# EFFECTS OF NEONATAL ADMINISTRATION OF DELTA-9-TETRAHYDROCANNABINOL AND/OR ESTRADIOL BENZOATE (EB) ON REPRODUCTIVE DEVELOPMENT AND FUNCTION OF THE MALE RAT

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## I. INTRODUCTION

There is continuing contention concerning the roles of delta-9-tetrahydrocannabinol (THC) in female and male mammalian reproductive physiology. Studies on the *in vivo* effects of THC, and other cannabinoids, show that cannabinoids may be antiestrogenic (1-3), or estrogenic (4-18), or have no effect on reproductive physiology (190).

In *in vitro* studies on female tissues it has been found that THC does not (3,20,21) and does (22,23) bind to receptors in the cytosol.

In some *in vivo* studies, THC may be antiestrogenic. In intact prepubertal female rodents, cannabis inhibits estradiol-induced uterine hypertrophy (5) and estradiol-induced increases in monoamine oxidase activity (1). In addition, feeding cannabis resin caused a decrease in uterine weights of immature and adult ovariectomized rats (23).

On the other hand, others have found that THC is estrogenic *in vivo*. In adult ovariectomized-adrenalectomized rats, Gordon et al. report that THC induces lordosis (11). Solomon et al. found that THC induces vaginal mucification and incipient cornification, as well as uterine hypertrophy and hyperplasia in adult ovariectomized rats (33).

There are others who find that THC has no effect in female mice. Virgo (19) reports that THC neither blocks nor induces implantation in mated female mice treated with THC alone or with THC plus progesterone.

In a review article, Block et al. discuss similarities in responses to estrogens and cannabinoids in men and male and female rodents (24). In men and male rodents, heavy chronic marijuana use or THC administration have the following estrogenic effects:

In men, marijuana use elicits gynecomastia (25), oligospermia and transient depression in plasma testosterone levels (24,26,10), which may be accompanied by depressed plasma LH levels (24). Depressed testosterone and gonadotropin levels, gynecomastia (27), anti-androgenic effects on secondary sex glands (18,19) and azoospermia (30) are also seen as a result of estrogen administration in men.

In male mice, chronic administration of THC induces arrest of spermatogenesis, regression of Leydig cell tissue and accessory sex glands (7). Chronic THC is found to depress LH secretion (4), stimulate development of male rat breast tissue (9,24) decrease sexual performance (31), increase adrenal weights (11), depress seminal vesicle weights and somatic growth (15) in male rats. Depressed somatic growth (32,33), increased adrenal weight (34), decreased gonadotropin secretion (35), and depressed seminal vesicle weights (36) are also seen in estrogen-treated male rats. These findings lend further support to the possibility that THC administration may have an estrogen-like action in adult male rodents.

In vivo responses to THC depend on several factors, including age and sex of the animal model. Since the effect on the development of reproductive structures and behavior of males treated neonatally with estradiol is well established, we used this model to examine the response of male neonates to THC and estradiol benzoate (EB) alone and in combination.

## II. MATERIALS AND METHODS

Two hundred Sprague-Dawley male pups obtained from Charles River were treated neonatally at 4 days of age with a single subcutaneous (s.c.) dose of either: (a) vehicle alone, (b) estradiol benzoate (EB) alone (0.5, 5.0, 10.0 mg/kg), (c) THC alone (20 mg/kg), or (d) EB plus THC (each dose of EB plus 20 mg/kg THC). Delta-9-THC was kindly supplied by the National Institute on Drug Abuse (NIDA). These animals were placed in one of 3 groups.

Group 1: At 39 days of age, 100 rats were autopsied after chloroform inhalation to examine the effects of treatment on body and reproductive organ weights, and on organ histology of prepubertalmales. Testes and seminal vesicles were excised and weighed at autopsy and immediately placed in modified Bouins solution and cut at 6 microns and stained with hematox-

ylin and eosin. The degree of damage to the seminiferous tubules was assessed using a modification of the method of Maqueo and Kincl (37). Because of the sexual immaturity of rats at 30 days of age, those tubules having spermatids, rather than free spermatozoa in the lumen, were counted. Approximately 1000-15000 tubules from each group were examined.

Group 2: Several animals from each treatment regimen were retained and autopsied at 60 days of age to examine body and reproductive weights and organ histology. When we found that the testes of 60 day old rats appeared to be near normal, we did a continuation study (Group 3).

Group 3: Several rats from each treatment group were retained and injected subcutaneously for 6 consecutive days (96-102 days of age) with the same substance(s) they received at 4 days of age. Three to 6 weeks after the last injection, these animals were examined for mating abilities from days 117-138. Each male rat was placed with proven female breeders. After each successful mating, the number of the pups were weighed and sexed at 21 days of age.

## III. RESULTS

In testes from vehicle-injected controls autopsied at 39 days of age, the seminiferous tubules have intact germinal epithelia. Spermatogonia are abundant, as are the more advanced stages of spermatogenesis. Spermatids are present in the majority of tubules and nearing the last stages of maturation. Interstitial connective tissue completely fills the spaces between seminiferous tubules, and Leydig cells for the most part, are present in small clusters of 3-8 cells between tubules. Histology is seen in Figure 1, weights of the testes and seminal vesicles are shown in Table I.

Compared to controls (Table I, Figures 1) testes removed from animals treated with graded doses of EB alone and sacrificed at 39 days of age are atrophied (Table I and Figures 3 and 4). The atrophied testicular germinal epithelium and interstitial tissue is accompanied by decrease diameter and spermatid content of the seminiferous tubules.

In testes removed from rats treated with EB the diameters of the germinal epithelium appear to narrow as the doses of EB are increased. Concomitant with the decreasing diameter is an increase in interstitial spaces. The amount of loose areolar connective tissue normally filling the interstitial space is reduced. Although the interstitial connective tissue has atrophied, Leydig cells seem comparable to the controls. As seen in Table II, there is a dose-response decrease in testes and seminal vesicle weights in CB-treated rats.

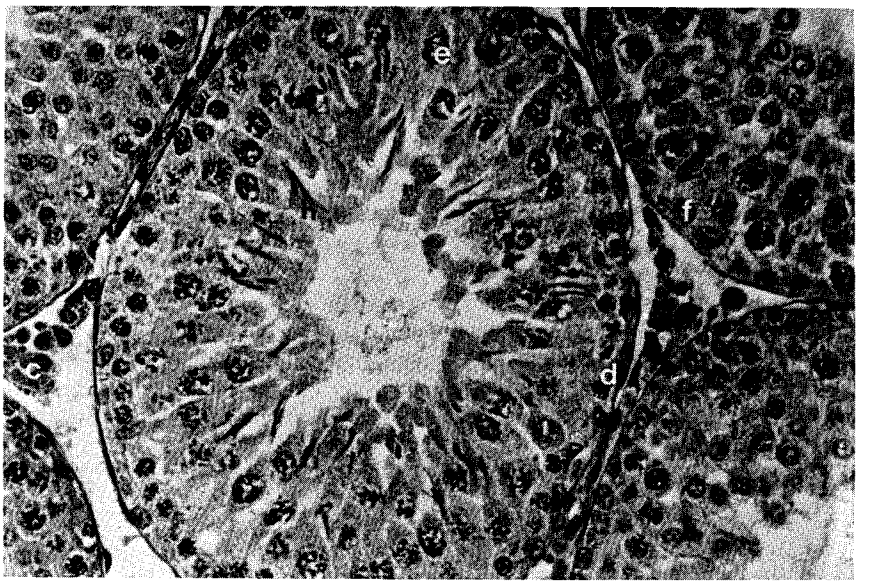


FIGURE 1. Testis: Control rat; labels: Leydig cells (c), spermatogonia (d), primary spermatocytes (e), Sertoli cells (f), spermatids (g), and spermatozoa (h) (400 X).

On the level of the light microscope, testes from 39 day old males treated with 20 mg/kg THC alone (Figure 2) are found to differ from controls only in the diameter of the seminiferous tubules. The germinal epithelium and the interstitial connective tissue all appeared similar to that of control animals. As seen in Table I, the testes and seminal vesicle weights of the THC-treated animals do not differ from those of controls. The testes of animals treated with combined doses of EB plus 20 mg/kg THC were more markedly affected than those treated with EB alone (Figures 5,6). In these doubly-injected rats, there was a sharp reduction in tubular diameters with an increasing amount of empty spaces between tubules. There was, primarily, a reduction in maturing spermatids (Figure 5). With the largest dose of EB (10 mg/kg) and THC, the entire organization of the testes is disrupted. Debris from degenerating germinal epithelium accumulates in the lumen of a large number of the tubules, and the outer periphery of many tubules lose their outlines (Figure 6). It appears that the majority of the cells at this dose level are moribund. However, spermatogenesis was observed in tubules from all groups, even those tubules with the most damage although in these there were no maturing spermatids.

TABLE I. Body and Organ Weights, and Number of Spermatid-Containing Seminiferous Tubules in Male Neonates Treated at 4 Days of Age and Sacrificed at 39 Days of Age

| Dose (mg/kg) | Mean[a] Body Weight (g) | Mean[a] Testes Weight[c] | Mean[a] Seminal Vesicle Weight[c] | Tubules with Spermatids[b] Total Tubules Counted | % | % of Control |
|---|---|---|---|---|---|---|
| Control | 182.1 (3.29) (10) | 961.11 (38.44) (10) | 100.38 (12.34) (10) | 690/1079 | 63.95 | 100.0 |
| 0.5 EB | 181.59 (6.76)+ (10) | 767.46 (42.01)* (10) | 67.76 (6.7)* (10) | 1010/1546 | 65.33 | 102.12 |
| 5.0 EB | 188.48 (4.11)++ (10) | 607.0 (45.63)** (10) | 52.80 (12.09)** (10) | 144/1314 | 10.96 | 17.14 |
| 10.0 EB | 170.57 (3.76)++ (10) | 552.06 (24.11)++ (10) | 40.7 (8.26)++ (10) | 42/1024 | 4.1 | 6.41 |
| 0.5 EB + 20.0 THC | 168.53 (4.62)* (10) | 849.58 (32.19)* (8) | 62.11 (7.8)** (9) | 383/1043 | 36.72 | 57.42 |
| 5.0 EB + 20.0 THC | 161.22 (8.1)# (9) | 506.46 (40.30)++ (8) | 53.0 (6.97)+ (8) | 272/1584 | 17.17 | 26.85 |
| 10.0 EB + 20.0 THC | 151.16 (9.75)*** (10) | 477.92 (57.50)++ (9) | 49.41 (8.98)*** (8) | 52/1440 | 3.61 | 5.65 |
| 20 THC | 190.42 (4.39) (10) | 902.64 (27.62) (10) | 103.4 (7.33) (10) | 832/1291 | 64.45 | 100.78 |

[a]Values represent means (+/- S.E.M.) The number below in ( ) represents the nubber of animals per group. Means were tested for significant differences using Student t's test. The symbols represent the following: * = $p < 0.05$; ** = $p < 0.25$; *** = $p < 0.01$; + = $p < 0.005$; ++ = $p < 0.001$; # = $p < 0.1$. Compared to controls, In only one dose 5.0 EB + the mean significantly different ($p < 0.01$) from the corresponding EB dose alone (5.0 EB).

[b]At least three animals per dose were examined. Approximately 1000-1500 tubules from each animal were assessed. [c]Units are in mg/100 g b.w.

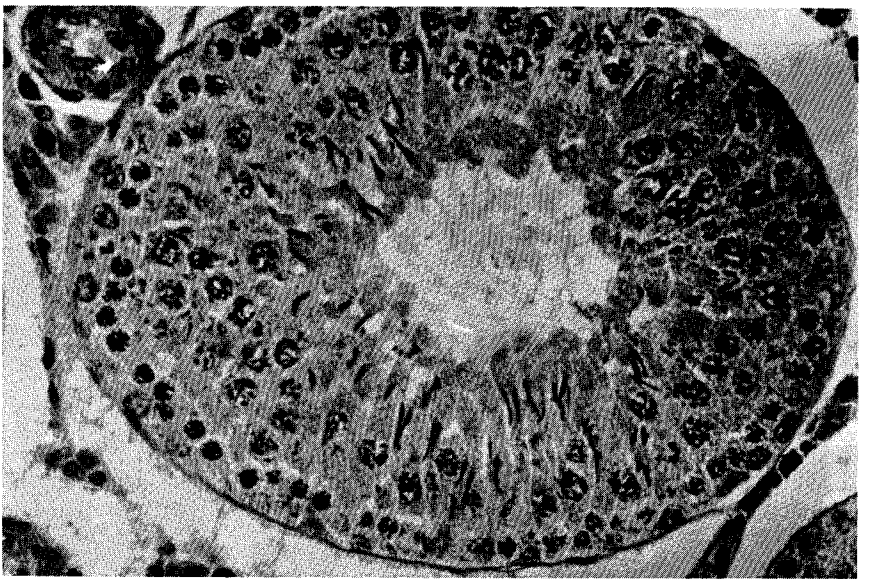

FIGURE 2. Testis: Rat treated with 20 mg/kg THC (400X).

The testes, seminal vesicles, and body weights (Table I), of 39 day old males are, with two exceptions, consistently less when THC and EB are administered simultaneously (See testes weights of 0.5 EB + 20 THC and seminal vesicle weights of 10.0 EB and 20 mg/kg THC). When animals are treated with EB plus THC, the testes are more adversely affected then when compared to animals treated with EB alone. In essence, as with the histological results, THC augments the response to EB.

Despite the normal appearance of the testes in light microscopic studies of 60-75 day old male rats treated neonatally with all doses of estrogen, there is a difference in their mating capacities when examined at 4 months of age after treatment. These four-month old rats were treated again at 96-102 days of age with the same substances they received at 4 days of age (See Materials and Methods, Group 3).

When rats are injected neonatally and at 4 months of age with low doses of EB (0.5 mg/kg) alone and 0.5 mg/kg EB in combination with THC (See Table II), the males sire litters (3/4 and 2/3 respectively), when mated three to six weeks after the last injection. On the other hand, when similarly treated animals are injected with larger doses of EB, 5 and 10 mg/kg, alone or in combination with THC, the males are unable to sire litters (Table II). Kincl et al. found that rats

TABLE II. Effect of Graded Doses of EB Alone or in Combination with 20 mg/kg THC on Mating Proficiency of Treated Males 2-6 Weeks After the Last of the Chronic Injections. (Group 3)

| Group | Male Treatment mg/kg Body Weight | Number of Males Mated | Females Giving Birth | Total Number In Litter | Average Weight Of Litter[a] | Number of Females In Litter | Average Weight of Females in Litter | Number of Males In Litter | Average Weight Of Males In Litter[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Vehicle control | 3 | 3 | 12 | 47.7 | 7 | 47.4 | 5 | 48.3 |
| | | | | 13 | 38.4 | 4 | 36.9 | 9 | 39.0 |
| | | | | 11 | 43.3 | 4 | 40.2 | 7 | 45.2 |
| 2 | 0.5 EB | 4 | 3 | 12 | 55.1 | 4 | 53.2 | 8 | 56.0 |
| | | | | 6 | 80.0 | 2 | 77.0 | 4 | 81.5 |
| | | | | 11 | 53.2 | 5 | 52.0 | 6 | 54.2 |
| 3 | 5.0 EB | 3 | 0 | - | - | - | - | - | - |
| 4 | 10.0 EB | 3 | 0 | - | - | - | - | - | - |
| 5 | 0.5 EB + 20 THC | 3 | 2 | 9 | 58.1 | 5 | 57.8 | 4 | 58.4 |
| | | | | 2 | 68.6 | 1 | 86.8 | 1 | 72.5 |
| 6 | 5.0 EB + 20 THC | 3 | 0 | - | - | - | - | - | - |
| 7 | 10.0 EB + 20 THC | 4 | 0 | - | - | - | - | - | - |
| 8 | | 3 | 3 | 13 | 44.5 | 7 | 46.7 | 6 | 48.5 |
| | | | | 13 | 45.1 | 5 | 46.7 | 8[b] | 44.2 |
| | | | | 10 | 54.8 | 4 | 51.2 | 6 | 57.2 |

[a]Pups were weighted at 21 days of age; weight in grams.
[b]One male pup was a runt.

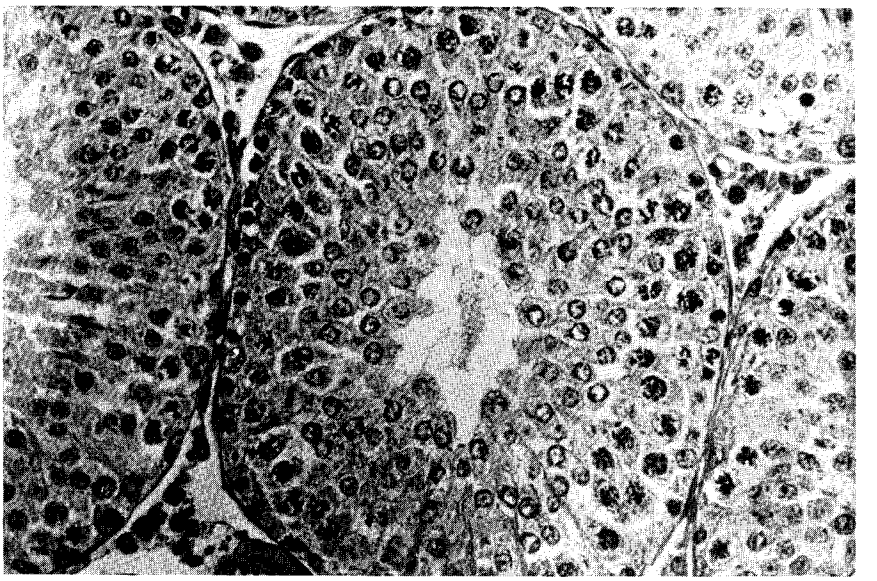

FIGURE 3. Testis: Rat treated with 0.5 mg/kg THC (400 X).

treated perinatally with estrogen were protected from development of testicular atrophy by neonatal administration of progesterone (38).

Brown-Grant et al. tested the fertility of his neonatally estrogen-treated males by placing a single male with a single pro-estrous female overnight (35). We mated our males in a similar fashion with proven female breeders at estrus.

When mating behavior was examined, we found that rats treated with THC alone showed increased mount and ejaculation latencies, and decreased total number of mounts and intromissions, and ejaculations.

## IV. DISCUSSION

The effects of estradiol benzoate on the maturation of the testis of neonatally-treated rats have been documented (35,37,39). The results of the present study are in agreement with these previous studies and indicate that the degree of abnormality in testis and seminal vesicle weights and testis histology is dependent on estrogen dose and age of animal at autopsy.

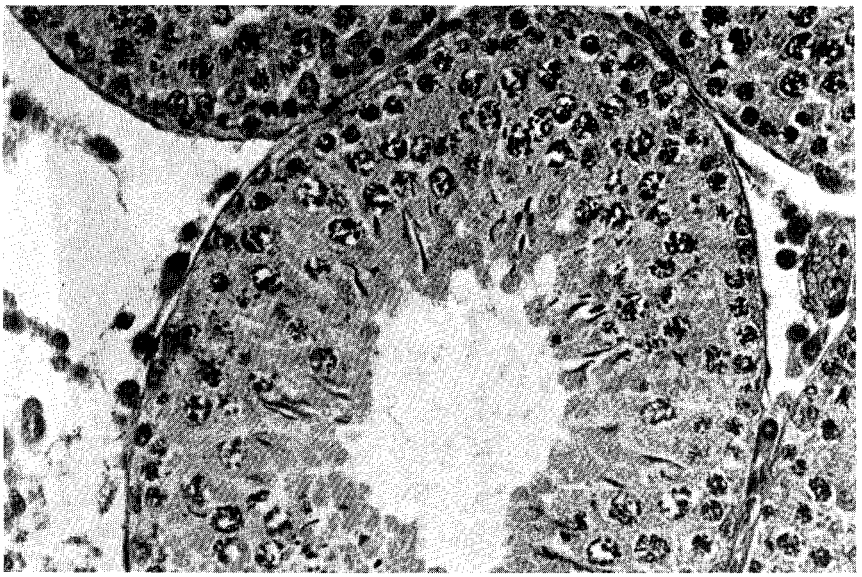

FIGURE 4. Rat treated with 10 mg/kg EB (400 X).

Maqueo and Kincl injected 5 day old animals with 6 to 24 mg/kg EB, autopsied the rats at 45-60 days of age and observed a dose-dependent pathology of the testis (37). Six mg/kg EB produced only slight changes, administration of 24 mg/kg EB caused a nearly complete degeneration of the germinal epithelium in 100% of the seminiferous tubules examined. Less severe atrophy was noted when EB was injected into rats 10 and 20 days old.

Brown-Grant et al. using a similar experimental design, also found degenerated tubules and decreased testes weights (35). When compared to controls, seminiferous epithelium had atrophied and testes and seminal vesicle weights were significantly reduced in EB-treated (25 mg/kg) animals autopsied at 40 days of age. In our experience, the weights of the seminal vesicles and testes were significantly reduced in rats treated at 4 days of age with 0.5 mg/kg EB, 5.0 mg/kg EB, and 10 mg/kg EB and examined at 39 days of age (Table I). Compared to controls, the number of spermatids in the tubules is decreased only with 5.0 and 10.0 mg/kg EB. The number and percent of tubules with spermatids was similar in controls and animals treated with 0.5 mg/kg EB (Table I). Histological examination shows that compared to control (Figure 1), the testes of animals treated with 0.5 mg/kg EB (Figure 3) appear similar to controls. The testes of animals treated with 5 mg/kg EB (no

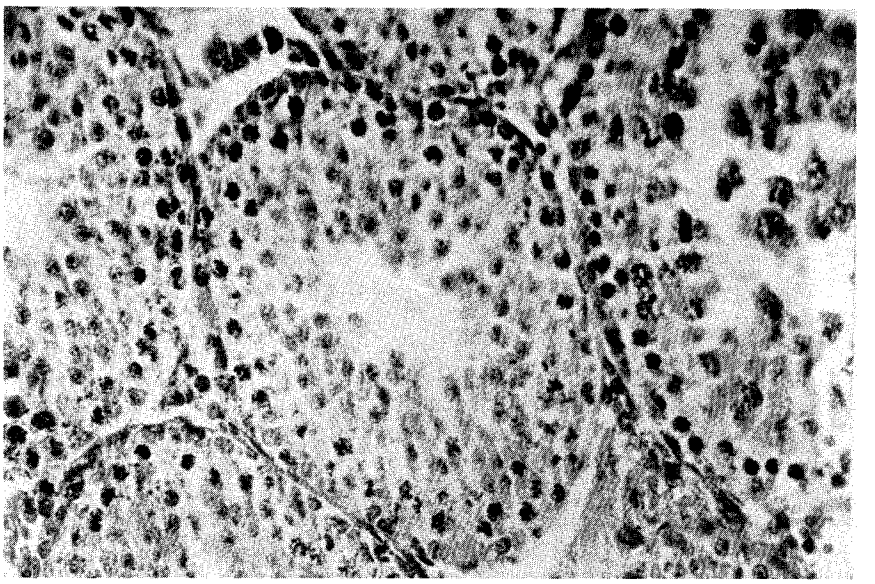

FIGURE 5. Rat treated with 5 mg/kg EB + 20 mg/kg THC (400 X).

figure shown) are less damaged than those treated with 10 mg/kg EB (Figure 4).

Additionally, Brown-Grant et al. observed that three-fourths of the animals sacrificed at 60 days of age had nearly all recovered from their neonatal treatments (35). No differences in the seminiferous epithelium were noted after 75 days. In our experience, 60 day old males treated with EB neonatally appeared to have near normal testes cytology with light microscopy.

The effects of direct THC administration to neonatal males on reproductive development have not been reported. However, Dalterio also found that male mice treated perinatally with THC increased mount latencies, and a decreased number of mounts and intromissions when tested as adults (40). Most studies have dealt with the effects of THC on prepubertal or adult rodents. Numerous reports have appeared indicating that THC caused a depression of testicular endocrine function of adult rats and other mammals (for review, see 24). The evidence, however, is far from conclusive. Cumulative doses greater than 1 gram administered over periods of 28-180 days led to small increases in testes weights (41,42), while with lower cumulative doses testes weights remained the same or were slightly lower than controls (4,43,44).

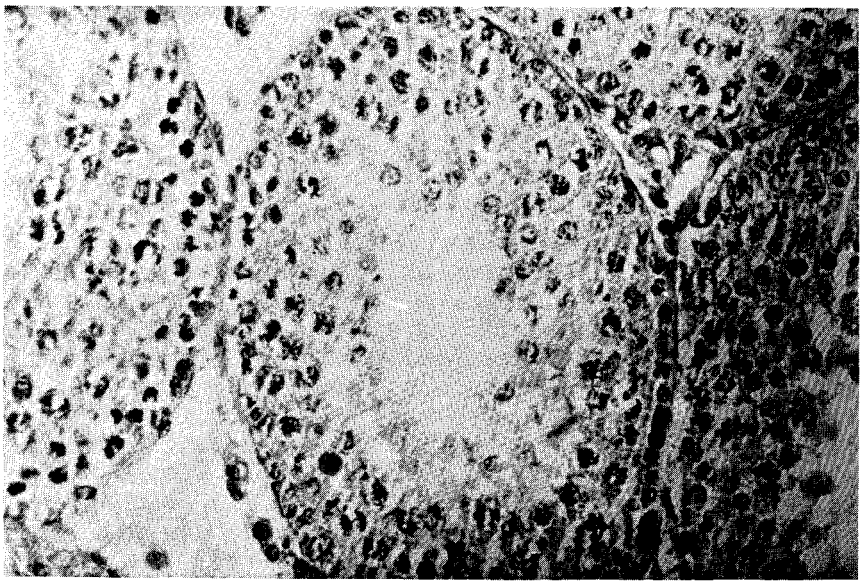

FIGURE 6. Rat treated with 10 mg/kg EB + 20 mg/kg THC (400 X).

In this study, a single injection of THC (20 mg/kg body weight) at 4 days of age reduced the seminiferous tubule diameter (Figure 2) when examined at 39 days of age. However, the histology of the testes of THC-treated rats was comparable to controls: 63.95% of the tubules from the treated rats contained spermatids; this is comparable to the control value of 64.45% (Table I). By day 60, tubule diameters had increased and were indistinguishable from controls. In another study, Dixit et al. administered 2 mg cannabis/day chronicallyto young adult mice and noted a significant decrease in the diameter of the seminiferous tubules (7). However, sixty-three days after the last dose of cannabis, the diameters were comparable to the controls.

The results of the mating studies show that a single dose of THC (20mg/kg at 4 days of age) had no effect on the ability of treated males to sire litters. All males treated with 20 mg/kg THC alone successfully mated with untreated females which became pregnant and gave birth 21-23 days after mating.

Despite the fact that 20 mg/kg THC alone had no observable effect on testes and seminal vesicles, weights and spermatid counts (Table I), or on testes cytology (Figure 4), the cytology of the seminal vesicles was affected by THC (see other paper in this volume). When 20 mg/kg THC, and EB (0.5, 5.0

and 10.0 mg/kg) are administered together, the effect of EB was markedly enhanced in a dose-related manner on both testes weights (Table I), as well as on testes histology (Figures 5,6).

## V. SUMMARY

Two hundred male rats were treated neonatally at 4 days of age with a single subcutaneous (s.c.) dose of either: (a) vehicle alone, (b) estradiol benzoate (EB) alone (0.5, 5.0, 10.0 mg/kg), (c) THC alone (20 mg/kg), or (d) EB plus THC (each dose EB plus 20 mg/kg THC). When some of these animals were autopsied at 39 days of age, there was a dose-response decrease in testes and seminal vesicle weights for EB-treated rats. The testes histology also shows a dose-response decrease in architecture to EB administration. Testes obtained from 39-day old rats treated with THC show no changes in weight or cytology when compared to controls. However, testes from animals treated with EB plus THC show that THC augments the effect of EB. By 60 days of age, testes cytology from all groups appeared normal by light microscope examination.

Some members of each group were treated for 6 consecutive days (days 92-106) with the same compound(s) they received at 4 days of age. These retreated rats were studied for reproductive behavior three to six weeks after the last injection. All of the males treated with vehicle alone or THC alone sired litters. However, sexual behavior was affected by administration of THC alone. Some of the animals treated with a low dose of (0.5 mg/kg) of EB alone and in combination with THC sired litters (3/4 and 2/3, respectively). None of the animals treated with higher doses (5.0 and 10.0mg/kg EB alone) or in combination with THC sired litters.

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## REFERENCES

1. Chakravarty, I., Sengupta, D., Bhattacharya, P. and Ghosh, J. J., Effect of cannabis extract on the uterine

monoamine oxidase activity of normal and estradiol treated rats, Biochem. Pharmacol. 25:377-378 (1975).
2. Chakravarty, I., Sengupta, D., Bhattacharya, P., and Ghosh, J. J., Effect of treatment with cannabis extract on the water and glycogen content of the uterus in normal and estradiol-treated rats, Toxicol. Applied Pharmacol. 34:513-516 (1975).
3. Okey, A. B., and Truant, G. S., Cannabis demasculinizes rats but is not estrogenic, Life Sciences 17:1111-1118 (1975).
4. Collu, R., Letarte, J., Leboeuf, G., and Ducharme, J. R., Endocrine effects of chronic administration of psychoactive drugs to prepubertal male rats. I: Delta-9-tetrahydrocannabinol, Life Sciences 16(4):533-542 (1975).
5. Dalterio, S., and Bartke, A., Perinatal exposure to cannabinoids alters male reproductive function in mice, Science 205:1420-1422 (1979).
6. Dixit, V. P., Arya, M., and Lottiya, N. K., The effect of chronically administered cannabis extract on the female genital tract of mice and rats, Endocrinologie 66:365-368 (1975).
7. Dixit, V. P., Sharma, V. N., and Lohiya, N. K., The effect of chronically administered cannabis extract on the testicular function of mice, Eur. Pharmacol. 26:111-114 (1974).
8. Gordon, J. H., Bromley, G. L. Gorski, R. A., and Zimmerman, E., Delta-9-tetrahydrocannabinol enhancement of lordosis behavior in estrogen-treated female rats, Pharmacol. Biochem. Behav. 8: 603-708 (1978).
9. Harmon, J. W., and Aliopoulios, M. A., Marijuana-induced gynecomastia: clinical and laboratory experience, Surg. Forum 25:423-425 (1974).
10. Kolodny, R.C., Lessin, P., Toro, G., Masters, W. H., and Cohen, S., Depression of plasma testosterone with acute marijuana administration, in "Pharmacology of Marihuana", Vol. 1 (M. C. Braude and S. Szara, eds.), pp. 217-225. Raven Press, New York, 1976.
11. Ling, G. M., Thomas, J. A., Usher, D. R., and Singhal, R. L., Effects of chronically administered delta-1-tetrahydrocannabinol and luteinizing hormone secretion, Prog. Brain Res. 39:331-338 (1973).
12. Marks, B. H., Delta-1-tetrahydrocannabinol and luteinizing hormone secretion, Prog. Brain Res. 39:331-338 (1972).
13. Merari, A., Barak, A., and Plaves, M., Effects of delta-1-tetrahydrocannabinol on copulation in the male rat, Psychopharmacol. (Berl) 28:243-246 (1973).
14. Nir, I., Ayalon, D., Tsafriri, A., Corodova, T., and Linder, H. R., Suppression of cyclic surge of luteinizing

hormone secretion and of ovulation in the rat by delta-9-tetrahydrocannabinol, Nature (London) 244:470-471 (1973).
15. Solomon, J., and Shattuck, D., Marihuana and sex, New Eng. J. Med. 291:309 (1974).
16. Solomon, J. Cocchia, M. A., Gray, R., Shattuck, D., and Vossmer, A., Uterotrophic effect of delta-9-tetrahydrocannabinol in ovariectomized rats, Science 192:559-561 (1976).
17. Solomon, J., and Cocchia, M. A., Is delta-9-tetrahydrocannabinol estrogenic? Science 195:905-906 (1977).
18. Solomon, J., Cocchia, M. A., and DiMarino, R., Effect of delta-9-tetrahydrocannabinol on uterine and vaginal cytology of ovariectomized rats, Science 195:875-877 (1977).
19. Virgo, B. B., The estrogenicity of delta-9-tetrahydrocannabinol (THC): THC neither blocks nor induces ovum implantation, nor does it effect uterine growth, Res. Comm. Chem. Path. Pharmacol. 25:65-77 (1979).
20. Okey, A. B., and Bondy, G. P., Delta-9-tetrahydrocannabinol and 17-beta-estradiol bind to different macromolecules in estrogen target tissue, Science 200:312-314 (1978).
21. Smith, R. G., Besch, N., and Besch, P. K., Inhibition of gonadotropin delta-9-tetrahydrocannabinol: mediation by steroid receptors? Science 204:325-327 (1979).
22. Rawitch, A. B., Schultz, G. S., Ebner, K. E., and Vardaris, R. M., Competition of delta-9-tetrahydrocannabinol with estrogen in rat uterine estrogen receptor binding, Science 197:1189-1191 (1977).
23. Shoemaker, R. H., and Harmon, J. W., Suggested mechanism for demasculinizing effects of marijuana, Fed. Proc. 36:345 (1977).
24. Block, E., Thysen, B., Morril, G., Gardner, E., and Fujimoto, G., Effects of cannabinoids on reproduction and development, Vitamins and Hormones 36:203-258 (1978).
25. Harmon, J. W., and Aliapoulis, M. A., Gynecomastia in marijuana users, N. Eng. J. Med. 287:936 (1972).
26. Kolodny, R. C., Master, W. H., Kolodner, R. M., and Toro, G., Depression of plasma testosterone levels after chronic intensive marijuana use, N. Eng. J.Med. 290:872-874 (1974).
27. Bronstein, I. P., and Shadaksharappa, K. S., Gynecomastia, in "Progress in Clinical Endocrinology" (Soskin, ed.), Greene and Stratton, New York, 1950.
28. Huggins, C., and Clark, P. J., Quantitative studies of prostatic secretions. II, J. Exp. Med. 72:747 (1940).
29. Huggins, C., Control of cancers of man by endocrinological methods, Cancer Res. 16:825-830 (1956).

30. Heckel, J. J., and Steinmetz, C. R., Production of azoospermia in man from use of estradiol benzoate (progynon B), Proc. Soc. Exp. Biol. Med. 46:174 (1941).
31. Merari, A., Barak, A., and Plaves, M., Effects of delta-1-tetrahydrocannabinol on copulation in the male rat, Psychopharmacol. (Berl) 28:243-246 (1973).
32. Bull, L., Hurley, W., Kennett, Tamplin, C., and Williams, W., Effects of sex hormones on feed intake in rats, J. Nutr. 104:968-975 (1974).
33. Shai, F., and Wallach, S., Interactions of estradiol and calcitonin on the rat skeleton, Endocrinol. 73:253-260 (1973).
34. Kitay, J. I., Pituitary-adrenal function in rat after gonadectomy and gonadal function replacement, Endocrinol. 73:253-260 (1963).
35. Brown-Grant, K., Fink, G., Grief, F., and Murray, M. A. F., Altered sexual development in male rats after oestrogen administration during the neonatal period, J. Reprod. Fert. 44:25-42 (1975).
36. Greep, R. O., and Jones, I. C., Steroid control of pituitary function, Rec. Prog. Hormone Res. 5:197-254 (1950).
37. Macque, M., and Kincl, F. A., Testicular histo-morphology of young rats treated with oestradiol-17-benzoate, Acta Endocrinol. Copehn. 46:25-30 (1964).
38. Kincl, F. A., and Maqueo, M., Prevention by progesterone of steroid-induced sterility in neonatal male and female rats, Endocrinol. 77:859-862 (1965).
39. Kincl, F. A., Pi, A. F., and Lasso, L. H., Effect of estradiol benzoate treatment in the newborn male rat, Endocrinol. 72:966-968 (1963).
40. Dalterio, S., Badr, F., Bartke, A., and Mayfield, D., Cannabinoids in male mice: effects on fertility and spermatogenesis, Science 216:315-316 (1982).
41. Rosekrantz, H., and Braude, M. C., Comparative chronic toxicities of delta-9-tetrahydrocannabinol administered orally be inhalation in rat, in "The Pharmacology of Marijuana" (M. C. Braude and S. Szara, eds.), pp. 517-584. Raven Press, New York, 1976.
42. Rosenkrantz, H., Sprague, R. A., Fleishmann, R. W., and Braude M. C., Oral delta-9-tetrahydrocannabinol toxicity in rats treated for periods up to 6 months, Toxicol. Appl. Pharmacol. 32:399-417 (1975).
43. Collu, R., Endocrine effects of chronic intraventricular administration of delta-9-THC to prepubertal and adult male rats, Life Sciences 18:223-230 (1976).
44. Harmon, J. W., Locke, D., Aliapoulious, M. A., and Macimdoe, J. H., Interference with testicular development by delta-9-THC, Surgical Forum 27:350-352 (1976).