Identification of a novel sex steroid binding protein

(receptors/sterophilin/chick oviduct)

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ABSTRACT We describe a cytoplasmic steroid binding protein in the chick oviduct which has intriguing characteristics. This protein binds [³H]estradiol with high affinity ($K_d = 30 \times 10^{-9}$ M) and limited capacity (300 fmol/mg of cytosol protein). It sediments at 8 S in low-salt sucrose density gradients and at 4 S in high-salt gradients. Unlike the estrogen receptor, however, this protein also binds progesterone, R5020, testosterone, and 5 α -dihydrotestosterone with similar affinities and in a competitive manner. Moreover, it is not translocated to the nucleus by the *in vivo* administration of these sex steroids. The protein is only present in estrogen-responsive tissue, and like the sex steroid receptors, its synthesis appears to be regulated by estrogen.

Current molecular models of steroid hormone action suggest that these hormones initiate their regulatory effects by interacting with stereospecific receptor proteins localized in the cytosol of target tissues. Steroid hormone-receptor complexes are believed to undergo an energy-dependent activation (1) that results in a conformational change and translocation into the nucleus, where regulatory interactions with the genome occur (1-4). In the past few years, this well-accepted hypothesis has become complicated by the discovery of steroid receptor heterogeneity (5, 6) and the demonstration of multiple steroid binding proteins within target tissues (7, 8). The role of these cytoplasmic hormone binding proteins is poorly understood, and the elucidation of their functions will be an important step in the understanding of steroid hormone action.

In addition to the classical estrogen receptor, Clark *et al.* (7) have reported the presence of a secondary cytoplasmic estrogen binding molecule (type II) in the rat uterus. This protein does not translocate into the nucleus after estradiol treatment. Moreover, type II has an affinity for [³H]estradiol ($K_d = 30 \times 10^{-9}$ M) considerably weaker than that of the classical rat uterus estrogen receptor ($K_d = 0.8 \times 10^{-9}$ M). A similar heterogeneity of estradiol binding molecules has been observed in mouse mammary cancer tissue (8). In contradistinction to type I and type II estrogen binding proteins in rodent target tissue cytosol, we have reported that human uterus and chick oviduct cytosols contain two high-affinity estrogen binding proteins, both of which have the characteristics of receptors (5). They have high affinities and specificity for estrogens, are present in limited cellular concentrations, and are found only in target tissue. Moreover, the chick oviduct estrogen receptors behave in sucrose gradients with characteristic profiles, translocate stoichiometrically from cytosol to nuclei *in vivo*, and appear to mediate a biological estrogenic response (9, 10). Preliminary evidence in the human uterus suggests that each of these two receptors may have independent physiologic functions involved in the regulation of menstrual cyclicity (6). For convenience, we refer to the two estrogen receptors as X and Y, the former repre-

senting the species with the higher affinity. This report describes the identification of a new estrogen binding protein, designated Z.

MATERIALS AND METHODS

Animals. Two-day-old White Leghorn pullets were purchased from Texas Animal Specialties (Humble, TX), provided with food and water ad lib, and raised in a constant temperature environment with a 13/11-hr light/dark schedule. At the chick age of 1 wk, estrogen stimulation was initiated by subcutaneous insertion of two silicone implants containing 25 mg of diethylstilbestrol (DES) each. Fourteen days after DES-implant insertion, the animals were considered fully stimulated. Periods of estrogen withdrawal, as indicated in the text, were begun after day 14 by removal of the DES implants through cutaneous incisions. In some experiments, chicks were injected subcutaneously 30 min before sacrifice with 2 mg each of estradiol, progesterone, and 5 α -dihydrotestosterone in sesame oil. All animals were killed by decapitation; then the oviduct and spleen tissues were rapidly excised and processed immediately.

Hormones. [2,4,6,7-³H]Estradiol (90-100 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), [1,2,6,7-³H]progesterone (89 Ci/mmol), and [1,2,4,5,6,7-³H]5 α -dihydrotestosterone (110 Ci/mmol) were purchased from Amersham, and their purity was confirmed by thin-layer chromatography on silica gel plates. Unlabeled DES, estradiol, cortisol, progesterone, testosterone, and 5 α -dihydrotestosterone were from Sigma. Tamoxifen was a gift from Stuart Pharmaceutical (Wilmington, DE). Promegestone (R5020) was purchased from New England Nuclear.

Chemicals. "Ultra-pure" Tris base, ammonium sulfate, and sucrose were obtained from Schwarz/Mann. Norit A charcoal, 1-thioglycerol, Scintiverse, and other analytical grade reagents were from Fisher. Dextran T 70 was from Pharmacia (Uppsala, Sweden). Bio-Rad supplied hydroxylapatite.

Preparation of Cytosol and Nuclear Extracts. Oviduct and spleen tissues isolated after hormonal stimulation or withdrawal were quickly excised, rinsed free of detectable blood in ice-cold 0.9% NaCl, and weighed. Cytosol and nuclear extracts were prepared as described (5). To obtain receptor-depleted cytosol, oviducts were excised 30 min after treatment with 2.0 mg each of DES, dihydrotestosterone, and progesterone in sesame oil. Oviduct cytosols prepared from hens or hormone-stimulated chicks were treated with dextran-coated charcoal for 5 min at 4°C to remove free steroids. The charcoal solution was prepared as described (5).

[³H]Estradiol Binding Site Titration. Cytosol preparations or redissolved 30% (NH₄)₂SO₄-precipitated, 0.4 M KCl-ex-

Abbreviations: DES, diethylstilbestrol; TESH, 10 mM Tris-HCl, pH 7.4/1.5 mM EDTA/12 mM thioglycerol.

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tracted nuclei were incubated in borosilicate glass tubes at 4°C for 18 hr with [³H]estradiol ranging from 60 pM to 60 nM. To assess total nonspecific binding, we simultaneously prepared a similar series of assay tubes containing a 100-fold molar excess of unlabeled estradiol. Nuclear preparations were titrated with [³H]estradiol in the same broad concentration range under exchange (30°C for 3 hr) conditions. [³H]Estradiol binding sites in purified whole nuclei were detected by using the nuclear exchange microassay as described (9). Otherwise, separation of bound from free hormone was accomplished by dextran/charcoal or hydroxylapatite assays. These data were plotted as described by Scatchard (11), providing estimates of the equilibrium dissociation constant, K_d , and concentration of binding sites, N_{max} . Complex Scatchard curves were resolved by the method of Rosenthal (12). Cellular concentrations of the binding proteins were estimated on the basis of 2.5 pg of DNA per chick cell nucleus (13). DNA was quantitated by the diphenylamine method (14).

Dextran-Coated Charcoal Adsorption Assay. This procedure was based on the method described by Korenman (15) and has been reported in detail by us (5).

Hydroxylapatite Adsorption Assay. A method modified from that of Erdos *et al.* (16), which has been described in detail (5) was used. An alternative, more rapid assay was also used. The hydroxylapatite-bound receptor sample was washed with 3 ml of buffer on a glass-fiber filter with a Millipore apparatus under low suction. The filters containing hydroxylapatite-protein complexes were dried, extracted with ethanol, and assayed for radioactivity. Although the washing was performed more quickly in this manner, no differences in results were observed, suggesting that dissociation of the ³H-labeled ligand did not result in an underestimation of binding sites.

Enzymatic Digestion Experiments. Chick oviduct cytosol samples (0.2 ml) were incubated with 100 µg of trypsin, pancreatic RNase, or pancreatic DNase I per ml or with an equal volume of 10 mM Tris·HCl, pH 7.4/1.5 mM EDTA/12 mM thioglycerol (TESH) containing only 5 mM MgCl₂ (control) for 15 min at 23°C. These samples were then incubated with increasing concentrations of [³H]estradiol and unlabeled estradiol to titrate binding sites as described above. Displaceable hormone binding was determined by the dextran/charcoal assay.

Preparation of Purified Oviduct Plasma Membrane Fractions. Chick oviducts were finely minced and homogenized on ice in a glass/Teflon homogenizer (35 strokes) in 8 vol of 5 mM Tris·HCl, pH 7.4 at 23°C/0.5 mM CaCl₂/0.25 M sucrose. The homogenization and isolation protocol used had been shown to provide the highest specific activities of the plasma membrane markers 5'-nucleotidase and alkaline phosphatase (17). Plasma membrane fractions were isolated and incubated for 2 hr at 4°C with 3–60 nM [³H]estradiol in the absence and presence of excess unlabeled estradiol. Each sample was filtered through a nitrocellulose disc, with a Millipore apparatus to separate membrane-bound from free ³H-labeled ligand. Specific binding was examined by Scatchard analysis.

Sucrose Density Gradient Centrifugation. A modification of the postlabeling method of Clark *et al.* (7) was used. Chicks stimulated using silastic-containing implants containing 25 mg of DES were then treated with DES (2.5 mg), progesterone (2 mg), and dihydrotestosterone (2 mg) to deplete the oviduct of their respective receptors. After 30 min, the chicks were killed, oviduct cytosol was prepared, and 0.2 ml was applied to linear gradients of 5–20% sucrose in TESH or in TESH containing 0.4 M KCl. The gradients were prepared in 5 ml of polyallomer tubes (Beckman) using a Buchler Densiflow IIC gradient former (Ft. Lee, NJ). The tubes were centrifuged at 200,000 × g for 18 hr in a Beckman SW 50.1 rotor at 2–4°C.

Each gradient was tapped from below and fractionated into 16 × 0.3 ml samples, which were directly collected into borosilicate glass tubes at 4°C. Each glass tube contained 15 pmol of [³H]estradiol in 0.2 ml of TESH. An additional series of 16 tubes also contained a 100-fold molar excess of unlabeled estradiol or progesterone for the estimation of nonspecific binding. Gradient samples fractionated into these tubes were incubated at 4°C for 18 hr prior to the addition of 0.3 ml of dextran-coated charcoal. These suspensions were incubated for 5 min at 0°C, and bound [³H] was determined as described for titration assays. Sedimentation coefficients of [³H]estradiol-binding components were estimated by comparison with [¹⁴C]ovalbumin (3.7 S) and human γ-[¹⁴C]globulin (7.0 S) standards prepared as described by Rice and Means (18).

RESULTS AND DISCUSSION

Fig. 1 shows a typical Scatchard (11) plot of [³H]estradiol binding in estrogen-withdrawn chick oviduct cytosol. The biphasic slope reflects the two estrogen receptors X and Y. Resolution of these curves by the graphical method of Rosenthal (12) gave equilibrium dissociation constants (K_d s) of 0.1 nM and 2 nM for X and Y, respectively. The LIGAND computer program of Munson and Rodbard (19) assigned K_d values of 0.05 and 0.6 nM to these two receptors. After estrogen administration *in vivo*, X and Y were translocated stoichiometrically into the nucleus, where their accumulation was highly correlated (r , 0.87; P ,

FIG. 1. Scatchard analyses of [³H]estradiol binding in chick oviduct cytosol. The binding characteristics of the estrogen receptors (X and Y) were evaluated by preparing cytosol from oviduct tissue isolated from estrogen-withdrawn chicks as described. (Inset) Analysis of Z binding was performed with oviduct cytosol isolated from chicks implanted with silastic tubing containing 25 mg of DES for 14 days. A fixed concentration of cytosol was incubated for 18 hr at 4°C with increasing concentrations of [³H]estradiol (specific activity, 90 Ci/mmol) ranging from 60 pM to 60 nM. Nonspecific binding was determined by performing the saturation analysis in the presence of a 100-fold molar excess of unlabeled estradiol. These Scatchard analyses were obtained by plotting the specifically bound label against the ratio of bound to free [³H]estradiol. Each point represents the mean of duplicate determinations. The separation of bound and free ligand by dextran-coated charcoal or hydroxylapatite methods yielded identical results. The figure shown was derived from data obtained using the dextran-coated charcoal method.

<0.001) with the induction of ovalbumin mRNA synthesis (9). These data suggest that X and Y are directly involved in mediating this physiological response. The Fig. 1 *Inset* shows the Scatchard analysis of [^3H]estradiol binding to hormone-stimulated oviduct cytosol. In this condition, receptors X and Y have been depleted from the cytoplasm, and binding component Z, having a K_d for estradiol of 30 nM, is clearly evident. This value differs significantly (P , <0.001) from the K_d s of X and Y. Identical K_d s and binding site concentrations of X, Y, and Z were measured when hydroxylapatite, rather than charcoal adsorption, was used to quantitate these estrogen-binding molecules. Furthermore, under identical assay conditions, chick spleen cytosol, chick serum, and ovalbumin demonstrated no displaceable [^3H]estradiol binding. These findings indicate that none of these estrogen-binding components are methodological artifacts.

The K_d s and cytoplasmic concentrations of X, Y, and Z were determined in oviducts from DES-stimulated or -withdrawn chicks, and from laying hens as shown in Table 1. All of these values were obtained under nonexchange conditions and, therefore, would not necessarily include estimates of occupied receptors. The concentration of Z was consistently greater than the X and Y receptor concentrations, and levels of Z were highest in chicks receiving pharmacologic doses of exogenous estrogen. The Z component can be further distinguished from the estrogen receptors by its refractoriness to precipitation by ammonium sulfate fractionation. Although chick progesterin and estrogen receptors can be precipitated by 30% ammonium sulfate with yields of 23–39% (21–23), no detectable Z activity could be recovered by such fractionation.

The oviduct cytosol Z molecule, found in estrogen receptor-depleted cytosol, differed significantly from X and Y with respect to its hormone specificity. Competition analyses at two different concentrations of competing hormones (Table 2) revealed that a variety of androgens and progestins inhibit [^3H]estradiol binding to Z as effectively as estradiol does. Interestingly, DES, a synthetic estrogen with a high affinity for estrogen receptors, and tamoxifen, a potent antiestrogen in the

Table 1. Characteristics of [^3H]estradiol-binding proteins in oviduct cytosol

		Affinity, $\times 10^{-9}$ M K_d	Capacity, fmol/mg protein	Sites/cell, no.
Stimulated chick	X(n=4)	ND	<3	<20
	Y(n=4)	1.9 $\pm$ 0.4	5 $\pm$ 1	72 $\pm$ 10
	Z(n=4)	30.0 $\pm$ 9.0	263 $\pm$ 125	3253 $\pm$ 692
Hen	X(n=3)	0.10 $\pm$ 0.03	3 $\pm$ 1	22 $\pm$ 1
	Y(n=3)	1.9 $\pm$ 0.1	8 $\pm$ 2	56 $\pm$ 3
	Z(n=3)	15.0 $\pm$ 2.0	78 $\pm$ 36	617 $\pm$ 202
60-hr withdrawn chick	X(n=7)	0.11 $\pm$ 0.03	59 $\pm$ 7	230 $\pm$ 22
	Y(n=7)	2.2 $\pm$ 0.3	157 $\pm$ 22	620 $\pm$ 70
	Z(n=3)	14.0 $\pm$ 2.0	314 $\pm$ 40	1564 $\pm$ 160

Scatchard analyses of [^3H]estradiol saturation were performed with final ^3H -labeled hormone concentrations ranging from 60 pM to 10 nM to quantitate the X and Y components. Curvilinear plots (e.g., Fig. 1) were corrected by the Rosenthal method (12) to determine K_d s and binding-site concentrations. Z was quantified by [^3H]estradiol saturation over the range of 3–60 nM. An average of 40 total assay points for each component was examined. These values represent the mean $\pm$ SE of the individual samples assayed (n). Cellular concentrations of these proteins were calculated on the basis that each oviduct cell contains 2.5 pg of DNA. Cytosol protein was quantitated by the method of Bradford (20). ND, not determined. X was undetectable in stimulated chick oviduct cytosol, hence no K_d value could be determined.

Table 2. Competitive binding analysis of Z

Competitor	[^3H]Estradiol bound	
	fmol/mg protein	% inhibition
10-fold excess		
Control	181	0
17 β -Estradiol	91	50
Testosterone	81	55
5 α -Dihydrotestosterone	83	54
Progesterone	88	51
Promegestone (R5020)	75	59
Diethylstilbestrol	158	13
Cortisol	138	24
Progesterone + testosterone*	87	52
Progesterone + 17 β -estradiol*	85	53
Testosterone + 17 β -estradiol*	81	55
100-fold excess		
Control	224	0
17 β -Estradiol	35	84
Testosterone	34	85
5 α -Dihydrotestosterone	26	88
Progesterone	18	92
Diethylstilbestrol	151	33
Cortisol	85	62
Tamoxifen	167	25

Cytosol was obtained from estrogen-stimulated chick oviducts and labeled with [^3H]estradiol (30 nM) in the presence of a 10- or 100-fold excess of radioinert competitors. Bound [^3H]estradiol and its percentage inhibition were determined by dextran-coated charcoal adsorption. Each value represents the mean of duplicate determinations.

*Combinations of two competitors were adjusted so that each unlabeled hormone was present in a 5-fold excess.

chick (24), were less effective competitors for Z binding. Competitive binding assays using combinations of unlabeled estradiol, progesterone, and testosterone confirmed that inhibition of [^3H]estradiol binding by these steroids was additive, suggesting competition for a common binding site with similar inhibition constants. To determine whether these hormones were competitive or noncompetitive inhibitors, Lineweaver–Burk (25) analyses were performed with 5–95 nM [^3H]estradiol in the absence or presence of 1 μM radioinert hormones. The results clearly showed that all were competitive inhibitors and that estradiol, progesterone, and dihydrotestosterone had identical inhibition constants (Fig. 2).

To ascertain whether Z was an artifact of [^3H]estradiol binding to chick progesterin or androgen receptors, we carried out Scatchard analyses of oviduct cytosol with [^3H]progesterone (89 Ci/mmol) and [^3H]dihydrotestosterone (110 Ci/mmol). Our results demonstrated that the concentrations of binding sites of these two receptors differed from that of Z and were 40,000 and 6,000 sites per cell, respectively, in agreement with previous reports (26, 27). The presence of these two receptors prohibited the quantitation of Z binding with radiolabeled progestins and androgens. However, when oviduct cytosol was isolated from chicks 30 min after the *in vivo* administration of 2 mg each of estrogen, dihydrotestosterone, and progesterone, Z was still present. Analyses of 0.4 M KCl nuclear extracts and examination of purified oviduct nuclei isolated from chicks after sex steroid treatments failed to show Z binding activity. These results confirmed that unlike receptors, Z was not translocated into nuclear fractions.

It is generally assumed that steroid hormones passively diffuse across cellular membranes by virtue of their lipophilic nature. However, some reports indicate that their transport may

FIG. 2. Lineweaver-Burk (double reciprocal) plots of Z binding activity in stimulated chick oviduct cytosol. Aliquots of cytosol were incubated with [3 H]estradiol at concentrations of 5–95 nM for 18 hr at 4°C. These reaction mixtures contained either buffer alone ($\circ$), or 1 μ M estradiol (E), progesterone (P), dihydrotestosterone (T), cortisol (F), or DES (D). Charcoal absorption was used to separate bound from free ligand.

be mediated by membrane-associated proteins (17, 28). We surmised that Z might represent a membrane-associated sex steroid-transport protein in oviduct cells. Pietras and Szego (17) have shown that the cytosolic redistribution of membrane-bound steroid binding proteins can be an artifact of homogenization. To examine this possibility, we used their buffers, homogenization, and isolation procedures to purify a fraction of oviduct tissue enriched in plasma membranes. Whereas these membranes bound [3 H]estradiol in a saturable manner (K_d , 300×10^{-9} M), the ligand was only displaced by estradiol and not by progesterone or testosterone. Hence, this fraction did not contain Z activity. Furthermore, Z remained localized in the cytosol fraction at its usual concentration, even when this alternative cell fractionation technique was employed.

Despite its distinction from the sex steroid receptors of chick oviduct cytosol, Z has some interesting similarities to these molecules. The Z macromolecule is heat labile, losing its hormone binding activity within 15 min if incubated at 45°C in the absence of hormone. Incubation of cytosol from estrogen-treated chicks in the presence of trypsin (100 μ g/ml) under mild conditions (15 min at 23°C) resulted in a 72–77% decrease in [3 H]estradiol binding to Z. Control incubations and incubations in the presence of similar concentrations of RNase and DNase caused no depletion of hormone binding. Therefore, the steroid binding moiety is protein in nature. The Z protein, like the sex steroid receptors, readily adsorbs to hydroxylapatite, indicating that its protein structure consists of exposed acidic residues similar to receptor proteins. A further important similarity of Z to the steroid receptor molecule is its tissue specificity. Like the receptors, Z is confined to oviduct tissue and is undetectable in serum or in spleen cytosol. Postlabeled sucrose density gradient analysis of receptor-depleted cytosol, obtained from

chicks treated with estrogen, progesterone, and dihydrotestosterone, was performed at low ionic strength and showed that Z sedimented predominately as an 8S complex (Fig. 3A). Z-

FIG. 3. Postlabeled sucrose density gradient analyses of Z binding activity in stimulated chick oviduct cytosol. Linear gradients containing 5–20% (wt/vol) sucrose in the absence (A) or presence (B) of 0.4 M KCl were prepared, centrifuged, and fractionated as described. Labeling of the Z protein was carried out at a final concentration of 30 nM [3 H]estradiol to determine total binding ($\bullet$). Nonspecific binding ($\circ$) was determined by the addition of a 100-fold molar excess of unlabeled estradiol. Similar nonspecific binding profiles were observed with the substitution of a 100-fold excess of unlabeled progesterone or when the cytosol sample was heated at 45°C for 15 min prior to centrifugation.

binding activity shifted to a 4S pattern in the presence of 0.4 M KCl (Fig. 3B).

These findings document a receptor-like steroid hormone binding protein in chick oviduct cytosol that has liberal hormone binding specificity. This protein (Z) binds estradiol, dihydrotestosterone, and progesterone with equal affinities. The characteristic of multispecific Z-ligand interactions implies that this protein may be a member of the "sterophilin" class, which we have described in other steroid hormone target tissues (*). It is of interest that an extravascular [³H]estradiol binding protein with characteristics very similar to Z was recently reported in the turtle oviduct (29). This protein likewise sedimented at 7 S in low-salt sucrose gradients and bound testosterone, progesterone, and estradiol with equal affinities, albeit with an apparently lower K_d (2.7×10^{-9} M) than the Z K_d (30×10^{-9} M).

One possible physiological function of Z is that it regulates the intracellular concentrations of the sex steroids in the oviduct. It has been shown by Corvol and Bardin (30) that the chicken does not possess a plasma binding protein corresponding to the sex hormone binding globulin found in a number of mammalian species, which has molecular properties similar to those of Z because it also has a common binding site for testosterone, dihydrotestosterone, and estradiol. In the absence of a serum sex hormone binding globulin, it is conceivable that an intracellular binding protein such as Z might regulate the equilibrium between free and bound sex steroids, thus controlling the levels of these steroids interacting with their respective intracellular receptors. Furthermore, the presence of such a protein would provide an intracellular environment which would inhibit rapid metabolism of the sex steroids. A rapid metabolism of these plasma steroids would otherwise be expected to occur in the absence of sex hormone binding globulin.

Another possible role for the Z protein is that it might represent a common receptor precursor protein. Palmiter *et al.* (31) have demonstrated that estradiol, testosterone, and progesterone act synergistically to optimize oviduct cytodifferentiation and ovalbumin production in the hormone-stimulated chick. The synergism of these steroids also has been suggested in the ovulatory physiology of laying hens (32). Estrogen, androgen, and progestin receptors have all been relatively well characterized in the chick oviduct, and their synthesis is amplified by the administration of estrogen (9, 26, 27, 33). The synthesis of Z also appears to be regulated by administration of estrogen. The programmed coordination of estrogen, androgen, and progestin action could be accomplished by the synthesis of a common receptor precursor protein. Fine tuning of such a regulatory system might involve differential posttranslational processing of such a precursor into mature receptor proteins, perhaps in a specific sex steroid-directed manner. Although we presently have no evidence from *in vitro* studies that Z can be

converted into a true steroid hormone receptor, this precursor-product relationship is currently under investigation.

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1. Jensen, E. V. & DeSombre, E. R. (1972) *Annu. Rev. Biochem.* **41**, 203-230.
2. O'Malley, B. W. & Means, A. R. (1974) *Science* **183**, 610-620.
3. Baulieu, E.-E. (1975) *Mol. Cell. Biochem.* **7**, 157-174.
4. Yamamoto, K. R. & Alberts, B. M. (1976) *Annu. Rev. Biochem.* **45**, 721-746.
5. Smith, R. G., Clarke, S. G., Zalta, E. & Taylor, R. N. (1979) *J. Steroid Biochem.* **10**, 31-35.
6. Gibbons, W. E., Buttram, V. C., Jr., Besch, P. K. & Smith, R. G. (1979) *Am. J. Obstet. Gynecol.* **135**, 799-803.
7. Clark, J. H., Hardin, J. W., Upchurch, S. & Eriksson, H. (1978) *J. Biol. Chem.* **253**, 7630-7634.
8. Watson, C. S. & Clark, J. H. (1980) *J. Receptor Res.* **1**, 91-111.
9. Taylor, R. N., Swaneck, G. E. & Smith, R. G. (1980) *Biochem. J.* **192**, 385-393.
10. Taylor, R. N. (1981) Dissertation (Baylor College of Medicine, Houston, TX).
11. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660-672.
12. Rosenthal, H. E. (1967) *Anal. Biochem.* **20**, 525-532.
13. Altman, P. L. & Dittmer, D. S., eds. (1972) *Biology Data Book* (Fed. Am. Soc. Exp. Biol., Bethesda, MD), 2nd Ed., pp. 390-391.
14. Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93.
15. Korenman, S. G. (1969) *Steroids* **13**, 163-177.
16. Erdos, T., Bessada, R. & Fries, J. (1969) *FEBS Lett.* **5**, 161-164.
17. Pietras, R. J. & Szego, C. M. (1979) *J. Steroid Biochem.* **11**, 1471-1483.
18. Rice, R. H., & Means, G. E. (1971) *J. Biol. Chem.* **245**, 831-832.
19. Munson, P. J. & Rodbard, D. (1979) *Endocrinology* **105**, 1377-1381.
20. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254.
21. Kuhn, R. W., Schrader, W. T., Smith, R. G. & O'Malley, B. W. (1975) *J. Biol. Chem.* **250**, 4220-4228.
22. Smith, R. G. & Schwartz, R. J. (1979) *Biochem. J.* **184**, 331-343.
23. Taylor, R. N. & Smith, R. G. (1979) *Biochem. Biophys. Res. Commun.* **91**, 136-142.
24. Sutherland, R., Mester, J. & Baulieu, E.-E. (1977) *Nature (London)* **267**, 434-435.
25. Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658-666.
26. Mester, J. & Baulieu, E.-E. (1977) *Eur. J. Biochem.* **72**, 405-414.
27. Harrison, R. W. & Toft, D. O. (1973) *Biochem. Biophys. Res. Commun.* **55**, 857-863.
28. Milgrom, E., Atger, M. & Baulieu, E.-E. (1973) *Biochim. Biophys. Acta* **320**, 267-283.
29. Salhanick, A. R., Vito, C. C., Fox, T. O. & Callard, I. P. (1979) *Endocrinology* **105**, 1388-1395.
30. Corvol, P. & Bardin, C. W. (1973) *Biol. Reprod.* **8**, 277-282.
31. Palmiter, R. D., Catlin, G. H. & Cox, R. F. (1973) *Cell Diff.* **2**, 163-170.
32. Shahabi, N. A., Norton, H. W. & Nalbandov, A. V. (1975) *Endocrinology* **96**, 962-968.
33. Toft, D. O. & O'Malley, B. W. (1975) *Endocrinology* **90**, 1041-1045.

* Taylor, R. N., Smith, R. G. & Corriere, J. N., Jr. (1981) American Urological Association, 1981 Annual Meeting, Abstr. 51, p. 104.