Br. J. Pharmac. (1986), **87**, 217–223

Oestrogenic and antioestrogenic actions in a series of triphenylbut-1-enes: modulation of prolactin synthesis *in vitro*

V.C. Jordan[1], R. Koch, S. Mittal & M.R. Schneider*

Department of Human Oncology and Pharmacology, Wisconsin Clinical Cancer Center, University of Wisconsin, Madison, Wisconsin 53792 U.S.A. and Chemistry and Pharmacy*, Naturwissenschaftliche Fakultat IV, University of Regensburg, Regensburg, Federal Republic of Germany

1 The oestrogenic and antioestrognic activities of a series of substituted derivatives of 1,1,2 triphenylbut-1-ene have been determined using primary cultures of rat pituitary gland cells to monitor prolactin synthesis *in vitro*.

2 The relative binding affinity of the agonists for the oestrogen receptor was consistent with their oestrogenic potency.

3 *Bis para* substitution at C_1 of 1,1,2 triphenylbut-1-ene with either phenolic or acetoxy groups produced partial agonists. The antioestrogenic properties were reversible by the incubation of cells with increasing concentrations of oestradiol.

4 The results lend support to a hypothetical single binding site model of oestrogen action, based upon an adaptation of Belleau's macromolecular perturbation theory.

Introduction

Hydroxylated derivatives of stilbene (Dodds *et al.*, 1983a, b) and triphenylethylene (Emmens, 1947) show potent oestrogenic activity in laboratory animals. However, the discovery by Lerner and coworkers (1958) that the triphenylethanol derivative MER25 (1-(4-βdimethylaminoethoxyphenyl)-1-phenyl-2-(4-methoxyphenylethanol) has antioestrogenic activity stimulated a search for a more potent compound for clinical application. Clomiphene (Z/E 2-chloro-1-(4-β-diethylaminoethoxyphenyl) -1,2-diphenyl ethylene) (Holtkamp *et al.*, 1960) and tamoxifen (Z-1(4-β-dimethylaminoethoxyphenyl) -1,2-diphenylbut-1-ene) (Harper & Walpole, 1967) are the result of that search. Clomiphene is used for the induction of ovulation in subfertile women (Huppert, 1979) and tamoxifen is used for the treatment of breast cancer (Furr & Jordan, 1984). The clinical use of derivatives of triphenylethylene has focused current interest on their mode of action and a systematic study of their molecular pharmacology (Jordan, 1984).

We have developed an assay system *in vitro* to study the structure-activity relationships of ligands that bind to the oestrogen receptor (Lieberman *et al.*, 1983a).

[1]To whom correspondence should be sent. (Department of Human Oncology).

Oestradiol increases prolactin synthesis in primary cultures of dispersed immature rat pituitary cells (Lieberman *et al.*, 1978) and nonsteroidal antioestrogens exhibit a competitive and reversible inhibition of oestradiol-stimulated prolactin synthesis (Lieberman *et al.*, 1983a, b). In earlier studies we defined three classes of compounds: agonist, partial agonists and antagonists (Jordan *et al.*, 1984; Jordan & Lieberman, 1984) and described a generalised receptor map of the oestrogen binding site (Jordan, 1984). In the current collaborative study we have focused upon the structure-activity relationships in a series of *para* hydroxy and acetoxy derivatives of triphenylbut-1-ene.

Methods

Cell culture and incorporation of radiolabelled precursors

Anterior pituitaries were enzymatically dispersed (Vale *et al.*, 1972) and plated in 35 mm dishes that had been treated with 0.001% poly(D)lysine. Culture media consisted of Dulbecco's modified Eagles medium (DMEM) buffered with 25 mM 4-(2-hydroxy ethyl)-1-piperazine ethane sulphonic acid and sup-

plemented with 10 μg of insulin per ml and containing 12.8% horse serum and 2.2% foetal calf serum. Sera were treated with dextran-coated charcoal to remove steroids (Horwitz *et al.*, 1976). After three cycles of stripping, the concentration of oestradiol per ml of serum able to be used for radioimmunoassay was <1 pg. Cells were seeded at a density of 2×10^5 per dish in serum-supplemented media and cultured at 37°C in an atmosphere of 95% air, 5% CO_2. Test compounds were dissolved in ethanol and diluted in the medium to a final ethanol concentration of 0.1–0.2% which was not detrimental to cell viability or function. Medium was changed on the 3rd day and cells harvested on the 6th day. At the end of the culture period, monolayers were rinsed with complete Hank's balanced salt solution and reincubated in Earle's minimial essential medium made 30 μM in leucine medium to which 10 μCi of [^{3}H]-leucine was added per ml. Monolayers were harvested into ice-cold 0.15 mol l^{-1} NaCl, 10 mmol l^{-1} sodium phosphate, pH 7.5, 10 mmol l^{-1} leucine, 1% Triton X-100 and 0.5% sodium deoxycholate. Cells were sonicated for 10 s at a power setting 2.5 using a cell disrupter (Kontes, Vineland, NJ) and centrifuged for 1 min in a microfuge (Beckman Instruments, Palo Alto, CA). The supernatant contained >90% of the trichloroacetic acid-insoluble radioactivity.

Analysis of total protein and prolactin synthesis

To determine total protein synthesis, aliquots of the supernatants were made 0.01% in bovine serum albumin and 10% in trichloroacetic acid. The acid-insoluble material was collected on glass fibre filters, washed, dried, and analysed for radioactivity after addition of scintillation fluid containing 9% NCS stabilizer + 1% H_2O. After 6 days of culture, the mean protein content in μg per dish seeded at 2×10^5 cells was 882 ± 38 in control cultures ($n = 4$). The protein content in cultures treated with various concentrations of oestradiol and/or test compounds did not differ significantly from this value (data not shown). Prolactin synthesis was determined in aliquots of the supernatant by immunoprecipitation with antiprolactin as previously described (Maurer *et al.*, 1976). To each sample, 2.5 μg of internally labelled [^{14}C]-prolactin was added as carrier and to monitor immunoprecipation efficiency. Immunoprecipitates were directly analysed for radioactivity after the addition of scintillation fluid containing 9% NCS and 1% H_2O.

Incorporation of radiolabel into total protein and prolactin was shown to be linear for 4 h and newly synthesized prolactin is not detectable in the medium until 90 min (Lieberman *et al.*, 1982). Thus, immunoprecipitation of prolactin after a 60 min labelling period provides an estimate of the total rate of prolactin synthesis. Results are expressed as disintegrations per min immunoprecipitable/disintegrations per min trichloracetic acid-insoluble × 100 (% prolactin synthesis).

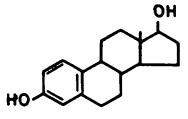
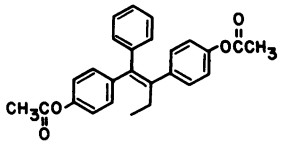
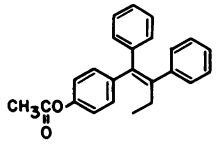
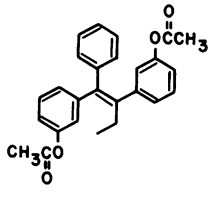
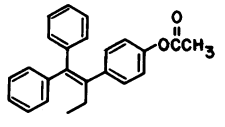
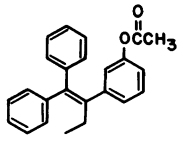
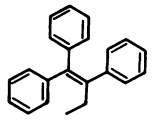
Inhibition of [^{3}H]-oestradiol binding in vitro

Uteri from 18–21 day-old rats were isolated and stored at − 70°C until use. Tissues were homogenized (2 × 10 s bursts with ice water cooling) with a Polytron tissue homogenizer (Brinkmann Instruments) in a ratio of 2 uteri ml^{-1} of buffer containing 10 mmol l^{-1} Tris-HCl, pH 7.4, 1.5 mmol l^{-1} EDTA, and 0.5 mmol l^{-1} dithiothreitol (TED). The homogenate was centrifuged for 1 h (4°C) at 100,000 *g* using an AH650 swinging bucket rotor in an OTD65 Sorvall ultracentrifuge. The supernatant (cytosol) was removed, taking care not to disturb the fat layer on the surface, and kept in an ice water bath before use. Aliquots of cytosol (200 μl) were incubated for 30 min at 30°C or 18 h at 4°C with different concentrations of oestradiol or test compounds administered in 10 μl of ethanol and 100 μl of [^{3}H]-oestradiol solution in TED buffer to give a final concentration of 5×10^{-9} mol l^{-1} [^{3}H]-oestradiol. Parallel incubations of cytosol (200 μl), [^{3}H]-oestradiol solution (100 μl), and either 10 μl of ethanol or 10 μl of ethanol containing diethylstilboestrol to give a final concentration of 5×10^{-6} mol l^{-1} diethylstilboestrol were used to determine the total specific binding of [^{3}H]-oestradiol by subtraction. Tubes were cooled in ice water for at least 15 min and then 500 μl of a suspension of dextran-coated charcoal (0.25% Norit A and 0.025% dextran in TED buffer) was added and allowed to stand for 20 min with occasional shaking. Tubes were centrifuged at 2000 *g* for 10 min at 4°C and the supernatants were decanted into vials containing scintillation fluid and analysed for radioactivity. The relative binding affinity was calculated from the following relationship. Relative binding = (molar concentration oestradiol for 50% inhibition of specific binding/molar concentration competitor for 50% inhibition of specific binding) × 100.

Materials

The derivatives of 1,1,2-triphenylbut-1-ene were synthesized and characterized at the University of Regensburg as previously described (Schneider *et al.*, 1982). Oestradiol-17β and buffer chemicals were obtained from Sigma Chemicals, St. Louis, MO. [4,5^{3}H]-leucine (sp. act. 52 Ci $mmol^{-1}$) and [6,7^{3}H]-oestradiol (sp. act. 51 Ci $mmol^{-1}$) and [6,7^{3}H]-oestradiol (sp. act. 51 Ci $mmol^{-1}$) were obtained from Amersham (Arlington Heights, IL) and were used for assays *in vitro* without further purification. Immature (18–21 day old) female rats of the Sprague-Dawley strain were obtained from Holtzman animal breeders, Madison,

Table 1 Summary of the relative binding affinities (RBA's) at different incubation temperatures and the potency (EC_{50}) of oestrogenic compounds to stimulate prolactin synthesis *in vitro*.

	Compound	RBA 4°C (18 h)	RBA 30°C (0.5 h)	EC_{50} (mol l^{-1})
1		100	100	6×10^{-12}
2		73	266	8×10^{-12}
3		21	31	1×10^{-10}
4		12	24	2×10^{-9}
5		1	3	1×10^{-8}
6		2	4	1×10^{-7}
7		0.01	0.2	3×10^{-7}

The EC_{50} is calculated as the concentration (M) of a compound to produce prolactin synthesis that is 50% of the maximum.

WI. Tissue culture media and sera were from Grand Island Biological Co. (Grand Island, NY).

Results

In the first experiments the relative binding affinity (RBA) of a series of mono- and di-acetoxy derivatives of 1,1,2-triphenylbut-1-ene was determined and the biological activity evaluated in the prolactin synthesis assay *in vitro*. The compounds listed in Table 1 were compared with oestradiol and were found to be full agonists of different potencies. The change in their RBAs and EC_{50} values were consistent, i.e. compounds with lower RBAs had lower potencies. 1,1,2-Triphenylbut-1-ene (Compound 7) was the least active of the compounds tested with a potency 1/50,000 of that of oestradiol (Compound 1). Di-*para* acetoxy substitution of the *trans* phenyl rings (1,2) (Compound 2) dramatically increased the potency of 1,1,2-triphenylbut-1-ene so that it became equivalent to that of oestradiol (EC_{50} 8×10^{-12} mol l^{-1}) (Compound 3). Mono-substitution of the phenyl ring at carbon 2 (Compound 5) had a reduced effect upon the oestrogenic potency (EC_{50} 1×10^{-8} mol l^{-1}). Similarly di-*meta* substitution of the *trans* 1,2 phenyl rings produced a dramatic decrease in oestrogenic potency (Compound 4) compared to the di-*para* substituted compound (Compound 2).

The *bis* 1,1. *para* acetoxy substitution of 1,1,2-triphenylbut-1-ene caused an increase in the RBA compared with mono-*para* acetoxy substitution (Figure 1). Receptor affinity was further improved with *bis* 1,1 phenolic substitution. However, the *bis* 1,1 substitution had a significant effect upon the oestrogenic activity of the compounds (Figure 2). Concentrations of 10^{-9} mol l^{-1} and greater were compared in the prolactin synthesis assay. The percentage of prolactin synthesis was equivalent with 1-*para*-acetoxy-phenyl-1,2-diphenylbut-1-ene and oestradiol; the concentrations were both at the top of the log concentration-response curves. In contrast, the *bis* 1,1 phenolic derivative of 1,1,2-triphenylbut-1-ene was less oestrogenic and the *bis* 1,1 acetoxy derivative was even less effective at inducing prolactin synthesis.

To avoid the possibility that the results illustrated in Figure 2 were not just a comparison of different parts of their respective concentration-response curve, lower concentrations were used. Mono- and tri-acetoxy derivatives were compared with oestradiol (Figure 3). The tri-substituted compound was a weakly active partial agonist whereas the mono-substituted compound was fully oestrogenic.

All of the prolactin synthesis assays were conducted after a six day incubation of the primary cell cultures with different concentrations of test compounds. To examine the possibility that the partial agonists may induce a full response at either an earlier or later time, a time experiment was conducted for up to 10 days. Oestradiol was compared with the *bis* 1,1 phenolic and

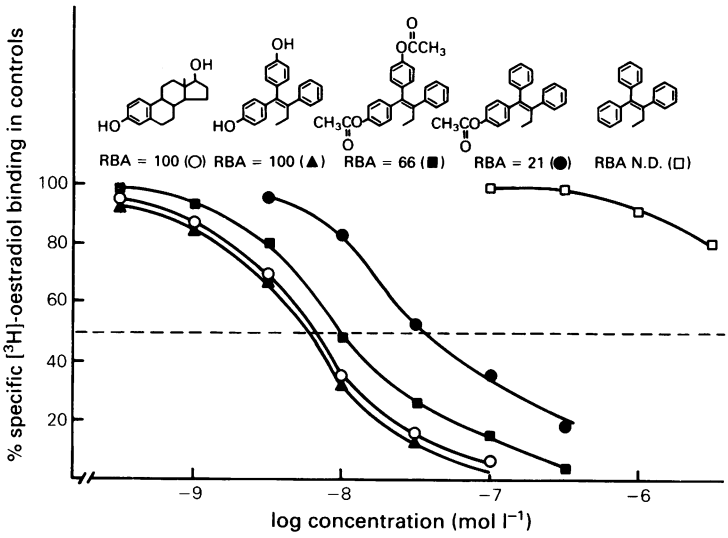


Figure 1 Inhibition of the binding of [^{3}H]oestradiol-17β (5×10^{-9} mol l^{-1}) to oestrogen receptors derived from immature rat uterine cytosol by substituted derivatives of 1,1,2-triphenylbut-1-ene. Incubations were undertaken at 4°C for 18 h. Specifically bound radioactivity is plotted as a percentage of the specifically bound radioactivity in control tubes, i.e., those with no competitive ligands. RBA = relative binding affinity.

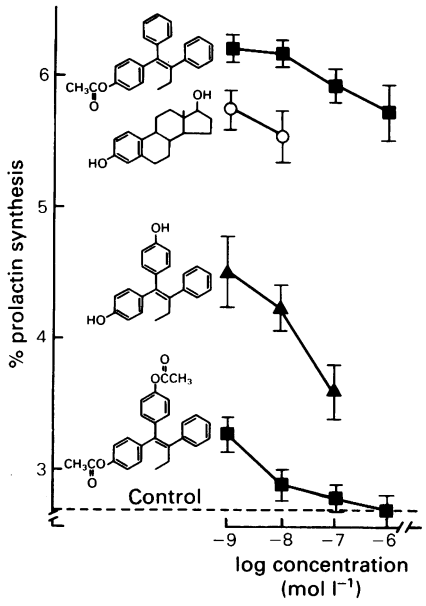


Figure 2 Effect of substituted derivatives of 1,1,2-triphenylbut-1-ene and oestradiol-17β on prolactin synthesis *in vitro*. Pituitary cells (2×10^5 per dish) were cultured for 6 days in medium containing the indicated concentrations of compounds. Control incubates contained medium alone. Values are means, with vertical lines showing s.e., for three cultures per point.

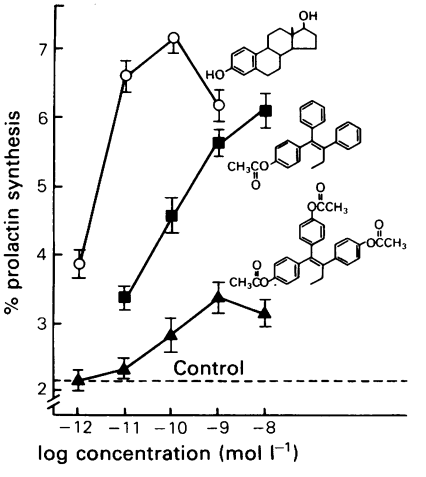


Figure 3 Effect of substituted derivatives of 1,1,2-triphenylbut-1-ene and oestradiol-17β on prolactin synthesis *in vitro*. The experiment was conducted as described in the legend of Figure 2.

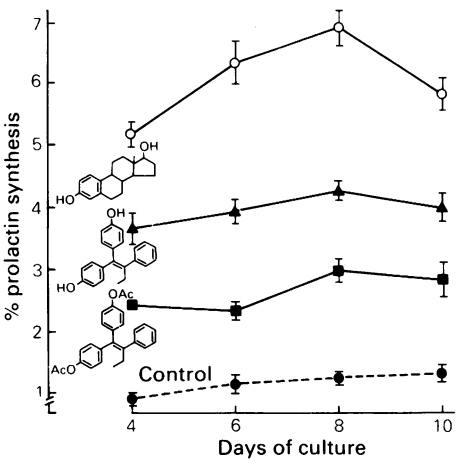


Figure 4 The effect of oestradiol-17β and derivatives of 1,1,2-triphenylbut-1-ene (1 nmol l^{-1}) on prolactin synthesis *in vitro*. Pituitary cells (2×10^5 per dish) were cultured for the indicated number of days. Control incubates contained medium alone. Values are means, with vertical lines showing s.e., for three cultures per point.

acetoxy compounds at a single concentration of 1 nmol l^{-1} (Figure 4). Each compound produced a consistent and reproducible induction of prolactin synthesis at 4, 6, 8 and 10 days. There was no evidence that the assays conducted at 6 days did not reflect the true efficacy of the respective partial agonists.

To ensure that *bis* 1,1-*para*-acetoxy-phenyl-2-phenylbut-1-ene was occupying the receptor and capable of antioestrogenic actions, a concentration-response assay was conducted with and without 1 nmol l^{-1} oestradiol. *Bis* 1,1-*para*-acetoxy-phenyl-2-phenylbut-1-ene was antioestrogenic (Figure 5) in a concentration-related manner. However, this effect was competitive and reversible as increasing concentrations of oestradiol reversed the antioestrogenic effect of 1 μmol l^{-1} *bis* 1,1 *para*-acetoxy-phenyl-2-phenyl-but-1-ene (Figure 6).

Discussion

The aim of the study was to describe the structure-activity relationships in a series of substituted derivatives of 1,1,2-triphenylbut-1-ene. These compounds were selected because the oestrogen triphenylbutene forms the nucleus of the antioestrogen tamoxifen. Several of the compounds have previously been screened for antitumor activity against human breast cancers transplanted into athymic mice (Schneider *et al.*, 1982). However, precise studies of structure-activity relationships are complex *in vivo* because of

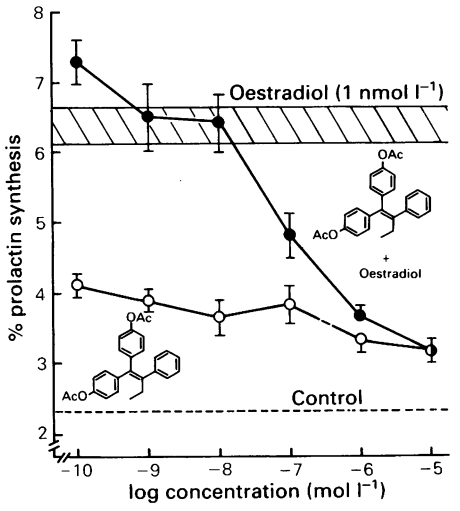


Figure 5 The inhibitory action of *bis, para*-acetoxyphenyl-2-but-l-ene on oestradiol-17β-induced prolactin synthesis *in vitro*. Pituitary cells (2×10^5 per dish) were cultured for 6 days in medium containing the indicated concentration of test compounds with, or without oestradiol-17β (1 nmol l^{-1}). Control incubates contained medium alone. Values are means, with vertical lines indicating s.e., for three cultures per point.

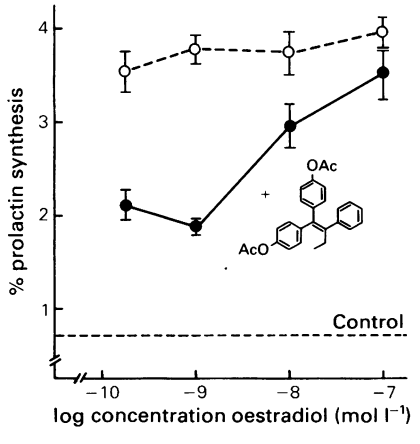


Figure 6 Competitive reversal of the inhibitory action of *bis,para*-acetoxy-phenyl-2-phenyl-but-l-ene on oestradiol-17β induced prolactin synthesis *in vitro*. Pituitary cells (2×10^5 per dish) were cultured for 6 days in media containing the indicated concentrations of oestradiol-17β with or without *bis,para*-acetoxyphenyl-2-phenyl-but-l-ene (1 μmol l^{-1}). Control incubates contained media alone. Values are means, with vertical lines indicating s.e., for three cultures per point.

metabolism. In fact, the correlation between the antitumor actions of the compounds and their activity in the prolactin synthesis assay is not good. This might be expected as the control mechanism for the regulation of breast cancer growth by high doses of oestrogens or antioestrogens is unknown. Indeed, both oestrogens and antioestrogens can inhibit breast cancer growth *in vivo* therefore it is an inappropriate endpoint to study the structural features necessary to modulate oestrogen regulated gene products, i.e. oestrogen action. Furthermore, the acetyl derivatives would be expected to be rapidly hydrolyzed *in vivo*. Although this is a possibility *in vitro* the contrasting pharmacology of *bis* 1,1. *para*-hydroxyphenyl-2-phenyl-but-l-ene and *bis* 1,1-*para*-acetoxy-phenyl-2-phenyl-but-l-ene argues against the suggestion.

The current experimental results support and extend the general receptor binding model previously described (Jordan, 1984). It is clear that *para* substitution with OH or acetoxy in the phenyl ring equivalent to the A ring of oestradiol is necessary for a high potency. Furthermore, potency was related to the RBA of the ligand for the receptor.

The single site receptor binding model proposed (Jordan, 1984) for the development of the intrinsic activity required for oestrogen action, depends upon a tertiary change in the structure of the receptor protein to fold around the binding ligand. In contrast an antioestrogen wedges into the ligand binding site to prevent the tertiary changes in the protein, and, as a result the complex has low intrinsic activity. Bisphenolic compounds produce partial agonist action (Jordan *et al.*, 1984; Jordan & Lieberman, 1984) and we have previously suggested that an adaptation of the macromolecule perturbation theory of Belleau (1964) might be used to explain the molecular events that occur at the ligand binding site of the oestrogen receptor. It was intriguing to find that acetylation of the bisphenolic compound decreases intrinsic oestrogenic activity. It is, therefore, tempting to speculate that acetylation results in an increase in steric hindrance. On the other hand, it is possible that the interaction of oestrogens and anti-oestrogens with the oestrogen receptor occurs through a two site allosteric model. Hahnel and coworkers (1973) proposed that the antioestrogen tamoxifen binds at an allosteric site to prevent the binding of oestradiol to the receptor. During the past decade, however, there has been little evidence offered to support an allosteric model. This in part is because the structure-activity relationships of the stilbenes and oestrogenic triphenylethylenes can be explained by a single receptor site model (Jordan *et al.*, 1985) and studies with [^{3}H]-oestradiol have assumed a single competitive binding site for oestrogens. The potency of the antioestrogen tamoxifen is increased by hydroxylation to 4-hydroxytamoxifen (Jordan *et al.*, 1977) whose binding affinity for the oestrogen recep-

tor is equivalent to that of oestradiol. This observation provides compelling support for a single ligand binding site. Nevertheless, the recent observation (Tate *et al.*, 1984) that polyclonal antibodies raised to the oestrogen receptor differentially reduce the binding of [^{3}H]-oestradiol to the unoccupied oestrogen receptor, whereas the binding of [^{3}H]-4-hydroxytamoxifen to the receptor is less affected, raises some interesting speculation. It was argued that these data may indicate an allosteric model. However, a single site model has been proposed based upon hypothetical differences in the conformation of the receptor during activation (Tate *et al.*, 1984). Only further investigation will resolve this issue.

This work was supported by United States Public Health Service Grant CA 32713.

References

BELLEAU, B. (1964). A molecular theory of drug action based on induced conformational perturbations of receptors. *J. med. Chem.*, **7**, 776–784.

DODDS, E.C., GOLBERG, L., LAWSON, W. & ROBINSON, R. (1983a). Oestrogenic activity of certain synthetic compounds. *Nature*, **141**, 247–248.

DODDS, E.C., LAWSON, W. & NOBLE, R.L. (1938b). Biological effects of the synthetic oestrogenic substance 4:4′-dihydroxy-α:β-diethylstilbene. *Lancet*, **i**, 1389–1391.

EMMENS (1947). Halogen-substituted oestrogens relaed to triphenylethylene. *J. Endocrinol.*, **5**, 170–173.

FURR, B.J.A. & JORDAN, V.C. (1984). The pharmacology and clinical uses of tamoxifen. *Pharmac. Ther.*, **25**, 127–205.

HAHNEL, R., TWADDLE, E. & RATAJCZAK, T. (1973). The influence of synthetic antioestrogens on the binding of tritiated oestradiol-17β by cytosols of human uterus and human breast carcinoma. *J. Steroid Biochem.*, **4**, 687–695.

HARPER, M.J.K. & WALPOLE, A.L. (1967). A new derivative of triphenyl-ethylene: effect on implantation and mode of action in rats. *J. Reprod. Fertil.*, **13**, 101–119.

HOLTKAMP, D.E., GRESLIN, S.C., ROOT, C.A. & LERNER, L.J. (1960). Gonadotropin inhibiting and antifecundity effects of chloramiphene. *Proc. Soc. exp. biol. Med.*, **105**, 197–201.

HORWITZ, K.B., COSTLOW, M.E. & McGUIRE, W.L. (1976). MCF-7: A human breast cancer cell line with estrogen, androgen, progesterone, and glucocorticoid receptors. *Steroids*, **26**, 785–795.

HUPPERT, L.C. (1979). Induction of ovulation with clomiphene citrate. *Fertil. Steril.*, **31**, 1–8.

JORDAN, V.C. (1984). Biochemical pharmacology of antiestrogen action. *Pharmac. Rev.*, **36**, 245–276.

JORDAN, V.C., COLLINS, M.M., ROWSBY, L. & PRESTWICH, G. (1977). A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J. Endocrinol.*, **75**, 305–316.

JORDAN, V.C. & LIEBERMAN, M.E. (1984). Estrogen-stimulated prolactin synthesis *in vitro*. *Molec. Pharmac.*, **26**, 279–285.

JORDAN, V.C., LIEBERMAN, M.E., CORMIER, E., KOCH, R., BAGLEY, J.R. & RUENITZ, P.C. (1984). Structural requirements for the pharmacological activity of nonsteroidal antiestrogens *in vitro*. *Molec. Pharmac.*, **26**, 272–278.

quirements for the pharmacological activity of nonsteroidal antiestrogens *in vitro*. *Molec. Pharmac.*, **26**, 272–278.

JORDAN, V.C., MITTAL, S., GOSDEN, B., KOCH, R. & LIEBERMAN, M.E. (1985). Structure-activity relationships of estrogens. *Environ. Health Perspect.*, (in press).

LERNER, L.J., HOLTHAUS, J.F., & THOMPSON, C.R. (1958). A non-steroidal estrogen antagonist 1-(p-2-diethylaminoethoxyphenyl) -1-phenyl-2-p-methoxyphenylethanol. *Endocrinology*, **63**, 295–318.

LIEBERMAN, M.E., GORSKI, J. & JORDAN, V.C. (1983a). An estrogen receptor model to describe the regulation of prolactin synthesis by antiestrogens *in vitro*. *J. biol. Chem.*, **258**, 4741–4745.

LIEBERMAN, M.E., JORDAN, V.C., FRITSCH, M., SANTOS, M.A. & GORSKI, J. (1983b). Direct and reversible inhibition of estradiol-stimulated prolactin synthesis by antiestrogen *in vitro*. *J. biol. Chem.*, **258**, 4734–4740.

LIEBERMAN, M.E., MAURER, R.A. & GORSKI, J. (1978). Estrogen control of prolactin synthesis *in vitro*. *Proc. natn. Acad. Sci. U.S.A.*, **75**, 5946–5949.

LIEBERMAN, M.E., MAURER, R.A., CLAUDE, P. & GORSKI, J. (1982). Prolactin synthesis in primary cultures of pituitary cells: regulation by estradiol. *Mol. Cell. Endocrinol.*, **25**, 277–294.

MAURER, R.A., STONE, R., & GORSKI, J. (1976). Cell-free synthesis of a large translation product of prolactin messenger RNA. *J. biol. Chem.*, **251**, 2801–2807.

SCHNEIDER, M.R., VON ANGERER, E., SCHOENENBERGER, H., MICHEL, R.TH. & FORTMEYER, H.P. (1982). 1,1,2-Triphenylbut-1-enes: relationship between structure, estradiol receptor affinity and mammary tumor inhibiting properties. *J. med. Chem.*, **25**, 1070–1077.

TATE, A.C., GREENE, G.L., DESOMBRE, E.R., JENSEN, E.V. & JORDAN, V.C. (1984). Differences between estrogen- and antiestrogen-estrogen receptor complexes from human breast tumors identified with an antibody raised against the estrogen receptor. *Cancer Res.*, **44**, 1012–1018.

VALE, W., GRANT, G., AMOSS, M., BLACKWELL, R. & GUILLEMIN, R. (1972). Culture of enzymatically dispersed antierior pituitary cells: Functional validation of a method. *Endocrinology*, **91**, 562–572.

(*Received June 12, 1985.*
Revised August 5, 1985.
Accepted August 17, 1985.)