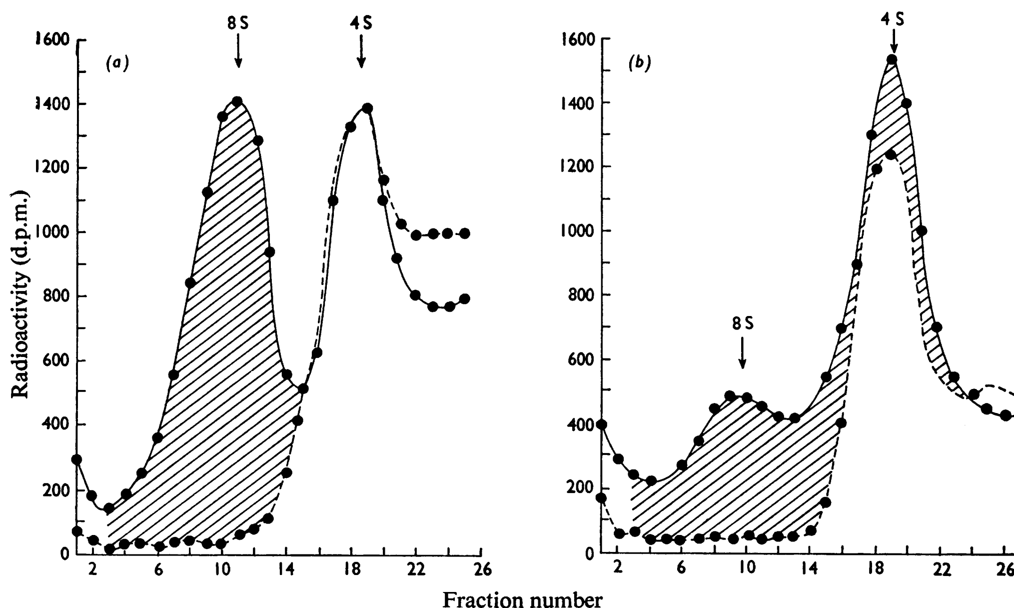


Fig. 4. *Sucrose-gradient analysis of oestradiol-17β binding in cytosol of testis and prostate*

Cytosols of testis (*a*) and prostate (*b*) were incubated with 2nM-[$^{3}$H]oestradiol-17β (●——●) or with 2nM-labelled plus 0.2μM-unlabelled oestradiol-17β (●----●). After charcoal pretreatment specific binding was determined by gradient centrifugation. Shading indicates specific binding. Sedimentation values have been determined as described in Fig. 2.

mined by sucrose-gradient centrifugation. The results are given in Table 3. The uterus of the female rat was used as a control tissue. All values are the mean of at least two determinations. The results show that a specific binding protein with sedimentation coefficient of 8S was present in liver, adrenal, pituitary, testis interstitial tissue, prostate and epididymis. Such a protein, however, could not be found in plasma, muscle, hypothalamus, seminal vesicle and seminiferous tubules. The amount of specific binding in the uterus was 0.24pmol/mg of protein. This is in agreement with the values obtained by other authors (Truong & Baulieu, 1971; Toft *et al.*, 1967). The largest amount of oestradiol-17β-binding macromolecules in the male rat was found in testis interstitial tissue and in the pituitary. In the experiments with prostate the value for specific binding of oestradiol-17β to receptors in the 8S area obtained after gradient centrifugation was lower than the specific binding determined by Sephadex chromatography. Gradient centrifugation after addition of excess of unlabelled oestradiol-17β to prostate cytosol showed, however, the presence of specific low-capacity oestradiol-17β binding in the 4S area (Fig. 4). In this respect specific receptors for oestradiol-17β in testis cytosol, which occurred only in the 8S area, may be different from receptors in prostate cytosol, which occurred in both the 8S and 4S areas.

In the kidney specific binding was demonstrated by using Sephadex chromatography and the charcoal technique. After gradient centrifugation it appeared that the specific binding was completely located in the 4S peak. However, it was not possible to find any specific binding in the kidney by using agar-gel electrophoresis. A possible explanation could be that there is a labile saturable protein with a 4S sedimentation coefficient.

## Discussion

Various methods can be used for the quantitative measurement of the amount of steroid specifically bound by macromolecules. In the present study the binding of oestradiol-17β by the cytoplasmic receptor in interstitial cells of the testis was analysed by using agar-gel electrophoresis, sucrose-gradient centrifugation, Sephadex chromatography and a charcoal-binding assay. The amounts of receptor-bound steroid found by these four methods did not differ significantly from each other. Jungblut *et al.* (1972) observed a similar agreement between agar-gel electrophoresis and Sephadex chromatography for the analysis of oestradiol-17β binding by cytosols of uterine tissue. However, with gradient centrifugation Jungblut *et al.* (1972) observed a much lower specific binding in uterine tissue. It was established

that all the non-specifically bound steroid dissociates during the agar-gel electrophoresis, whereas in our study some non-specific binding was always present. In uterine tissue the concentration of specific binding proteins is much higher than in testis tissue and therefore small amounts of non-specifically bound steroid might not have been detected. Another reason for the observed difference could be that Jungblut *et al.* (1972) determined specific binding as the difference between the binding before and after heating. In our study the amount of specifically bound steroid was estimated by subtracting the amount of bound steroid after incubation with labelled and unlabelled oestradiol-17β from the amount after incubation of cytosol with labelled hormone only.

The present results, showing some further characteristics of the receptor in the interstitial cells of the testis, established the protein nature and temperature-sensitivity of the oestradiol-17β-binding macromolecule. They were similar to results found for uterus receptor (Toft & Gorski, 1966). Previous studies (Brinkmann *et al.*, 1972) showed that the testis receptor has a high affinity for oestradiol-17β ($K_a$ $10^{10}$M$^{-1}$). The steroid specificity of the receptor was high; oestriol, oestrone, testosterone and dihydrotestosterone showed a low degree of competition for the binding sites, whereas diethylstilboestrol and oestradiol-17α demonstrated relatively high competitive activities.

In the male rat specific binding of oestradiol-17β appears to occur in several tissues (Table 3). In addition to the testis interstitial tissue specific binding was observed in liver, adrenal, pituitary, prostate and epididymis, but not in seminiferous tubules, plasma, kidney, skeletal muscle, seminal vesicle and hypothalamus.

The occurrence of an oestradiol-17β receptor in the adrenal was not observed in other studies (Jungblut *et al.*, 1967; Stumpf, 1969). Chobanian *et al.* (1968), however, showed a high oestradiol-17β uptake in dog adrenal. In the liver of calf and rat no specific uptake of oestradiol-17β could be demonstrated by Jungblut *et al.* (1967) and Stumpf (1969). Rao & Talwar (1969) on the other hand showed retention of oestradiol-17β in the liver of the female rat, which might suggest the presence of an oestradiol-17β receptor. The existence of an oestradiol-17β receptor in the rat epididymis has not previously been reported. The presence of an androgen receptor in the epididymis has been reported (Blaquier & Calandra, 1973), so that the epididymis could be another example of the simultaneous occurrence of different oestrogen and androgen receptors in a single tissue. Jungblut *et al.* (1971) have already demonstrated very clearly the existence of individual androgen and oestrogen receptors in calf prostate and seminal vesicle. We also observed an 8S oestradiol-17β receptor in the prostate. However, in the seminal vesicle we were not able to find any specific oestradiol-17β binding. In the kidney indications were obtained for the existence of a labile saturable protein with a sedimentation coefficient of 4S, that dissociates during agar-gel electrophoresis.

The occurrence of an 8S oestradiol-17β receptor in the pituitary of the female rat is well known (Vertes & King, 1971; Eisenfeld, 1970; Mowles *et al.*, 1971). In the male rat Clark *et al.* (1972) demonstrated a nuclear oestradiol-17β receptor in the pituitary but not in the hypothalamus. This is in agreement with the results of our present study. Oestradiol-17β can be bound by cytosol of the hypothalamus of the ovariectomized female rat (Vertes & King, 1971; Eisenfeld, 1970; Mowles *et al.*, 1971). Oestradiol-17β uptake by the hypothalamus of the castrated male rat was established by radioautography (Attramadal, 1970). Injections of testosterone (Vertes & King, 1971; Tuohimaa & Johansson, 1971) lower the oestradiol-17β binding in the hypothalamus of the female rat. Therefore the endogenous high concentrations of androgens in the male rat could be a possible explanation for the fact that it was impossible to demonstrate a receptor in the hypothalamus. Another explanation might be that the presence of the aromatizing system (Massa *et al.*, 1972) in the hypothalamus of the male rat causes a higher endogenous oestradiol-17β concentration that could mask the presence of an oestradiol-17β receptor.

The physiological meaning of the oestradiol-17β receptors in the different tissues of the male rat is not yet clear. It has been suggested that the presence of specific steroid-binding macromolecules in the cytoplasm is a prerequisite for steroid-hormone action (Jensen & de Sombre, 1972). To what extent the occurrence of a receptor in these different tissues implies a steroid-induced transformation of the cytoplasmic receptor and a transfer of steroid to specific receptor sites on the chromatin in the nucleus remains to be investigated.

Oestradiol-17β is present in the male rat. The concentration in testis tissue is 44.6pg/g of testis (de Jong *et al.*, 1974). It is not known whether this concentration is sufficiently high to initiate any biological action, nor is anything known about the concentration of oestradiol-17β in the other tissues containing specific receptors for this steroid.

Under certain conditions there might be a cooperative effect of several steroids on a target tissue. Palmiter & Haines (1973) have reported the effect of oestradiol-17β, progesterone and dihydrotestosterone and their receptors on protein synthesis in one cell type of the chick oviduct. Apparently all three steroids need to be present for maximal stimulation of the synthesis of some proteins in the oviduct. Hence the possibility may be considered that the oestradiol-17β receptors observed in different tissues of the male rat might have a similar positive or

negative co-operative effect with androgen receptors on induced protein synthesis.

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