Europ. J. Cancer Vol. 16, pp. 1141–1148.

0014-2964/80/0901-1141 $02.00/0

The Binding of Tamoxifen to Human Mammary Carcinoma Cytosol

R. L. SUTHERLAND and L. C. MURPHY

Ludwig Institute for Cancer Research, University of Sydney, N.S.W. 2006, *Australia*

Abstract—*Interactions between tamoxifen and cytoplasmic binding sites from both ER+ and ER− human mammary carcinoma biopsies were investigated directly using* [^{3}H] *tamoxifen. Cytosol preparations were incubated for* 16 hr *at* 4°C *with* 1.25 nM [^{3}H] *tamoxifen and increasing concentrations of unlabelled tamoxifen* (1.25 nM–5 μM). *Protein bound* (*B*) *and unbound* (*U*) *tamoxifen were separated by charcoal adsorption, and the affinity constants and binding site concentrations were calculated from measured values of B and U by a non-linear, least squares regression analysis. A saturable, high affinity* (K_d = 6.0 ± 1.6 nM), *tamoxifen binding site was present in ER+ but not ER− tumours. This site was present at* 8.6 *times the concentration of the high affinity oestradiol receptor site measured in the same tissue. Competition studies performed under similar assay conditions demonstrated that tamoxifen completely inhibited* [^{3}H] *oestradiol binding to its saturable binding site in ER+ tumours with a relative binding affinity* 0.87 ± 0.35% *that of oestradiol. However, when* [^{3}H] *tamoxifen and cytosol were incubated with unlabelled oestradiol at concentrations as high as* 10 μM *there was no inhibition of* [^{3}H] *tamoxifen binding to its saturable binding site. It is concluded that under the present* in vitro *assay conditions tamoxifen is bound predominantly by a high affinity, saturable binding site in ER+ human mammary carcinoma cytosol that is distinct from the classical oestrogen receptor site.*

INTRODUCTION

TAMOXIFEN [1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] is a synthetic non-steroidal compound which shows varying degrees of oestrogen agonist and antagonist activity depending upon the experimental conditions under which it is tested [1–3]. The antioestrogenic properties of this compound have led to its clinical use in the treatment of a number of human malignant tumours especially oestrogen receptor positive (ER+) metastatic breast cancer [4, 5]. Despite this widespread clinical use little is yet known of the molecular modes of action of tamoxifen. However, studies in a number of experimental systems including: the rat uterus [6–10], chick oviduct [2, 11], DMBA induced mammary carcinoma [12–15], and the oestrogen responsive human mammary carcinoma cell line, MCF-7, [3, 16, 17] have indicated that tamoxifen exerts its effects, at least in part, via the specific oestrogen receptor proteins of oestrogen responsive cells.

Accepted 13 February 1980.

The majority of data relating to tamoxifen binding by oestrogen receptors have been obtained from indirect binding studies employing tritiated oestradiol [6, 7, 15, 17–21]. With the recent synthesis and availability of tritiated tamoxifen it is now possible to investigate directly the binding of this compound to specific binding components in normal and neoplastic tissues. A recent article in this Journal documented the binding of tamoxifen to cytosol preparations from human and rat mammary carcinoma and rat uterus [22]. These authors demonstrated that oestradiol and tamoxifen were mutually competitive for binding sites in DMBA induced rat mammary carcinoma and that these compounds were bound to the same number of binding sites in the three tissues studied. It was concluded that in addition to non-specific binding, tamoxifen was bound only to the classical cytoplasmic oestrogen receptor site [22]. Results from this laboratory are in general agreement with these findings as far as rat uterus is concerned and we have no data on the DMBA induced rat mammary carcinoma. However, the results reported by Nicholson *et al.* [22] for

tamoxifen binding to human mammary carcinoma cytosol are at variance with those of this laboratory. In this paper we report data which illustrate that in addition to binding to the oestrogen receptor tamoxifen is bound to another high affinity, saturable binding site in ER+ human mammary carcinoma cytosol which appears to be distinct from the classical oestrogen receptor site.

MATERIALS AND METHODS

Materials

[2,4,6,7-^{3}H] Oestradiol (85–110 Ci/mmole) was purchased from the Radiochemical Centre, Amersham, Bucks, U.K. Unlabelled oestradiol-17β was from Sigma, St. Louis, Missouri, U.S.A. [^{3}H] Tamoxifen (19.5 Ci/mmole) and unlabelled tamoxifen base were gifts from I.C.I. Pharmaceuticals, Alderley Park, Cheshire, U.K. Radioactive tamoxifen was stored at −20°C in the dark as a 4 μM solution in methanol. To reduce adsorption of tamoxifen to surfaces, all solutions were prepared in buffers containing 0.2% BSA to give a final concentration of 0.1% BSA in the reaction medium. In addition all aqueous solutions of tamoxifen were prepared and stored in polypropylene tubes. All assays were performed in 4 ml disposable tubes of the same material.

Preparation of cytoplasmic fractions

All procedures were conducted at 2–4°C unless otherwise stated. Human mammary tumour biopsies, which had been stored at −70°C, were thawed, chopped into fine pieces with a scalpel, and homogenized in 10 volumes (w/v) of buffer using 3 × 30 sec bursts with a Polytron (Kinematica Luzern, Switzerland). The homogenization buffer was 10 mM Tris–HCl, pH 7.4 containing 1.5 mM EDTA and 0.5 mM dithiothreitol. A low speed supernatant was prepared by an 800 ***g*** × 10 min centrifugation. A subsequent centrifugation at 105,000 ***g*** for 60 min yielded the cytosol which was used in all binding studies following dilution to a protein concentration of 3–4 mg/ml with homogenization buffer.

Determination of binding parameters

(*a*) *Assay conditions.* In order to derive estimates for the concentration of protein bound, [B], and unbound, [U], ligand (oestradiol or tamoxifen) over a wide range of ligand concentrations, 100 μl of cytosol was incubated with 50 μl tritiated ligand and 50 μl unlabelled ligand for 16 hr at 4°C. For estimation of the oestradiol binding parameters (equilibrium dissociation constant, K_d, and binding site concentration, C) a final concentration of 0.25 nM [^{3}H] oestradiol and final unlabelled oestradiol concentrations in the range of 0.25 nM–1 μM were employed. The comparable concentrations of [^{3}H] tamoxifen and unlabelled tamoxifen were 1.25 nM, and 1.25 nM–5 μM, respectively. Following incubation, bound and unbound ligand were separated by a 30 min incubation at 4°C with 500 μl of 0.5% charcoal–0.05% dextran in homogenization buffer, and centrifuged at 1500 ***g*** for 10 min. A 500 μl aliquot of the supernatant was then counted in 5 ml of scintillation fluid (4g Omnifluor, 330 ml Triton X-100, 670 ml toluene) in a LKB liquid scintillation spectrometer using external standardization. The molar concentrations of bound ligand, [B], were calculated according to the formula:

$$[B] = \frac{\text{dis/min bound}}{\text{dis/min total}} \times [T]$$

where [T] is the molar concentration of ligand per assay tube. Unbound ligand concentration, [U], was calculated from $[U] = [T] - [B]$.

(*b*) *Calculation.* In order to calculate the oestradiol and tamoxifen binding properties of human mammary tumour cytosol, measured values of [B] and [U] were fitted to a model of the form

$$[B] = \sum C[U]/(K_d + [U]).$$

This assumes that oestradiol and tamoxifen are bound according to the law of mass action by a number of non-interacting binding sites of binding capacity, C, and dissociation constant, K_d. Under the present experimental conditions only one saturable binding site for both oestradiol and tamoxifen could be detected and thus the data were fitted to the equation:

$$[B] = C[U]/(K_d + [U]) + K_{ns}[U]$$

where C and K_d relate to the concentration and affinity of the saturable binding site and K_{ns} is a measure of the non-specific binding [23, 24]. The parameters C, K_d and K_{ns} were fitted to the experimental data using an iterative, non-linear least-squares regression analysis as previously described [23, 24].

In addition to estimates of K_{ns} obtained from the above analysis, non-specific binding was also calculated from the linear regression of [B] on [U], at concentrations of ligand in excess of that required for saturation of the high affinity binding component [25, 26].

Competition studies

To determine the ability of tamoxifen to compete for oestradiol binding sites and vice versa, tracer [^{3}H] oestradiol (0.25 nM) or [^{3}H] tamoxifen (1.25 nM) was incubated with cytosol and increasing concentrations of oestradiol or tamoxifen for 16 hr at 4°C. Protein-bound and unbound ligand were separated by charcoal adsorption as described above and the data plotted as percent tracer bound versus log ligand concentration. The relative binding affinity was calculated from the amount of ligand required to displace 50% of the tracer [27].

RESULTS

Direct saturation analysis studies

In order to clearly define both the specific (saturable) and non-specific binding of oestradiol and tamoxifen to both ER+ and ER− human mammary carcinoma cytosols the relationship between [B] and [U] for each ligand was studied over a 10,000-fold concentration range of total ligand. At low total ligand concentrations i.e., less than 2 nM for oestradiol and less than 20 nM for tamoxifen, cytosolic preparations from ER+ tumours demonstrated curvilinear relationships between [B] and [U] indicating the presence of saturable binding components for both ligands (Figs. 1A and 1B). At higher total ligand concentrations the relationship between [B] and [U] became linear, and remained so over the concentration range studied (data not shown), as would be expected when the high affinity saturable binding components were saturated and only non-specific binding components were being measured [23, 24]. When cytosol preparations from ER− tumours were studied the relationship between [B] and [U] was linear for both ligands even at low ligand concentrations illustrating the absence of saturable binding components for tamoxifen, as well as oestradiol in ER− tumours (Figs. 1A and 1B).

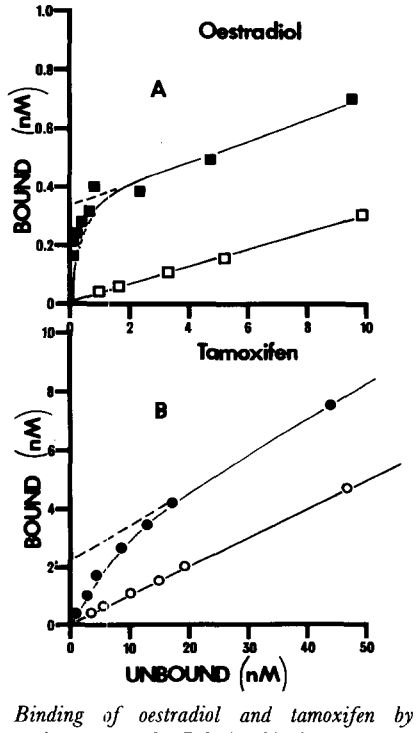

Fig. 1. Binding of oestradiol and tamoxifen by human mammary carcinoma cytosols. Relationship between protein-bound and unbound ligand concentrations. Cytosol fractions were incubated with a constant amount of tracer ligand and increasing concentrations of unlabelled ligand for 16 hr *at* 4°C. *B and U were separated by charcoal adsorption and the data plotted as [B] vs [U]. (A) Oestradiol binding to ER+ (■) and ER− (□) tumour cytosols at low total ligand concentrations, i.e., less than* 10 nM. *(B) Tamoxifen binding to ER+ (●) and ER− (○) tumour cytosols at low total ligand concentration, i.e., less than* 50 nM.

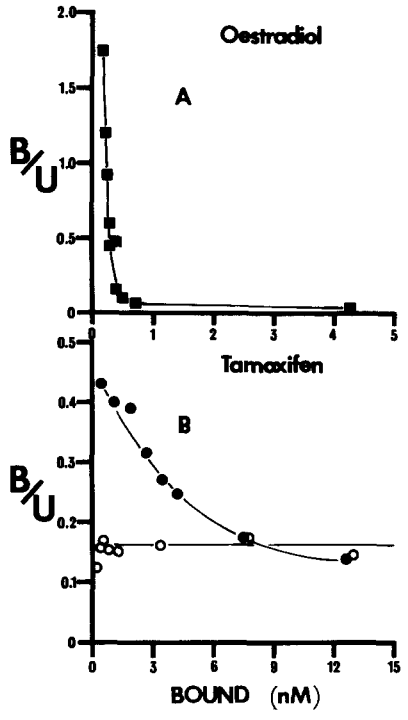

Fig. 2. Scatchard plots of oestradiol and tamoxifen binding to human mammary carcinoma cytosols. Cytosol was incubated with tracer ligand and increasing concentrations of unlabelled ligand for 16 hr *at* 4°C, *B and U were separated by charcoal adsorption and the binding parameters, C,* K_d *and* K_{ns}, *calculated as outlined in 'Materials and Methods'. Data are presented as Scatchard plots and the calculated regression lines are shown. (A) Oestradiol binding to an ER+ (■) tumour cytosol. (B) Tamoxifen binding to ER+ (●) and ER− (○) tumour cytosol.*

Table 1. Binding parameters for the interactions between oestradiol, tamoxifen and human mammary carcinoma cytosols

Ligand	Tissue	K_d (nM)	C (nM)	K_{ns}
Oestradiol	ER+	0.18±0.07	0.17±0.05 (119±41)	0.05±0.01
	ER−	—	—	0.06±0.02
Tamoxifen	ER+	6.04±1.60	1.47±0.50 (1023±348)	0.12±0.02
	ER−	—	—	0.14±0.03

The parameters are defined in 'Materials and Methods'. Binding capacity, C, is expressed as nmole/litre of reaction mixture which is a 2 fold dilution of the cytosol. The values in parenthesis are binding capacities expressed as fmole/mg protein. Non-specific binding, K_{ns}, is a unitless parameter [24]. Data are presented as mean±S.E.M. of 5 replicates/group.

Closer appraisal of the curves for oestradiol and tamoxifen binding to ER+ tumour cytosols, illustrated in Figs. 1A and 1B, revealed that the number of saturable binding sites for tamoxifen was considerably greater than that for oestradiol. Such a conclusion is based on the observation that if the linear portion of the curve of [B] versus [U] is extrapolated to the ordinate a first approximation of the total number of saturable binding sites can be obtained. When this was done with the data presented in Fig. 1, the approximations of total saturable binding sites were 0.32 and 2.5 nM for oestradiol and tamoxifen, respectively. These observations were confirmed when the measured [B] and [U] values were analysed by the computer methods described in 'Materials and Methods'. Both the oestradiol and tamoxifen binding were best fitted by a model consisting of one saturable binding component and non-specific binding. This is illustrated in Fig. 2 where the data are plotted according to the Scatchard transformation and the calculated non-linear regression lines are drawn. Replicate estimates of the binding parameters illustrated that the saturable tamoxifen binding sites were present at 8.6 times the concentration of saturable oestradiol binding sites (the cytoplasmic oestrogen receptor) and that the affinity of tamoxifen for its saturable site was about 34-fold less than the affinity of oestradiol for the oestrogen receptor (Table 1). Since the mean concentration of oestrogen receptors in the group

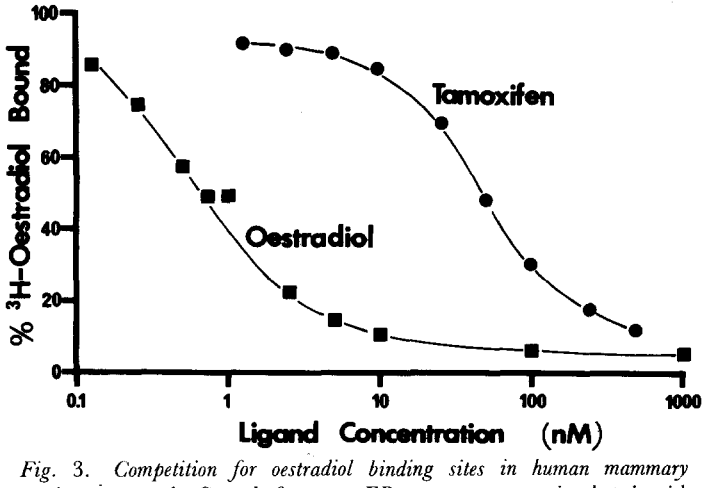

Fig. 3. Competition for oestradiol binding sites in human mammary carcinoma cytosol. Cytosol from an ER+ tumour was incubated with 0.25 nM [^{3}H] *oestradiol and increasing concentrations of unlabelled oestradiol* (0.25–100 nM) *or tamoxifen* (10 nM–1 μM) *for* 16 hr *at* 4°C. *The protein-bound ligand was then separated by charcoal adsorption. Data are presented as per cent* B/B_0 vs log *of the added ligand concentration, where* B = dis/min *bound at each level of unlabelled ligand and* B_0 = dis/min *bound in the absence of unlabelled ligand.*

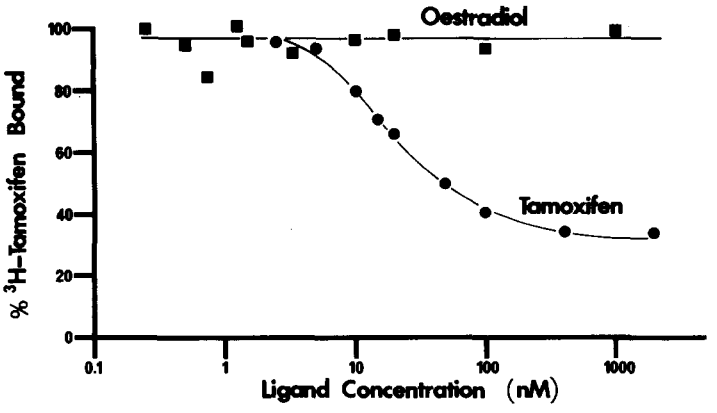

Fig. 4. Competition for tamoxifen binding sites in human mammary carcinoma cytosol. Cytosol from an ER+ tumour was incubated with 1.25 nM [^{3}H] *tamoxifen and increasing concentrations of unlabelled oestradiol or tamoxifen for* 16 hr *at* 4°C *and the protein-bound ligand separated by charcoal adsorption. Data are presented as in* Fig. 3.

of ER + tumours was 119 fmole/mg protein the mean concentration of saturable tamoxifen binding sites was 1.02 pmole/mg protein. Non-specific binding of tamoxifen was 2.3 times that of oestradiol measured in the same samples. No saturable tamoxifen binding sites were detected in any of the ER − tumours studied (Table 1).

Indirect competition studies

Competition studies employing a fixed amount of tracer oestradiol and increasing concentrations of unlabelled oestradiol or tamoxifen, demonstrated that tamoxifen could completely inhibit the binding of oestradiol to the cytoplasmic oestrogen receptor of ER + tumours (Fig. 3) with a mean (± S.E.M.) relative binding affinity 0.87 ± 0.35% that of oestradiol. Such a result is compatible with tamoxifen binding to the oestrogen receptor. Neither oestradiol nor tamoxifen could inhibit the binding of [^{3}H] oestradiol to ER − tumour cytosols confirming the non-specific nature of such binding.

When attempts were then made to inhibit the binding of tritiated tamoxifen to its saturable binding site with increasing concentrations of oestradiol, no inhibition was observed even when unlabelled oestradiol was present at a 10,000-fold excess over tracer tamoxifen (Fig. 4). This result illustrates that under the present *in vitro* assay conditions little or no tracer tamoxifen was bound to the oestrogen receptor and that the saturable tamoxifen binding site does not bind oestradiol.

DISCUSSION

Despite the widespread clinical use of tamoxifen in the treatment of human breast cancer there is little data on the interaction of this drug with neoplastic tissue from the human mammary gland. Indeed at the time of writing we were aware of only one publication where the interactions of tamoxifen with such a tissue had been investigated directly using tritiated tamoxifen [22]. There have, however, been a number of earlier reports where the interactions of tamoxifen with human mammary carcinoma cytosol had been investigated indirectly using tritiated oestradiol as the label [18, 28, 29]. These latter studies illustrated that tamoxifen could inhibit the binding of tritiated oestradiol to the oestrogen receptor in a dose-dependent manner but with an affinity considerably lower than that of oestradiol i.e., in the range 0.4–1.0% that of oestradiol [18, 20, 28, 29]. Such estimates of the relative binding affinity of tamoxifen for the oestrogen receptor are significantly lower than those found in some other oestrogen target tissues. For example, studies carried out under identical assay conditions in this laboratory have yielded estimates for the relative binding affinities of tamoxifen in rat uterus, chick oviduct, and human mammary carcinoma cytosols of 13, 5 and 0.9%, respectively. (Sutherland and Foo, unpublished data). These differences could be due to a number of factors including: tissue and species differences in the structure of the oestrogen binding site, different degrees of tamoxifen degradation and

metabolism *in vitro* in different tissue cytosols, different ratios of receptor binding to non-specific binding in different tissues, and the presence of additional tamoxifen binding components which reduce the availability of tamoxifen for binding to the classical oestrogen receptor site. At this stage it is the latter possibility which we favour most strongly since the data reported above clearly demonstrate the presence of a high affinity, saturable tamoxifen binding site in the cytosol of ER+ tumours (Figs. 1, 2 and 4; Table 1). The affinity and concentration of this site are such that, at the level of labelled tamoxifen used in the current *in vitro* studies, the majority of this ligand will be bound to the saturable anti-oestrogen binding site at the expense of the oestrogen receptor. Indeed, in computer simulation studies (Sutherland and Milthorpe, unpublished data) we have estimated that, under the experimental conditions described in Fig. 4, less than 5% of the tritiated tamoxifen is bound to the oestrogen receptor. This probably explains the inability of high concentrations of unlabelled oestradiol to inhibit [^{3}H] tamoxifen binding to ER+ mammary tumour cytosols (Fig. 4).

The data presented herein are not incompatible with tamoxifen binding to the oestrogen receptor in human mammary carcinoma. Indeed the competition data presented in Fig. 3 support such an hypothesis and are in agreement with data reported by others [18–20, 28, 29]. The fact that we did not observe tamoxifen binding to oestrogen receptors in our direct binding studies, an observation at variance with that of Nicholson *et al.* [22], may be explained by the concentration range over which the assays were performed. Not only did the latter workers employ lower ligand concentrations but they also used 4–5-fold higher protein concentrations [22]. The overall effect of this experimental design would be to facilitate estimates of bound and unbound ligand concentrations at much lower degrees of saturation than we report here. If the oestrogen receptor has a higher affinity for tamoxifen than the antioestrogen binding site, it is likely that oestrogen receptor binding would constitute a greater percentage of total tamoxifen binding at these reduced ligand concentrations, and may therefore be detected on Scatchard plots.

In conclusion we have described the presence of a novel, high affinity, saturable anti-oestrogen binding site in ER+ but not ER− human mammary carcinoma cytosols. This site which is specific for the synthetic non-steroidal anti-oestrogens and does not bind natural or synthetic oestrogens, androgens or progesterone is also present in a number of other oestrogen target tissues ([30], Sutherland, Murphy, Foo, Green, Whybourne and Krozowski, to be published). The affinity and concentration of this site are such that it must play a major role in the intracellular binding of tamoxifen. Elucidation of the roles, if any, of this binding site in mediating the antitumour activity of tamoxifen in human breast cancer awaits further experimentation.

Acknowledgements—We thank I.C.I. Ltd., for their gifts of [^{3}H] tamoxifen and tamoxifen base. The assistance of Dr. B. K. Milthorpe in preparing the data analysis for the computer facilities in the Institute is gratefully acknowledged. Professor M. H. N. Tattersall's continual interest, encouragement and critical comment is ackowledged with gratitude. We are indebted to our colleagues, Michael Green, Terry Foo and Anne Whybourne for their help and criticism.

REFERENCES

1. M. J. K. Harper and A. L. Walpole, Contrasting endocrine activities of *cis* and *trans* isomers in a series of substituted triphenylethylenes. *Nature* (*Lond.*) **212,** 87 (1966).
2. R. Sutherland, J. Mester and E. E. Baulieu, Tamoxifen is a potent 'pure' antioestrogen in chicken oviduct. *Nature* (*Lond.*) **267,** 434 (1977).
3. K. B. Horwitz, Y. Koseki and W. L. McGuire, Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogens. *Endocrinology* **103,** 1742 (1978).
4. G. Leclercq and J. C Heuson, Therapeutic significance of sex-steroid hormone receptors in the treatment of breast cancer. *Europ. J. Cancer* **13,** 1205 (1977).
5. H. Mouridsen, T. Palshof, J. Patterson and L. Battersby, Tamoxifen in advanced breast cancer. *Cancer Treat. Rev.* **5,** 131 (1978).

6. J. SKIDMORE, A. L. WALPOLE and J. WOODBURN, Effect of some triphenylethylenes on oestradiol binding *in vitro* to macromolecules from uterus and anterior pituitary. *J. Endocr.* **52,** 289 (1972).
7. V. C. JORDAN and L. J. DOWSE, Tamoxifen as an anti-tumour agent: effect on oestrogen binding. *J. Endocr.* **68,** 297 (1976).
8. V. C. JORDAN, C. J. DIX, L. ROWSBY and G. PRESTWICH, Studies on the mechanism of action of the nonsteriodal antioestrogen tamoxifen (I.C.I. 46, 474) in the rat. *Molec. cell. Endocr.* **7,** 177 (1977).
9. Y. KOSEKI, D. T. ZAVA, G. C. CHAMNESS and W. L. MCGUIRE, Estrogen receptor translocation and replenishment by the antiestrogen tamoxifen. *Endocrinolgy* **101,** 1104 (1977).
10. F. CAPONY and H. ROCHEFORT, High-affinity binding of the antioestrogen [^{3}H] tamoxifen to the 8S oestradiol receptor. *Molec. cell. Endocr.* **11,** 181 (1978).
11. J. MESTER, C. GEYNET, N. BINART and E. E. BAULIEU, Retarded administration of an antagonist stops steroid hormone action. *Biochem. biophys Res. Commun.* **79,** 112 (1977).
12. R. I. NICHOLSON and M. P. GOLDER, The effect of synthetic antioestrogens on the growth and biochemistry of rat mammary tumours. *Europ. J. Cancer* **11,** 571 (1975).
13. V. C. JORDAN, Effect of tamoxifen (I.C.I. 46, 474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Europ. J. Cancer* **12,** 419 (1976).
14. R. I. NICHOLSON, M. P. GOLDER, P. DAVIES and K. GRIFFITHS, Effects of oestradiol-17β receptors in DMBA-induced rat mammary tumours. *Europ. J. Cancer* **12,** 711 (1976).
15. R. I. NICHOLSON, P. DAVIES and K. GRIFFITHS, Effects of oestradiol-17β and tamoxifen on nuclear oestradiol-17β receptors in DMBA-induced rat mammary tumours. *Europ. J. Cancer* **13,** 201 (1977).
16. M. LIPPMAN, G. BOLAN and K. HUFF, The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res.* **36,** 4595 (1976).
17. K. B. HORWITZ and W. L. MCGUIRE, Nuclear mechanisms of estrogen action. Effects of estradiol and anti-estrogens on estrogen receptors and nuclear receptor processing. *J. biol. Chem* **253,** 8185 (1978).
18. R. HÄHNEL, E. TWADDLE and T. RATAJCZAK, The influence of synthetic antiestrogens on the binding of tritiated estradiol-17β by cytosols of human uterus and human breast carcinoma. *J. Steroid Biochem.* **4,** 687 (1973).
19. V. C. JORDAN and S. KOERNER, Tamoxifen (I.C.I. 46, 474) and the human carcinoma 8S oestrogen receptor. *Europ. J. Cancer* **11,** 205 (1975).
20. W. POWELL-JONES, D. A. JENNER, R. W. BLUMEY, P. DAVIES and K. GRIFFITHS, Influence of antioestrogens on the specific binding *in vitro* of [^{3}H] oestradiol-17β by cytosols of rat mammary tumours and human breast carcinomata. *Biochem. J.* **150,** 71 (1975).
21. M. LIPPMAN, G. BOLAN and K. HUFF, The effects of androgens and antiandrogens on hormone-responsive breast cancer in long-term tissue culture. *Cancer Res.* **36,** 4610 (1976).
22. R. I. NICHOLSON, J. S. SYNE, C. P. DANIEL and K. GRIFFITHS, The binding of tamoxifen to oestrogen receptor proteins under equilibrium and non-equilibrium conditions *Europ. J. Cancer* **15,** 317 (1979).
23. E. E. BAULIEU and J. P. RAYNAUD, A "proportion graph" method for measuring binding systems. *Europ. J. Biochem.* **13,** 293 (1970).
24. R. L. SUTHERLAND and M. W. SIMPSON-MORGAN, The thyroxine-binding properties of serum proteins. A competitive binding technique employing Sephadex G-25. *J. Endocr.* **65,** 319 (1975).
25. R. L. SUTHERLAND, M. R. BRANDON and M. W. SIMPSON-MORGAN, Effect of ionic strength and ionic composition of assay buffers on the interaction of thyroxine with plasma proteins. *J. Endocr.* **66,** 319 (1975).
26. R. L. SUTHERLAND and M. R. BRANDON, The thyroxine-binding properties of rat and rabbit serum proteins. *Endocrinology* **98,** 91 (1976).
27. S. G. KORENMAN, Relation between estrogen inhibitory activity and binding to cytosol of rabbit and human uterus. *Endocrinology* **87,** 1119 (1970).
28. M. LIPPMAN, G. BOLAN, M. MONACO, L. PINKUS and L. ENGEL, Model systems for the study of estrogen action in tissue culture. *J. Steroid Biochem.* **7,** 1045 (1976).

29. M. Tanaka, K. Abe, S. Ohnami, I. Adachi, K. Yamaguchi and S. Miyakawa, Tamoxifen in advanced breast cancer; response rate, effect on pituitary hormone reserve and binding affinity to estrogen receptor. *Jap. J. clin. Oncol.* **8,** 141 (1978).
30. R. L. Sutherland and M. S. Foo, Differential binding of antiestrogens by rat uterine and chick oviduct cytosol. *Biochem. biophys. Res. Commun.* **91,** 183 (1980).