Estrogen Receptor Binding to Isolated Nuclei. A Nonsaturable Process†

Gary C. Chamness,‡ Arthur W. Jennings, and William L. McGuire*

ABSTRACT: Binding sites for estrogen receptor in isolated nuclei from several target and nontarget tissues are shown to be unsaturable, even when the nuclei have bound several times more receptor than found at maximal *in vivo* stimulation of uterus. Previous indications of saturability resulted from varying concentrations of total cytosol protein, which at higher levels depresses receptor binding. When cytosol protein is held constant, nuclear binding becomes strictly proportional to the total receptor concentration. Partially purified receptor also yields the same result. In addition, an excess of receptor charged with unlabeled estradiol does not compete with labeled estradiol-charged receptor for binding sites. In agreement with results from intact cells, we therefore conclude that the nuclear acceptor sites for estrogen receptor are present in large numbers and bind receptor with relatively low affinity.

On entering a target cell, estrogens are bound by a receptor protein in the cytoplasm (Jensen and DeSombre, 1972; Gorski *et al.*, 1968). At 25 or 37° but not at 4°, most of the receptor-bound estrogen molecules quickly enter the cell nucleus, as revealed by autoradiography after injection of [^{3}H]estradiol *in vivo* or after incubation of target tissues with the hormone *in vitro* (Jensen *et al.*, 1968, 1969a; Stumpf, 1969). One temperature-dependent step appears to be the transformation of estrogen-charged receptor in the cytoplasm to a form which is capable of activating nuclear RNA polymerase (Brecher *et al.*, 1970; Mohla *et al.*, 1972); the nuclear binding form of *un*-transformed receptor is different from that found in nuclei *in vivo* (Chamness *et al.*, 1973). Entry of estrogen into the cell is also strongly dependent on temperature (Williams and Gorski, 1971), as is the binding of receptor to isolated chromatin (McGuire *et al.*, 1972). The appearance of receptor in the nucleus is correlated with the early synthesis of at least one new acidic protein in the cytoplasm (Notides and Gorski, 1966; Mayol and Thayer, 1970; Katzenellenbogen and Gorski, 1972) followed by ribosome production and increased RNA and protein synthesis (Hamilton, 1971).

The mechanism by which estrogen-charged receptor acts within the nucleus has received extensive study without being fully clarified. Early experiments showed the nuclear receptor to be firmly associated with the nuclear particulate fraction, from which it could be at least partly released with 0.3 M salt (DeSombre *et al.*, 1967; Puca and Bresciani, 1968). When nuclear components were fractionated, the receptor remained associated with the chromatin complex (King *et al.*, 1966; Shyamala and Gorski, 1969). This suggested that the receptor might function by binding to certain highly specific sites (termed "acceptor sites") in the chromatin, thus activating associated clusters of genes. Binding of receptors for androgen and estrogen to isolated chromatin was also found (Mainwaring and Peterken, 1971; Steggles *et al.*, 1971) and appeared to be tissue specific. Further examining this concept, a number of experiments showed cell-free binding of chick oviduct progesterone receptor either to intact oviduct chromatin or to chromatin selectively fractionated and reconstituted, with the conclusion that an acidic protein fraction designated AP3 was chiefly responsible (Steggles *et al.*, 1971; Spelsberg *et al.*, 1972). Less extensive experiments with estrogen receptor achieved similar results, but interpretation of all of these chromatin binding experiments was limited by variations in the chromatin composition (Steggles *et al.*, 1971) and by the low incubation temperatures employed (McGuire *et al.*, 1972). Schrader *et al.* (1972) showed that while one subunit of the progesterone receptor has a high affinity for intact chromatin, another possesses an equal affinity for free DNA. Other laboratories have reported a strong and apparently saturable binding of the estrogen receptor to DNA alone (Clemens and Kleinsmith, 1972; Yamamoto and Alberts, 1972; Toft, 1972; Musliner and Chader, 1972; King and Gordon, 1972). Glucocorticoid receptors have also been reported to bind to free DNA with an affinity in general agreement with physiological effects (Baxter *et al.*, 1972), so that several pieces of evidence support a role for DNA itself in receptor attachment. Still another suggestion has come from very recent work showing apparently tissue specific association of estrogen and androgen receptors with ribonucleoprotein particles from prostate and uterine nuclei, respectively (Liao *et al.*, 1973).

Because of these inconsistent results from various nuclear subfractions, we returned to an investigation of the binding of receptor to unfractionated nuclei. Using nuclei from several rat tissues under a variety of conditions of incubation with receptor, we found that estrogen receptor binding was not restricted to target nuclei (Chamness *et al.*, 1973). In contrast with earlier reports (Jensen *et al.*, 1969b; Musliner *et al.*, 1970; Gschwendt and Hamilton, 1972) there were no apparent quantitative or qualitative differences in binding of estrogen receptor to target and nontarget nuclei.

We have now investigated the binding properties of the nuclear acceptor sites. As suggested earlier, receptor might be bound to a limited number of sites with very high affinity, therefore remaining at a given site for an extended time during which transcription of associated genes would be somehow activated. This binding model we designate type I. Type I sites would be saturable at physiological receptor levels, and competition for binding should be demonstrable. Alternatively, a

† From the Department of Medicine, The University of Texas Medical School at San Antonio, San Antonio, Texas 78284. *Received July 26, 1973.* Supported in part by The National Cancer Institute (CA11378 and G-72-3862), The American Cancer Society (BC-23C), and The Robert A. Welch Foundation.

‡ Postdoctoral Fellow of the National Cancer Institute.

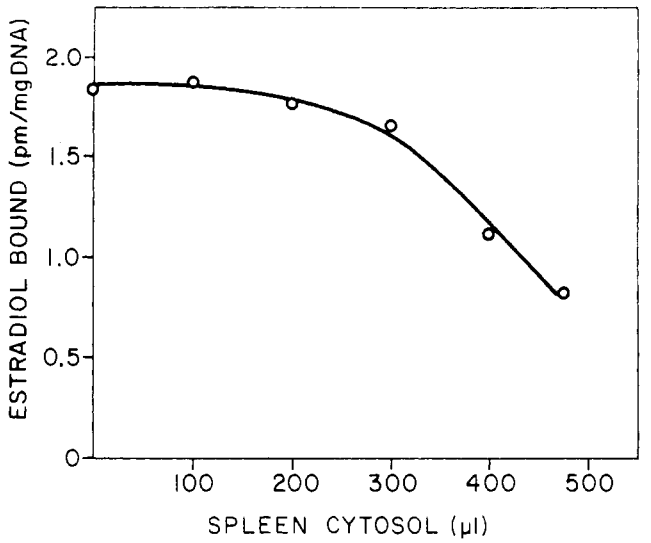

FIGURE 1: Effect of cytosol concentration on the nuclear binding of uterine receptor. 25 μl of uterine cytosol in TE/0.15, charged with [^{3}H]estradiol and treated with DCC, was incubated 15 min at 25° with crude uterine nuclei (30 μg of DNA per pellet) and various volumes of spleen cytosol in TE/0.15, made up to a final volume of 500 μl with buffer.

relatively large number of lower affinity sites could also explain the observed nuclear binding. We will designate this model type II. In this case, saturation and competition would not be observed.

Many previous experiments, including our own, seemed to show that binding of receptor to isolated nuclei or nuclear components was a saturable process, indicating type I binding. Competition, however, has not been reported. And the experiments of Williams and Gorski (1972a,b) on intact rat uterus *in vivo* or *in vitro* showed no type I saturation of nuclear binding even at maximum physiological levels of charged receptor. With this evidence for type II binding *in vivo*, we set out to reexamine the binding of receptor to isolated nuclei. We are now presenting new experiments showing that cell-free binding of estrogen receptor to nuclei is all or nearly all to type II nuclear acceptor sites. There is no evidence of type I binding in these systems.

Experimental Section

Preparation of Cytosol. Tissues were removed from mature female rats ovariectomized at least 2 days previously. After removal of necrotic areas in the case of tumors, the tissues were either placed on ice for immediate use, or frozen in liquid nitrogen for storage at −76° and then thawed at 4° at the time of use. All further operations were at 4° unless noted. The tissues were minced with scissors and homogenized in 4 volumes of buffer using a glass–glass homogenizer. The homogenate was centrifuged 50 min at 105,000*g* and the cytosol removed from between the floating fat layer and the pelleted particulate fractions. Those cytosols to be charged with estradiol were incubated at 4° with [^{3}H]-17β-estradiol (96 Ci/mmol) at a final concentration of 4.5×10^{-9} M. For some uses free estradiol was removed by dextran-coated charcoal (DCC), as previously described (Chamness and McGuire, 1972). Buffers were T (0.01 M Tris-HCl (pH 7.4)), TE (T with 1.5 mM EDTA), or TE/0.15 (TE with 0.15 M KCl), as indicated.

Partial Purification of Receptor. For purification, receptor was prepared from transplantable MtTW5 rat pituitary tumors originally obtained from Dr. Vincent Hollander and grown in female Wistar-Furth rats. This tumor has been shown to possess estrogen receptors with properties indistinguishable from those of rat uterus (McGuire *et al.*, 1973). A cytosol was prepared in TE/0.15 and charged with estradiol for 60 min at 4°. Saturated ammonium sulfate in TE was added over at least 30 min to a final 30% saturation. The solution was stirred slowly for an additional 30 min and the precipitate was pelleted at 20,000*g* for 20 min. The pellets were redissolved in 0.1 volume TE/0.15, and any residual precipitate was removed by centrifugation at 105,000*g* for 30 min. Purification was typically 10–15-fold in terms of cpm of estradiol bound per mg of protein, and recovery was 50–60%.

TABLE 1: Depression of Estrogen Receptor Binding to Uterine Nuclei by Various Cytosols.[a]

Diluent	Receptor Bound (pmol/mg of DNA)	% Decrease
Buffer (TE/0.15)	1.01	0
	0.95	
Spleen cytosol	0.42	49
	0.58	
Uterine cytosol (uncharged)[b]	0.63	40
	0.58	
MtTW5 cytosol (uncharged)[b]	0.55	44
	0.55	
MtTW5 cytosol (heat denatured)[c]	0.51	46
	0.54	
Bovine serum albumin (10 mg/ml)	0.60	40
	0.56	

[a] Procedure as in Figure 1, with 475 μl of diluent added to 25 μl of charged uterine cytosol and a uterine nuclear pellet containing 50 μg of DNA, in duplicate. [b] Cytosol prepared as before but not treated with [^{3}H]estradiol. [c] Uncharged cytosol heated to 65° for 10 min and centrifuged before use.

Preparation of Nuclei. Kidney, spleen, pituitary tumor (MtTW5), and liver nuclei were prepared in the following manner. The test tissues were removed from Wistar-Furth rats, chilled, and weighed. The tissues were minced and homogenized in 5–10 volumes of buffer 1 (2.2 M sucrose–0.01 M Tris-HCl (pH 7.4)–0.1 mM EDTA–1 mM $MgCl_2$) with 5-sec bursts of a Polytron PT-10 at a power setting of 5. The homogenates were filtered through two layers of cheesecloth and centrifuged in a SW-27 rotor for 90 min at 96,300*g*. The pellets were gently resuspended in TE/0.15 and filtered through organza cloth. The DNA content of the purified nuclear suspensions was determined according to Burton (1956), except that for rapid determination incubation was 2 hr at 60° rather than overnight at 30° with appropriate standards. Examination of the Trypan Blue stained nuclei under a Unitron inverted phase contrast microscope showed fully intact nuclei with very little particulate contamination and few cytoplasmic tags. Using 6 pg of DNA per nucleus (Davidson, 1969), all of the DNA present could be accounted for by the number of intact nuclei as determined in a hemocytometer. After incubation as described below, most nuclei remained intact but there was a slight increase in particulate contamination.

Due to the differences in the tissues and the smaller amounts

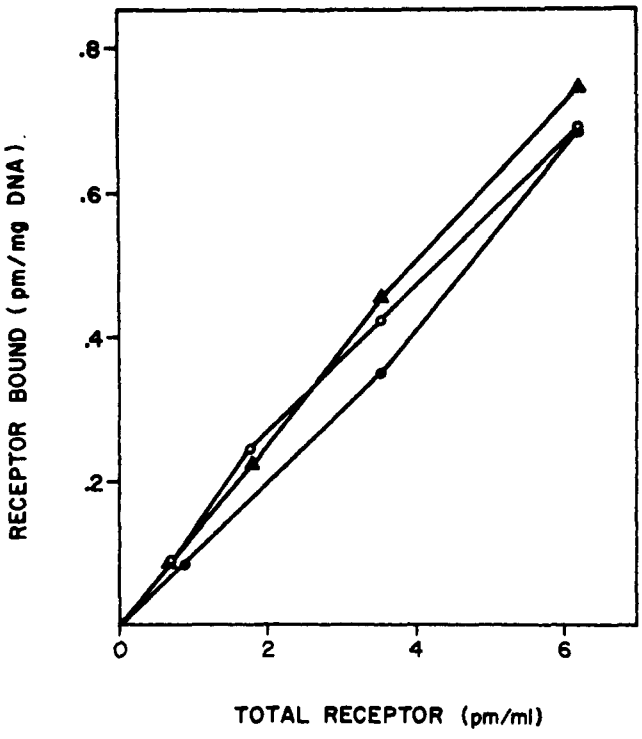

FIGURE 2: Binding of partially purified receptor to purified nuclei of several tissues. Aliquots of receptor in TE/0.15 partially purified as described under the Experimental Section were incubated with purified nuclei from uterus (●), spleen (▲), and kidney (○) for 30 min at 25°, after nuclear preparations were diluted to give 700 μg of DNA per pellet using the rapid DNA determination given earlier. The final volume of 500 μl was made up with spleen cytosol subjected to the same purification procedure.

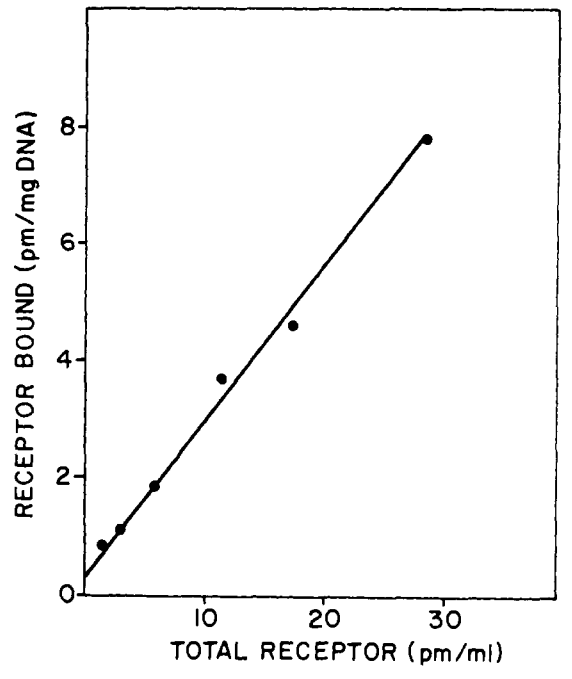

FIGURE 3: Binding of partially purified receptor to uterine nuclei. The procedure followed Figure 2, except that the incubation was for 15 min and used crude uterine nuclei, 110 μg of DNA per pellet, with a more concentrated receptor preparation.

of tissue available, a modified procedure was used to isolate pure rat uterine nuclei. Uteri were removed, cleaned of adipose tissue, and kept on ice in 0.25 M sucrose–0.001 M $MgCl_2$–0.01 M Tris (pH 7.4). The uteri were weighed, split open, and minced completely. Two to four uteri were homogenized in 10 volumes of 0.5 M sucrose–0.005 M $MgCl_2$–0.01 M Tris (pH 7.4) with three 15-sec bursts of a Polytron PT-10 at a power setting of 3. The homogenate was cooled between each burst and filtered through one layer of organza cloth (100 mesh); the trapped material was rehomogenized twice. The Polytron was rinsed with 5 volumes of buffer by a short burst and the rinse was filtered through organza cloth. The filtrate was centrifuged at 2000*g* for 10 min and the supernatant removed. The centrifuge tube was drained and the pellet resuspended in 8 ml of buffer 1 with the Polytron PT-10. The resuspended pellet was then filtered through two layers of organza cloth into 14 ml of SW 27.1 cellulose nitrate tubes and then underlaid with buffer 1 to fill the tube. The tubes were centrifuged at 96,300*g* for 60 min and the supernatant was decanted. The nuclear pellet was gently resuspended by vortexing in TE/0.15 and centrifuged at 2000*g* for 10 min to remove sucrose. The nuclei were again resuspended in the desired volume of TE/0.15 and their purity and integrity were confirmed microscopically as described earlier.

Crude nuclei were obtained from the pellet after preparation of cytosol by rehomogenizing in at least 10 volumes of buffer and centrifuging 20 min at 105,000*g*. The washed pellet was again homogenized in buffer, divided into an appropriate number of tubes, and recentrifuged to provide aliquots suitable for incubation with receptor.

Incubation of Nuclei with Receptor. Nuclei were suspended in 0.5 ml of receptor solution and incubated as indicated; 4 ml of chilled buffer (T or TE/0.15, whichever was used in the incubation) was then added, and the suspension was vigorously shaken before centrifugation at 105,000*g* for 30 min, 4°. Washings did not further reduce bound counts significantly. The pellet was rehomogenized in 0.5 ml of buffer and 0.2 ml was dissolved in 1.0 ml of NCS solubilizer (Amersham-Searle) for radioactivity determination in 10 ml of toluene–2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene. Another 0.2 ml was used for DNA assay (Burton, 1956), and the results were recorded as pmoles of estradiol bound per mg of DNA.

Results

Effect of Nonreceptor Components of Cytosol on Nuclear Binding. Previous experiments (Chamness *et al.*, 1973) produced binding curves suggesting an approach to saturation of nuclear sites. But since these experiments involved incubation of nuclei with various dilutions of estrogen-charged target tissue cytosol in buffer, we questioned whether some nonreceptor component of the cytosol at higher concentrations might depress binding and create the semblance of saturation. We therefore incubated nuclei with fixed amounts of estrogen-charged uterine receptor and varying amounts of cytosol from a nontarget tissue. Figure 1 shows that higher levels of cytosol do indeed depress nuclear binding, so that previous indications of saturability must be considered artifactual. The same phenomenon is produced by varying amounts of uterine or MtTW5 cytosol, either uncharged or heat inactivated, with a fixed amount of charged uterine receptor, thus showing that the effect is not limited to cytosol from a particular tissue and that it does not depend on the action of heat-labile enzymes (Table I). Even bovine serum albumin at a total protein concentration close to that of the cytosols causes a similar effect. To control for this effect in the experiments presented below, all cytosol receptor dilutions have been made with another cytosol rather than with buffer alone. To be sure that this

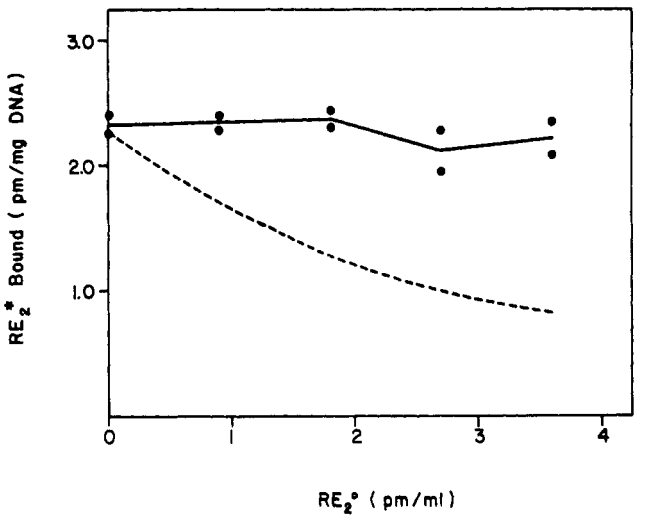

FIGURE 4: Competition for receptor binding to nuclei. Pituitary tumor MtTW5 cytosol was prepared in T buffer, and portions were charged 30 min at 25° with either [^{3}H]estradiol or unlabeled estradiol before DCC treatment. Purified tumor nuclei (70 μg of DNA per pellet) were incubated at 25° for 15 min with 100 μl (final concentration 0.9 pm/ml) of labeled receptor (RE*) and 0–400 μl of unlabeled receptor (RE°), the volume being made up to 500 μl with uncharged cytosol which had been inactivated by heating to 60° for 30 min.

precaution introduces no new artifact, experiments have also been performed using partially purified receptor preparations.

Binding of Partially Purified Receptor to Nuclei of Several Tissues. Nuclei were purified from a number of different rat tissues as described above. Increasing amounts of partially purified, estradiol-charged receptor were added to fixed aliquots of nuclei, and the volume in each case was adjusted to 0.5 ml with spleen cytosol treated in parallel with the receptor-containing tumor cytosol. The amount of receptor bound per mg of DNA after 30 min at 25° is plotted for three such experiments in Figure 2.

It is clear that uterus, spleen, and kidney nuclei do not differ in their ability to bind estrogen receptor. The same results were found for nuclei from all other tissues examined, including liver, MtTW5 pituitary tumor, and R3230AC mammary tumor. This confirms earlier results (Chamness *et al.*, 1973) which showed equal binding of unpurified receptor to nuclei of both target and nontarget tissues.

It is also clear that saturation of nuclei by receptor was not approached, since the amount of bound receptor remained strictly proportional to the receptor concentration added throughout the range employed. We next considered whether higher concentrations of receptor might still reveal saturation of nuclear binding. Uterine nuclei were therefore incubated with a very concentrated preparation of receptor. The result appears in Figure 3. Using the value 6 pg of DNA/nucleus in the rat (Davidson, 1969), 1 pmol/mg of DNA corresponds to about 3600 molecules of bound estradiol per nucleus, so that the 8 pmol/mg of DNA attained here represents two to five times more receptor bound to the nuclei than found at *maximum* stimulation *in vivo* (Anderson *et al.*, 1973; Clark *et al.*, 1972; Williams and Gorski, 1973). There is no sign of approaching saturation.

The same linearity of uptake to varying hyperphysiological levels was found using either crude or partially purified receptor with crude or purified nuclei under a variety of conditions, as long as similarly treated cytosol preparations which do not contain charged receptor (such as spleen cytosol or uncharged or 65° inactivated uterus or MtTW5 cytosol) were used as diluents for the receptor preparations. Earlier hints of saturability were apparently due to omitting this precaution.

Absence of Competition for Nuclear Binding of Receptor. The apparent absence of saturation of nuclear capacity for receptor predicts that even large amounts of estradiol-charged but unlabeled receptor should not competitively inhibit the binding of a smaller amount of receptor labeled with [^{3}H]estradiol. To test this prediction, fixed amounts of labeled receptor from MtTW5 tumor were incubated with tumor nuclei in the presence of increasing amounts of competing unlabeled receptor. There was no decrease in binding of the labeled receptor even with a fourfold excess of the competitor and maximum physiological binding levels (Figure 4). For comparison, the figure includes a theoretical competition curve derived using 2×10^{-10} M as K_d. The same results were obtained also with uterine nuclei and uterine receptor, showing no evidence of competition.

The absence of competition for nuclear binding has been confirmed in our laboratory by recent experiments of R. E. Shepherd *et al.* (in preparation) in which even hyperphysiological injections of estradiol *in vivo* failed to reduce cell-free receptor binding to nuclei prepared from the animals 1 hr after injection.

Discussion

The results presented here show no evidence of a limited number of high affinity acceptor sites (type I) for binding estrogen receptor in isolated nuclei. Though we cannot exclude the presence of a very few type I sites, it is clear that at least the great majority of receptor molecules found in the nucleus are associated with type II binding. Since this total number of nuclear acceptor molecules is directly correlated with the physiological estrogen response of the cell (Anderson *et al.*, 1973; Katzenellenbogen and Gorski, 1972), it seems probable that type II binding is involved in determining this response. These conclusions support recent findings *in vivo* and in incubated tissues that estrogen, glucocorticoid, or progesterone receptor binding in nuclei is strictly proportional to the amount of charged receptor in the cytoplasm, *i.e.*, that there is no saturation of nuclei by the amount of receptor available in intact cells (Williams and Gorski, 1972b; Funder *et al.*, 1973; Rao *et al.*, 1973).

Evidence against a substantial number of specific, high affinity acceptor sites in the nucleus does not preclude a transcriptional level of action for the charged estrogen receptor. The concentration of charged receptor by nuclei has been well demonstrated by autoradiography and by cell fractionation. Stimulation of RNA polymerase by receptor in isolated nuclei (Mohla *et al.*, 1972) and the very rapid induction of synthesis of a presumed messenger for uterine specific protein (DeAngelo and Gorski, 1970) both support direct transcriptional effects of the receptor. But many conceivable direct actions of receptor in the nucleus, *e.g.*, enzymatic modification of a specific component of chromatin, would not reveal themselves by type I binding to particular sites. Other methods may be necessary to expose such actions.

We have also substantiated the earlier conclusion that the ability to bind estrogen receptor under cell-free conditions is not unique to nuclei from target cells. *In vivo*, only target cells possess the receptor in their cytoplasm, and hormone responsiveness is thus limited to these tissues, whatever the potential of their nuclei. Though the RNA polymerase stimulation has been reported to be absent in isolated non-

target nuclei (Mohla *et al.*, 1972), it is not known whether these nuclei might have some other responses to estradiol if the receptor were provided.

References

Anderson, J. N., Peck, E. J., Jr., and Clark, J. H. (1973), *Endocrinology 92*, 1488.

Baxter, J. D., Rousseau, G. G., Benson, M. C., Garcea, R. L., Ito, J., and Tomkins, G. M. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 1892.

Brecher, P. I., Numata, M., DeSombre, E. R., and Jensen, E. V. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 249.

Burton, K. (1956), *Biochem. J. 62*, 315.

Chamness, G. C., Jennings, A. W., and McGuire, W. L. (1973), *Nature (London) 241*, 458.

Chamness, G. C., and McGuire, W. L. (1972), *Biochemistry 11*, 2466.

Clark, J. H., Anderson, J. N., and Peck, E. J., Jr. (1972), *Science 176*, 528.

Clemens, L. E., and Kleinsmith, L. J. (1972), *Nature (London), New. Biol. 237*, 204.

Davidson, J. N. (1969), The Biochemistry of the Nucleic Acids, 6th ed, London, Methuen, p 325.

DeAngelo, A. B., and Gorski, J. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 693.

DeSombre, E., Hurst, D., Kawashima, T., Jungblut, P., and Jensen, E. V. (1967), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 26*, 536.

Funder, J. W., Feldman, D., and Edelman, I. S. (1973), *Endocrinology 92*, 1005.

Gorski, J., Toft, D., Shyamala, G., Smith, D., and Notides, A. (1968), *Recent Progr. Horm. Res. 24*, 45.

Gschwendt, M., and Hamilton, T. H. (1972), *Biochem. J. 128*, 611.

Hamilton, T. H. (1971), *in* The Biochemistry of Steroid Hormone Action, Smellie, R. M. S., Ed., London, Academic Press, p 49.

Jensen, E. V., and DeSombre, E. R. (1972), *Annu. Rev. Biochem. 41*, 203.

Jensen, E. V., DeSombre, E. R., Jungblut, P. W., Stumpf, W. E., and Roth, L. J. (1969a), *in* Autoradiography of Diffusible Substances, Stumpf, W. E., and Roth, L. J., Ed., New York, N. Y., Academic Press, p 81.

Jensen, E. V., Numata, M., Smith, S., Suzuki, T., Brecher, P. I., and DeSombre, E. R. (1969b), *in* Communication in Development, Lang, A., Ed., New York, N. Y., Academic Press, p 151.

Jensen, E. V., Suzuki, T., Kawashima, T., Stumpf, W. E., Jungblut, P. W., and DeSombre, E. R. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 632.

Katzenellenbogen, B. S., and Gorski, J. (1972), *J. Biol. Chem. 247*, 1299.

King, R. J. B., and Gordon, J. (1972), *Nature (London), New Biol. 240*, 185.

King, R. J. B., Gordon, J., Cowan, D. M., and Inman, D. R. (1966), *J. Endocrinol. 36*, 139.

Liao, S., Liang, T., and Tymoczko, J. L. (1973), *Nature (London), New Biol. 241*, 211.

Mainwaring, W. I. P., and Peterken, B. M. (1971), *Biochem. J. 125*, 285.

Mayol, R. F., and Thayer, S. A. (1970), *Biochemistry 9*, 2484.

McGuire, W. L., DeLaGarza, M., and Chamness, G. C. (1973), *Endocrinology* (in press).

McGuire, W. L., Huff, K., and Chamness, G. C. (1972), *Biochemistry 11*, 4562.

Mohla, S., DeSombre, E. R., and Jensen, E. V. (1972), *Biochem. Biophys. Res. Commun. 46*, 661.

Musliner, T. A., and Chader, G. J. (1972), *Biochim. Biophys. Acta 262*, 256.

Musliner, T. A., Chader, G. J., and Villee, C. A. (1970), *Biochemistry 9*, 4448.

Notides, A., and Gorski, J. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 230.

Puca, G. A., and Bresciani, F. (1968), *Nature (London) 218*, 967.

Rao, B. R., Wiest, W. G., and Allen, W. M. (1973), *Endocrinology 92*, 1229.

Schrader, W. T., Toft, D. O., and O'Malley, B. W. (1972), *J. Biol. Chem. 247*, 2401.

Shyamala, G., and Gorski, J. (1969), *J. Biol. Chem. 244*, 1097.

Spelsberg, T. C., Steggles, A. W., Chytil, F., and O'Malley, B. W. (1972), *J. Biol. Chem. 247*, 1368.

Steggles, A. W., Spelsberg, T. C., Glasser, S. R., and O'Malley, B. W. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1479.

Stumpf, W. E. (1969), *Endocrinology 85*, 31.

Toft, D. O. (1972), *J. Steroid Biochem. 3*, 575.

Williams, D., and Gorski, J. (1971), *Biochem. Biophys. Res. Commun. 45*, 258.

Williams, D., and Gorski, J. (1972a), *Acta Endocrinol. (Copenhagen), Suppl. 168*, 420.

Williams, D., and Gorski, J. (1972b), *Proc. Nat. Acad. Sci. U. S. 69*, 3464.

Williams, D., and Gorski, J. (1973), *Biochemistry 12*, 297.

Yamamoto, K. R., and Alberts, B. M. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2105.