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# Nucleo-Cytoplasmic Relationships of Oestrogen Receptors in Rat Liver during the Oestrous Cycle and in Response to Administered Natural and Synthetic Oestrogen

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Oestrogen receptors were measured in the cytosolic and purified nuclear fractions of rat liver. Both cytosolic and nuclear receptors bind oestrogen with high affinity ($K_d$ = 1.47 and 2.28 nM respectively) and specificity similar to that of receptors in other oestrogen-target tissues such as the uterus. During the 4-day oestrous cycle the receptor content and distribution between cytosol and nucleus did not vary; in particular, the content of nuclear receptor did not appear to fluctuate in concert with known cyclic changes in the concentration of plasma oestrogen. Injection of 50 $\mu$g of oestradiol-17$\beta$ or 10 $\mu$g of ethynyloestradiol resulted in a 4–6-fold increase in the nuclear receptor content, with a concomitant decrease in the unoccupied-receptor content of cytosol 1 h after injection. The nuclear receptors present after injection bind oestrogens with similar affinity ($K_d$ = 2.78 nM) and specificity to receptors present in uninjected animals. The administration of lower doses of either oestrogen was less effective in producing increases in nuclear receptor content. Hence there is apparently substantial translocation of receptor to the nucleus in response to hyperphysiological doses of oestrogen, but not to the physiological changes in plasma oestrogen concentrations during the oestrous cycle. The response to exogenous oestrogens is discussed in relation to the clinical use of synthetic oestrogens and progestogens.

It has been recognized for some time that the mammalian liver is responsive to the action of oestrogen (Song *et al.*, 1969). Although it is not recognized as a classical target organ for the sex hormones as are, for example, the tissues of the female reproductive tract, the liver has been shown to contain proteins with certain characteristics of the oestrogen receptor (Chamness *et al.*, 1975; Eisenfeld *et al.*, 1976). There is as yet no compelling evidence, as there is for the avian liver (Bieri-Bonniot *et al.*, 1977; Jost *et al.*, 1978), for oestrogen-induced changes in transcription in the mammalian liver; nevertheless it is widely accepted that oestrogen administration to rats results in altered liver function, including increases in the concentration of certain plasma proteins, e.g. renin substrate (Menard *et al.*, 1973; Eisenfeld *et al.*, 1977). The latter effect has been demonstrated directly by using perfused liver preparations (Nasjletti & Masson, 1972).

The administration of oestrogens to women results in disturbances in the concentration of several plasma proteins (Seal & Doe, 1969). It has also been reported that in users of oral contraceptives liver metabolism is altered (Stokes & Wynn, 1971; Rossner *et al.*, 1971; Conrad *et al.*, 1972). Clearly the modulating effect of oestrogens on human liver function requires further studies, in view of the widespead use of oestrogen/progestogen combinations as contraceptives and in hormonal replacement therapy.

Our interest in the side-effects of sex hormones has led us to investigate extensively the characteristics of the oestrogen-receptor system in the rat. In this paper we report on the identification of the oestrogen receptor in both cytosol and nuclear fractions from rat liver. Further, we have examined the nucleo–cytoplasmic relationships of these receptors during the oestrous cycle and on administration of oestradiol-17$\beta$ and ethynyloestradiol.

## Experimental

### *Materials*

[2,4,6,7(*n*)-$^3$H]Oestradiol-17$\beta$ (sp. radioactivity 100 Ci/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Sephadex LH-20



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was from Pharmacia (G.B.) Ltd., London W5, U.K. Butyl-PBD [5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] was from Ciba, Horsham, Sussex, U.K. 17α-Ethynyloestradiol (17α-ethynyl-1,3,5-oestratriene-3,17β-diol) and oestradiol-17β [1,3,5(10)-oestratriene-3,17β-diol] were supplied by Sigma (London), Chemical Co., Poole, Dorset, U.K.

*Monitoring the oestrous cycle*

Female Wistar rats were bred in our laboratories in the Institute of Neurology. Groups of 40 mature females aged 80–85 days were maintained for 1 week on a 12h-light/12h-dark schedule before use. Progress of rats through the oestrous cycle was followed by means of vaginal smears taken on several successive days, including the day of experiment. The rats displayed a 4-day oestrous cycle.

Animals were killed by decapitation at 12:00h on the day of experiment and livers from animals in the same phase were pooled.

*Oestrogen-injected animals*

Groups of mature female rats (80–85 days old) received subcutaneous injections of oestradiol-17β and ethynyloestradiol (1–100μg) dissolved in propylene glycol (0.1ml), and control groups received the vehicle alone. Animals were decapitated 1h after injection.

*Preparation of liver fractions*

Livers were removed rapidly, chilled in ice, then forced through a tissue press of 1mm pore size. All procedures were carried out at 4°C. A 5g sample was removed and suspended in 4vol. of TSC buffer [10mM-Tris/HCl (pH7.4)/0.25M-sucrose/1mM-$CaCl_2$]. Homogenization was carried out in a Potter–Elvehjem-type homogenizer by using four up-and-down strokes, and the homogenate was passed through four layers of cheesecloth.

A crude nuclear pellet was obtained by centrifugation of the homogenate at 1200*g* for 10min, and further centrifugation of the supernatant at 200000*g* for 60min provided the cytosol fraction. The nuclear pellet was washed with TSC buffer (4 × 25ml) before receptor determinations.

*Assay of cytosol oestrogen receptors*

Scatchard-plot analysis of oestrogen binding to liver cytosol was carried out by incubating 0.2ml portions of cytosol in duplicate with various concentrations of [$^3$H]oestradiol (0.5–40nM) with and without a 50-fold excess of diethylstilboestrol for 18h at 0–4°C. The samples were then chromatographed on columns (6cm × 0.6cm) of Sephadex LH-20 (Ginsburg *et al.*, 1974, Thrower *et al.*, 1976). The [$^3$H]oestradiol–receptor complex was eluted in the void volume, whereas free [$^3$H]oestradiol was retarded in the gel. The void volume was collected and added to 10ml of a scintillation cocktail of 4.5g of butyl-PBD in 1 litre of toluene for determination of radioactivity 24h later in a Beckman LS 330 spectrometer with an efficiency of 46%.

Duplicate measurements agreed to within 5%, and non-specific binding was less than 20% of total bound radioactivity.

This method allows measurement of unoccupied receptor sites. For routine measurements of cytosol receptors, a saturating concentration of 10nM-[$^3$H]oestradiol was employed.

Comparison of oestrogen binding to cytosols prepared in TSC and TED [10mM-Tris/HCl (pH7.4)/1.5mM-EDTA/1mM-dithiothreitol] buffers was carried out with cytosol fractions prepared in their respective buffers from 5g samples of tissue taken from a pool of four to six livers.

Studies of binding specificity were carried out by adding 0.5μM of various non-radioactive steroids to the cytosols together with [$^3$H]oestradiol. For measurements on perfused livers animals were anaesthetized with diethyl ether and perfused with 0.9% NaCl introduced into the heart through the left ventricle after incising the right atrium, before extraction of the tissue.

Oestrogen binding to liver cytosols was also analysed by affinity chromatography on spheroidal-hydroxyapatite columns (Booth *et al.*, 1977) by the method described by Thrower & Lim (1980). Portions of cytosol were incubated on the columns, washed and then further incubated with 0.5–20nM-[$^3$H]oestradiol with and without a 50-fold excess of diethylstilboestrol at 0–4°C for 18h.

*Dextran/charcoal treatment of cytosols*

Portions (0.5ml) of dextran-coated charcoal in suspension (0.5% charcoal, 0.05% Pharmacia dextran T70 suspended in 10mM-Tris/HCl, pH7.4) were centrifuged at 2000*g* for 10min at 4°C. The pellet was washed with 1ml of buffer to remove fine dextran particles, re-centrifuged and 2ml portions of cytosol were added to the pellets. The samples were vortex-mixed, left on ice for 30min, then centrifuged at 2000*g* for 10min and the supernatants removed for measurement of receptor content as described above.

*Assay of nuclear oestrogen receptors*

In order to analyse oestrogen binding to the nuclear fractions of liver, triplicate 0.2ml samples of the nuclear suspension were incubated at 37°C for 30min with different concentrations of [$^3$H]oestradiol (0.25–40nM) with and without a 50-fold excess of diethylstilboestrol at each concentration (adapted from Anderson *et al.*, 1972). Under these conditions endogenous oestradiol bound to the receptor is exchanged with [$^3$H]oestradiol and

available binding sites are filled with the labelled steroid.

A saturating concentration of 10nM-[$^3$H]oestradiol in the presence and absence of 0.5$\mu$M-diethylstilboestrol was used for routine measurements of total nuclear receptor content. After incubation, the nuclear samples were treated with 1ml of buffer A (TSC buffer containing 0.2% Triton X-100 and 1% bovine serum albumin), mixed and kept at 4°C for 7min before centrifuging at 1200*g* for 5min. The pellets were then washed with 2 × 1ml of buffer B (TSC buffer containing 1% bovine serum albumin) and once with TSC buffer. The pellets were then transferred with 0.8ml of distilled water into vials containing 10ml of butyl-PBD/toluene scintillation fluid as previously described for determination of radioactivity. Triplicate determinations agreed to within 10–15%.

Purified nuclei were prepared by the dense-sucrose method of Chauveau *et al.* (1956) and the nuclei were resuspended in TSC buffer. The incubation conditions for measurement of nuclear oestrogen receptors in both normal and oestrogen-injected animals were determined by incubating nuclear samples (0.2ml) in triplicate at various temperatures for different times (0.5–2h). Under the optimal incubation conditions used for total nuclear-receptor measurements (37°C for 30min), the receptor breakdown was calculated to be 23% and nuclear receptor measurements were corrected accordingly.

The specificity of steroid binding to nuclear oestrogen receptors was determined by adding 0.5 and 1$\mu$M of various non-radioactive steroids to triplicate 0.2ml portions of nuclear suspension containing 10nM-[$^3$H]oestradiol. Controls contained 10nM-[$^3$H]oestradiol alone.

*Purity of nuclei*

The liver nuclei examined under an electron microscope appeared intact, with the chromatin undisturbed. The detergent Triton X-100 removed most of the nuclear outer membrane. The electron micrographs were interpreted as containing nuclei essentially free of contamination by other cellular particles. This is consistent with the findings of Hubert *et al.* (1962) and Hymer & Kuff (1963) in their examination of rat liver nuclei prepared with Triton X-100.

*DNA and protein determinations*

The DNA content of homogenates and nuclear fractions was determined by the Schmidt–Thannhauser procedure with modifications recommended by Munro & Fleck (1966). DNA recovery in the nuclear suspensions averaged 50% and all nuclear-receptor measurements were corrected for loss. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

## Results and Discussion

*Characterization of cytosol oestrogen binding*

In target tissues, oestrogens bind to specific cytosol receptors, which are subsequently translocated to the nucleus. The interaction of the receptor with chromatin results in tissue-specific responses (Buller & O'Malley, 1976; Gorski & Gannon, 1976). The oestrogen binding in cytosol and nuclear preparations from adult female liver was therefore investigated initially. All results stated in the text are expressed as means ± S.E.M., with the numbers of determinations in parentheses.

The preparation of the liver nuclear fractions involved the use of medium containing sucrose (TSC buffer; see the Experimental section). Because it has been suggested that this results in a lower yield of cytosol oestrogen receptors (King & Mainwaring, 1974), the binding of oestrogens to cytosol prepared in either TSC buffer or TED buffer containing no sucrose was compared. TED buffer was used in previous investigations of uterine receptors (White *et al.*, 1978) as well as of liver oestrogen receptors (Eisenfeld *et al.*, 1976). The use of either TSC or TED buffer made no significant difference to determinations of the dissociation constant ($K_d$) which were respectively 1.47 ± 0.26 (5) and 1.28 ± 0.20 (3)nM or to the concentration of oestrogen receptor [4.10 ± 0.44 (5) and 4.02 ± 0.52 (3)pmol/g wet wt.]. The $K_d$ values were similar to those reported for liver cytosol (Powell-Jones *et al.*, 1976) and to those of oestrogen receptors present in 'classical' target tissues (King & Mainwaring, 1974; White *et al.*, 1978). The concentration of receptor was also similar to that reported by others, who used a different isolation procedure (Aten *et al.*, 1978). We also found that perfusion of the liver before tissue fractionation altered neither the $K_d$ [2.17 ± 0.32 (3)nM] nor the yield of receptor [3.90 ± 0.29 (3)pmol/g wet wt.]. In subsequent experiments tissue was therefore directly prepared in TSC buffer for the simultaneous determination of cytosol and nuclear receptor concentrations.

Measurements of the content of cytosol receptor were made at 4°C with different periods of incubation. Maximal values were obtained at 2h, with no further changes at later times.

An alternative method for measuring the cytosol oestrogen receptors, involving spheroidal-hydroxyapatite chromatography (Booth *et al.*, 1977), was also used. This allows much of the non-specific binding and perhaps degradative and metabolizing enzymes to be separated from the specific binding component (Thrower & Lim, 1980), thus achieving a partial purification of the oestrogen receptor (see the Experimental section). The dissociation constant [$K_d$ = 1.70 ± 0.21 (3)nM] and concentration of

receptor [4.24 ± 0.58 (3) pmol/g of tissue] were similar to those obtained by using Sephadex LH 20 (cf. above values). These results suggest that whatever metabolism at 4°C does occur, measurements of receptor in unfractionated liver cytosol are not affected.

The specificity of oestradiol binding to receptor was determined by using a range of competitors (Table 1*a*). Only oestradiol-17$\beta$ and the synthetic oestrogen diethylstilboestrol were effective competitors, providing further evidence that the high-affinity limited-capacity binding of oestradiol in liver cytosol is due to interaction with specific oestrogen receptors.

*Characterization of nuclear oestrogen binding*

The limited data available about oestrogen binding in liver nuclear preparations (Aten *et al.*, 1978) do not include information on the affinity of ligand binding ($K_d$). The binding of [$^3$H]oestradiol to liver nuclear fractions prepared in TSC buffer was therefore characterized and compared with that of the corresponding cytosol fractions. Specific binding over a range of [$^3$H]oestradiol concentrations was examined by Scatchard analysis. In experiments where the detergent Triton X-100 was not included in the first post-incubation wash, a curvilinear plot was obtained (Fig. 1*a*); the existence of a high-affinity component [$K_d = 1.91 \pm 0.20$ (3) nM] was shown by extrapolation as described by Chamness *et al.* (1975). The lower-affinity component of the specific nuclear oestrogen binding could be removed by inclusion of Triton X-100 in the preparation buffer (see the Experimental section). The dissociation constant, 2.28 ± 0.29 (5) nM (Fig. 1*b*), was similar to that derived from Fig. 1(*a*). The binding capacity of the high-affinity component in the presence and absence of detergent was 0.19 ± 0.02 (5) and 0.18 ± 0.02 (3) pmol/g wet wt. respectively. Detergent treatment was therefore used in routine measurements of nuclear oestrogen-receptor content at saturating concentrations of oestradiol.

The binding of oestradiol to the nuclear preparation was examined in the presence of other steroids (Table 1*b*). Only oestradiol-17$\beta$ and diethylstilboestrol were effective competitors, as also observed with the cytosol receptor (Table 1*a*). The $K_d$ value and these competition studies suggest that oestrogen binding in rat liver nuclei is to a high-affinity specific oestrogen receptor with similar properties to that found in the cytosol.

Maximal binding of [$^3$H]oestradiol to nuclear receptors was observed after incubation at 37°C for 30 min; at this temperature the half-life of the receptor was approx. 90 min (Table 2). At 4°C a considerable proportion of the nuclear receptors became complexed with radioactive oestradiol; similar observations have been made for the nuclear receptors of mammary tumours (Zava & McGuire, 1977) and the female rat hypothalamus (White & Lim, 1978).

Studies of incubation conditions were made after the administration of ethynyloestradiol to promote

Table 1. *Specificity of the liver cytosol and nuclear oestrogen receptor*

(*a*) Samples of liver cytosol (0.2 ml) from uninjected animals were incubated at 4°C for 18 h with 10 nM-[$^3$H]-oestradiol and 0.5 $\mu$M of each of the competitors listed. Controls contained [$^3$H]oestradiol alone. The samples were assayed as described in the Experimental section. Duplicate determinations were carried out in each experiment, and results for three separate experiments are given. (*b*) Samples of liver nuclear suspension (0.2 ml) from uninjected animals were incubated at 37°C for 30 min in the presence of 10 nM-[$^3$H]oestradiol and 0.5 $\mu$M non-radioactive competitors as indicated. Controls contained radioactive steroid alone. Triplicate determinations were carried out for each group and the result for three separate experiments are given. Livers from five to seven animals were pooled in each experiment. (*c*) Groups of four to six animals were injected with 100 $\mu$g of ethynyloestradiol each and killed 1 h later. Samples of liver nuclear suspension (0.2 ml) were incubated with 10 nM-[$^3$H]oestradiol alone and in the presence of the competitors as described in (*b*). Triplicate determinations were carried out in each experiment and the results for three separate experiments are given.

| | Radioactivity bound (c.p.m/0.2 ml samples) | | |
|---|---|---|---|
| | | Nuclear suspensions | |
| Competitor added | (*a*) Cytosol ($\times 10^{-2}$) | (*b*) Uninjected rats | (*c*) Injected rats |
| (*a*) Control | 217 ± 26 | 1280 ± 52 | 3109 ± 62 |
| (*b*) Diethylstilboestrol | 45 ± 8 | 747 ± 16 | 804 ± 56 |
| (*c*) Oestradiol-17$\beta$ | 44 ± 7 | 668 ± 16 | 694 ± 47 |
| (*d*) Testosterone propionate | 232 ± 27 | 1408 ± 28 | — |
| (*e*) Dihydrotestosterone | 205 ± 20 | 1175 ± 47 | 2470 ± 84 |
| (*f*) Progesterone | 242 ± 25 | 1127 ± 38 | 2133 ± 48 |
| (*g*) Dexamethasone | 237 ± 20 | 1175 ± 48 | 2776 ± 161 |
| (*h*) Cortisol | 236 ± 20 | 1179 ± 46 | 2788 ± 42 |

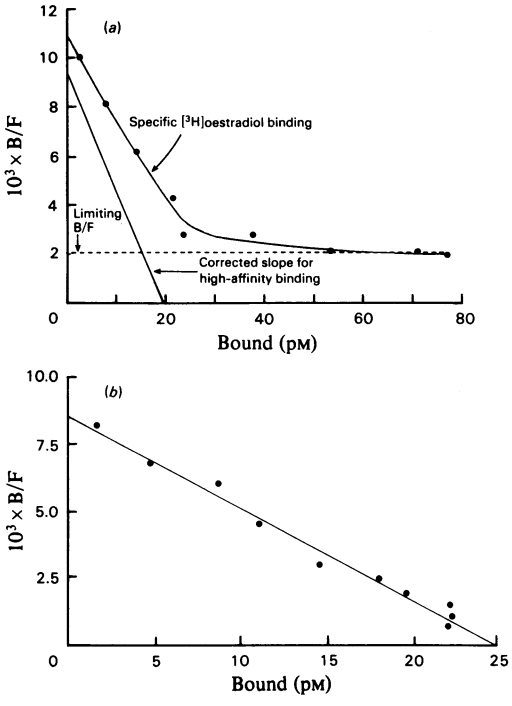


Fig. 1. *Scatchard analysis of oestrogen binding to nuclear fractions in the (a) absence and (b) presence of detergent treatment*

(*a*) Portions of nuclear suspension (0.2ml) were incubated with various concentrations of [$^3$H]oestradiol with or without a 50-fold excess of diethylstilboestrol at 37°C for 30min. The samples were then processed as described in the Experimental section with the omission of Triton X-100 from the buffer in the first post-incubation wash. The specific binding of steroid was determined at each concentration as explained in the Experimental section, and this was plotted against the bound/free (B/F) ratio. The curvilinear plot obtained indicates both a high- and a lower-affinity binding of [$^3$H]oestradiol to these fractions. The slope for high-affinity binding corrected as described by Chamness *et al.* (1975) is also shown. (*b*) Portions of nuclear suspensions (0.2ml) were incubated in triplicate at 37°C for 30min with various concentrations of [$^3$H]oestradiol (0.25–40nM) with and without a 50-fold excess of diethylstilboestrol. The specific binding at each concentration of radioactive steroid was measured, as previously explained, and the dissociation constant ($K_d$) was obtained from the plot of steroid bound against B/F ratio.

increases in nuclear receptor content. In these nuclear preparations the optimal incubation conditions were also found to be 37°C for 30min and these were used in subsequent experiments.

*Oestrogen receptors during the oestrous cycle*

In oestrogen-target tissues the intracellular distribution of receptor varies in relation to fluctuations in plasma oestrogen concentrations throughout the oestrous cycle (White *et al.*, 1978; Myatt *et al.*, 1978). The nature of the oestrogenic response is dependent on the target tissue; for example part of the uterotrophic response involves an increase in uterine wet weight at pro-oestrus, when nuclear oestrogen-receptor content is maximal. There was no variation in liver wet weight throughout the oestrous cycle (Table 3); similarly DNA content and cytosol protein content remained constant throughout the cycle.

There was no significant change in the total receptor content (Table 3). This contrasts with observations in other target tissues, where total receptor content undergoes cyclic variation (White *et al.*, 1978). There was also no variation in the distribution of receptor between the cytosol and nuclear fractions (Table 3). This is in marked contrast with the receptor distribution in target tissues, where an increase in nuclear receptor content occurs at pro-oestrus, when plasma oestrogen concentration is maximal.

The concentration of cytosol oestrogen receptor in liver was similar to that reported by others using randomly cycling animals (Aten *et al.*, 1978). However, in order to eliminate the possibility that cytosol-receptor measurements were affected by changes in the endogenous concentration of oestrogens throughout the oestrous cycle, preparations were preincubated with dextran-treated charcoal before assay for receptor content. Prior treatment with dextran-treated charcoal did not alter the receptor content in cytosol at any phase of the oestrous cycle (Table 4).

*Effect of exogenous oestrogen on receptor distribution*

The effect of oestrogen administration on liver oestrogen-receptor content and distribution was then examined. Because of the lack of variation in receptor content throughout the oestrous cycle, which may reflect a homeostatic mechanism operating to regulate hepatic oestrogen concentration, randomly cycling animals were used.

The oestrogen receptor was translocated from the cytosol into the nucleus in response to exogenous oestrogens (Fig. 2). The effects of the natural oestrogen oestradiol-17$\beta$ and the synthetic compound ethynyloestradiol were different. Ethynyloestradiol was more effective in depleting the cytosol receptor content than was oestradiol-17$\beta$. A dose of 50$\mu$g of oestradiol-17$\beta$ was required to deplete the cytosol receptor content by 80%, whereas only 10$\mu$g of ethynyloestradiol produced a similar effect. This

Table 2. *Incubation conditions for measuring nuclear oestrogen receptor*

Portions (0.2 ml) of nuclear suspensions were incubated with 10 nM-[$^3$H]oestradiol with or without 0.5 $\mu$M-diethylstilboesterol at different temperatures for various times and the specific oestrogen receptor contents determined as explained in the Experimental section. Triplicate determinations were carried out for each point, and the complete experiments were repeated three times. For the measurements on oestrogen-injected animals, groups of four to six rats were injected with 100 $\mu$g of ethynyloestradiol and killed after 1 h.

| | | | Oestrogen-receptor content (fmol/ml of nuclear suspension) | |
|---|---|---|---|---|
| Temp (°C) | Time (h) | Nuclei from ... | Uninjected animals | Oestrogen-injected animals |
| 0–4 | 1 | | 19.2 ± 2.0 | 3.6 ± 1.5 |
| 25 | 1 | | 21.8 ± 2.4 | 30.4 ± 1.5 |
| 25 | 2 | | 17.9 ± 4.1 | 35.6 ± 0.5 |
| 30 | 1 | | 21.8 ± 1.8 | 38.3 ± 3.4 |
| 30 | 2 | | 20.6 ± 4.7 | 30.7 ± 5.6 |
| 37 | 0.5 | | 22.4 ± 2.6 | 87.3 ± 4.2 |
| 37 | 1 | | 15.7 ± 1.8 | 66.3 ± 7.5 |
| 37 | 2 | | 12.5 ± 1.7 | 37.8 ± 5.8 |

Table 3. *Wet weight, cytosol protein, DNA and oestrogen-receptor content and distribution during the oestrous cycle*

Measurements of oestrogen-receptor content of cytosol and nuclear fractions, tissue wet weight, DNA and cytosol protein were made on the four successive days of the oestrous cycle. The results are from five separate experiments with four to six animals per group. Student's *t* tests were applied to the data and none of the measurements was significantly different from the others (two-tailed tests).

| | Pro-oestrus | Oestrus | Metoestrus | Dioestrus |
|---|---|---|---|---|
| (*a*) Wet wt. (g) | 8.8 ± 0.2 | 8.0 ± 0.2 | 8.6 ± 0.2 | 8.8 ± 0.3 |
| (*b*) DNA content (mg/g wet wt.) | 1.9 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.1 | 2.1 ± 0.2 |
| (*c*) Cytosol protein (mg/g wet wt.) | 68 ± 2.3 | 72 ± 2.6 | 67 ± 3.4 | 69 ± 3.6 |
| (*d*) Total receptor (pmol/g wet wt.) | 4.2 ± 0.3 | 4.1 ± 0.5 | 3.5 ± 0.4 | 4.1 ± 0.4 |
| (*e*) Cytosol receptor (pmol/g wet wt.) | 4.0 ± 0.2 | 3.8 ± 0.5 | 3.3 ± 0.3 | 3.9 ± 0.4 |
| (*f*) Nuclear receptor (pmol/g wet wt.) | 0.21 ± 0.02 | 0.24 ± 0.02 | 0.19 ± 0.02 | 0.18 ± 0.02 |

Table 4. *Comparison of specific cytosol receptor content with and without dextran/charcoal treatment of cytosols*

At each cycle stage, samples of cytosol were treated with dextran/charcoal, as explained in the Experimental section, and were then assayed for cytosol receptor content along with the untreated cytosols. Duplicate determinations were made in each of three oestrous-cycle experiments.

| | Specific oestrogen receptor content (pmol/ml of cytosol) | |
|---|---|---|
| Cycle stage | With dextran/charcoal | Without dextran/charcoal |
| Pro-oestrus | 0.81 ± 0.04 | 0.84 ± 0.02 |
| Oestrus | 0.75 ± 0.04 | 0.73 ± 0.03 |
| Metoestrus | 0.73 ± 0.05 | 0.74 ± 0.06 |
| Dioestrus | 0.72 ± 0.06 | 0.72 ± 0.05 |

is probably because oestradiol is more rapidly metabolized than ethynyloestradiol.

Maximal depletion of cytosol receptor was observed with a 50 $\mu$g dose of each oestrogen, but the extent of receptor depletion was greater for ethynyloestradiol than for oestradiol-17$\beta$.

The increase in the concentration of nuclear receptor after oestrogen stimulation was different for both oestrogens and reflected to some extent the observed differences in cytosol depletion. Thus the maximal concentration of nuclear receptor was achieved with a 10 $\mu$g dose of ethynyloestradiol, compared with 50 $\mu$g of oestradiol-17$\beta$. Higher doses of oestrogen are required to translocate the liver oestrogen receptor compared with that of the uterus (Aten *et al.*, 1978), and this was attributed to

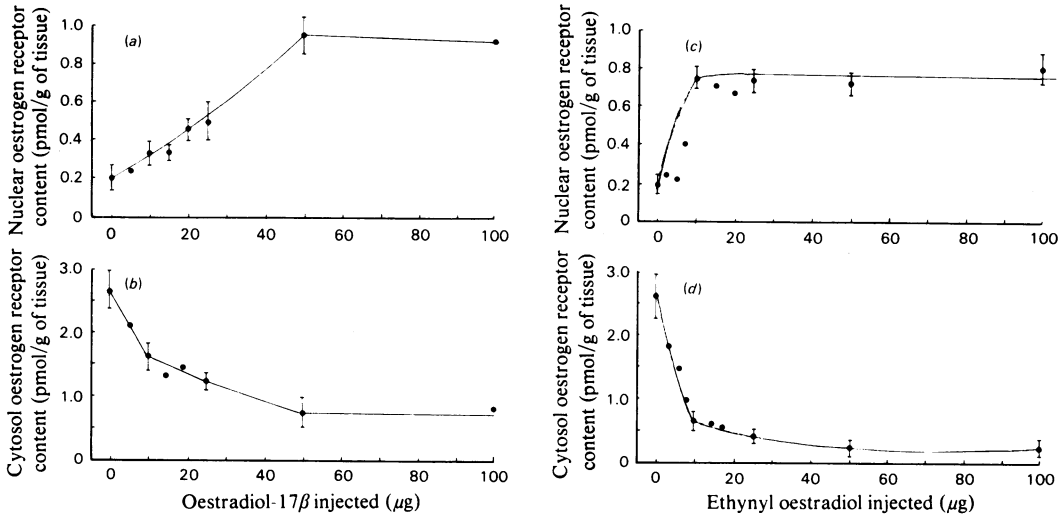


Fig. 2. *Oestrogen-receptor distribution in response to injected oestrogens*

Groups of four to six rats were injected subcutaneously with various doses (1–100μg) of oestradiol-17β (*a*,*b*) or ethynyloestradiol (*c*,*d*) and were killed 1 h later. The oestrogen-receptor content of the nuclear and cytosol fractions was determined as explained in the Experimental section. Results are expressed as the means ± S.E.M. for three separate determinations, and the absence of error bars indicates a mean value for two separate determinations. Oestradiol-injected rats: (*a*), nuclear; (*b*), cytosol; ethynyloestradiol-injected rats: (*c*) nuclear; (*d*), cytosol.

metabolism of the steroid in the liver.

There is an apparent loss of total receptor content (cytosol and nuclear) associated with the translocation of cytosol receptor into the nucleus. The 10μg dose of ethynyloestradiol depleted the cytosol receptor content by about 2.0pmol/g of tissue, whereas the corresponding increase in nuclear oestrogen receptor was only about 0.6pmol/g of tissue.

Similarly, in response to the administration of 50μg of oestradiol-17β there was an apparent receptor loss of 1.3pmol/g of tissue. Our cytosol-receptor measurements are equivalent to those reported by Aten *et al.* (1978), who used an exchange assay procedure, and it is unlikely that they are underestimations caused by either masking of cytosol receptor by endogenously bound ligand or the lowering of the specific radioactivity of [$^3$H]-oestradiol by endogenous steroid. A possible explanation is that after oestrogen stimulation a certain proportion of the nuclear receptor is processed and not detectable 1 h after injection (Aten *et al.*, 1978) and that this processing is required for the initiation of some other cellular events. Such a relationship between oestrogen-receptor processing and the induction of progesterone receptors has been demonstrated in MCF-7 human breast-cancer cells (Horwitz & McGuire, 1978).

The equilibrium dissociation constant ($K_d$) of the nuclear receptor binding after the injection of 100μg of ethynyloestradiol was 2.78 ± 0.27 (5)nM, which is similar to that for nuclear oestrogen binding in control animals. The Scatchard analysis, a straight-line plot, suggested a single high-affinity binding component. Competition studies (Table 1*c*) showed that oestradiol-17β and diethylstilboestrol were most effective in suppressing nuclear binding of radioactive oestradiol. In contrast with uninjected animals, there was some inhibition of nuclear [$^3$H]oestradiol binding by dihydrotestosterone (27%) and progesterone (42%). The significance of such changes in specificity is unknown.

## *Conclusions*

The characteristics of oestrogen receptors of mammalian liver are similar to those of the uterus and other target tissues. There is a high-affinity limited-capacity oestrogen-specific receptor in both nuclear and cytosol fractions.

However, there appear to be differences in the interaction of oestrogens with their receptors in liver, as indicated by no detectable change in the nuclear and cytosol receptor content during the oestrous cycle. In particular, the increase in plasma oestrogen at pro-oestrus was not associated in liver with depleted cytosol receptor content or an increased nuclear receptor content, as is observed, for example, in uterus, where the increase is 4–5-fold (White *et al.*, 1978).

Liver is the main site for the conversion and

deactivation of steroids, and it is possible that rapid metabolism of oestrogens serves also to regulate the interaction of the steroid with its receptors. The constancy of the nuclear receptor content during the cycle implies a limited but sustained stimulation of the hepatic genome by oestrogen, assuming that the presence of nuclear oestrogen receptors is associated with oestrogenic responses in this tissue. In contrast, other oestrogen-target tissues are synchronized in their receptor-mediated responses to the cyclic fluctuations in the plasma content of oestrogens.

Increases in nuclear receptor content concomitant with depletions of cytosol receptor content are, however, induced *in vivo* by hyperphysiological doses of the oestrogens oestradiol-17$\beta$ and ethynyloestradiol. High concentrations of steroid can apparently overcome the normal regulatory/inhibitory mechanisms, thus allowing oestrogens, or their metabolites, to interact with their receptors. In particular, a lower dose of ethynyloestradiol effected maximal receptor translocation, which might be related to the decreased metabolism of the 17$\alpha$-ethynylated oestrogen (Helton & Goldzieher, 1977). Further work done in our laboratory (W. Marr, M. Elder & L. Lim, unpublished work) has shown that long-term nuclear retention of oestrogen receptors results from injections of ethynyloestradiol, but not of the natural oestrogen. In uterus, these long-term nuclear receptors are related to true uterine growth, whereas short-term retention of nuclear receptors results in limited uterotrophic responses (Clark & Peck, 1976).

In target tissues, part of the oestrogenic response involves new mRNA and protein synthesis and, if this also applies to the mammalian liver, as has been shown for avian liver, then the presence of nuclear receptors and their increase on oestrogen administration could be consistent with the concept of a receptor-mediated response to the hormone in this tissue. Thus the translocation of receptor could be salient to the problem of liver dysfunction caused by the clinical use of synthetic oestrogens. Reports of clinical trials on oral-contraceptive users have revealed that the extent of liver dysfunction depends not only on the dose of oestrogen but also on the dose and type of progestogen employed in combined oral-contraceptive pills (Wynn *et al.*, 1979).

We have therefore continued to investigate the interaction of oestrogens with their receptors in liver and have extended the study to include two progestogens, d-norgestrel (D-13$\beta$-ethyl-17$\alpha$-ethynyl-17$\beta$-hydroxygon-4-en-3-one) and norethisterone (19-nor-17$\alpha$-ethynyl-17$\beta$-hydroxyandrost-4-en-3-one) acetate. It is hoped that the information gained from this work will further elucidate the effects of synthetic steroids used in oral contraceptives on liver function.

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