et al., submitted for publication). Thus, the induced ribosomes may stabilize the poly(A)(+)-RNA synthesized immediately after hormone administration and thereby maintain translation of the induced mRNA and the physiological response.

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Nuclear Estradiol Receptor in the Adult Rat Uterus: a New Exchange Assay†

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ABSTRACT: A protamine exchange assay has been developed to measure uterine nuclear estrogen receptor in mature rats exposed to estradiol (E). After ovariectomized-adrenalectomized mature rats are injected with E, estrogen receptor (RnE) is extracted from uterine nuclei with 0.6 M potassium chloride, diluted, and quantitatively precipitated with protamine sulfate. The precipitate is subjected to a ligand exchange with radiolabeled estradiol (E*), with or without unlabeled diethylstilbesterol, to determine nonspecific binding. At 37 °C complete exchange of E* for E in RnE is observed at 2.5 h; virtually no receptor degradation occurs up to at least 5 h. Exchange does not occur at 4 °C. Using the protamine assay, the depletion of cytoplasmic estrogen receptor (Rc) and the accumulation of RnE were studied at various doses of E at specific time points. Increasing doses of E result in a decrease of Rc with an equal increase of RnE. At the highest dose of E (10 μg) Rc is completely depleted within 10 min, by 6 h it is 25% replenished, and by 24 h returns to slightly above control levels. Within 10 min after the injection, RnE increases to 80-90% of the original cytoplasmic level of receptor (~2-3 pmol/mg of DNA or ~1.5 pmol/100 mg of uterus). At 6 h RnE is 75% depleted and it is completely absent at 24 h. The protamine assay permits precise quantitative studies of nuclear estrogen receptor and avoids the problems of receptor degradation and excessive nonspecific binding often found in exchange reactions at elevated temperatures.

Estrogen administration to mature ovariectomized-adrenalectomized rats results in rapid binding of hormone to specific receptor molecules in the cytoplasm of target organs. This is followed by rapid nuclear accumulation of the receptor-hormone complex (Gorski et al., 1968; Anderson et al., 1973; Giannopoulous and Gorski, 1971; Tchernitchin and Chandross, 1973). Well defined uterotrophic responses such as RNA and protein synthesis (Hamilton, 1971), increased uterine weight, and increased cell division (Anderson et al., 1974) are all associated with nuclear entry of the receptor-hormone complex.

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Various techniques have been developed to measure both cytoplasmic and nuclear receptors in parallel with the cellular responses evoked after exposure to estradiol. Unfilled cytoplasmic estrogen receptor sites (Rc)[1] are assayed by incubating radioactive estradiol (E*) with a cytosol preparation and removing nonreceptor-bound E* with charcoal (McGuire and De La Garza, 1973), or precipitating Rc with protamine (Steggles and King, 1970; Chamness et al., 1975), or demonstrating 8S RcE* binding peaks in a sucrose gradient (Gorski et al., 1968). Filled cytoplasmic sites (RcE) have been measured by direct exchange of E* for E in RcE (Katzenellenbogen et al., 1973) or on protamine precipitates (Chamness et al., 1975).

In the nucleus most receptor molecules are filled with E (RnE); measurement of RnE requires an exchange reaction with a large excess of E* at elevated temperature directly in the crude nuclear pellet (Anderson et al., 1972) or in a salt extract of the nuclear pellet (Rochefort and Capony, 1973). Alternatively, direct ethanol extraction of the nuclear pellet following E* administration has been utilized (Gorski et al., 1968).

The nuclear-exchange reactions have proved valuable in studying RnE in immature rat uteri but we find these assays less satisfactory in mature rat uteri. In our hands, the major difficulties of the exchange reaction in crude nuclear pellets have been instability of RnE at elevated temperatures, high nonspecific E* binding, and variable replication. To circumvent these difficulties, we have adapted a protamine exchange assay recently developed in our laboratory for RcE (Chamness et al., 1975) to the measurement of RnE. In this report, we will validate the nuclear protamine assay and use it to examine charged nuclear estrogen receptor in the mature rat uterus under controlled hormonal conditions.

Materials and Methods

Preparation of Rats. Female Sprague Dawley (S.D.) (Holtzman) or Wistar Furth (W.F.) rats (age 4–6 months) were ovariectomized and adrenalectomized (O-A) under ether anesthesia 24–72 h prior to use. Rats were maintained on regulation rat chow and 0.9% saline ad lib.

Rats were injected intraperitoneally with estradiol in 1 ml of 10% ethanol–0.9% saline. Controls received vehicle alone. Rats were then sacrificed by cervical dislocation. The uteri were rapidly removed (within 1 min), cleaned of adhering fat and mesentery, and immediately frozen in liquid nitrogen (within 3 min after removal from animal).

Preparation of Cytoplasmic and Nuclear Extracts. The frozen uteri were pulverized with a Thermovac tissue pulverizer (Thermovac Industries) and immediately placed in a 15-ml tube over ice. All procedures, unless otherwise stated, were carried out at 0–4 °C. To 200–350 mg of tissue, 1 ml of TED buffer (0.01 M Tris-HCl, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.4) was added and the tissue was homogenized with a Brinkman Polytron PT-10-ST at speed 3.5 for three 10-s intervals. This was the optimum homogenization condition for virtually complete cellular disruption and minimal nuclear shearing as detected by phase microscopy. The homogenate was centrifuged at 800*g*. The pellet was twice resuspended in TED buffer and recentrifuged at 800*g*. The three supernatants were combined and centrifuged at 105 000*g* for 30 min at 4 °C to remove cytoplasmic debris. The supernatant (undiluted cytosol preparation) was then treated with dextran-coated charcoal (DCC) (Chamness et al., 1973) to remove free hormone.

The washed nuclear–myofibrillar pellet was extracted three times with 1 ml of 0.6 M TKED buffer (0.01 M Tris-HCl, 0.6 M potassium chloride, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 8.5). The first two extractions were allowed to incubate for 20 min at 4 °C and were followed by centrifugation at 800*g* for 10 min. The supernatants from extracts 1 and 2 were combined and centrifuged at 105 000*g* for 30 min at 4 °C. To the remaining pellet was added 1 ml of 0.6 M TKED buffer, the tube was vortexed vigorously, and the suspension was centrifuged at 105 000*g* for 30 min at 4 °C. The two supernatants were then combined and used for the nuclear protamine assay. The pellets from the two 105 000*g* spins were saved for DNA analysis.

Cytoplasmic and Nuclear Protamine Assay. The cytoplasmic protamine assay was performed according to the method of Chamness et al. (1975) with the following modifications. The protein concentration of the DCC-treated cytosol extract was determined by a ratio of optical densities at 260 and 280 nm according to the method of Layne (1957) and then diluted with TED buffer to a concentration of 1.5 mg/ml. Protein concentration was later more accurately determined by the method of Lowry et al. (1951).

Protein content of the nuclear extract was determined by the same procedure and was accordingly diluted with TED buffer, pH 7.4, to a protein concentration of 0.25 mg/ml, thus reducing the salt concentration. Tubes used in the cytoplasmic and nuclear assays were prepared according to the method of Chamness et al. (1975).

Cytosol (200 μl) and nuclear (500 μl) extracts were incubated 3–5 min with 250 μl of 1 mg/ml of protamine sulfate (Lilly) and then were centrifuged at 800*g* for 10 min. The supernatant was removed and the presence of specific estrogen receptors in the precipitate was determined by the addition of 2 nM E* (17β-[^{3}H]estradiol 100 Ci/mmol, New England Nuclear) (total binding) or 2 nM E* containing 200 nM diethystilbestrol (DES) (nonspecific binding) in a volume of 500 μl TED buffer. The assay was performed in triplicate. Specific estrogen receptor sites were determined by subtracting nonspecific binding from total binding.

Cytoplasmic and nuclear estrogen receptor can theoretically exist as free proteins (Rc and Rn) or proteins charged with E (RcE and RnE). Specific, uncharged receptors, Rc and Rn, were assayed in the protamine pellet by incubating with E* or E* + DES at 4 °C for 4–24 h. Total receptors (Rc + RcE or Rn + RnE) were assayed in the protamine pellet by incubation with E* or E* + DES at 4 °C for 1 h followed by 30 °C for 5 h for total cytoplasmic receptor and 37 °C for 2.5 h for total nuclear receptor. The difference between the assay for total receptor (30 or 37 °C) and the assay for uncharged receptor (4 °C) yielded the values for RcE and RnE.

Estradiol Exchange Assay of the Nuclear–Myofibrillar Pellet. The exchange assay of the nuclear–myofibrillar pellet was performed according to the method of Anderson et al. (1972) with minor modifications. The washed nuclear pellet was resuspended in TED buffer, pH 7.4, and gently redispersed. The homogenate was then diluted with TED buffer to give an estimated 20–30 μg of DNA/assay tube. To determine total hormone binding, E* in 500 μl of TED buffer was added to a series of 3–5 tubes; to an equal series of tubes was added a 100-fold excess of DES for determination of nonspecific binding. Specific nuclear sites were determined by subtracting

[1] Abbreviations used are: Rc, RcE, unfilled and filled cytoplasmic estrogen receptor sites; Rn, RnE, unfilled and filled nuclear estrogen receptor sites; E, estradiol; E*, radioactive estradiol; O–A, ovariectomized–adrenalectomized; DCC, dextran-coated charcoal; DES, diethylstilbestrol; EDTA, (ethylenedinitrilo)tetraacetic acid.

TABLE I: Extraction of Radiolabeled Estradiol from Nuclear Pellet of Rat Uterus.[a]

Extract	cpm	% of Total
1	121 500	51
2	60 500	26
3	14 700	6
Nonextractable[b]	39 810	17
Total	236 510	100

[a] Two mature O-A rats were injected with 1 μg of E* (34 μCi/μg of E*) and sacrificed after 30 min. The uteri were pooled and the nuclear pellet was prepared and sequentially extracted three times with 2 ml of 0.6 M TKED buffer, pH 8.5, as described in the text. [b] Radioactivity that was resistant to salt extraction was extracted from the nuclear pellet with 3 ml of ethanol which was dried in a scintillation vial then counted as described in the text.

nonspecific from total nuclear binding. Following incubation the pellets were washed and extracted as previously described (Anderson et al., 1972).

DNA Determinations. DNA for both the protamine and nuclear exchange assays was determined according to the diphenylamine method of Burton (1956) using calf thymus DNA as a standard. Insignificant amounts (<1%) of DNA were found in the cytoplasmic debris from the 105 000*g* pellet and no DNA could be detected in the nuclear salt extract.

Results

Development of Protamine Assay for Nuclear Estrogen Receptor. Following injection of radiolabeled estradiol into mature O-A female rats the amount of estrogen receptor in the uterine nucleus can be measured by direct extraction of E*. We therefore injected mature O-A rats with 1 μg of E* and determined the efficiency of RnE* extraction and subsequent protamine precipitation. Three sequential extractions of the uterine nuclear pellet with 0.6 M KCl buffer consistently remove 80 ± 5% of E* (Table I). More exhaustive extractions fail to remove the 10–20% of residual radioactivity that remain tightly bound to the nuclear pellet. We do not yet know whether this 10–20% of salt-resistant radiolabel remaining in the nuclear-myofibrillar pellet represents specific estrogen receptor sites.

Although we found 0.6 M KCl to be optimal for extraction of RnE*, we anticipated this salt concentration might be too high for effective protamine precipitation. We therefore studied protamine precipitation of RnE* as a function of salt concentration. Figure 1 shows that 0.6 M KCl interferes with precipitation of RnE*, but that dilution to 0.1 M KCl or below permits essentially complete precipitation. All subsequent protamine precipitations of salt-extractable nuclear estrogen receptor were therefore carried out in buffer diluted to less than 0.1 M KCl content. To further show that the E* is indeed protein bound, we analyzed the nuclear extract on a 0.4 M KCl sucrose gradient (data not shown). Essentially all of the radioactivity sedimented as a 5S peak which was completely abolished upon addition of protamine sulfate.

Having established conditions for extracting and precipitating RnE, we next showed that protamine-precipitated RnE can be fully quantitated by ligand exchange with E* at elevated temperatures. RnE* was extracted from uterine nuclei of rats injected in vivo with E* of *low* specific activity. The uterine nuclear extract was adjusted to the appropriate protein concentration, and fractions of the extract were precipitated with

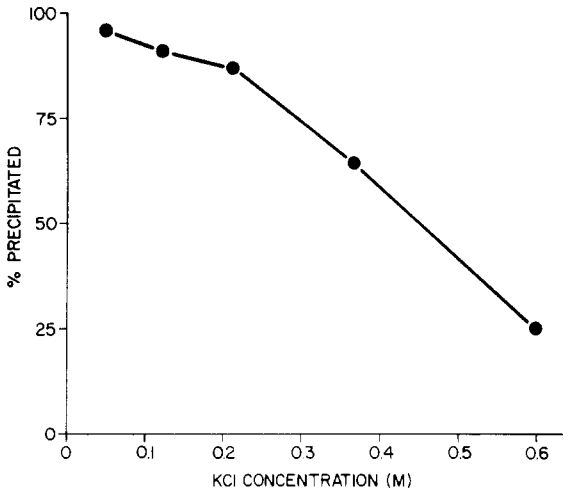

FIGURE 1: Precipitation of charged nuclear receptor (RnE*) with protamine at various salt concentrations. O-A rats were injected with 1 μg of E* and RnE* extracted from the uterine nuclear pellet according to Materials and Methods. RnE* was then diluted to a protein concentration of 0.25 mg/ml at 0.06, 0.11, 0.2, 0.35, and 0.6 M KCl concentrations. Three 500-μl fractions at each salt concentration were precipitated with 250 μl of 1 mg/ml of protamine sulfate. The percent of the total radioactivity precipitated with protamine was then determined. Each value represents the mean of triplicate determinations.

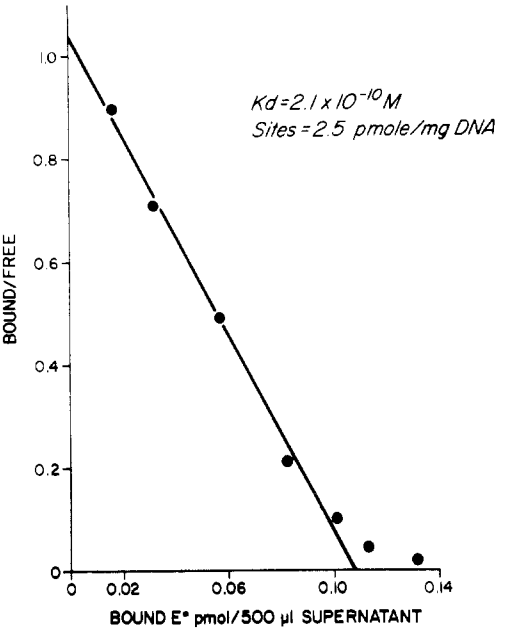

FIGURE 2: Scatchard plot of specific E* exchange with protamine-precipitated nuclear extract. Two mature O-A rats were injected with 10 μg of E and the uterine nuclear extract (RnE) was prepared as described in the text. RnE-protamine pellets were incubated in triplicate with E* (1×10^{-11} to 1×10^{-8}) in parallel with an equal series of tubes containing E* + DES (10^{-6} M) to determine nonspecific binding. Exchange was carried out at 37 °C for 2.5 h. Each point represents the mean of three determinations for specific E* binding. Nonspecific binding was less than 10%.

protamine. Nuclear receptor sites were determined either from ethanol extraction of the protamine-precipitated radioactivity of *low* specific activity or by ligand exchange in an equal series of protamine pellets (37 °C, 2.5 h) with E* of *high* specific activity. As seen in Table II, the number of charged nuclear sites are equivalent when assayed by either procedure.

We further demonstrated that RnE is an estrogen receptor of high affinity and low capacity. Rats were injected with 10

TABLE II: Comparison of Charged Nuclear Sites (RnE) by E* Injection and Nuclear Protamine Assay.[a]

	Nuclear Protamine Assay (pmol/mg of DNA)			E* Extraction (pmol/mg of DNA)
Uterus	RnE + Rn (37 °C)	Rn (4 °C)	RnE (37 − 4 °C)	RnE*
1	1.560	0.164	1.396	1.360
2	1.340	0.142	1.198	1.020
3	2.320	0.400	1.920	2.020

[a] Three mature O-A rats were injected with 1.0 μg of E* (34 μCi/μg of E*—low specific activity). After 30 min, the uteri were removed and RnE* was extracted from the nuclear-myofibrillar pellet according to Materials and Methods. RnE* was protamine precipitated and the supernatant was discarded. From some tubes, E* was extracted with ethanol and the radioactivity was counted. To a parallel series of tubes was added 2 nM E* (340 μCi/μg of E*—high specific activity) with or without 100-fold excess DES. These were incubated at 37 °C, 2.5 h and 4 °C, 4 h. Each value represents the mean of triplicate determinations for each uterus.

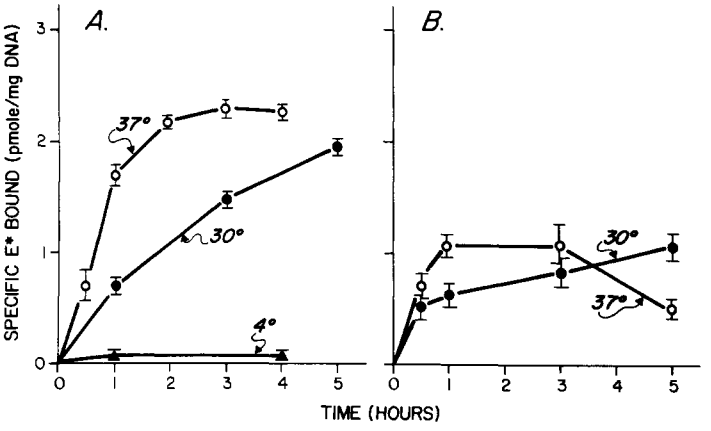

FIGURE 3: Exchange of E* for protamine-precipitated nuclear extract (A) and crude nuclear pellets (B) as a function of time and temperature. Four mature O-A rats were injected with 10 μg of E. After 30 min, the rats were sacrificed and their uteri were removed, pooled, frozen, pulverized, and then halved. Tissue in each tube was then prepared for the respective assays according to the text. The washed crude nuclear pellet was diluted with TED buffer to yield 30–50 μg of DNA/0.5 ml. The nuclear extract was diluted with TED buffer to the same volume. Fractions (0.5 ml) of the diluted nuclear extract and washed crude nuclear pellet were assayed for charged estrogen receptor with 2 nM E* (with or without 100-fold excess DES) by procedures described in the text. Figure 3A represents a 5-h time course of E* exchange for charged nuclear receptor by the protamine assay at 4, 30, and 37 °C. Figure 3B characterizes the exchange of E* for charged nuclear receptor by the direct exchange assay at 30 and 37 °C. All points represent the mean ± SE of three determinations for protamine and five determinations for direct exchange. Nonspecific binding by the protamine sulfate assay was consistently less than 10% and 30–50% by the direct exchange (data not shown).

μg of E to translocate all estrogen receptor. Fractions of the uterine nuclear extract were precipitated with protamine and the pellets were incubated with increasing doses of E* at 37 °C for 2.5 h. Complete exchange and saturation of E* for E in the RnE–protamine pellets occurs at about 2 nM E* with only about 5–10% nonspecific binding (data not shown). Scatchard analysis of the binding data (Figure 2) indicates that the protamine-precipitated nuclear extract does indeed have a high affinity (K_d = 0.2 nM) for E* as well as a saturable number of E* binding sites (2.5 pmol/mg of DNA). In subsequent experiments we therefore used 2 nM E* for all single point exchange assays, since it allows complete saturation of RnE with minimal nonspecific binding.

In our hands, the older exchange procedures for measuring charged nuclear receptor in mature rat uteri have been subject to receptor losses, high nonspecific estradiol binding, and variable replication at the elevated temperatures used for exchange. To illustrate these problems, we next compared the nuclear protamine assay to the direct nuclear exchange assay (Anderson et al., 1972). Uteri were removed from mature O-A rats 30 min after injection of 10 μg of E. Equal portions of thoroughly mixed uterine tissue were used for comparison of the two assays. Equivalent fractions of protamine-precipitated nuclear extract or crude nuclear pellet were incubated over a 5-h time course with 2 nM E* (with or without 100-fold excess DES) at 4, 30, and 37 °C. Figure 3A shows that there is a saturable exchange of E* for E in the RnE–protamine pellets within 3 h at 37 °C and 5 h at 30 °C; essentially no exchange occurs at 4 °C. Over the 5-h time course, protamine-precipitated RnE is stable at 30 and 37 °C; even after 20 h at 30 °C, specific E* binding is 85% of the maximum binding at 30 °C for 5 h (data not shown). Total E* binding at saturation is 2.0–2.3 pmol/mg of DNA, while nonspecific binding is less than 10% and variability in triplicates is negligible.

In contrast, we find that E* binding by direct exchange is complete within 1 h at 37 °C but requires 3–5 h for saturation at 30 °C (Figure 3B). The number of saturable E* binding sites at 30 and 37 °C are approximately 1 pmol/mg of DNA or about half the number of sites measured by the nuclear protamine assay (Figure 3A). Nonspecific binding ranges from 30 to 50% and variability in replicate samples is somewhat greater than seen in the nuclear protamine assay.

When the same comparisons were made in a different group of rats using 2 nM E* as before for the nuclear protamine assay but 13 nM E* instead of 2 nM E* for the direct exchange assay, we found that the direct exchange assay has even higher nonspecific binding (70–90%), high variability in replicate samples, and a disparity in the number of saturable E* binding sites at 30 and 37 °C (data not shown). Nevertheless, even the highest E* binding (37 °C, 3 h) was still only about half that measured by the nuclear protamine assay. Because of the extremely high nonspecific binding (70–90%) when incubates contain E* above 2 nM, we feel a better comparison of the two assays was made at 2 nM E*.

Giannopoulous et al. (1971) first reported equivalence between loss of cytoplasmic estrogen binding sites and gain in nuclear estrogen binding sites when rat uteri were incubated in vitro with increasing concentrations of E*. Anderson et al. (1973) showed, using the direct nuclear exchange assay in *immature* rat uteri, that charged nuclear estrogen receptor increases to a finite level with increasing doses of E. We developed the nuclear protamine assay in part to study the subcellular distribution of cytoplasmic and nuclear estrogen receptor in mature rat uteri as a function of various E doses. Figure 4 shows that 30 min after mature O-A rats are injected with increasing doses of E there is a dose-related decline in Rc

TABLE III: Subcellular Distribution of Estrogen Receptor with Time after in Vivo Exposure to Estradiol.[a]

Time After Injection	Specific E* Binding (pmole/mg DNA)				Total E* Binding (Rc + RcE + Rn + RnE) (pmole/mg DNA)	% of Control
	Rc	RcE	Rn	RnE		
0 min	3.20	0	0.24	0	3.44	100
10 min	0.12	0.37	0.10	2.07	2.66	77
20 min	0.07	0.21	0.15	2.31	2.74	79
40 min	0.11	0.34	0.12	2.16	2.73	79
6 h	0.80	0	0.27	0.63	1.70	49
24 h	3.60	0	0.42	0.25	4.26	124

[a] Mature O-A rats were injected with 10 μg of E or saline (control time 0) and sacrificed at 10, 20, min, 6 and 24 h. Cytoplasmic (Rc and RcE) and nuclear (Rn and RnE) sites were then determined according to Materials and Methods. Each value represents the mean of at least two experiments with one rat per experiment and triplicate samples for each determination.

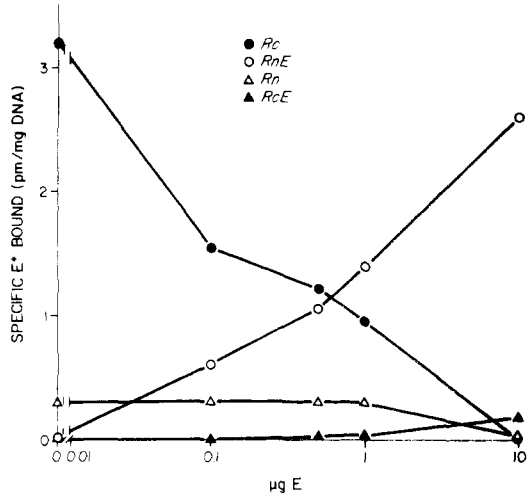

FIGURE 4: In vivo distribution of cytoplasmic and nuclear receptor sites as a function of estradiol dose. Mature O-A rats were injected with estradiol (0.1, 0.5, 1, or 10 μg of E) or saline (control). Uteri were removed 30 min after injection, and cytoplasmic (Rc and RcE) and nuclear (Rn and RnE) sites were determined according to Materials and Methods. Each value represents the mean of at least two experiments (2 rats/experiment) with triplicate determinations per experiment. (●) Free cytoplasmic (Rc); (○) charged nuclear (RnE); (▲) charged cytoplasmic (RcE); (△) free nuclear (Rn).

with a parallel and almost equivalent accumulation of RnE. No significant changes are seen in RcE or Rn.

In immature rat uterus, the amount of charged estrogen receptor in the nucleus rises quickly after an injection of E and then gradually declines (Anderson et al., 1973). Using the new protamine assay, we have found a similar time course for RnE in the mature rat uterus. Table III shows that after a 10-μg injection of E into mature O-A rats there is a striking alteration in the subcellular distribution of estrogen receptor. Rc is completely depleted within 10 min at which time RnE increases to 80% of the original control levels of Rc. From 10-40 min RnE remains stable, but declines by 6 h and is almost completely absent by 24 h. Restoration of Rc begins within 6 h (25% of control) and is 124% of control by 24 h.

Discussion

We have shown that a nuclear protamine assay can be used to fully quantitate salt-extracted nuclear estrogen receptor in the mature rat uterus. Charged and free nuclear receptor sites are easily distinguished, since there is no exchange of E* for E in RnE-protamine pellets at 4 °C but there is a quantitative exchange within 3 h at 37 °C (Figure 2).

Comparing the nuclear protamine assay to the direct nuclear exchange (Anderson et al., 1972), we find that the former assay is more quantitative, significantly reduces nonspecific binding and receptor degradation at elevated temperatures, and also improves variability in replicate samples (Figure 3AB). That the direct nuclear exchange only measures about half (1-1.3 pmol of RnE/mg of DNA) of the nuclear receptor sites actually present is consistent with Mester and Baulieu (1975) but at variance with Clark et al. (1972) who showed that RnE in adult cycling rats was 2-3 pmol/mg of DNA after injection of 10 μg of E. This discrepancy might be due, however, to the difference in preparation of uteri. We utilized frozen uteri from adrenalectomized-ovariectomized rats, whereas Clark et al. (1972) used fresh uteri from adult cycling rats. Although in our hands the direct exchange assay is less applicable in mature rat uteri than in immature rat uteri, we do find that, in cell cultures containing estrogen receptor where nonspecific estrogen binding is low, the direct exchange assay equals the nuclear protamine assay in its ability to quantitate total charged nuclear sites. Since the nuclear protamine assay works equally well in a variety of tissues studied, we believe it to be more universal in its applicability.

Although the cytoplasmic estrogen receptor undergoes conformational changes upon nuclear entry (Jensen et al., 1969), the affinity of the estrogen receptor for estradiol is not altered (Figure 2) in this transition. Using the nuclear protamine assay, the K_d of RnE is about 0.2 nM (Figure 2), identical to that found for Rc (Chamness et al. (1975)).

It was previously reported (Anderson et al., 1973) that in immature rat uteri physiological concentrations of E elicit a maximal uterotrophic response. Yet at these concentrations of hormone, estrogen receptor is not completely translocated. Similarly, we find that in mature rat uteri physiological doses of E (0.1-1 μg) only transport about half of Rc into the nucleus (Figure 4) in close agreement with previous studies on adult cycling rats (Clark et al., 1972). This suggests that uterine cells contain excess or spare cytoplasmic receptors, and that the limits of uterine responsiveness might be due to a critical number of nuclear sites that interact with RnE (Anderson et al., 1973).

After entering the nucleus, RnE probably activates the transcription of specific regions along the genetic template. Once these regions are activated RnE appears to be no longer required for stimulation, since the immature uterine cells shift to RnE degradation (Anderson et al., 1973) and resynthesis of Rc (Sarff and Gorski, 1971; Katzenellenbogen, 1975). Results with the new protamine assay are compatible with this concept, showing that after mature rats are exposed to estradiol

RnE remains in the nucleus and then gradually disappears by 24 h, at which time Rc is completely restored. Since the total number of cytoplasmic and nuclear receptor sites after 6-h exposure to E is only about 50% of control Rc (Table III), it suggests that replenishment of Rc is not simply due to recycling of receptor from the nucleus to the cytoplasm but rather involves active synthesis of new receptor molecules. This agrees with the finding that cycloheximide blocks appearance of the new receptor sites (Sarff and Gorski, 1971; Cidlowski and Muldoon, 1974; Katzenellenbogen, 1975).

In conclusion, we have developed a convenient nuclear exchange assay that has enabled us to quantitatively monitor the metabolic fate of nuclear estrogen receptor at different times after various doses of estradiol in the mature rat uterus. The new assay shows that estrogen binding and nuclear retention of estradiol receptor in the mature rat uterus are quite similar to that reported for the immature rat uterus (Anderson et al., 1972, 1973). Preliminary work has shown that with certain modifications the new nuclear exchange assay may have general usefulness for measuring other nuclear hormone receptors.

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