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In Vitro Nuclear Translocation of the Estrogen Receptor in the Hepatic Parenchymal Cell from Male Rats

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SUMMARY

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Isolated, purified, viable parenchymal cells from adult male rat liver were characterized for the distribution of the estrogen receptor. Demonstration of the cytosol estrogen receptor required its partial purification by fractionation with ammonium sulfate. Crude cytosol from the isolated cells contained high levels of the recently described, male-specific, nonreceptor, steroid binding protein. The estrogen receptor found in the partially purified cytosol fraction of parenchymal cells was at a level near that found in similar preparations from whole liver. The properties of these partially purified cytosol estrogen receptors closely resembled those of cytosol estrogen receptors from whole liver of male or female rats, or from rat uterus. The distribution of the two classes of cytosol binding sites for estrogens was then studied after exposure of the cells to ethinyl estradiol. After *in vitro* incubation with ethinyl estradiol, cytosol and nuclear estrogen receptors were determined by exchange assay with [^{3}H]estradiol. Cytosol estrogen receptor levels were rapidly diminished, while receptor levels in highly purified nuclei were rapidly increased. In contrast, levels of the nonreceptor, male-specific sex steroid binder in cytosol were not diminished following exposure of the cells to ethinyl estradiol. There was a similar steroid specificity (during exchange with [^{3}H]estradiol) of cytosol estrogen receptors of cells not exposed to estrogen and of nuclear estrogen receptors of cells exposed to ethinyl estradiol for 30 min. The levels of occupied receptors calculated by exchange assay at the time of maximal occupation of the nuclear receptors compared well with the value obtained after incubating [^{3}H]ethinyl estradiol with the cells. The data are consistent with estrogen receptor translocation from the cytoplasmic to nuclear compartment in the isolated parenchymal cell from adult male rat liver. Several differences were found comparing this translocation process in hepatic parenchymal cells from male rats to results obtained previously using this cell type from female rats. In male liver cells, the onset of translocation was slower, the nuclear retention of the receptor longer, and the half maximal concentration of ethinyl estradiol required for nuclear translocation approximately two-fold higher than in female liver cells.

INTRODUCTION

Cytosol of mammalian liver contains high affinity, estrogen-specific binding sites. These binding sites are very similar, both quantitatively and qualitatively, to the classical estrogen receptor in uterine cytosol (1, 2). Livers from male as well as female rats contain estrogen receptors. It has not yet been possible to measure the estrogen receptor in crude cytosol prepared from male rat liver because of the presence of high quantities of an unusual sex steroid-binding protein. Measurement of the cytosol estrogen receptor requires partial purification with ammonium sulfate at 30% of saturation (3, 4).

This laboratory has described the presence of nuclear estrogen receptor complexes in the liver following exposure to estrogens both *in vivo* and *in vitro*. After a subcutaneous injection of 17α-ethinyl estradiol (EE_2)[1] in adult female rats, the liver cytosol estrogen receptor

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[1] Abbreviations used are: EE_2, 17,α-ethinylestradiol; E_2, 17,β-estradiol; BSA, bovine serum albumin; LDH, lactic acid dehydrogenase; 30% AS ppt, a redissolved precipitate of cytosol protein after fractionation with ammonium sulfate at 30% of saturation.

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appears to be translocated to liver nuclei (5). In addition, after incubation of EE_2 with isolated, purified parenchymal cells from adult female rat liver, the receptor translocation process also occurs with marked similarities to the process *in vivo* (6). After incubation of $[^3H]E_2$ with adult female rat liver slices *in vitro*, nuclear receptor-bound $[^3H]E_2$ and the catechol estrogen 2-[^{3}H]hydroxyestradiol can be demonstrated (7).

The purpose of this work was to study the estrogen receptor translocation process *in vitro* in purified, viable male rat liver parenchymal cells. Since adult male rat liver contains an estrogen receptor that is apparently identical to that found in adult female rat liver (3) but also contains high levels of a nonreceptor binder (4) and a much greater metabolic activity for estrogens (8), it was of interest to study estrogen receptor translocation in this new system. Such a comparison of estrogen receptor translocation in male versus female rat liver parenchymal cells could provide insight into factors modulating the receptor translocation process. The only other estrogen receptor-containing tissues that have been studied relative to a male–female comparison have been in brain regions and in the pituitary gland. An estrogen receptor has been described for various brain regions and the pituitary gland from male and female rats with no apparent sexual differences. No sex differences in estrogen metabolism, nonreceptor sex steroid-binding proteins (such as α-fetoprotein), or estrogen receptor translocation parameters for these tissues have been demonstrated at present (9). Isolated parenchymal cells from male or female liver may prove to be an interesting system for making such male–female comparisons.

MATERIALS AND METHODS

The methods used in liver cell preparation, incubation, and fractionation, as well as in estrogen-binding studies of the estrogen receptor, and male-specific sex-steroid binding protein have been described in detail (4, 6). Only deviations from these procedures will be specified.

Animals, liver cell preparation, and liver cell incubation. Mature, intact male rats (200–300 g CD strain) were obtained from Charles River. Rats were anesthetized, and liver cells were prepared by the collagenase perfusion technique and incubated in suspension as previously described (6). When rats weighing 300 g were used, an elevated concentration of collagenase (75 mg/100 ml) was used.

Characterization of cell properties. Cell viability was assessed on the basis of trypan blue exclusion, LDH release, and [^{14}C]leucine incorporation into protein (6). Cell viability averaged 94%, DNA content 2.40 mg/g cell wt, LDH content 630 U/g cell wt, and protein content 200 mg/g cell wt. These values were similar to those previously reported for cells from female rat liver (6).

Preparation of partially purified cytosol and purified nuclei. Cytosol and 30% AS ppt cytosol were prepared (6). The recovery of protein in the partially purified cytosol averaged 5%. Highly purified nuclei were prepared by sedimentation in dense sucrose containing 0.2% Triton X-100 as described (5, 6) and the DNA recovery was 20%.

Assays of male-specific cytosol binding sites for $[^3H]E_2$. The male-specific, cytosol $[^3H]E_2$-binding sites were assayed with 10^{-6} M $[^3H]E_2$ (4). Cells were homogenized as described (6) but buffer containing 10 mM Tris, 1.5 mM EDTA, and 1 mM NaN_3 (pH 7.4) was used for homogenization (4).

$[^3H]E_2$ exchange assays and estrogen receptor binding by $[^3H]EE_2$. $[^3H]E_2$ exchange assays were conducted on 30% AS ppt cytosol and purified nuclear fractions with 30 nM $[^3H]E_2$ with and without 1 μM diethylstilbestrol at 30° and 0°. 30% AS ppt cytosol was gel filtered and bound radioactivity was measured. Nuclei were first solubilized using buffered 5 M urea, 2 M NaCl and then gel filtered 15 min later using a 1 M NaCl elution buffer (5, 6). Specific binding is expressed as the difference in binding without and with diethylstilbestrol present. Specific binding measured after 0° incubations is considered to measure unoccupied receptors, while that measured after 30° incubations is a measure of total receptors. Occupied receptors are calculated as the difference of total and unoccupied receptor levels (5, 6). Levels of nuclear receptor are corrected for the DNA recovery during nuclear preparation. Levels of cytosol receptor are corrected after assuming a 60% recovery of the receptor after 30% AS ppt (as was found with the female (6)). Assays of binding of radioactive estrogen in cell fractions after cell incubations with $[^3H]EE_2$ were performed as described (6).

Experiments using EE_2 injections in intact rats. In some experiments, intact rats were injected with EE_2 in propylene glycol or with the vehicle itself. In these cases, rats were injected and sacrificed as previously described. Livers were fractionated into nuclei and 30% AS ppt cytosol and subjected to $[^3H]E_2$ exchange assays (5).

Thin layer chromatography radioactivity after in vitro $[^3H]E_2$ binding assays. The identity of radioactivity after $[^3H]E_2$ exchange assays, or cytosol $[^3H]E_2$-binding assays was examined by thin layer chromatography on silica gel using chloroform–ethyl acetate (80:20) (4, 6).

RESULTS

Stability of the parenchymal cell preparation. The parenchymal cell suspension used in this study appeared to be quite stable during at least the first 4 hr of incubation. Figure 1 shows that the percentage of cells excluding trypan blue, the cellular LDH content, and the apparent rate of protein synthesis were stable for 4 hr. The presence of 10^{-5} M EE_2 during the incubations had no effect on any of these three parameters of cell viability. Figure 2 shows that the total number of partially purified cytosol estrogen receptors did not change during 2 hr of cell incubation. The cytosol also contained high levels of the male-specific, moderate capacity sex steroid-binding protein, which did not change during 2 hr of incubation. The low level of total nuclear estrogen receptors observed without preincubating the parenchymal cells with estrogen was unchanged after 2 hr.

Exchange assay of binding by $[^3H]E_2$ in liver cell fractions. Estrogen receptors in partially purified cytosol and nuclear fractions were determined *in vitro* after cell disruption and fractionation. Cells had been previously incubated with EE_2 or no steroid for various times. In

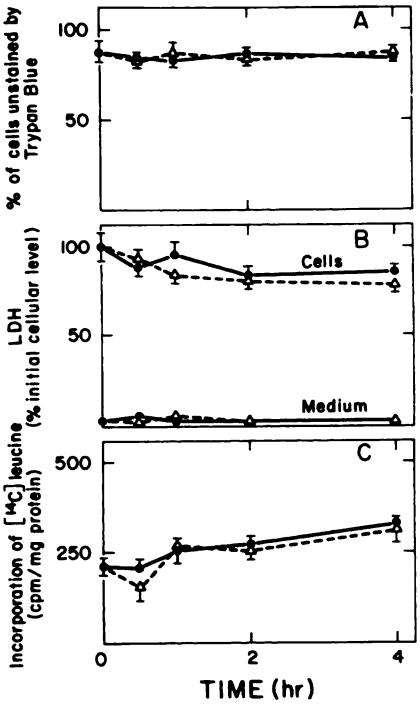

FIG. 1. *Viability parameters of isolated liver cells incubated in the absence (solid lines) or presence of (broken lines) 10^{-5} M EE_2 from 0 to 4 hr*

(A) Averages ± SE of triplicate determinations of percentage of parenchymal cells remaining unstained by trypan blue. One hundred cells were counted for each determination. (B) Averages ± SE of triplicate determinations of lactic dehydrogenase (LDH) activity in the incubation medium and in cell extracts. All data are expressed as a percentage of the activity in a zero time cell extract. (C) Averages ± SE of triplicate determinations of cpm of [^{14}C]leucine incorporated into protein. Cells were preincubated for the indicated time, and then resuspended and incubated in fresh medium containing [^{14}C]leucine for 10 min.

cells not treated with EE_2, the estrogen receptor was mostly in the cytosol and unoccupied. Following exposure to high concentrations of EE_2, the receptors were mostly in the nucleus and occupied. Table 1 shows the specific binding by [^{3}H]E_2 in partially purified cytosol or purified nuclei after using various concentrations of [^{3}H]E_2 during exchange; 5 nM [^{3}H]E_2 gave about half-maximal levels of specific binding for cytosol estrogen receptor of untreated cells or nuclei of EE_2-treated parenchymal cells. No statistically significant increase in specific binding sites for [^{3}H]E_2 occurred from 30 to 50 nM [^{3}H]E_2.

Figure 3 shows the steroid specificity of binding by [^{3}H]E_2 during exchange by the partially purified estrogen receptor from cells not incubated with EE_2 or by the nuclear receptor from cells incubated with 10^{-5} M EE_2 for 30 min. In both fractions, diethylstilbestrol, E_2, and EE_2 were the best competitors; estriol and estrone competed less well, while dihydrotestosterone and progesterone did not compete. The stability of the [^{3}H]E_2-bound complexes was examined by first preincubating either fraction with [^{3}H]E_2 and then adding an excess of diethylstilbestrol. Very little of the binding of [^{3}H]E_2 was removed from either fraction by a 1-hr "chase" with diethylstilbestrol at 0°.

Comparison of exchange and direct [^{3}H]EE_2 assays of estrogen receptors. Table 2 presents a comparison between a [^{3}H]E_2 receptor exchange assay using nonradioactive EE_2 incubations with cells and an assay using direct incubation of [^{3}H]EE_2 with the cells. The concentration of EE_2 in each case was 10^{-7} M and the incubation time was 30 min. The direct [^{3}H]EE_2 incubations included replicate incubations of cells with 10^{-5} M diethylstilbestrol. The direct [^{3}H]EE_2 incubations were also exposed to a 10^{-5} M EE_2 "trap" during the cell pellet washes and the subsequent homogenization to assess if binding occurred before rather than during or subsequent to homogenization. The two assay techniques gave sim-

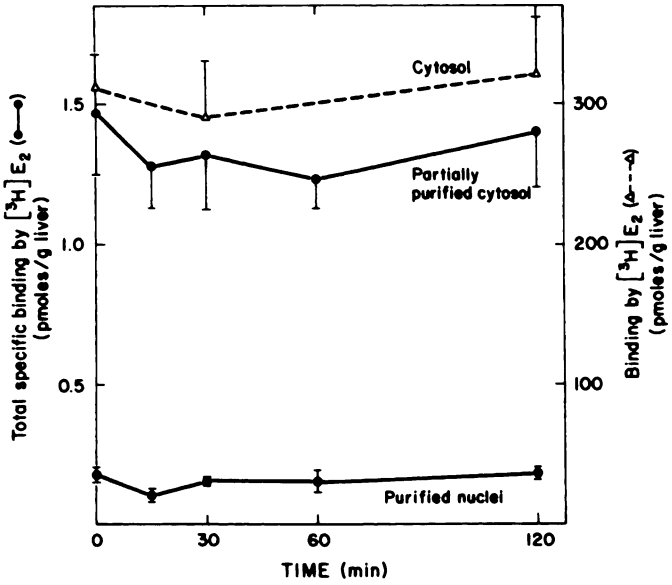

FIG. 2. *Total, specific cytosol and nuclear estrogen receptor and nonreceptor, male-specific estrogen binding sites in parenchymal cells incubated without estrogens*

Cells were incubated for the indicated times and then estrogen receptors were determined by [^{3}H]E_2 exchange assay in 30% AS ppt cytosol and purified nuclear fractions. Male-specific, nonreceptor, cytosol binding sites were also determined (note the high levels of binding in unfractionated cytosol). Data are averages of triplicate determinations ± SE.

TABLE 1

Effect of varying in vitro [^{3}H]E_2 concentrations for measuring specific binding by liver fractions after various cell treatments

Total, specific binding of [^{3}H]E_2 by partially purified cytosol and purified nuclear fractions. Cells were incubated with either no hormone or with 10^{-5} M EE_2 for 30 min. The cells were then washed and fractionated. The fractions were subjected to an [^{3}H]E_2 exchange assay containing various concentrations of [^{3}H]E_2 with or without 10^{-6} M diethylstilbestrol. Data are the averages ± SE of triplicate determinations. Cell preparations from two different rats were used for the control and EE_2-treated cells.

[^{3}H]E_2 concentration *in vitro*	Total [^{3}H]E_2-specific binding sites			
	Nuclei		Partially purified cytosol	
	0 time, No EE_2	30 min, 10^{-5} M EE_2	0 time, No EE_2	30 min, 10^{-5} M EE_2
(nM)	*(pmol/g liver)*			
5	0.023 ± 0.005	0.51 ± 0.01	1.82 ± 0.11	0.12 ± 0.01
30	0.091 ± 0.010	0.85 ± 0.03	2.26 ± 0.17	0.45 ± 0.13
50	0.092 ± 0.012	0.96 ± 0.10	2.09 ± 0.29	0.46 ± 0.07

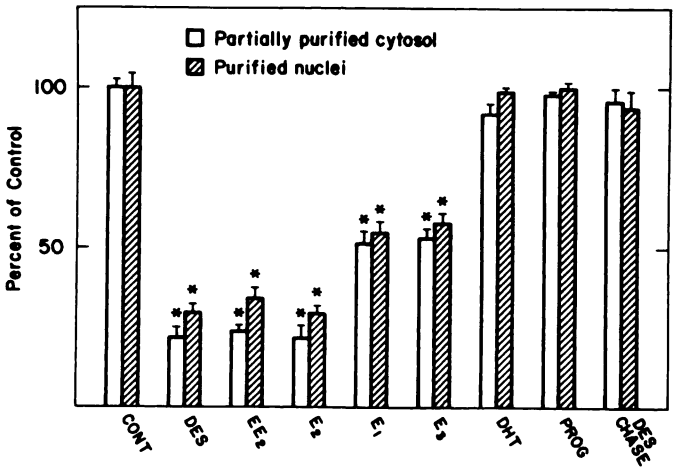

FIG. 3. *Specificity of [^{3}H]E_2-binding sites in partially purified cytosol (from cells incubated with no steroid) or purified nuclei (from cells incubated with 10^{-5} M EE_2 for 30 min)*

Fractions were incubated under exchange assay conditions with 30 nM [^{3}H]E_2 and either no nonradioactive hormone (CONT), or 10^{-6} M of the following hormones: diethylstilbestrol (DES), ethinyl estradiol (EE_2), estradiol (E_2), estrone (E_1), estriol (E_3), dihydrotestosterone (DHT) or progesterone (PROG). For DES CHASE, fractions were incubated with 30 nM [^{3}H]E_2 under exchange conditions to load binding sites, and then incubated with 10^{-6} M DES for 1 hr at 0°. The values for control binding were 1.53 ± 0.04 pmol/g for the 30% AS ppt and 0.71 ± 0.03 pmol/g for the nuclei. * $p < 0.001$ comparing control levels of binding with those in the presence of the nonradioactive hormones. Data are averages of four determinations ± SE.

ilar measurements of the estrogen-specific occupied binding sites in partially purified cytosol and nuclear fractions. In addition, the 10^{-5} M EE_2 trap did not significantly reduce binding by [^{3}H]EE_2 in either fraction from the direct [^{3}H]EE_2 experiment.

Receptor distribution following EE_2 incubation with parenchymal cells. Figure 4 shows a plot of the concentration of estrogen-specific nuclear and partially purified cytosol binding sites following incubations of cells with various concentrations of EE_2 for 30 min. Total, specific binding of [^{3}H]E_2 by partially purified cytosol was one-half maximally depleted at about 0.2 μM EE_2. Both total and occupied nuclear binding sites for [^{3}H]E_2 increased with a one-half maximal concentration of about 10^{-6} M EE_2. Most of the nuclear sites were occupied.

Time course of binding to estrogen receptors. Figure 5A shows a time course of binding by [^{3}H]E_2 to nuclear and cytosol receptors after incubation of cells with 10^{-7} M EE_2. Total, specific binding of [^{3}H]E_2 by partially purified cytosol was maximal at 0 time, and 60% depleted by 15 min. Nuclear total and occupied [^{3}H]E_2-specific binding sites were maximal at 15 min and remained elevated for at least 2 hr.

Figure 5B shows a similar time course using 10^{-5} M EE_2. Total, specific binding of [^{3}H]E_2 by partially purified cytosol was maximally depleted (to 30% of initial) by 30 min. Nuclear levels of total and occupied estrogen-specific binding sites were maximal at 30 min and remained elevated for at least 2 hr.

Figure 5C shows a similar time course using 10^{-4} M EE_2 (in media containing 2% BSA). Cytosol estrogen receptors were maximally depleted by 15 min, while nuclear estrogen receptors were maximally accumulated by 30 min. The level of nuclear receptors remained elevated for at least 2 hr.

Effect of BSA in the medium on estrogen receptor

TABLE 2

Comparison of [^{3}H]E_2 exchange assay and direct assay of binding by [^{3}H]EE_2

Cells were incubated with either 10^{-7} M EE_2 or [^{3}H]EE_2 30 min. Cells incubated with non-radioactive EE_2 were then subjected to an exchange assay. Cells incubated with [^{3}H]EE_2 either contained no other hormone, or 10^{-5} M diethylstilbestrol during incubation. Each of these incubations was divided into two groups. One group was washed and homogenized as usual. The other was washed and homogenized in the presence of 10^{-5} M EE_2 (Trap). [^{3}H]EE_2-specific binding is expressed as the difference between macromolecular binding by radioactivity in the absence and presence of DES. Data are the averages of triplicate experiments ± SE.

Fraction	Specific binding by [^{3}H]E_2 exchange		Specific binding by [^{3}H]-EE_2 incubations with cells	
			No EE_2 Trap	EE_2 Trap
		(pmol/g liver)	*(pmol/g liver)*	
Nuclei	Total	0.31 ± 0.02		
	Occupied	0.16 ± 0.02	0.18 ± 0.02	0.17 ± 0.02
Partially purified cytosol	Total	0.62 ± 0.07		
	Occupied	0.38 ± 0.11	0.43 ± 0.05	0.40 ± 0.03

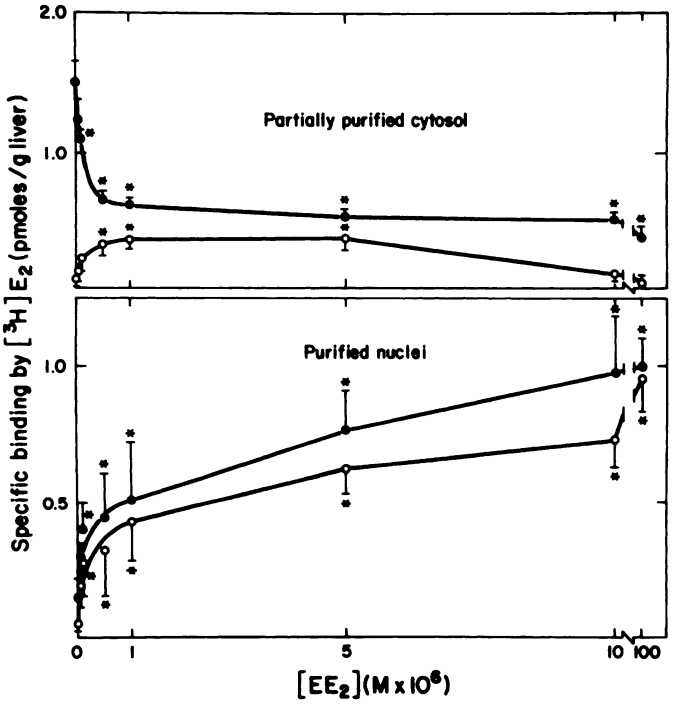

FIG. 4. *Effect of the dose of EE_2 on cytosol receptor depletion and nuclear receptor accumulation*

Cells were incubated for 30 min with various concentrations of EE_2, then fractionated and subjected to exchange assays. Filled circles represent total [^{3}H]E_2-specific binding sites and open circles represent occupied [^{3}H]E_2-specific binding sites. Each point is the average of at least three determinations ± SE and lines are drawn as a visual aid only in this and subsequent figures. * $p < 0.05$ comparing binding by [^{3}H]E_2 with fractions from cell incubations in the absence and presence of EE_2.

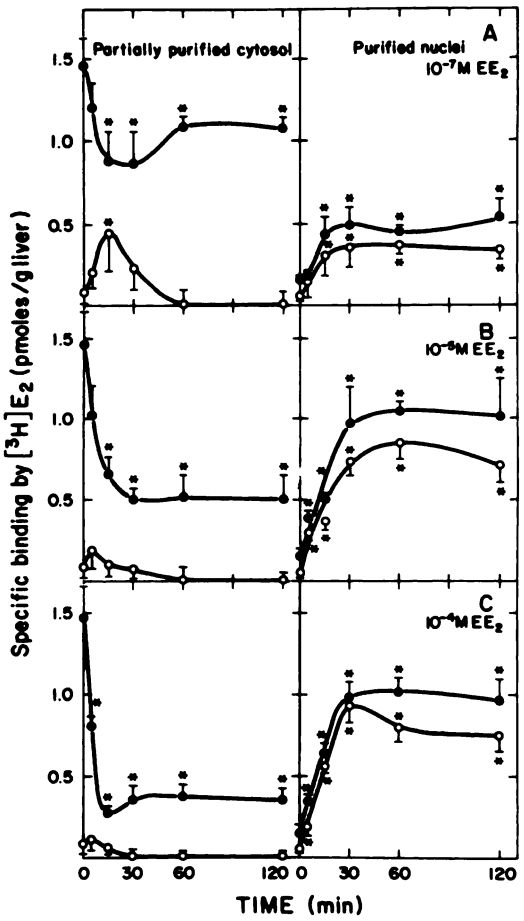

FIG. 5. *Time course of cytosol receptor depletion and nuclear receptor accumulation after incubating cells with (A) 10^{-7} M EE_2, (B) 10^{-6} M EE_2, or (C) 10^{-4} M EE_2 for various times*

Cells were then fractionated and subjected to exchange assays. Filled circles represent total [^{3}H]E_2-specific binding sites and open circles represent occupied [^{3}H]E_2-specific binding sites. * $p < 0.05$ comparing 0 time binding by [^{3}H]E_2 with binding after the time indicated. Points are averages of at least 3 determinations ± SE.

levels following exposure to EE_2. Since BSA was used in some of the cellular incubations to solubilize the high EE_2 concentrations, experiments were performed using 10^{-5} M EE_2, with or without 2% BSA, and a 30-min cell incubation time. The presence of the BSA did not appear to affect the levels of total or occupied estrogen receptor in the nucleus or partially purified cytosol under these conditions (Table 3).

Identification of the male-specific sex steroid-binding protein and effects of EE_2 on its level. Figure 6 shows the steroid specificity of the male-specific sex steroid-binding sites in cytosol of cells not exposed to EE_2. Estriol, E_2 and EE_2 were the best competitors, dihydrotestosterone and estrone competed less well, while diethylstilbestrol did not compete. Binding by [^{3}H]E_2 was mostly removed by a 1-min "chase" with E_2 at 0°.

Table 4 shows the results of experiments examining the levels of the two cytosol [^{3}H]E_2-binding proteins in adult male rat liver after exposure to EE_2 *in vivo* or *in vitro* using isolated parenchymal cells. In contrast to the cytosol estrogen receptor, levels of which are substantially diminished following exposure to EE_2 (coincident with increased levels of the nuclear receptor), the levels

TABLE 3

Effect of 2% bovine serum albumin (BSA) in the medium on cellular estrogen receptor levels

Cells were incubated with 10^{-5} M EE_2 in the presence or absence of 2% BSA for 30 min. Then the cells were washed and fractionated. Total and occupied receptor was determined by exchange assay using [^{3}H]E_2. Data are averages ± SE of triplicate determinations.

In vitro incubation condition	Binding of [^{3}H]E_2 to estrogen receptors			
	Nuclei		Partially purified cytosol	
	Total	Occupied	Total	Occupied
	(pmol/g)			
10^{-5} M EE_2 30 min No BSA	1.21 ± 0.08	0.83 ± 0.09	0.42 ± 0.15	0.0 ± 0.15
10^{-5} M EE_2 30 min 2% BSA	1.01 ± 0.11	0.70 ± 0.11	0.43 ± 0.11	0.0 ± 0.11

of the male-specific sex steroid-binding protein were not diminished following the same treatment.

DISCUSSION

The preparation of purified, isolated liver parenchymal cells used in this study appeared to be viable using the three parameters mentioned. In addition, these measurements did not reveal any toxicity of the high doses of EE_2 used for most of this study. The initial level of estrogen receptors was apparently 50% of the *in vivo* level, and it remained stable during 2 hr of culture. The initial level of the male-specific sex steroid-binding protein was approximately 50% of the *in vivo* level, and its level also remained unchanged during 2 hr of culture. The reason for the slightly reduced level of these binding proteins *in vitro* is unknown, but parallels the slightly reduced level

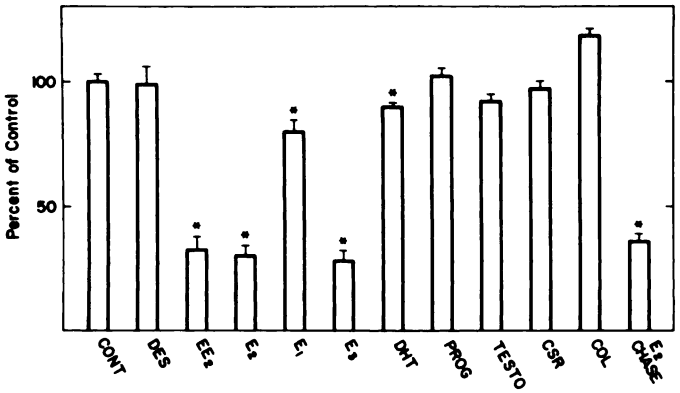

FIG. 6. *Specificity of binding by [^{3}H]E_2 to cytosol (male-specific nonreceptor binding sites)*

Cytosol was prepared from untreated cells and assayed for cytosol binding sites as previously described (4). Cytosol was incubated with 10^{-6} M [^{3}H]E_2 and either no nonradioactive hormone (CONT) or 10^{-5} M concentrations of the following hormones: diethylstilbestrol (DES), ethinyl estradiol (EE_2), estradiol (E_2), estrone (E_1), estriol (E_3), dihydrotestosterone (DHT), progesterone (PROG), testosterone (TESTO), corticosterone (CSR), or cortisol (COL). For E_2 CHASE, cytosol was incubated with 10^{-6} M [^{3}H]E_2 for 1 hr, and then 10^{-5} M E_2 was added for 1 min at 0° before separating bound and free steroid. The value for control binding was 310 ± 10 pmol/g liver. * $p < 0.05$ comparing control levels of binding with those in the presence of the nonradioactive hormones. Data are averages ± SE of four determinations.

TABLE 4

Effect of exposure to EE_2 on the level of estrogen receptor and male-specific binding protein in unfractionated cytosol

Isolated parenchymal cells were incubated with either no EE_2 or with 10^{-5} M EE_2 for 30 min, and then fractionated and assayed for estrogen receptor and the nonreceptor cytosol estrogen binder. Intact rats were injected with 100 μg EE_2 or with vehicle and sacrificed 1 hr later. Their livers were fractionated and assayed for estrogen receptor and the nonreceptor cytosol estrogen binder. Estrogen receptors were determined by exchange assay and are expressed as total specific sites. The male-specific nonreceptor cytosol binding sites for [^{3}H]E_2 were determined as described (4). Data are the averages ± SE of triplicate determinations.

Type of preparation	Treatment	Binding of [^{3}H]E_2 by various fractions		
		Total estrogen receptor in nuclei	Total estrogen receptor in partially purified cytosol	Binding by cytosol
		(pmol/g liver)		
Isolated parenchymal cells (*in vitro* incubations)	No EE_2	0.15 ± 0.01	1.07 ± 0.12	290 ± 40
	10^{-5} M, EE_2 30 min	0.71 ± 0.02	0.43 ± 0.04	290 ± 60
Intact rats (*in vivo* injections)	Vehicle	0.18 ± 0.02	3.16 ± 0.20	380 ± 30
	100 μg, EE_2 1 hr	1.18 ± 0.06	0.98 ± 0.14	530 ± 80

of estrogen receptor found in female rat liver parenchymal cells (6). This is the first demonstration of the estrogen receptor and the male-specific sex steroid-binding protein in the male hepatic parenchymal cell. (Previous studies used whole liver, which contained 25% nonparenchymal cells (10).)

The [^{3}H]E_2 exchange assay used in this study appeared to be a valid measure of nuclear and cytoplasmic estrogen receptors. The concentration of [^{3}H]E_2 that was used apparently saturated the estrogen-specific binding sites under the conditions studied. In addition, receptor quantitation using direct labeling of nuclear and cytoplasmic estrogen receptors with [^{3}H]EE_2 under cell culture conditions compared favorably with exchange assay quantitation for the incubation condition studied. Receptor quantitation after direct [^{3}H]EE_2 labeling and then homogenization of cells in the presence of unlabeled EE_2 suggests that unlike the case with uterine organ culture, there was little radioactive estrogen that associated with receptor during or after homogenization (11).

The partially purified cytosol or nuclear estrogen-specific binding sites appear to be classical estrogen receptors, not resembling the Type II sites in the rat uterus (12–15). Evidence in favor of this suggestion was the relatively slow dissociation kinetics both of the nuclear and 30% AS ppt cytosol [^{3}H]E_2 receptor complexes under "chase" conditions. In addition, the 30% AS ppt cytosol sites were mostly depleted after EE_2 treatment and the receptors appearing in the nucleus were mostly occupied. The nuclear receptor sites were also occupied by estrogen after a direct [^{3}H]EE_2 incubation with liver cells. The estrogen receptors described in the present system were indistinguishable (using the criteria of steroid specificity, saturability, and slow dissociation rate) from estrogen receptors found in isolated parenchymal cells from female rat liver (6). The receptor specificity profile found in the present study is consistent with that of female hepatic estrogen receptors in a 40% AS ppt preparation of cytosol using a variety of concentrations of competing steroids (16). The apparent affinity of E_2 (K_d 0.17 nM) was approximately equal to that of diethylstilbestrol, and greater than that of estrone and estriol. Dihydrotestosterone and progesterone did not compete for binding by [^{3}H]E_2 (16).

The time course studies indicated that an increase in nuclear binding sites occurred after apparent decrease in the level of cytosol sites. Using a range of EE_2 concentrations, half-maximal depletion of cytosol binding sites and accumulation of nuclear binding sites occurred at approximately the same concentration of EE_2. The maximal quantity of nuclear binding sites present after incubation of cells was close to the quantity of depleted cytosol binding sites for the three EE_2 concentrations studied. Both the cytosol and nuclear [^{3}H]E_2-specific binding sites have properties of classical estrogen receptors, and the disappearance of cytosol receptor and appearance of nuclear receptor appear to be closely related for high EE_2 doses. These results imply that the cytosol receptor becomes occupied with estrogen and then associates with the nucleus. In contrast, the male-specific sex steroid-binding protein did not appear to be depleted from the cytosol following exposure to EE_2 *in vivo* or *in vitro*.

In vitro, as previously noted with *in vivo* treatment, very high levels of EE_2 were required to translocate the liver receptor relative to the uterine receptor (5). *In vivo* injections of estrogens have also been used to induce plasma renin substrate increases in rats (17). This response is probably liver mediated (18) and requires much higher doses of estrogens than those necessary for a uterotrophic response (17). This laboratory has shown that higher estrogen doses for binding by the liver estrogen receptor may be due to the rapid metabolism of estrogens by the liver (rather than the receptor K_d, which is 0.1 nM in liver (3) and uterus (19)) and may explain the dose differences in estrogen effects (7).

Estrogen receptor translocation in isolated parenchymal cells from male rat liver differed from previous results with cells from female rat liver. First, nuclear translocation in male rat liver cells required 30 min to reach maximum at 10^{-5} M EE_2, in contrast to the 15 min found for female rat liver cells (6). The reason for this is unknown, but it may be due to the presence of the rapidly binding, male-specific sex steroid binder (4), or to the more rapid sex hormone metabolic activity found in male rat liver (8). Second, the concentration of EE_2 required for one-half maximal nuclear translocation of the estrogen receptor in male liver cells is approximately two times that in female liver cells (6). This observation also has been made in comparing injected doses of EE_2 required for half-maximal nuclear translocation of the hepatic estrogen receptor in male or female rats.[2] The reason for this phenomenon is unknown, but possibly represents an effect of the male-specific sex steroid binder (4) or elevated estrogen metabolic activities in male rat

liver (8). It is probably not due to intrinsic differences in the estrogen receptor in male versus female rats since the K_d of the estrogen receptor for [^{3}H]E_2 in both sexes is the same (3). Third, in the male liver cells the nuclear receptor appears to remain at elevated levels for a longer time than in the female liver cells. After 1 hr of exposure to 10^{-5} M EE_2, occupied nuclear receptors had not decreased below maximal in male liver cells, but were down to 25% of maximal in female liver cells (6). This observation also has been made *in vivo* using injections of EE_2 in male or female rats.[2] The reason for this phenomenon is unknown, but may involve prolonged intracellular retention of EE_2 or an active metabolite by the male-specific sex steroid binder (4), a different chromatin acceptor(s) or intrinsic differences in the nuclear receptor "processing" mechanism comparing male and female rat liver. It is possible that metabolites of EE_2 may bind and translocate the receptor (7). This question has not yet been evaluated in the present system.

A principal reason for studying the mammalian liver estrogen receptors has been their potential role in modifying various liver functions including the synthesis of some plasma proteins (20). Such a direct liver–estrogen interaction may contribute to some of the side effects of the oral contraceptive estrogens in women (21). Estrogen treatment of men (as in therapy of prostatic carcinoma) has resulted in an increase in thrombosis (22, 23). The study of estrogen interactions with male hepatic estrogen receptors may ultimately provide insight into the mechanism of this side effect in men.

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[2] Aten, R. F., M. J. Weinberger and A. J. Eisenfeld, unpublished observations.

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