# A High-Affinity Oestrogen-Binding Protein in Cockerel Liver Cytosol

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In contrast with several earlier reports, cytosol from cockerel liver contains a significant concentration of a protein that binds oestradiol with high affinity. To demonstrate the activity, certain alterations in the conventional method of preparation of cytosol must be made. Homogenization in sucrose-containing buffer at pH 8.4 in the presence of proteinase inhibitors and rapid fractionation of the cytosol with $(NH_4)_2SO_4$ enables demonstration of a single class of oestradiol-binding sites with a $K_d$ of about 1 nM and specificity only for oestrogens. The concentration is about 300 sites per cell in liver from 2-week-old cockerels. Oestradiol treatment *in vivo* decreases the number of exchangeable cytosol oestradiol-binding sites by about 80% for 1–4 h, after which time it is gradually restored. Gel filtration of the cytosol preparation in the presence of high salt concentrations reveals that most of the oestradiol-binding activity is in high-molecular-weight aggregates, but a mild trypsin treatment generates a specific binding protein with an approximate mol.wt. of 40 000. This protein may be an oestrogen receptor.

The mechanism of oestrogen regulation of gene expression is a fundamental problem which has received considerable attention in recent years. In the conventional model for oestrogen action, the hormone binds with high affinity to a receptor protein in the cytoplasm of the target cell, and the receptor-hormone complex moves to the nucleus, where it initiates specific changes in transcription (Jensen *et al.*, 1974; O'Malley & Means, 1974). The growth response of the mammalian uterus has been a particularly useful model system for the study of receptor properties and regulation (Gorski & Gannon, 1976), and the induction of egg-white proteins in chick oviduct has been a fruitful model for quantification of hormone-induced specific mRNA molecules (Chan & O'Malley, 1976; Schimke *et al.*, 1975). In both of these tissues, however, oestrogen treatment results in massive growth and differentiation. Most of the studies in oviduct are carried out on the secondary response, after prior treatment with large amounts of oestrogen. Oestrogen induction of the synthesis of egg-yolk protein (vitellogenin) by avian or amphibian liver is a model for the study of oestrogen action which has the particular advantage that highly specific transcriptional changes can be measured accurately in both the primary and the secondary responses (Tata, 1976; Deeley *et al.*, 1977; Baker & Shapiro, 1978). We have been studying oestrogen receptors in avian liver with the long-term goal of understanding their role in the regulation of the vitellogenic response (Lazier, 1975, 1978; Lazier & Alford, 1977).

The generally accepted criteria for an oestrogen receptor are that it is present in target tissues in a limited number of sites, that it exhibits hormone-specific, high-affinity binding, and that its activity and intracellular distribution are consistent with the hormonal status of the animal (King & Mainwaring, 1974). Liver nuclei from oestrogen-treated immature chickens, roosters or laying hens contain a substantial concentration of specific, high-affinity oestradiol-binding activity generally considered to be 'receptor' (Mester & Baulieu, 1972; Gschwendt & Kittstein, 1974; Lazier, 1975; Joss *et al.*, 1976; Lebeau *et al.*, 1977). The prediction that such oestrogen-receptor sites would be found in the liver cytosol of untreated immature chicks or roosters could not be substantiated by using conventional techniques (Gschwendt, 1975a; Lazier & Alford, 1977; Lebeau *et al.*, 1977). However, Gschwendt (1977) has characterized a high-affinity oestrogen-binding protein in embryonic-chicken liver cytosol. Here we report conditions that allow detection of a receptor-like oestrogen-binding activity in a cytosol fraction from cockerel liver. Some of these data have been reported in a preliminary abstract (Lazier *et al.*, 1978).

## Materials and Methods

### Chemicals

[2,4,6,7-$^3$H]Oestradiol-17$\beta$ (98.5 Ci/mmol) was obtained from New England Nuclear Corp., Montreal, Quebec, Canada. The labelled oestradiol, supplied in solution in benzene/ethanol (9:1, v/v), was evaporated to dryness and redissolved in ethanol. Dilutions were made in buffer (10 mM-Tris/HCl/1.5 mM-EDTA, pH 7.4). Radiochemical purity was monitored

by t.l.c. on Polygram silica-gel sheets from Macherey–Nagel and Co., Düren, Germany, by using the solvent system chloroform/acetone/ethanol (89:10:1, by vol.). Unlabelled steroids were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., as were the marker proteins used in gel filtration. Sephacryl-S200 (superfine grade) was obtained from Pharmacia (Montreal, Quebec, Canada). Materials for liquid-scintillation counting were obtained from New England Nuclear Corp.

### Animals and injections

Cobb cockerels were obtained from a local hatchery, maintained in quarters lighted for 12h daily and fed on a standard diet. They were used for experiments when body weight reached $100 \pm 10$ g. Oestradiol-17$\beta$ in propylene glycol (25 mg/ml) was injected intraperitoneally at a dose of 2.5 mg/100 g. The cockerels were starved overnight before being killed.

### Preparation of cell fractions

Liver was rinsed three times with cold 0.9% NaCl, weighed, and minced in buffer A (2.5 ml/g). Buffer A contained 0.33 M-sucrose, 3 mM-MgCl$_2$, 10 mM-$\alpha$-thioglycerol and 20 mM-Tris/HCl, pH 8.4. Phenylmethanesulphonyl fluoride and benzamidine were freshly prepared in ethanol solutions and added to the buffer (10 $\mu$l/ml of buffer A) just before use (final concentrations of 0.3 and 1 mM respectively) (Lazier, 1978). After gentle homogenization (four strokes in a glass/Teflon homogenizer) the liver preparation was centrifuged at 800$g$ for 20 min to remove nuclei and debris, followed by 90 min at 105000$g$. The supernatant was removed, avoiding the fat layer, and was immediately subjected to (NH$_4$)$_2$SO$_4$ precipitation by the slow addition of 0.5 vol. of saturated (NH$_4$)$_2$SO$_4$ in 10 mM-Tris/HCl, pH 7.4. After thorough mixing for 15 min the precipitate was removed by centrifugation at 30000$g$ for 20 min. The pellet was drained well and dissolved in buffer B (10 mM-Tris/HCl/1.5 mM-EDTA/0.5 M-KCl/10 mM-thioglycerol, pH 7.4) at a protein concentration of about 10 mg/ml.

Nuclei and 0.5 M-KCl extracts of nuclei were prepared as described previously (Lazier, 1978).

Protein was determined by using the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, CA, U.S.A.). DNA was determined by the method of Burton (1970).

### Measurement of [$^3$H]oestradiol-binding activity

The dissolved pellets after (NH$_4$)$_2$SO$_4$ precipitation were assayed for [$^3$H]oestradiol-binding activity essentially by the exchange assay developed by Sutherland & Baulieu (1976) for chick oviduct cytosol. Before assay, the preparations were incubated with 0.1 vol. of charcoal/dextran suspension (5.0% charcoal, 0.5% dextran in 10 mM-Tris/HCl/1.5 mM-EDTA, pH 7.4) for 15 min at 2°C. This served to remove

endogenous free oestrogen. It was not a necessary step for preparations from control cockerels, but it was essential in order to observe exchange of [$^3$H]-oestradiol in preparations from oestradiol-treated cockerels. After removal of charcoal by centrifugation at 3000$g$ for 10 min, 0.3 ml portions of the supernatant were incubated in duplicate with [$^3$H]oestradiol in final concentrations of 0.2–80 nM. Parallel tubes containing the [$^3$H]oestradiol plus a 100-fold excess of unlabelled diethylstilboestrol were included for determination of non-specific binding. Incubation was for 16 h at 2°C, unless otherwise indicated in the text. After incubation an equal volume of charcoal/dextran suspension (0.25% charcoal, 0.025% dextran in 10 mM-Tris/HCl/1.5 mM-EDTA, pH 7.4) was added. After intermittent mixing for 30 min at 2°C, the charcoal was sedimented and the supernatants were added to Aquasol II (New England Nuclear Corp.) and counted for radioactivity as described previously (Lazier, 1978).

Assay of salt-soluble nuclear [$^3$H]oestradiol-binding activity was as documented by Lazier (1978).

### Gel filtration

The fraction precipitated by (NH$_4$)$_2$SO$_4$ from cytosol was incubated for 16 h at 2°C with 10 nM-[$^3$H]oestradiol in the absence or presence of 0.1 $\mu$M-diethylstilboestrol, then treated with 0.1 vol. of 5% charcoal/0.5% dextran suspension for 15 min at 2°C. After sedimentation of the charcoal, the supernatant was applied to a calibrated column (26 cm × 1.5 cm) of Sephacryl-S200 (superfine grade; Pharmacia). Elution was with buffer B at a flow rate of 36 ml/h. Portions of each fraction were added to Aquasol II and counted for radioactivity. Mild trypsin treatment of the cytosol (NH$_4$)$_2$SO$_4$-precipitated preparation was carried out as described by Gschwendt (1977), except that the fraction incubated with [$^3$H]oestradiol was treated with charcoal before the incubation with trypsin at 10°C.

### Results

#### Concentration and binding affinity of the cytosol oestrogen-binding protein

In early experiments examining unfractionated cockerel liver cytosol for high-affinity [$^3$H]oestradiol binding, we detected copious quantities of bound hormone, but found less than 5% suppression of total binding by inclusion of 100–1000-fold excess of unlabelled oestradiol-17$\beta$ or diethylstilboestrol in the assay mixture. In view of the possibility that a putative cytosol receptor was labile, owing to proteinase activity in the liver preparations, we raised the pH and cation concentration of the homogenization buffer such that the pH of the homogenate was 7.4. If homogenization was carried out in a Tris/HCl buffer of pH 7.4, the resulting homogenate pH was

between 5 and 6. In addition, the proteinase inhibitors phenylmethanesulphonyl fluoride and benzamidine were added to the buffer before the cells were disrupted (Lazier, 1978). A further consideration was that some workers assaying oestradiol cytosol receptor in rat liver routinely subjected cytosol to $(NH_4)_2SO_4$ precipitation before assay (Eisenfeld *et al.*, 1977; Beers & Rosner, 1977). Not only could this serve to remove proteinases, but also it could remove a considerable amount of lower-affinity oestrogen-binding proteins (Dower, 1977). Finally, a fortuitous observation was that higher diethylstilboestrol-suppressible binding was found if the cockerels were starved for 12–18 h before assay. This resulted in preparations that were much less fatty.

Fig. 1 shows the total [$^3$H]oestradiol binding, the non-specific and the net saturable binding in an $(NH_4)_2SO_4$ pellet from cockerel liver cytosol incubated for 16 h at 2°C. A preliminary experiment had
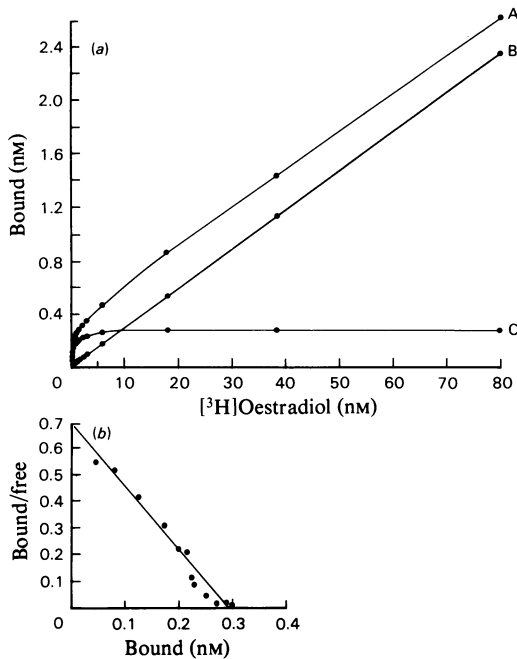
shown that apparent equilibrium was reached after about 4 h of incubation, and that binding was linear with protein concentration up to a concentration of 20 mg/ml. The specific binding curve in Fig. 1 shows that over a very wide range of [$^3$H]oestradiol concentrations only a single class of saturable binding sites can be detected. Although non-specific binding makes a marked contribution to total bound radioactivity at higher [$^3$H]oestradiol concentrations, it is much decreased at the lower concentrations. Cockerel serum contains a component that binds [$^3$H]oestradiol in a non-saturable fashion up to a hormone concentration in the $\mu$molar range (C. Lazier, unpublished work). This may contribute to the so-called non-specific binding in the liver preparations. The Scatchard plot corresponding to the binding data in Fig. 1 shows an equilibrium dissociation constant ($K_d$) of 0.4 nM and a binding-site concentration of 18.2 fmol/mg of protein.

In ten experiments with different preparations of cytosol from Cobb cockerels $K_d$ values of 0.4–2.6 nM were obtained. The concentration range was 16–33 fmol/mg of protein. Within an experiment less variation was found: for example, for separate assays on liver from each of 16 birds the mean concentration of cytosol sites was $19.2 \pm 2.6$ fmol/mg of protein, or $416 \pm 36$ fmol/g of liver, or about 300 sites/cell. Similar values were obtained for cockerels of the Comet or White Leghorn breeds.

Essentially all of the high-affinity [$^3$H]oestradiol-binding protein was precipitated at 0–33% saturation with $(NH_4)_2SO_4$. Very little diethylstilboestrol-suppressible binding was found in the fractions precipitated at 33–45% or 45–66% saturation with the salt. In such behaviour the cockerel cytosol [$^3$H]oestradiol-binding protein is similar to typical



Fig. 1. [$^3$H]Oestradiol-binding characteristics of the $(NH_4)_2SO_4$ preparation from cockerel liver cytosol
The $(NH_4)_2SO_4$ preparation (0.3 ml) was incubated with various concentrations of [$^3$H]oestradiol in the absence or presence of a 100-fold excess of unlabelled diethylstilboestrol for 16 h at 2°C. The bound fractions were determined by the charcoal/dextran method described in the Materials and Methods section. (a) Binding saturation curve: A, total binding; B, non-specific binding; C, net specific binding (A−B). (b) Scatchard plot of the specific-binding data.

Table 1. *Specificity of [$^3$H]oestradiol binding by chick liver cytosol*
A portion (0.3 ml) of the $(NH_4)_2SO_4$ pellet of cytosol in buffer B was incubated for 16 h at 2°C with 2 nM-[$^3$H]oestradiol in the absence or presence of a potential competitor at a concentration of 200 nM. Bound [$^3$H]oestradiol was measured as described in the Materials and Methods section.

| | [$^3$H]Oestradiol bound (c.p.m.) | Inhibition (% of that by oestradiol-17$\beta$) |
|---|---|---|
| None | $9420 \pm 103$ | — |
| Oestradiol-17$\beta$ | $5710 \pm 28$ | 100 |
| Oestradiol-17$\alpha$ | $6250 \pm 56$ | 85.6 |
| Oestrone | $5620 \pm 118$ | 102.4 |
| Oestriol | $6120 \pm 69$ | 88.9 |
| Diethylstilboestrol | $5790 \pm 51$ | 97.3 |
| Progesterone | $9350 \pm 102$ | 2.0 |
| Dihydrotestosterone | $8900 \pm 22$ | 14.1 |
| Cortisol | $9210 \pm 151$ | 5.8 |

steroid receptors (Puca *et al.*, 1975; Mainwaring & Irving, 1975) and is clearly different from the non-receptor hepatic steroid-binding protein in hen liver, which is preferentially precipitated at 55–70% saturation with $(NH_4)_2SO_4$ (Dower, 1977).

## Hormone specificity of binding and identity of the bound oestrogen

The hormone specificity of the [$^3$H]oestradiol binding is shown in Table 1. At 100-fold excess, the non-oestrogens do not compete with [$^3$H]oestradiol binding. Oestradiol-17$\beta$, oestrone and diethylstilboestrol are equally effective competitors, and oestriol and oestradiol-17$\alpha$ exhibit partial competition. At 10-fold excess, oestradiol-17$\alpha$ and oestriol are only 50% as potent as the stronger oestrogens. These relative affinities are similar for the soluble nuclear oestrogen receptor in cockerel liver (Lazier & Alford, 1977).

Since liver is a well-known site of oestradiol metabolism, the question of the actual identity of the specifically bound $^3$H-labelled compound was addressed. To minimize the problem of non-specific binding the concentration of 0.25 nM-[$^3$H]oestradiol was chosen, where binding not suppressible by a 100-fold excess of diethylstilboestrol is less than 20% of the total. The $(NH_4)_2SO_4$-precipitated cytosol fraction was incubated for 2h at 2°C or at 25°C, charcoal-treated at 2°C and the supernatants containing the bound [$^3$H]oestradiol were extracted with $3 \times 5$vol. of ether. Analysis by t.l.c. of the ether extracts showed that the large majority of the labelled
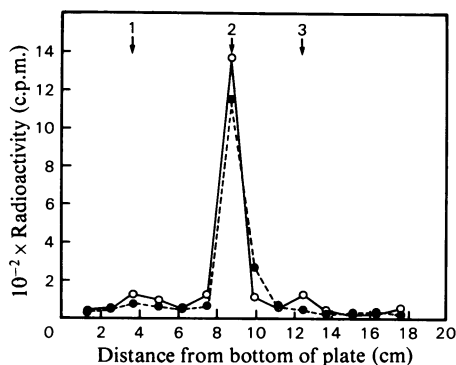
steroid chromatographed with authentic oestradiol-17$\beta$ (Fig. 2). In each case, however, a small fraction of the radioactive material remained water-soluble (13% on incubation at 2°C, 24% at 25°C). This could reflect formation of water-soluble metabolites and could give the impression of non-specific binding.

## Depletion of the cytosol oestradiol-binding protein after oestrogen treatment in vivo

The criteria of high-affinity and specific binding of [$^3$H]oestradiol in the cockerel liver $(NH_4)_2SO_4$-precipitated cytosol fraction fit those required for a receptor. It is also essential to demonstrate depletion of the cytoplasmic sites after injection of oestradiol *in vivo*. In order to be sure that occupied as well as unoccupied binding sites were being assayed in the cytosol fraction, the completeness of the exchange reaction had to be assessed. This was done by first incubating a $(NH_4)_2SO_4$-precipitated cytosol preparation from an untreated cockerel with a high concentration of unlabelled oestradiol-17$\beta$ (10 nM) in order to saturate all of the high-affinity sites, followed by charcoal treatment at 2°C to remove unbound hormone. [$^3$H]Oestradiol (10 nM) was then added in the absence or presence of 0.1 $\mu$M-diethylstilboestrol, and the preparation was incubated at 2°C for 16h followed by various times at 25°C in order to effect exchange of bound unlabelled oestradiol by the labelled hormone. A corresponding control preparation, not presaturated with unlabelled oestradiol, was similarly
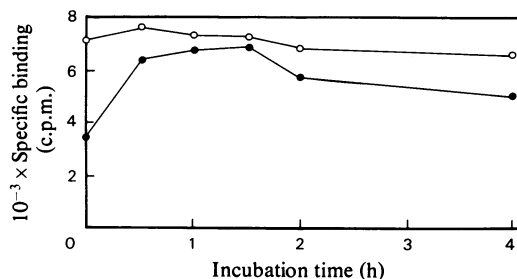


Fig. 2. *Thin-layer-chromatographic analysis of the ether-soluble bound fraction*

[$^3$H]Oestradiol (0.25 nM) was incubated for 2h with the cytosol $(NH_4)_2SO_4$ fraction (1.0 ml) at 25°C (○) or at 2°C (●). The incubation mixtures were then charcoal-treated, extracted with $3 \times 5$vol. of ether and the extracts were chromatographed on t.l.c. plates as described in the Materials and Methods section. The numbers indicate the positions of authentic steroid standards: 1, oestriol; 2, oestradiol; 3, oestrone.



Fig. 3. *Exchange of presaturated oestradiol-binding sites*

A portion (8.0 ml) of the cytosol $(NH_4)_2SO_4$ preparation was preincubated for 4h at 2°C with 10 nM-oestradiol-17$\beta$ (unlabelled) and an otherwise identical preparation was preincubated without the hormone. The samples were charcoal-treated with 0.1 vol. of 5.0% charcoal/0.5% dextran for 15 min at 2°C, and the supernatants incubated with 10 nM-[$^3$H]oestradiol in the absence or presence of 0.1 $\mu$M-diethylstilboestrol for 16h at 2°C followed by various times at 25°C. At each time indicated duplicate 0.3 ml samples were taken for charcoal treatment and determination of specific [$^3$H]oestradiol binding as described in the Materials and Methods section. ●, Presaturated $(NH_4)_2SO_4$ fraction; ○, non-presaturated control.
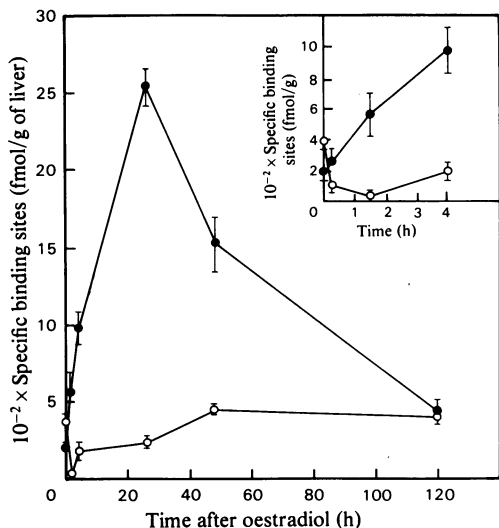
Fig. 4. *Depletion of the high-affinity oestradiol-binding sites from cytosol and the increase in soluble nuclear oestrogen receptor after oestradiol treatment in vivo*
Cockerels were injected with oestradiol and livers were removed at various times thereafter and homogenized in buffer A in the presence of proteinase inhibitors. The cytosol $(NH_4)_2SO_4$ fractions and the soluble nuclear extracts were prepared as described in the Materials and Methods section. The cytosol fractions were charcoal-treated at 2°C and assayed for specific [³H]oestradiol binding by exchange for 16h at 2°C, followed by 1h at 25°C. Assay of the nuclear receptor was as described by Lazier (1978). The results are the means ± S.E.M. for duplicate determinations on separate preparations from each of four animals per group. The inset shows binding-site distribution at early times after oestradiol injection. ○, Cytosol $(NH_4)_2SO_4$ fractions; ●, soluble nuclear receptor.

treated. This assay was essentially based on that developed by Sutherland & Baulieu (1976) for oestrogen receptors from chick oviduct. The results in Fig. 3 show that exchange of the presaturated sites is complete by 1–2h at 25°C, and that the number of binding sites detected is very close to that found in the control preparation which had not been presaturated with oestradiol-17β. It is noteworthy that about half of the occupied sites exchange during the 16h incubation at 2°C. Exchange assays at 37°C revealed considerable instability of the binder: only half of the sites found on exchange for 1h at 25°C were detectable at the higher temperature (results not shown).

Fig. 4 shows the effect of intraperitoneal injection of oestradiol on the concentration of high-affinity exchangeable oestrogen-binding sites in the cockerel liver cytosol $(NH_4)_2SO_4$-precipitated fraction. Maxi-

mum depletion (over 80%) was seen at 1.5h and restoration to control values was gradual over the next 48h. Fig. 4 expresses the binding-site concentration in terms of fmol/g of tissue, and similar depletion is seen when results are expressed in terms of fmol/mg of protein or fmol/μg of DNA. Fig. 4 also shows the concentration of salt-soluble nuclear oestrogen receptor in nuclear pellets from the same liver preparations from which the cytosol fractions were prepared. At the early stages after oestrogen treatment the increase in the soluble nuclear receptor more or less paralleled the decrease in the high-affinity binding protein in cytosol. At later stages the concentration of the soluble nuclear receptor was many times that of the cytosol binder in control chicks. It should be borne in mind that the salt-soluble nuclear receptor does not represent total nuclear oestrogen receptor: a proportion of the nuclear binding is in an insoluble fraction (Schneider & Gschwendt, 1977; Lebeau et al., 1977). We were unable to measure reproducibly total nuclear oestrogen receptor by exchange in the nuclei prepared by the method used for demonstration of the high-affinity binding protein in cytosol. Salt extraction of the nuclei yielded highly reproducible results.

### Physical properties of the cytosol high-affinity oestradiol-binding protein

Preliminary experiments indicated that the cockerel liver cytosol oestradiol-binding protein was not reproducibly stable in sucrose-density-gradient centrifugation, apparently owing to variable aggregation. Gel filtration of the $(NH_4)_2SO_4$-precipitated cytosol fraction on columns of Sephacryl-S200 yielded much more consistent results (Figs. 5a and 5b). In the presence of salt, most of the bound [³H]oestradiol was eluted in a fraction corresponding to very high-molecular-weight or aggregated protein, with a shoulder corresponding to bound hormone in the mol.-wt. region of 40000–70000. Although salt treatment of crude cytoplasmic receptor fractions often leads to production of apparently non-aggregated receptor species, Erdos et al. (1971) note that partly purified fractions from uterine cytosol often tend to aggregate, even in the presence of high salt concentrations.

Mild trypsin treatment of the cockerel liver preparation (Gschwendt, 1977) gave rise to a pronounced peak of bound [³H]oestradiol that was eluted close to marker ovalbumin (mol.wt. 43000). Fig. 5(b) shows that a 100-fold excess of diethylstilboestrol suppressed labelling of both the trypsin-generated and high-molecular-weight peaks, indicating that both represent specific binding of oestradiol. The size of the trypsin-treated oestradiol-binding component reported here is very close to that found by Gschwendt (1977) in a similar fraction from embryonic-chick liver. In addition, the trypsin-treated salt-soluble
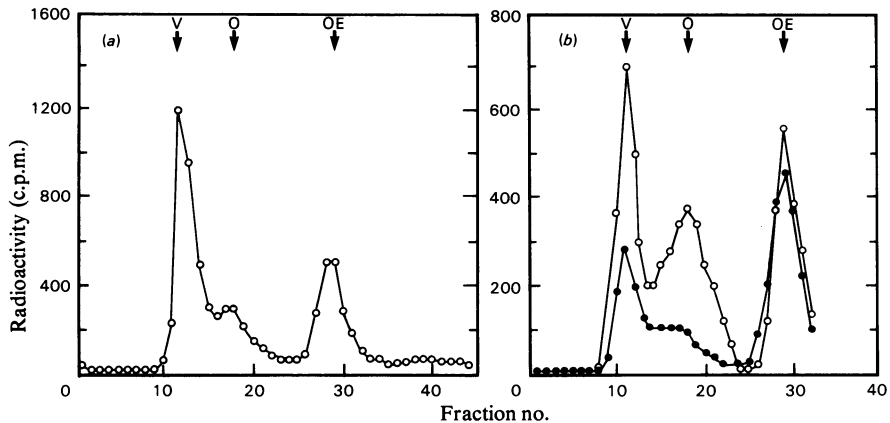
Fig. 5. *Gel filtration of the cytosol* $(NH_4)_2SO_4$ *fraction*

The cytosol $(NH_4)_2SO_4$ fraction was incubated for 16h at 2°C with 10nM-[³H]oestradiol, charcoal-treated, and applied to a column of Sephacryl-S200 as described in the Materials and Methods section. (*a*) ○, Control cytosol $(NH_4)_2SO_4$ preparation; (*b*) ○, trypsin-treated preparation; ●, trypsin-treated preparation that had been preincubated with 10nM-[³H]oestradiol and 0.1 μM-diethylstilboestrol. V, void volume; O, ovalbumin marker; OE, free [³H]oestradiol.

nuclear receptor partitioned on gel filtration at about the same position as the trypsin-treated cytosol binding protein both in embryos (Gschwendt, 1977) and in cockerels (H. W. Murdock & C. B. Lazier, unpublished work).

## Discussion

The high-affinity oestradiol-binding protein described here fits several of the criteria which are generally used to define an oestrogen 'receptor' (Williams, 1974; King & Mainwaring, 1974). The specificity, the binding affinity and the binding-site concentration as well as the observed loss of the sites from cytosol after oestrogen treatment *in vivo* strongly suggest, although they do not prove, that the binder is indeed a receptor. Further direct evidence on nuclear uptake of the binder and ultimately on its biological activity *in vitro* will be required for definitive proof.

The concentration of the cockerel cytosol oestrogen binder is much less than that of the cytosol receptor in the uterus of immature or ovariectomized rats (King & Mainwaring, 1974). It is, however, comparable with that found by some workers for a high-affinity oestradiol-binding activity in cytosol from liver of the adult rat (Chamness *et al.*, 1975; Viladin *et al.*, 1975), and about half that found by some others (Eisenfeld *et al.*, 1977; Beers & Rosner, 1977). Oviduct cytosol from immature chicks contains about three times the number of binding sites found here for cockerel liver (Sutherland & Baulieu, 1976; Sutherland *et al.*, 1977). These comparisons neglect consideration of heterogeneity of cell types in the different tissues. If vitellogenin is induced by

oestradiol only in liver parenchymal cells (Tata, 1976; Wachsmuth & Jost, 1976), the cytosol receptor concentration in these cells might therefore be at least twice as high as that reported here for whole liver.

Liver from chick embryos at day 19 of development appears to contain a 3–5-fold higher concentration of cytoplasmic high-affinity oestrogen-binding sites than does liver from the hatched chick (Gschwendt, 1977; C. B. Lazier, unpublished work). This apparent peak in binding-site concentration at day 19 does not seem to bear any obvious relationship to the ability of exogenous oestradiol to induce vitellogenin synthesis (Lazier, 1978). It may be related instead to developmental events in liver that are independent of oestrogen responsiveness, or it may reflect ill-understood technical problems in the assay of the binding protein, resulting in underestimation of the concentration of sites in cockerel liver.

Regardless of whether or not complete recovery of high-affinity oestrogen-binding sites from cockerel liver cytosol is presently being achieved, it is clear that a binding activity that has many properties of a typical oestrogen receptor can be detected, and that earlier speculation that the mode of action of oestrogen in avian liver was independent of a cytosol receptor mechanism is not justified (Sheridan, 1975; Gschwendt, 1975*a*; Lazier & Alford, 1977). The dynamics of regulation of receptor concentrations in avian liver may differ somewhat from that in other target tissues. In rat uterus, for example, the accumulation of nuclear receptor sites after a single dose of oestrogen appears to reflect a rapid, stoicheiometric transfer of cytoplasmic receptor sites (Lan & Katzenellenbogen, 1976). In avian liver, there seems to be initial translocation of the small number of

cytoplasmic sites to the nucleus, followed by marked nuclear receptor accumulation by a mechanism involving either induced synthesis and/or stabilization of the protein (Schneider & Gschwendt, 1977). Non-steroidal anti-oestrogens appear to be more purely antagonists of oestrogen action in liver than in uterus, possibly because they block the mechanism of nuclear receptor accumulation in the former tissue (Gschwendt, 1975b; Lazier & Alford, 1977), but permit a substantial increase in nuclear receptor in the latter tissue (Clark et al., 1974).

A further consideration with regard to the role of cytoplasmic oestrogen receptor in avian liver is that very high doses of oestradiol in propylene glycol are necessary to stimulate a prolonged increase in nuclear oestrogen-receptor concentration and apparently consequent vitellogenin production (Lazier, 1975; Joss et al., 1976; Schneider & Gschwendt, 1977; Burns et al., 1978). The dose generally used leads initially to a very high blood oestradiol concentration (over 100nM), which is far in excess of that which should be needed to saturate the cytosol high-affinity oestrogen-binding sites described here. However, the intrahepatic concentration of oestradiol reached is not known, and it seems likely that the non-receptor hepatic steroid-binding protein (Dower & Ryan, 1976; Dower, 1977) plays an important role in regulating the available concentration of intracellular oestradiol. Experiments using oestrogen implants, or constant infusion of more physiological doses of the hormone over various periods, may lead to better understanding of the relationship between blood oestrogen, receptor saturation and regulation of the vitellogenic response.

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