Estrogen-Receptor Interaction

Estrogenic hormones effect transformation of specific receptor proteins to a biochemically functional form.

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One of the challenging problems confronting biological scientists has been the manner in which hormones serve as regulators of biochemical processes in tissues of higher animals. For most types of endocrine agents the physiologic effects have been known for many years, but only recently has insight been gained concerning the detailed biochemical mechanisms by which some of these actions are mediated.

Two general patterns of hormone-cell interaction have so far been recognized. In the first, illustrated by epinephrine and by many types of peptide hormones, the hormone reacts with membrane-bound nucleotide cyclase systems to stimulate the conversion of a nucleoside triphosphate, such as adenosine triphosphate (ATP), to the corresponding 3′5′-monophosphate, for example, cyclic adenosine monophosphate (cAMP) (*1*). The cyclic nucleotide then serves as a "second messenger," reacting with appropriate cellular entities to deliver and amplify the regulatory signal. In the second pattern of interaction, which appears operative with the various types of steroid hormones (*2*), the hormone enters the cell and binds to a specific extranuclear "receptor" protein, characteristic of the responsive or "target" cell. The resulting steroid-protein complex then migrates to the nucleus where specific RNA synthesis is initiated or accelerated, leading in the case of the estrogenic hormones to eventual tissue growth. With the estrogens, and probably with other steroid hormones as well, hormone induced translocation to the nucleus involves an alteration of the receptor protein, a phenomenon we have called "receptor transformation" (*2*).

In this article we summarize current knowledge about the interaction of estrogens with receptor proteins in hormone responsive organs, such as uterus, and the relation of receptor transformation to early biochemical responses elicited in the cell. More detailed information with original references is provided in recent reviews (*3*).

Estrogen Binding in Target Tissues

The presence in estrogen responsive tissues of characteristic hormone-binding components, now called estrogen receptors or estrophiles, was first indicated by the striking affinity of these tissues for the hormone. After administration of physiologic amounts of tritiated estradiol (Fig. 1) to immature rats (*4*) or tritiated hexestrol to young goats and sheep (*5*), the uterus, vagina, and anterior pituitary take up and retain radioactive hormone from the blood, against a large concentration gradient (Fig. 2). Despite extensive metabolism of estradiol in the animal giving rise to a variety of metabolites in the blood, only unchanged estradiol is taken up by the uterus of the immature rat (*6*) or mouse (*7*). The inference that estradiol binds to receptor substances and initiates uterine growth without itself undergoing chemical conversion has proved consistent with much subsequent evidence and served to direct investigative attention away from earlier considerations that had linked estrogen action to steroid metabolism.

Evidence that the uterotrophic action of estradiol depends on its binding to receptors was first provided by experiments with specific binding inhibitors. Certain substances, such as ethamoxytriphetol (MER-25, Richardson-Merrell), clomiphene, nafoxidine (Upjohn-11,100), and CI-628 (Parke-Davis) (Fig. 1), which had been known (*8*) to inhibit the uterotrophic action of estradiol, were shown to prevent the characteristic uptake of estrogens by target tissues in vivo (*9, 10*). When varying doses of nafoxidine are administered to immature rats receiving tritiated estradiol, there is a quantitative correlation between reduction of hormone uptake by the uterus and inhibition of its growth response (*10*). In contrast to the foregoing agents, actinomycin D and puromycin, which also block the uterotrophic action of estradiol (*11*), do not decrease the uptake and binding of the hormone (*10*), suggesting that these antagonists act at later stages in a sequence of biochemical events in which the hormone-receptor interaction is an early if not the initial step.

When excised uterine tissue is exposed to dilute solutions of tritiated estradiol at physiologic temperatures in vitro, a hormone-receptor interaction occurs that shows the principal characteristics of that observed in the whole animal (*12, 13*). Comparison of hormone uptake in the presence and absence of those antagonists that prevent hormone-receptor association in vivo (*13, 14*) permits distinction between specific interaction and the nonspecific binding that estradiol shows with target and nontarget tissues alike (Fig. 3). The system in vitro has provided a valuable tool for studying various features of the hormone-receptor interaction in whole tissues, including its dependence on sulfhydryl groups (*15*) and the influence of temperature to be discussed later.

The foregoing studies of the fate of physiologic amounts of estrogenic hormones with intact mammalian tissues in vivo and in vitro, confirmed and extended by many other careful investigations (*3*), served to establish the principal characteristics of the interaction of estrogens with specific receptors in target cells and provided a basis for evaluating the significance of estrogen binding phenomena observed with broken cell systems where artifacts of nonspecific binding can occur. As more detailed investigations were conducted, it became evident that the interaction of estradiol with uterine cells is not a simple association of the

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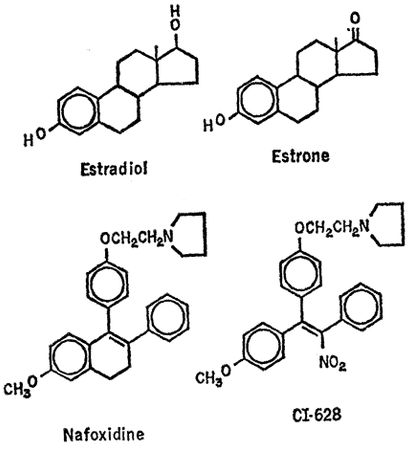
Fig. 1. Structures of the estrogens, estradiol and estrone, and two anti-estrogens, nafoxidine (Upjohn-11,000) and CI-628 (Parke-Davis).



hormone with a single binding site. As described below, radioactive hormone was found to be localized in two separate regions of the uterine cell. Moreover, determination of estradiol incorporation as a function of time and administered dose indicated two distinct binding phenomena in target but not in nontarget tissues: initial uptake, not saturable even at considerably hyperphysiologic doses of hormone, and retention, saturable at physiologic hormone doses (*16*). Study of the relation between these two processes and two binding sites led to the formulation of a two-step mechanism which has come to provide a model for the interaction of other classes of steroid hormones with their respective target tissues.

Estrogen-Receptor Complexes

When uterine homogenates from estradiol-treated rats are subjected to differential centrifugation, the incorporated steroid appears in two cellular fractions (*16, 17*). Most of the hormone is found in the nuclei, with a smaller amount (20 to 30 percent) present in the high-speed supernatant or cytosol fraction. The predominance of nuclear binding, controversial in early reports (*17*), is confirmed by autoradiographic studies (*13, 18*), in which a dry-mount procedure is used (*19, 20*) that minimizes steroid translocation during tissue processing (Fig. 4).

The estradiol taken up by rat uterus is associated with a different form of the receptor substance in the cytosol than in the nucleus. The application (*21*) of ultracentrifugation in sucrose density gradients for characterizing estrogen-receptor complexes provided a valuable means for distinguishing between different forms of the receptor. By this technique the radioactive hormone in the cytosol was found to sediment as a discrete band (*21*) with a coefficient originally reported as 9.5*S* but later shown to be close to 8*S* (Fig. 5A). In sodium or potassium chloride of concentration above 0.2*M*, the 8*S* complex is reversibly dissociated into subunits (*22*), originally called 5*S* but later shown (*23*) to sediment at about 4*S*, just behind bovine plasma albumin (Fig. 5B).

The estradiol bound in the nucleus appears to be associated with chromatin (*24*). The nuclear hormone can be solubilized, unaccompanied by DNA, by extraction with 0.3*M* KCl at *p*H 7.5 (*13*) or, more effectively, by 0.4*M* KCl at *p*H 8.5 (*25*), to yield an estradiol-receptor complex which, in the presence of salt, sediments at about 5*S*, that is, slightly faster than bovine plasma albumin. On removal of salt, the nuclear complex usually aggregates to an 8*S* to 9*S* form but can lose this property on aging or partial purification. As shown in Fig. 5B, the nuclear complex is readily distinguished from the cytosol complex by careful ultracentrifugation in salt-containing sucrose gradients (*23*); this difference in sedimentation rate provided the first criterion for recognizing the phenomenon of receptor transformation.

Recent experiments described below indicate that the sedimentation coefficients of the cytosol and nuclear complexes, determined in salt-containing sucrose gradients, are close to 3.8*S* and 5.2*S*, respectively. For simplicity, the approximate values of 4*S* and 5*S* are used in this article.

The 8*S* estradiol-receptor complex, or its 4*S* subunit, forms directly in the cold when estradiol is added to the cytosol fraction of uteri not previously exposed to hormone (*13, 26*). Thus, the receptor content of the cytosol is easily estimated by adding sufficient

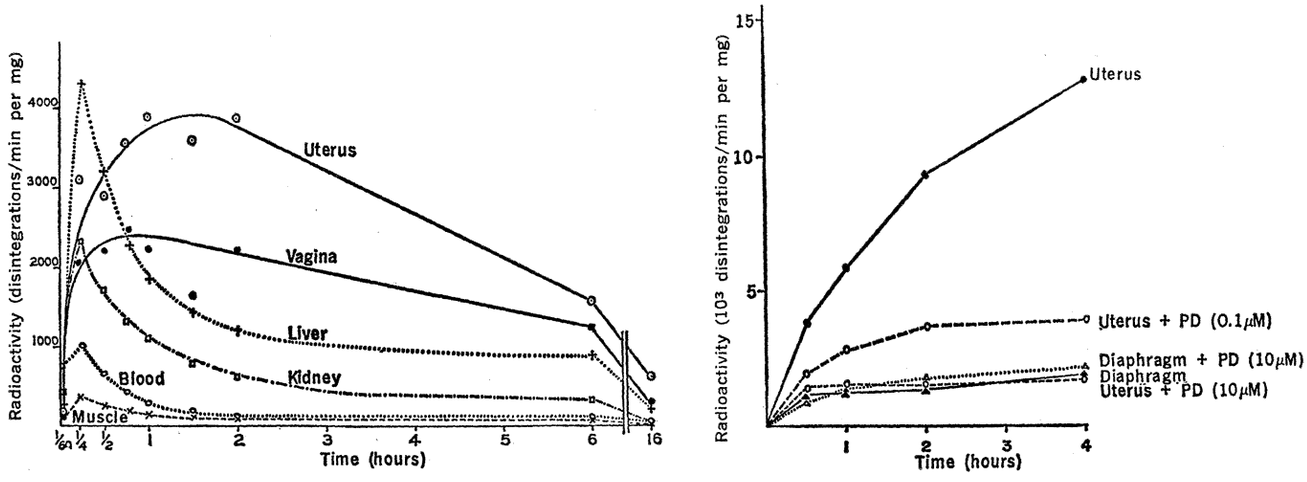


Fig. 2 (left). Uptake of tritiated estradiol by various tissues of immature rats receiving a single subcutaneous injection of 0.098 μg (11.5 μc) of [6,7-^{3}H]estradiol in 0.5 ml of saline. Total radioactivity expressed as disintegrations per minute per milligram of dry tissue or per 5 μl of blood. Because blood contains a mixture of radioactive metabolites but uterus and vagina incorporate only estradiol, the ratio of estradiol concentration between uterus and blood is about 500 to 1. Details in (*4*). Fig. 3 (right). Uptake of [^{3}H]estradiol by uterine and diaphragm tissue of immature rats after incubation in 0.12 n*M* [^{3}H]estradiol (57 c/mmole) at 37°C in Krebs-Ringer-Henseleit buffer, *p*H 7.3, in the presence and absence of different concentrations of an estrogen antagonist, CI-628 (PD) [from Jensen *et al.* (*56*)].

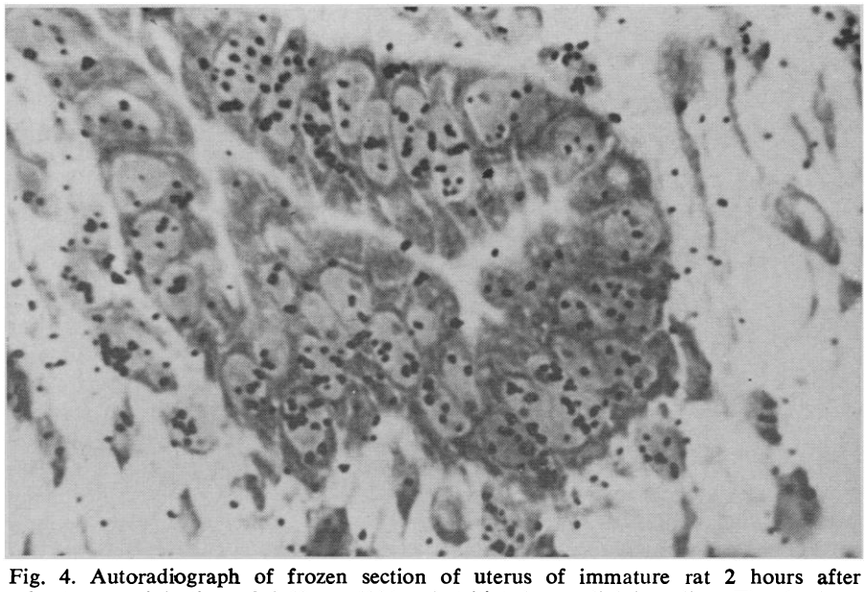


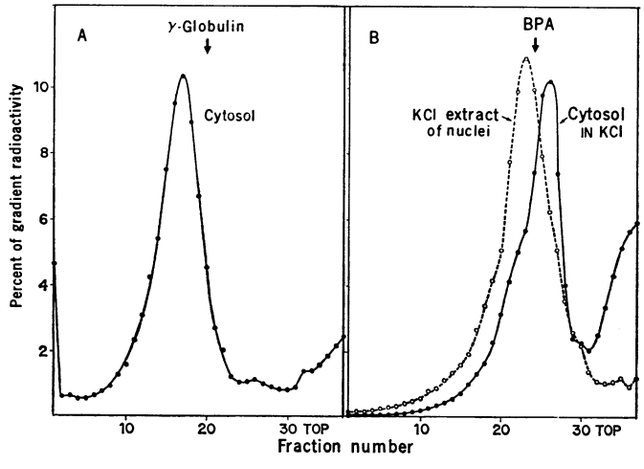
Fig. 4. Autoradiograph of frozen section of uterus of immature rat 2 hours after subcutaneous injection of 0.63 μg (131 μc) tritiated estradiol in saline. The 1-micrometer section showing epithelial gland and lamina propria was exposed to the emulsion 42 days and stained with methyl green-pyronin. Predominantly nuclear localization is also seen in myometrium. Reproduced from Stumpf and Roth (*20*), courtesy of Plenum Press.

tritiated estradiol to saturate the binding sites and determining the radioactivity present in the 8*S* sedimentation peak. This interaction of estradiol with the cytosol receptor is prevented by the presence of nafoxidine or CI-628.

From the sensitivity of their complexes to proteases but not to nucleases, the estrogen binding substances of both cytosol (*21*) and nucleus (*2, 25*) appear to be mainly protein in composition. They are somewhat unstable in crude extracts, tending to aggregate and to decompose during storage or attempted purification. Addition of calcium ions to the salt-dissociated complex of uterine cytosol, prepared in the presence of ethylenediaminetetraacetic acid (EDTA), yields a stabilized 4.5*S* form of the binding unit that is resistant to aggregation and does not revert to the 8*S* state on removal of salt (*27, 28*). This stabilization appears to result from the activation by calcium of an enzyme, present in uterine cytosol and rather unfortunately called "receptor transforming factor," that acts on the receptor protein to destroy its ability to aggregate in low salt concentrations (*28*).

Though noncovalent, the binding of estradiol to receptor proteins of uterine tissue is remarkably strong; association constants varying from 10^9 to $10^{12} M^{-1}$ have been reported for the cytosol complex (*3*). This tight binding appears to result from a slow rate of dissociation (*29*); once formed, the complex does not readily lose estradiol in the cold except by receptor decomposition or denaturation.

By a combination of salt precipitation, gel filtration, ion-exchange chromatography, and disc gel electrophoresis, microgram quantities of both the calcium-stabilized complex from calf uterine cytosol and the 5*S* complex extracted from nuclei have been obtained in apparently pure form; these purified products show clear differences in mobility on gel electro-



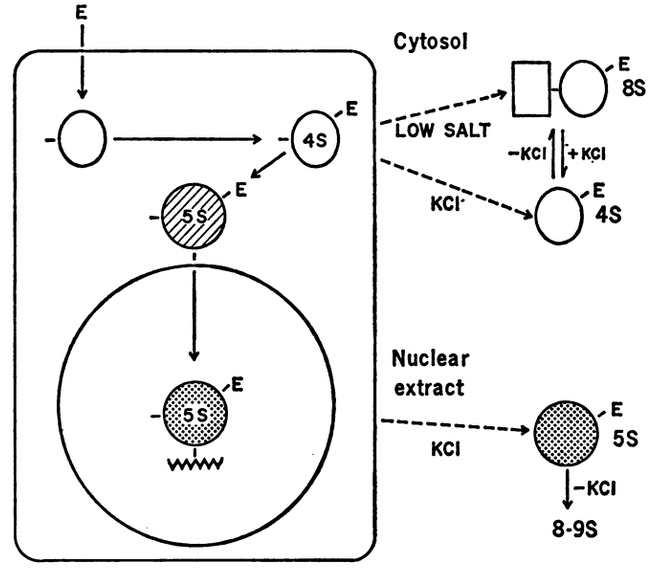


Fig. 5 (left). Sedimentation patterns of radioactive estradiol-receptor complexes of cytosol in tris-EDTA buffer [10 m*M* tris-(hydroxymethyl)aminomethane, 1.5 m*M* EDTA, *p*H 7.4] and nuclear extract (0.4*M* KCl in tris-EDTA, *p*H 8.5) from uteri of immature rats 1 hour after subcutaneous injection of 0.1 μg (20.8 μc) [^{3}H]estradiol. To saturate its receptor capacity, the cytosol fraction was made 5 n*M* with additional tritiated estradiol. Centrifugation was carried out at 2°C for 12 hours; (A) at 308,000*g* in 10 to 30 percent sucrose containing *p*H 7.4 tris-EDTA; (B) at 284,500*g* in 5 to 20 percent sucrose containing 0.4 m*M* KCl in tris-EDTA, *p*H 8.5. Globulin and BPA indicate positions of markers for 7.0*S* (bovine γ-globulin) and 4.6*S* (bovine plasma albumin) fractions. Fig. 6 (right). Schematic representation of interaction pathway of estradiol (*E*) in uterine cell. Diagram at left indicates uterine cell with extranuclear estradiol-receptor complex undergoing transformation and entering nucleus to bind to chromatin. Diagrams at right indicate sedimentation properties of complexes extracted from the cell.

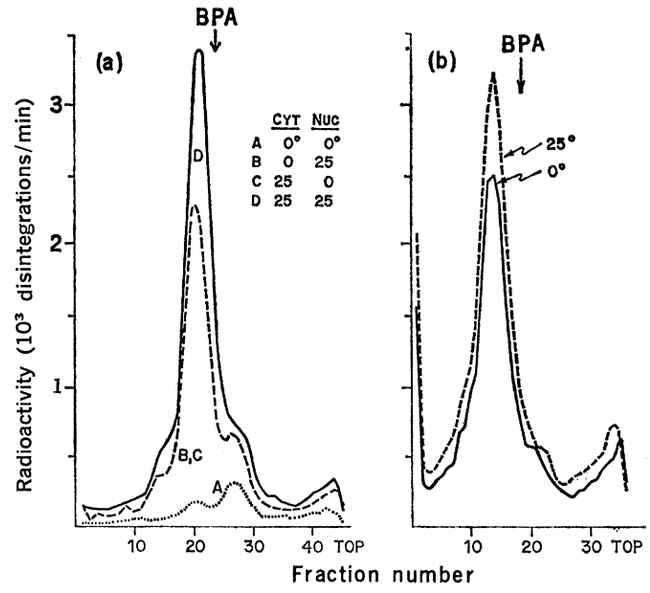


Fig. 7. Comparison of native and transformed complexes from calf endometrium cytosol in their binding to sucrose-purified nuclei from calf endometrium. Nuclei were incubated for 60 minutes at either 0° or 25°C: (a) with cytosol that had previously been incubated with 5.6 n*M* [^{3}H]estradiol for 45 minutes at either 0° (A, B) or 25°C (C, D); or (b) with redissolved ammonium sulfate precipitate from an unheated mixture of [^{3}H]estradiol and cytosol (final concentration of [^{3}H]estradiol, 5.6 n*M*). The separated nuclei were extracted with 0.4*M* KCl, and the extracts centrifuged on salt-containing sucrose gradients. Successive 100 microliter fractions of the gradient were counted. BPA indicates the position of bovine plasma albumin marker. Details in (*30*) and (*42*).

phoresis as well as in sedimentation properties (*30*). With partially purified complexes of calf uterine cytosol, estimates of 200,000 and 5.8 for molecular weight and isoelectric point, respectively, were found for the 8*S* complex, compared to 75,000 and 6.4 for the calcium-stabilized 4.5*S* unit (*27*); in another study, respective values of 238,000 and 6.2 for the 8*S* complex and 61,000 and 6.6 plus 6.8 for the 4.5*S* unit were obtained (*28*).

Two-Step Interaction Mechanism

A major advance in the understanding of the interaction of steroid hormones with target cells came with the recognition that the estradiol-receptor complex of the uterine nucleus is derived from the cytosol by a temperature-dependent process in which association with the hormone activates the cytosol receptor protein to migrate to the nucleus (Fig. 6). This two-step interaction pathway is supported by evidence from a variety of experiments.

A relation between the two intracellular sites of estrogen localization was first indicated by observations that a given dose of nafoxidine in vivo inhibits cytosol and nuclear binding in the rat uterus to the same degree and that there is a difference in saturability between estradiol uptake and its retention (*16*). These findings led to the suggestion that the cytosol protein, which is present in reserve amounts and binds spontaneously with estradiol, might serve as an "uptake receptor," that delivers the hormone to the nucleus for retention (*13*). In the autumn of 1967, the concept of a two-step mechanism, in which the cytosol complex is translocated to the nucleus, was put forth independently at the Laurentian Hormone Conference and at a meeting of the National Academy of Sciences. One proposal (*31*) was based on the temperature dependent shift of extranuclear to nuclear radioactivity with accompanying loss of cytosol binding in rat uteri previously exposed to tritiated estradiol at 0°C in vitro, while the other (*32*) arose from similar observations of temperature-induced redistribution of estradiol within the uterine cell, as well as the absolute requirement for cytosol receptor in the formation of the nuclear complex and the temporary depletion of cytosol receptor that follows administration of estradiol in vivo. These mutually supportive pieces of experimental evidence are elaborated below. Participation of uterine cytosol in nuclear binding was suggested independently (*33*) by the observation that, on incubation with tritiated estradiol, uterine nuclei take up more radioactivity when the incubation is with uterine cytosol rather than with buffer alone.

Subsequent to the first proposals that the 5*S* nuclear complex is derived from the 8*S* cytosol complex by a temperature-dependent process, it was recognized that the cytosol receptor contains 4*S* hormone-binding subunits and that these can undergo estrogen-induced, temperature-dependent conversion to a 5*S* form, as described in the next section. The sequence in Fig. 6 represents a modification of the original scheme in accordance with this additional knowledge.

More recent studies by various investigators, summarized with specific references in (*2*) and (*3*), have demonstrated that target tissues for other classes of steroid hormones contain specific extranuclear receptor proteins that bind with the hormone and then migrate to the nucleus. Thus, a two-step or perhaps a three-step (*34*) mechanism, similar to that elucidated for the estrogens, appears to provide a general model for the interaction of steroid hormones with their respective target cells.

Probably the most significant evidence supporting the two-step interaction mechanism is the dependence of nuclear binding on the presence of the cytosol receptor. In contrast to the 8*S* protein, which is present in uterine cytosol and reacts directly with estradiol to form a complex (*13, 26*), there is no detectable 5*S* binding protein in nuclei of uteri that have not been exposed to hormone (*13, 32*). No 5*S* complex is obtained either by addition of estradiol to an extract of uterine nuclei or by direct treatment of the nuclei themselves, although it is readily produced (Fig. 7a) when estradiol is incubated either with uterine homogenates or with isolated nuclei in the presence of the cytosol fraction (*23, 32, 35*). Formation of the nuclear complex is a temperature dependent phenomenon that proceeds at an appreciable rate between 25° and 37°C. Prior heating of the cytosol to 45°C destroys estradiol binding by the 8*S* receptor and eliminates its ability to

produce 5*S* complex on subsequent incubation with nuclei (*32*).

In whole uterine tissue, nuclear binding is likewise temperature dependent. After immature rat or calf uteri are exposed to dilute solutions of tritiated estradiol at 37°C, the intracellular distribution pattern of uterine radioactivity, as determined by fractionation and by autoradiography, is similar to that seen after hormone administration in vivo (*13, 18*). But if the tissue is treated with hormone at 2°C, the major portion (70 to 75 percent) of the radioactive steroid appears as 8*S* complex in the cytosol fraction (*31, 32, 36*) and is seen in the extranuclear region on autoradiography (*32*). When such uteri, rich in extranuclear 8*S* complex, are warmed briefly to 37°C, redistribution of the steroid takes place within the tissue to yield predominantly nuclear bound steroid, extractable as 5*S* complex. These observations imply that radioactive estradiol, which can associate with the extranuclear receptor in the cold, is transferred to the nuclear binding site by a second process that does not proceed readily at low temperature.

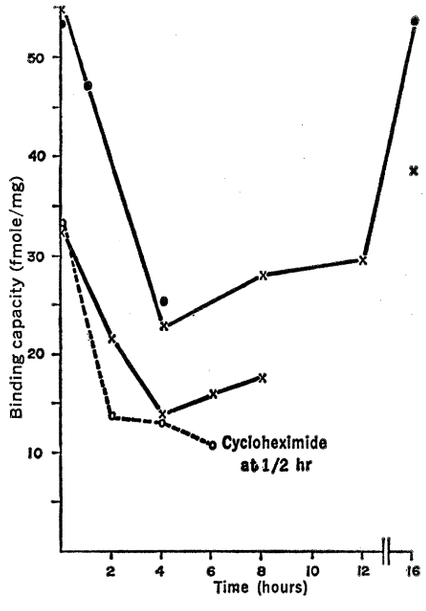


Fig. 8. Estradiol binding capacity (determined from the radioactivity of the 8*S* sedimentation peak and expressed as femtomoles per milligram fresh tssue) of cytosol fractions of immature rat uteri excised at different times after subcutaneous injection of 0.1 μg [^{3}H]estradiol in saline. Upper curve is a composite of two experiments; lower curves a third experiment in which some animals (○ - - - ○) received 0.2 mg cycloheximide intraperitoneally in 0.2 ml of saline 30 minutes prior to the injection of estradiol. Cytosols for each time point were prepared from pooled uteri from ten rats; before they were layered on the sucrose gradient, excess [^{3}H]estradiol (10 to 20 n*M*) was added to saturate the binding capacity. Details in (*23*).

As estradiol reacts with uterine cells to become localized in the nucleus, the extranuclear receptor protein temporarily disappears. It was found that the total receptor content of rat uterine cytosol is less after a large dose of estradiol than after a smaller one (*32*) and that after administration of physiologic amounts of hormone there is a progressive fall in cytosol receptor content for about 4 hours (Fig. 8), after which the 8*S* protein is gradually replenished (*23, 37*). This restoration appears to involve new synthesis of receptor protein, inasmuch as it can be blocked by the administration of cycloheximide at the proper time. With a massive dose of estradiol (5 μg), 90 percent of the receptor disappears within 15 minutes (*2*). Similar depletion of extranuclear receptor accompanies interaction of uteri with estradiol in vitro (*35, 36, 38*), consistent with its utilization to generate the nuclear complex.

The depletion of extranuclear receptor is four to five times greater than can be accounted for by the estradiol present in the nucleus at 4 hours (*2*). This observation indicates that nuclear turnover of estradiol in vivo must be fairly rapid. It also suggests the possibility that on leaving the nucleus estradiol may encounter more cytosol receptor and repeat the interaction cycle, so that each hormone molecule may induce the translocation of several receptor molecules to the nucleus.

Receptor Transformation

After the demonstration that incubation of uterine nuclei with an estradiol-cytosol mixture gives rise to an extractable 5*S* hormone-receptor complex, indistinguishable from that obtained in whole tissue, it was first assumed that the new complex was formed in the nucleus (*23*). But subsequent observations that conversion of the 4*S* receptor to a 5*S* form does not require the presence of nuclei (*2, 39*) and that only the transformed form of the complex is taken up by isolated uterine nuclei to stimulate their RNA polymerase activity (*30, 40*) suggest that the estrogen-induced alteration of the cytosol complex accompanies or even precedes its migration to the nucleus and may be a prerequisite for receptor translocation as well as for biological action.

Uterine cytosol warmed in the presence but not the absence of estradiol yields a hormone-receptor complex that sediments in sucrose density gradients containing salt at approximately the same rate as the nuclear complex (Fig. 9). This transformation in cytosol shows the same characteristics as those associated with incubations carried out in the presence of nuclei (*2, 39*). The reaction, which takes place only slowly in the cold, proceeds readily at 25° to 37°C and is accelerated with increasing *p*H over the range 6.5 to 8.5, as well as by the presence of salt. It is retarded slightly by calcium, magnesium, or manganese ions, and more strongly by EDTA. Under the same conditions that estradiol is effective, estrone does not induce the formation

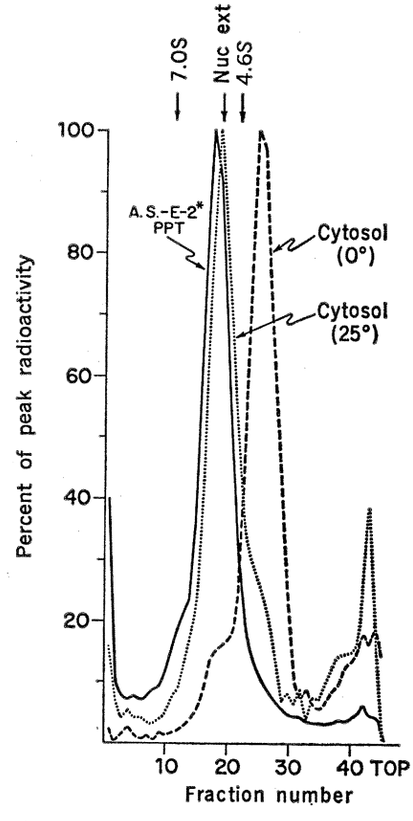


Fig. 9. Sedimentation patterns, in salt-containing sucrose gradients, of radioactive estradiol-receptor complexes of calf uterine cytosol, incubated with 3.8 n*M* [^{3}H]estradiol for 90 minutes at either 0° or 25°C, and of redissolved ammonium sulfate precipitate (*A.S.-E-2* PPT*) from cytosol containing 10 n*M* [^{3}H]estradiol. 4.6*S* and 7.0*S* indicate respective positions of bovine plasma albumin and γ-globulin markers; *Nuc. ext.*, that of complex extracted by 0.4*M* KCl from nuclei of calf uteri previously incubated with [^{3}H]estradiol in vitro. Details in (*42*).

of the 5S complex, either in the presence or absence of nuclei (*2, 30*), although it has been found to do so when it is present in higher concentrations (*41*).

Recently it was observed that ammonium sulfate precipitation of the estradiol-receptor complex of calf uterine cytosol, prepared in the absence of EDTA, is accompanied by conversion of the complex to a more rapidly sedimenting form suggestive of the transformed state (*42*). The complex from the redissolved precipitate sediments in sucrose density gradients containing salt slightly faster than that obtained by warming uterine cytosol with estradiol (Fig. 9). By careful comparison with 4.6S (bovine plasma albumin) and 7.0S (commercial bovine γ-globulin) markers, the following sedimentation coefficients can be calculated (*42*) for the receptor proteins of calf uterus: native cytosol, 3.8S; transformed (25°C) cytosol, 5.3S; nuclear extract, 5.2S; and ammonium sulfate precipitate, 5.5S. Whether these small but reproducible differences in sedimentation behavior among the various preparations are due to variations in the molecular milieu or whether they represent subtle differences in the hormone-receptor complexes themselves is uncertain.

In contrast to the temperature-dependent transformation in uterine cytosol, which takes place only if the receptor protein is complexed with the estrogenic hormone, the alteration that accompanies ammonium sulfate precipitation proceeds rapidly in the cold and does not require estrogen. When the uncomplexed receptor of calf uterine cytosol is precipitated with ammonium sulfate, and tritiated estradiol is then added to the redissolved precipitate, the same 5.5S complex is produced as when the receptor is precipitated in the presence of estradiol. Similar production of a more rapidly sedimenting form is observed when the native cytosol complex is subjected to other processes of partial purification, such as filtration through Sephadex G-25, preparative ultracentrifugation in sucrose density gradients, or dialysis, suggesting that uterine cytosol may contain a substance of small molecular weight that maintains the receptor protein in its native form. Although the significance of these observations is not yet clear, they provide clues for further investigation relative to the chemical basis of the transformation phenomenon.

Binding to Nuclei

The transformed and native forms of the estradiol-receptor complex of uterine cytosol differ markedly in their affinities for uterine nuclei (Fig. 7a). When incubated with estradiol-cytosol mixtures, sucrose-purified uterine nuclei show little incorporation of the native complex at 0°C, in contrast to a large uptake at 25°C where transformation to the 5S form accompanies the nuclear incorporation. But if the estradiol-cytosol mixture is first warmed to 25°C to effect receptor transformation, subsequent incubation with nuclei at either 0° or 25°C results in a substantial uptake of the transformed complex.

Thus, receptor transformation appears to be a prerequisite for binding of the hormone-receptor complex to isolated uterine nuclei and probably represents the temperature-dependent process associated with nuclear incorporation in whole uterine tissue. Similar conclusions have been reached in recent studies of the effect of temperature on the binding of the estradiol-receptor complex to isolated uterine chromatin (*42a*).

The estradiol-receptor complex obtained by ammonium sulfate precipitation resembles the transformed cytosol complex in its ability to bind to purified uterine nuclei at either 0° or 25°C (Fig. 7b). It is evident that the alteration that leads to increased sedimentation rate, whether induced by warming with estradiol or by salt precipitation, also results in enhanced affinity for nuclei.

Rat uterine chromatin has been reported to bind more uterine cytosol complex in the cold than does chromatin from liver, spleen, or lung (*43*), and earlier studies have indicated somewhat greater uptake of complex at 25°C by crude nuclear preparations from uterus than by those from diaphragm, liver, or kidney (*2, 23*). These observations suggest that uterine nuclei contain specific "acceptor" sites with affinity for the estrogen-receptor complex. In more recent experiments, however, we find that sucrose-purified nuclei from calf uterus, liver, and thymus do not show any marked difference in their incorporation of transformed estrogen-receptor complex when compared in terms of their DNA contents. However, the effect of the bound complex on nuclear RNA synthesis is highly tissue-specific, as discussed later.

Transformed Receptor and Nuclear RNA Synthesis

The fact that estradiol moves to the nucleus together with the receptor protein might imply that the receptor system is simply a transport mechanism to deliver the hormone to its eventual site of action. On the other hand, it has been suggested (*2*) that the steroid-protein complex, or even the receptor protein itself, may be an important factor in some nuclear process and that the function of the hormone is to effect conversion of the protein to an active form that can enter the nucleus. Evidence supporting this concept is provided by the ability of the transformed but not the native estradiol-receptor complex to enhance RNA synthesis in isolated uterine nuclei.

Numerous investigations (*44*) have demonstrated that increased biosynthesis of RNA in the rat uterus is an early response to the administration of estrogen, although there has been some controversy as to how soon a bona fide effect on overall RNA synthesis can be detected. Indirect evidence suggests that production of messenger RNA for a specific soluble protein in the rat uterus is an especially early event (*45*). It was also found (*46*) that the RNA polymerase activity of nuclei isolated from uteri of immature or castrated rats is increased two- to threefold by administration of estradiol to the animal 1 to 4 hours before the uteri are excised, but that direct treatment of the nuclei with hormone has no effect. An important advance in our knowledge was provided by the discovery (*47*) that direct stimulation of nuclei from heifer endometrium is possible if treatment with estradiol is conducted in the presence of endometrial cytosol containing the receptor protein. It was also observed that the RNA polymerase activity of heifer endometrium nuclei or of the enzyme prepared from these nuclei could be enhanced by adding a mixture of estradiol and certain uterine fractions direcly to the polymerase assay system (*48*).

We have found that susceptibility of RNA synthesis to stimulation by the estradiol-receptor complex is a specific characteristic of uterine nuclei and that to produce this activating effect the receptor must be in the transformed state (*40*). Purified nuclei from immature rat uteri incorporate less labeled nucleotide into RNA than do kidney

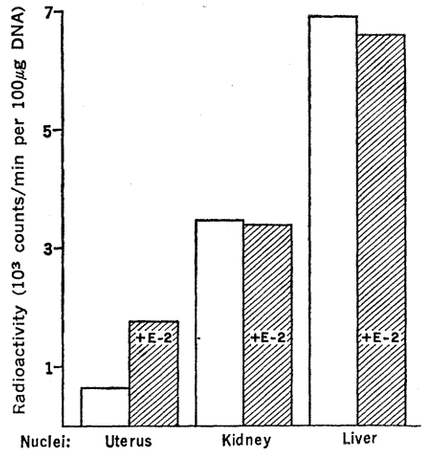


Fig. 10. Synthesis of RNA in nuclei, isolated in 2.2*M* sucrose from various rat tissues, after incubation at 25°C for 30 minutes with rat uterine cytosol (in 2.2*M* sucrose, 1 m*M* $MgCl_2$) in the presence and absence of 10 n*M* estradiol (E-2). After incubation, the nuclei were separated and resuspended in 0.32*M* sucrose for assay of magnesium-dependent RNA polymerase. Results expressed as counts per minute of radioactive nucleotide incorporated per 100 μg of nuclear DNA. Details in (*40*).

or liver nuclei (Fig. 10). After incubation at 25°C for 30 minutes with uterine cytosol containing estradiol, subsequent RNA synthesis is increased nearly threefold in uterine nuclei, whereas there is no enhancement of the already high synthetic capacity of kidney or liver nuclei after incubation with the hormone, either in uterine cytosol or in their own cytosols. Thus, nuclei from the hormone-dependent tissue appear to possess a specific deficiency in RNA synthetic capacity that can be alleviated by the estradiol-receptor complex of uterine cytosol.

Stimulation of uterine nuclei requires that the estradiol-receptor complex be converted from the 4*S* to the 5*S* form (Table 1). Nuclei from calf endometrium are activated by incubation with estradiol and endometrium cytosol at 25°C, where receptor transformation takes place readily, but not at 0°C, where it does not. However, if the estradiol-cytosol mixture is first warmed to effect transformation of the receptor, the resulting 5*S* complex can stimulate nuclei on incubation at either 0° or 25°C. Estrone, which binds to the native receptor protein but does not induce its transformation at the concentration used, does not cause nuclear stimulation under conditions where estradiol is effective. The 5*S* estradiol-receptor complex, extracted from calf uterine nuclei previously incubated with estradiol and uterine cytosol, resembles the transformed cytosol complex in stimulating uterine nuclei on incubation at either 0° or 25°C.

The redissolved dialyzed ammonium sulfate precipitate of uterine cytosol, containing the 5.5*S* form of the receptor, likewise can enhance the RNA polymerase activity of uterine nuclei, whether or not the receptor protein is complexed with estradiol (*42*). Because similar stimulation is seen with ammonium sulfate precipitates from cytosols of certain nontarget tissues, as well as from uterine cytosol previously heated to 50°C to destroy specific estradiol binding, the earlier conclusion that the uncomplexed 5.5*S* receptor protein can itself influence RNA synthesis must be held in abeyance until the action of purified preparations of this protein can be evaluated.

The enhancement of RNA synthetic capacity of uterine nuclei when they interact with transformed estradiol-receptor complex is considerably greater than that which would correspond to new messenger RNA for a limited number of specific protein species. Thus, the relation of this polymerase activation to early uterine responses to estradiol, such as the formation of "induced protein" (*45, 49*), is not clear. But the fact that a similar increase in nuclear RNA polymerase activity results from the administration of estradiol to the whole animal (*46*) provides reassurance that the stimulation of nuclei in vitro is of physiologic relevance.

How the hormone-receptor complex acts to enhance nuclear RNA synthesis needs elucidation. At least part of the effect appears to involve some action other than that on chromatin template activity. It is known (*50*) that the magnesium-dependent RNA polymerase activity of mammalian nuclei prepared in 2.2*M* sucrose can be separated into two fractions: bound enzyme, firmly associated with chromatin, and soluble

Table 1. Effect of transformed as opposed to native estradiol-receptor complex on RNA synthesis in calf endometrium nuclei. Purified nuclei from calf endometrium, prepared in 2.2*M* sucrose containing 1 m*M* $MgCl_2$, were incubated for 30 minutes (series A and B) or 45 minutes (series C) with endometrial cytosol in sucrose containing 10 n*M* estradiol or estrone, or with an extract of crude calf uterine nuclei previously exposed for 1 hour at 25°C to uterine cytosol containing 10 n*M* estradiol. Before incubation the nuclear extract in 0.4*M* KCl was concentrated (Diaflo XM-50 membrane) and then diluted with sucrose to a receptor concentration equivalent to that of the cytosol giving a KCl concentration of 35 m*M*. In series A, experiments 5 and 6, receptor transformation was effected prior to incubation with nuclei by warming the estradiol-cytosol mixture at 25°C for 30 minutes. After incubation, the nuclei were separated and resuspended in 0.32*M* sucrose for assay of magnesium dependent RNA polymerase by measuring the incorporation of uridine monophosphate, [^{3}H]UMP, from tritiated uridine triphosphate as described (*40*). Results are mean values of seven replicate determinations after subtraction of blanks from similar assays with 0.12*M* EDTA; estimated standard deviations of the mean are indicated. Control values were obtained with cytosol without steroid, except in series C, experiments 3 to 6, where heat-inactivated (50°C, 15 minutes) nuclear extract was used for the controls.

Exp.	Incubation		[^{3}H]UMP into RNA	
	Addition to nuclei	Temperature (°C)	Picomoles per 100 μg of DNA	Percent of control
		Series A		
1	Cytosol	0	4.63 ± 0.24	Control
2	Cytosol + estradiol	0	4.42 ± 0.22	95
3	Cytosol + estradiol	25	10.23 ± 0.87	220
4	Heated cytosol*	0	3.69 ± 0.37	Control
5	Heated cytosol + estradiol*	0	10.04 ± 0.54	270
6	Heated cytosol + estradiol*	25	8.80 ± 1.33	240
		Series B		
1	Cytosol	25	2.06 ± 0.13	Control
2	Sucrose† + estradiol	25	1.76 ± 0.15	85
3	Cytosol + estradiol	25	3.46 ± 0.11	170
4	Cytosol + estrone	25	2.16 ± 0.13	105
5	Cytosol + estradiol	0	2.15 ± 0.17	105
		Series C		
1	Cytosol	25	2.84 ± 0.26	Control
2	Cytosol + estradiol	25	5.57 ± 0.89	195
3	Heated nuclear extract‡	25	2.56 ± 0.34	Control
4	Nuclear extract	25	5.95 ± 0.87	235
5	Heated nuclear extract‡	0	2.67 ± 0.41	Control
6	Nuclear extract	0	4.72 ± 0.25	175

* Heated at 25°C for 30 minutes to transform receptor. † Homogenization medium: 2.2*M* sucrose containing 1 m*M* $MgCl_2$. ‡ Heated at 50°C for 15 minutes to destroy receptor.

enzyme, which is extracted from the nuclei by 0.32*M* sucrose. Both types of RNA polymerase activity are enhanced after incubation of endometrial nuclei with the estradiol-cytosol mixture (*40*). Inasmuch as the soluble enzyme is assayed with exogenous template, its increased activity is independent of uterine chromatin and appears to reflect an influence on the enzyme system itself. This conclusion is in agreement with the estradiol-receptor complex being able to cause stimulation when added directly to the polymerase assay mixture (*48*). It is also consistent with the observation (*51*) that the increased incorporation of precursor into RNA in uterine nuclei isolated from estrogen-treated rats results from the synthesis of longer RNA chains, rather than more chains as would be the case if the hormone acted to make new template sites available.

General Significance of Receptor Transformation

As previously noted, all classes of steroid hormones appear to interact with their target cells by similar mechanisms involving temperature dependent translocation to the nucleus of an initially formed extranuclear hormone-receptor complex. For other steroids, hormone mediated transformation of the cytosol receptor in the absence of nuclei has been demonstrated by a physical criterion (isoelectric point) only for the dihydrotestosterone receptor of rat prostate (*52*). However, changes in biochemical and nuclear binding properties suggest that glucocorticoid (*53, 54*) and progesterone (*55*) receptors undergo hormone induced, temperature dependent activation prior to their action in the target cell nucleus. A similar requirement for transformation has been postulated for aldosterone receptors in the kidney cell (*34*). It appears likely that receptor transformation will prove to be an important step in the biological action of all types of steroid hormones.

Recognition of the significance of receptor transformation serves to focus investigative attention on two important questions: the physicochemical basis of the transformation process, and the biochemical mechanism by which the transformed complex influences RNA synthesis in uterine nuclei. The eventual isolation of the native and transformed receptor proteins in amounts sufficient to permit comparison of their compositions and structures should provide insight into the nature of the transformation phenomenon, leading to a clearer understanding of the role of the hormone in this key step in estrogen action.

Summary

The interaction of estradiol with uterine cells involves the association of the hormone with an extranuclear receptor protein, followed by temperature dependent translocation of the resulting complex to the nucleus. During this process, the steroid binding unit of the protein undergoes an alteration, called "receptor transformation," that can be recognized by an increase in its sedimentation rate from 3.8*S* to 5.2*S*, and by its acquisition of the ability to bind to isolated uterine nuclei and to alleviate a tissue specific deficiency in the RNA synthesizing capacity of such nuclei.

Receptor transformation can be effected in the absence of nuclei by warming uterine cytosol with estradiol. This preparation of transformed complex resembles that extracted from nuclei both in its sedimentation rate (5.3*S*) and in its ability to bind to uterine nuclei and augment RNA synthesis, properties that are not shown by the native complex. It is proposed that receptor transformation is an important step in estrogen action and that a principal role of the hormone is to induce conversion of the receptor protein to a biochemically functional form.

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Computing in China: A Travel Report

Computer technology advances rapidly in China with no external aid.

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In July 1972, at their invitation, we visited the People's Republic of China for 3 weeks to tour computer facilities and to discuss computer technology with Chinese experts in Shanghai and Peking. Officially, the trip was seen by the Chinese in two lights: as a step in reestablishing the long-interrupted friendship between the two nations and as a step in opening channels for technical dialogue. We, less bound to national purposes than our hosts, had our many individual motives for taking part, not least among them curiosity.

The project was initiated by Severo Ornstein, who, on an informal basis, got in touch with a small number of interested computer scientists at universities and research institutions in the United States and subsequently applied for visas on behalf of the group to the Embassy of the People's Republic of China in Canada. After a 9-month period of discouraging silence, the embassy, in April 1972, transmitted an invitation from the Chinese government for a delegation of six scientists and their wives to visit China for 3 weeks in July, with all expenses within China to be covered by the host country.

While no group of six can fairly represent the young and as yet loosely organized field of computer science, our group did bring interests and competence in computer systems and computer design, programming languages, systems theory, management science, artificial intelligence, and computer science education. A representative of the computer manufacturing community with more competence than ours in computer components and device engineering would have been a valuable addition to the group (*1*).

We were received in China with open arms. A greater cordiality and solicitude, a more earnest effort to satisfy our wishes (within practical and policy limits) would be hard to imagine. "You have brought your wives; next time you must bring your children too," they said. Egalitarian convictions in no way inhibited them from offering their American guests, on a very full and

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