Estrogen-binding Protein in Mouse and Rat Adrenal Glands*

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Cytosol from rat and mouse adrenal glands has been shown to contain a macromolecule which binds estradiol, but not corticosterone or testosterone. The receptor-estrogen complex has a sedimentation coefficient of 8 S when centrifuged on low salt sucrose gradients. Centrifugation on 0.4 M KCl gradients results in an 8 S → 4 S transformation. The estrogen-binding entity is, at least partially, a protein since no receptor-estrogen complex can be detected on sucrose gradients after trypsin digestion. On gel chromatography on Sephadex G-200 the 8 S form is eluted in the void volume, whereas the 4 S form is included in the gel matrix and elutes as a protein with M, of 95,000 and a Stokes radius of 41 Å. Estradiol binding to the cytosol receptor at 0° is very rapid and the constant Kd calculated from Scatchard plots is 2 to 4 × 10^-9 M-1. With intact adrenals at 25° the hormone is bound and translocated to the nucleus; the subcellular distribution of the receptor-estrogen complex is 85% in the nucleus and 15% in the cytosol. Sucrose gradient analysis of the nuclear KCl extracts reveals the presence of a receptor-estrogen complex with a sedimentation coefficient of 5 S. No significant differences in the characteristics of the estrogen-binding protein in the two animal species were observed. One adrenal cell contains approximately 1200 estrogen binding sites. The presence of an adrenal estrogen-binding protein with properties similar to those of other steroid receptors in steroid-responsive tissues provides a basis for an understanding of some of the observed effects of sex steroids on the physiology of the adrenal gland.

Numerous studies over the past four decades have provided evidence suggesting that in a number of mammals adrenal function is influenced by the gonads. For instance in the male rat, castration causes hypertrophy of the zona fasciculata and zona reticularis; this effect is inhibited by administration of estrogens (1). In contrast in the female rat oophorectomy results in a decrease in adrenal size (2). In general, in most animal species the adrenal weight is greater in the female than in the male (3). Concomitant with such anatomical changes in the adrenals are alterations in adrenal physiology, such as corticosterone production and secretion. Studies on humans with gonadal dysgenesis (4) led to similar observations. It is currently accepted that sex hormones affect various components of the pituitary-adrenocortical axis (5-8) and more recently a direct relationship of the thyroid gland to some of the estrogen effects on adrenal function has been demonstrated (9). Pituitary adrenocorticotropic hormone secretion (10), production and secretion of adrenocortical steroids (11, 12), corticosteroid binding to plasma proteins (13), and hepatic steroid metabolism (14) are some of the sites at which estrogens exert their effects; any of the above may ultimately alter the amount and chemical identity of corticosteroids circulating in the blood. Thus, alterations in the production and secretion of adrenal steroids as reflected by measurement of their concentration in blood does not necessarily imply direct effect of estrogens on the adrenal gland. Particular attention has, therefore, been given to the study of the effects of estrogens on adrenal enzymes catalyzing reactions of steroid metabolism, such as 5α-reductase, Δ3β-hydroxysteroid dehydrogenase, Δ5-isomerase, and 30α-hydroxysteroid dehydrogenase (6, 9, 11, 15-18). A direct effect of estrogens on the intra-adrenal steroid reductive pathways has been demonstrated (5, 9, 18).

It is well known that the cytoplasm of estrogen-responsive cells contains a specific estrogen receptor protein (19). Following estrogen binding to this receptor, the complex is translocated to the nucleus where it interacts with chromatin. As a result of this nuclear binding a series of biochemical reactions is initiated which eventually culminate in the observed tissue response to hormonal stimulation. By analogy, it was postulated that some of the observed estrogen effect on the function of the adrenal gland could be mediated by similar estrogen-binding proteins. A preliminary report from this laboratory (20) provided evidence for the existence of a specific estrogen receptor in the adrenal cytosol of the mouse. This report will further establish the presence of a specific estrogen-binding protein in both mouse and rat adrenal glands.

MATERIALS AND METHODS

Unless otherwise stated all chemicals used were reagent grade. [2,4,6,7-3H]Estradiol (96 Ci/mmol) was purchased from Amersham/Searle. Nonradioactive steroids were obtained from Steraloids. The purity of all labeled and unlabeled steroids used was determined by thin layer chromatography as previously described (21).

Female 21- to 23-day-old Sprague-Dawley rats and female 25-day-old Swiss albino mice were obtained from Charles River Breeding Laboratories and used 18 h after shipment. Animals were killed by cervical dislocation and the adrenals were quickly removed and placed in ice-cold Buffer A (0.01 M Tris/HCl, 0.0015 M EDTA, 0.005 M dithiothreitol, 10% glycerol, pH 7.6 at 4°).

Cytosol Preparation—Adrenals from 60 mice or 25 rats were homogenized in 2 ml of ice-cold Buffer A using a Duall glass-glass homogenizer. The pestle was gently driven by hand and five strokes.

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yielded the homogenate. The latter was centrifuged at 105,000 × g for 45 min at 2° and the cytosol (supernatant) was removed from between the floating fat layer and the crude nuclear pellet. Protein concentration of the cytosol was determined by the method of Lowry et al. (22) using bovine serum albumin standards and adjusted to the desired protein concentration with Buffer A. DNA concentration in the nuclear pellet was determined by the method of Burton (23), using calf thymus DNA as a standard.

Binding of [3H]Estradiol to Cytosol Receptor - Aliquots of cytosol were incubated for 18 h at 0° with an equal volume of Buffer A containing the appropriate concentration of [3H]estradiol with and without a 100-fold excess of nonradioactive competitor. At the end of the incubation a slurry of dextran-coated charcoal consisting of 0.3% acid-washed Norit A and 0.03% dextran in Buffer A was added to the cytosol incubations (500 μl) and, after 90 min at 0° with intermittent mixing, the samples were centrifuged at 800 × g for 10 min and the radioactivity in the supernatant was counted using Instagel scintillation fluid (Packard). Counting efficiency for tritium was 90%. No loss of estrogen binding sites by adsorption of receptor-estrogen complex to the charcoal under these conditions occurred since the addition of unlabeled estradiol. This value was subtracted from the binding obtained for the incubations without [3H]estradiol only; the difference between the two represents [3H]estradiol bound to high affinity, low capacity binding sites.

Sucrose Gradient Sedimentation - Crystalline sucrose, ribonucleic acid-free (Schwartz/Mann) was dissolved in Buffer A with and without 0.6 M KCl at pH 6.0. Linear 5 to 20% sucrose density gradients of 18.5 ml were prepared in polyallomer tubes using a Beckman density gradient former and kept at 0-4° for 1 h before use. Two-tenths milliliter of [3H]estradiol-labeled cytosol was layered on the gradient and centrifuged for 46,000 rpm in an SW 36 rotor for 16 to 19 h at 2°. 14C-Labeled bovine serum albumin (24) was included as a marker with each gradient. Individual 0.1-ml fractions were collected from the bottom and their radioactivity counted.

Nuclear Binding of Receptor - Estrogen Glands - Intact adrenal glands (15 pairs/ml) were incubated in Medium M-199, pH 7.6, containing 2.38 g of Hepes (4-2-hydroxyethyl-l-piperazineethanesulfonic acid) and 0.350 g of sodium bicarbonate/liter for 25°. The [3H]estradiol concentration was 1 × 10⁻⁸ M. A parallel incubation with a 100-fold excess of added unlabeled estradiol was carried out to determine nonspecific binding. At the end of the incubation the glands of the two incubations were rinsed three times with ice-cold Buffer A and homogenized in the same buffer in presence of an added excess of unlabeled estradiol (1 × 10⁻⁴ M). The latter competitively inhibits any further binding of [3H]estradiol to receptor sites during the homogenization procedure (35). Tissue homogenization was performed as described above. The homogenate was centrifuged at 800 × g for 10 min and the resulting supernatant subjected to further centrifugation at 105,000 × g to yield the cytosol fraction. The 800 × g nuclear pellet was washed by centrifugation three times with Buffer A, extracted for 12 h at 37° with absolute ethanol, and radioactivity in the extracts counted. Aliquots of the washed 800 × g nuclear pellet were suspended in 9 volumes of ice-cold 0.6 M KCl/Buffer A, pH 8.5, and transferred to a Dounce all-glass homogenizer. The suspension was gently homogenized every 10 min followed by centrifugation at 105,000 × g for 30 min. The resulting supernatant containing the extracted nuclear receptor [3H]estradiol complex was subjected to gradient analysis on 0.6 M KCl sucrose gradients as described above.

Gel Filtration - Sephadex G-200 was prepared in Buffer B (0.10 M Tris/HCl, 0.0015 M EDTA, 0.0005 M diethiothreitol, pH 7.6, at 4°) and allowed to swell for 5 h at 90°. After removal of fine particles by suction, a standard Pharmacia K 15/30 glass column was packed with gel to a total volume of 56 ml. Column equilibration and sample elution were performed at 5° using a constant downward flow (12 ml/h) and 1-ml fractions were collected. Standard molecules used for molecular weight determination were rabbit muscle aldolase, bovine pancreas chymotrypsinogen A, and bovine serum albumin (Pharmacia).

RESULTS

Identification of Specific Estrogen-binding Protein ("Receptor") in Cytosol of Mouse and Rat Adrenals - It is currently accepted that estrogen receptor-proteins can be traced by their ability to bind radioactive estrogen. The presence of a receptor [3H]estradiol complex in the soluble fraction of mouse and rat adrenals homogenates should thus result in a discrete radioactivity peak when homogenates are subjected to sucrose gradient ultracentrifugation. The results of one such experiment are shown in Fig. 1. When mouse adrenal cytosol was incubated with [3H]estradiol at 0° and, after treatment with dextran-coated charcoal, centrifuged on linear 5 to 20% sucrose gradients, a radioactivity peak corresponding to a macromolecule with a sedimentation coefficient of 8 S was found. Binding specificity and saturability was demonstrated by incubating aliquots of cytosol with a constant concentration of radioactive estradiol in presence of a 100-fold excess of unlabeled estradiol, testosterone, and corticosterone, respectively. The radioactivity peak corresponding to the 8 S region was completely suppressed by the addition of unlabeled estradiol, but not testosterone and corticosterone. Chemically, the estrogen-binding component appeared to be a protein, since incubation of cytosol with [3H]estradiol followed by 3 h digestion at 0° with trypsin did not result in a discrete radioactivity peak on sucrose density gradients. When cytosol containing receptor [3H]estradiol was centrifuged on sucrose gradients prepared in high salt (0.6 M KCl) a conversion from the 8 S to the 4 S form was observed (Fig. 2). Results essentially identical with those shown in Figs. 1 and 2 were obtained with cytosol of rat adrenals (data not shown).

Gel Filtration - Several attempts to further characterize the estrogen-binding protein of adrenal cytosol preparations by gel chromatography were made. In these experiments glycerol was omitted from all buffers. Rat and mouse adrenal cytosol was labeled for 1 h at 0° with 1 × 10⁻⁴ M [3H]estradiol in absence or presence of an excess of nonradioactive estradiol. Most of the free and nonspecifically bound [3H]estradiol was removed by dextran-coated charcoal. In order to avoid cytosol

![Fig. 1. Sucrose gradient centrifugation of "low salt" [3H]estradiol-labeled cytosol from mouse adrenals homogenized in Buffer A, pH 7.6. Cytosol was incubated for 2 h at 0° with 1 × 10⁻⁴ M [3H]estradiol in absence (○) or in presence of a 100-fold excess of nonradioactive estradiol (Δ), corticosterone (●), or testosterone (□). At the end of the incubation the samples were treated with dextran-coated charcoal and 0.2-ml aliquots (1.4 mg of protein) layered on 5 to 20% linear sucrose gradients in Buffer A and centrifuged for 19 h at 50,000 rpm in a Beckman SW 56 rotor. 14C-Labeled bovine serum albumin (14C-BSA) was included as 4.6 S marker protein in all gradients.](http://www.jbc.org/)

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Fig. 2. Salt-induced 8 S → 4 S conversion of receptor·estradiol complex. Mouse adrenal cytosol was prepared in Buffer A and labeled with [3H]estradiol in absence (○, C) or presence (△) of an excess of unlabeled estradiol. After treatment with charcoal, 0.2-ml aliquots (1 mg of protein) were centrifuged on linear 5 to 20% sucrose gradients in Buffer A for 16 h at 48,000 rpm in an SW 56 rotor. Centrifugation on high salt gradients (●) gives a radioactivity peak in the 4 S region. Centrifugation on low salt gradients (○) gives a radioactivity peak in the 8 S region. 14C-BSA, 14C-labeled bovine serum albumin.

Fig. 3. Chromatography of "low salt" [3H]estradiol-labeled rat adrenal cytosol. Cytosol prepared in Buffer A was labeled with [3H]estradiol (1 × 10−8 M) and treated with dextran-coated charcoal as described in the text. One milliliter (6.0 mg of protein) was applied to a Sephadex G-200 column (K 15/30). The gel was equilibrated and eluted with Buffer B. Calibration data: V0 = 20 ml; Vt = 56 ml. A, B, and C refer to the elution volumes of aldolase (27 ml), bovine serum albumin (30.6 ml), and chymotrypsinogen A (36 ml).

In the previous section we have shown that upon centrifugation on high salt sucrose density gradients the 8 S receptor·estradiol complex was transformed into a 4 S sedimenting entity (Fig. 2). Since it is reasonable to assume that such alteration in sedimentation rate is related to changes in protein-protein interactions resulting from modifications of environmental conditions, experiments were carried out to establish whether or not the larger molecular complexes generated in low salt buffer could be transformed to smaller molecular weight entities by use of 0.4 M KCl buffer. Only rat adrenals were used in these experiments. Tissue from 25 animals was homogenized in 1 ml of Buffer A and following centrifugation at 800 × g for 10 min the supernatant was diluted with an equal volume of 0.8 M KCl/Buffer A. Centrifugation at 105,000 × g for 30 min yielded the high speed supernatant (cytosol). Aliquots (0.9 ml) of the latter were added to 0.1 ml of 0.4 M KCl/Buffer A containing [3H]estradiol with and without an added excess of unlabeled estradiol. The final concentration of the radioactive hormone was 1 × 10−8 M. The conditions for incubation and subsequent treatment with charcoal have been described above. One-milliliter aliquots (6 mg of protein) of estrogen-labeled cytosol were chromatographed on Sephadex G-200 columns equilibrated with 0.4 M KCl/Buffer B and eluted with this buffer. Column calibration with protein standards was performed before and after each cytosol run under identical conditions. The elution patterns obtained are plotted in Fig. 4. The subtraction of the radioactivity profile of cytosol labeled in presence of an excess of nonradioactive estradiol (Graph 2) from that of cytosol incubated only with [3H]estradiol (Graph 1) results in the graph shown as a dashed line. The small peak which is eluted in the void volume probably consists of nontransformed 8 S receptor·estradiol complex or aggregates thereof. The included peak with a maximum at 26 ml represents specifically bound...
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radioactivity and is thought to correspond to that form of receptor-estrogen complex which upon sucrose gradient analysis in high salt medium has a sedimentation coefficient of 4 S (Fig. 2). Using the column calibration data given in Fig. 4 one can calculate that the peak at 26 ml coincides with a macromolecule with a $M_0$ of 95,000 and a Stokes radius of 41 A. Although smaller subunits of estrogen receptors have been identified (26) at the moment we do not attribute particular significance to the shoulder at 29 ml and to the small peak at about 33 ml. It should be emphasized that the dotted line was calculated by subtraction of elution profiles obtained by chromatographing on the same gel first the sample labeled with $[^3H]$estradiol (Graph 1) and then after approximately 5 h the sample labeled in presence of an excess of nonradioactive estradiol (Graph 2). Thus, "aging" of the second sample together with possible variations in the column properties between experiments might result in small shifts in elution Profile 2. Consequently small peaks and shoulders on the dashed graph are not necessarily suggestive of individual specific estrogen-binding entities. The above experiment was repeated three times. In each instance a minor peak at $V_e$ and a major peak between protein markers A and B was obtained; in contrast the elution volumes of smaller shoulders and peaks were not reproducible. As in the previous experiment (Fig. 3) the elution profile shown on Fig. 4 indicates that a considerable amount of free estradiol is eluted at the end of the run. Since the cytosol had been treated with charcoal, only a small amount of free estradiol was present when the sample was applied to the column. Gel chromatography was performed in steroid-free medium under nonequilibrium conditions; the dissociation of $[^3H]$estradiol from its binding protein will therefore result in free radioactivity eluting at the end of the column. The quantity of this free radioactivity will be smaller for the sample which does not contain $[^3H]$labeled estrogen-receptor complex (Graph 2). Thus, upon subtraction from Graph 1, a peak in the 47-ml region is obtained which does not represent specifically bound estradiol.

Determination of Binding Affinity of Cytosolic Receptor Protein for Estradiol—A common feature of steroid receptor-proteins is the high equilibrium association constant ($K_a$) for the reaction (receptor) + (steroid) $\rightleftharpoons$ (receptor-steroid). We therefore performed several experiments in order to determine the $K_a$ of the adrenal estrogen receptor. Cytosol was prepared in Buffer A as described and 0.1-ml aliquots were added to 0.1 ml of the same buffer containing the appropriate concentrations of $[^3H]$estradiol or $[^3H]$estradiol plus a 100-fold excess of unlabeled estradiol. Specific binding was determined by use of the dextran-coated charcoal technique. Preliminary experiments were performed to assess the time required for equilibrium binding to occur in those cytosol incubations containing the lowest concentration of radioactive estradiol ($1 \times 10^{-10}$ M). It was found that within 6 h at 0°C maximum binding was attained. Furthermore the stability of the receptor-estradiol complex was measured and, as shown in Fig. 5, estradiol is rapidly bound to the receptor and no significant loss of receptor-estradiol complex occurs between 6 and 16 h of incubation. Routinely, equilibrium binding curves were generated by incubating cytosol receptor preparations for 16 h at $[^3H]$estradiol molarities ranging from $1 \times 10^{-10}$ to $1 \times 10^{-9}$ M. A typical plot of binding data of one such experiment is given in Fig. 6. A hyperbolic curve was obtained. Linear transformation of the hyperbolic binding curve according to the method of Scatchard (27) is shown in the inset; from the slope a $K_a$ of $2.4 \times 10^{-8}$ M$^{-1}$ was calculated; from the intercept on the abscissa approximately 20 fmol of estradiol/mg of protein were specifically bound. Several equilibrium binding experiments were performed and no significant differences between rat and mouse adrenal cytosol were found: the $K_a$ ranged from 2.4 to 3 $\times 10^{-8}$ M$^{-1}$; the total number of estradiol binding sites per mg of protein ranged from 25 to 30 fmol. One rat adrenal gland contained approximately 3 times the amount of receptor sites present in the mouse adrenal (5 fmol of estradiol bound versus 1.5 fmol). However, on a DNA basis the same concentration of binding sites was found, since the rat adrenal contained 18 $\mu$g of DNA versus 5.7 $\mu$g of DNA in the mouse. Similarly, the wet weight of rat adrenals was 3 times that of the mouse (10 mg versus 3.2 mg).

Subcellular Distribution of Receptor-Estradiol Complex and Characterization of Nuclear Receptor—Since it is postu-

Fig. 4. Gel chromatography of "high salt" $[^3H]$estradiol-labeled rat adrenal cytosol. Cytosol prepared in Buffer A/KCl buffer was labeled with $[^3H]$estradiol ($1 \times 10^{-8}$ M) in presence (Graph 2) or absence (Graph 1) of an excess of unlabeled estradiol and treated with dextran-coated charcoal as described in the text. One milliliter (6.0 mg of protein) was applied to a Sephadex G-300 column (K 15/30). The gel was equilibrated and eluted with Buffer B containing 0.4 M KCl. The dashed line is obtained by subtracting the values of Graph 2 from those of Graph 1. Calibration data: $V_e$ = 16 ml; $V_s$ = 56 ml. A, B, and C refer to the elution volumes of aldolase (23 ml), bovine serum albumin (28 ml), and chymotrypsinogen A (36 ml).

Fig. 5. Time course of $[^3H]$estradiol binding to mouse adrenal cytosol receptor. Cytosol was incubated at 0°C with $1 \times 10^{-8}$ M $[^3H]$estradiol with and without an excess of nonradioactive estradiol for the indicated periods of time. Samples were treated with charcoal as described. Only specific binding is shown in the graph.
was extracted with 0.6 M KCl. Parallel incubations with an added excess of unlabeled estradiol were carried out to determine nonspecific binding. At the end of the incubation the samples were treated with charcoal as described. The binding data represent specifically bound estradiol. The inset represents a Scatchard plot of the same data. The intercept on the abscissa corresponds to 20 fmol/mg of protein.

It was found that at saturating concentration of estradiol (2 x 10^-9 M) 85% of the specifically bound [3H]estradiol is concentrated in the cell nucleus, since 1.5 fmol/adrenal were in the nuclear fraction and 0.3 fmol/adrenal in the cytoplasm. The cytosol of one mouse adrenal gland contained approximately 65 µg of protein; the binding site concentration reported above was 25 to 30 fmol/mg of protein. Thus the cytosol-binding capacity of one gland should be in the range of 1.6 to 2 fmol. This correlates with the total binding found at 25°C (1.8 fmol) and substantiates the assumption that, as in other estrogen-responsive tissues, for the estrogen receptor protein interacts with some nuclear component in order to promote transcriptional events. We have also found that the estrogen·receptor protein binds with high affinity to form a stable receptor·estradiol complex: ultracentrifugation on low salt sucrose gradients (Fig. 1) reveals that this complex has a sedimentation coefficient of 8 S. Binding specificity was demonstrated by competition experiments with unlabeled estradiol, testosterone, and corticosterone: only estradiol inhibited binding of [3H]estradiol. When the cytosol prepared and labeled in hypotonic buffer was centrifuged on 0.6 M KCl sucrose gradients, complete conversion of the 8 S complex to a 4 S sedimenting entity was observed (Fig. 2). The estrogen binding moiety is, at least partially, a protein since a radioactivity peak was absent in sucrose gradients of estrogen-labeled cytosol preparation digested with trypsin. Gel filtration experiments showed that, based on the calibration data with protein standards of known molecular weight, the 8 S estrogen receptor has a Mr > 200,000 since it is eluted in the void volume. However, 8 S to 4 S transformation induced by 0.4 M KCl resulted in dissociation of the 8 S receptor into smaller entities with a Mr = 95,000 and a Stokes radius of approximately 41 A (Fig. 4). Although meaningful comparisons of these parameters with those reported for purified or partially purified steroid receptor-proteins from other tissues are not possible at the moment due to the heterogeneity of our preparations, it appears that the adrenal receptor consists of subunits. The data presented in Figs. 1 to 4 show that the receptor·estradiol complex undergoes considerable dissociation when subjected to sucrose gradient centrifugation or gel chromatography. Both systems represent a nonequilibrium situation in which the receptor molecule migrates into steroid-free media; upon dissociation of estrogen, reassociation with the labeled ligand is prevented by sedimentation or gel exclusion. Since the cytosol preparations analyzed in these experiments had been treated with charcoal, we conclude that the unbound [3H]estradiol recovered on top of the sucrose gradients or eluted in the internal volume of the Sephadex gel columns is mainly derived from dissociated receptor·estradiol complex. Our finding that addition of glycerol to all buffers used resulted in higher yields of receptor might reflect either a

**DISCUSSION**

As mentioned in the introduction, it appears that gonadal hormones have a direct effect on the physiology of the adrenal gland. Since it is well recognized that binding of steroid hormones to a specific intracellular receptor protein is a prerequisite for their action on target tissues, we attempted to identify and characterize the estrogen receptor protein in the rat and mouse adrenal.

![FIG. 6. Equilibrium binding of [3H]estradiol to the mouse cytosol receptor. Aliquots of cytosol were incubated at 0° for 16 h with the indicated concentrations of [3H]estradiol. The final protein concentration was 9.6 mg/ml. Parallel incubations with an added excess of unlabeled estradiol were carried out to determine nonspecific binding. At the end of the incubation the samples were treated with charcoal as described. The binding data represent specifically bound estradiol. The inset represents a Scatchard plot of the same data. The intercept on the abscissa corresponds to 20 fmol/mg of protein.](http://www.jbc.org/)

![FIG. 7. Sucrose gradient centrifugation of the nuclear receptor·estradiol complex. Intact mouse adrenal glands were incubated at 25° with [3H]estradiol in presence or absence of unlabeled estradiol as described in the text. Nuclear 0.6 M KCl extracts were layered on linear 5 to 20% sucrose gradients prepared in 0.6 M KCl and centrifuged for 18 h at 46,000 rpm in an SW 56 rotor. • total binding; □, nonspecific binding.](http://www.jbc.org/)
stabilizing effect of this reagent on the receptor molecule itself or an inhibitory effect on the dissociation of the receptor-estrogen complex. The kinetic and equilibrium-binding data presented in Figs. 5 and 6 are typical for protein-ligand interactions of high affinity. Estradiol binding is rapid and saturable, with a very high equilibrium association constant (2 to $4 \times 10^{7}$ M$^{-1}$).

When the dynamics of hormone-cell interaction were investigated in the intact tissue, it was found that upon estradiol binding to the cytosol receptor the complex formed is translocated to the nucleus. Since in these experiments we have shown that the total number of estrogen binding sites recovered in nuclear and cytosolic subcellular fractions closely approximated that in adrenal cytosol preparations labeled with estrogen at 0", we conclude that upon binding to the hormone translocation of receptor-estrogen complex to the nucleus occurred. At saturating concentrations of estradiol the subcellular distribution was 85% in the nucleus and 15% in the cytosol. Finally, sucrose gradient analysis of nuclear KCl extracts revealed that this translocation is associated with a conformational change of the estrogen-binding protein, which now has a sedimentation coefficient of 5 S (Fig. 7).

Studies on the general mechanism of action of steroid hormones (28, 29) have shown that steroids bind to a specific cytoplasmic receptor with subsequent translocation of the complex into the nucleus where it binds to chromatin and initiates RNA synthesis. The experiments described in this paper demonstrate that in the adrenal gland also the initial event of estradiol action involves binding to a cytoplasmic receptor protein with subsequent nuclear translocation of the complex. Further studies will have to be awaited in order to determine whether these reactions are followed by chromatin binding and induction of DNA synthesis.

Since the rat adrenal gland contains approximately 18 $\mu$g of DNA, and one cell, approximately 7 pg of DNA, one gland consists of 2.5 $\times$ 10$^{10}$ cells. Furthermore, we reported here that one gland has an average of 5 fmol of estradiol binding sites. Thus, using Avogadro's number, one can calculate that one adrenal cell contains 1200 estrogen binding sites. This concentration is relatively low if compared with data from other estrogen target tissues (30, 31). However, it should be kept in mind that the adrenal gland consists of a variety of cell types with different physiological functions. It is possible that only certain cell populations are modulated by estrogens in their physiological activity and are those containing estrogen receptors. Consequently the calculated cellular concentration of estrogen binding sites would increase considerably to approach levels comparable to those of cells from other estrogen target tissues. A generalization of this concept would imply that the traditional classification of tissues into target and nontarget tissue based exclusively on receptor concentration/organ wet weight or total DNA might be misleading, since cell heterogeneity is not taken into account. For example, it has been demonstrated that kidney and heart muscle, previously used as control nontarget tissues for estrogens (32, 33), contain specific estrogen receptors (34, 35). Finally, the concept that a target tissue is always characterized by the presence of a receptor for the specific hormone under investigation is rather restrictive. For instance, a variety of effects of sex steroids on the adrenal gland are mediated through the pituitary and thyroid gland and do not require an adrenal estrogen-receptor.

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Note Added in Proof—Similar results have been obtained by Cutler et al. and will appear in Cutler, G. B. et al. (1978) Endocrinology, in press.

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