Evidence for Hormonal Imbalance after Methadone Treatment in Pregnant and Pseudopregnant Rats (41663)

QUANG Q. BUI, MINHTAM B. TRAN, AND WILLIAM L. WEST

Department of Pharmacology, College of Medicine, Howard University, Washington, D.C. 20059

Abstract. The effects of methadone (METH) on the plasma estriol level and hormonal target tissues' cyclic nucleotides (cAMP and cGMP) were investigated in pregnant and pseudopregnant rats. In the pregnant animals, METH (5 mg/kg/day), given once daily from Days 6 to 15 of gestation, significantly reduced the maternal body weight gain in association with an increase in the number of dams bearing resorptions (56%) and a significant reduction in fetal body weight (33%). An inhibition of the plasma estriol level by METH was observed on Day 9 of gestation. Stimulation of the sympatho-adrenal axis and hypothalamo-pituitary axis by acute METH administration was observed and correlated with a significant increase in the levels of cyclic nucleotides in the uterus and adrenal glands of pregnant rats. However, tolerance to METH effects on cyclic nucleotide levels developed by Day 15 of gestation. METH also depressed the fetal cyclic nucleotide levels on Days 12 and 15 of gestation. These findings suggest that METH had pronounced effects on hormonal secretion during pregnancy, and hormonal transport to or hormonal production by the fetuses. In contrast, METH did not exhibit any adverse effects on the hormonal and cyclic nucleotide levels of pseudopregnant rats with deciduoma formation; a model for the maternal compartment. These latter findings may reflect METH's adverse effects on the fetal compartment, and suggest the use of pseudopregnancy as a model to distinguish adverse drug effects between these compartments.

Methadone, a narcotic analgesic, was synthesized as a substitute for morphine in 1943 (1). However, since its discovery, the clinical uses of methadone have extended far beyond its use as an analgesic and it is now accepted world wide as maintenance therapy for heroin addiction in humans.

Numerous adverse effects have been observed in patients on methadone therapy (2, 3), and during pregnancy, methadone is reported to produce fetal loss, neonatal death, and low birth weight in infants born to methadone-treated mothers (4–6). The latter reports suggest that regular use of methadone during pregnancy may have adverse effects on the offspring, including short and long term consequences. However, clinical reports such as these are difficult to interpret because prenatal care was not controlled and some of the observed effects may be due to the overall poor health or to a hormonal imbalance in the mothers. In this regard, methadone has been shown to decrease the plasma levels of estrogens, follicle stimulating hormone (FSH), luteinizing hormone (LH) (7, 8), adrenocorticotrophin hormone (ACTH), and plasma cortisol levels (9, 10).

The purpose of this study was to explore the hypothesis that administration of an analgesic dose of methadone during pregnancy may cause hormonal imbalance which may lead to adverse effects on conception, fetal development, and/or parturition. The plasma estriol levels and the levels and the ratios of cyclic nucleotides were monitored in several hormonal target tissues since they may indicate alterations in physiological responsiveness. Further, pseudopregnant rats with deciduoma formation were also used in an attempt to distinguish between the adverse effects of methadone in the maternal and fetal compartments.

Materials and Methods. *Animals.* Mature and naive Sprague–Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing from 200 to 250 g were used. Rats were individually housed in hanging cages under a controlled photoperiod of 12 hr light per day and temperature (25°C) with food (Ralston Purina Co., St. Louis, Mo.) and tap water *ad libitum.* All female rats were subjected to daily vaginal lavage 7 days per week to determine the stage of their estrous cycle. Only rats that exhibited at least two normal, 4- or 5-day estrous cycles were selected.

Pseudopregnancy. Pseudopregnancy was

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induced by two separate electric stimulations of the uterine cervix (11) using a Yeda Cervical Stimulator (Rohovot, Israel). The first stimulation was performed on the afternoon of the proestrus day (Day 0) followed by a second stimulus 24 hr later, on estrus day (12). The intensity of the electric stimulus was 15 V, 30 msec/pulse at regular intervals of 6 msec. The day when the microscopic examination of the vaginal lavage showed leukocytic infiltration followed by cornification was designated as Day 1 of pseudopregnancy (PSP). Daily smears were then made to monitor the persistence of the PSP status of each animal. On Day 5 of PSP (13), induction of decidual tissue was performed via a midventral laparotomy in lightly etherized animals and experimental decidualization was initiated by scratching the antimesometrial surface of the endometrium with a burred needle and inserting a silk thread (silicone-treated black, Davis Geck silk No. 0-1081-61) into the left uterine horn. The contralateral horn was not traumatized and served as control. The decidual cell reaction was confirmed at autopsy by gross appearance of the uterine horn and by removing and weighing the deciduoma.

Pregnancy. Virgin rats, selected randomly, were housed with males overnight for mating. Copulation was verified the next morning by the presence of a vaginal plug and a vaginal smear showing motile sperm. In this investigation, the day the copulatory plug was found was considered as Day 1 of gestation. Pregnant rats were then separated and housed individually in clear polypropylene cages with stainless-steel tops.

All animals were weighed and observed daily for indications of toxicity from the beginning of the experiment until sacrifice.

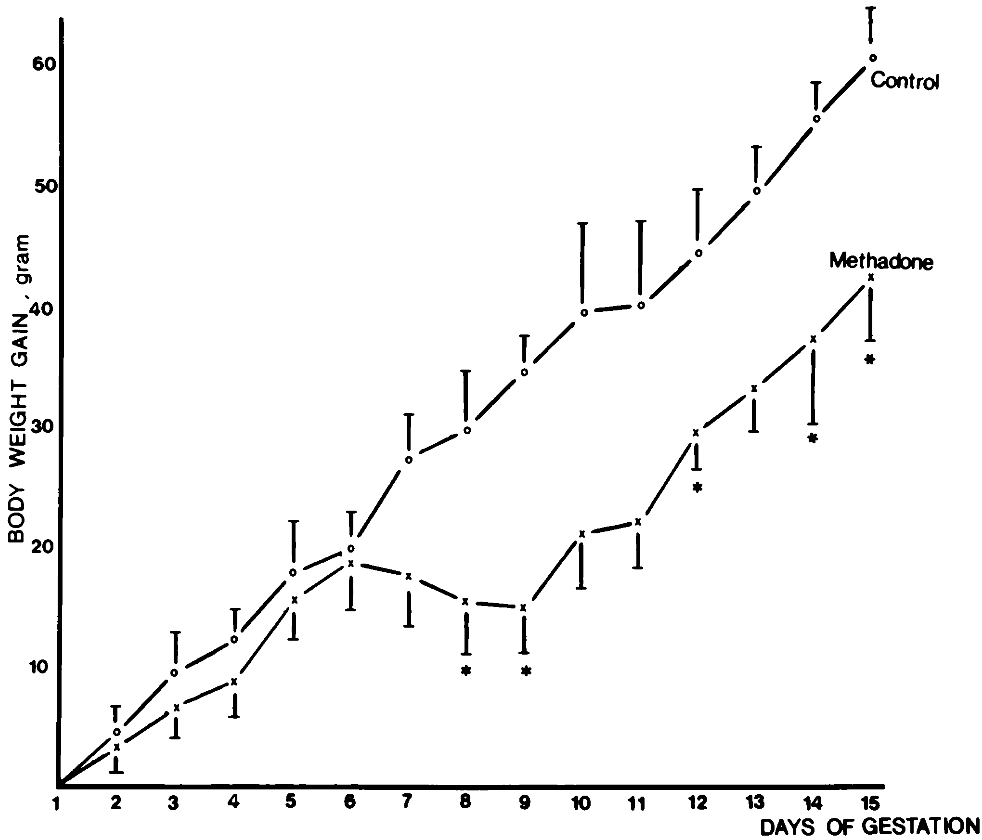

FIG. 1. Effect of subcutaneous injection of methadone (5 mg/kg/day). From Days 6–15 of gestation on body weight gain of CD-1 rats. Saline-treated pregnant animals served as controls. Each point represents a mean value of at least eight animals. Bars represent the SEM. * Denotes statistically different from the corresponding control.

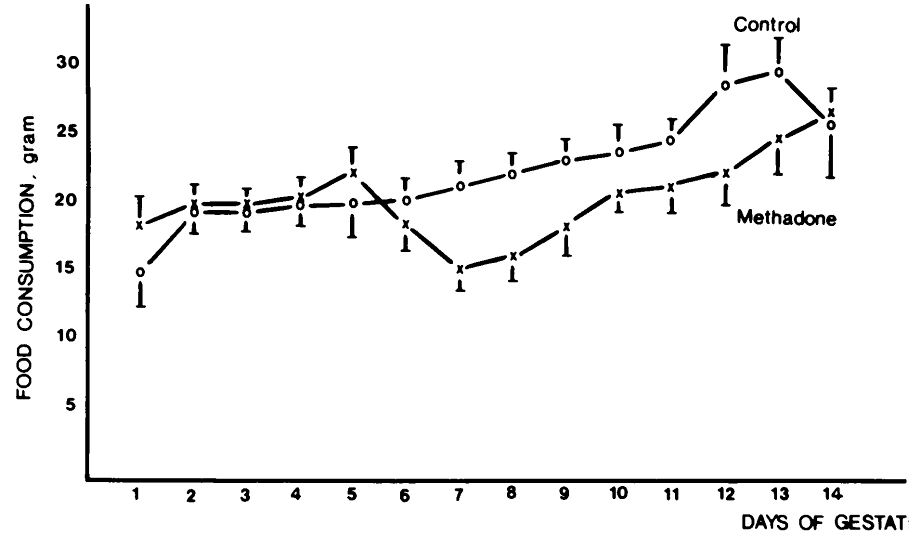

FIG. 2. Effect of subcutaneous injection of methadone (5 mg/kg/day) or saline from Days 6–15 of gestation on daily food consumption of pregnant rats. Each point represents a mean value of at least eight animals. Bars represent the SEM. * Denotes statistically different from the corresponding control.

Treatment. Methadone (Dolophine HCl, Eli Lilly Co., Indianapolis, Ind.) was given daily, subcutaneously (5 mg/kg), to both pseudopregnant and pregnant rats in a constant volume of physiological saline (10 ml/kg). The dose of methadone was adjusted daily according to the body weight variations of the animals. The daily dose of 5 mg/kg was considered since this dose did not produce catatonia and would be least likely to produce

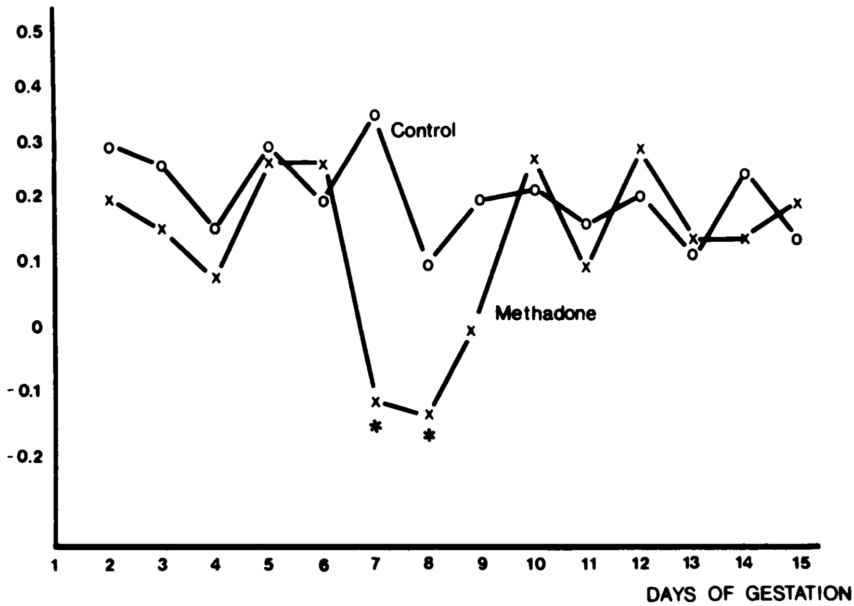

FIG. 3. Food efficiency index of pregnant rats treated with saline or methadone (5 mg/kg/day) from Days 6–15 of gestation. Food efficiency index is the ratio of daily body weight increased over daily food consumption. * Denotes statistically different from the corresponding control.

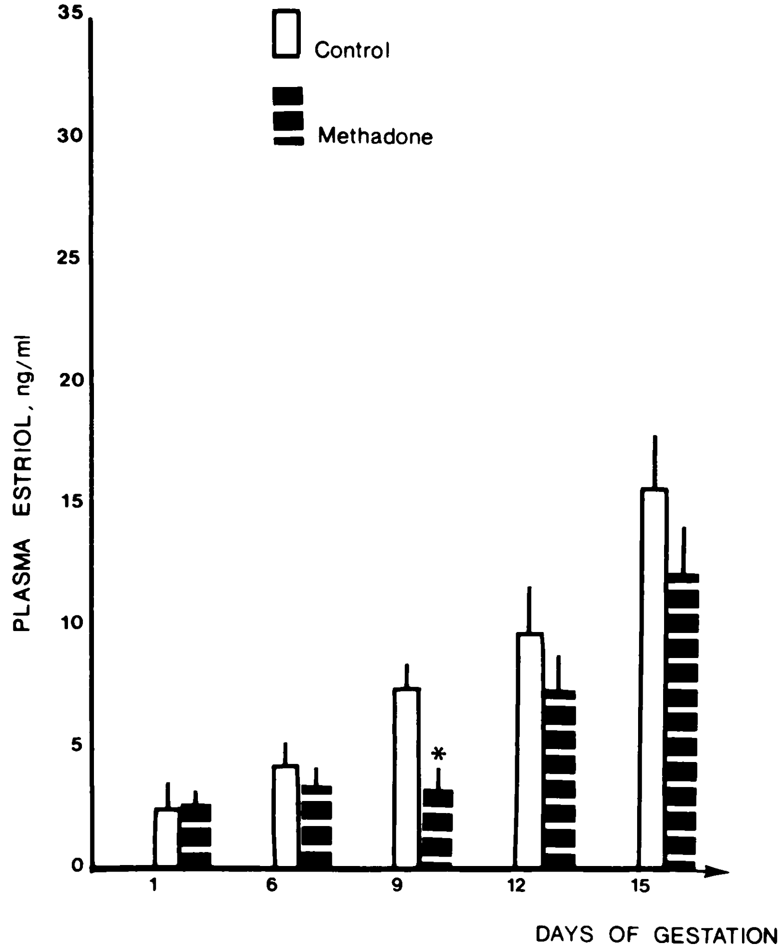

FIG. 4. Plasma total estriol levels of pregnant rats treated with saline or methadone from Days 6–15 of gestation. Total estriol levels were determined by radioimmunoassay techniques. All animals were sacrificed 1 hr after the last injection on Days 6, 9, 12, and 15 of gestation. Each bar represents the mean ± SEM of four animals. * Denotes statistically different from the corresponding control.

dependence on the narcotic. Pregnant and pseudopregnant animals treated with saline (10 ml/kg) served as controls.

Methadone or saline was administered from Days 6 to 15 of gestation and from Days 5 to 14 of PSP to pregnant and pseudopregnant rats, respectively. Sacrifice of pregnant animals was performed on Days 6, 9, 12, and 15 of gestation and that of pseudopregnant rats on Days 5, 8, 11, and 14 of PSP.

Blood sampling and assay of serum estriol level. All animals were sacrificed 1 hr after the last injection by decapitation. Blood samples were collected and allowed to clot at 5°C, serum was separated by centrifugation, collected, and stored at −20°C until assayed. Since hormonal levels are subject to a diurnal rhythm, all samples were collected at the same time (10 AM). Serum estriol levels were determined by radioimmunoassay (Amersham Corporation, Arlington Heights, Ill.). All determinations were made in triplicate.

Tissues sampling and assays of cyclic nucleotides. Upon sacrifice, the uterus of the pregnant animals were exposed and the number of corpora lutea, implantations, and resorptions were recorded. Cyclic nucleotides (cAMP and cGMP) were determined from the uterus, placenta, fetuses (when applicable), and adrenal glands of pregnant animals and from

TABLE I. REPRODUCTIVE STATUS OF PREGNANT RATS TREATED WITH SALINE OR METHADONE (5 mg/kg/Day) OBSERVED ON DAY 15 OF GESTATION

	Vehicle control (N = 8)	Methadone (N = 9)
Mean copora lutea/ litter[a]	15.0 ± 1.72	15.3 ± 1.36
Mean implantation/ litter	12.9 ± 1.98	13.6 ± 1.55
Number of resorptions	2/103	7/122
Number of dams with resorption	1/8 (13%)	5/9 (56%)*
Mean of live fetuses/ litter	12.6 ± 2.05	12.8 ± 1.60
Mean fetal weight (grams)	0.4 ± 0.04	0.25 ± 0.02*

[a] Mean ± SEM.
* Significantly different from control values at $P < 0.05$.

the uterus of pseudopregnant rats. All tissues were removed, immediately placed on dry-ice, and stored at −20°C until assayed. The cyclic nucleotide assays were performed by radioimmunoassay techniques (Amersham Corporation, Arlington Heights, Ill.). All determinations were made in triplicate.

Statistics. All values are expressed as the mean ± SEM. Intergroup differences were assessed by the Student's t test or analysis of variance followed by Newman–Keuls exam where appropriate with a significance level set at $P < 0.05$.

Results. *Pregnant animals.* Although methadone did not significantly affect the maternal body weight gain on a daily basis, statistically significant differences were evident on Days 8 and 9 of gestation, and toward the end of the experiment ($P < 0.05$) as is shown in Fig. 1. The daily food consumption was comparable between the control and treated group (Fig. 2). The food efficiency index is shown in Fig. 3 and significant differences ($P < 0.05$) were noted only on Days 7 and 8 of gestation. No differences in the number of corpora lutea, implantations, resorptions, and viable fetuses were found between the two

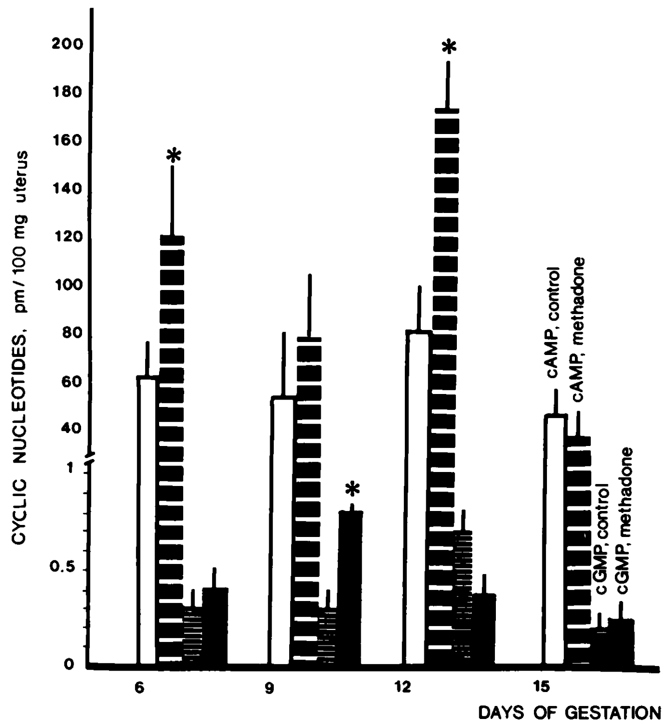

FIG. 5. Pregnant rats were treated with either saline or methadone from Days 6–15 of gestation. The uterine levels of cAMP and cGMP were assayed by radioimmunoassay techniques on Days 6, 9, 12, and 15 of gestation. Each bar represents the mean ± SEM of four animals. * Denotes statistically different from the corresponding control.

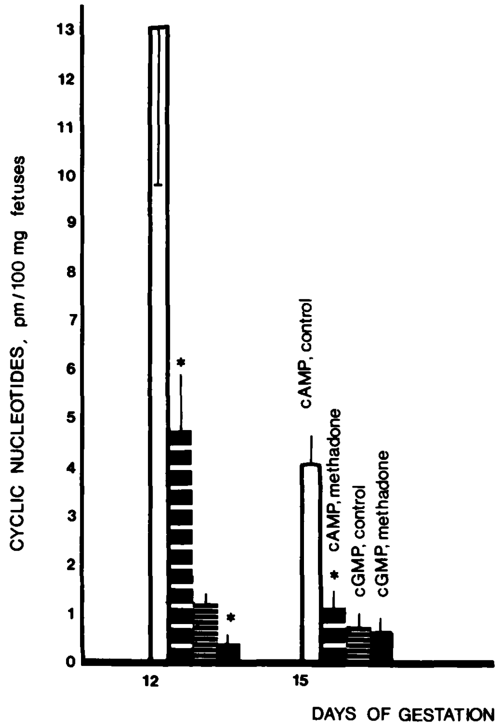

FIG. 6. Pregnant rats were treated with either saline or methadone from Days 6–15 of gestation. The fetal levels of cAMP and cGMP were determined by radioimmunoassay techniques on Days 12 and 15 of gestation. Each bar represents the mean ± SEM of four animals. * Denotes statistically different from the corresponding control.

groups (Table I). However, an increase in the number of dams bearing resorptions (from 13 to 56%, Table I) as well as a decrease in fetal body weight (33% reduction of vehicle-control fetal body weight) were noted. No significant differences were observed in the weight of the maternal adrenal glands, uterus, kidneys, liver, and placentae (data not shown).

The plasma estriol level in the vehicle control pregnant rats doubled on Day 6 of gestation and gradually increased as pregnancy progressed (Fig. 4). Administration of methadone significantly decreased estriol level only on Day 9 of gestation.

The effects of methadone on the uterine cyclic nucleotides are shown in Fig. 5 and a significant increase in cAMP was observed on Days 6 and 12 of gestation, and that of cGMP recorded on Day 9 of gestation. All uterine cyclic nucleotide levels converged by Day 15 of gestation. In contrast to the increase in the levels of cyclic nucleotides observed in the uterus, methadone significantly reduced the fetal level of cAMP and cGMP (Fig. 6). The cyclic nucleotides were also measured from the adrenal glands (Fig. 7) and on Day 6 of gestation, after a single injection of methadone, 60 and 40% increases were observed for both cAMP and cGMP, respectively. However, after repeated administration (once daily) cAMP significantly decreased by 43 and 58% ($P < 0.05$) by Days 9 (once daily for 3 consecutive days) and 12 (once daily for 6 consecutive days) of gestation, respectively. The level of cAMP returned to vehicle control values by Day 15 of gestation (one daily dose for 10 consecutive days). A profound effect on cGMP was observed by Day 15 of gestation with a 84% decrease ($P < 0.01$).

Pseudopregnant animals. In contrast to an increase in estriol level observed in pregnant animals, the estriol level in the methadone-treated pseudopregnant animals was comparable to vehicle control animals throughout pseudopregnancy (Fig. 8). No change from the vehicle control in uterine cyclic nucleotides (cAMP and cGMP) were observed in methadone-treated pseudopregnant animals (Fig. 9).

Discussion. The experimental design intended to avoid the induction of maternal addiction in rats in order to observe whether adverse effects, if any, could occur to a narcotic in an analgesic dose. However, the dosing paradigm selected does not rule out addiction after a single dose but rather takes into consideration the dose–response relationship of this phenomena.

Methadone (5 mg/kg/day) reduced pregnant maternal weight gain as compared to that of vehicle control mothers. However, the food efficiency index was affected only in the early part of gestation (Fig. 3), a finding which suggests that malnutrition is not the only factor in reducing maternal weight gain. In this regard, Ford and Rhines (14) indicated that the weight gain of pregnant methadone-treated rats was significantly less than their pair-fed controls. The reduced maternal weight gain was associated with a reduction in fetal weight as observed on Day 15 of gestation. Our findings indicate that the adverse effects on fetal weight are likely to occur during early gestation coinciding with the decrease in food efficiency

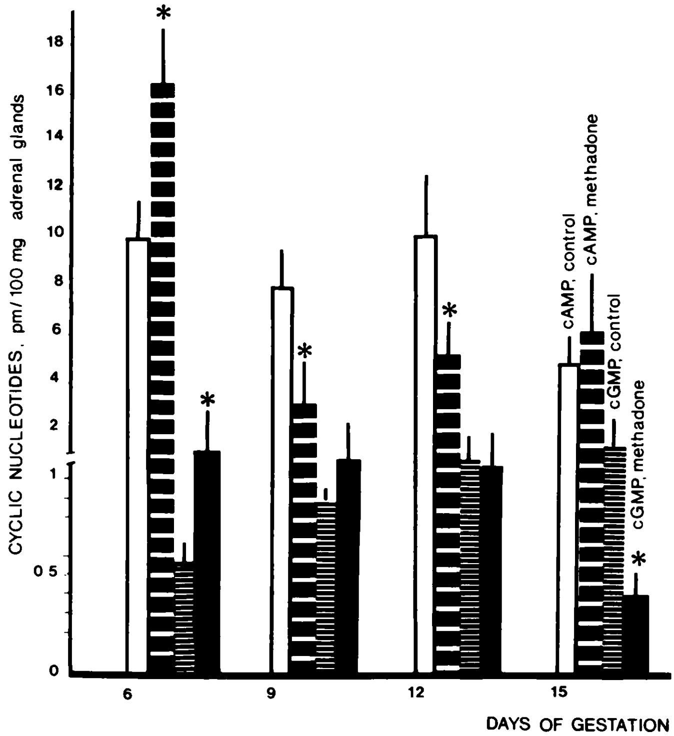

FIG. 7. Pregnant rats were treated with either saline or methadone from Days 6–15 of gestation. The cyclic nucleotide levels of the adrenal glands were determined by radioimmunoassay on Days 6, 9, 12, and 15 of gestation. Each bar represents the mean ± SEM of four animals. * Denotes statistically different from the corresponding control.

index observed at that stage. Therefore, the offspring may not recover from this profound effect which could result in low birth weight observed in both human (5, 6) and animal (15–18) studies.

The estrogen levels were estimated in this study after being enzymatically converted to estriol. Plasma estriol levels were measured during pregnancy since nine-tenths of the estrogen secreted by the fetoplacental unit consist of estriol. Estriol thus may serve as an index to recognize signs of fetal distress (19). The increase in plasma estriol level observed on Day 6 of gestation (day of implantation; Fig. 4) correlated with an increase in uterine blood flow and hyperemia. This correlation has been established and documented (20). After implantation has occurred, the gradual increase in estriol level is mainly due to its production from the fetoplacental unit. The reduction in the estriol level observed on Day 9 of gestation in the methadone-treated rats might signal that the viability of the offspring is adversely affected and may indicate an increase in the number of dams bearing resorptions. This finding further reinforces the suggestion that the effects of methadone on fetal weight most likely occur during an early stage of pregnancy.

The estriol level in the pseudopregnant rats also attained a peak on Day 5 of pseudopregnancy, the expected time of blastocyst implantation (19, 20). In contrast to a gradual rise in estriol levels observed during pregnancy, the estriol levels remained constant throughout pseudopregnancy. Constant estradiol levels in pseudopregnant rats have also been reported by Garris (21). The constant and high levels of estriol found during pseudopregnancy may be due to an immediate and maximum response of the uterus to both the electrical and physical stimuli used in inducing pseudopregnancy with deciduomata formation. Failure of methadone to reduce the estriol lev-

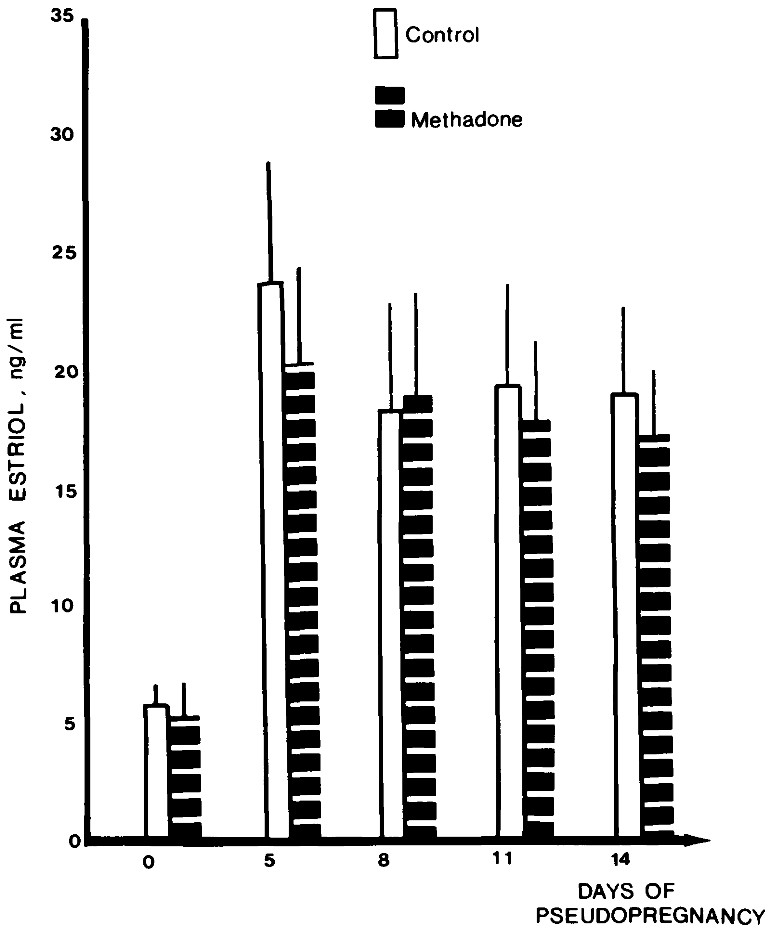

FIG. 8. Pseudopregnant rats were treated with either saline or methadone (5 mg/kg/day) from Days 5–14 of pseudopregnancy. The plasma total estriol levels were determined by radioimmunoassay techniques on Days 0, 5, 8, 11, and 14 of pseudopregnancy. Each bar represents the mean ± SEM of four animals.

els in pseudopregnant animals suggests a profound effect of methadone on the fetal compartment.

Administration of methadone increases the uterine level of cAMP during the early stages of pregnancy (Days 6 to 12 of gestation). This rise in cAMP is probably due to the stimulation of both the sympatho-adrenal axis and the hypothalamo-pituitary axis in releasing cathecolamines and hormones which act via activation of adenylate cyclase enzyme. Acute administration of opiates and opioid peptides had been shown to increase the concentration of adrenocorticotrophin hormone (ACTH) (22), prolactin, and growth hormone (23–25).

In contrast, failure of methadone to influence the uterine level of cAMP on Day 15 of gestation, after one daily injection for 10 consecutive days, may be due to decreased ACTH (9, 10, 26), plasma FSH (27, 29), and to an inhibition of LH release (29, 30) after repeated exposure to the narcotic. In addition, a development of tolerance to the narcotic by the sympatho-adrenal axis was suggested by Nakaki *et al.* (31). These two effects may explain the lack of an effect on the uterine level of cAMP as observed. However, the fetal cyclic nucleotide levels are still depressed by Day 15 of gestation and might result from an interference with the hormonal transport to, or hormonal production in, the fetal compartment. Indirect support for the latter was evident by the low levels of estriol on Day 9 of gestation.

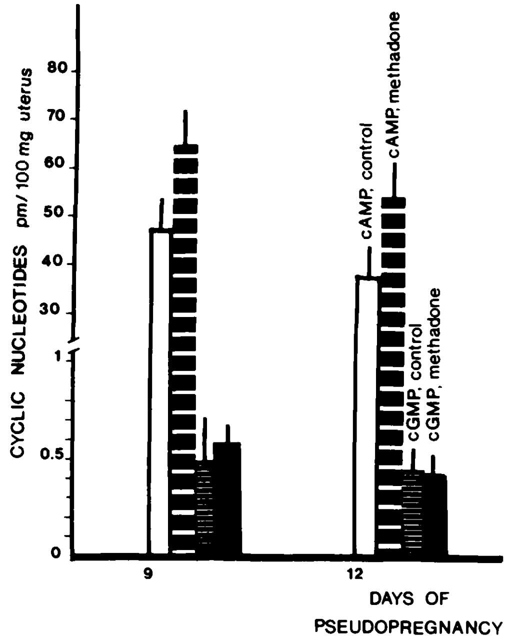

FIG. 9. Pseudopregnant rats were treated with either saline of methadone (5 mg/kg/day) from Days 5–14 of pseudopregnancy. The uterine levels of cyclic nucleotides were determined by radioimmunoassay techniques on Days 9 and 12 of pseudopregnancy. Each bar represents the mean ± SEM of four animals.

The levels of uterine cAMP in the pseudopregnant rats are much lower than those of the pregnant animals. This may be due to the high level of estriol found during pseudopregnancy. In fact, high levels of estrogens are known to inhibit the release of pituitary hormones which exert its effects on the uterus via adenylate cyclase. Nevertheless, our data indicate that methadone administration can stimulate the sympatho–adrenal axis and hypothalamo–pituitary axis of pregnant rats as evidenced by an increase in the uterine cAMP when compared to control values. However, no significant differences were found in the pseudopregnant uterus suggesting that the stimulation of the hypothalamopituitary axis by methadone could be antagonized, in part, by the presence of a high level of estriol.

Our findings suggest that acute or subchronic administration of methadone during pregnancy might interfere with the proper hormonal balance of the mothers and lead to reproductive adverse effects as observed by many investigators (15–18). The effects observed during pseudopregnancy with deciduomata formation reflect what is happening in the maternal compartment, and suggest that methadone must exert its adverse effects mainly in the fetal compartment leading to growth retardation associated with perinatal opiate administration. It can also be proposed that the pseudopregnant rat may be useful as a model to distinguish adverse drug effects between the maternal and fetal compartments.

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