ular biological techniques to this neuronal pathway. Brain cells that produce prolactin may concentrate estrogen from the circulation, since pituitary lactotrophs concentrate tritiated estradiol (*16*). Estrogen acts directly on lactotrophs to increase greatly the synthesis of prolactin (*17*) by increasing the synthesis of messenger RNA for prolactin (*18*). If such an estrogen-induced stimulation of prolactin synthesis occurs in the brain, this peptidergic neuronal system of cell bodies in the mediobasal hypothalamus and fibers in the dorsal midbrain could be a potent cell population mediating some of the behavioral effects of estrogen on the brain.

RICHARD E. HARLAN
BRENDA D. SHIVERS
DONALD W. PFAFF

Rockefeller University,
New York 10021

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9. Each rat was given 5 μg of estradiol benzoate subcutaneously. The following day, in tests of lordosis [see (*13*)], rats scoring less than 1.0 received 5 μg of estradiol benzoate, rats scoring 1.0 to 1.8 received 2.5 μg of estradiol benzoate, and rats scoring 2.0 or more received none of the drug. The following day, the rats were given progesterone or were infused with peptides.
10. Rats were tested by manual stimulation consisting of vigorous stroking of the flanks followed by grasping the flanks and perineum. The degree of dorsiflexion of the back was rated on a 4-point scale: 0, no lordosis; 1, slight; 2, moderate; and 3, maximum lordosis. Each rat was stimulated five times at intervals of a few seconds, and the average score of these five stimulations was used as the score for the rat at the indicated time-point. The inter-observer correlation (Spearman rank correlation coefficient) in lordosis reflex scores obtained by manual stimulation (performed by one author and scored by the same author and one other author) is 0.98, $N = 35$ tests. The intra-observer correlation (test-retest repeatability) is 0.96, $N = 23$ pairs of tests conducted 15 to 20 minutes apart on 23 different rats. For details see D. W. Pfaff, M. Montgomery, C. Lewis, *J. Comp. Physiol. Psychol.* **91**, 134 (1977); and (*8*).
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Tolerance Develops to the Disruptive Effects of Δ^9-Tetrahydrocannabinol on Primate Menstrual Cycle

Abstract. *Long-term exposure of sexually mature female rhesus monkeys* (Macaca mulata) *to thrice weekly injections of Δ^9-tetrahydrocannabinol resulted in a disruption of menstrual cycles that lasted for several months. This period was marked by an absence of ovulation and decreased basal concentrations of gonadotropin and sex steroids in the plasma. After this period, normal cycles and hormone concentrations were reestablished. These studies demonstrate that in rhesus monkeys subjected to long-term treatment with Δ^9-tetrahydrocannabinol tolerance develops to the disruptive effects of the drug on the menstrual cycle.*

The effects of marijuana and its principal psychoactive component, Δ^9-tetrahydrocannabinol (THC), on gonadal function in the female rhesus monkey have been studied in our laboratories. During the follicular phase of the menstrual cycle, monkeys treated with THC (2.5 mg/kg; day 1 to day 18) fail to ovulate and show decreased concentrations of estrogen and gonadotropins in the plasma. When exogenous gonadotropins are administered to THC-treated monkeys, ovulation is restored and normal luteal function follows (*1*). When monkeys are treated with THC (2.5 mg/kg) daily during the luteal phase of the menstrual cycle, the daily progesterone concentrations and the length of the luteal phases are no different from those in control groups. In addition, THC does not impair the normal pattern of response of the corpus luteum to increasing doses of human chorionic gonadotropin as measured by increases in serum progesterone concentrations (*2*). However, THC-treated monkeys exhibit abnormal menstrual cycles after THC treatment during the luteal phase (*3*). These results are consistent with studies from other laboratories that show that THC inhibits ovulation in rats (*4*) and rabbits (*5*) by a reversible effect on gonadotropin secretion.

We designed the present study to examine the effects of long-term THC treatment on the menstrual cycle. Five female rhesus monkeys (*Macaca mulata*) with normal menstrual cycles were used. Ovulation was detected by monitoring the concentrations of estrogen, luteinizing hormone (LH), and progesterone in the plasma and by laparoscopic examination (*2*). Daily swabs from the vagina were used to detect the onset of vaginal bleeding and duration of menses. Each monkey was followed for one control cycle and one cycle with vehicle treatment before being treated with THC. On day 1 of the third cycle the monkeys began thrice weekly injections of THC at doses of 2.5 or 1.25 mg/kg. The injections were continued for a total of 230 days or until two consecutive ovulatory cycles were observed. The injections were given on a Monday, Wednesday, and Friday schedule (at noon), and blood was sampled immediately before each THC injection.

The THC, which was obtained in solution in absolute ethanol (*6*), was prepared for injection by evaporating the ethanol under a constant stream of nitrogen gas. The residue was homogenized in Emulphor (polyethoxylated vegetable oil) in saline. The final concentration represented 10 percent Emulphor and 90 percent saline. The drug or vehicle was administered intramuscularly. A dose of 2.5 mg of THC per kilogram of body weight in monkeys is equivalent to mod-

erate to heavy use of marijuana (five to six joints per day, three times per week) (*7*).

Concentrations of THC in the blood were measured by radioimmunoassay where adequate serum was available after hormone measurements (*8*). The average maximum concentration of THC in the blood was 300 ng/ml at 60 minutes after injection. The blood concentration decreased to an average of 20 ng/ml by 12 hours, and remained at this level until the next dose at 48 hours. These parameters did not change significantly throughout the studies. The average maximum concentration of THC in the blood during the anovulatory cycles was 409 ng/ml (range, 288 to 542 ng/ml) and 378 ng/ml (range, 250 to 640 ng/ml) after menstrual cycles were restored. These concentrations decreased (at 48 hours after drug injection) to 15.3 ng/ml (range 12.1 to 16.5 ng/ml) during the anovulatory cycles and 18.8 ng/ml (range, 13.0 to 24.0 ng/ml) after menstrual cycles were restored. These results agree with other reports indicating that metabolic tolerance does not play an important role in the tolerance that develops to physiological changes with long-term THC treatment (*9, 10*).

Hormone concentrations and ovulation were normal in all the monkeys during the control and vehicle cycles. Cycle lengths were within normal limits for the colony. After the THC injections began, none of the monkeys ovulated or showed normal hormone concentrations. The duration of the drug effects (days until next normal menstruation) were 135, 110, and 103 days for the monkeys treated with the 2.5 mg/kg dose (Fig. 1). After tolerance developed to the effects of THC on the menstrual cycle, normal cycles were reestablished, ovulation occurred, and hormone concentrations reached normal levels.

In previous studies with rhesus monkeys, marked increases in prolactin concentrations occurred during anovulatory periods after short-term treatment with THC (*3*). These studies indicated that the changes in prolactin might be related to the disruptive effects of THC on the primate menstrual cycle. Prolactin does not fluctuate rhythmically during the menstrual cycle in normal rhesus monkeys, but there is considerable individual variation in prolactin concentrations (*11*). In the present study, we measured prolactin in blood samples obtained three times per week during control and vehicle cycles and during the long-term drug treatment. Since vehicle administration had no effect on prolactin, we compared the prolactin concentrations measured before THC administration (during control and vehicle cycles), during the period of disruption, and during the two cycles after the development of tolerance to the effects of THC (Fig. 2). Although the average concentrations of prolactin appeared to decrease during the period of disruption for monkeys 922C, 103C, and 237C, comparisons for the five monkeys of values before and during long-term treatment indicated no significant effects of drug treatment. It is clear, however, that the increases in prolactin observed previously after the discontinuation of short-term THC treatment do not occur after long-term treatment with THC.

Since both gonadotropins and sex ste-

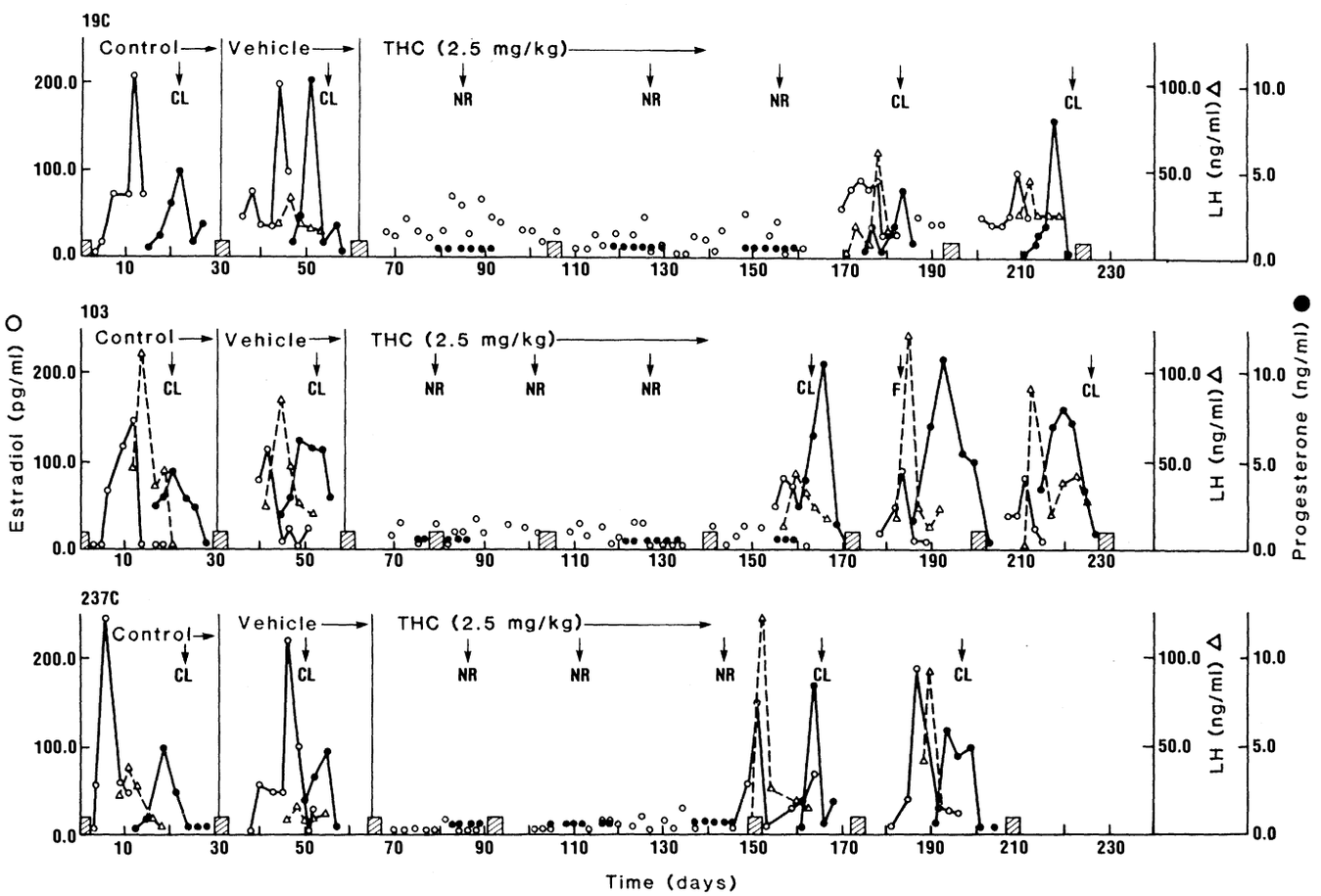

Fig. 1. The development of tolerance to the effect of long-term treatment with THC in the menstrual cycles of rhesus monkeys. The monkeys were monitored for at least one control cycle and one cycle with vehicle treatment. Thrice weekly injections with THC began on day 1 of the third cycle and continued for 230 days or until two consecutive ovulatory cycles were observed. Normal cycles of hormone production and ovulation were disrupted for 135, 110, and 101 days with the 2.5 mg/kg dose; but were restored with continued THC treatment. Arrows show dates of laparoscopy. Abbreviations: *CL*, corpus luteum; *F*, follicle; and *NR*, no ovarian activity. Hatched bars show dates of menses.

roids are at low levels during the period of disruption of the menstrual cycle by THC, the drug probably has a direct suppressive effect on hypothalamic or pituitary activity. Most studies of the effects of THC on reproduction indicate that the major pharmacologic site of action is central. Some investigators, however, suggest direct effects at the gonadal level. Ayalon *et al.* (*12*) suggested that THC inhibits the response of the rat ovary to exogenous LH and proposed that THC competitively inhibits ovarian prostaglandin synthesis. Burstein *et al.* (*13*) demonstrated that THC decreases progesterone production by rat luteal cells in vitro by inhibiting cholesterol esterase. On the contrary, studies in rats (*4*), rabbits (*5*), and monkeys (*3*) show that ovulation and normal steroid levels can be obtained when gonadotropins are placed in THC-treated animals. The present study shows that normal menstrual cycles can be reestablished even during continued treatment with high doses of THC. These results suggest that the inhibitory effect of THC on reproduction is not mediated by a direct effect on gonadal steroidogenesis—a hypothesis that is supported by studies showing that THC and marijuana extract have no effect on basal progesterone production by dispersed luteal cells from rhesus monkeys (*14*). The highest concentrations of THC used in these studies in vitro (50 μM) was approximately 20-fold greater than the maximum concentration of THC in the blood of the same monkeys after they received a single dose of THC (2.5 mg/kg).

The present study demonstrates that the menstrual cycle of rhesus monkeys develops tolerance to the inhibitory effect of THC and that normal cycles are reestablished during the long-term treatment with the drug.

The mechanisms for this tolerance are not known. Tolerance develops to other pharmacologic effects of THC including euphoria and tachycardia. Behavioral tolerance to the drug has been reported in rhesus monkeys and was also observed in the present study. Our data, 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 (*9, 10*). It seems likely that tolerance to the reproductive effects of THC is due to adaptation of neural mechanisms in the hypothalamus rather than to increased metabolism of the drug.

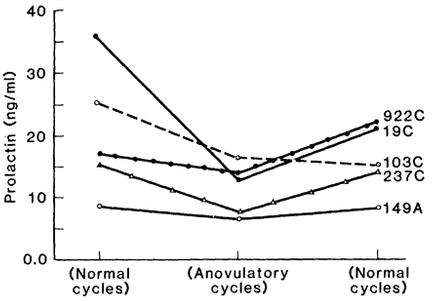

Fig. 2. Prolactin concentrations before and during treatment of five rhesus monkeys with THC. The thrice weekly dosage was 2.5 mg/kg (19C, 103C, 237C) or 1.25 mg/kg (149A and 922C). Hormone measurements were made on blood samples obtained before each drug injection. Each point represents the average prolactin concentration of approximately 24 samples obtained before THC administration (during control and vehicle cycles) and approximately 48 and 24 samples obtained during the disrupted (anovulatory) and normal cycles when THC was being administered. These sample numbers are approximations because the cycle durations varied among the monkeys.

Our data are consistent with a clinical study of young women who regularly used marijuana (*15*). These women experienced changes in menstrual cycles associated with decreased prolactin concentrations. 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.

CAROL GRACE SMITH
RAMONA G. ALMIREZ
JEFFREY BERENBERG
Department of Pharmacology and
Division of Clinical Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814
RICARDO H. ASCH
Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio 78284

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