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## Cannabinoids in Male Mice: Effects on Fertility and Spermatogenesis

Abstract. *Exposure of adult male mice to cannabinoids is associated with a reduction in fertility and an increased incidence of chromosomal abnormalities. These effects are evident not only in the treated mice, but also in their untreated male offspring.*

Marijuana and its major psychoactive component, $\Delta^9$-tetrahydrocannabinol (THC), have been reported to alter reproductive functions in several species of laboratory animals and in humans (*1*). In humans, marijuana exposure reduces the sperm count and may result in abnormal or absent acrosomal morphogenesis, incomplete condensation of chromatin in the sperm heads, and inhibition of sperm maturation (*2*). Exposure of male rats to cannabinoids significantly alters the sex ratio of their offspring (*2*). Long-term exposure of male mice to crude marijuana extract (CME) arrests spermatogenesis, causes Leydig cell regression, and results in significant increases in the number of ring and chain translocations in germ cells (*3*).

In the present study, we examined the effects of long-term ingestion of THC, cannabinol (CBN), or cannabidiol (CBD) on fertility and on the meiotic chromosomes of the dividing germ cells in adult male mice. In addition, we determined whether exposure of these males to cannabinoids affects reproduction in their $F_1$ offspring.

The subjects were obtained from our colony of randomly bred mice, which are derived from two inbred strains, Dw/Wf and YS/Ch WF-dw, and from a randomly bred stock, CD-1. Cannabinoids (50 mg/kg) were administered orally three times a week for 5 weeks to groups of 18 mice. On the basis of a body-surface conversion factor of 12 for mice, this dose corresponds to an oral dose of about 4 mg/kg in humans—the equivalent of three marijuana cigarettes containing 1 percent THC (*4*). In mice this dose of THC affects behavior for about 5 hours, which is comparable to the duration of the behavioral effects of a single marijuana smoking episode by a human (*5*). The males were housed with adult females from the third through fifth weeks of treatment and during the first and fourth weeks after treatment. Half of the pregnant females in each group were killed between days 15 and 19 of gestation; the remaining females were allowed to deliver their pups and to raise them. We recorded the number of corpora lutea, resorptions, dead fetuses, viable fetuses, live births, stillbirths, and postnatal deaths and the percentage of females impregnated. The $F_1$ male offspring were weaned at 21 days of age and housed, four to a cage, until adulthood (60 to 80 days), when their reproductive status was assessed.

Six weeks after treatment, the males were bled by cardiac puncture under ether anesthesia for radioimmunoassay of plasma testosterone (*5*). Body and testicular weights were recorded, and testes were randomly sampled from each group and prepared for cytogenetic evaluation.

To specifically evaluate the effects of cannabinoids on germ cells without the confounding effects of mating, four groups of ten males each were treated with sesame oil, the previously described dose of THC or CBN, or CME (25 mg/kg) daily for 5 days. The mice were killed 50 to 60 days after treatment, both testes were removed, and the meiotic chromosomes were prepared in accordance with the method described by Evans *et al.* (*6*).

In an additional experiment, groups of six adult male mice received a single dose of THC or CBN (100 mg/kg), CME (50 mg/kg), or oil (40 μl). The animals were killed 14 days later and the meiotic chromosomes were prepared for study. A minimum of 50 plates showing the chromosomes in diakinesis or metaphase 1 were examined, and all chromosomal abnormalities were recorded.

In the final experiment, the $F_1$ male offspring were tested for fertility, as had been their cannabinoid-treated sires. Female mice that failed to become pregnant were remated with fertile males to verify their fertility. Each $F_1$ male was given the opportunity (during a 1-week cohabitation period) to mate with at least three different females.

Males repeatedly exposed to CBD impregnated significantly fewer females than did control males (Table 1). Also, significantly more prenatal and postnatal deaths resulted from impregnation by CBD-exposed males. Repeated exposure to THC or CBN significantly increased

Table 1. Effect of repeated cannabinoid exposure on fertility, testicular weight, and plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in adult male mice. Values not given as percentages are mean ± standard errors. There were 18 mice per group.

| Treatment | Impregnation rate (%) | Prenatal loss* (%) | Postnatal loss (%) | Weight of testes (mg) | Testosterone (ng/ml) | LH (ng/ml) | FSH (ng/ml) |
|---|---|---|---|---|---|---|---|
| Oil | 80 | 19 | 5 | 332 ± 10 | 9.2 ± 2.9 | 21 ± 4 | 1103 ± 148 |
| THC | 73 | 37† | 10 | 310 ± 10 | 12.3 ± 3.4 | 28 ± 8 | 1190 ± 117 |
| CBD | 60† | 44† | 26† | 299 ± 12 | 7.8 ± 2.1 | 25 ± 7 | 1300 ± 108 |
| CBN | 73 | 50† | 4 | 288 ± 9‡ | 6.1 ± 1.5‡ | 59 ± 4§ | 1604 ± 74§ |

*Refers to pregnancies in which there was evidence of mortality in utero. †Significantly different from corresponding value for oil-treated mice at $P < .05$ (chi-square test). ‡$P < .05$ (analysis of variance and Duncan's test). §$P < .01$.


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Table 2. Chromosomal analysis of primary spermatocytes from cannabinoid-treated male mice. Values are expressed as percentages. There were ten mice per group.

| Treatment | Number of cells analyzed | Univalent chromosomes | Translocation | | | Aneuploidy | | Polyploidy (4N) |
|---|---|---|---|---|---|---|---|---|
| | | | Ring IV | Chain IV | Chain III | Monosomy | Trisomy | |
| Oil | 500 | 4.07 | 0.54 | 0.27 | 0.00 | 0.00 | 0.27 | 3.88 |
| CME (25 mg/kg) | 500 | 1.44 | 1.91* | 3.35* | 0.48* | 2.39* | 4.30* | 5.74* |
| THC (50 mg/kg) | 500 | 15.06* | 3.12* | 1.42* | 0.00 | 4.26* | 9.66* | 5.40* |
| CBN (50 mg/kg) | 500 | 5.05 | 5.05* | 1.08* | 0.36* | 3.97* | 4.33* | 3.97 |

*Significantly different from corresponding value for oil-treated mice at $P < .05$ (chi-square test).

the percentage of pregnancies with fetal loss, but did not affect impregnation rate or postnatal survival. Testicular weight and plasma testosterone concentration were lower in CBN-treated males than in controls 6 weeks after treatment, while plasma gonadotropins were higher. Cytogenetic studies revealed chromosomal abnormalities consistent in frequency with the data reported in Table 2.

In the experiment designed to assess the effects of cannabinoids on genetic material, chromosomal rearrangements (ring and chain translocations) were significantly more frequent in the cannabinoid-treated males than in control males (Table 2). In addition, in cannabinoid-exposed males there was a marked increase in the incidence of aneuploidy, which might have resulted from nondisjunction at a preceding cell division. Several preparations were characterized by a scarce number of cells in metaphase 1 (*7*). There was also a significantly higher frequency of polyploidy in preparations from the THC- and CME-treated mice than in preparations from the controls.

In THC-treated animals the frequency of unpaired sex chromosomes at metaphase 1 (15.06 percent) was significantly higher than in the controls (4.07 percent). Treatment with a single dose of THC, CBN, or CME resulted in depletion of cells in diakinesis or metaphase 1 (Table 2). Fifty percent of the CME-treated animals showed this deficit, while only 33 and 16.5 percent of the THC- and CBN-treated animals, respectively, were affected.

All the $F_1$ male offspring of the CBD-exposed and control sires successfully impregnated the females with whom they were paired. In addition, the resulting litters were normal by the standards of our breeding colony (9 to 12 pups per litter and less than 10 percent fetal mortality). However, among the offspring of the THC- and CBN-treated sires, 36 and 21 percent, respectively, were either infertile or failed to produce normal litters. In litters sired by two $F_1$ males from THC-exposed fathers, one contained an exencephalic fetus and the other a pup (delivered alive at autopsy on day 19 of gestation) with exencephaly, spina bifida, and exteriorized intestines. Testes from the most severely affected group of $F_1$ males were examined cytogenetically. Chromosomal rearrangements were observed in two of the eight animals evaluated. They were associated with small testis size.

These experiments indicate that brief or repeated exposure to psychoactive and nonpsychoactive components of marijuana affects spermatogenesis and fertility in male mice. These effects are not rapidly reversible and may be accompanied by chromosomal rearrangements and by changes in testicular weight and in the concentration of testosterone and gonadotropin. It is not known whether the reduction in fertility and the chromosomal abnormalities are a reuslt of (i) retention of cannabinoids in testicular tissue (*8*) and subsequent local action on gametogenesis or (ii) a reduction in pituitary or gonadal endocrine function. However, the present data indicate that fertility changes may occur in the presence or absence of endocrine correlates. It is doubtful that general toxicity resulting from cannabinoid exposure affected these results, since in our experience no adult male mice have died as a result of oral administration of cannabinoids at these doses. Furthermore, we did not see any significant changes in body weight or other evidence of toxicity in this study.

The evidence that the untreated $F_1$ male offspring exhibited reduced fertility and chromosomal abnormalities markedly similar to those in their drug-exposed sires strongly suggests that the effects of cannabinoids on reproduction are transmissible. It should be emphasized that the $F_1$ male offspring were survivors from matings in which there was evidence of subfertility or perinatal loss, and thus were not the offspring most seriously affected by the cannabinoid exposure of their sires. In addition, undetected chromosomal changes in the form of minor deletions, duplications, or inversions may have been induced as a result of the treatment with cannabinoids, and these may have contributed to the reduced fertility of offspring carrying such chromosomal aberrations. Thus, it is possible that cannabinoids induce mutational changes in the polygenic system which reduce the fertility of $F_1$ offspring. The chromosomal analysis of treated mice and their male offspring and the occurrence of congenital birth defects indicate that cannabinoids are capable of inducing chromosomal aberrations in germ cells and of producing genetic mutations.

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