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METABOLISM OF TAMOXIFEN AND ITS UTEROTROPHIC ACTIVITY*†

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Abstract—Tamoxifen is an estrogen agonist in mouse uterus, a partial estrogen agonist/antagonist in rat uterus, and a pure estrogen antagonist in chicken oviduct. Tamoxifen metabolism was examined both *in vitro* and *in vivo* to determine if differences in the species response to this drug resulted from the differential formation of metabolites with estrogenic or antiestrogenic activity. Animals were given a subcutaneous injection of [^{3}H]tamoxifen, and 4 or 24 hr later tamoxifen and its metabolites were extracted from tissues and separated by TLC. The profiles of metabolites extracted from the livers of these species were qualitatively similar; the principle metabolite was 4-hydroxytamoxifen, which comprised 27, 14, and 16% of the radioactivity from mouse, rat, and chicken livers, respectively, at 24 hr. Tamoxifen, however, was the principal compound extracted from all three livers. Metabolites extracted from mouse and rat uteri were the same ones obtained from liver, although their abundance (relative to tamoxifen) was much lower in uteri than in liver. Metabolite E and bisphenol, two tamoxifen derivatives that we believed might account for the uterotrophic effect of tamoxifen in the mouse, were found not to be formed in either liver or uterus. Tamoxifen metabolism was also studied *in vitro* using liver microsomes from these same species; The same metabolites were formed *in vitro* as *in vivo*, although their relative abundances were lower *in vitro*. No clear-cut differences in metabolism were seen that would account for the disparate pharmacological effects of tamoxifen in these species.

Interest has been focused on determining the molecular mechanism of action of the non-steroidal antiestrogens because these drugs are used clinically in the treatment of hormone-dependent breast cancer [1]. Tamoxifen, a triphenylethylene derivative, is thought to inhibit estrogen action by competing with this hormone for the estrogen receptor [2]. An understanding of the mechanism by which tamoxifen exerts its antiestrogenic effects is complicated by the fact that tamoxifen has different pharmacological properties in different species. Thus, tamoxifen functions as a complete estrogen antagonist in the chicken oviduct [3], as a partial estrogen agonist/antagonist in the rat uterus [4], and as a full estrogen agonist in the mouse uterus [5].

One possible explanation for the disparate action of tamoxifen in these species would be the differential formation in mice, rats, and chickens, of tamoxifen metabolites that have different estrogenic or antiestrogenic properties. Tamoxifen is antiestrogenic in a cell culture system in which no metabolism of this compound occurs [6], suggesting that the antiestrogenic properties of this drug are not dependent on its metabolism. The dimethylaminoethyl portion of the tamoxifen side chain is necessary for its antiestrogenic properties in the rat uterus; removal of this portion of the molecule converts tamoxifen to a compound that is fully estrogenic (i.e. uterotrophic) [7]. This compound has been identified in dog bile [8], where it was designated metabolite E, and tamoxifen has been reported [9] to be estrogenic in this species.

Our working hypothesis was that tamoxifen is an antiestrogenic compound that is converted into an estrogenic derivative, possibly metabolite E, in those species in which it is uterotrophic. Therefore, we compared the metabolism of tamoxifen *in vitro*, using liver microsomes from mice, rats, and chickens, as well as *in vivo* in these same species. We now report that metabolite E was not formed either *in vitro* or *in vivo* in any of these species, and that no significant differences in tamoxifen metabolism were observed that would account for its disparate pharmacological effects in mice, rats, and chickens.

MATERIALS AND METHODS

Materials. *trans*-[ring-^{3}H] Tamoxifen (sp. act. 19.9 Ci/mmole; 97% radiochemically pure) and Z-4-hydroxy-[ring-3,5-^{3}H]tamoxifen (sp. act. 42 Ci/mmole; radiochemically pure) were gifts of ICI, plc, Macclesfield, England, and Amersham, England. The *trans* isomers of tamoxifen, 4-hydroxytamoxifen, metabolite X (*N*-desmethyltamoxifen), metabolite E, and metabolite Y were also gifts of ICI, plc. The bisphenol derivative of tamoxifen was a gift from Dr. Martin Schneider, University of Regensburg, Federal Republic of Germany. The 4-chloro derivative of tamoxifen was a gift from Dr. E. R. Clark, Department of Pharmacology, University of Leeds,

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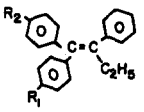

COMPOUND	R_1	R_2
TAMOXIFEN	H	$O-CH_2-CH_2-N(CH_3)_2$
N-DESMETHYLTAMOXIFEN (METABOLITE X)	H	$O-CH_2-CH_2-NHCH_3$
TAMOXIFEN N-OXIDE	H	$O-CH_2-CH_2-N^+(O^-)(CH_3)_2$
METABOLITE Y	H	$O-CH_2-CH_2-OH$
METABOLITE E	H	OH
4-HYDROXYTAMOXIFEN (METABOLITE B)	OH	$O-CH_2-CH_2-N(CH_3)_2$
4-CHLOROTAMOXIFEN	Cl	$O-CH_2-CH_2-N(CH_3)_2$
BISPHENOL	OH	OH

Fig. 1. Structures of compounds that are discussed in the text.

England. Tamoxifen-*N*-oxide was synthesized from tamoxifen free base by the method of Foster *et al.* [10]. The structures of all of these compounds are given in Fig. 1. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase (type XII), NADP, and glucuronidase type H-1 (with associated sulfatase activity) were purchased from the Sigma Chemical Co., St. Louis, MO. Whatman LK5DF thin-layer chromatography plates were purchased from the ANSPEC Co., Ann Arbor, MI.

Animals. Female mice (outbred ICR strain) and rats (outbred Sprague–Dawley strain) were purchased from Sasco/King Animal Laboratories, Inc., Oregon, WI. Chickens (white Leghorn) were purchased from the Poultry Science Department, University of Wisconsin. All animals were given food and water *ad lib.*

Uterotrophic activity tests. Adult female ICR mice (10 weeks old) were ovariectomized under ether anesthesia and used 8 days later. Test compounds were dissolved in peanut oil as previously described [7]; all injections were given subcutaneously in a volume of 0.1 ml. Mice were injected with the test compounds for 3 days; on day 4 the mice were killed, their uteri were removed, intraluminal fluid was pressed out of the tissue, and the uteri were weighed.

Preparation of microsomes. Animals were killed by cervical dislocation (mice, rats) or decapitation (chickens). Livers were removed, weighed, and homogenized in 3 vol. of 0.15 M KCl. The homogenate was centrifuged at 10,000 *g* for 20 min at 4°, and the resulting post-mitochondrial supernatant fraction was then centrifuged for 1 hr at 100,000 *g* (4°). The cytosol was decanted and the microsomal pellet was rinsed twice and then resuspended in 0.25 M sucrose to a concentration equivalent to 2 g wet weight liver/ml sucrose. Microsomes were either assayed immediately for their capacity to metabolize [^{3}H]tamoxifen or were frozen at −70° for later assaying.

Metabolism of [^{3}H]*tamoxifen* in vitro. Our method was based on the assay of Robertson *et al.* [11]. The 250-μl reaction mixture contained [^{3}H]tamoxifen (in 2.5 μl ethanol), microsomes (in 100 μl 0.25 M sucrose), buffer, and an NADPH-generating system; initial assay concentrations were: 0.16 μM [^{3}H]-tamoxifen (1.8×10^6 dpm), 80 mM $NaPO_4$ buffer, pH 6.9 (37°), 10 mM $MgCl_2$, 1 mM nicotinamide, 10 mM glucose-6-phosphate, 0.3 mM NADP, and 0.5 units glucose-6-phosphate dehydrogenase. The reaction was started by adding the [^{3}H]tamoxifen to the test tube, which was then incubated in a 37° water bath with shaking. The reaction was ended after 1 hr by the addition of 1 ml ethanol, the contents of the tube were mixed, and the precipitate was removed by centrifugation. Greater than 96% of the radioactivity was found in the supernatant fraction, an aliquot of which was analyzed by thin-layer chromatography (TLC) as described below.

Metabolism of [^{3}H]*tamoxifen and* [^{3}H]4-*hydroxytamoxifen* in vivo. Solutions of [^{3}H]tamoxifen and [^{3}H]4-hydroxytamoxifen for injection were prepared in peanut oil as previously described [7]. Mice and rats were given subcutaneous injections behind their necks, chickens under their wings. Animals were killed at appropriate time points, and their livers and uteri (or oviduct) were removed, weighed, and homogenized in either 3 vol. (livers) or 10 vol. (uteri) of 0.15 M KCl. An aliquot of the homogenate was mixed with 4 vol. of ethanol, and the precipitate was removed by centrifugation. The ethanolic supernatant fraction, which contained greater than 95% of the radioactivity, was placed in a second test tube; the ethanol was removed under a nitrogen stream, and the residual H_2O was removed by lyophilization. A small volume of ethanol was then added to the tube, and the radioactivity was resolubilized by placing the tube in a sonicator bath for 5 min. Nonsoluble materials were removed by centrifugation, and an aliquot of the supernatant fraction was analyzed by TLC as described below.

Analysis of tamoxifen metabolites in bile was also performed. Bile was removed with a syringe from mouse or chicken gall bladders, mixed with 4 vol. of ethanol, and, after centrifugation to remove the precipitate, an aliquot of the supernatant fraction was analyzed by TLC. In some experiments, an aliquot of the supernatant fraction was placed in a second test tube, ethanol and H_2O were removed in turn by evaporation and lyophilization, and the lyophilizate was resuspended in 0.5 ml of 0.1 M sodium citrate buffer, pH 5.0 (37°), containing 0.1% sodium azide. Sigma type H-1 glucuronidase/sulfatase (5 mg) was added to the tube, which was then incubated for 40 hr at 37°, with an additional 5 mg of enzyme being added at 15 and at 25 hr. The reaction was ended by adding 2 ml ethanol to the tube, the precipitate was removed by centrifugation, and radioactivity in the supernatant fraction was analyzed by TLC.

Chromatographic analysis of tamoxifen metabolites. Aliquots of radioactivity extracted from *in vitro* microsome incubation mixtures, tissue homogenates, or bile were spotted along with authentic standards of tamoxifen and its metabolites on Whatman LK5DF TLC plates. Solvent systems used for development of the plates and the R_f values of the standards are listed in Table 1. The plates were dried

Table 1. R_f values for tamoxifen and metabolites separated by TLC

Compound	R_f values Solvent system* A	B
Tamoxifen	0.60	0.44
4-Hydroxytamoxifen	0.15	0.18
Metabolite X (*N*-desmethyltamoxifen)	0.27	0.26
Metabolite E	0.35	0.52
Metabolite Y	0.41	0.57
Bisphenol	0.05	0.24
Tamoxife-*N*-oxide	0.00	0.03

* The solvent systems used were: (A) toluene–triethylamine–methanol–piperidine–acetonitrile (90:10:1:1:0.5); (B) chloroform–methanol–conc. ammonium hydroxide (95:5:0.25). Solvent system B was adapted from Ruenitz and Toledo [12].

after development, and the standards were located with u.v. light. The silica gel was then removed from the plates in small sections and the radioactivity in it was quantified using ACS tritium scintillation liquid (Amersham) by liquid scintillation spectrometry (35% counting efficiency). Extraction efficiency of radioactivity from the silica gel was greater than 98%.

RESULTS

Our first experiment was to determine what effect *para*-hydroxylation or chlorination had on the uterotrophic activity of tamoxifen in the mouse. We found that 4-hydroxytamoxifen was a more potent uterotrophic agent than tamoxifen, which in turn was more potent than 4-chlorotamoxifen (Fig. 2). The same rank order of uterotrophic potencies of these compounds was observed in the rat by Allen *et al.* [13], although in that species all three compounds were only weakly uterotrophic. These same workers also showed that 4-hydroxytamoxifen has a much higher affinity for the estrogen receptor *in vitro* than either tamoxifen or 4-chlorotamoxifen, which have similar affinities. In addition, Fromson *et al.* [8] have shown that tamoxifen is metabolized in the mouse and rat to form 4-hydroxytamoxifen. Taken together, these data suggest that the dose–response curve of tamoxifen lies to the left of the 4-chlorotamoxifen curve in both species because tamoxifen is activated by hydroxylation *in vivo* to the more potent 4-hydroxytamoxifen; 4-chlorotamoxifen cannot be activated *in vivo* in an analogous fashion because the 4-chloro substitution renders the molecule resistant to metabolic *para*-hydroxylation. Since all three compounds elicit the same maximal response, these data also suggest that metabolism of this portion of the tamoxifen molecule affects its potency but do not determine if a compound will be an estrogen agonist or antagonist. Therefore, we concentrated our metabolism studies on the dimethylaminoethoxy side-chain portion of tamoxifen.

Uterotrophic assay of metabolite E. Our working hypothesis was that tamoxifen is uterotrophic in mice because it is converted *in vivo*, by cleavage of a portion of the dialkylaminoethoxy side chain, into an estrogenic derivative, designated metabolite E.

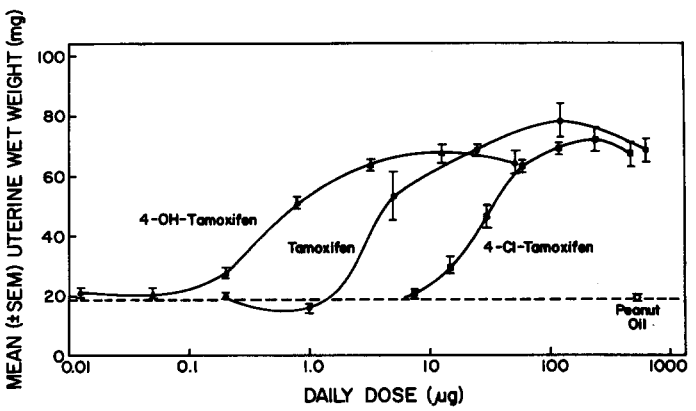

Fig. 2. Uterotrophic activity of tamoxifen, 4-hydroxytamoxifen, and 4-chlorotamoxifen in ovariectomized mice. Uterine weight tests were performed as described in Materials and Methods. There were at least five mice per group. Mice treated with 0.3 μg estradiol had a mean (±S.E.M.) uterine wet weight of 90.7 ± 4.2 mg.

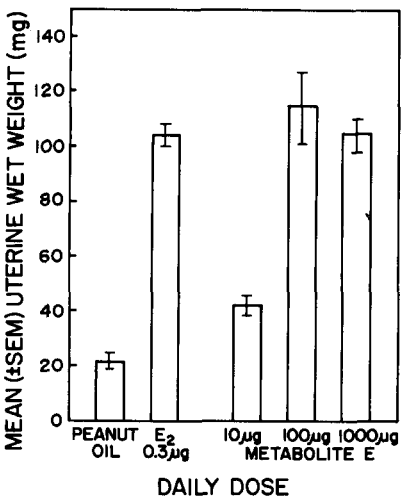

Fig. 3. Uterotrophic activity of metabolite E in the 3-day ovariectomized-mouse uterine weight test. The assay was performed as described in Materials and Methods; peanut oil was the vehicle in which the test compounds were administered; E_2 is 17-β estradiol.

Therefore, we tested the capacity of this metabolite to elicit a uterotrophic response in ovariectomized mice (Fig. 3). Metabolite E was fully uterotrophic in mice, with a potency roughly equal to that of tamoxifen. Having determined that metabolite E was uterotrophic, we then examined the metabolism of [^{3}H]tamoxifen *in vitro* and *in vivo* to determine if this metabolite was selectively formed in those species in which tamoxifen is estrogenic.

Tamoxifen metabolism in vitro. The liver is the primary site in the body of drug biotransformation [14]; therefore, we compared the capacity of liver microsomes from mice, rats, and chickens to metabolize [^{3}H]tamoxifen (Fig. 4). Very little tamoxifen metabolism was observed with mouse or rat microsomes, with 4-hydroxytamoxifen and tamoxifen-*N*-oxide being the principal metabolites formed. The low level of tamoxifen metabolism was not due to an inactive enzyme preparation because these microsomes were active in an aminopyrine-*N*-demethylase assay (data not shown). Robertson *et al.* [11] have also reported only low levels of tamoxifen metabolism by rat liver microsomes. Metabolism of [^{3}H]-tamoxifen by mouse or rat microsomes was not increased by changing the incubation time or substrate concentration, by removing nicotinamide from the buffer, or by replacing the microsomes with liver cytosol (data not shown).

Chicken liver microsomes, in contrast to rodent microsomes, were very active in metabolizing tamoxifen (Fig. 4). The principal metabolite formed was 4-hydroxytamoxifen, which confirms the finding of Borgna and Rocherfort [15].

Metabolite E was not generated in detectable amounts by liver microsomes from mice, rats, or chickens, suggesting that this metabolite was not responsible for the uterotrophic activity of tamoxifen in rodents. Drug metabolism *in vitro*, however, is not always a good predictor of metabolism *in vivo*; therefore, we examined the metabolism of [^{3}H]-tamoxifen in mice, rats, and chickens.

Tamoxifen metabolism in vivo. The doses of tamoxifen administered in these experiments were chosen from literature values for effective uterotrophic (mouse) or antiuterotrophic (rat, chicken) doses of tamoxifen. Tissues from which metabolites were extracted were the uterus, which is an estrogen target tissue, and the liver, which is also an estrogen target tissue (especially in chickens) and which we presume is the primary organ for tamoxifen metabolism.

Tamoxifen metabolism in the mouse. The major metabolite extracted from mouse liver 24 hr after a subcutaneous injection of 10 μg [^{3}H]tamoxifen was 4-hydroxytamoxifen, which was also a major metabolite in the uterus (Fig. 5). Tamoxifen was the principal compound extracted from both tissues; this was not unexpected given the long reported half-life of tamoxifen *in vivo* [8] and the low levels of metabolism observed *in vitro* (Fig. 4). Similar metabolite profiles were obtained with tissue extracts prepared at a 4-hr time point (data not shown). No metabolite E was observed in extracts from liver or uterus at 4 or 24 hr.

Endogenous estrogens will compete with tamoxifen and its metabolites for estrogen receptors; this could reduce the uptake of these compounds into estrogen target tissues. Therefore, we injected 10 μg [^{3}H]tamoxifen (50 μCi) into ovariectomized adult mice and 24 hr later extraced the radioactivity from liver and uterus. Thin-layer chromatograms of radio-

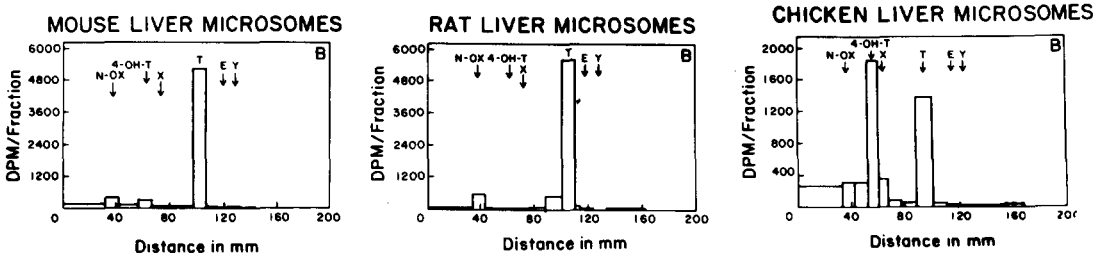

Fig. 4. Metabolism of [^{3}H]tamoxifen *in vitro* by liver microsomes. Assays were performed as described in Materials and Methods; the B in each panel of these thin-layer chromatograms refers to solvent system B that was used to develop the TLC plates. The position of the metabolite standards is indicated by the arrows. Abbreviations: T, tamoxifen; N-ox, tamoxifen *N*-oxide; X, *N*-desmethyltamoxifen (metabolite X); E, metabolite E; Y, metabolite Y; and 4-OH-T, 4-hydroxytamoxifen (metabolite B).

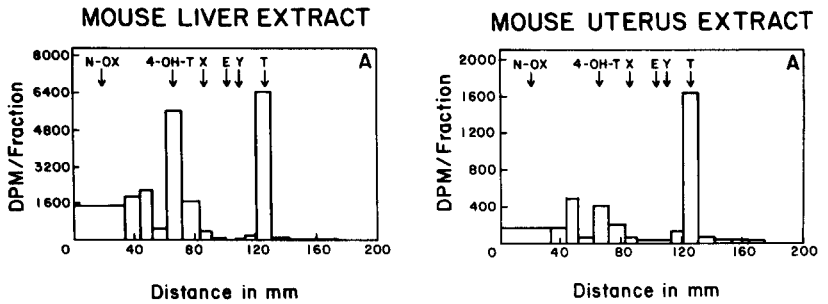

Fig. 5. Thin-layer chromatograms of radioactivity extracted from the tissues of an adult female mouse 24 hr after subcutaneous injection of 10 μg [^{3}H]tamoxifen (50 μCi). The letter in the upper right corner of each panel indicates the solvent system used to develop the plate (see Table 1).

activity extracted from these tissues were virtually identical, however, to those obtained from normal mice (data not shown).

To rule out the possibility that the metabolites seen in these experiments were artifactually generated by our extraction procedure, we added [^{3}H]tamoxifen to liver homogenate (prepared from an untreated mouse), and then extracted and analyzed the radioactivity. Tamoxifen was the sole compound observed in the chromatograms (data not shown), indicating that the metabolites observed in Fig. 5 were not artifacts formed during the extraction process.

We also considered the possibility that tamoxifen may alter its own metabolism (via cytochrome P-450 enzyme induction) because the mouse uterotrophic assay requires three consecutive daily injections of tamoxifen. However, the profiles of metabolites extracted from the liver and uterus of a mouse treated with unlabeled tamoxifen for the 3 days before injection of [^{3}H]tamoxifen were virtually identical to those profiles obtained from the non-pretreated mice in Fig. 5 (data now shown).

Since metabolite E was originally isolated from dog bile [8], we examined mouse bile for the presence of this compound (Fig. 6). Virtually all of the radioactivity extracted from bile remains at the point of application on the thin-layer plate; we presume this material to be glucuronide and sulfate conjugates of tamoxifen metabolites. Digestion of this material with glucuronidase and sulfatase revealed the presence of a number of polar metabolites; metabolite E, however, was not observed in bile either before or after enzymatic digestion.

Tamoxifen metabolism in the rat. The pattern of tamoxifen metabolism in the rat (Fig. 7) was similar to that observed in the mouse. Tamoxifen was the principal compound extracted from both liver and uterus 24 hr after the subcutaneous administration of 50 μg [^{3}H]tamoxifen. Again, this is consistent with the long half-life of tamoxifen in this species [8]. The major metabolite in the liver was 4-hydroxytamoxifen, which was also a major metabolite in the uterus. Metabolite E was not observed in any of the chromatograms above the background level of radioactivity. Results qualitatively similar to these were observed at a 4-hr time point (data not shown).

Tamoxifen metabolism in the chicken. Tamoxifen metabolism in chicken liver was much more extensive than in either mice or rats, as might have been expected from our *in vitro* data. The principal metabolite extracted 24 hr after the subcutaneous injection of 8 mg [^{3}H]tamoxifen was 4-hydroxytamoxifen (Fig. 8). Metabolite E was not observed in either of the radiochromatograms above the background level of radioactivity. Chicken bile was also examined for the presence of metabolite E (Fig. 9); this compound was not seen in biliary extracts either before or after enzymatic digestion.

Metabolism studies in vivo *with 4-hydroxytamoxifen.* Jordan *et al.* [2] have shown that 4-hydroxytamoxifen, like tamoxifen, is a fully uterotrophic drug in the mouse. Since 4-hydroxytamoxifen was

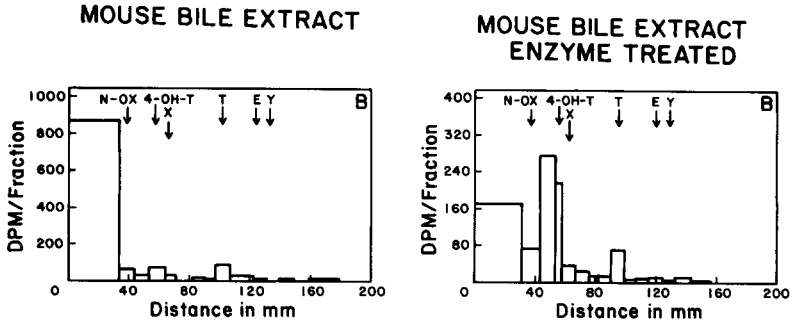

Fig. 6. Thin-layer chromatograms of radioactivity extracted from the bile of the mouse in Fig. 3. In the panel labeled "enzyme treated", the radioactive materials extracted from bile were digested with sulfatase and glucuronidase as described in Materials and Methods.

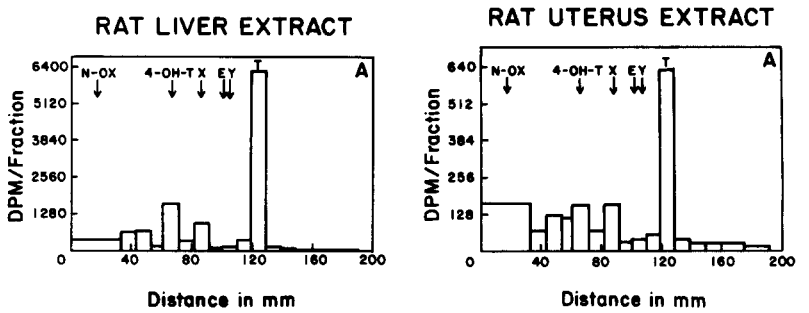

Fig. 7. Thin-layer chromatograms of radioactivity extracted from the tissues of an adult female Sprague–Dawley rat 24 hr after subcutaneous injection of 50 μg [^{3}H]tamoxifen (50 μCi).

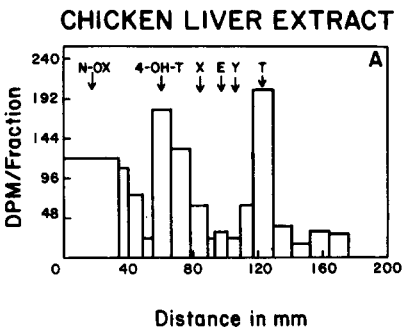

Fig. 8. Thin-layer chromatograms of radioactivity extracted from the liver of an immature chicken 24 hr after subcutaneous injection of 8 mg [^{3}H]tamoxifen (50 μCi).

the principal tamoxifen metabolite in mice, rats, and chickens, we considered the possibility that this compound was an intermediate in a two-step process that would give rise to an estrogenic metabolite. Hydroxylation of tamoxifen, followed by side-chain cleavage, would yield a bisphenolic tamoxifen derivative (Fig. 1) that would be predicted to be estrogenic (since it lacks the side chain that is thought to be required for antiestrogenicity) and to have a high affinity for the estrogen receptor (as a result of the first hydroxylation step). This compound, referred to as bisphenol (Fig. 1), has been synthesized [11], and was reported to be estrogenic in the rat, and to have a high affinity for the estrogen receptor; however, this compound has never been shown to be a tamoxifen metabolite *in vivo*. This compound was uterotrophic in mice (Fig. 10), with a potency intermediate to that of tamoxifen and 4-hydroxtamoxifen. Having established that this compound was uterotrophic, we administered a subcutaneous injection of 10 μg [^{3}H]4-hydroxytamoxifen to a mouse and 24 hr later killed the animal, extracted the radioactivity from liver and uterus, and analyzed this materal by TLC for the presence of bisphenol (Fig. 11). Most of the radioactivity extracted from these tissues was 4-hydroxytamoxifen. Although bisphenol appears to be present in those chromatograms developed with solvent system A, no bisphenol was observed in chromatograms developed with solvent system B. Thus, the radioactivity that co-migrates without bisphenol standard in solvent system A is not bisphenol, but some other unidentified metabolite(s). Similarly, Robertson *et al.* [11] reported finding unidentified metabolite(s) in rat uterus extracts that co-migrated with bisphenol in their TLC solvent system. Bisphenol was not observed in bile extracts from this mouse (either before or after enzymatic digestion), nor in liver or uterus extracts from rats given an injection of [^{3}H]-4-hydroxytamoxifen (data not shown).

DISCUSSION

Our working hypothesis was that tamoxifen is an antiestrogenic compound that exhibits uterotrophic activity in rodents because it is converted into an

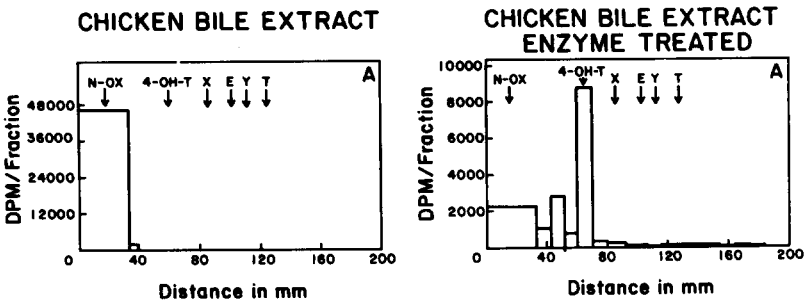

Fig. 9. Thin-layer chromatograms of radioactivity extracted from the bile of the chicken in Fig. 8. In the panels labeled "enzyme treated", the radioactive materials extracted from bile were digested with sulfatase and glucuronidase as described in Materials and Methods.

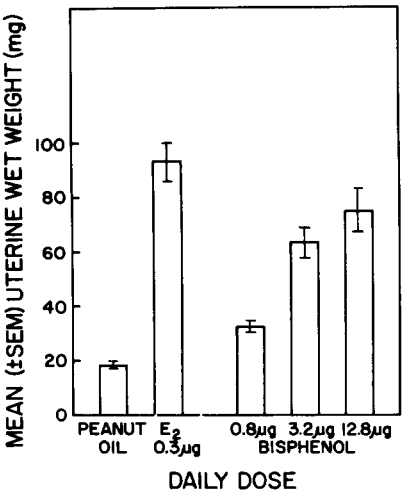

Fig. 10. Uterotrophic activity of the bisphenol derivative of tamoxifen in ovariectomized mice. Uterine weight tests were performed as described in Materials and Methods. There were five mice per group.

estrogenic metabolite *in vivo*. We focused our attention on metabolite E because (1) this metabolite has been shown to have estrogenic activity in rat pituitary cells *in vitro* [16] and in rat uterus *in vivo* [7], whereas tamoxifen is antiestrogenic in these systems, and (2) because this compound was identified as a tamoxifen metabolite in a species (dog) [8] in which the parent drug is estrogenic [9]. In addition, tamoxifen *N*-oxide [17], metabolite Y [18, 19], and metabolite X [18, 19] have all been shown to have antiestrogenic activity either *in vivo* or *in vitro*. Our data indicate that metabolite E was not formed from tamoxifen in either mice, rats or chickens and, therefore, was not responsible for the uterotrophic activity of tamoxifen in rodents. Bisphenol has been reported to be uterotrophic in the rat [11] and we found that it was uterotrophic in mice (Fig. 10); however, bisphenol was not formed in rodents from 4-hydroxytamoxifen (or tamoxifen) in appreciable amounts and, therefore, does not account for the estrogenic effects of tamoxifen in this species.

The principal metabolite of tamoxifen in all three species was 4-hydroxytamoxifen; this agrees with the findings of Fromson *et al.* [8], Borgna and Rochefort [15], and Robertson *et al.* [11]. This compound has the same biological properties as tamoxifen: it is fully uterotrophic in mice [2], a partial estrogen agonist/antagonist in the rat [20], and a complete estrogen antagonist in the chicken [21]. Similarly, both tamoxifen and 4-hydroxytamoxifen have the same biological properties *in vitro*, i.e. they both inhibit the estrogen-stimulated increase in prolactin synthesis in rat pituitary cells [6, 16]. Tamoxifen is less potent than 4-hydroxytamoxifen both *in vitro* and *in vivo*; this is presumably because it has a much lower affinity for the estrogen receptor [2].

A number of unidentified metabolites were observed in small amounts in our chromatograms. For these compounds to have significant biological effects *in vivo*, they would have to possess a very high affinity for the estrogen receptor to compete with 4-hydroxytamoxifen, which has a very high affinity for the estrogen receptor from the mouse (S.D. Lyman and V. C. Jordan, unpublished data) and the rat [22]. In addition, these unidentified metabolites are seen in the tissue extracts from all three species, and although we have no evidence that these metabolites are identical in mice, rats, and chickens, it is unlikely that they are responsible for the disparate activity of tamoxifen in these species.

The question of why tamoxifen exhibits different

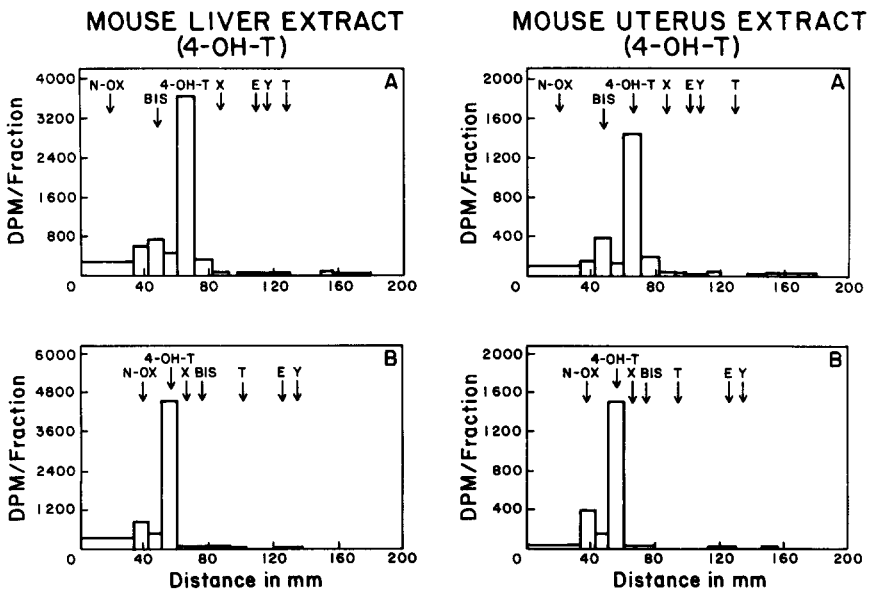

Fig. 11. Thin-layer chromatograms of radioactivity extracted from the pooled tissues of two adult female mice 24 hr after the subcutaneous injection of 10 μg [^{3}H]4-hydroxytamoxifen (50 μCi) to each animal. BIS is an abbreviation for the bisphenol derivative of tamoxifen (see Fig. 1).

biological effects in mice, rats, and chickens is still unresolved. We have assumed in our studies that the biological effects of tamoxifen, be they estrogenic or antiestrogenic, are mediated through binding to the estrogen receptor. This assumption is based on two observations: (1) that tamoxifen and its metabolites inhibit the binding of tritiated estradiol to estrogen receptors prepared from mouse uterus [5], rat uterus [2], and chicken oviduct [23], and (2) that tamoxifen is capable of blocking the uptake of [^{3}H]estradiol into estrogen target tissues in the mouse [5] and rat [24]. We believe that the disparate biological activity of tamoxifen in mice, rats, and chickens results from differences in the way in which the ligand–estrogen receptor complex interacts with nuclear acceptor sites. Jordan *et al.* [25] have shown that virtually all tamoxifen-translocated estrogen receptors were salt extractable from rat uterus nuclei, whereas a portion of estradiol-translocated estrogen receptors were not salt extractable. This suggests a different binding of the ligand–receptor complexes to chromatin. Similarly, Evans *et al.* [26] presented evidence that receptor–antiestrogen complexes bind with a lower affinity to non-specific double-stranded DNA than do receptor–estrogen complexes. Species differences in estrogen receptors clearly exist, since monoclonal antibodies to human estrogen receptor cross-react with receptors purified from rat uteri, but not from chicken oviduct [27]. Tate *et al.* [28] recently demonstrated that antibodies raised against estrogen receptors could detect differences between estrogen and antiestrogen–estrogen receptor complexes. We believe that monoclonal antibodies will be useful tools in probing the complexities of receptor–ligand interactions with nuclear acceptor sites.

It has also been suggested, however, that the antiestrogenic effects of tamoxifen may be mediated through binding to antiestrogen binding sites that are distinct from the estrogen receptor [29, 30] or through an interaction with the calcium binding protein calmodulin [31]. We are currently conducting experiments to determine if the biological properties of tamoxifen are correlated with binding to these other intracellular sites. It is possible that the uterotrophic effects of tamoxifen are mediated through one binding site, whereas the antiuterotrophic effects are mediated through a second site.

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