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Instability of the Uterine Estrogen Receptor under *in Vitro* Conditions†

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ABSTRACT: The stability of the estrogen receptor in excised uteri during *in vitro* incubation at various temperatures in a medium which is devoid of estrogen has been examined. The uterine estrogen receptor is very unstable in culture conditions with an 85% loss in diethylstilbestrol-competeable, high-affinity estrogen binding in the cytoplasmic fraction after 2 hr at 30–37°. Attempts to demonstrate the leakage of specific binding sites into the incubation medium or the translocation of receptor to the nuclear compartment of uterine tissue were without success. The presence of retinal in the incubation medium enhanced the rate of loss of receptor from uterine cytoplasm; however, hydrocortisone acetate did not prevent the loss of receptor under *in vitro* conditions. The present results suggest that the uterine estrogen receptor is very unstable at elevated temperatures in the absence of estrogen and that this instability may explain the failure of previous attempts to demonstrate physiologic responses of the uterus *in vitro*.

Since the recognition that estrogens exert their effects on target tissues *via* a specific protein, the estrogen receptor (R),[1] many attempts have been made to correlate target tissue responses with the levels of receptor–estrogen complex (RE) present in the target tissue. A great deal of effort has been expended on the development of an *in vitro* system for relating physiologic response to the level of RE in uterine cells. However, with the exception of the induction of an estrogen-dependent uterine protein (IP) by Gorski and coworkers (Katzenellenbogen and Gorski, 1972), all attempts at the development of an *in vitro* system which is responsive to physiologic concentrations of estrogens have failed (Mueller *et al.*, 1958; Hecter *et al.*, 1966; Rao and Talwar, 1972).

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[1] Abbreviations used are: R, the high affinity estrogen binding protein or receptor; E, estradiol; RE, the complex of estradiol with the estrogen receptor; TE, a buffer containing 10 mM Tris and 1.5 mM EDTA at pH 7.4; TKM, a buffer containing 40 mM Tris, 100 mM KCl, and 4 mM $MgCl_2$ at pH 7.2.

We have recently related both early (Anderson *et al.*, 1973) and late (Anderson *et al.*, 1972a) responses of the uterus to levels of nuclear RE in uterine cells *in vivo* and have been interested in developing an *in vitro* system. However, recent reports have suggested that uteri incubated *in vitro* undergo conspicuous cytologic changes (Ljungkvist and Terenius, 1969; Rao *et al.*, 1972). This morphologic instability suggests that the lack of *in vitro* responses in uterine tissue might arise from an instability of the estrogen receptor under *in vitro* conditions. It has been previously reported that the receptor is stable under *in vitro* conditions (Giannopoulos and Gorski, 1971) and that the turnover of the estrogen receptor *in vivo* or *in vitro* proceeds with a half-life of about 138 hr (Sarff and Gorski, 1971). However, Katzenellenbogen and Gorski (1972) have reported that the capacity of excised uteri to synthesize IP in response to estrogen stimulation decreases with the time of incubation in an estrogen-free medium. In view of our correlation of nuclear levels of receptor–estrogen complex (RE) to uterine responsivity *in vivo* (Anderson *et al.*, 1972a, 1973) and the loss of the IP response with *in vitro* incubation in the absence of estrogen (Katzenellenbogen and Gorski, 1972), we have reexamined the stability of the estrogen receptor in excised uteri during *in vitro* incubation at various temperatures in estrogen-free media.

Experimental Procedure

Materials. Immature female Purdue-Wistar rats (21–22 days old) were employed in all experiments unless otherwise indicated. All animals were maintained in a controlled environment of 70–72°F with a relative humidity of 45–55% and a light–dark cycle consisting of 13 hr of light and 11 hr of darkness. Food and water were given *ad lib* and all animals were sacrificed between 9:00 a.m. and noon.

[6,7-^{3}H]Estradiol (40–46 Ci/mmol) was obtained from New England Nuclear Corporation and examined for radiopurity by thin-layer chromatography. Eagle's HeLa medium (Difco) was employed without modification unless otherwise indicated.

Preparation, Incubation, and Fractionation of Excised Uteri. Rats were killed by cervicle dislocation. The uteri were quickly excised, stripped of fat and mesentery, and weighed on a torsion balance. Control uteri which were not exposed to *in vitro* incubation (zero time) were placed in TE or TKM buffer (see below) on ice for the immediate determination of specific estrogen binding while uteri to be incubated *in vitro* for various time periods (1, 2, or 4 hr) were placed in 10-ml glass vials containing 3 ml of Eagle's medium through which O_2–CO_2 (95:5%) was bubbled for 15 sec. In most instances two uteri were employed in each incubation flask or control. The experimental vials were incubated on ice at 0–4 or at 30 or 37° and under O_2–CO_2 in a Dubnoff shaker. At the termination of incubation, uteri were washed three times with ice-cold buffer (TE or TKM) and homogenized in all-glass Kontes homogenizers using a motor driven pestle (1 uterus/ml of buffer). Cytoplasmic fractions were prepared by centrifugation at 48,000*g* for 10 min.

Assay of Cytoplasmic Receptor. The cytosol or cytoplasmic fraction was assayed for the estrogen receptor by three different procedures: a modification of the pellet binding assay of Clark and Gorski (1969) in which alumina (150 mg) replaced the glass pellets, a gel filtration assay essentially the same as that of Sarff and Gorski (1971), and the charcoal adsorption procedure of Sanborn *et al.* (1971). A 0.04 M Tris-HCl–0.1 M KCl–0.004 M $MgCl_2$ buffer at pH 7.2 (TKM) was employed for the pellet binding assay while a 0.01 M Tris-HCl–1.5 mM EDTA buffer at pH 7.4 (TE) was employed for the gel filtration and charcoal adsorption procedures. For each assay procedure two series of assay tubes were used: one series containing 0.25–1 uterine equivalent of cytosol plus 1.3×10^{-8} M [^{3}H]estradiol while the second series contained the same quantities of cytosol and estradiol plus diethylstilbestrol at 1.3×10^{-6} M. In this manner the specific diethylstilbestrol-competeable binding of estrogen can be ascertained by difference.

Assay of the Nuclear Receptor–Estrogen Complex. The determination of diethylstilbestrol-competeable estrogen binding sites in the nuclear fraction employed the [^{3}H]estradiol exchange assay (Anderson *et al.*, 1972b). Briefly, the assay procedure consists of the incubation of washed nuclear fractions from incubated uteri at 37° for 30 min in the presence of 1.3×10^{-8} M [^{3}H]estradiol alone or 1.3×10^{-8} M [^{3}H]estradiol plus 1.3×10^{-6} M diethystilbestrol. After incubation, the fractions were washed, and the [^{3}H]estradiol was extracted with 3 ml of ethanol and measured in a scintillation counter. The amount of specifically bound [^{3}H]estradiol is determined by subtraction of nonspecifically bound [^{3}H]estradiol ([^{3}H]estradiol bound in the presence of a 100-fold excess of diethylstilbestrol) from total [^{3}H]estradiol binding in the absence of diethylstilbestrol.

Assay of Phosphohexose Isomerase. Phosphohexose isomerase was assayed by the method of Bodansky (1954) using the assay kits available from Sigma Chemical Co. (St. Louis, Mo.). Enzyme activity is expressed in micrograms of product per minute per milligram of uterus or microgram of product per minute per milligram of soluble protein.

Electrophoretic Analysis. Samples (10, 50, and 75 μl) of the incubation media were added to 0.2 ml of 30% sucrose and applied to 6-mm diameter gels containing 7.5% acrylamide. Samples of crystalline bovine serum albumin (10 μg) were analyzed simultaneously with the samples of media. Electrophoresis was essentially according to Davis (1964). The gels consisted of a 0.5-cm stacking gel, pH 6.7, and a 6.0-cm running gel, pH 8.9. Electrophoresis was conducted at 3 mA/gel for 1.5–2.0 hr at 5° in a 5 mM Tris-38 mM glycine buffer (pH 8.4).

Gels were stained with either Buffalo Black (0.5% w/v) or Fast Green (1% w/v) in 7% acetic acid for either 2 hr or overnight at room temperature. Destaining was accomplished in a horizontal electrophoretic destaining apparatus (Gelman Co.). Densitometry of stained gels was performed with a Gilford Gel Scanner using either 650 (Buffalo Black) or 625 mμ (Fast Green).

General Procedures. Aliquots (0.2 ml) from the gel filtration and charcoal adsorption assay were added to 3 ml of 100% ethanol in scintillation vials. Scintillation fluid (10 ml) [99.5% toluene–0.45% 2,5-diphenyloxazole–0.05% 1,4-bis[2-(5-phenyloxazolyl)]benzene] was added and [^{3}H]estradiol was determined at 30–33% efficiency with a Packard 3375 liquid scintillation spectrometer. The 3-ml ethanol extract from the pellet binding assay was handled in a like manner. Protein was determined by the procedure of Lowry *et al.* (1951). All values for [^{3}H]estradiol binding, protein content, and enzyme activity are expressed either on a per milligram of uterus basis or have been normalized for a 30-mg uterus.

Results

Effect of Temperature and Duration of in Vitro Incubation on Uterine Content of Estrogen Receptor. In order to assess the

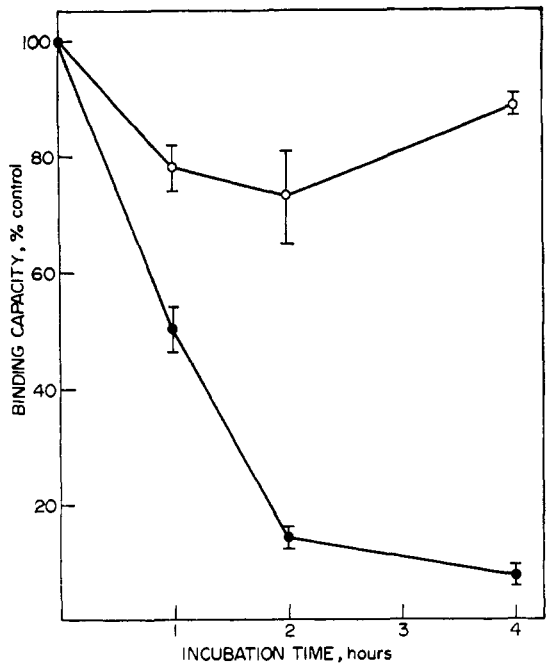

FIGURE 1: Effect of incubation time and temperature on cytoplasmic receptor. Diethylstilbestrol-competeable cytoplasmic binding sites were determined after 1, 2, or 4 hr of incubation in the absence of exogenous estradiol by the charcoal adsorption procedure (see Experimental Procedure). *In vitro* incubations were at 0–4° (○) or at 30 or 37° (●). Each point represents the mean ± the standard error of the mean of three–six determinations.

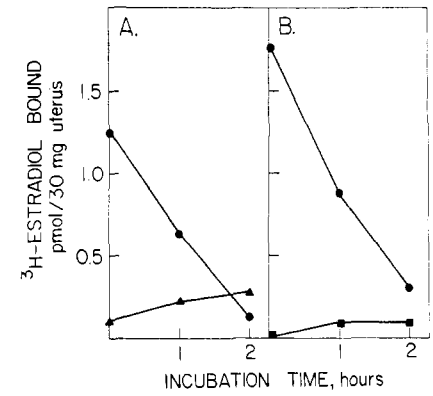

FIGURE 2: (A) Determination of nuclear binding sites after *in vitro* incubation. Diethylstilbestrol-competeable nuclear (▲) and cytoplasmic (●) binding sites were determined after 1 and 2 hr of incubation at 37° in the absence of estradiol. The assay of cytoplasmic sites was *via* the pellet binding assay. Nuclear sites were determined using the [^{3}H]estradiol exchange procedure (see Experimental Procedure). The data have been normalized for a 30-mg uterus. (B) Examination of incubation medium for estrogen binding sites. Diethylstilbestrol-competeable binding sites were determined for one uterine equivalent of the cytoplasmic fraction (●) and the incubation medium (■) by the gel filtration procedure (see Experimental Procedure). The data have been normalized for a 30-mg uterus.

effect of *in vitro* incubation on the uterine estrogen receptor, uteri were incubated on ice (0–4°) and at elevated temperatures (30 or 37°) for 1, 2, and 4 hr in Eagles medium under O_2–CO_2. After incubation, the capacity of the cytoplasmic fraction to bind estrogen in a specific diethylstilbestrol-competeable manner was determined using the charcoal adsorption assay or the gel filtration procedure (see Experimental Procedure). The results in Figure 1 represent the mean ± the standard error of the mean for three–six determinations per point. It is apparent that at elevated temperatures (30 or 37°) the capacity of uterine cytosol to bind estrogen in a specific diethylstilbestrol-competeable manner is drastically reduced when compared to controls (zero time) or uteri incubated on ice (0–4°).[2] At the end of 4 hr at 30 or 37°, specific binding of estradiol is about 7% of control uteri. In analogous experiments (not shown), the addition of glutamine (2 mM), penicillin G (100 U/ml), and/or hypophysectomized lamb serum (10% v/v) was without effect in preventing the depletion of specific binding capacity at elevated temperatures.

Analysis of Nuclei and Media for Uterine Estrogen Receptor. The disappearance of cytoplasmic receptor following *in vitro* incubation prompted us to search for the receptor among the various compartments of the incubation system. Figure 2A shows the analysis of cytoplasmic (circles) and nuclear compartments (triangles) for specific diethylstilbestrol-competeable binding following 1 and 2 hr of incubation at 37°. Cytoplasmic sites were determined *via* the pellet binding assay of Clark and Gorski (1969) while nuclear sites were determined with the [^{3}H]estradiol exchange assay (see Experimental Procedure and Anderson *et al.*, 1972b). It is readily apparent that the disappearance of cytoplasmic receptor cannot be explained in terms of the translocation of receptor from the cytoplasmic to the nuclear compartment. Thus, after 2 hr at 37°, cytoplasmic sites were reduced by about 1.1 pmol while nuclear sites increased by about 0.15 pmol.

Figure 2B shows the analysis of the cytoplasmic fraction (circles) and the incubation medium (squares) for diethylstilbestrol-competeable binding sites. In these experiments four uteri were incubated in 2 ml of media at 37° and one uterine equivalent of cytosol or media was assayed for estrogen binding activity *via* the gel filtration procedure. The procedure for the assay of media binding was adequate to detect the leakage of as little as 0.2 pmol of binding sites/uterus. Cytoplasmic sites were reduced by 1.5 pmol/30 mg of uterus in 2 hr at 37° while diethylstilbestrol-competeable sites in the media remained essentially unchanged. These results indicate that the receptor does not leak into the incubation medium or, if leakage does occur, that the receptor is inactivated within the time interval employed.

Leakage of Proteins into the Incubation Medium. To examine the possibility that proteins might leak into the incubation medium, uteri were incubated at 0–4 and 37° for 1–4 hr. The media were then assayed for protein by the procedure of Lowry *et al.* (1951) and the cytoplasmic fraction was assayed for diethylstilbestrol-competeable estrogen binding by the charcoal adsorption procedure. Figure 3 summarizes the results for cytoplasmic binding sites (circles) and media protein (triangles) after incubation at 0–4° (closed figures) and at 37° (open figures). There is a rapid loss of protein from the uterine tissue into the medium up to 1 hr followed by a gradual loss thereafter. It is evident from these results that protein does leak into the medium; however, the results of Figure 2B suggest that receptor protein does not leak out to any significant extent, at least not in an active form.

An examination of the nonspecific as well as specific binding of estradiol in the cytoplasmic fraction after 4 hr at 0–4 and 37° suggests the nature of the protein which leaks into the medium. Thus, Figure 4 shows the specific, diethylstilbestrol-

[2] The increase in specific binding of estradiol at 0–4° which is observed between 2 and 4 hr of incubation in Figures 1 and 4 is statistically significant. We are unable to offer any explanation for this phenomenon at this time.

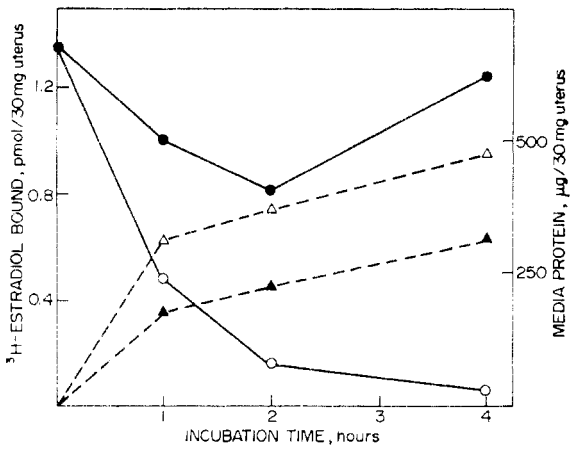

FIGURE 3: Leakage of protein into incubation media. Incubation media were analyzed for protein as described in the Experimental Procedure after 1, 2, or 4 hr in the absence of exogenous estradiol at 0–4° (▲) and 37° (△). Cytoplasmic high-affinity sites were determined by the charcoal adsorption procedure for uteri which were incubated in the absence of exogenous estradiol at 0–4° (●) and at 37° (○).

competeable (open bars) and nonspecific, noncompeteable (hatched bars) binding of estrogen to the cytosol of control uteri and uteri incubated at 0–4 and 37° for 4 hr. Nonspecific binding in the uterus is largely attributable to serum binding proteins (Peck *et al.*, 1973) and the loss of nonspecific binding with incubation suggests that serum albumins leak into the medium during *in vitro* incubation.

To examine the nature of the protein lost to the medium, uteri were incubated in Eagles medium (5 uteri/2 ml) for 4 hr at 0–4 and 37°, and the medium was subsequently analyzed by polyacrylamide gel electrophoresis. Figure 5 shows the results of such an analysis and compares the gel patterns from the incubation media with a 10-µg sample of bovine serum albumin. These results confirm the loss of serum albumin from the uterus which was suggested from binding studies (see above).

Effect of Incubation at 37° on Protein Content and Phosphohexose Isomerase Activity. In view of the rapid loss of diethylstilbestrol-competeable estrogen binding sites from the cytoplasm following *in vitro* incubation in the absence of estrogen, the cytoplasmic fraction was examined for total protein and a specific cytoplasmic marker, phosphohexose isomerase. Table I shows the effect of 1 and 2 hr of exposure to *in vitro* conditions at 37° on the activity of phosphohexose isomerase and the level of soluble protein in the cytoplasmic fraction. The loss of protein (about 500 µg/30 mg of uterus) is largely accounted for by leakage into the medium (see Figure 3). Thus, leakage at 37° for 2 hr is about 375 µg/30 mg of uterus (Figure 3) or 75% of that lost from the cytoplasmic fraction (Table I).

Phosphohexose isomerase activity decreases by about 20% following 2 hr of *in vitro* incubation at 37° (Table I). This value represents the loss of intracellular, cytoplasmic enzyme activity during *in vitro* incubation whereas the total soluble protein in the cytoplasmic fraction represents both inter- and intracellular compartments. Again, these results support the

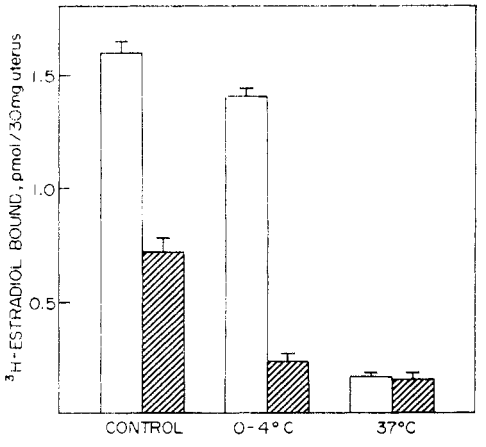

FIGURE 4: Nonspecific and diethylstilbestrol-competeable binding sites after *in vitro* incubation. Uteri were incubtaed in the absence of estradiol at 0–4 and 37° for 4 hr and the diethylstilbestrol-competeable (open bars) and nonspecific (hatched bars) [^{3}H]estradiol binding was determined for the cytoplasmic fraction by the charcoal adsorption procedure (see Experimental Procedure). The control consists of uteri not exposed to *in vitro* incubation. Each value represents the mean ± the standard error of the mean of four–six determinations.

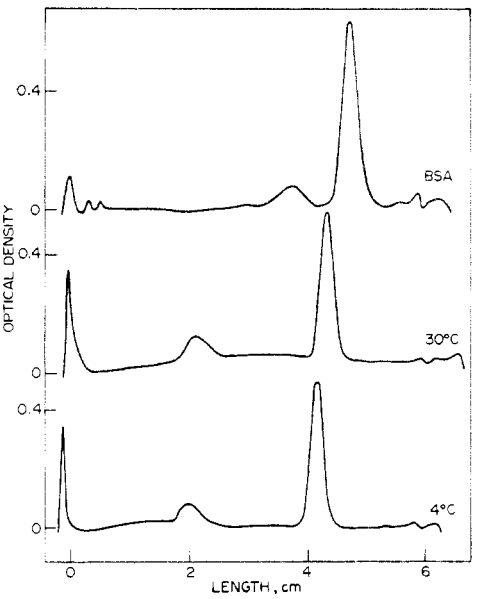

FIGURE 5: Electrophoretic analysis of media proteins. Samples of media were removed after the incubation of five uteri in 2 ml of media for 4 hr in the absence of exogenous estradiol at 0–4 or 37° and electrophoresed as described in the Experimental Procedure.

TABLE I: Effect of *in Vitro* Incubation on Protein Content and Phosphohexose Isomerase Activity.[a]

Duration of Incubation (hr)	Phosphohexose Isomerase Act.		Protein	
	µg/min per mg of Uterus	% Control	µg/mg of Uterus	% Control
0	3.2 ± 0.2	100	41 ± 6	100
1	2.8 ± 0.2	86	31 ± 3	75
2	2.6 ± 0.2	80	24 ± 2	58

[a] Uteri were incubated at 37° for 1 or 2 hr and both soluble protein and phosphohexose isomerase activity were assayed as described in the Experimental Procedure. All values represent the mean ± standard deviation of the mean of three–four determinations.

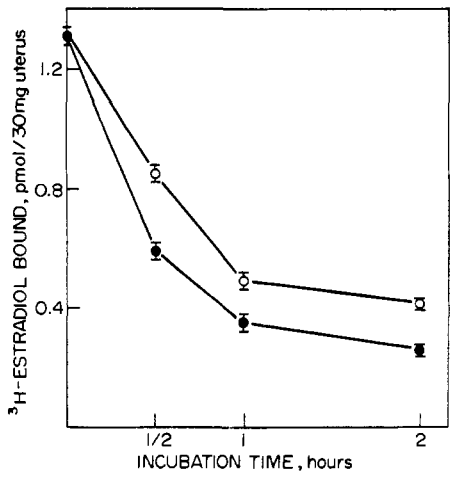

FIGURE 6: Effect of retinal on receptor stability. Uteri were incubated in the absence of estradiol at 30° in the presence (●) or absence (○) of retinal (5 μg/ml). Diethylstilbestrol-competeable binding sites were determined by the charcoal adsorption procedure (see Experimental Procedure). Each point represents the mean ± the standard error of the mean of three–four determinations.

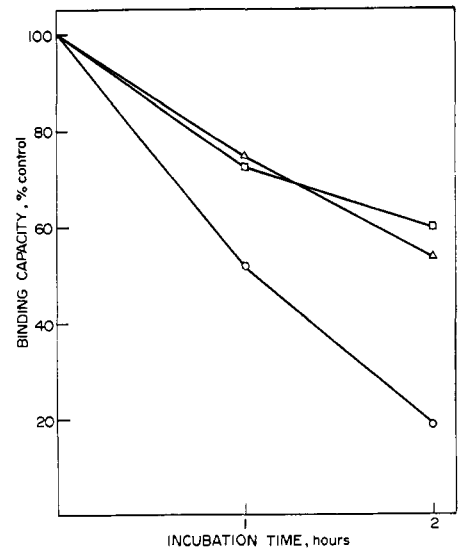

FIGURE 7: Strain differences and receptor stability. Diethylstilbestrol-competeable [^{3}H]estradiol binding sites were determined for Purdue-Wistar (○), Sprague-Dawley (□), and Holtzman rats (△) by the charcoal adsorption procedure. Each point represents the mean of two–three determinations.

suggestion that the bulk of the protein lost is intercellular serum albumins.

Retinal and the Stimulation of Receptor Disappearance. The cytologic changes observed upon *in vitro* incubation together with the suggestion of Szego (1971) that lysosomes may function as the interceptor and deliverer of estrogens in estrogen target tissues prompted us to examine the rate of loss of the estrogen receptor from the cytoplasmic fraction in the presence of compounds which stabilize and labilize lysosomes. For this purpose we chose retinal which has been demonstrated to labilize lysosomes (Dingle and Lucy, 1962) and hydrocortisone acetate which has been demonstrated to be an antagonist of the vitamin A derivatives and a stabilizer of lysosomes (Fell and Thomas, 1961). Figure 6 shows the disappearance of diethylstilbestrol-competeable estradiol binding from the cytosol of uteri incubated at 30° in the presence (closed circles) or absence (open circles) of retinal (5 μg/ml). The values are the mean ± standard error of the mean of four determinations. It is apparent that retinal increases the rate of loss of specific estrogen binding in uterine tissue. In addition, the presence of hydrocortisone acetate (1 mg/ml) and retinal (5 μg/ml) in the incubation medium resulted in a loss curve identical with that of the control (results not shown). Hydrocortisone acetate alone (1 mg/ml) did not stabilize the diethylstilbestrol-competeable binding above the control values (open circles, data not shown).

Strain Differences and the Instability of the Uterine Estrogen Receptor. In view of the paradoxical nature of our results when compared with those of Gorski (Giannopolous and Gorski, 1971; Sarff and Gorski, 1972), we examined the possibility that strain differences among experimental animals might account for the disparity in our results. Purdue-Wistar, Sprague-Dawley, and Holtzman rats were employed to examine potential strain differences. Figure 7 shows the loss curve obtained over 2 hr at 30° for Purdue-Wistar (circles), Sprague-Dawley (squares), and Holtzman (triangles) rats. Although there are quantitative differences in the rate of loss, the loss is quite significant in all strains examined. In all of these studies diethylstilbestrol-competeable binding was determined *via* the charcoal adsorption procedure and all values are the mean of two–three determinations.

Discussion and Conclusions

The present results indicate that the estrogen receptor of the rat uterus is quite unstable under *in vitro* incubation conditions. The loss of high-affinity, low capacity binding sites for estradiol from the cytoplasmic fraction when incubated *in vitro* at elevated temperatures cannot be explained in terms of the translocation of receptor to the nuclear fraction (Figure 2A) or by the leakage of receptor from uterine tissue into the incubation medium (Figure 2B). However, the present results demonstrate that large quantities of protein leak into the incubation medium (Figure 3) and that this protein is largely accounted for by serum albumin (Figures 4 and 5).

The present results also indicate that the estrogen receptor is much more labile than at least one cytoplasmic marker protein, phosphohexose isomerase. Thus, in 2 hr at 37° in the absence of estrogens, the cytoplasmic receptor is reduced by about 85% while the level of phosphohexose isomerase is decreased by about 20%.

The extreme lability of the cytoplasmic estrogen receptor suggests that it might be associated with, or highly susceptible to, lysosomal activity. However, while the rate of loss of cytoplasmic receptor was increased by retinal, we were unable to demonstrate a stabilization of cytoplasmic receptor with hydrocortisone acetate.

The loss of cytoplasmic, diethylstilbestrol-competeable binding activity might result from physical alterations of the receptor which lead to a decrease in the affinity of the receptor for estrogen. In the present studies, we have been concerned with the high-affinity, low capacity binding of estrogen in uterine cytosol and have employed a concentration of [^{3}H]-estradiol, 1.3×10^{-8} M, which is sufficient to saturate this species of receptor. The present data do not eliminate the possibility that the affinity of the estrophilic binding site for estrogen has been decreased. Such an alteration might, in fact, explain the unusually high concentrations of estradiol (up to 2.5×10^{-5} M) required to produce feeble responses of uteri *in vitro* (Aizawa and Mueller, 1961; Mayol and Thayer, 1970).

It is remarkable how closely the present results on the loss of the cytoplasmic high-affinity estrogen receptor parallel the

loss of capacity of excised uteri to respond to estradiol stimulation after various periods of incubation in estrogen-free media (Katzenellenbogen and Gorski, 1972). The similarity in these results, that is, the parallel nature of the decreases, would seem to establish once again the absolute requirement for the cytoplasmic estrogen receptor for a physiologic response and to demonstrate the relationship between the amount of receptor available and the degree of responsivity of the target tissue. A similar observation has recently been made with the androgen receptor; that is, androgen insensitivity in pseudohermaphroditic male rats has been correlated with the absence of the androgen receptor (Gehring *et al.*, 1971; Milin and Roy, 1973). In addition, we have recently demonstrated a similar relationship with the estrogen receptor using a reinjection schedule for the depletion of cytoplasmic receptor (J. N. Anderson, J. H. Clark, and E. J. Peck, Jr., unpublished).

The present results do not agree with the reports of Giannopoulos and Gorski (1971) and Sarff and Gorski (1971) that the uterine cytoplasmic receptor is stable under *in vitro* incubation conditions in the absence of estrogen. We are unable to reconcile the present results with those of Giannopolous and Gorski (1971). However, we are able to suggest a possible explanation for some of the results of Sarff and Gorski (1971). The absence of free [^{3}H]estradiol in the sucrose density gradient patterns of Sarff and Gorski (1971) suggests that the concentration of [^{3}H]estradiol employed in those experiments was not sufficient to saturate the receptor. If a minimal estimate of 1.0 pmol of receptor/uterus is made, then under these conditions about 2.5×10^{-8} M receptor with a K_d at 0–4° of about 2×10^{-10} M (Sanborn *et al.*, 1971) was exposed to 9.2×10^{-9} M [^{3}H]estradiol. Under conditions such as these where total binding sites are in great excess over their K_d, *i.e.*, $R_T \gg K_d^R$, essentially all of the small molecular weight ligand (in this case, estradiol) will be complexed with the binding sites, *i.e.*, RE = E_T. Therefore, if inadequate amounts of steroid are available, large quantities of receptor may be unsaturated. Thus, for the sucrose density gradients of Sarff and Gorski, it would seem that assay conditions were not saturating with respect to estradiol and that at most about 37% of the cytoplasmic receptor could be saturated. Information is not available to carry out a similar calculation on the other assays employed by Sarff and Gorski. We can only conclude that the present data, obtained under conditions where estradiol is saturating for a high-affinity, low-capacity binding site and in which specific, diethylstilbestrol-competeable binding is measured, suggest that the uterine estrogen receptor is very unstable when uteri are incubated at elevated temperatures in the absence of estradiol. This instability is greater than that of other cytoplasmic markers and may result either from the accessibility of the receptor to lysosomal attack or from an alteration in the affinity of the receptor for estrogens.

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