High affinity binding of anti-oestrogen to the chick liver nuclear oestrogen receptor

Catherine B. LAZIER* and V. Craig JORDAN†
*Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7, and †Department of Human Oncology, The University of Wisconsin, Madison, WI 53792, U.S.A.

(Received 21 April 1982/ Accepted 10 May 1982)

Tamoxifen is a potent inhibitor of specific oestrogen-induced yolk protein synthesis by chicken liver. The oestriadiol receptor in salt extracts of liver nuclei from oestrogen-treated chicks has a $K_p$ for oestriadiol of $0.7 \pm 0.2\,\text{nm}$. Tamoxifen and its metabolite, monohydroxytamoxifen, compete for binding to the salt-soluble nuclear receptor with $K_i$ values of 2.6 and 0.1 nm respectively. The anti-oestrogens show much less inhibition of $[^3\text{H}]$oestriadiol binding when assays are carried out using intact nuclei. The competition by unlabelled oestriadiol for $[^3\text{H}]$oestriadiol binding to receptor is identical in both salt extracts and intact nuclei. This suggests that intact nuclei contain components which bind anti-oestrogens, but not oestriadiol. While tamoxifen and desmethyltamoxifen will readily dissociate from the salt-soluble nuclear oestrogen receptor, monohydroxytamoxifen does not dissociate under the conditions generally used for exchange assays. A modified assay was developed in which 60–70% of monohydroxytamoxifen-bound sites were shown to be exchangeable for $[^3\text{H}]$oestriadiol. Soluble receptor preparations were first incubated in a 1.7% charcoal suspension at $37^\circ\text{C}$ for 15 min before assay of specific oestriadiol binding. This technique was used in examining the effects of tamoxifen and monohydroxytamoxifen given in vivo on the nuclear oestrogen receptor concentration. Despite their 30-fold difference in binding affinity for the receptor, both anti-oestrogens increase nuclear receptor levels to about the same degree. When given with oestriadiol, both compounds have the same apparent partial inhibitory effect on the oestrogen-induced increase in nuclear receptor. These data are consistent with the metabolic hydroxylation of tamoxifen before binding to the hepatic oestrogen receptor.

Non-steroidal anti-oestrogens can be classified as compounds that inhibit the binding of oestriadiol to the oestrogen receptor and, possibly as a result, prevent the full expression of oestrogenic responses in target tissues. As yet no precise molecular mechanism has been described for anti-oestrogen action although several hypotheses have been suggested. Anti-oestrogens have been observed to inhibit cytoplasmic oestrogen receptor replenishment (Clark et al., 1974), to interfere with processing of nuclear oestrogen receptor (Horwitz & McGuire, 1978), and to produce some qualitative or quantitative defects in receptor activation and translocation (Rochfort & Borgna, 1981; Hayes et al., 1981; Katzenellenbogen et al., 1981). Overall, the structurally specific anti-oestrogen-oestrogen receptor complex appears to have a lower intrinsic activity than the oestriadiol-oestrogen-receptor complex, particularly for stimulating proliferative processes (Jordan et al., 1977a).

Generation of a convincing general theory for the mechanism of anti-oestrogen action is frustrated by the species-specific effects of these compounds. The so-called anti-oestrogens exhibit full oestrogenic properties in short term tests in the mouse uterus (Terenius, 1971), some oestrogenic responses (e.g. progesterone receptor synthesis; Jordan & Prestwich, 1978) and anti-oestrogenic responses (e.g. inhibition of mitosis; Jordan & Dix, 1979) in the rat uterus, and apparently universal anti-oestrogenic responses in the chick oviduct (Sutherland et al., 1977; Sutherland, 1981).

Tamoxifen $([Z]-2-\{p-(1,2\text{-diphenylbutyl}\text{-1-ethylamine})\text{-phenoxyl\text{-NN-dimethylethylamine}}$, an anti-oestrogen used in the treatment of breast cancer (Patterson, 1981), has several metabolites, monohydroxytamoxifen $([Z]-2-\{p-(4\text{-hydroxyphenyl})-2\text{-phenyl-}$

Abbreviation used: Tes, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulphonic acid.
but - 1 - enyl[phenoxy] - NN - dimethylethylamine] (Jordan et al., 1977b) and desmethyltamoxifen (Adam et al., 1979) that are anti-oestrogens in their own right. The metabolites may play an important role in the mechanism of action of tamoxifen because the administration of \(^{3}H\)tamoxifen results in the localization of monohydroxy-\(^{3}H\)tamoxifen in target cell nuclei (Borgna & Rochefort, 1981) and derivatives of tamoxifen that cannot undergo metabolic conversion to monohydroxytamoxifen have attenuated agonistic and antagonistic actions in the rat (Allen et al., 1980).

Tamoxifen, as well as nafoxidine and CI-628, are extraordinarily potent inhibitors of specific oestrogen-induced egg yolk protein synthesis in the chick liver (Gschwendt, 1975; Lazier & Alford, 1977; Capony & Williams, 1980). However, earlier studies have shown that neither nafoxidine nor CI-628 competes to a significant degree with oestriadiol binding to the liver nuclear oestrogen receptor, the presumed effector of oestrogen action (Gschwendt, 1975; Lazier & Alford, 1977). We now find that tamoxifen, and particularly its monohydroxylated derivative, exhibit high affinity binding for this receptor.

Materials and methods

Animals and injections

White leghorn cockerels of about 100g body weight were used for all experiments. Oestradiol-17\(\beta\) and/or anti-oestrogen was dissolved in propylene glycol/ethanol (1:1, v/v) at the concentrations indicated in the text. Injections (0.2ml/100g body weight) were given intramuscularly.

Preparations of liver fractions

Nuclei were purified from liver by centrifugation in glycerol-containing buffers using methods based on those described by Snow et al. (1978). Briefly, minced liver was homogenized at 0\(^\circ\)C in 10vol. of buffer containing 10mm-Tris/HCl (pH 7.5 at 25\(^\circ\)C)/10mm-NaCl/1.5mm-MgCl\(_2\)/50% (v/v) glycerol. After centrifugation at 6000g for 15min the crude nuclear pellet was washed twice with the homogenization buffer supplemented with detergents (0.05% Triton X-100 and 0.1% NP-40). The resulting pellet was washed twice in TGM buffer [10mm-Tris/HCl (pH 7.4 at 25\(^\circ\)C)/1mm-MgCl\(_2\)/25% (v/v) glycerol] and was suspended in TGM buffer at a concentration of about 1.5mg of DNA/ml. For preparation of salt extracts, the nuclei were sedimented from suspension, and a volume of buffer B [0.5m-KCl/1.5mm-EDTA/10mm-Tes (pH 7.4)/10mm-monothioglycerol] equal to the original suspension volume was added, giving a final KCl concentration of 0.4m. After vigorous vortexing, the salt extract was frozen, thawed and centrifuged at 37000g for 20min. The chromatin fraction was prepared from the purified nuclei by washing three times in 10mm-Tris/HCl, pH 7.4, containing 10mm-MgCl\(_2\) and 10mm-monothioglycerol, swelling for 18h in the same solution, followed by sedimentation at 10000g for 10min and one additional wash and final suspension in TGM buffer.

Assay of \(^{3}H\)oestriadiol binding activity

The oestrogen receptor content of intact nuclei and of chromatin was measured by exchange at 30\(^\circ\)C (Snow et al., 1978). Incubation of nuclei or chromatin (100\(\mu\)l) was carried out in a total volume of 0.3ml containing TGM buffer (100\(\mu\)l), TE buffer [0.01m-Tris/HCl (pH7.5)/1.5mm-EDTA/10mm-monothioglycerol] (85\(\mu\)l), \(^{3}H\)oestriadiol in TGM buffer, 10\(\mu\)l (final concn. 8nm), and 5\(\mu\)l of ethanol or diethylstilboestrol (final concn. 800nm) or anti-oestrogen as indicated in the text. After incubation, washing of the nuclei or chromatin pellet was performed with TM buffer [0.01m-Tes (pH 7.5)/1mm-MgCl\(_2\)].

Binding activity of the nuclear salt extracts was determined by a modification of the charcoal adsorption technique reported previously (Lazier, 1978). The extracts were usually stripped of endogenous steroid by preincubation at 37\(^\circ\)C for 15min with an equal volume of 0.5% charcoal/0.05% Dextran T70 in buffer B. After removal of the charcoal by centrifugation the extracts were incubated in a total volume of 0.3ml at 30\(^\circ\)C for 30min with \(^{3}H\)oestriadiol (8nm) in the absence or presence of a 100-fold excess of unlabelled diethylstilboestrol, cooled and charcoal-treated (as above) for 30min at 0\(^\circ\)C. Samples of the supernatant (0.4ml) were counted for radioactivity in Aquasol II (New England Nuclear) at an efficiency of about 45%.

Anti-oestrogens

Because of the photosensitivity of monohydroxytamoxifen, solutions in ethanol were freshly prepared before each experiment. The anti-oestrogens were supplied by ICI, Macclesfield, Cheshire, U.K.

Results and discussion

Binding of tamoxifen and its derivatives to the liver oestrogen receptor in vitro

Fixed concentrations of tamoxifen and monohydroxytamoxifen were incubated with nuclear salt extract and increasing concentrations of \(^{3}H\)oestradiol. The binding data were analysed by the classical Lineweaver–Burk method. Fig. 1 shows that both anti-oestrogens exhibit apparently competitive inhibition of \(^{3}H\)oestradiol binding. The \(K_i\) values for
Binding of anti-oestrogen to nuclear oestrogen receptor

Fig. 1. Lineweaver–Burk plot of anti-oestrogen inhibition of [\(^3\)H]oestradiol binding to the soluble nuclear oestrogen receptor

Salt extract was prepared from liver nuclei of chicks which had been given oestradiol (25 mg/kg) 18 h earlier. Specific binding of increasing concentrations of [\(^3\)H]oestradiol was determined in the absence of anti-oestrogen (○) and in the presence of 25 nM-tamoxifen (▲) or 2.5 nM-monohydroxytamoxifen (△).

Fig. 2. Competition of anti-oestrogens for the oestrogen receptor in intact nuclei and chromatin as compared with salt extracts

Specific binding of 8 nM-[\(^3\)H]oestradiol was determined in the absence or presence of increasing concentrations of anti-oestrogens in: ○, salt extracts; Q, intact nuclei; ▲, chromatin. The individual assays are detailed in the Materials and methods section. Competition was by: (a), desmethyltamoxifen; (b) oestradiol-17β; (c), monohydroxytamoxifen; (d), tamoxifen.

monohydroxytamoxifen binding are 0.10 ± 0.06 nM (s.d., four preparations) and for tamoxifen are 1.5–3.6 nM (two preparations). This indicates an approx. 30-fold difference in the relative affinities of the two anti-oestrogens for the receptor. The \(K_D\) for oestradiol is 0.73 ± 0.21 nM (s.d., for four preparations). Thus the affinity of monohydroxytamoxifen for the oestrogen receptor is several-fold greater than that of oestradiol itself.

Competition of the anti-oestrogens for the oestrogen receptor was also assessed in assays in which a fixed, saturating, concentration of [\(^3\)H]oestradiol was incubated with increasing concentrations of unlabelled anti-oestrogen. In addition, we compared the competitive potential of the anti-oestrogens for receptor in intact purified nuclei and in salt extracts of the nuclei. This was of interest because studies on regulation of the nuclear oestrogen receptor had been carried out in both types of preparation (Snow et al., 1978; Lazier & Haggarty, 1979).

The concentrations of unlabelled oestradiol or anti-oestrogen necessary to give 50% inhibition of [\(^3\)H]oestradiol binding were determined and the relative binding affinities, representing the ratio of the oestrogen concentration divided by the anti-oestrogen concentration \(\times 100\) (Fig. 2, Table 1) were determined. The concentration of unlabelled oestradiol required for 50% inhibition of binding of 8 nM-[\(^3\)H]oestradiol was 7 nM, which is close to the predicted value. Fig. 2(b) shows that this is the case for oestradiol competition both in nuclear salt extracts and in intact nuclei. It will be seen however that the anti-oestrogens display quite different inhibitory activity in salt extracts and in intact nuclei.

In salt extracts, a tamoxifen concentration of 79 nM gives 50% inhibition, and 1.6 nM-monohydroxytamoxifen produces the same effect. This
Table 1. Relative binding affinities of anti-oestrogens and oestradiol for the oestrogen receptor in salt extracts or intact nuclei from chick liver

<table>
<thead>
<tr>
<th>Salt extracts</th>
<th>Temperature</th>
<th>Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Monohydroxytamoxifen</td>
<td>360</td>
<td>440</td>
</tr>
<tr>
<td>Desmethyltamoxifen</td>
<td>ND</td>
<td>28</td>
</tr>
</tbody>
</table>

Relative binding affinity

Table 2. Exchange of anti-oestrogens and oestradiol bound to the soluble nuclear oestrogen receptor: conventional methods

Salt extract from liver nuclei from oestrogen-treated chicks was prepared and charcoal-treated as described in the Materials and methods section. Samples were preincubated with 20 nm-unlabelled oestradiol or anti-oestrogen for 30 min at 30°C. After cooling, the samples were treated with charcoal (0.5% final concn.) for 15 min at 0°C, and the supernatants were assayed for specific oestradiol binding by incubation at the indicated temperature for 30 min with 8 nm-[3H]oestradiol in the absence or presence of 800 nm-diesthylstilboestrol.

<table>
<thead>
<tr>
<th>Specific binding (fmol/100μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preincubation addition</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
</tr>
<tr>
<td>Monohydroxytamoxifen</td>
</tr>
<tr>
<td>Tamoxifen</td>
</tr>
<tr>
<td>Desmethyltamoxifen</td>
</tr>
<tr>
<td>Ethanol (vehicle)</td>
</tr>
</tbody>
</table>

Exchange characteristics of the anti-oestrogens

Considering the extremely high affinity of monohydroxytamoxifen for the salt-soluble nuclear oestrogen receptor, it was imperative to demonstrate that the conditions of an exchange assay used to assess nuclear oestrogen receptor concentration after treatment in vivo with anti-oestrogens actually were sufficient to permit dissociation of the ligand and subsequent binding of [3H]oestradiol. Furthermore, the apparent very high non-receptor binding of the anti-oestrogen in the isolated nuclei suggests that a reservoir of such ligand may be present in nuclei of anti-oestrogen-treated animals. Such ligand could dissociate during an exchange assay with [3H]oestradiol, and could inhibit binding of the labelled ligand to receptor sites and give a false impression of diminished receptor concentration. Earlier studies on the effect of anti-oestrogen treatment in chick liver or oviduct did not demonstrate that the exchange assay was thus adequate (Lazier & Alford, 1977; Binart et al., 1979; Lazier et al., 1981; Lebeau et al., 1981).

In order to investigate exchange conditions, charcoal-treated salt extracts from liver nuclei of oestrogen-treated chickens were incubated with sufficient unlabelled anti-oestrogen or oestradiol to saturate the oestrogen receptor. The samples were treated with varying concentrations of charcoal suspension at different temperatures in order to remove unbound and possibly some, or all, of the bound ligand. The efficacy of the charcoal step was judged by comparison of [3H]oestradiol binding of the presaturated charcoal-treated extracts with that...
of the appropriate controls. Table 2 shows that the conventional charcoal treatment (0.5% charcoal for 15 min at 0°C) (Sutherland & Baulieu, 1976; Lazier & Haggarty, 1979) permits quantitative detection of oestrogen receptor sites previously bound with oestradiol, tamoxifen or desmethyltamoxifen. However, sites previously bound with monohydroxytamoxifen are not detectable, possibly due to ineffective removal of all the non-receptor-bound ligand and as well to a very low dissociation rate for monohydroxytamoxifen from the oestrogen receptor. Increasing the concentration to 1.7% and the temperature of the charcoal to 37°C permits assay of the majority of sites previously bound to monohydroxytamoxifen (Table 3, A and B). The recovery is 60–70%, and is not improved by further increases in charcoal concentration, incubation temperature or time. Limited preincubation at 37°C has a stimulating effect on subsequent [3H]oestradiol binding (Table 3, B); however, prolonged preincubation at 37°C in the presence of high charcoal concentrations has a deleterious effect on the receptor. Other methods of promoting the exchange of tightly bound ligands such as the use of chaotropic agents (Sica et al., 1981), were not found to be useful in this system.

The effect of anti-oestrogens in vivo on nuclear oestrogen receptor

Earlier reports showed that nafoxidine, CI-628 or tamoxifen treatment of chicks resulted in an increase in the concentration of nuclear oestrogen receptor which was quantitatively considerably less than, and temporally delayed, compared with that evoked by a similar dose of oestradiol (Gschwendt, 1975; Lazier & Alford, 1977; Lazier et al., 1981). In the case of tamoxifen, it is clear that this compound has a sufficient rate of dissociation from the oestradiol receptor to permit assay under the usual exchange conditions. However, if tamoxifen is metabolized to the monohydroxylated form in vivo and the latter compound is actually that which is bound to the oestrogen receptor, then the usual assay conditions would not be sufficient for quantitative assay. We now find that a relatively brief (4 h) exposure to tamoxifen or to monohydroxytamoxifen does give rise to a 4–6-fold increase in the nuclear oestrogen receptor concentration, as assayed in salt extracts by the modified charcoal treatment procedure (Table 4). No such increase was found by using the nuclear exchange assay. The experiments in vitro suggest that the exchange assays with intact nuclei are not valid reflections of the actual nuclear receptor concentrations because of high non-receptor binding of anti-oestrogen. Furthermore, it is unlikely that monohydroxytamoxifen bound to the receptor would dissociate under the conditions used. The measurements of receptor concentration made by the modified charcoal treatment technique with the salt extracts more likely give a truer picture of actual receptor levels. These figures are still limited by the 60–70% efficiency of the exchange reaction for monohydroxytamoxifen. Thus, the values given for nuclear receptor concentration in monohydroxytamoxifen-treated chicks and in the tamoxi-
Table 4. Effect of injection in vivo of anti-oestrogens on the apparent concentration of nuclear oestrogen receptor in salt extracts and in intact nuclei

Oestradiol or anti-oestrogen was administered intramuscularly at the dose indicated. After 4 h liver nuclei and salt extracts were prepared and assayed for specific [3H]oestradiol binding activity. The salt extracts were charcoal-treated before assay by incubation with 1.7% charcoal suspension for 15 min at 37°C. The data represent the mean ± s.d. for duplicate determinations on at least three preparations per group.

<table>
<thead>
<tr>
<th>Treatment (4 h)</th>
<th>Salt extracts</th>
<th>Intact nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol (3 mg/kg)</td>
<td>0.46 ± 0.07</td>
<td>0.40 ± 0.12</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0.05 ± 0.04</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Tamoxifen (2 mg/kg)</td>
<td>0.22 ± 0.04</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Tamoxifen (6 mg/kg)</td>
<td>0.27 ± 0.04</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Monohydroxytamoxifen (2 mg/kg)</td>
<td>0.20 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Monohydroxytamoxifen (6 mg/kg)</td>
<td>0.24 ± 0.02</td>
<td>0.07 ± 0.02</td>
</tr>
</tbody>
</table>

Fig. 3. Time course of the effect of tamoxifen and monohydroxytamoxifen treatment in vivo on the soluble nuclear oestrogen receptor

Chicks were injected with oestradiol (Δ, 3 mg/kg) or with tamoxifen (●) or monohydroxytamoxifen (○) (each 6 mg/kg). Salt extracts of liver nuclei were prepared at various times after injection and [3H]oestradiol binding activity was measured in extracts which had been pretreated with 1.7% charcoal suspension at 37°C for 15 min. Results are the means ± s.d. for duplicate determinations on preparations from each of three animals.

Fig. 4. Effects of tamoxifen and monohydroxytamoxifen given with oestradiol in vivo on the apparent concentration of nuclear oestrogen receptor

Tamoxifen (open symbols) or monohydroxytamoxifen (filled symbols) were given simultaneously with oestradiol (3 mg/kg) at the molar dose ratio indicated on the abscissa. After 4 h, liver nuclei and nuclear salt extracts were prepared. [3H]Oestradiol binding activity in intact nuclei was assayed by exchange at 30°C and the salt extracts were pretreated with 1.7% charcoal suspension for 15 min at 37°C prior to assay at 30°C. ●● Salt extracts; Δ, ● intact nuclei. Results given are the means ± s.d. for duplicate determinations of preparations from each of three animals.

Monohydroxytamoxifen-treated chicks could be 30–40% low depending on the degree of metabolic hydroxylation.

The time course of the anti-oestrogen-induced increase in the nuclear oestrogen receptor concentration is shown in Fig. 3. Unlike in earlier experiments where higher doses and conventional exchange techniques were used (Lazier et al., 1981), we now find that tamoxifen injection produces a relatively rapid and sustained effect. Similar kinetics are found for monohydroxytamoxifen, while oestradiol injection results in a peak in nuclear receptor concentration at 4 h followed by gradual decline.
These results most likely reflect differential clearance rates for oestradiol and anti-oestrogens in the chicken (Binart et al., 1979).

Fig. 4 shows the effect of different doses of the two anti-oestrogens given with oestradiol for 4h in vivo on the accumulation of nuclear oestrogen receptor. Both the nuclear exchange and the modified salt extract assays were used. Tamoxifen and monohydroxytamoxifen produce a similar degree of apparent inhibition. This is especially pronounced when binding is assessed by the assay with intact nuclei. Although the results may be artifactual in terms of true inhibition of nuclear receptor accumulation, they suggest that the metabolic transformation of tamoxifen to the higher affinity ligand is taking place.

For a mixed dose of anti-oestrogen and oestradiol the amounts of nuclear receptor-specific binding of anti-oestrogen and oestradiol would be proportional to their relative affinities for the receptor and to their intrahepatic concentrations. Thus, for a molar dose ratio of 1.5:1 (anti-oestrogen:oestrogen) it is possible that the majority of the intranuclear oestrogen receptor sites would be bound to monohydroxytamoxifen. If, as pointed out earlier, some 30-40% of these sites are not detectable in the salt extract assay, then it is obvious that there is little true inhibition of accumulation of nuclear receptor. Furthermore, the estimate that monohydroxytamoxifen constitutes the majority of nuclear-bound ligand at a molar dose ratio of 1.5:1 is consistent with observations on the striking anti-oestrogenic capacity of tamoxifen. Under these conditions, over 70% inhibition of oestradiol-induced synthesis of the apoprotein B of very low density lipoprotein is found (Capony & Williams, 1980).

Very high affinity binding of monohydroxytamoxifen relative to tamoxifen has been reported for the oestrogen receptor from chick oviduct (Binart et al., 1979) and from rat and calf uterine tissues (Jordan et al., 1977b; Borgna & Rochefort, 1980; Rochefort et al., 1979). There is some controversy as to whether or not metabolism is an obligatory step in the anti-oestrogenic activity of tamoxifen. According to Binart et al. (1979) little monohydroxytamoxifen is found in chick oviduct after tamoxifen injection and no conversion occurs in the tissue in vitro, suggesting that hydroxylation is not a prerequisite. On the other hand, Borgna & Rochefort (1981) report that both oviduct and liver readily hydroxylate tamoxifen and conclude that hydroxylation is likely to be an important feature in its action. Derivatives of tamoxifen which cannot undergo hydroxylation have been tested for uterotrophic effects in the rat (Allen et al., 1980). Although greatly attenuated, the derivatives retain some antagonistic and agonistic potential, demonstrating that, in this case, metabolic hydroxylation is an advantage, but not an absolute prerequisite, for anti-oestrogen action. A similar situation appears to hold in the case of the human breast tumour cell line, MCF-7 (Horwitz et al., 1978).

The very high affinity of monohydroxytamoxifen for the liver nuclear oestrogen receptor makes it an interesting and potentially useful compound for further study. Our results underline the necessity for thorough investigation of exchange assay conditions for anti-oestrogens and their metabolites in order to assess their effects on nuclear receptor accumulation. With one possible exception (Hayes et al., 1981), it appears that reports of defective oestrogen receptor translocation by anti-oestrogens could be reinterpreted in terms of high affinity binding of the administered compound or a metabolite interfering with the nuclear exchange assay (Gschwendt, 1975; Lazier & Alford, 1977; Lazier et al., 1981; Binart et al., 1979; Lebeau et al., 1981).

Technical assistance by Shirley Sangster is gratefully acknowledged, as is the participation of Paul Fraser and Terry Ackles in early stages of this work. Grant support to C. B. L. was from the Medical Research Council of Canada and the National Cancer Institute of Canada. The anti-oestrogens were gifts to V. C. J. from ICI Ltd.

References


Vol. 206

1982