contrast, flutamide, although suppressing somewhat the formation of the labelled 3S complex, was not as effective as compound Sch 16423, suggesting that the activity of flutamide in vivo may be partially due to its conversion to compound Sch 16423.

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**Biochemistry of Tamoxifen Therapy in Breast Cancer**

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Studies on the mechanism of action of the anti-oestrogen tamoxifen (1-p-β-dimethylaminoethoxyphenyl-trans-1,2-diphenylbut-1-ene), also known clinically as Nolvadex and originally as ICT 46474, have shown that the compound ultimately causes regression of hormone-dependent human (Cole et al., 1971) and rat (Nicholson & Golder, 1975; Jordan & Dowse, 1976) mammary tumours, despite producing preliminary effects in the rat similar to those produced by oestradiol-17β (Nicholson et al., 1976, 1977a). Regressions occurred principally in those tumours containing oestrogen-receptor proteins (Manni et al., 1976; Jordan, 1975; Nicholson et al., 1978). It is supposed, therefore, that tamoxifen acts primarily through the oestrogen-receptor system, although the precise sequence of events that leads to the expression of its anti-oestrogenic activity has not been fully defined. Compounds that, however, interfere with the ability of the tissue to bind oestradiol-17β, process its message, or bind to the receptor and induce a new message, may result in cellular atrophy or cell death.

**Interaction of tamoxifen with oestrogen-receptor proteins**

A consideration of the structures of tamoxifen and oestradiol-17β does not reveal many similarities. Nevertheless several groups of workers have demonstrated that tamoxifen can interfere with the high-affinity binding of oestradiol-17β to its specific cytoplasmic receptor protein in both human (Jordan & Koerner, 1975; Powell-Jones et al., 1975) and rat mammary tumours (Nicholson et al., 1977a). The anti-oestrogen apparently competes with oestradiol-17β for its specific 8S binding protein (Nicholson et al., 1978). Tamoxifen associates with approximately the same number of binding sites as does oestradiol-17β, but binds with about 5% of its affinity (Nicholson et al., 1978a,b; Capony & Rochefort, 1978). The compounds also show a similar specificity of binding (Nicholson et al., 1978b; Capony & Rochefort, 1978). These data suggest a common binding site for oestradiol-17β and tamoxifen.

**Translocation of the tamoxifen-receptor complex and early transcriptional events**

It is clear from the above data that tamoxifen can interact and compete with oestradiol-17β for its specific cytoplasmic binding protein, thereby reducing both the amount, or possibly the quality of oestradiol-17β–receptor complex formed and subsequently
translocated to the nucleus. Terenius (1970) suggested that this might form the molecular basis for anti-oestrogenicity. More recent studies have, however, indicated that, although the initial association of the anti-oestrogen with the oestrogen receptor is of prime importance, it forms only part of a complex sequence of events by which tamoxifen affects tumour growth (Nicholson et al., 1977b). It can be demonstrated that tamoxifen, like oestradiol-17β, can instigate a dose-dependent translocation of the oestrogen receptor to dimethylbenz[a]anthracene-induced tumour nuclei and exists there as a 4–5S form. This results in an initial depletion of both total and accessible cytoplasmic oestradiol-17β-binding sites (Nicholson et al., 1976). The transfer of ligand-bound receptor into nuclei is paralleled by a rapid increase in activity of RNA polymerase B (Nicholson et al., 1977b).

It appears, therefore, that tamoxifen and oestradiol bound to the cytoplasmic oestradiol-17β-binding protein enter the nucleus and associate with chromatin acceptor sites, thus affecting the rates of transcription of the DNA. Tamoxifen is able to elicit further oestrogen-like actions that result in elevated concentrations of cytoplasmic oestradiol-17β- (Nicholson et al., 1976) and progesterone-binding sites. It would seem reasonable to assume that the secondary effects observed in the cytoplasm are a direct consequence of an interaction of the tamoxifen–receptor complex with chromatin, resulting in the production of mRNA species coding for specific proteins, including the receptors for oestradiol-17β and progesterone. These events are in keeping with the known weak oestrogenic properties of tamoxifen (Harper & Walpole, 1967).

Late effects of tamoxifen

Tamoxifen, unlike oestradiol-17β, is unable to sustain cytoplasmic oestrogen-receptor concentrations, which decrease appreciably in tumour tissue on continued tamoxifen therapy (Nicholson & Golder, 1975), The reasons for this are not clear. It may represent a complete failure of the tumour to synthesize oestrogen-receptor proteins, resulting from the eventual blockage of some specific gene function by the anti-oestrogen–receptor complex. Alternatively, the complex may decrease the efficiency of transcription of that gene, thereby initiating only limited receptor synthesis. High and maintained plasma concentrations of tamoxifen in the animal (Gaskell et al., 1978) makes any differentiation of these alternatives difficult, since newly synthesized receptor would be sequestered by tamoxifen and subsequently transferred to the nucleus, thus maintaining the concentration of tamoxifen–receptor complexes at key sites on chromatin and depleting the cytoplasm of oestrogen receptors. Limited receptor synthesis may also explain the eventual recovery of tissues exposed to anti-oestrogens, since a fall in the plasma and tissue concentrations of the anti-oestrogens would be commensurate with the binding of endogenous oestrogens to newly synthesized receptors (Katzenellenbogen & Ferguson, 1975).

The apparent failure to replenish oestrogen-binding sites has also been suggested to be responsible for the antagonistic actions of anti-oestrogens (Clark et al., 1974) by breaking the sequence of events by which oestrogens act. Experiments performed at the time of low oestrogen-receptor concentrations support this concept. It can be shown that under these conditions dimethylbenz[a]anthracene-induced mammary tumour nuclei have a reduced ability to take up [3H]oestradiol-17β, specifically into a 4–5S binding form. Furthermore, these tumours have a decreased capacity to synthesize oestrogen receptors after oestradiol-17β administration (Nicholson et al., 1977a), an essential step in the growth processes of oestrogen target tissues. In addition, Powell-Jones et al. (1975) have demonstrated that minces of mammary tumours preincubated with tamoxifen do not form nuclear [3H]oestradiol-17β–receptor complexes as readily as untreated tumours. These data suggest that the tumour is in an oestradiol-17β non-responsive state, a condition that is consistent with the tumour regressions that follow tamoxifen administration.

Decreased cytoplasmic oestrogen-receptor concentrations are not necessarily a prerequisite for tumour regression. It can be shown that the administration of low doses of tamoxifen (10 μg/day), although they initiate breast tumour regressions, do not
substantially reduce cytoplasmic oestrogen-receptor concentrations. These tumours, which contain nuclear anti-oestrogen–receptor complex, also retain their capacity to accumulate nuclear $[\text{H}]$oestradiol-17β-binding proteins. Administration of high doses of oestradiol-17β (1 μg/day) in combination with tamoxifen (10 μg/day) to animals bearing tumours regressing as a result of tamoxifen treatment (10 μg/day) reversed the regression response. It would appear, therefore, that although reduced cytoplasmic concentrations of the oestrogen receptor are not totally necessary for tumour regression, they aid the status quo.

Nuclear binding of the tamoxifen–receptor complex

The nature of the interaction between the tamoxifen–receptor complex and chromatin remains unclear, as does the failure of tamoxifen to promote a full oestrogenic response. The possibility exists that the rapid dissociation rate of tamoxifen from the receptor (Nicholson et al., 1978b; Capony & Rochefort, 1978) may influence these properties. This, however, seems unlikely, since, while tamoxifen is oestrogenic in the mouse and anti-oestrogenic in the rat (Harper & Walpole, 1967; Terenius, 1971) it shows identical dissociation rates from uterine oestrogen-receptor preparations (R. I. Nicholson, unpublished work). Also the cis isomer of tamoxifen, ICI 47699, binds to the receptor with a much lower affinity than tamoxifen, yet is oestrogenic in the rat (Harper & Walpole, 1967). Alternatively, the binding of tamoxifen to the receptor may cause conformational changes in the receptor different from those produced by oestradiol-17β, thus affecting nuclear binding of the receptor and promoting inefficient transcriptional events. The relative merit of the latter possibility awaits the purification of the receptor.

Significance of tamoxifen metabolism to the therapy of breast cancer

The existence of metabolites of tamoxifen was first demonstrated by Fromson and his colleagues in man and also in laboratory animals (Fromson et al., 1973a,b). The principal metabolite was identified in each instance as being a monohydroxylated derivative of tamoxifen, metabolite B (1-p-/3-dimethylaminoethoxyphenyl-trans-1-p-hydroxyphenyl-2-phenylbut-1-ene). This compound, possessing potent anti-oestrogenic activity in the rat (Jordan et al., 1978), can be detected in plasma of tamoxifen-treated animals 4 days after the initiation of treatment, although its concentration at this time is reduced in comparison with that of tamoxifen. Interestingly, metabolite B, like tamoxifen, can displace oestradiol-17β from its specific cytoplasmic 8S binding component in both human (Jordan et al., 1978) and rat mammary tumour tissue. Competitive binding studies indicate that metabolite B has a much higher affinity for the rat mammary tumour oestrogen receptor than has tamoxifen (Nicholson et al., 1978b). An approximate 50% inhibition of $[^\text{H}]$oestradiol-17β (5 nmol/litre) binding was achieved by 5 nmol of oestradiol-17β/litre, 50 nmol of metabolite B/litre and 300 nmol of tamoxifen/litre, The possibility therefore exists that metabolite B, although present in plasma at a lower concentration than tamoxifen, may effectively sequester available oestrogen receptor and play a supportive, or possibly primary, role in the therapy of breast cancer. Indeed Jordan et al. (1978) indicated that tamoxifen, at a dose that was inactive via the subcutaneous route, was active as an anti-oestrogen via the oral route, implying a metabolic conversion had taken place to activate the drug. In addition, studies in vitro indicate that the metabolite B receptor, once formed, is inherently more stable than the tamoxifen–receptor complex (Nicholson et al., 1978b). Thus, if the absolute concentration of the nuclear anti-oestrogen–receptor complex is the determinant feature of an anti-oestrogenic response, the relative stabilities of the receptor complexes may partially explain the quantitative differences in potencies between tamoxifen and metabolite B.


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Some Clinical Aspects of Anti-Hormonal Therapy

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