EFFECTS OF CANNABINOIDS ON SPERMATOGENESIS IN MICE: IN VIVO AND IN VITRO STUDIES

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I. INTRODUCTION

The mechanism of action of marijuana on somatic and germinal cells is controversial. An area of concern is whether cannabinoids act directly or indirectly on the cellular system. A direct effect may involve interaction with cell membranes (10,11), macromolecular synthesis (1,12,15,18,22), DNA replication (2) and the mitotic mechanism (13,14). An indirect effect on these systems may be mediated via metabolites of the cannabinoids as well as via alterations of hormonal levels.

Numerous studies have revealed the potential adverse effects of marijuana on reproductive function. Reduced fertility, aberrations of the repertoire of reproductive behaviors and altered hormonal levels have been reported in rodents and primates. There is increasing evidence that cannabinoids can interfere, either directly or indirectly, with testicular function. Our laboratory is interested in determining the effects of marijuana on spermatogenesis. Spermatogenic differentiation is an intricate process involving neurohormonal control and an as yet undefined control of gene expression in the seminiferous tubule. Cannabinoids may interfere with the hormonal regulation of spermatogenesis at the hypothalamic, pituitary or testicular level. Alternatively, cannabinoids

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may exert some of their effects directly of the differentiating spermatogenic cells. Thus a brief review of the effects of cannabinoids on spermatogenesis and macromolecular synthesis in somatic and germinal cells is pertinent.

A. In Vivo Effects of Cannabinoids on Spermatogenesis

1. Cytogenetic Studies. Treatment of hybrid male mice genotype (C57BL X C3H)F_1 with cannabinol (CBN; 10 mg/kg), cannabidiol (CBD; 10 mg/kg) and delta-9-tetrahydrocannabinol (THC; 10 mg/kg) for 5 days led to an increased incidence of ring and chain translocations in pachytene spermatocytes (25). The animals were sacrificed 16 days after the last treatment and, thus, pachytene spermatocytes scored were assumed to be at the spermatogonium stage when exposed to the cannabinoids.

Exposure of male mice to cannabinoids has been reported to lead to a reduction in fertility and an increased incidence of chromosomal abnormalities (3). Treatment of mice orally with THC (50 mg/kg), CBN (50 mg/kg) or crude marijuana extract (CME; 25 mg/kg) daily for 5 days led to an increase of chromosomal abnormalities in pachytene spermatocytes 50 to 60 days later. The abnormalities included ring and chain translocations, aneuploidy (THC, CBN or CME treatment), univalent chromosomes (THC treatment) and polyploidy (CME or THC treatment). Treatment of mice with a single dose of THC or CBN (100 mg/kg) or CME (50 mg/kg) led to a reduction of cells in diakinesis or metaphase I. Thus it appears that exposure of spermatogonia to cannabinoids in vivo can lead to chromosomal abnormalities, while exposure to primary spermatocytes may retard their progression to metaphase I and diakinesis.

2. Sperm Morphology. The induction of abnormal sperm head morphology by cannabinoid treatment has been reported by Zimmerman and co-workers (24). Treatment of hybrid male mice genotype (C57BL X C3H)F_1 for 5 consecutive days with THC (5 and 10 mg/kg) and CBN (10 and 25 mg/kg) led to a statistically higher incidence of abnormal sperm than the vehicle (dimethylsulfoxide; DMSO) treated controls. Since the mice were sacrificed 35 days after the last treatment, sperm scored were assumed to be in the primary spermatocyte stage when exposed to the cannabinoids.

Hembree and co-workers (5) reported that heavy use of marijuana caused a reduction of total sperm count and of concentration of sperm in the ejaculate of human subjects but did not significantly affect the percentage of sperm displaying normal morphology. However, in a later study (6), they reported a reduction of total sperm count and concentration which was accompanied by a decrease in the number of sperm

with normal morphology. No evidence for a hormonal effect was observed and their studies suggested a direct effect of marijuana on the germinal epithelium during spermiogenesis. Similar conclusions were drawn from studies (7) using rats, in which cauda epididymal sperm from rats exposed to marijuana smoke displayed a higher incidence of dissociated heads and tails. This study also reported a reduction of epididymal sperm count and histological abnormalities of the seminiferous tubules, including aberrant cellular associations andearly release of spermatocytes and spermatids.

Incomplete or absent acrosomal differentiation has been reported in sperm from chronic hashish users (8). In the same study, forms of sperm were found in the ejaculates that suggested the occurrence of spermatidic maturation arrest in these chronic hashish users.

The results of these various studies suggest that in vivo exposure to cannabinoids may interfere with spermatogenic differentiation during the primary spermatocyte or spermatid stages. It has been suggested that the occurrence of abnormal sperm head morphology may serve as an assay method for screening potentially mutagenic agents (20). However, other studies have shown that abnormal sperm morphology in mice does not result from chromosome translocation nor from chromosomal imbalance within the haploid cell (21). The mechanism of induction of abnormal sperm head morphology remains to be elucidated.

B. In Vitro Effects of Cannabinoids on Macromolecular Synthesis

Several studies have suggested that cannabinoid effects on macromolecular synthesis are mediated by the availability of macromolecular precursors. A cannabinoid-induced reduction of the intracellular pool of particular precursors (eg., thymidine or uridine or their nucleotide forms) can lead to a quantitatively similar reduction of incorporation of these precursors into macromolecules (eg., DNA or RNA). This has been demonstrated in HeLa cells (15,18), human lymphocytes (4), rat testicular slices and cell suspensions (9) and in Tetrahymena pyriformis (23). It has been suggested that micromolar concentrations of cannabinoids may inhibit intracellular precursor pools by dissolving into membranes, altering their conformation and as a consequence, inhibiting membrane-bound transport systems (4).

II. IN VITRO AND IN VIVO EFFECTS OF CANNABINOIDS ON URIDINE METABOLISM IN PURIFIED SPERMATOGENIC CELLS

The present studies are part of a program to investigate the effects of specific cannabinoids on spermatogenic differentiation. In view of the reports on the effects of cannabinoids on spermatogenic differentiation and macromolecular synthesis in somatic cells and in mixed rat testicular cells, we decided to investigate whether cannabinoids affect macromolecular synthesis in purified populations of spermatogenic cells. Spermatogenic cells were obtained from mice and homogeneous populations of pachytene primary spermatocytes and round spermatids were obtained by sedimentation at unit gravity. The ability of cells from cannabinoid-treated miceto incorporate uridine in culture into the acid-insoluble fraction was compared to incorporation by cells isolated from vehicle-treated mice to access in vivo cannabinoid effects. In vitro cannabinoid effects were investigated by studying the metabolism of uridine by pachytene spermatocytes and round spermatids (isolated from untreated mice) exposed to cannabinoids in culture.

A. General Methodology

1. Spermatogenic Cell Separation and Culture. Asingle-cell spermatogenic cell suspension was prepared from the testes of mice following the methodology of Bellvé and co-workers (17). An enriched Krebs-Ringer buffer was used for all cell manipulations. Male CD-1 mice were obtained from Canadian Breeding Laboratories, Montreal, at 8-10 weeks of age. Spermatogenic cell suspensions were obtained by sequential collagenase (CLS; Worthington Biochemicals, Mississauga, Ont.) and trypsin (Bovine pancreas, type III, Sigma Chemical Co.,St. Louis, MO) digestion of decapsulated testes. Following gentle pipetting and filtration through Nitex monofilament nylon cloth (80 mcm mesh;Tet Kressilk) and Sephadex G-25 (Pharmacia Fine Chemicals, Dorval, Que.), the cells were fractionated by sedimentation at unit gravity on the basis of size using a STA-PUT apparatus (SP-240, Johns Scientific, Toronto, Ont.). Cells were sedimented for 90 minutes at room temperature (22°C) into a gradient of 2-6% Percoll (Pharmacia Fine Chemicals, Dorval, Que.) in 1% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, MO). Subsequently, 12 ml fractions were collected and fractions containing the desired cell types (as judged by morphology under Nomarski differential interference contrast optics) were pooled. Population of

round spermatids contained 85-90% round spermatids; cell populations were ready for culture within 6 hours of sacrifice.

Cells were cultured in Eagle's minimum essential medium with Earle's balanced salt solution, supplemented with 0.1 mM non-essential amino acids, 1mM sodium pyruvate, 2 mM glutamine, 50 U/ml penicillin base and 50 U/ml streptomycin base. Culturing was carried out in an atmosphere of 5% CO_2 in air at 33°C in Falcon plastic petri dishes in a volume of 2-3 ml; cell concentrations were 2 X 10^4 cells/ml and 4 X 10^4 cells/ml for pachytene spermatocytes and round spermatids respectively. The viability of cells in culture was determined by trypan blue dye exclusion and by the ability of cells to incorporate [^{3}H]-uridine into the acid-soluble fraction. Cells treated with drug vehicle (DMSO) or 5 mcM THC excluded trypan bluefor over 4 hours (viability > 85%) and vehicle-treated cells continued to incorporate [^{3}H]-uridine after 6 hours of culture.

2. Cannabinoids. All cannabinoids were provided byThe National Institute on Drug Abuse, U. S. A., through the Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario.

For in vivo studies, THC, supplied dissolved in dehydrated ethanol (200 mg/ml), was suspended in a propylene glycol:Tween 80:saline (10:1:89) vehicle and 0.1 ml was administered i.p.. Vehicle treatment contained 95% ethanol instead of the THC solution. In multiple treatment experiments, the THC treatments were spaced 24 hours apart and the mice were sacrificed 2 hours after the final treatment.

For in vitro studies, the cannabinoids, dissolved in ethanol (200 mg/ml), were diluted in DMSO before addition to the culture medium. Control cells received an identical volume of DMSO, the final concentration of DMSO never exceeding 0.35%.

3. Acid-insoluble incorporation of [^{3}H]-uridine. Cells were incubated in the presence of 10mcCi/ml [5-^{3}H]-uridine (25-30 Ci/mmol; NET 174, New England Nuclear, Lachine, Que.) for 2 hours. The incubation was stopped by placing the cells on ice and then precipitated overnight in ice-cold 10% trichloroacetic acid. The acid-insoluble fraction was collected on glass fiber filters and radioactivity was determined by liquid scintillation spectrometry.

4. Acid-soluble incorporation of [^{3}H]-uridine. Cells were incubatedin the presence of 10 mcCi/ml [5-^{3}H]-uridine (25-30 Ci/mmol; NET 174, New England Nuclear, Lachine, Que.) for 2 hours. The incubation was stopped by adding an equal volume of ice-cold phosphate-buffered saline. The cells were collected onto glass fiber filters and washed by gentle suction and then precipitated for 45 minutes in ice-cold 0.5 N perchloric acid. The acid-soluble and acid-insoluble pools were

then collected by filtration through another glass fiber filter. An aliquot or the soluble pool was counted by liquid scintillation spectrometry to determine the total radioactivity in the soluble pool. The rest of the soluble pool was neutralized with alamine-chloroform and fractionated on a DEAE-cellulose anion-exchange column. The column was eluted with an increasing linear gradient of ammonium bicarbonate (0.002 M, pH 8.3 to 0.15 M, pH 8.6) and collected using a peristaltic pump and an automatic fraction collector with a drop counter. Uridine, uridine monophosphate (UMP), uridine diphosphate (UDP) and uridine and uridine triposphate (UTP) standards were used to ascertain the separation as determined by the optical density (lambda = 260 nm). Radioactivity in the nucleoside and nucleotide pools of labelled cells was determined by liquid scintillation spectrometry.

B. In Vitro Cannabinoid Effects: Acid-insoluble Incorporation of [^{3}H]-Uridine

In order to determine the effects of in vitro cannabinoid treatment on macromolecular synthesis, cells were incubated in the presence of [^{3}H]-uridine and 1, 3 or 5 mcM THC, CBN or CBD for 2 hours. Acid-insoluble incorporation of radioactivity was then determined following overnight precipitation. In vitro cannabinoid treatment suppressed the incorporation of radioactivity by pachytene spermatocytes and round spermatids isolated from untreated mice. Acid-insoluble incorporation of radioactivity was significantly suppressed in pachytene spermatocytes by treatments of THC (3 and 5 mcM; 67% and 79% suppressions, respectively), CBN (5 mcM; 85% suppression) and CBD (5 mcM; 67% suppression) (Fig. 1a). In round spermatids, acid-insoluble incorporation of radioactivity was significantly suppressed by treatments of THC (1, 3 and 5 mcM; 39%, 72% and 58% suppressions, respectively), CBN (3 and 5 mcM; 46% and 72% suppressions, respectively) and CBD (3 and 5 mcM; 44% and 67% suppressions, respectively) (Fig. 1b).

C. In Vitro THC Effects: Acid-soluble Incorporation of [^{3}H]--Uridine

1. Total Soluble Incorporation of Radioactivity. Soluble incorporation of radioactivity was assessed by culturing cells isolated from untreated mice for 2 hours in the presence of [^{3}H]-uridine and 5 mcM THC. Acid-soluble radioactivity in 5 mcM THC-treated pachytene spermatocytes was reduced in 4 experiments by 37, 44, 10 and 38% (mean = 32%) as compared to vehicle (DMSO) treated cells. In 3 experiments, acid-soluble

radioactivity in 5 mcM THC-treated spermatids was reduced by 32, 13 and 58% (mean = 34%) as compared to vehicle (DMSO) treated cells.

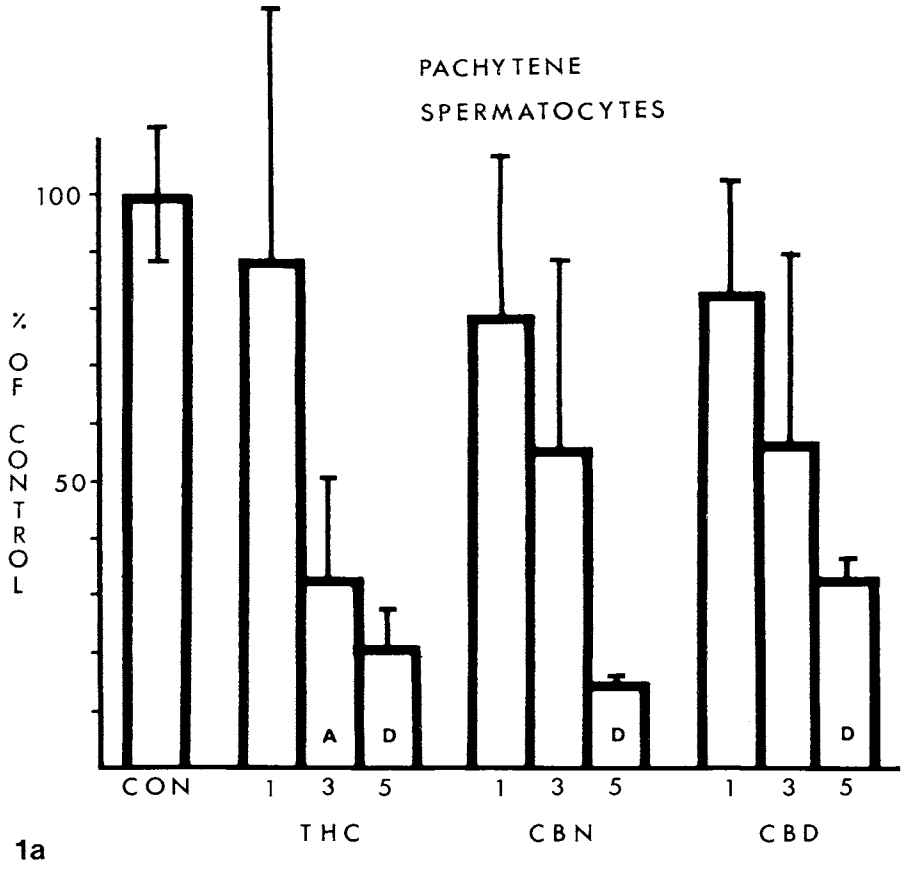
2. Fractionation of Uridine Nucleoside and Nucleotide Radioactivity. The soluble pool of radioactivity was fractionated into uridine nucleoside and nucleotide radioactivity by DEAD-cellulose anion-exchange chromatography. In general, 2 hour treatment of pachytene spermatocytes and round spermatids with 5 mcM THC led to reductions in the nucleoside and nucleotide pools as compared to pools from vehicle (DMSO) treated cells (Table I). The amount of radioactivity in the UDP and UTP pools of both cell types was low, being undetectable in most cases. The data suggest a reduction in the uptake of exogenous [^{3}H]-uridine and a suppression of subsequent [^{3}H]-uridine phosphorylation by THC-treated cells.

D. In Vivo THC Effects: Acid-insoluble Incorporation of [^{3}H]-Uridine

The ability of cells isolated from mice two hours after the last THC treatment to incorporate [^{3}H]-uridine in vitro

TABLE I. Acid-soluble Radioactivity in Pachytene Spermatocytes and Round Spermatids

	CPM/10^4 CELLS	
	Uridine	Nucleotides
Pachytene Spermatocytes		
CONTROL	7406	189
5 mcM THC	4920	-
CONTROL	3719	361
5 mcM THC	2543	42
CONTROL	3933	212
5 mcM THC	3215	-
Round Spermatids		
CONTROL	5801	39
5 mcM THC	5100	-
CONTROL	5170	579
5 mcM THC	5113	167



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was used as an indicator of in vivo THC-treatment effects. Mice were treated with a single THC dosage of 10 or 20 mg/kg, or dosages of 10 mg/kg on 5 consecutive days. None of the treatment schedules induced a reduction in the acid-insoluble incorporation of radioactivity by pachytene spermatocytes and round spermatids isolated from THC-treated mice as compared to cells isolated from vehicle-treated mice.

III. CONCLUDING REMARKS

In general, these studies have demonstrated that in vitro treatment of isolated pachytene spermatocytes and round spermatids with THC, CBN, or CBD (1, 3 or 5 mcM) interferes with uridine metabolism in these cells. The concomitant reduction

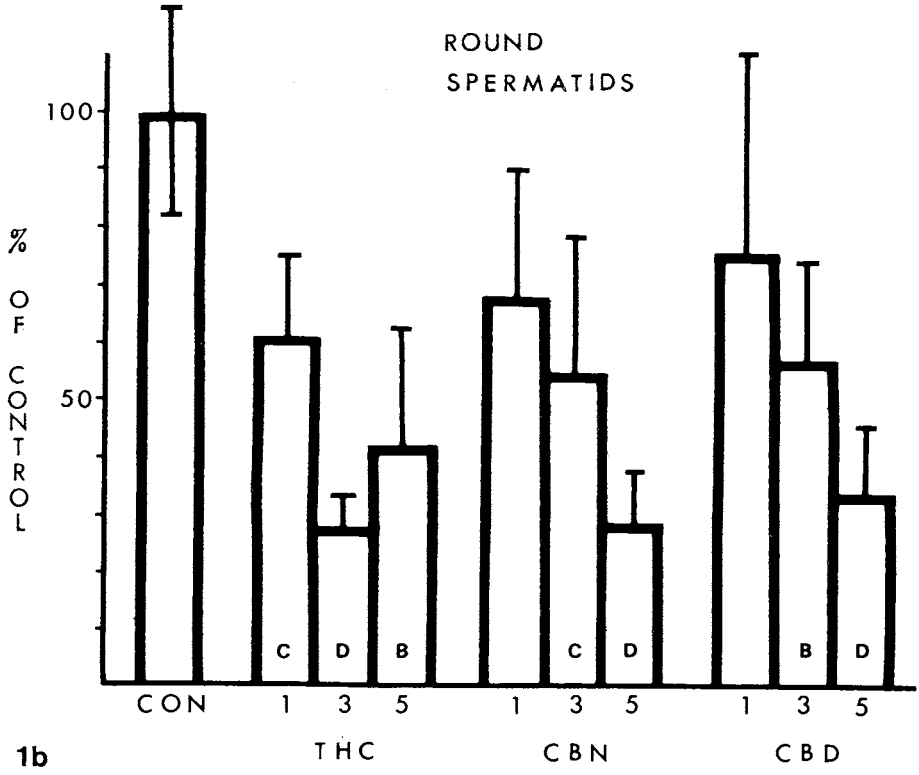

FIGURE 1. Acid-insoluble incorporation of radioactivity (+/- standard error of the mean, n = 3) by a: pachytene spermatocytes, and b: round spermatids treated in vivo with [^{3}H]-uridine and 1, 3 or 5 mcM THC, CBN or CBD for 2 hours. (Student's t-test, one-tail: A: $p < 0.05$; B: $p < 0.01$; C: $p < 0.025$; D: $p < 0.005$.)

of acid-insoluble incorporation of uridine and oftheuptake and phosphorylation of uridine, suggests that cannabinoid inhibition of macromolecular synthesis may be a secondary consequence of a reduction of the intracellular pool of macromolecular precursors. Such "non-specific" inhibitions of macromolecular synthesis by psychoactive (THC) as well as non-psychoactive (CBN and CBD) cannabinoids have been demonstrated in HeLa cells (15), rat testicular cells (9) and Tetrahymena pyriformis (23). The significance of these results in relation to spermatogenic differentiation is unclear. Since spermatogenic differentiation has not been demonstrated in vitro,

a direct cannabinoid effect on the differentiation has not been demonstrated *in vitro*, a direct cannabinoid effect on the differentiation of spermatogenic cells is difficult to ascertain. *In vivo*, pachytene spermatocytes and round spermatids are the stages most active in RNA and protein synthesis during spermatogenesis (16). Interference with these processes could lead to changes in the kinetics, efficiency or fidelity of the orderly processes of spermatogenic differentiation and gene expression. The results of the present *in vitro* studies are consistent with the hypothesis (19) that cannabinoid interference with spermatogenic differentiation, leading to morphologically or chromosomally abnormal sperm, may be partially mediated by interference with macromolecular synthesis.

In vivo treatment of mice with THC in the present studies did not inhibit the incorporation of [^{3}H]-uridine by isolated pachytene spermatocytes and round spermatids. Similar dosages of THC (10 mg/kg for 5 consecutive days) have been shown to lead to the induction of morphologically abnormal sperm when pachytene spermatocytes were exposed to the drug *in vivo* and to an increase of ring and chain translocations in pachytene spermatocytes when spermatogonia were exposed to the drug *in vivo* (25). While it is possible that *in vivo* THC treatment has no effect on uridine metabolism in pachytene spermatocytes and round spermatids *in situ*, it is possible that such an effect does occur but could not be measured by the present experimental design. The suppressive effect on uridine incorporation might be short-lived and may have been dissipated during the cell separation procedure; 5 to 6 hour elapsed between sacrifice and cell culture. An alternative hypothesis is that *in vivo* THC treatment does not lead to a gross alteration of uridine metabolism, but does suppress the synthesis or processing of specific RNA or protein molecules.

In conclusion, it should be noted that the lack of a demonstrable *in vivo* THC effect in the present study requires us to be extremely cautious in the interpretation of our *in vitro* results. Further studies are required to determine whether a cannabinoid-induced inhibition of overall macromolecular synthesis or an inhibition of the synthesis of specific macromolecules may interfere with spermatogenic differentiation. We propose that analysis of macromolecules labelled *in situ* in spermatogenic, Sertoli and Leydig cells from cannabinoid-treated mice is required.

REFERENCES

1. Blevins, R. D., and Regan, J. D., delta-9-Tetrahydrocannabinol. Effect on macromolecular synthesis in humans

and other mammalian cells, Arch. Toxicol. 35:127-135 (1976).

2. Carchman, R. A., Harris, L. S., and Munson, A. E., The inhibition of DNA synthesis by cannabinoids, Cancer Res. 36:95-100 (1976).
3. Dalterio, S., Badr, F., Bartke, A., and Mayfield, D., Cannabinoids in male mice: Effects on fertility and spermatogenesis, Science 216:315-316 (1982).
4. Desoize, B., Leger, C., Jardillier, J-C., and Nahas, G., Inhibition by THC of thymidine transport: A plasma membrane effect, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 145-159. Pergamon Press, New York, 1979.
5. Hembree, W. C., Zeidenberg, P., and Nahas, G. G., Marijuana's effects on human gonadal function, in "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G.G. Nahas, ed.), pp. 521-532. Springer-Verlag, New York, 1976.
6. Hembree, W. C., Nahas, G. G., Zeidenberg, P., and Huang, H. F. S., Changes in human spermatozoa associated with high dose marijuana smoking, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 429-439. Pergamon Press, New York, 1979.
7. Huang, H. F. S., Nahas, G. G., and Hembree, W. C., Effects of marijuana inhalation on spermatogenesis of the rat, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 419-427. Pergamon Press, New York, 1979.
8. Issidorides, M. R., Observations in chronic hashish users, Nuclear aberrations in blood and sperm and abnormal acrosomes in spermatozoa, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 377-388. Pergamon Press, New York, 1979.
9. Jakubovic, A., and McGeer, P. L., Biochemical changes in rat testicular cells in vitro produced by cannabinoids and alcohol: Metabolism and incorporation of labelled glucose, amino acids and nucleic acid precursors, Toxicol. Appl. Pharmacol. 41:473-486 (1977).
10. Mellors, A., Cannabinoids: Effects on lysosomes and lymphocytes, in "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G. G. Nahas, ed.), pp. 283-298. Springer-Verlag, New York, 1976.
11. Mellors, A., Cannabinoids and membrane-bound enzymes, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 329-342. Pergamon Press, New York, 1979.
12. McLean, D. K., and Zimmerman, A. M., Action of delta-9-tetrahydrocannabinol on cell division and macromolecular

synthesis in division-synchronized protozoa, Pharmacol. 14:307-321 (1976).
13. Morishima, A., Henrish, R. T., Jou, S., and Nahas, G. G., Errors of chromosome segregation induced by olivetol, a compound with the structure of the C-ring common to cannabinoids: Formation of bridges and multipolar divisions, in "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G. G. Nahas, ed.), pp. 265-271. Springer-Verlag, New York, 1976.
14. Morishima, A., Milstein, M. M., Henrich, R. T., and Nahas, G. G., Effects of marijuana smoking, cannabinoids and olivetol on replication of human lymphocytes: Formation of micronuclei in "Pharmacology of Marihuana", Vol. 2 (M. C. Braude and S. Szara, eds.), pp. 711-722. Raven Press, New York, 1976.
15. Mon, M. J., Haas, A. E., Stein, J. L. and Stein, G. S., Influence of psychoactive and nonpsychoactive cannabinoids on cell proliferation and macromolecular biosynthesis in human cells, Biochem. Pharmacol. 30:31-43 (1981).
16. Monesi, V., Geremia, R., D'Agostino, A., and Boitani, C., Biochemistry of male germ cell differentiation in mammals: RNA synthesis in meiotic and postmeiotic cells, Curr. Top. Dev. Biol. 12:11-36 (1978).
17. Romrell, L. J., Bellvé, A. R. and Fawcett, D. W.: Separation of spermatogenic cells by sedimentation velocity, Dev. Biol. 49:119-131 (1976).
18. Stein, G. S., Mon, M. J., Haas, A. E., Jansing, R. L., and Stein, J. L., Cannabinoids: The influence on cell proliferation and macromolecular biosynthesis, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 171-208. Pergamon Press, New York, 1979.
19. Tilak, S. K., and Zimmerman, A. M., The effects of delta-9-tetrahydrocannabinol on spermatogenesis, J. Cell Biol. 87:148a (1980).
20. Wyrobek, J. A., and Bruce, W. R., Chemical induction of sperm abnormalities in mice, Proc. Nat. Acad. Sci. U.S.A. 72:4425-4429 (1975).
21. Wyrobek, A. J., Heddle, J. A., and Bruce, W. R., Chromosomal abnormalities and the morphology of mouse sperm heads, Can. J. Genet. Cytol. 17:675-681 (1975).
22. Zimmerman, A. M., and McLean, D. K., Action of Narcotic and hallucinogenic agents on the cell cycle, in "Drugs and the Cell Cycle" (A. M. Zimmerman, G. M. Padilla and I. L. Cameron, eds.), pp. 67-94. Academic Press, New York, 1973.
23. Zimmerman, A. M., and Zimmerman, S. B., The influence of marijuana on eukaryote cell growth and development, in "Marihuana: Chemistry, Biochemistry and Cellular Effects"

(G. G. Nahas, ed.), pp. 195-205. Springer-Verlag, New York, 1976.

24. Zimmerman, A. M., Bruce, W. R., and Zimmerman, S., Effects of cannabinoids on sperm morphology, Pharmacol. 18:143-148 (1979).
25. Zimmerman, A. M., Zimmerman, S., and Ray, A. Y., Effects of cannabinoids on spermatogenesis in mice, in "Marihuana: Biological Effects" (G. G. Nahas and W. D. M. Paton, eds.), pp. 407-418. Pergamon Press, New York, 1979.