TOLERANCE TO THE REPRODUCTIVE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL: COMPARISON OF THE ACUTE, SHORT-TERM, AND CHRONIC DRUG EFFECTS ON MENSTRUAL CYCLE HORMONES

Carol Grace Smith, Ramona G. Almirez
Pamela M. Scher, Riccardo H. Asch

Department of Pharmacology
Uniformed Services University of the Health Sciences
Bethesda, Maryland
and
Department of Obstetrics and Gynecology
University of Texas Health Science Center
San Antonio, Texas

I. INTRODUCTION

The reproductive effects of marijuana and delta-9-tetrahydrocannabinol (THC) have received much attention from the scientific community for the past several years. Early clinical reports indicated that chronic marijuana use may be associated with decreased hormone levels and infertility. Later studies failed to confirm these findings. Studies in laboratory animals clearly demonstrate that THC has pronounced effects on reproductive hormones and on ovulation and spermatogenesis, and have provided much information on how these effects are produced. These studies have not, however, provided much insight into the apparent discrepancy in the pronounced drug effects reported in laboratory animal studies and the less impressive effects of absence of disruptive effects reported in clinical studies. Since much of our knowledge of human reproductive physiology has been obtained from studies in laboratory animals, it is difficult to ascribe these differences to species variations. It is more likely that the discrepancies in laboratory animal studies and the clinical studies are based on differences in experimental designs and

failure to consider the role of the development of drug tolerance in the conclusions of these studies.

The sexually mature rhesus monkey is one of the best experimental animal models for extrapolating to the human reproductive system. The female commonly has a 28-day menstrual cycle that is controlled by negative and positive feedback mechanisms between gonadal steroids and pituitary gonadotropins similar to those found in the human menstrual cycle. Specific amount of marijuana can be administered to these animals and reproductive parameters can be examined directly. The purpose of this review is to summarize the effects of acute, short-term (less than 30 days), and chronic treatment with THC on the primate menstrual cycle. Particular emphasis will be place on the studies that examine the development of tolerance to the reproductive effects of THC.

II. RESULTS

A. Acute Effects of THC on Menstrual Cycle Hormones

It is now well established that THC decreases the circulating levels of FSH and LH in various species (1-4) including primates of both sexes (5). This inhibition in ovariectomized rhesus monkeys ranges from 50 to 80% of basal levels and lasts for up to 24 hours after a single dose (dose dependent). This inhibitory effect can be reversed by the administration of the hypothalamic gonadotropin releasing hormone (GnRH) indicating a hypothalamic site of action for the THC (6,7).

Acute administration of THC also causes a decrease in the circulating levels of prolactin for up to 180 minutes in both male and female rhesus monkeys (8) and in other species (9). This effect on prolactin again indicates a hypothalamic site of action for THC.

The acute effects of THC on menstrual cycle hormones have been studied in the rhesus monkey using both *in vivo* and *in vitro* techniques (10). In the *in vivo* studies, THC (2.5 mg/kg) or vehicle (3% Tween 80 in saline) was administered by an intramuscular injection to rhesus monkeys on day 20, 21 or 22 of the menstrual cycle. Progesterone levels were measured at 6 hour intervals for the first 24 hours after treatment. THC caused a significant decrease in progesterone levels during this 24 hour period. This decease was reversed by the administration of human chorionic gonadotropin (HCG) at 6 hours after THC administration (Figure 1). This stimulatory effect on HCG could be observed as early as 30 minutes after injection, and normal progesterone levels were observed from 180 minutes to 48 hours after the HCG injection. These

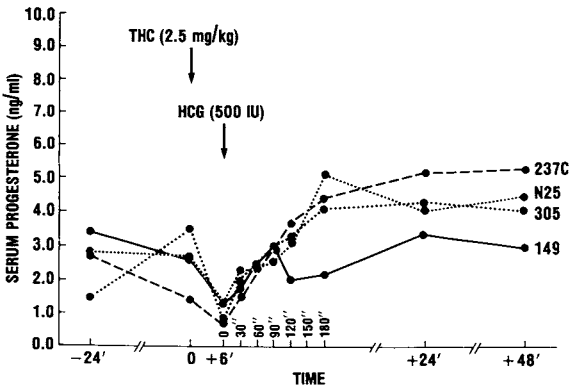

FIGURE 1. The effect of THC (2.5 mg/kg) on progesterone levels during the luteal phase in rhesus monkeys and the reversal of the effect of THC by injection of HCG six hours after the drug injection (cf. ref. 10).

results indicate that the acute effect of THC on progesterone levels is mediated by an indirect effect on pituitary gonadotropins rather than direct effects on the ovarian synthesis and secretion of progesterone.

This conclusion is supported by in vitro studies of basal progesterone production by dispersed cells from the rhesus monkey corpus luteum. The corpora lutea were surgically removed from the ovaries six to eight days after ovulation. These dispersed cells will continue to produce progesterone for several hours without adding gonadotropin to the medium. THC or marijuana extract was added to the cell suspension to a final concentration of up to 50 mcM. Figure 2 shows an example of the production or progesterone by these cells. Neither THC nor marijuana extract had any effect on progesterone production at any concentration up to 50 mcM (limit of drug solubility). However, a number of other in vitro studies with cannabinoids have shown that these drugs disrupt gonadal steroidogenesis, protein and nucleic acid synthesis, glucose utilization, prostaglandin synthesis, and cyclic AMP concentrations in various species including mice, rats and pigs and in various tissues including Leydig cells, granulosa cells and luteal cells (11-15). Clearly, the weight of evidence points to a pronounced in vitro effect of THC on gonadal function.

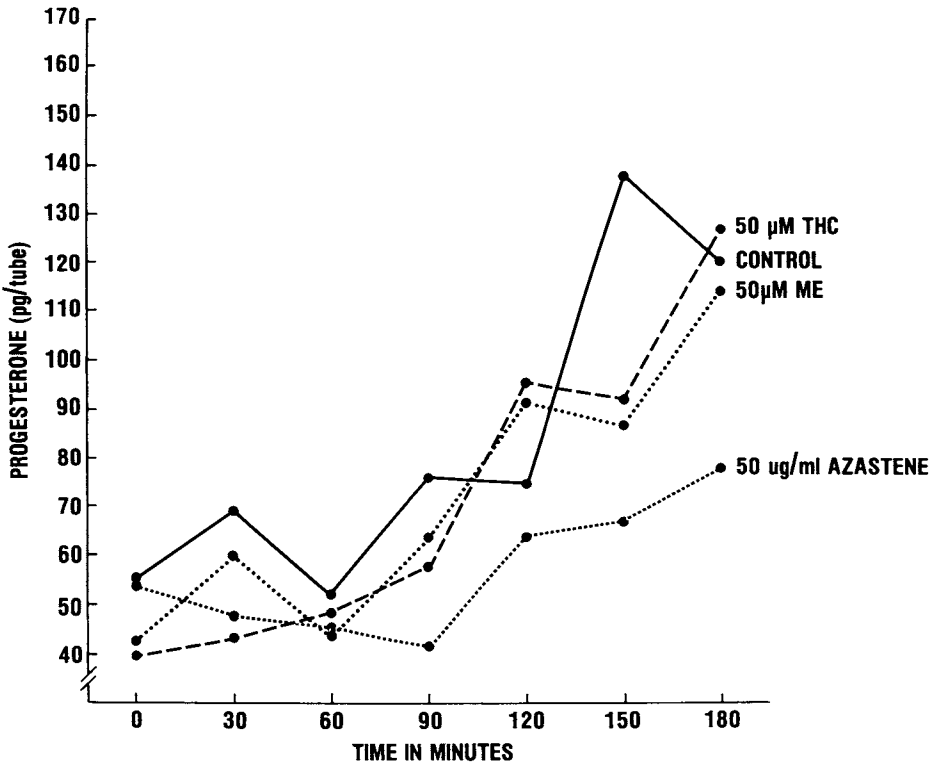

FIGURE 2. Progesterone production by dispersed luteal cells. Neither THC nor marijuana extract showed any effect on progesterone production at concentrations of up to 50 mcM (limit of drug solubility) when compared to control incubations (cf. ref. 10). Azastene (a competitive inhibitor of 3-beta-hydroxysteroid dehydrogenase) was used as a positive control in these studies.

This generates a conflict between the in vivo studies that show no direct effect on gonadal steroid production with these in vitro results; therefore, special attention to the details of the in vitro methods is warranted. All of the cannabinoids studied in these in vitro systems are poorly water soluble. If the solubility of the compound is exceeded, the actual tissue level may be greater than the concentration added to the medium. Unlike the studies with dispersed luteal cells from rhesus monkeys, most of the other in vitro systems use tissues that require the addition of a gonadotropin to the incubation medium to stimulate steroid production. The integrity of the in vitro environment and the function of the poly-

peptide hormones added to the incubations may be compromised in the presence of these drugs. Until adequate information is available on the actual levels of these drugs in the media and in the tissues and on the chemical identity of the drugs throughout the incubation period, the conclusions drawn from all in vitro studies will remain tentative.

B. Short-term Effects of THC on Menstrual Cycle Hormones

The effects of short-term administration of THC on the menstrual cycle in the rhesus monkey has been studied in our laboratories. One such study examined the effect of THC administration on follicular development and ovulation (16). The rhesus monkeys used in these studies had normal menstrual cycles. The first day of menses was designated as day 1 of the cycle, and ovulation normally occurs on about day 15 (average day 15 +/- 1 day S. D.). In order to determine whether THC administration would affect ovulation in these monkeys, THC (2.5 mg/kg) or vehicle (3% Tween 80 in saline) was injected daily from day 1 to day 18 of the cycle. Blood was drawn daily from day 8 to day 18 of the cycle and then every other day until the occurrence of menses. The hormones that were measured included total estrogens, LH, and progesterone. Serial laparoscopies were done twice weekly to observe follicular maturation, ovulation, and corpus luteum formation. None of the five THC-treated monkeys exhibited estrogen rises of LH surges, and none of the THC-treated monkeys ovulated before the next menses. A typical response is shown in Figure 3. The period of disruption that followed the short-term THC treatment ranged from 55 to 145 days. After this period, normal hormone levels and ovulation were observed in all monkeys. This study shows that the inhibitory effect of THC on gonadotropin secretion is sufficient to disrupt ovulation and that the subsequent disruption of themenstrual cycle may last as long as several months. When exogenous gonadotropins were administered to monkeys treated with THC during the first 18 days of the cycle, ovulation was restored and normal luteal function followed (Figure 4). The successful induction of ovulation and normal luteal function inthis study using exogenous gonadotropins in the presence of anti-ovulatory doses of THC clearly supports the hypothesis of a central action of the drug rather than an action directly at the gonad. These results are consistent with studies from other laboratories that show that THC treatment causes an inhibition of ovulation in rats (7) and rabbits (3) by a reversible inhibition of gonadotropin secretion.

Similar results were obtained in studies in which THC was administered to rhesus monkeys during the luteal phase of

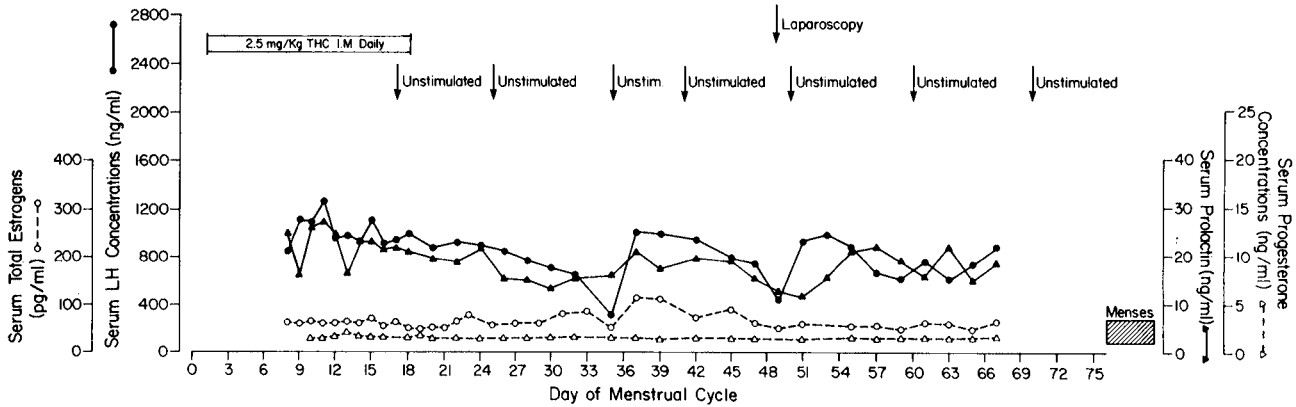

FIGURE 3. The effect of short-term treatment with THC during the follicular phase of the menstrual cycle. This figure shows a typical response of a female monkey to daily treatment with THC (2.5 mg/kg I.M. daily) from day 1 to day 18 of the menstrual cycle. Drug treatment caused a disruption of the normal cyclic pattern of hormones and an inhibition of ovulation (cf. ref. 16). The effect was observed for several months following discontinuation of drug treatment.

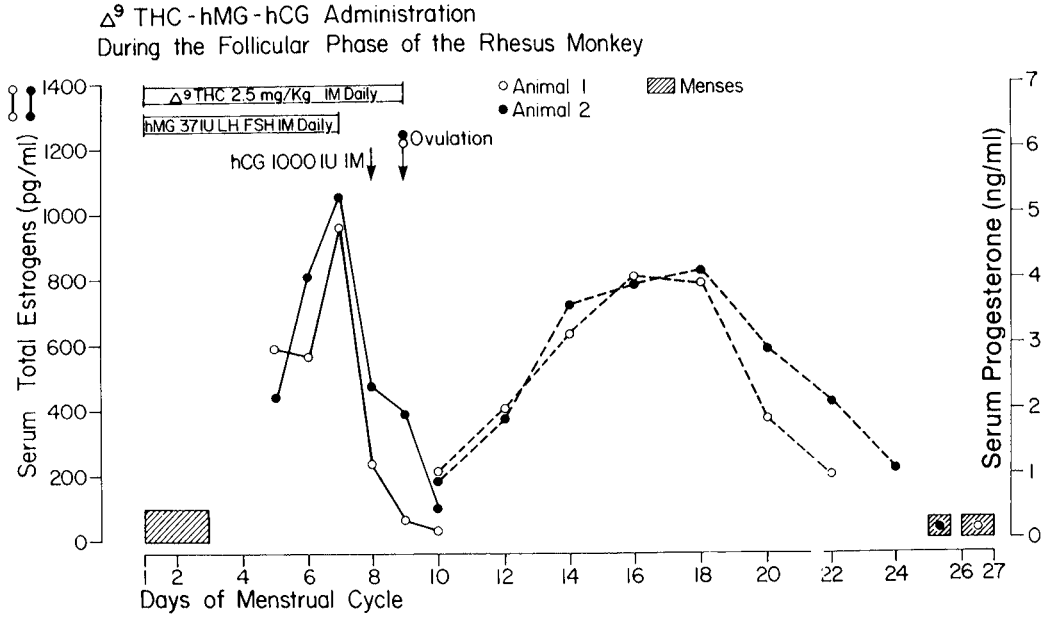

FIGURE 4. Induction of ovulation by the administration of exogenous gonadotropins during short-term treatment with antiovulatory doses of THC in two rhesus monkeys. These monkeys were given hMG (human menopausal gonadotropin) to induce ovarian follicular maturation and hCG (human chorionic gonadotropin) to induce ovulation. The figure shows the original estrogen rise associated with follicular growth and the normal progesterone use during the luteal phase in both THC-treated monkeys.

normal ovulatory cycles (17). The daily administration of THC (2.5 mg/kg) had no effect on serum progesterone levels or on the length of the luteal phase. A typical response is shown in Figure 5. Again, the post-treatment period was marked by an absence of normal levels of estrogens, gonadotropins, and progesterone. The prolactin levels recorded during the post-treatment period were 4 to 5 times greater than prolactin levels in normal ovulatory cycles. A separate study was done in which increasing doses of HCG were administered from Day 6 to Day 10 after ovulation (Day 6, 30 I.U.; Day 7, 60 I.U.; Day 8, 90 I.U.; Day 9, 180 I.U.; Day 30, 360 I.U.). This treatment with HCG during the luteal phase in control animals resulted in augmentation of the progesterone levels to 4-5 times greater than those of control cycles. The daily injections of THC (2.5 mg/kg) had no effect on the HCG-induced progesterone rise when compared to either vehicle treatment or control responses. These results show that THC has no direct effect on luteal function during normal cycles. Further, THC has no direct effect on corpus luteum function stimulatedby HCG administration. The lack of an effect of THC on HCG-stimulated corpus luteum function is particularlyimportant, since this experimental condition mimics progesterone secretion in early pregnancy.

C. Chronic Effects of THC on the Primate Menstrual Cycle

Drug tolerance can be defined as a decrease in pharmacologic response that results from prior exposure to the drug. This phenomenon has been reported with repeated administration of THC in man and laboratory animals to the behavioral and cardiac effects of THC administration. Tolerance can develop by several mechanisms including metabolic tolerance or an increased metabolic clearance of the drug from the body. Cellular or adaptive tolerance occurs when the organ system through homeostatic mechanisms loses its sensitivity to the drug's actions. Either of these mechanisms could be involved in the development of tolerance to the reproductive effects of THC. The chronic studies described here were designed to study the effects of chronic THC on the primate menstrual cycle and to examine the mechanisms involved in the development of tolerance. The study was designed to continue drug treatment for at least one year or until tolerance developed and normal cycles were restored.

Five female rhesus monkeys with normal menstrual cycles were used in this study. Ovulation was detected by monitoring plasma estrogen, LH and progesterone levels and by laparoscopic examination. Daily vaginal swabbings were utilized to detect the onset of vaginal bleeding and duration of menses.

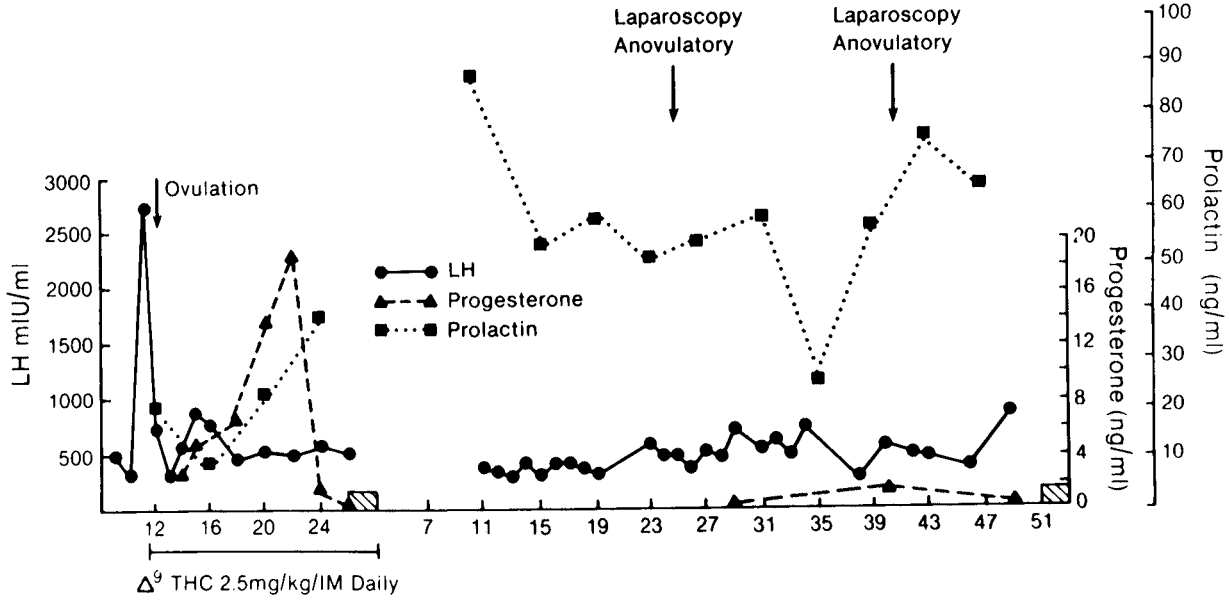

FIGURE 5. The effect of short-term treatment with THC during the luteal phase of the mensural cycle. This figure shows a typical response of a female rhesus monkey to daily treatment with THC from the day of ovulation until the occurrence of menses (hatched bars). Normal progesterone levels were observed during the drug treatment and the luteal phase was of normal length. These monkeys do not ovulate following the luteal phase treatment with THC. This post-treatment period was marked by an absence of normal estrogens, gonadotropins, and progesterone. Prolactin levels were 4 to 5 times greater than those observed in normal ovulatory cycles (cf. ref. 17).

Each monkey was followed for one control cycle and one vehicle treatment cycle before the THC treatment began. On day 1 of the third cycle the monkeys began receiving thrice weekly injections of 2.5 mg/kg THC or 1.25 mg/kg THC. The injections were given on a M-W-F schedule (at noon), and blood sampled on each treatment day immediately before injection.

THC was obtained from the National Institute on Drug Abuse in solution in absolute ethanol. The ethanol was evaporated under a constant stream of nitrogen gas, and the residue was homogenized in Emulphor (polyethoxylated vegetable oil and ethanol) in saline. The drug or vehicle was administered by an intramuscular injection. The blood level of THC obtained with the 2.5 mg/kg dose of THC given 3 times per week in monkeys is equivalent to moderate to heavy use of marijuana (5-6 joints per day; three times per week). Blood levels of THC were measured by RIA where adequate serum was available after hormone measurements (19). The maximum blood level of THC was an average of 300 ng/ml at 60 minutes after injection. The blood level had decreased to an average of 20 ng/ml by 12 hours, and this trough level was maintained until the next dose at 48 hours. These parameters did not change significantly throughout the studies.

All monkeys exhibited normal hormone levels and ovulation during the control and vehicle cycles. Cycle lengths were within normal limits for the colony. After the drug injections began, none of the monkeys ovulated or showed normal hormone levels. The duration of the drug effects (days until next normal menstruation) are shown on Table I. After the tolerance to the drug effects developed, normal cycles were reestablished. Ovulation was again detected laparoscopy and normal hormone levels were observed.

Vehicle administration produced no significant change in prolactin levels. The comparisons shown in Figure 6 were made between pre-treatment prolactin values from control and vehicle cycles (sample size ca. 24 values/monkey); treatment prolactin values during the period of disruption (sample size ca. 48 values/ monkey); and prolactin values during the two cycles after tolerance (sample size 24 values per monkey). There appears to be some decrease in the average prolactin levels during the period of disruption produced by the chronic drug treatment for the three monkeys treated with the 2.5 mg/kg dose of THC (19C; 103C; 237C). Whether this is due to direct drug effects or is secondary to disrupted menstrual cycles cannot be determined. It is clear, however, that the previously observed elevations in prolactin levels following discontinuation of short-term drug treatment are not observed with chronic drug treatment. Some recovery in the average prolactin level was observed after tolerance developed and normal menstrual cycles were restored in these three monkeys.

TABLE I. The Effect of Chronic Treatment with Delta-9-Tetrahydrocannabinol on the Menstural Cycle in Rhesus Monkeys (see Footnote, opposite page)

	CONTROL CYCLE		VEHICLE CYCLE		CHRONIC THC TREATMENT			
					DISRUPTED CYCLES		TOLERANCE	
ANIMAL NO. (THC mg/kg)	DURATION *	OVARIAN ** ACTIVITY	DURATION	OVARIAN ACTIVITY	DURATION	OVARIAN ACTIVITY	DURATION	OVARIAN ACTIVITY
19C (2.5mg/kg)	30	Ov-CL	31	Ov-CL	135	NR	30	Ov-CL
103C (2.5mg/kg)	30	Ov-CL	28	Ov-CL	110	NR	29	OV-CL
237C (2.5mg/kg)	30	Ov-CL	34	Ov-CL	103	NR	35	Ov-CL
149A (1.25mg/kg)	25	Ov-CL	28	Ov-CL	100	NR	30	Ov-CL
922C (1.25mg/kg)	25	Ov-CL	22	Ov-CL	70	NR	29	Ov-CL

* Number of days from first day of menses until next normal mense for each cycle or period of time.

**Ovulation (Ov) and corpus luteum (CL) formation or no ovarian activity (NR) as verified by hormone levels and laparoscopy.

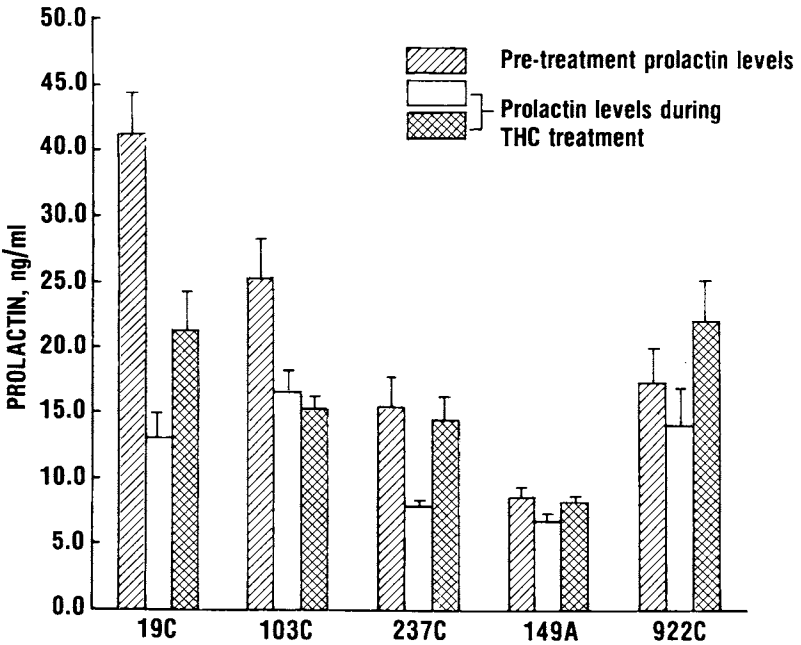

FIGURE 6. Prolactin levels during pretreatment and drug treatment periods for 5 rhesus monkeys treated thrice weekly with 2.5 mg/kg of THC (19C, 103C, 237C) or 1.25 mg/kg of THC (149C and 922C). Hormone measurements were made on blood samples obtained each day before the drug or vehicle was administered. Each bar represents the average (mean +/- SEM) for prolactin levels for each monkey for approximately 24 samples during the anovulatory (open bars) and normal cycles (cross-hatched bars) during chronic drug treatment. These sample numbers are approximations because the cycle durations varied among the monkeys.

TABLE I. (Footnote) In these studies, monkeys were followed for at least one control and one vehicle treatment cycle (hormone levels, laparoscopy, and dates of menses). On the first day of the third cycle, THC injections began, with each monkey receiving an injection on M-W-F of each week. Hormone levels and ovulation were disrupted for the period of time shown for each monkey. Normal cycles were restored with continued THC treatment showing the development of tolerance to THC.

III. DISCUSSION

This chronic study demonstrates the disruptive effects of chronic THC administration on the primate menstrual cycle. Since both gonadotropins and sex steroids are at basal levels during the period of disruption, it is likely that there is a direct suppression of hypothalamic/pituitary activity. This study also demonstrates that with chronic drug treatment tolerance develops to the inhibitory effect of THC and normal cycles are reestablished.

The mechanism for the tolerance to the effect of THC is not known. Tolerance develops to other pharmacologic effects of THC including euphoria and tachycardia. Behavioral tolerance has been reported in rhesus monkeys and was observed in the present study. Preliminary data from this study and complete pharmacokinetic studies in man and laboratory animals indicate that increased drug metabolism or clearance is not a major factor in the development of tolerance (19). It is likely that the tolerance that develops to the reproductive effects of THC is due to adaption of neural mechanisms in the hypothalamus rather than to increased metabolism of the drug.

The results of the present study are consistent with the one clinical study of young women who regularly used marijuana (20). These women experienced changes in menstrual cycles associated with decreased prolactin levels. However, the development of tolerance and return to apparently normal menstrual cycles may mean that normally fertile young women who use marijuana regularly may not notice much change in their menstrual cycles. Drug effects may be more obvious during adolescence, in young women who have some other menstrual irregularities, or if pregnancy occurs. The present studies also demonstrate that the development of tolerance must be considered as a variable in reproductive studies in young men and women who use marijuana and may help to explain some of the conflicting data in human and laboratory animal studies.

REFERENCES

1. Marks, B. H., delta-1-tetrahydrocannabinol and luteinizing hormone secretion, Prog. Brain Res. 39:331 (1973).
2. Dalterio, S., Bartke, A., Roberson, C., Watson, D., and Burstein, S., Direct and pituitary-mediated effects of THC and cannabinol on the testis, Pharmacol. Biochem. Behav. 8:673 (1978).
3. Besch, N. R., Smith, C. G., Besch, P. K., and Kaufman, R. H., The effect of marijuana (delta-9-tetrahydrocannabi-

nol) on the secretion of luteinizing hormone in the ovariectomized rhesus monkey, Amer. J. Obstet, Gynecol. 128:635 (1977).

4. Asch, R. H., Fernandez, E. O., Smith, C. G., and Pauerstein, C. J., Precoital single doses of delta-9-tetrahydrocannabinol blockovulation in therabbit, Fertil. Steril. 31:331 (1979).
5. Smith, C. G., Besch, N. F., and Asch, R. H., Effects of marihuana on the reproductive system, in "Advances in Sex Hormone Research", (J. A. Thomas and R. Singhal, eds.), p. 273. Urban and Schwartzenberg, Baltimore, MD, 1980.
6. Smith, C. G., Besch, N. F., Smith, R. G., and Besch, P. K., Effect of tetrahydrocannabinol on the hypothalamic-pituitary axis in the ovariectomized rhesus monkey, Fertil. Steril. 31:331 (1979).
7. Nir, I., Ayalon, D., Tsafriri, A., Cordova, T., and Lindner, H. R., Suppression of the cyclic surge of luteinizing hormone secretion and of ovulation in the rat by delta-1-tetrahydrocannabinol, Nature 243:470 (1973).
8. Asch, R. H., Smith, C. G., Siler-Knodr, T. M., and Pauerstein, C. J., Acute decreases in serum prolactin concentrations caused by delta-9-tetrahydrocannabinol in non-human primates, Fertil. Steril. 32:571 (1979).
9. Kramer, J., and Ben-David, M., Prolactin suppression by (-)-delta-9-tetrahydrocannabinol (THC): involvement of serotonergic and dopaminergic pathways, Endocrinol. 102: 452 (1978).
10. Almirez, R. G., Smith, C. G., and Asch, R. H. The effects of marijuana extract and delta-9-tetrahydrocannabinol on luteal function in the rhesus monkey, Fertil. Steril. 38:000 (1982).
11. Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., and Lindner H. R., Acute effect of delta-1-tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in the rat, Neuroendocrinol. 23:31 (1977).
12. Burstein, S., Hunter, S., and Shoupe, T. S., Cannabinoid inhibition of rat luteal cell progesterone synthesis, Res. Commun. Chem. Pathol. Pharmacol. 24:413 (1979).
13. Dalterio, S., Bartke, A., and Burstein, S., Cannabinoids inhibit testosterone secretion by mouse testes in vitro, Science 196:1472 (1976).
14. Reich, R., Laufer, N., Lewysohn, O, Cordova, T., Ayalon, D., and Tsafriri, A., (1982) In vitro effects of cannabinoids on follicular function in the rat, Biol. Reprod. 27:223 (1982).
15. Jakubovic, A., and McGreer, P. L., In vitro inhibition of protein and nucleic acid synthesis in rat testicular tissue by cannabinoids, in "Marijuana: Chemistry, Biochemis-

try, and Cellular Effects", (G. G. Nahas, W. D. M. Paton, and J. Idanpaan-Heikkila, eds.), p. 223. Springer-Verlag, New York, 1976.

16. Asch, R. H., Smith, C. G., Siler-Knodr, T. M., and Pauerstein, C. J., Effects of delta-9-tetrahydrocannabinol during the follicular phase of the rhesus monkey (macaca mulatta), J. Clin. Endocrinol. Metab. 52:50 (1981).
17. Asch, R. H., Smith, C. G., Siler-Knodr, T. M., and Pauerstein, C. J. Effects of delta-9-tetrahydrocannabinol administration on gonadal steroidogenic activity in vivo, Fertil. Steril. 32:576 (1979).
18. Lemberger, L., and Rubin, A., Cannabis: Role of metabolism in the development of tolerance, Drug Metab. Rev. 8(1):59 (1978).
19. Cook, C. E., Seltzman, H. H., Schindler, V. H., Tallent, C. R., and Chin, M. M., The analysis of cannabinoids in biological fluids, in "Cannabinoid Assays in Body Fluids" (R. L. Hawks, ed.). NIDA Research Monograph, No. 42, U.S. Government Printing Office, Washington, D. C., 1982.
20. Bauman, J. E., Marijuana and the female reproductive system. Testimony before the Subcommittee on Criminal Justice of the Committee on the Judiciary, U. S. Senate, in "Health Consequences of Marijuana Use", p. 85. U.S. Government Printing Office, Washington, D. C., 1980.