in the 'cumulative administrative costs' between the multi-investigator grant and the 'principal investigator' grant when both the Foundation and the university are considered *together*."

The integrated research programs of the IBP were an experiment. It seems that little is to be gained at this point by debating whether they were a good experiment or a bad experiment. The complexity of the programs defies a simple yes/no answer, and the conclusions reached by individuals examining the data are likely to be correlated with personal experiences and individual preferences. The important observation is that attainment of certain scientific objectives dealing with understanding ecosystems do require large programs. None of the reviews and evaluations have suggested that programs larger than $0.5 million should never be undertaken. Some problems will necessitate programs of this size. Both the mistakes that were made and the mechanisms that tended to work well need to be identified, discussed, and further evaluated.

A detailed analysis of the management successes and failures of the biome programs is still needed. The management structures were a significant part of the IBP experiment, and the evidence may soon be lost as management staff and leaders are siphoned off to other assignments. We feel the scientific community should set about the important task of making concrete suggestions on the management mechanisms to be encouraged in future studies of a similar integrated nature. Certainly EDFB (and the other biome program planners) made mistakes, and some negative conclusions are quite correct. But the reasons for failure or success are *not* dealt with by Mitchell *et al.* and still critically need analysis.

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*A reply to the preceding comment is incorporated in the reply by Downhower and Mayer to letters from Gibson and Blair in this issue (Letters, page 822).*

## Is Delta-9-Tetrahydrocannabinol Estrogenic?

Several authors have reported that various cannabis preparations demasculinize male rodents (*1–4*), depress plasma testosterone levels in men (*5*), and may lead to gynecomastia (breast growth in males) (*6*). Solomon *et al.* (*7*) claim that delta-9-tetrahydrocannabinol ($\Delta^9$-THC) has estrogenic properties; this would be an attractive and simple explanation for the mechanism by which cannabinoids demasculinize or feminize (or both). Unfortunately, the contention that $\Delta^9$-THC has estrogen-like properties is not supported by the bulk of experimental evidence.

Prior to the submission of their report by Solomon *et al.*, our laboratory reported that cannabis (in high doses) demasculinizes male rats but is *not* estrogenic (*2*). In our experiments oral administration of cannabis resin (54 percent $\Delta^9$-THC, 19 percent cannabidiol, 14 percent cannabinol) at doses between 0 and 250 parts per million in the diet caused linear dose-related *decreases* in uterine weight, both in ovariectomized adult female rats and in prepubertal female rats. Uterine weight was decreased even when corrected for the severe loss in body weight which occurred at higher doses. Decreased uterine weight in nonovariectomized adult female rats given chronic oral $\Delta^9$-THC has been reported by Thompson *et al.* (*3*). In preliminary experiments we administered cannabis resin by intraperitoneal injection (as done by Solomon *et al.*); the results were erratic and unreliable, since intraperitoneal injection of large doses of $\Delta^9$-THC leads to inflammation of abdominal organs. Intraperitoneal injection is not a suitable mode of administration for studying the possible uterotrophic effects of $\Delta^9$-THC.

Although all three $\Delta^9$-THC doses used by Solomon *et al.* "had [statistically] significant uterotrophic effects," the data lack one important characteristic necessary for a satisfactory estrogen bioassay; that is, uterine weight did not increase

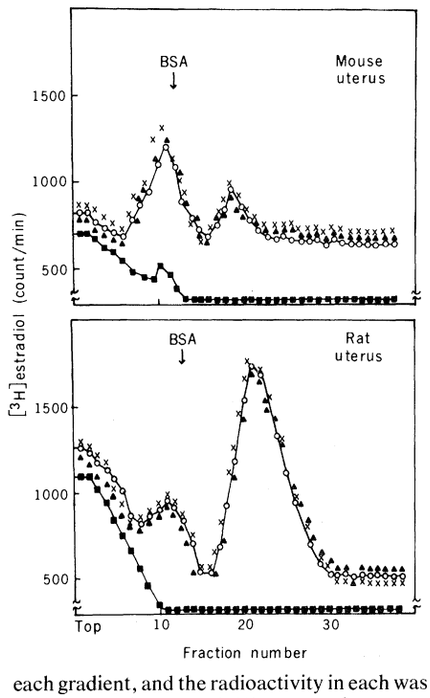


Fig. 1. Lack of effect of delta-9-tetrahydrocannabinol or cannabis resin on [$^3$H]estradiol-17$\beta$ binding to uterine cytosol receptor. Uteri from sexually mature C3H mice (top panel) or 21-day-old Wistar-strain rats (bottom panel) were homogenized in three times their volume of TED buffer (0.01*M* tris-HCl, 0.015*M* ethylenediaminetetraacetic acid, 0.001*M* dithiothreitol; *p*H 7.4). All procedures were done at 0° at 4°C. The homogenates were centrifuged at 4000*g* for 15 minutes, then the supernatant was removed and centrifuged at 105,000*g* for 1.5 hours to obtain a supernatant (cytosol) fraction. Cytosol (about 3.5 mg of protein per milliliter) was incubated overnight with $2 \times 10^{-9}M$ [$^3$H]estradiol-17$\beta$ in the presence or absence of potential competitors for estrogen receptor sites. After incubation the samples were mixed with an equal volume of dextran-coated charcoal (0.5 percent charcoal, 0.05 percent dextran, in TED buffer) to remove unbound steroid. Charcoal was sedimented by centrifugation at 4000*g* for 15 minutes; then samples (0.5 ml) of the supernatants were layered onto linear sucrose gradients (5 to 20 percent) prepared in TED buffer. Gradients were centrifuged at 40,000 rev/min for 22 hours in a Beckman SW.41 rotor at 4°C. Forty 300-$\mu$l fractions were collected from each gradient, and the radioactivity in each was measured by liquid scintillation counting. Bovine serum albumin (*BSA*; 4.6*S*) was used as a marker to determine approximate sedimentation coefficients of the radioactive peaks. Sedimentation peaks for the two samples illustrated correspond to previous reports (*11*) that uterine cytosol receptor sediments predominantly at the "8*S*" in immature rats (bottom panel) and nearer "4*S*" in sexually mature animals (top panel). Competitors were added in 10 $\mu$l of ethanol; an equal quantity of ethanol was added to the control tubes. (○) Binding in control (no competitor); (■) extinction of binding by incubation in the presence of $10^{-7}M$ diethylstilbestrol, a potent synthetic estrogen. Binding to either "4*S*" or "8*S*" receptor is unaltered by incubation in the presence of $10^{-5}M$ THC (x) (95.6 percent pure, NIMH lot No. SC 75518) or by the presence of cannabis resin (54 percent THC; 19 percent cannabidiol; 14 percent cannabinol) equivalent to $10^{-5}M$ $\Delta^9$-THC (▲). Cytosol also was incubated with $10^{-3}M$ $\Delta^9$-THC (data not shown); when that concentration was added to cytosol, a visible flocculence occurred which clarified during overnight shaking. Binding peaks in these samples were equivalent to the control.

linearly with increasing $\Delta^9$-THC doses. By $t$-test, the responses to 1 versus 10 mg of $\Delta^9$-THC per kilogram are not significantly different ($t = 1.606$; d.f. = 12; $P > .10$); uteri from rats treated with 10 mg of $\Delta^9$-THC per kilogram are significantly *smaller* than the 2.5 mg/kg group ($t = 2.305$; d.f. = 12; $P < .05$). It is not unusual for known estrogens to induce nonlinear increases in uterine growth when doses are excessive; however, the maximum uterine growth in any $\Delta^9$-THC–treated group was far below that caused by 2 $\mu$g of the "positive control," estradiol benzoate, per kilogram. At best, the assay reported by Solomon *et al.* represents a poor choice of $\Delta^9$-THC doses or insufficient numbers of animals or of doses. Stimulation of uterine growth does not per se prove that a substance is estrogenic; even androgens are uterotrophic if given in sufficient amounts (*8*).

We have further tested cannabis resin and $\Delta^9$-THC for possible estrogenic activity by in vitro competition for estrogen-receptor sites. It is well known that estrogens (both natural and synthetic) stimulate true uterine growth by first binding to specific cytoplasmic receptor proteins (*9*). Thus, a sensitive in vitro test for potential estrogens can be done by determining whether the substance competes with [$^3$H]estradiol-17$\beta$ for specific, high-affinity binding sites in uterine cytosol. As shown in Fig. 1, neither cannabis resin nor $\Delta^9$-THC has any effect on [$^3$H]estradiol-17$\beta$ binding, even when the cannabinoids are added to the limit of their solubility. Thus, the cannabinoids which demasculinize when given in vivo have no estrogenic properties as assessed by receptor studies on two different rodent species. It might be argued that cannabinoids are metabolized in vivo to estrogenic forms. Many hydroxylated metabolites of cannabinoids have been identified in the rat (*10*), but none of the major metabolites seems to have free hydroxyl or phenolic groups at both extremities of the molecule, as occurs in known estrogens (*11*). Failure of uteri to grow upon oral $\Delta^9$-THC administration (*2, 3*) also does not support the idea that significant amounts of estrogenic metabolites might be formed in vivo. As for other indications that $\Delta^9$-THC is estrogenic, Solomon *et al.* state that "Vaginal smears obtained from rats treated with THC alone . . . were not uniform; however, in general, . . . the epithelial cells, some of which were cornified, were increased over those of the controls." It is difficult to accept this as evidence that $\Delta^9$-THC is estrogenic without seeing data upon which the statement is based.

Solomon *et al.* also raise the specter of estrogen-induced cancers in regard to $\Delta^9$-THC. This seems groundless. Even if their report of $\Delta^9$-THC-stimulated uterine growth could be taken as evidence that $\Delta^9$-THC is estrogenic, $\Delta^9$-THC obviously would be a much weaker estrogen than the "natural" hormone, estradiol. Their own data show that 2 $\mu$g of estradiol benzoate per kilogram per day stimulates twice the increase (over control) in uterine weight that 2.5 mg of $\Delta^9$-THC per kilogram per day produces.

"THC is estrogenic" was a useful hypothesis to attempt to explain its effects on reproductive function. It is refuted by the experimental evidence.

Whether demasculinization and/or feminization by $\Delta^9$-THC occurs in humans still is being argued [(*5*) versus (*12*)]. Attention should now be focused on alternative mechanisms by which cannabinoids might cause reproductive pathologies. These could range from altered pituitary function (*4*) through liver disease (*13*) to effects at the level of DNA (*14*) or interaction of several of these with genetic predisposition. It is very unlikely that the demasculinizing and/or feminizing effects of cannabis are due to simple estrogen-like action.

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Okey and Bondy comment on several aspects of our report (*1*). Some points are well taken. In measuring the uterotrophic effect of $\Delta^9$-THC, we did not use estrogen bioassays. Rather, we used estrogen bioassay techniques. In addition, Okey and Bondy are correct in their assessment of our data: $\Delta^9$-THC is a much weaker estrogen-like compound than estradiol benzoate. Other of their comments are based on inaccurate statements and inappropriate comparisons between the study by Okey and Truant (*2*) and our report (*1*). Okey and Truant examined the effect of oral administration of cannabis resin on uterine weights of prepuberal female rats, and adult rats treated 6 weeks after ovariectomy. We examined the effect of intraperitoneal administration of $\Delta^9$-THC on uterine weights of adult rats treated on the day of ovariectomy (*1*).

Okey and Bondy write "the contention that $\Delta^9$-THC has estrogen-like properties is not supported by the bulk of experimental evidence." They support this statement by the finding of Okey and Truant (*2*) that oral administration of cannabis resin causes a dose-related decrease in uterine weight of intact and ovariectomized rats, even when uterine weights are "corrected for the severe loss in body weight which occurred at high doses." They corroborate that finding with a reference to Thompson *et al.* (*3*) who found decreased uterine weight in nonovariectomized adult female rats given chronic oral cannabinoids.

It should be pointed out that Thompson *et al.* (*3*) studied the chronic oral toxicity of cannabinoids. They (*3*) used doses "chosen specifically to induce toxicity in rats: 50 to 500 mg/kg/day of $\Delta^9$THC or 150 to 500 mg/kg/day of crude marihuana extract for 119 consecutive days." Thompson *et al.* found that "prostate, uterus, ovary and spleen absolute and relative weights were both decreased, while absolute and relative weights of the adrenal were usually increased. The most severe and persistent organ weight changes occurred in the prostate, adrenals and spleen; changes in weights for the uterus and ovary occurred less frequently."

It is difficult to compare the results obtained from studies using oral administration of cannabis resin (*2, 3*) or of large

doses of $\Delta^9$-THC (*3*) with those from studies using intraperitoneal administration of comparatively low doses of $\Delta^9$-THC. The differences in results might be due to the differences in route of administration, or in dosage, or to components in cannabis resin which are not found in $\Delta^9$-THC (*4*, p. 196).

The species, as well as the route of administration, the dose, and the compound, may be involved in the response of the uterus to cannabis. Dixit *et al.* (*5*) reported that the uterine weights of intact female rats injected intraperitoneally with 5 mg per day (25 mg/kg) of cannabis extract for 64 days were not significantly different from those of controls. However, in this same study (*5*), the uterine weights of intact adult mice injected intraperitoneally with 1 mg per day (40 mg/kg) of cannabis extract for 64 days were significantly decreased as compared with controls.

Further, Okey and Bondy write that when Okey and Truant "administered cannabis resin by intraperitoneal injection (as done by Solomon *et al.*); the results were erratic and unreliable, since intraperitoneal injection of large doses of $\Delta^9$-THC leads to inflammation of the abdominal organs. Intraperitoneal injection is not a suitable mode of administration for studying the possible uterotrophic effects of $\Delta^9$-THC."

In reference to this statement, three facts should be noted. First, we did not administer cannabis resin. We injected $\Delta^9$-THC. Second, intraperitoneal administration of 0.2 ml of $\Delta^9$-THC in doses of 1, 2.5, and 10 mg $kg^{-1}$ $day^{-1}$ for 14 days did not result in inflammation. Third, it is generally agreed (*4*, p. 238; *6*) that in chronic studies on laboratory animals, intraperitoneal administration of $\Delta^9$-THC is effective.

In measuring the effect of route of administration on the distribution of [$^{14}$C]$\Delta^9$-THC, Mantilla-Plata *et al.* (*6*) found that: "Total radioactivity of [$^{14}$C]-$\Delta^9$THC in all tissues was lowest after p.o. or s.c. administration, higher after i.p. and highest after i.v. Unfortunately, the i.v. route of administration is impractical in long-term studies and consequently the i.p. route remains as the alternative."

In addition, Okey and Bondy write that "the data lack one important characteristic necessary for a satisfactory estrogen bioassay; that is, uterine weight did not increase linearly with increasing doses of $\Delta^9$-THC."

Our report was concerned with the qualitative effect of $\Delta^9$-THC on uterine weight gain in ovariectomized rats. The data demonstrate that administration of $\Delta^9$-THC at 1, 2.5, or 10 mg $kg^{-1}$ $day^{-1}$ for 14 days induces an increase in uterine weight which is statistically significant ($P < .01$, $P < .001$, and $P < .001$, respectively, compared to ovariectomized controls). The lack of a linear uterine weight response to $\Delta^9$-THC does not negate the uterotrophic activity of $\Delta^9$-THC.

In fact, when ovariectomized rats are treated with estrogens, the uterine weight response curves may or may not be linear. Linearity depends on the estrogen (*7*), the dose range (*8*), and the age of the animal (*9*). When spayed rats are treated with estrone or estradiol, the dose response curve is linear (*8*); the dose responses obtained with estriol and 16 epi-estriol are nonlinear (*7*). A linear uterine weight response to estradiol is obtained in immature ovariectomized rats, but not in mature ovariectomized rats given high doses of estradiol (*8–10*).

As Okey and Bondy state: "It is not unusual for known estrogens to induce nonlinear increases in uterine growth when doses are excessive; however, the maximum uterine growth in any $\Delta^9$-THC–treated group was far below that caused by 2 $\mu$g of the 'positive control,' estradiol benzoate, per kilogram." The fact that $\Delta^9$-THC was not as active as 2 $\mu$g of estradiol benzoate does not negate the estrogen-like effect of $\Delta^9$-THC as measured in uterine weight gain. Indeed, it is known that some estrogens are comparatively weak in inducing uterine weight gain in ovariectomized rats (*7*).

Okey and Bondy also write "none of the major metabolites [of cannabinoids] seems to have free hydroxyl or phenolic groups at both extremities of the molecule, as occurs in known estrogens." While the metabolism of cannabinoids is not completely understood (*11*), it should be pointed out that several steroidal (*8*) and nonsteroidal compounds (*12*) which lack free hydroxyl or phenolic groups at both extremities of the molecule induce uterine weight gain in ovariectomized rats.

Further evidence for the estrogenic activity of $\Delta^9$-THC comes from histologic examination of uterine and vaginal tissues and vaginal smears obtained from control ovariectomized rats, or from spayed rats treated with 2 $\mu$g of estradiol benzoate per kilogram per day or with 1, 2.5, or 10 mg of $\Delta^9$-THC per kilogram per day for 14 days (*13*).

In sum, Okey and Bondy are concerned with the lack of linearity obtained with administration of $\Delta^9$-THC. When ovariectomized rats are treated with estrogens, the uterine weight curves may or may not be linear, depending on the estrogen (*7*), the dose range (*8*), and the age of the animal (*9*). The estrogenic effect of $\Delta^9$-THC in ovariectomized rats, as measured by uterine weight gain and vaginal smear analysis, is reflected in uterine hyperplasia and hypertrophy and proliferation of vaginal epithelium (*13*).

Lastly, Okey and Bondy write ". . . $\Delta^9$-THC obviously would be a much weaker estrogen than the 'natural' hormone, estradiol." While estradiol is not the only "natural" hormone (*14*), we are in complete agreement with Okey and Bondy. Delta-9-THC is a much weaker estrogen-like compound than estradiol benzoate.

*Note added in proof*: Since our work is concerned with the in vivo effects of $\Delta^9$-THC on reproductive tissue, it is unnecessary to comment further on the in vitro studies of Okey and Bondy other than to say that their failure to show that $\Delta^9$-THC competes for estrogen receptor sites is not confirmed by the work of others (*15*).

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