Serum immunoreactive parathyroid hormone and aluminum concentrations were determined in the 39 patients who had been on dialysis for 1 to 91 months (Fig. 1). Parathyroid hormone and aluminum concentrations were positively correlated in these patients ($r = .62$, $P < .001$). Gastrointestinal absorption of aluminum hydroxide has been demonstrated among dialysis patients and ranges from 100 to 568 mg/day (*13*). In addition, elevated concentrations of aluminum have been found in the serum of dialysis patients (*12*). These data and those presented here seem to indicate that among dialysis patients exogenous aluminum, in the presence of severely elevated parathyroid hormone, can result in increased gastrointestinal absorption and altered tissue distribution of aluminum.

This conclusion is supported by the high net gastrointestinal absorption demonstrated in rats treated with parathyroid hormone and by the high tissue aluminum concentrations seen in whole brain, gray matter, muscle, bone, and kidney in these animals. These increases, in the absence of increased liver and white matter aluminum, argue against a nonspecific gastrointestinal effect alone and are consistent with a specific effect of parathyroid hormone on both the uptake and the distribution of aluminum in these tissues. The distribution of aluminum in the tissues of these animals is strikingly similar to the distribution of aluminum in dialysis patients who died of a fatal encephalopathy (*7*). The significant correlation of serum parathyroid hormone and aluminum in humans suggests that both Alzheimer's disease and dialysis encephalopathy may be explained in part by parathyroid hormone acting on orally ingested aluminum.

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Competition of Δ^9-Tetrahydrocannabinol with Estrogen in Rat Uterine Estrogen Receptor Binding

Abstract. *Direct competition experiments with Δ^9-tetrahydrocannibinol (Δ^9-THC) and estradiol in binding assays with rat uterine cytosol estrogen receptors showed that Δ^9-THC was a weak, but nevertheless significant, competitor for binding to cytoplasmic estrogen receptors. These data support, at the molecular level, the observations that Δ^9-THC has a weak estrogenic activity (at least the ability to bind to estrogen receptors). Moreover, estrogen-like binding suggests that Δ^9-THC, acting at the level of estrogen receptor, causes a primary estrogenic effect rather than an indirect or secondary phenomenon.*

There has been increasing speculation (*1*) and some experimental support (*2, 3*) for an estrogen-like biological activity associated with heavy, long-term marihuana smoking in man and with the administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) in male rodents. Because most of the data have been obtained from experiments in vivo where specific hormone-sensitive organs were examined for stimulation (*2, 3*) or repression (*4*), the conflicting results may be due to indirect effects in the whole animal.

The reports of uterine stimulation in castrated female rats by Solomon *et al.* (*3*), and of lowered levels of luteinizing hormone, follicle-stimulating hormone, and testosterone in men by Kolodny and co-workers (*2, 5*) after the long-term administration of Δ^9-THC have also focused attention on the estrogenic nature of the drug. Since it has been demonstrated that Δ^9-THC may be transferred across the placenta to the developing fetus (*6*), and that it appears in the milk of lactating animals (*7*), the question of deliterious effects on fetal and newborn offspring must be addressed. Estrogen and estrogenic compounds may play an important part in the development of several forms of carcinoma in human females and their offspring (*8, 9*), and they are potential suppressors of male hormones and of androgen-dependent tissues (*4*). Moreover, it has recently been reported that 4*S* estrogen receptors occur in both human prostate (*10*) and rat testes (*11*).

To test the hypothesis that Δ^9-THC acts as an estrogen in a direct experiment, we have examined the extent to which Δ^9-THC competes with estrogen for sites on rat uterus estrogen receptors in vitro. We have also conducted binding experiments with ^{14}C-labeled Δ^9-THC on rat uterus cytosol to determine the components that can bind Δ^9-THC.

Virgin female (Fisher 344) rats, approximately 60 days old, were decapitated. Their uteri were removed, pulverized in liquid nitrogen, and then homogenized in 10 m*M* tris, 1.5 m*M* EDTA, and 0.5 m*M* dithiothreitol (DTT) at *p*H 7.4 with a Sorvall Omni-Mixer at setting 7 for two 15-second bursts; the suspension was centrifuged at 100,000*g* for 30 minutes at 4°C. The supernatant solution (100 μl; 2 to 4 mg of protein per milliliter) was incubated with increasing amounts of ^{3}H-labeled 17β-estradiol (0.076 to 1.52 pmole; specific activity, 43 c/mmole) for 2 hours at 4°C, and the receptor-bound [^{3}H]estradiol was separated from the free and weakly bound steroid by a 20-minute incubation with dextran-coated charcoal. The charcoal was prepared from a 0.5 percent suspension of Norite A (Sigma) and 0.05 percent dextran (Sigma) in buffer in the usual manner (*12*). The nonspecifically bound [^{3}H]-estradiol not removed by the dextran-coated charcoal was determined by prior incubation (30 minutes) of the uterine cytosol with a 300- to 1000-fold excess of unlabeled 17β-estradiol (556 pmole) relative to ^{3}H-labeled estradiol and measuring the remaining bound estrogen.

The nonspecific binding ranged from about 3 percent of the total bound counts in the case of the lowest levels of labeled estrogen (high ratios of unlabeled to labeled hormone) to a maximum of about 15 percent at the higher levels of labeled

estrogen used in this study (Fig. 1A). The estrogen receptor as defined here represents specific and saturable binding sites which are determined by subtracting the nonsaturable and nondisplaceable [^{3}H]estradiol binding from the total [^{3}H]estradiol binding. Characterization of the specific binding by Scatchard analysis (*13*) (Fig. 1B) gave a straight line with linear regression correlation coefficient of .96. Analysis of the Scatchard data indicates strong binding with a K_d of $9.9 \times 10^{-10} M$ and a stoichiometry of 560 fmole of estradiol specifically bound per milligram of cytosol protein. While the estrous cycle status or other factors in a given rat might affect the level of estrogen receptor and hence the stoichiometry of this interaction, we have assumed that the intrinsic binding properties of those estrogen receptors available for binding or free exchange with added estrogen or other competing ligand remain constant and, therefore, that relative competition should be independent of receptor level.

Competition for binding to the estrogen receptor was performed by substituting the test compounds for the unlabeled estradiol in the mixture prior to the addition of the [^{3}H]estradiol. As shown in Table 1, prior incubation with unlabeled 17β-estradiol blocked the binding of 84.4 percent of the total bound ^{3}H-labeled 17β-estradiol. The remaining bound counts (13.8 percent) were interpreted as nonspecific binding. A comparable level of Δ^9-THC blocked the binding of 22.3 percent of the specifically bound estradiol. Increasing the concentration of Δ^9-THC ten times inhibited 22.0 percent of the specific binding. The major metabolite of Δ^9-THC, 11-OH-Δ^9-THC, blocked the binding of 12.5 percent of the specifically bound estradiol when it was present at comparable concentrations, and blocked the binding of 19.1 percent of the specifically bound estrogen when its concentration was increased to ten times that of the unlabeled estradiol. Four other steroids were tested at concentrations of 40 to 60 μM. An average of 4.4 percent inhibition of the specific binding of estrogen was observed when the nonestrogenic steroids were present at even 100 times the concentration of the unlabeled estradiol or ten times the highest concentration of the cannabinoid derivatives, thus indicating that the competition seen with Δ^9-THC is not merely a reflection of "steroid carbon mass." These data are consistent with the earlier reports of Wittliff *et al.* (*14*) and McGuire and DeLaGarza (*15*) for similar groups of nonestrogenic steroids, even though the concentrations of potential competitors we used were from 20 to 60 times higher than they examined.

Table 1. Competition of Δ^9-THC with estrogen in rat uterine estrogen receptor binding.

Competitor	Amount of competitor added to assay (ng)	Total [^{3}H]estrogen bound after incubation with competitor (%)	Specifically bound estrogen displaced * (%)
None †	0.41 [^{3}H] Estradiol	100	
17β-Estradiol ‡	150	13.8 ± 2.8	100
Δ^9-THC ‡	150	81.1 ± 7.2	22.3 ± 8.2
Δ^9-THC ‡	1,500	81.3 ± 6.9	22.0 ± 8.3
11-OH-Δ^9-THC ‡	150	89.3 ± 3.1	12.5 ± 3.8
11-OH-Δ^9-THC ‡	1,500	83.5 ± 5.0	19.1 ± 5.7
Hydrocortisone §	13,830	94.1 ± 1.5	7.0 ± 1.7
Testosterone acetate §	16,650	96.9 ± 3.1	3.7 ± 3.6
Progesterone §	20,550	99.6 ± 0.9	0.4 ± 1.0
Cholesterol §	18,150	94.5 ± 2.7	6.5 ± 3.1

* Corrected for nonspecific binding. † This was a 100 percent control. ‡ Data presented are average values taken from duplicates in four experiments. Reported values are the average of eight points ± standard deviation. § Averages of duplicate samples in single experiments ± the range.

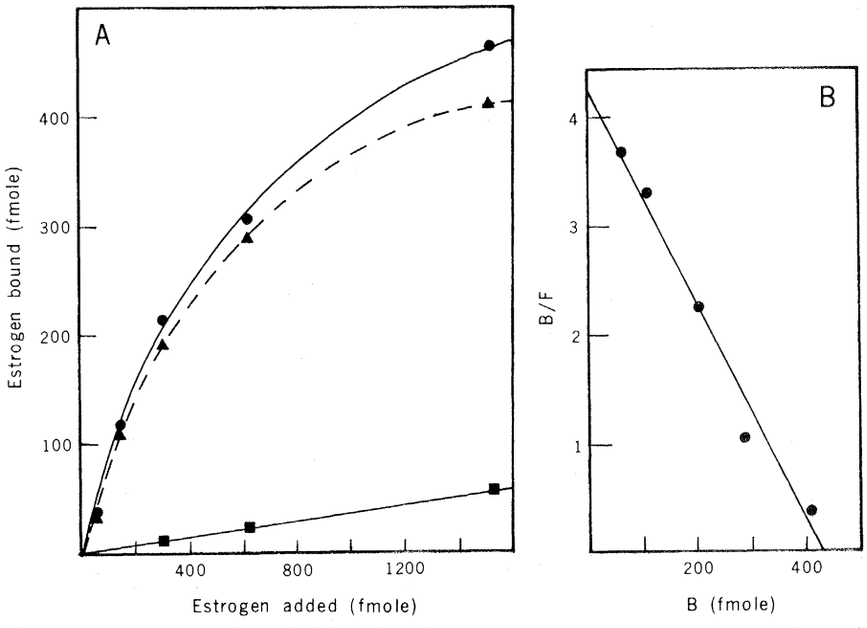

Fig. 1. (A) The amount of estradiol bound per 0.1 ml of uterine cytosol plotted against the total amount of estradiol added. Circles represent total binding, squares represent nonspecific binding (not displaceable by excess unlabeled estradiol), and the triangles represent specific binding. (B) Scatchard analysis of the binding of estradiol to rat uterine cytosol (*B/F* indicates the ratio of bound to free estradiol). The correlation coefficient for the linear regression fit to the data was .96; K_d was calculated as $9.9 \times 10^{-10} M$ and the stoichiometry of binding was 567 fmole of estrogen bound per milligram of cytosol protein.

The amount of nonspecific binding of [^{3}H]estradiol was unaffected by prior incubation of rat uterine cytosol with unlabeled estradiol and Δ^9-THC, suggesting that the inhibition of [^{3}H]estradiol binding by Δ^9-THC was not due to a change in nonspecific binding of [^{3}H]estradiol.

To ensure that tests were performed with comparable concentrations of compounds, the solubilities of 17β-estradiol and Δ^9-THC were determined in the estrogen receptor assay system. Portions of rat diaphragm muscle cytosol (2 to 4 mg of protein per milliliter) were incubated for 2 hours at 4°C with ^{3}H-labeled 17β-estradiol or with ^{14}C-labeled Δ^9-THC (10.5 mc/mmole) at the highest concentration (556 pmole) used in the competitive study, and then filtered through 0.2-μm-pore filters. The filtrates were counted in Bray's liquid scintillation mixture. The total amounts of material in solution and bound to the soluble cell constituents in the filtrate were nearly identical for both the estradiol (113.0 ± 9.9 ng of soluble constituents per milliliter of assay mix) and the Δ^9-THC (113.6 ± 7.0 ng per milliliter). This suggests that the effects seen in the com-

petitive binding assay were not distorted as a result of significant differences in the solubilities of the compounds, and explains why the high dose of Δ^9-THC (1500 ng) did not further inhibit the estrogen binding. It further suggests that concentrations of Δ^9-THC in excess of 150 ng/0.1 ml ($4.8 \times 10^{-6} M$) exceed saturation and are probably not useful in such a system in vitro.

Direct binding of Δ^9-THC with estrogen receptor was demonstrated by sucrose density gradient centrifugation. Rat uterine cytosol (100 μl) was incubated as before with ^{14}C-labeled Δ^9-THC (200 μl; specific activity, 10.5 mc/mmole) and [^{3}H]estradiol (50 μl, 4.3 c/mmole) separately or together. After incubation with dextran-coated charcoal and centrifugation in order to remove free ligand, the samples were applied to sucrose density gradients (5 to 30 percent) prepared in 10 mM tris, pH 7.4, containing 1.5 mM EDTA and 0.5 mM DTT and centrifuged at 4°C for 10 hours at 300,000g. Fractions (ten drops) were collected and analyzed for ^{3}H and ^{14}C by means of liquid scintillation counting. Bovine serum albumin served as an internal marker (see Fig. 2). The [^{3}H]estradiol sedimented as a single peak (4S) which was eliminated by prior incubation with 500-fold excess of unlabeled estradiol. The ^{14}C-labeled Δ^9-THC sedimented as two peaks, 4S and 10.4S. The apparent 10.4S form is attributed to aggregates containing Δ^9-THC and appeared only in experiments in which Δ^9-THC was used. The 10.4S peak contained both Δ^9-THC and estradiol in the case of experiments in which both labeled Δ^9-THC and labeled estradiol were used. Prior incubation with an equal amount of unlabeled Δ^9-THC caused a significant reduction of radioactivity in the 4S peak. Simultaneous incubation of [^{3}H]estradiol and ^{14}C-labeled Δ^9-THC with rat uterine cytosol produced a 4S peak labeled with both ^{3}H and ^{14}C and a 10.4S peak labeled primarily with ^{14}C-labeled Δ^9-THC, but containing some estradiol (we assume this to be due to aggregated receptor–Δ^9-THC complexes interacting with estradiol-receptor complex). While the low specific activity of the ^{14}C-labeled Δ^9-THC (10.5 mc/mmole) relative to the [^{3}H]estradiol (43 c/mmole) and the resulting chemical composition of the mixtures necessary made a reverse competition experiment difficult, at least a partial inhibition of the binding of ^{14}C-labeled Δ^9-THC to the 4S component by prior incubation with estradiol was observed.

Taken together, our data demonstrate

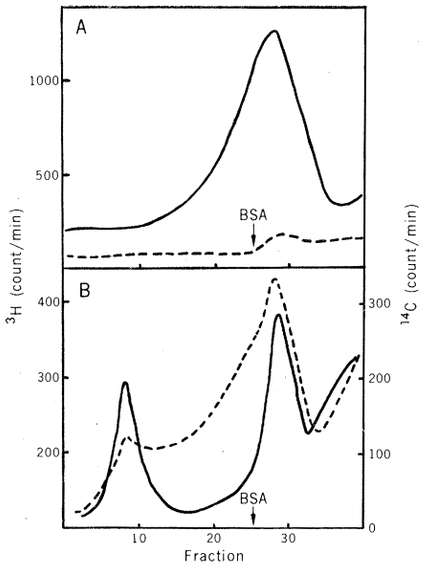

Fig. 2. Sucrose density gradient sedimentation patterns of Δ^9-THC and estrogen receptor complexes. (A) Sucrose density gradients (5 to 30 percent at pH 7.4) were run for 10 hours at 4°C. Gradients contained 100 μl of rat uterine cytosol with [^{3}H]estradiol (——) or cold estradiol (500-fold excess) plus [^{3}H]estradiol (- - - -). (B) Identical gradient to (A) but containing uterine cytosol plus both [^{3}H]estradiol and ^{14}C-labeled Δ^9-THC. The solid lines indicate ^{14}C counts per minute per fraction due to the protein-bound cannabinoid. The dashed line indicates ^{3}H counts per minute per fraction due to the protein-bound estrogen. The more rapidly sedimenting (10S) peak was observed only in gradients containing Δ^9-THC and is believed to be an aggregated form of estrogen receptor complex induced by Δ^9-THC. (*BSA*, bovine serum albumin.)

the capability of Δ^9-THC, and to a lesser extent 11-OH-Δ^9-THC, to compete with estradiol for binding to cytoplasmic estrogen receptors. The ability of the drug to occupy estrogen receptor appears to be limited under the conditions reported here (a maximum of 22 percent of the specific binding sites). It is clear, however, that the compounds responsible for the primary psychoactive effect of marihuana show significant estrogenic binding affinity.

These findings are opposite to those reported by Okey and Truant (*16*) who used a cannabis resin preparation to examine Δ^9-THC inhibition of estrogen binding to cytosol receptors. We have no explanation for their failure to observe competition between their cannabis resin preparation and estrogen receptor. These workers also reported that their cannabis resin had no effect on uterine weight (*16*) and, more recently, Okey and Bondy (*17*), using pure Δ^9-THC, obtained a similar result. It may not be appropriate to compare the effects of pure Δ^9-THC in vitro with those observed with cannabis resin (a mixture of many cannabinoid compounds and related natural products). Moreover, both Solomon *et al.* (*3, 18*) and Shoemaker and Harmon (*19*) provide data consistent with our observations.

While the demonstration of chemical binding to estrogen receptors (as judged from competitive displacement and direct binding experiments) is not, in itself, sufficient evidence to prove estrogenic activity in the full biological sense, recent reports by Solomon *et al.* (*3, 18*) of a uterotropic effect of Δ^9-THC in castrated female rats and by Kolodny *et al.* (*2*) of lowered testosterone levels suggest that this competitive binding may have biological significance.

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