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Effects of Lead on Luteal Function in Rhesus Monkeys[1]

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ABSTRACT

Exposure to lead in the workplace or home environment has been implicated as a cause of decreased fertility in women. In a previous study, as part of our effort to determine effects of lead in primates, female rhesus monkeys were exposed to lead acetate in drinking water (n=10) or provided water with no added lead (n=7) for 33 mo. Lead was administered at levels between 2 and 8 mg/kg/day, with doses adjusted to keep blood lead values near a target of 70 μg/dl (observed mean ± SEM = 68.9 ± 6.54 μg/dl). Blood lead concentrations in control animals were < 10 μg/dl. No significant differences were detected between control and experimental animals in body weight, hematocrit, or general health. Female monkeys receiving lead exhibited longer and more variable menstrual cycles and shorter menstrual flow. In the present study, circulating amounts of progesterone (P_4) were determined to evaluate luteal function during the final 7 mo of treatment with lead. Several characteristics were altered as a result of lead treatment: circulating amounts of P_4 were reduced as indicated by relative units of area under the concentration-time curve, maximal amounts of P_4 were reduced, and P_4 levels were > 1 ng/ml on fewer days. There were no significant differences between groups in mean percent of anovulatory cycles. Therefore, although chronic treatment with the levels of lead used in this study did not prevent ovulation, luteal function was suppressed. These results extend previous observations of adverse effects of lead on ovarian activity and fertility in monkeys.

INTRODUCTION

Reproductive biologists are concerned with lead because of its toxic effects on the reproductive system. Lead has long been recognized as a toxicant (Hamilton and Hardy, 1983), and much effort has been devoted to reducing the use of lead in our daily lives. Nevertheless, exposure to lead is still prevalent in the form of atmospheric lead from vehicular exhaust, lead solder in water pipes, and lead used in various industries. Lead has damaging effects on fertility, pregnancy, and fetal development in women living near or working in factories using lead (Rom, 1976; Hamilton and Hardy, 1983). Reproductive anomalies range from sterility, menstrual disturbances, premature rupture of the amniotic membrane, premature delivery, and decreased birth weight to chromosomal aberrations, macrocephaly, miscarriage, stillbirths, and early death of offspring (Forni et al., 1980; Nordstrom et al., 1979; Fahim et al., 1976; Panova, 1972).

Results from controlled animal studies indicate that lead has an equally wide-ranging and profound effect on several aspects of reproductive function as well as on embryonic development. For example, studies have linked oral lead exposure to delayed vaginal opening in immature female rats (Kimmel et al., 1980). Adult female mice and rats exhibited irregularity of estrous cycles and anestrus when given lead by various routes (oral: Hilderbrand et al., 1973; s.c. injection: Lach and Srebro, 1972; s.c. injection: Der et al., 1974). Cessation of menses, reduced sex-skin color, and decreased numbers of secondary and tertiary follicles were observed in female rhesus monkeys injected with doses of lead that produced overt toxicity (Vermande-Van Eck and Meigs, 1960). Female rhesus monkeys exposed to subclinical levels of lead in their drinking water showed

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significantly longer and more variable menstrual cycles as well as reduced frequency of menstrual cycles and shorter duration of menses (Laughlin et al., 1987). Lead poisoning in macaques, induced by administering lead acetate i.m. daily during pregnancy, resulted in abortion (Tachon et al., 1983). Similar findings in rodents suggest that lead acts to reduce the number of offspring born to mothers receiving lead by different routes during pregnancy (oral: Stowe and Goyer, 1971; Schroeder and Mitchner, 1971; Jacquet et al., 1977; Odenbro and Kihlstrom, 1977; and i.v.: McClain and Becker, 1975). Reduced reproductive success in mice treated with lead i.v. has been associated with the preimplantation period of embryonic development (Wide and Nilsson, 1977).

Specific effects of lead on the ovary have been reported in rats and monkeys (Vermande-Van Eck and Meigs, 1960; Stowe and Goyer, 1971; Hilderbrand et al., 1973). In monkeys, the results were flat, white, atretic ovaries with no dominant follicles. Follicular cysts were noted in rats. In both species, decreased follicular development was apparent. However, to date, the effect that lead exerts on the ovarian hormones has been demonstrated only in pregnant mice. At the time of blastocyst implantation (Day 5), examination of hormone levels concomitant with lead exposure showed lower than normal progesterone (P_4) levels and degenerated corpora lutea. By Day 6, estrogen (E_2) levels were also below control values (Jacquet et al., 1977; Wide and Nilsson, 1977; Odenbro et al., 1982). Administration of E_2 and P_4 on Day 5 reversed the inhibitory effects of lead on implantation (Wide, 1980). However, the mechanism of action has not been clarified. It has been proposed that lead inhibits secretion of steroid hormones (Jacquet, 1977) and/or alters actions of these hormones by reducing receptor concentrations (Wide and Nilsson, 1979; Wide and Wide, 1980). Alternatively, it has been suggested that lead interferes with ovarian steroid receptors in the uterus by inhibiting binding and thus interfering with implantation (Young et al., 1977).

The present study was undertaken to characterize patterns of circulating levels of P_4 in female rhesus monkeys in which treatment with lead resulted in aberrant menstrual cycles (Laughlin et al., 1987). In particular, we wished to determine if ovulation was occurring and to assess adequacy of luteal function.

MATERIALS AND METHODS

Subjects were 17 feral-born female rhesus monkeys (*Macaca mulatta*), 10 experimental and 7 control. The two groups were similar in age, number of previous pregnancies, and parental experience. The experimental animals had been treated with lead during 3 periods of approximately 14 mo each in 1976–1977, 1978–1979, and 1980–1981 (in conjunction with breeding experiments), and again for 33 mo in 1982–1984 (in conjunction with behavioral studies). Lead acetate was added to distilled water and treatments were at approximately the same levels of lead intake for each of the four exposures (see Laughlin et al., 1987). We report here on the last 7 mo of the fourth exposure, from April through October 1984. Estimated age of the subjects at that time was 16–20 yr.

Lead was administered to the experimental subjects 5 days/wk. Amounts were adjusted weekly to maintain circulating blood lead levels near a target of 70 μg/dl. Control levels were < 10 μg/dl. Doses required to maintain this level for individual monkeys ranged from 2 to 8 mg/kg/day with a mean dose of 5.4 mg/kg/day, 5 days/wk. A target level of 70 μg/dl was chosen as the highest amount that would result in no central nervous system alterations nor produce clinical symptoms such as appetite loss, weight loss, or hematocrit depression. Animals were weighed every 2 wk. Animals were checked 6 days/wk for the presence of menstrual blood, and the first day of bleeding was defined as Day 1 of the menstrual cycle.

Blood samples were obtained from each monkey twice a month for analysis of lead and zinc protoporphyrin (ZPP). All samples were analyzed for ZPP; however, because blood lead values were stable, only 3 samples (selected at approximately equal intervals) from experimental subjects and 1 sample from controls were analyzed for blood lead levels. Hematocrit volume, measured from the same blood samples, was determined only once. Blood samples for P_4 analysis were taken 3 times/wk (M, W, F) between 0800 and 1000 h beginning on Day 10 of the menstrual cycle, to coincide with ovulation and the beginning of the luteal phase. If menstruation had not occurred by Day 30, frequency of sampling was reduced to once a week.

Blood lead levels were analyzed by atomic absorption spectrophotometry at the Harlow Primate Laboratory or at the Wisconsin State Laboratory of Hygiene in Madison, WI. ZPP levels were determined with a hematofluorometer (AVIV, Lakewood, NJ), and hematocrit levels were measured by capillary centrifugation at the Harlow Primate Laboratory.

Plasma concentrations of P_4 were determined by a radioimmunoassay differing from that originally reported by Clark et al. (1978) in that 1) the antibody

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(Holly Hill Biologicals, Inc., Hillsboro, OR) was raised in rabbits against a conjugate of progesterone-11-succinyl bovine serum albumin and its specificity was verified to be adequate for unchromatographed samples; 2) 20 µl of plasma was assayed; and 3) the range of the standard curve was 4 – 250 pg, with an average 50% inhibition point of 23.8 ± 2.0 pg (mean ± SD). Parallelism was confirmed between the plasma samples and the standard curve. The average intra- and interassay coefficients of variation were 9.8% and 13.8%, respectively; the extraction efficiency was 82.3% ± 4.0%; and the average minimal sensitivity of the assay was 2.5 ± 0.4 pg.

Plasma P_4 levels were graphed using an Interactive Digital Plotter to give a profile over time (days) for each animal (Fig. 1 and 2). Relative area under the curve was measured from each profile with a Terak 89510/10a Graphic Computer System (Trerak Corp., Scottsdale, AZ).

Statistical analysis of differences between experimental and control groups for the measures of blood lead and body weight was performed with Student's *t*-test. Group mean differences in P_4 were analyzed by ANOVA for variables with repeated measured and otherwise with Student's *t*-test. All results have been presented as the mean ± SEM. In addition, individual values for plasma P_4 (both the area under the curve and the maximum height) were analyzed against lead dose, blood lead level, or ZPP by regression analysis on a computer program (Cricket Graph, Cricket Software, Inc., Philadelphia, PA). For all statistics, a p value <0.05 was considered to be significant.

RESULTS

Effects of Lead Exposure on General Health

All animals maintained stable body weights during the study, and there was no significant difference between control (7.08 ± 0.36 kg) and treated (7.33 ± 0.77 kg) females. The mean blood lead level of experimental animals was 68.9 ± 6.54 µg/100 ml; values for the control females were <10 µg/100 ml. Concentrations of ZPP were significantly greater for treated animals (107.4 ± 12.0 µg/dl blood) than for control animals (14.7 ± 14.4 µg/dl blood). Hematocrit values ranged from 36% to 44% and did not differ significantly between groups. There was no significant correlation between lead intake and blood lead level (r=0.57) or between lead intake and ZPP concentration (r=0.56) among the experimental monkeys. This was not surprising given that lead intake was adjusted to maintain equivalent blood lead levels across all the treated monkeys, thus restricting the range of correlation.

Relationship between Menstrual Cycles and Plasma Progesterone Patterns for Individual Subjects

Menstrual cycles were considered anovulatory when menstruation was preceded by a maximum amount of P_4 lower than 1.0 ng/ml. There was no significant difference between control and experimental subjects in the mean percent of anovulatory cycles (31% ± 8.7 vs. 40% ± 12.3, respectively).

Time-line graphs are shown for females which exemplify the most regular, average, and least regular patterns of P_4 profiles for control (Fig. 1) and experimental (Fig. 2) groups. Figure 1a shows the P_4 curve for the control subject (Con 7 in Table 1) with the most regular pattern; Con 5 in Table 1 shows a similar curve, indicating regular and ovulatory cycles for both of these control monkeys across all 7 mo of the study. A control animal (Con 4) that had ovulatory cycles in spring and fall, but not in summer, is shown in Figure 1b; Con 2 also exhibited this pattern. The curve for 1 of the 3 control animals with the lowest or least frequent P_4 peaks is shown in Figure 1c (Con 1). The monkey represented in Figure 1c showed ovulatory cycles in the spring but none in summer, and it did not resume ovulation by the end of the study in fall. Con 6 exhibited this overall pattern, having only one P_4 peak, of about 3 ng/ml, in the spring. Con 3 had a pattern similar to that of Con 1, except for the occurrence of an additional ovulatory cycle in fall.

In contrast to the control subjects, only one experimental animal, Pb6, showed a pattern of regular ovulatory cycles similar to that of the most regular control subject represented in Figure 1a. Nevertheless, P_4 peaks for this monkey were lower than for the most regular controls.

Three additional experimental monkeys (Pb8, Pb9, and Pb10) showed ovulatory cycles in both spring and fall but not in summer (Fig. 2a). P_4 peaks for these 3 monkeys also were markedly lower than for the controls in Figure 1, a and b.

Five experimental monkeys (Pb1, Pb2, Pb3, Pb4, and Pb7) had curves similar to the curve for Pb7 (shown in Fig. 2b), a monkey that had neither the most regular nor

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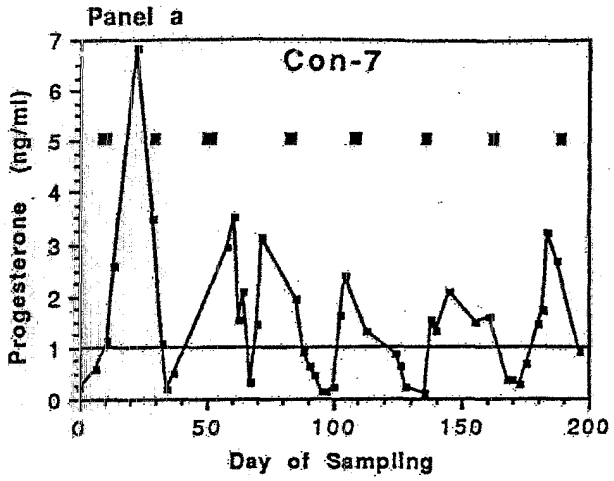
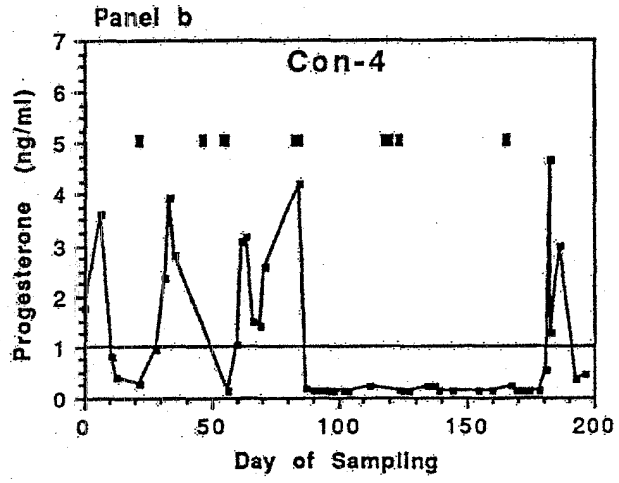




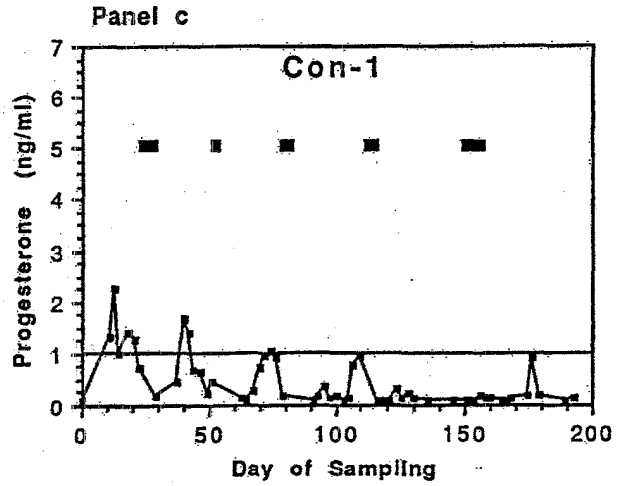
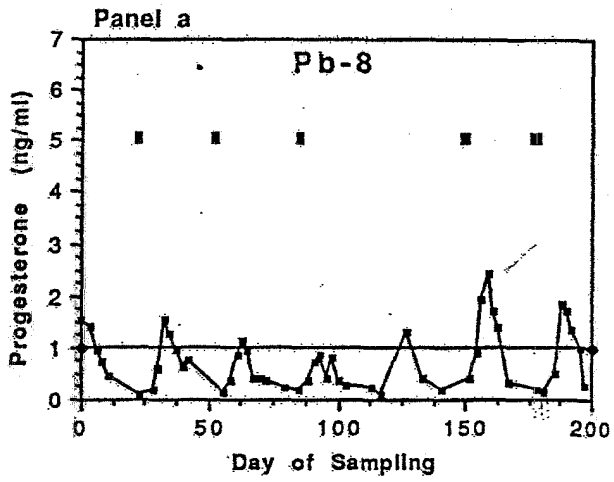
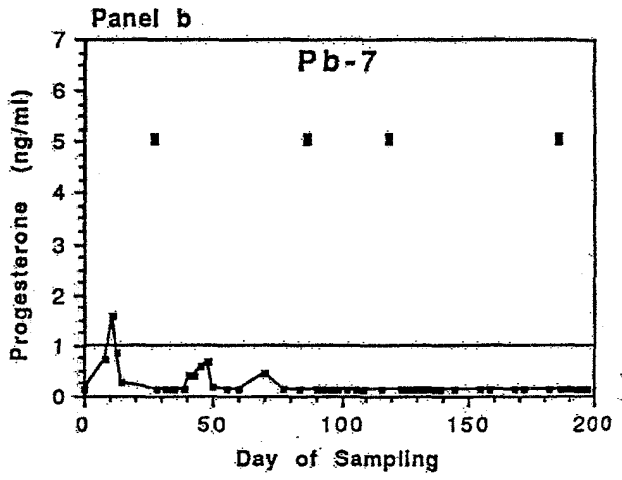


FIG. 1. Progesterone profiles for control subjects for the 7 mo of the study. *Panel a*: an example of most regular pattern; *Panel b*: an example of an average pattern; *Panel c*: an example of least regular pattern. *Solid blocks* correspond to observed menstrual bleeding.





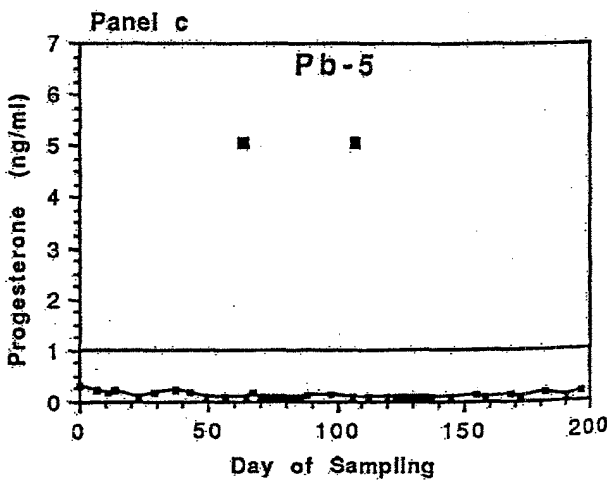


FIG. 2. Progesterone profiles for experimental subjects for the 7 mo of the study. *Panel a*: an example of most regular pattern; *Panel b*: an example of an average pattern; *Panel c*: an example of least regular pattern. *Solid blocks* correspond to observed menstrual bleeding.

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TABLE 1. Summary of data on individual subjects, shown as means and standard errors of repeated measures per subject.

Subject	Body weight (kg) (n = 14)[a]	Pb dose (mg/kg/day)	PbB (μg/dl blood) (n = 3)[a]	ZPP (μg/dl blood) (n = 15)[a]	Total # of menstrual periods, P_4 peaks ( ), & ovulatory P_4 peaks	P_4 peak heights, ng/ml; and mean area/Day[b] Overall	Spring	Summer	Fall
Pb-1	5.59	5.0	59.3	69.6	4	0.49	1.27	0.12	0.09
	0.33		11.9	15.49	(1), 1	(12.2)	(31.3)	(1.3)	(4.0)
Pb-2	6.80	6.0	96.3	99.9	6	0.61	1.53	0.21	0.09
	0.28		31.3	10.82	(2), 1	(12.1)	(28.3)	(5.3)	(2.8)
Pb-3	9.10	7.0	68.3	187.6	2	–	1.22	0.63	–[d]
	0.44		19.1	16.80	(2), 2	–	(25.7)	(0.0)	–
Pb-4	7.12	5.0	43.7	114.9	4	0.73	1.75	0.16	0.28
	0.38		9.3	20.07	(2), 2	(20.3)	(48.5)	(4.9)	(7.6)
Pb-5	7.77	8.0	43.7	148.5	2	–	–	0.05	–
	0.18		20.5	17.96	(0), 0	–	–	(0.5)	–
Pb-6	6.02	3.0	94.0	92.9	5	2.69	1.88	3.56	2.62
	0.32		59.7	17.72	(6), 6	(39.4)	(37.3)	(66.4)	(14.4)
Pb-7	8.42	7.0	58.0	118.2	4	0.32	0.97	0.00	0.00
	0.57		16.4	16.88	(2), 1	(7.4)	(22.1)	(0.0)	(0.0)
Pb-8	5.86	4.0	60.3	56.0	6	1.43	1.40	0.88	2.00
	0.23		15.5	8.52	(7), 6	(38.1)	(41.3)	(20.7)	(52.2)
Pb-9	6.78	2.0	99.0	96.0	6	1.60	1.72	1.64	1.44
	0.42		42.8	20.93	(6), 6	(25.3)	(44.4)	(2.1)	(29.4)
Pb-10	7.34	7.0	66.3	89.9	7	1.60	1.07	0.67	3.06
	0.73		25.0	18.70	(6), 4	(27.4)	(24.0)	(46.7)	(40.8)
Con-1	9.11	NA	4.0[c]	18.9	5	1.11	1.84	.067	0.81
	0.21		(1/5/84)	2.29	(5), 3	(23.1)	(51.0)	(13.3)	(5.1)
Con-2	9.31	NA	1.0	14.9	6	1.49	3.12	1.21	0.15
	0.40		(2/7/85)	2.15	(3), 3	(37.3)	(88.6)	(22.8)	(0.6)
Con-3	7.21	NA	4.0	16.8	5	–	1.48	0.37	–
	0.15		(7/5/84)	2.93	(3), 3	–	(25.2)	(4.24)	–
Con-4	4.46	NS	3.0	12.3	7	3.50	3.73	2.34	4.44
	0.21		(10/4/84)	2.99	(5), 5	(65.8)	(126.3)	(34.3)	(37.8)
Con-5	6.82	NA	1.0	8.5	8	2.81	2.83	2.09	3.52
	0.25		(10/17/85)	2.03	(7), 7	(60.0)	(52.3)	(43.2)	(84.6)
Con-6	5.05	NA	3.0	20.6	4	–	2.95	0.10	–
	0.43		(4/12/84)	3.42	(1), 1	–	(55.2)	(2.4)	–
Con-7	9.36	NA	3.0	16.0	9	3.68	4.75	2.51	3.78
	0.53		(10/6/83)	3.89	(8), 8	(54.4)	(74.7)	(49.0)	(39.6)

[a]n = Minimum number of scores averaged per subject, with mean (upper) and SD (lower).

[b]For each subject, mean P_4 peak height is shown on the first line as an open number, and mean P_4 area is shown on the second line in parentheses; data are based on 3/wk sampling. All numbers are mean/season/subject.

[c]One blood sample was assayed per control subject, taken on the date in parentheses. All experimental blood lead values were determined from blood sampled between 4/1/84 and 11/2/84.

[d]No 3/wk sampling in seasons in which the subject failed to show menstrual bleeding.

the least regular pattern of P_4 profiles of these 5. Note the small ovulatory peak in spring, followed by an anovulatory cycle, with no further ovulatory cycles during the summer and fall months. This subject had no detectable levels of P_4 during the summer and fall. The other 4 monkeys showed small increases of P_4 in summer and fall, although none of the increases exceeded 0.3 ng/ml. One monkey (Pb3) had one ovulatory cycle in late spring and another in early summer. A second monkey (Pb1) had only one ovulatory peak, and that occurred in spring. A third monkey (Pb2) had one ovulatory cycle in spring and one in summer. The fourth monkey (Pb4) had an ovulatory and an anovulatory cycle in spring and a small anovulatory peak in summer; the two anovulatory peaks were not associated with menstrual bleeding.

Finally, one experimental monkey (Pb5) exhibited the curve shown in Figure 2c. This monkey had very little P_4 secretion and only two periods of menstrual bleeding during the entire 7 mo of the study, both occurring in the summer months. It is pertinent that this monkey received the highest lead dose of all (8 mg/kg/day) and had the second highest mean ZPP level, yet had the lowest mean blood lead level in the group. By contrast, the animal receiving the lowest lead dose of all (Pb9 at 2 mg/kg/day) had a mean ZPP level in the midrange and the highest mean blood lead level; she had a P_4 pattern virtually identical to that in Figure 2a, with low peaks and with six ovulatory cycles over the 7 mo of the study. In addition, the experimental monkey with the most regular cycles (not shown) also received

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TABLE 2. Effects of lead on progesterone measures.

Group	n	Area under curve (relative units)*	Peak height (ng/ml)*	Days above 1 ng/ml
Control	7	6.82 (1.11)	2.81 (0.31)	10.54 (1.44)
Lead	10	4.07[a] (0.74)	1.79[b] (0.24)	6.06[b] (0.635)

[a]Significantly different from controls by 2-tailed *t*-test; $p<0.05$.

[b]Significantly different from controls by 2-tailed *t*-test; $p<0.01$.

*Numbers are means (upper) and SEM (in parentheses).

one of the lowest doses of lead (3 mg/kg/day) and had a ZPP and blood lead level in the midrange.

Plasma Progesterone Levels

Four different characteristics of P_4 were analyzed to assess differences between control and experimental monkeys: 1) number of P_4 peaks (a peak was determined to be a rise in P_4 levels above 1 ng/ml as determined from the P_4 profile of each animal); 2) height of P_4 peaks; 3) area under the curve; and 4) number of days when P_4 was above 1 ng/ml. There were no significant differences in actual numbers of P_4 peaks between the two groups. However, in other characteristics, clear differences between groups were noted. Table 2 compares P_4 levels of treated and control animals. Animals exposed to lead had only 60% of the P_4 values of control animals, which was manifested in a significantly smaller area under the curve ($t=2.16$, $p<0.05$) and significantly shorter peak heights of P_4 ($t=2.63$, $p<0.01$). Monkeys exposed to lead also had significantly fewer days per cycle when P_4 levels were 1 ng/ml ($t=3.10$, $p<0.01$).

For all animals in both groups, the frequency of blood sampling was 3/wk starting on Day 10 of the cycle, but was reduced to 1/wk following Day 30 of the cycle if no menstruation occurred in that interval (see *Materials and Methods*). To ensure that the method of sampling did not affect these analyses, the P_4 data were analyzed further using only those calendar periods for each subject in which blood samples were taken 3 times/wk. This assured exactly the same sampling density for both the experimental and control groups. The P_4 curves during only these more frequent sampling periods were then analyzed by measuring each subject's P_4 peak height (ng/ml) and mean P_4 area (digitizer units per day); P_4 area was expressed per day to compensate for variation in the durations of the 3-wk sampling periods. The regression analyses for lead intake, blood lead levels, and ZPP levels are shown by season in Table 3.

From the regression analyses (Table 3), it can be seen that the P_4 areas declined significantly with increasing lead intake, increasing blood lead levels, and increasing levels of ZPP. The P_4 peak heights were more variable. They declined significantly with increasing lead intake and were not significantly correlated with blood lead level. The correlation between peak height and ZPP level approached significance ($p<0.10$). In summary, these regressions demonstrated that higher lead intake was associated with reduced P_4 areas and lower P_4 heights. Furthermore, area and height each correlated about equally with lead intake, but area correlated more strongly than did height when regressed against blood lead level and ZPP.

The seasonal effect on P_4 peak height was tested by ANOVA with lead treatment as a between-subjects variable (lead vs. control) and season as a within-subject variable (3 seasons). Four subjects failed to exhibit menstrual bleeding within some season, thereby precluding a period of 3/wk sampling during that season. Each subject had missing points in fall, and one treated subject also had a missing point in summer. Since 2 subjects were experimental and 2 were control, suggesting no undue treatment bias, those subjects with missing data points were eliminated from the ANOVA. This avoided the uncertainty of estimating missing data points. Thus, the ANOVA was run on the 13 subjects for which overall P_4 means are shown in Table 1.

The ANOVA on P_4 height shows that the main effect of lead treatment was significant ($F=7.10$, $df=7,11$; $p=0.022$), the seasonal effect was significant ($F=6.70$, $df=2,22$; $p=0.005$), and the interaction was nonsignificant ($F=1.61$, $df=2,22$; $p=0.22$). Analysis on the measure of P_4 area gave similar results. Thus these results gave no support for treating the effect of lead as differential across the three seasons.

In summary, the experimental monkeys had reduced secretion of P_4 as shown by lower P_4 area under the curve over the 7 mo of the study. P_4 secretion varied by season, but not at differential rates for the two groups.

DISCUSSION

In this study, chronic exposure to lead clearly had a detrimental impact on the luteal phase of ovarian func-

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TABLE 3. Linear regressions on lead intake, on blood lead level, and on ZPP for the two progesterone measures, maximum peak height, and total area, as quantified for only those time periods at which blood was sampled 3 times/wk.

Lead intake and related measures (n = 13 subjects; see text)	Peak area (digitizer units/day)			Maximum peak height (ng/ml)		
	Intercept	Slope	r	Intercept	Slope	r
Lead intake (mg/kg/day)	46.7	-4.46	-.67[a]	2.51	-0.27	-.68[a]
PbB (μg/dl)	45.5	-0.27	-.56[b]	2.27	-0.01	-.43
ZPP (μg/dl blood)	52.1	-0.30	-.70[a]	2.40	-0.01	-.51[c]

[a] $p<0.01$.

[b] $p<0.05$.

[c] $p<0.10$.

tion. Significant differences were found between control and experimental subjects in three of the four measures of P_4 secretion used to evaluate luteal function: area under the curve, peak heights of P_4, and mean number of days per ovulatory cycle when P_4 was above 1 ng/ml. In all three of these measures, females exposed to lead showed significantly reduced amounts of P_4 secreted by the ovary. The fourth measure of progesterone activity, total number of P_4 peaks, as well as number of anovulatory cycles, did not yield differences between control and lead-treated animals. Although blood lead and ZPP levels were elevated during treatment, the monkeys exhibited no overt signs of toxicity. Hematocrit values and body weights of the treated animals did not differ from control animals. Thus, these effects of lead on luteal function occurred in the absence of overt effects on the general health of the experimental females.

Although there were seasonal changes in P_4 activity, as is typical of monkeys housed in laboratory environments (Hutz et al., 1985; Daily and Neill, 1981), the patterns of change were similar for both control and treated monkeys. That is, although peak height and area of P_4 changed across the seasons, the magnitude of the change was not different for the two groups of monkeys. In the previous study, probability of cycles of normal length also declined during summer and fall compared to spring, but not differentially for experimental and control monkeys (Laughlin et al., 1987). Further, there was substantial cycle activity during the months of increased cycle length, even for the treated monkeys.

These effects on ovarian cycle extend findings from the previous study with the same animals in which lead-treated monkeys were found to have fewer cycles, longer and more variable intercycle intervals, and fewer days of vaginal bleeding per cycle than control monkeys (Laughlin et al., 1987). Three of these four alterations became apparent only after lead exposure had been continued for many years, while the fourth, duration of vaginal bleeding, was disrupted by lead during the initial year of intake. All four effects of lead on menstrual cycles remained apparent for at least 1 yr after lead intake was discontinued following a total of approximately 6 yr of lead administration. The longer and more variable intercycle intervals observed in the previous study suggest that lead has effects on the follicular stage of the cycle, since the postovulatory period is relatively constant regardless of total cycle length (Reid et al., 1972). Results of the present study, obtained during the last 7 mo of lead intake, extend these effects of lead to include luteal function. Although menstruation and progesterone activity do not cease in response to prolonged chronic exposure to lead, both are clearly attenuated, which could result in reduced fertility.

Whether the alteration in luteal function demonstrated in the present study was due to direct effects of lead upon the ovary or to changes in the hypothalamic-pituitary axis is not clear. Some studies suggest the possibility of direct effects of lead on ovaries. In rhesus monkeys, treatment with lead resulted in reduced numbers of secondary and tertiary follicles and increased follicular atresia (Vermande-Van Eck and Meigs, 1960). Ovarian weight (Der et al., 1974) and number of corpora lutea (Hilderbrand, et al., 1974) were reduced in rats treated with lead. Also, fish exposed to low levels of lead showed reduced ovarian weight (Katti and Sathyanesan, 1983; Sastry and Agrawal, 1979).

Alternatively, other reports suggest that lead may act at the level of the hypothalamus and pituitary. For example, sexual maturation, thought to be mediated by the hypothalamus (Firlit and Schwartz, 1977), was delayed in female rodents exposed chronically to low lead

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levels (Der et al., 1974; Grant et al., 1980; Kimmel et al., 1980). It has been demonstrated that high levels of lead result in abolition of the surge of follicle-stimulating hormone (FSH) in prepubertal rats (Petrusz et al., 1979). This surge normally occurs at 15 days of age (Kragt et al., 1972; Ramirez, 1973; Uilenbrock et al., 1976; Kronibus and Wuttke, 1977) and is required for normal puberty. The possibility that lead interferes with FSH surges suggests that suppression of this increase during the follicular phase of the menstrual cycle in the present study could have been responsible for the deficient luteal function noted (Nass et al., 1979). Thus, although our demonstration of decreased P_4 levels concomitant with alterations in menstrual cycles clearly implies ovarian dysfunction, multiple loci within the hypothalamic-pituitary-ovarian axis may be mediating this action of lead.

This study points out the subtlety with which lead can act. Exposure to lead at levels that do not cause overt toxicity can nevertheless exert demonstrably negative effects upon ovarian function.

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