o,p'-DDT as an Estrogen: An Evaluation of Its Ability to Compete with ^{3}H-Estradiol for Nuclear Estrogen Receptor Sites in the Quail Oviduct

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Several species of birds have been reported to develop thin-shelled eggs after being fed either DDT or its metabolite DDE (BITMAN et al., 1969; HEATH et al., 1969; and PORTER and WIEMEYER, 1969). Eggshell thinning, and as a consequence increased breakage, resulting from environmental exposure to DDT has been suggested as the primary cause of declines in a number of species of wild birds (RATCLIFF, 1967). The mechanism of action of DDT is not known. o,p'-DDT, and to a lesser extent p,p'-DDT, DDE and DDD, illicit a uterotrophic response in mammals and birds. This response is similar to the action of estrogen in that increases in wet weight, ^{14}C-glucose uptake and glycogen content occur in the uterus and oviduct after treatment (BITMAN et al., 1969; and WELCH et al., 1969). If DDT acts as an estrogen the hormonal control of reproductive function might be impaired. The purpose of this study was to evaluate the ability of DDT, DDE and DDD to compete with ^{3}H-estradiol ($^{*}E_2$) for specific nuclear estrogen receptor binding sites in the quail oviduct.

Methods

Sixteen week-old female Japanese quail (Coturnix coturnix japonica) were obtained from Lab Associates (Kirkland, WA.). The birds were housed in individual cages and received food and water ad. lib. Laying hens were sacrificed by decapitation and their oviducts excised, stripped of connective tissue and placed in cold TEG buffer (0.01 M Tris-HCl, 1.5 mM EDTA in 10% glycerol at pH 7.4). Unless stated otherwise all subsequent steps were performed at 0-4°C. Tissues were homogenized using a motor driven teflon-glass homogenizer (100mg wet wt. tissue/ml buffer). The homogenate was centrifuged at 800g for 10 min. The crude nuclear pellet was washed with buffer, centrifuged and then resuspended in TEG buffer.

Aliquots of the nuclear suspension were dispensed into two sets of tubes (A and B). The A tubes contained various concentrations of ($^{*}E_2$) and were used to determine the total amount of radioactivity that bound to nuclei. The B tubes contained the same concentrations of $^{*}E_2$ plus a 100

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fold excess of non-radioactive estradiol (E_2) and were used to determine non-specific binding. Various concentrations of diethylstilbestrol (DES), estrone (E_1), progesterone (P), testosterone (T), o,p'-DDT, p,p'-DDT, o,p'-DDE and o,p'-DDD were added to other tubes to test the ability of the various compounds to compete with ^{3}H-estradiol for specific estrogen binding sites.

The nuclear-receptor was quantitated using the direct ^{3}H-estradiol exchange assay (ANDERSON et al., 1972). The following modifications in the conditions of the assay were made to maximize exchange in oviduct: an incubation volume of 0.5 ml was used and the incubation was conducted at a temperature of 30°C for 4 hr.

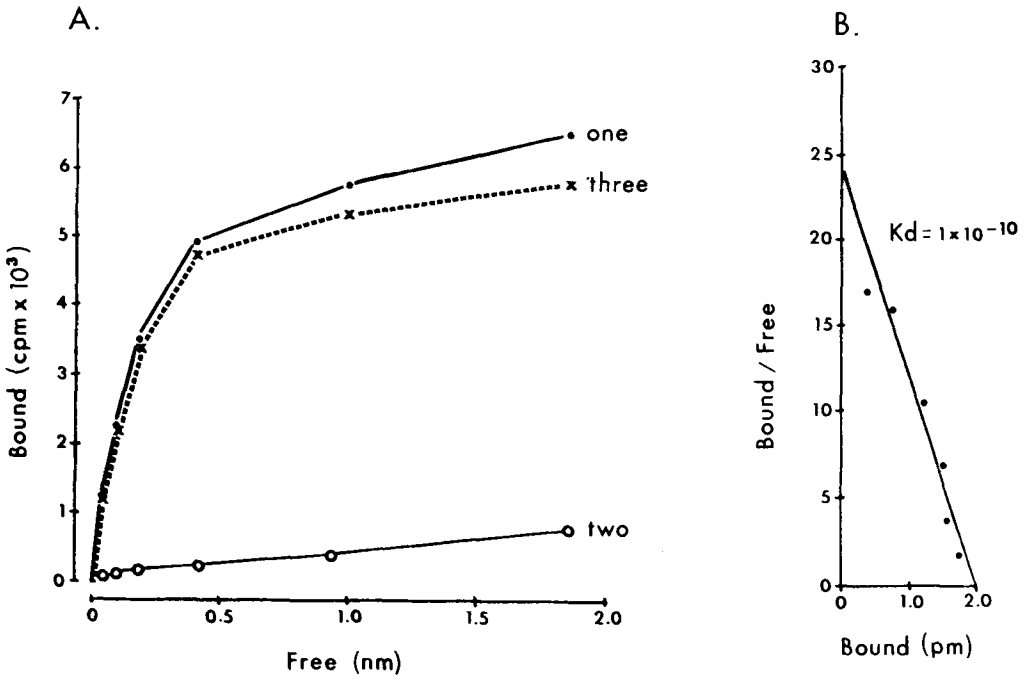

Figure 1. A) A representative $^*E_2$ saturation curve. Nuclei were prepared and incubated with $^*E_2$ as described in the text. Line # one represents the total binding of $^*E_2$ to nuclei. Line # two represents the non-specific component. Line # three represents the specific binding of $^*E_2$ to nuclei and was calculated by subtracting Line # two from Line # one.
B) Line # three from A) plotted according to Scatchard (1949).

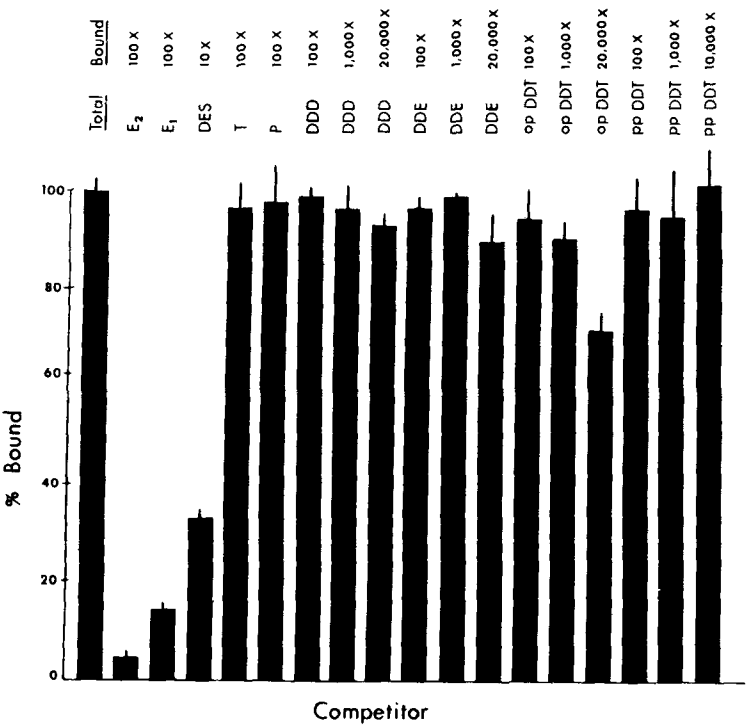

Figure 2. The ability of a variety of compounds to compete with $^{*}E_2$ for nuclear receptor sites. The compounds to be tested were added simultaneously with 1 NM $^{*}E_2$ and radioactivity bound to nuclei determined by the exchange assay. The bar labeled "total bound" represents the $^{*}E_2$ that bound to nuclei in the absence of competition. The value above each of the other bars refer to the molar excess of competitor. Each value represents the average of at least three determinations. The vertical lines equal one standard deviation.

Results and Discussion

Estrogen target tissues contain a limited number of high affinity estrogen receptor sites which can be quantitated by nuclear exchange saturation analysis (ANDERSON et al., 1972). A typical saturation curve for oviduct nuclei is shown in Fig. 1. The results show the presence of a single class of saturable, high affinity $^{*}E_2$ binding sites. The ability of a variety of estrogenic and non-estrogenic compounds to compete with $^{*}E_2$ for the receptor sites is shown in Fig. 2. Both steroid (E_1 and E_2) and non-steroid (DES) estrogens competed effectively with $^{*}E_2$ for binding sites but the non-estrogenic steroids, T and P, did not compete. This result shows that the $^{*}E_2$ binding sites exhibit structural specificity. Of the test compounds (o,p'-DDT, p,p'-DDT, o,p'-DDE and o,p'-DDD) only o,p'-DDT competed significantly with $^{*}E_2$ at the concentrations used. A

large molar excess (20,000x) was required before o,p'-DDT competed with $^{*}E_2$ indicating that the affinity of o,p'-DDT for estrogen receptor is weak. This result would suggest that any estrogenic response induced by o,p'-DDT would be due to "swamping" the receptor sites and, therefore, would not represent a true hormone specific response. It is of course possible, as has been suggested by BITMAN et al. (1969), that a metabolic product of o,p'-DDT is more estrogenic than the parent compound.

Acknowledgments

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