Br. J. Pharmac. (1979), **65**, 167–173

THE BINDING OF [^{3}H]-OESTRADIOL-17β IN THE IMMATURE RAT UTERUS DURING THE SEQUENTIAL ADMINISTRATION OF NON-STEROIDAL ANTI-OESTROGENS

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1 The binding of [^{3}H]-oestradiol-17β (0.08 μg) in the uterus, vagina, liver and heart of immature female rats has been studied *in vivo* and the effect of daily administrations of the non-steroidal anti-oestrogens, tamoxifen and monohydroxytamoxifen, on the 2 h accumulation of [^{3}H]-oestradiol-17β in the uterus has been determined.

2 Doses of tamoxifen (8 μg daily) or monohydroxytamoxifen (1.28 μg daily), which have previously been found to antagonize completely the uterotrophic activity of oestradiol-17β (0.08 μg daily), did not significantly reduce the total uterine binding of 0.08 μg [^{3}H]-oestradiol-17β administered on day 4 of a 3-day uterine weight test.

3 The simultaneous administration of tamoxifen (8 μg) or monohydroxytamoxifen (1.28 μg) with [^{3}H]-oestradiol-17β (0.08 μg) on day 3 of a uterine weight test did not significantly reduce the total uterine binding of oestradiol-17β. The binding of [^{3}H]-oestradiol-17β was reduced if monohydroxytamoxifen or tamoxifen was administered 4 h before the oestradiol.

4 Tamoxifen (8 μg daily) or monohydroxytamoxifen (1.28 μg daily) did not prevent the translocation of [^{3}H]-oestradiol (0.08 μg) to the uterine cell nucleus on day 3 of a 3-day uterine weight test.

5 The measurement of total nuclear oestrogen receptors by an exchange assay technique demonstrated a higher concentration of oestrogen receptors in anti-oestrogen-treated animals compared with controls.

6 Since the administration of anti-oestrogenic doses of non-steroidal anti-oestrogens during a 3-day uterine weight test did not inhibit the total binding of oestradiol in the uterus, or affect the translocation of the steroid to the nucleus, the mechanism of action of non-steroidal anti-oestrogens over the range of the partial agonist dose-response curve must involve an interaction, or competition of oestradiol-17β- and anti-oestrogen-oestrogen receptor complexes for sites within the nucleus.

Introduction

Oestradiol-17β is believed to stimulate uterine growth by binding to proteins (oestrogen receptors) located in the cell cytoplasm. The steroid-receptor complex which results is transformed (the molecular form of the protein changes from 4S to 5S) and is then translocated to the nucleus (Jensen & DeSombre, 1973) where increased RNA polymerase activity is initiated (Hamilton, 1968). As a result, uterine RNA and protein levels increase (Aizawa & Mueller, 1961) and the depleted cytoplasmic oestrogen receptors are replenished by resynthesis (Sarff & Gorski, 1971). Cell division occurs 24 to 48 h after the initial stimulus.

Non-steroidal anti-oestrogens have been shown to compete with oestradiol-17β for the oestrogen receptor *in vitro* (Korenman, 1970; Skidmore, Walpole & Woodburn, 1972) and exchange assay techniques which can determine the concentration of ligand-filled oestrogen receptors (Anderson, Clark & Peck, 1972), have been used to demonstrate that anti-oestrogens can translocate oestrogen receptors from the cytoplasm to the nucleus (Clark, Anderson & Peck, 1973; Katzenellenbogen & Ferguson, 1975; Capony & Rochefort, 1975; Jordan, Dix, Rowsby & Prestwich, 1977b). The administration of a single large dose of non-steroidal anti-oestrogen to immature rats produces a prolonged depletion of the cytoplasmic oestrogen receptor pool, an effect which was originally interpreted as a specific inhibition of the oestrogen

0007-1188/79/020167-07 $01.00

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receptor resynthesis process which ultimately renders the tissue refractory to future oestrogenic stimuli (Clark, Peck & Anderson, 1974).

In earlier papers we have shown that small, yet anti-oestrogenic doses of the non-steroidal anti-oestrogen, tamoxifen, do not inhibit the replenishment of cytoplasmic oestrogen receptors or in fact completely deplete the cytoplasmic oestrogen receptor pool (Jordan, *et al.*, 1977b). The depletion of cytoplasmic oestrogen receptors appears to be directly related to the administered dose of the anti-oestrogen (Jordan *et al.*, 1977b; Jordan, Rowsby, Dix & Prestwich, 1978). The aim of the present experiments was to extend our observation that uterine oestrogen receptors are available for oestradiol binding *in vivo* during the administration of non-steroidal anti-oestrogens in an anti-oestrogenic immature rat uterine weight test.

Methods

The anti-oestrogens used were tamoxifen (ICI 46,474; *trans* 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene) and monohydroxytamoxifen (1-(4-β-dimethylaminoethoxyphenyl)-1-(4-hydroxyphenyl)-2-phenylbut-1-ene). [6,7-^{3}H]-oestradiol-17β (sp. act. 41 Ci/*m*mol) was obtained 98% pure dissolved in benzene/ethanol (9:1 v/v) from New England Nuclear Corp. and was used without further purification.

All subcutaneous injections were made in 0.1 ml arachis oil. Oily solutions of non-steroidal anti-oestrogens were prepared by taking aliquots from freshly prepared stock ethanolic solutions, adding the required volume of arachis oil and evaporating the ethanol, under a stream of N_2, on a warm (60°C) water bath. Oily solutions of [6,7-^{3}H]-oestradiol-17β were prepared by evaporating aliquots to dryness, adding a few drops of absolute ethanol and, after adding the required volume of arachis oil, evaporating the ethanol as described above.

Immature female rats (35 to 50 g) of the Alderley Park strain were used throughout.

Distribution of [6,7-^{3}*H*]-*oestradiol*-17β

Rats were randomized into groups of six. Each animal received a single dose of 0.08 μg [6,7-^{3}H]-oestradiol-17β which was injected subcutaneously into the dorsal skin at the back of the neck. Animals were killed by stunning and cervical dislocation at 0.5, 1, 2, 4 and 8 h after injection. Uteri, vaginae, a piece of liver and the apex of the heart were dissected out, blotted to remove blood before weighing on a torsion balance. Tissues were dried overnight in a 40°C oven and reweighed. Samples were wrapped in 1 inch squares of paper tissue and individually burnt in a Packard Tri-Carb Tissue Oxidiser. The resulting tritiated water was collected into 10 ml tritium scintillator (6 g butyl PBD [2(4′-1-butylphenyl)-5-4″biphenylyl-1,3,4-oxadiazole], 100 g naphthalene, 45 ml methanol, 135 ml toluene, 720 ml dioxan) and counted for 5 min in a Beckman LS 330 Liquid Scintillation Spectrometer. Counting efficiency was determined by an automatic external standard.

Effects of tamoxifen and monohydroxytamoxifen on the uterine uptake of [6,7-^{3}*H*]-*oestradiol*-17β in vivo

Rats were randomized into groups, each of six animals. Rats received daily subcutaneous injections of tamoxifen (2, 8 or 32 μg) or monohydroxytamoxifen (0.32, 1.28 or 5.12 μg) on three consecutive days. Control animals were injected with arachis oil (0.1 ml). On day 4, 24 h after the last injection, each animal received a single injection of 0.08 μg [6,7-^{3}H]-oestradiol-17β. Two hours later animals were killed, uteri were dissected out and processed as described above.

To determine whether non-steroidal anti-oestrogens inhibit the binding of oestradiol on day 3 of the uterine weight test, rats (6 rats/group) were injected with tamoxifen (8 μg) or monohydroxytamoxifen (1.28 μg) on two consecutive days and then on day 3 tamoxifen or monohydroxytamoxifen was injected and 0.08 μg [6,7-^{3}H]-oestradiol was injected simultaneously or 4 h later. Control rats received daily injections of arachis oil (0.1 ml) before [^{3}H]-oestradiol and another group received injections of anti-oestrogens on two consecutive days and was not injected with anti-oestrogen when the [^{3}H]-oestradiol-17β was injected. Uteri were dissected out 2 h after the administration of [^{3}H]-oestradiol and processed as described before.

The results were expressed as fmol [^{3}H]-oestradiol/uterus or fmol [^{3}H]-oestradiol per mg wet weight and dry weight of tissue. Statistical comparisons between group means were made by Student's *t* test.

Determination of oestradiol binding in uterine nuclei

Thirty rats were randomized into three groups each of ten. Groups received injections of tamoxifen (8 μg) or monohydroxytamoxifen (1.28 μg) on two consecutive days. The control group received injections of arachis oil (0.1 ml). On day 3, each rat was injected with 0.08 μg [6,7-^{3}H]-oestradiol-17β and killed 2 h later. The uteri were dissected out and 2 uteri were pooled from groups and homogenized in STED buffer (sucrose 0.25 mol/l, Tris 0.01 mol/l, disodium edetate (EDTA) 0.0015 mol/l and dithiothreitol 0.0005 mol/l, pH 7.4) with an Ultraturrax tissue homogenizer cooled in ice/water. The homogenates were centrifuged at 2300 *g* for 30 min (4°C) in an MSE Superspeed 18. Supernatants were discarded and the crude

nuclear pellets were each extracted with 1 ml absolute ethanol for 10 min at 22°C. Samples were centrifuged at 2000 *g* for 20 min (6°C) and supernatants were added to 10 ml tritium scintillator and counted as before.

In a separate experiment three groups of ten rats were injected with tamoxifen (8 μg), monohydroxytamoxifen (1.28 μg) or arachis oil (0.1 ml) on two consecutive days. On day 3 all animals received an injection of 0.08 μg non-radioactive oestradiol-17*β* and then were killed 2 h later. Pooled uteri (2) were homogenized as described above and the homogenates were centrifuged at 100 *g* for 10 min (4°C). The supernatants were stored and the crude pellet was resuspended in 1.5 ml STED buffer and the procedure was repeated twice more. The pellet was stored for DNA determination according to the methods described by Burton (1956). The pooled supernatants were centrifuged at 800 *g* for 10 min (4°C) and the pellet nuclei were then resuspended in 1.6 ml STED buffer and 800 μl was used for the determination of nuclear occupied oestrogen receptors by [^{3}H]-oestradiol exchange. Our procedure to determine the concentration of oestrogen receptors in the nucleus was an adaptation of the method described by Anderson *et al.* (1972) and has been published in detail elsewhere (Jordan *et al.*, 1977b). The total uterine DNA values were calculated by adding together the crude pellet DNA value and the total DNA value for the nuclear suspension. The results of the [^{3}H]-oestradiol exchange on an aliquot of the nuclear suspension were normalised to whole uterine values and the results were represented as fmol [^{3}H]-oestradiol binding per uterus.

Results

Distribution of [6,7-^{3}*H*]-*oestradiol*-17*β*

The administration of 0.08 μg [6,7-^{3}H]-oestradiol to immature female rats resulted in the rapid accumulation of radioactivity in the uterus and vagina (oestrogen target tissues) with only low levels of radioactivity accumulating in liver and heart (non-target tissues). The levels of radioactivity in uteri and vaginae remained high during an 8 h period after injection whereas the levels in liver and heart muscle were comparably small at 8 h.

Uterine radioactivity was maximal at 2 h and this time interval was used for the determination of uterine [^{3}H]-oestradiol levels in all subsequent experiments.

Effect of tamoxifen and monohydroxytamoxifen on the uterine uptake of [6,7-^{3}*H*]-*oestradiol*-17*β* in vivo

Tamoxifen and monohydroxytamoxifen both produced a significant increase in immature rat uterine wet weight (Table 1). A significant rise in uterine wet and dry weights occurred at all three dose levels, although there were no significant differences between

Table 1 Effect of (a) tamoxifen and (b) monohydroxytamoxifen on the binding of [^{3}H]-oestradiol

Daily dose of compound (μg)	*Uterine wet wt. (mg)*	*Uterine dry wt. (mg)*	[^{3}*H*]-*oestradiol binding (fmol/mg wet wt. tissue)*	[^{3}*H*]-*oestradiol binding (fmol/mg dry wt. tissue)*	[^{3}*H*]-*oestradiol binding (fmol/uterus)*
(a) Tamoxifen					
Control	40.1 ± 5.74	7.61 ± 0.83	4.45 ± 0.35	23.2 ± 2.43	184.97 ± 41.35
2	60.2 ± 4.86*	10.66 ± 0.91*	4.21 ± 0.38	23.7 ± 1.93	257.32 ± 42.20
8	65.1 ± 3.10**	12.55 ± 0.76**	2.95 ± 0.31***	15.4 ± 1.69*	189.57 ± 16.11
32	71.3 ± 2.53***	13.80 ± 0.73***	1.42 ± 0.18***	7.48 ± 1.01**	100.53 ± 11.41
(b) Monohydroxytamoxifen					
Control	47.63 ± 3.91	8.03 ± 0.60	5.00 ± 0.48	29.79 ± 3.32	241.83 ± 34.91
0.32	66.83 ± 2.74**	12.48 ± 0.68***	4.15 ± 0.34	22.31 ± 1.96	275.17 ± 18.80
1.28	71.03 ± 3.58**	13.45 ± 0.63***	2.37 ± 0.58***	12.58 ± 0.53***	170.46 ± 14.14
5.12	64.32 ± 1.65**	12.46 ± 0.44***	1.62 ± 0.18***	8.37 ± 0.96***	104.27 ± 12.11**

Mean uterine wet and dry weights of rats treated with different daily doses of tamoxifen or monohydroxytamoxifen for 3 days. On day 4 animals were injected with 0.08 μg [6,7-^{3}H]-oestradiol-17*β* and killed 2 h later. Levels of radioactivity in the uteri were determined as fmol [^{3}H]-oestradiol binding per mg wet or dry weight of uterus and fmol [^{3}H]-oestradiol per uterus. All compounds were administered subcutaneously in 0.1 ml arachis oil. Results represent mean ± s.e. mean (6 rats per group).

Statistically significant change from control determined by Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the anti-oestrogen treated groups. The uterine binding of [6,7-^{3}H]-oestradiol-17β of animals treated with tamoxifen or monohydroxytamoxifen was expressed in three ways: fmol [^{3}H]-oestradiol per mg dry or wet weight and fmol [^{3}H]-oestradiol per uterus (Table 1). The dose-related inhibition of [^{3}H]-oestradiol binding by the anti-oestrogens was similar whether the results were expressed as fmol [^{3}H]-oestradiol per mg dry or wet weight of uterus. In the animals treated with monohydroxytamoxifen, only the group treated with 5.12 μg daily produced a significant ($P < 0.01$) decrease in [^{3}H]-oestradiol binding compared with controls if the results were expressed as fmol [^{3}H]-oestradiol per uterus. Similarly the highest dose of tamoxifen (32 μg daily) produced a significant decrease ($P < 0.01$) in oestradiol binding compared with the low dose (2 μg daily) tamoxifen-treated group but because of the spread of results in the control group none of the tamoxifen-treated groups was statistically different from the untreated value. The total uterine binding of [^{3}H]-oestradiol in groups treated with either 2 μg or 8 μg tamoxifen daily was not significantly different.

The simultaneous administration of either monohydroxytamoxifen or tamoxifen with [^{3}H]-oestradiol to antioestrogen pretreated animals did not inhibit the total uterine binding of [^{3}H]-oestradiol. No statistical difference was observed between the total uterine binding of [^{3}H]-oestradiol in control, anti-oestrogen pretreated or antioestrogen pretreated and simultaneous anti-oestrogen injected groups (Figure 1). The administration of anti-oestrogens 4 h before the [^{3}H]-oestradiol resulted in a significant decrease in binding (tamoxifen, $P < 0.01$; monohydroxytamoxifen, $P < 0.001$) compared with the related anti-oestrogen pretreated control.

Determination of oestradiol binding in uterine nuclei

The administration of tamoxifen (8 μg) or monohydroxytamoxifen (1.28 μg) for 2 days did not significantly alter the accumulation of [^{3}H]-oestradiol in rat uterine nuclei. Control, tamoxifen- and monohydroxytamoxifen-treated groups contained 263 ± 23.5, 267 ± 27.5 and 248 ± 5.3 fmol [^{3}H]-oestradiol (±s.e. mean) per uterus respectively. In contrast with these findings, groups previously treated with tamoxifen (8 μg) or monohydroxytamoxifen (1.28 μg) had significantly (both, $P < 0.001$) higher levels of exchangeable nuclear oestrogen receptors than controls (Figure 2).

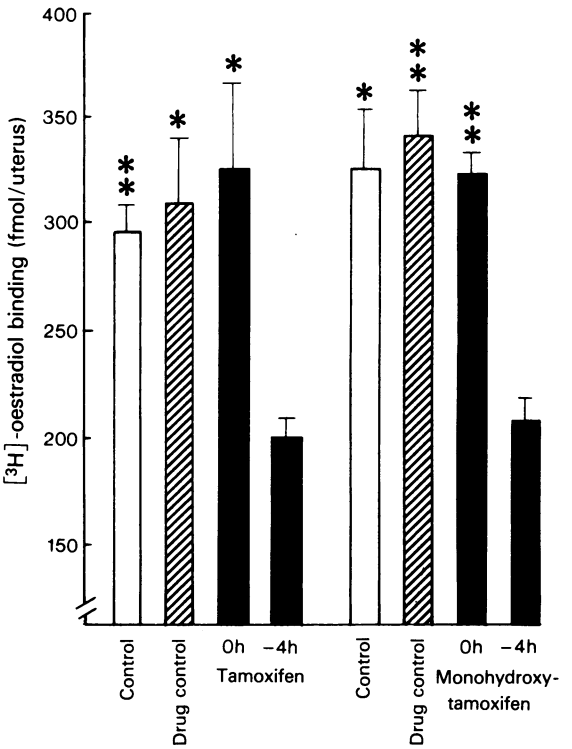

Figure 1 The effect of the administration of tamoxifen (8 μg, s.c.) or monohydroxytamoxifen (1.28 μg, s.c.) simultaneously with or 4 h before the administration of [6, 7-^{3}H]-oestradiol-17β (0.08 μg, s.c.) on the total uterine levels of radioactivity determined 2 h later. Animals were treated with tamoxifen on the two previous days and control animals received arachis oil. The results are mean values ($n = 6$); vertical lines indicate s.e. mean. Comparison of −4h antioestrogen treated groups with other values by Student's t-test; * $P < 0.01$; ** $P < 0.001$.

Discussion

Large doses of non-steroidal anti-oestrogens produce a prolonged depletion of the cytoplasmic oestrogen receptor pool with a prolonged elevation of nuclear oestrogen receptor concentrations (Clark *et al.*, 1973; Katzenellenbogen & Ferguson 1975; Ruh & Baudendistel, 1977). Originally it was suggested (Clark *et al.*, 1974) that non-steroidal anti-oestrogens inhibited the resynthesis of cytoplasmic oestrogen receptors but now several studies have demonstrated that anti-oestrogens can replenish the cytoplasmic oestrogen receptor pool (Capony & Rochefort, 1975; Jordan *et al.*, 1977b; Koseki, Zava, Chamness & McGuire, 1977a). It is therefore more likely that the prolonged depletion of the cytoplasmic oestrogen receptor pool is the result of a continuing translocation of resynthesized receptor to the nucleus by binding ligands in the blood. This view is consistent with

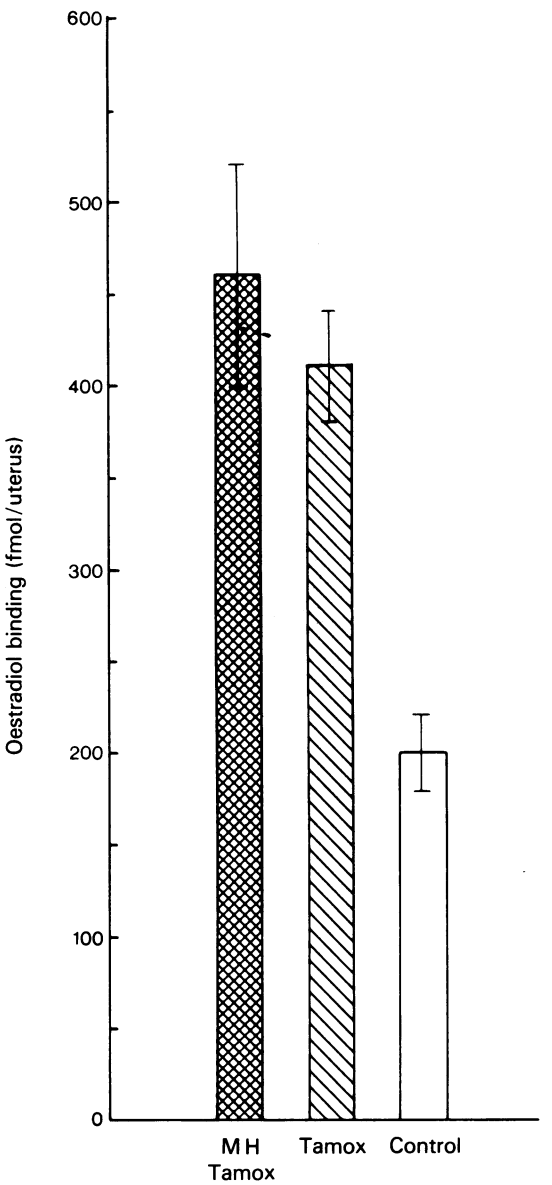

Figure 2 The effect of tamoxifen (Tamox, 8 μg) monohydroxytamoxifen (MHTamox, 1.28 μg) on the levels of exchangeable oestrogen receptors in immature rat uterine nuclei. Animals (10 rats per group) were treated with tamoxifen or monohydroxytamoxifen on two consecutive days. Control (10 rats) received arachis oil. On day 3, all animals received an injection of oestradiol-17β (0.08 μg, s.c.) and 2 h later animals were killed. For further details see Methods. Results represent mean values ($n = 5$); vertical lines show s.e. mean. Treatments vs control, $P < 0.001$ by Student's t test.

the known long biological half life of non-steroidal anti-oestrogens (Fromson, Pearson & Bramah, 1973; Katzenellenbogen, Katzenellenbogen, Ferguson & Kranthammer, 1978). Furthermore, since long acting steroidal (Katzenellenbogen, Ferguson & Lan, 1977) and non-steroidal oestrogens (Ruh & Baudendistel, 1977) can deplete the cytoplasmic oestrogen receptor pool for a prolonged period, the loss of cytoplasmic oestrogen receptors is unlikely to be the primary mechanism of action of non-steroidal anti-oestrogens.

Rather than rely upon supramaximal doses of non-steroidal anti-oestrogens to study anti-oestrogenic mechanisms it was our aim to use small, yet effectively anti-oestrogenic doses of tamoxifen and monohydroxytamoxifen in order to study the uterine binding of oestradiol-17β in the 3 day immature rat uterine weight test. The results demonstrate that the total uterine binding of [^{3}H]-oestradiol-17β is unimpaired in animals that had been treated on three consecutive days with monohydroxytamoxifen (1.28 μg) or tamoxifen (8 μg). The doses have previously been shown to produce complete antagonism of the uterotrophic effects of oestradiol-17β (0.08 μg daily) (Jordan, Collins, Rowsby & Prestwich 1977a). However, as the dose of the anti-oestrogen was increased the binding of [^{3}H]-oestradiol-17β was reduced. This is consistent with earlier observations that the depletion of the cytoplasmic oestrogen receptor pool (determined *in vitro*) in the immature rat is directly related to the administered dose of an anti-oestrogen (Jordan *et al.* 1977b; Jordan *et al.*, 1978). Although oestrogen receptors are clearly available to bind [^{3}H]-oestradiol at the end of a uterine weight test it was possible that the simultaneous administration of oestradiol and an anti-oestrogen during the test may result in the anti-oestrogen reaching the target tissue first and occupying all the cytoplasmic oestrogen receptors. Oestrogen antagonism would then result from a denial of oestradiol binding. The results show that the simultaneous administration of non-steroidal anti-oestrogens and oestradiol does not prevent the initial binding of oestradiol in the uterus or inhibit the translocation of oestrogen receptors to the nucleus. Therefore, oestradiol can bind within the uterus during an immature rat uterine weight test, but the oestradiol cannot stimulate a full uterotrophic response. This result is consistent with a previous report (Katzenellenbogen & Ferguson, 1975) that oestradiol can translocate oestrogen receptors to the uterine cell nucleus after the administration of non-steroidal anti-oestrogens but the nuclear oestradiol-oestrogen receptor complexes are biologically inactive. It is possible therefore that the elevated levels of nuclear anti-oestrogen-oestrogen receptor complex observed in the present and previous studies (Clark *et al.* 1973; Capony & Rochefort, 1975; Katzenellenbogen & Ferguson, 1975; Jordan, *et al.* 1977b; Koseki *et al.*, 1977a) may inhibit the

oestradiol-oestrogen receptor complex from interacting at an as yet unidentified nuclear acceptor site. Oestrogen antagonism would therefore occur at a nuclear site.

Tamoxifen and monohydroxytamoxifen probably both increase uterine wet and dry weight by translocating oestrogen receptors to the nucleus. Unlike oestrogens though, non-steroidal anti-oestrogens can only stimulate a small increase in uterine DNA content (Jordan, *et al.*, 1977b; Clark, Dix, Jordan, Prestwich & Sexton 1978; Dix & Jordan, 1978). This is consistent with the observation that the non-steroidal anti-oestrogens CI628 (Kang, Anderson & DeSombre, 1975), tamoxifen (Clark *et al.*, 1978) and monohydroxytamoxifen (Dix & Jordan, 1978) can stimulate luminal epithelial hypertrophy with virtually no increase in endometrial mitoses. Although cell division does not increase, anti-oestrogen-stimulated increases in uterine proteins have been reported e.g. progesterone receptors (Koseki, Zava, Chamness & McGuire 1977b; Jordan & Prestwich, 1977; 1978) and ornithine decarboxylase (Bulger & Kupfer, 1977). The increase in protein synthesis without an increase in cellularity is probably the reason why the total uterine binding of [^{3}H]-oestradiol after tamoxifen (8 μg daily × 3) or monohydroxytamoxifen (1.28 μg daily × 3) is not different from the untreated controls whilst the binding of steroid expressed per unit weight is reduced. The result implies that if oestrogen receptors are resynthesized to the cell requirements, then only a proportion are available to bind [^{3}H]-oestradiol since others are being occupied by anti-oestrogen and translocated to the nucleus. The proportion of available cytoplasmic oestrogen receptors is reduced in relation to other cytoplasmic proteins. Similarly in the ovariectomized rat, the daily administration of a dose of tamoxifen (50 μg) known to be antioestrogenic (Jordan & Koerner, 1976) and inhibit the growth of hormone-dependent dimethylbenzanthracene-induced rat mammary tumours (Jordan & Jaspan, 1976), does not reduce the total concentration of cytoplasmic oestrogen receptors when expressed per mg uterine DNA (Koseki *et al.*, 1977a). However, the effect of tamoxifen on the uterus is dose-related since larger doses will deplete the uterine cytoplasmic oestrogen receptor pool (Jordan, 1976b; Kurl & Morris, 1978).

Although we have shown oestradiol can bind in the uterus during a pharmacological test for antioestrogenic activity it is clearly an advantage to deny oestrogen binding by the chronic administration of anti-oestrogens (Roy, Mahesh & Greenblatt, 1964; Perry, DiPasquale, Ferguson, Pozzi & Rassaert, 1973; Jordan, 1976a; Jordan & Dowse, 1976). However, the reason why the anti-oestrogen-oestrogen receptor complexes within the nucleus are unable to provoke cell division is as yet unexplained. Future studies of the properties of the oestradiol-oestrogen receptor complex and the anti-oestrogen-oestrogen receptor complex *in vivo* and *in vitro* may provide evidence to explain the differences in their biological activities.

We would like to thank Dr Dora Richardson for the gift of tamoxifen and monohydroxytamoxifen and ICI Ltd., (Pharmaceuticals Division) for the grants to support this study.

References

AIZAWA, Y. & MUELLER, G.C. (1961). The effects *in vivo* and *in vitro* of estrogens on lipid synthesis in the rat uterus. *J. biol. Chem.*, **236**, 381–386.

ANDERSON, J., CLARK, J.H. & PECK, E.J. (1972). Oestrogen and nuclear binding sites. Determination of specific sites by [^{3}H]-oestradiol exchange. *Biochem. J.*, **126**, 561–567.

BULGER, W.H. & KUPFER, D. (1977). Inhibition of the 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichlorethane (*o*, -p DDT) and oestradiol-mediated induction of rat uterine ornithine decarboxylase by prior treatment with *o*, *p* DDT, oestradiol and tamoxifen. *Archs Biochem. Biophys.*, **182**, 138–146.

BURTON, K. (1956). The conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.*, **62**, 315–323.

CAPONY, F. & ROCHEFORT, H. (1975). *In vivo* effect of antiestrogens on the localisation and replenishment of estrogen receptor. *Mol. Cell. Endocr.*, **3**, 233–251.

CLARK, E.R., DIX, C.J., JORDAN, V.C., PRESTWICH, G. & SEXTON, S. (1978). A comparison, at the cellular and subcellular levels, of the effects of tamoxifen and oestradiol benzoate on the immature rat uterus. *Br. J. Pharmac.*, **62**, 442P–443P.

CLARK, J.H., ANDERSON, J.N. & PECK, E.J. (1973). Estrogen receptor anti-estrogen complex-atypical binding by uterine nuclei and effects on uterine growth. *Steroids*, **22**, 707–718.

CLARK, J.H., PECK, E.J. & ANDERSON, J.N. (1974). Oestrogen receptors and antagonism of steroid action. *Nature, Lond.*, **251**, 446–448.

DIX, C.J. & JORDAN, V.C. (1978). Contrasting subcellular responses to monohydroxytamoxifen and oestradiol benzoate in the immature rat uterus. *Br. J. Pharmac.*, **64**, 375–376P.

FROMSON, J.M., PEARSON, S. & BRAMAH, S. (1973). The metabolism of tamoxifen (ICI 46,474) 1. In laboratory animals. *Xenobiotica*, **3**, 693–710.

HAMILTON, T.H. (1968). Control by estrogen of genetic transcriptions and translation. Binding to chromatin and stimulation of nucleolar RNA synthesis are primary events in early estrogen action. *Science, N.Y.*, **161**, 649–661.

JENSEN, E.V. & DESOMBRE, E.R. (1973). Estrogen receptor interaction. *Science, N.Y.*, **182**, 126–134.

JORDAN, V.C. (1976a). Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Eur. J. Cancer*, **12**, 419–424.

JORDAN, V.C. (1976b). Antiestrogenic and antitumor properties of tamoxifen in laboratory animals. *Cancer Treat. Rep.*, **60**, 409–419.

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

JORDAN, V.C., DIX, C.J., ROWSBY, L. & PRESTWICH, G. (1977b). Studies on the mechanism of action of the non-steroidal anti-oestrogen tamoxifen (ICI 46,474) in the immature rat. *Mol. Cell. Endocr.*, **7**, 177–192.

JORDAN, V.C. & DOWSE, L.J. (1976). Tamoxifen as an anti-tumour agent: effect on oestrogen binding. *J. Endocr.*, **68**, 297–303.

JORDAN, V.C. & JASPAN, T. (1976). Tamoxifen as an anti-tumour agent: oestrogen binding as a predictive test for tumour response. *J. Endocr.*, **68**, 453–460.

JORDAN, V.C. & KOERNER, S. (1976). Tamoxifen as an anti-tumour agent: Role of oestradiol-17β and prolactin. *J. Endocr.*, **68**, 305–311.

JORDAN, V.C. & PRESTWICH, G. (1977). An investigation by sucrose density gradient analysis of the interaction of ligands with steroid hormone receptors pp. 245–262 In *Multiple Molecular Forms of Steroid Hormone Receptors*. ed. Agarwal, M.K. Amsterdam: Elsevier, North Holland.

JORDAN, V.C. & PRESTWICH, G. (1978). Effect of non-steroidal anti-oestrogens on the concentration of rat uterine progesterone receptors. *J. Endocr.*, **76**, 363–364.

JORDAN, V.C., ROWSBY, L., DIX, C.J. & PRESTWICH, G. (1978). Dose related effect of non-steroidal antioestrogens and non-steroidal oestrogens on the measurement of cytoplasmic oestrogen receptors in the rat and mouse uterus. *J. Endocr.*, **78**, 71–81.

KANG, Y.H., ANDERSON, W.A. & DESOMBRE, E.R. (1975). Modulation of uterine morphology and growth by estradiol-17β and an estrogen antagonist. *J. cell. Biol.*, **64**, 682–691.

KATZENELLENBOGEN, B.S. & FERGUSON, E.R. (1975). Anti-estrogen action in the uterus: biological ineffectiveness of nuclear bound estradiol after anti-estrogen. *Endocr.* **97**, 1–12.

KATZENELLENBOGEN, B.S., FERGUSON, E.R. & LAN, N.C. (1977). Fundamental differences in the action of estrogens and antiestrogens on the uterus: comparison between compounds with similar duration of action. *Endocr*, **100**, 1252–1259.

KATZENELLENBOGEN, B.S. KATZENELLENBOGEN, J.A., FERGUSON, E.R. & KRANTHAMMER, N. (1978). Anti-estrogen interaction with uterine estrogen receptors. *J. biol. Chem.*, **253**, 697–707.

KORENMAN, S.G. (1970). Relation between estrogen inhibiting activity and binding to cytosols of rabbit and human uterus. *Endocr.*, **87**, 1119–1123.

KOSEKI, Y., ZAVA, D.T., CHAMNESS, G.C. & MCGUIRE, W.L. (1977a). Estrogen receptor translocation and replenishment by the antiestrogen tamoxifen. *Endocr.*, **101**, 1104–1110.

KOSEKI, Y., ZAVA, D.T., CHAMNESS, G.C. & MCGUIRE, W.L. (1977b). Progesterone interaction with oestrogen and antioestrogen in the rat uterus—receptor effects. *Steroids*, **30**, 169–178.

KURL, R.N. & MORRIS, I.D. (1978). Differential depletion of cytoplasmic high affinity oestrogen receptors after the *in vivo* administration of the antioestrogens, clomiphene, MER 25 and tamoxifen. *Br. J. Pharmac.*, **62**, 487–493.

PERRY, K., DIPASQUALE, G., FERGUSON, E., POZZI, G. & RASSAERT, C.L. (1973). Actions of an estrogen antagonist in intact and ovariectomized rats. *Contraception*, **7**, 231–242.

ROY, S., MAHESH, V.B. & GREENBLATT, R.B. (1964). Effect of clomiphene on the physiology of reproduction in the rat. III. Inhibition of uptake of radioactive estradiol by the uterus and pituitary of immature rat. *Acta. Endocr.*, **47**, 669–675.

RUH, T.S. & BAUDENDISTAL, L.J. (1977). Different nuclear bindings of sites for antiestrogen and estrogen receptor complexes. *Endocr.*, **100**, 420–426.

SARFF, M. & GORSKI, J. (1971). Control of estrogen binding protein concentration under basal conditions and after estrogen administration. *Biochemistry*, **10**, 2557–2563.

SKIDMORE, J.R., WALPOLE, A.L. & WOODBURN, J. (1972). Effect of some triphenylethylenes on oestradiol binding *in vitro* to macromolecules from uterus and anterior-pituitary. *J. Endocr.*, **52**, 289–298.

(Received March 8, 1978.
Revised September 21, 1978.)