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Prenatal or Lactational Exposure of Male Rats to Lead Acetate. Effect on Reproductive Function

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Lead is an environmental pollutant which has received much attention (Mushak 1992), partly because of the particular sensitivity of children to this element. As regards the consequences of exposure to lead during fetal life or childhood, epidemiological studies have so far focused on its neuropsychological effects (for review, see Goyer 1993) and little is known about the consequences of fetal or childhood exposure for reproduction. With respect to animals, the reproductive toxicity of lead in males exposed during prenatal life or the suckling period has only been considered in a few studies. Four such studies concerned the rat, the most current model of lead toxicity for male reproduction; two of studies considered the long term effects (i.e. during adulthood) of moderate *in utero* lead exposure (McGivern et al. 1991, Coffigny et al. 1994), another covered the prenatal and neonatal periods and focused on the possible impact of lead intoxication on steroidogenesis before weaning (Wiebe et al. 1982), while the remaining study dealt with pituitary hormone level at the end of lead gavage in newborns (Petrusz et al. 1979). None of these investigations compared the effects of exposure during prenatal life to those of exposure via lactation, or the early effects (at about weaning time) to the long-term consequences during adulthood. Because of the paucity of data on these points, we conducted two experiments: in one, rats were exposed to lead prenatally, and in the other via maternal milk. In both cases male reproductive function at weaning and adulthood was examined.

MATERIALS AND METHODS

Thirty-nine female Sprague-Dawley rats, primigravidae from timed mating, were obtained from IFFA CREDO (France). Upon arrival on day 1 of gestation, the pregnant rats were randomly allocated to 3 treatment groups and housed individually. They were maintained under standard laboratory conditions (21±1 °C, 12 h light-dark cycle) and fed ad libitum. Fourteen females (group 1) were given a solution of distilled water containing 7 g/l lead acetate as drinking water. HCl at a final concentration of 10^{-2} M was added to the solution to preclude the precipitation of lead salts. The 25 other pregnant females, comprising group 2 (11 females) and group 3 (14 control females) were given distilled water with HCl at the same concentration. Just before birth, the fetuses of 3

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dams in groups 1 and 3 were decapitated and trunk blood was collected in heparinized tubes for lead measurement. Blood from 5 to 7 fetuses was pooled for each sample. At birth, the 11 remaining prenatally exposed litters in group 1 were cross-fostered by the 11 untreated dams in group 2. The pups from group 2 dams were cross-fostered by the dams of group 1, which continued to be given 0.7% lead acetate in their drinking water; these group 2 pups were thus exposed to lead during the suckling period. The pups from group 3 dams (control group) were cross-fostered by dams in the same group. All litters were standardized to 10 pups apiece. On day 22 post partum, the rats were weaned and males and females were separated after ear punching for treatment determination. They were given tap water ad libitum until they were killed.
To determine the evolution of blood lead levels, blood was collected from the pups in group 1 by cardiac puncture in heparinized tubes on post partum days 5, 12, 22, 26, 32, and 97, from group 2 pups, on days 22, 26, 32, 56, and 97 , and from group 3 pups, on days 5, 12, 22, 26, 32, 56 and 97. Lead was also measured on pooled blood from rats of the same litter on days 5 and 12, and then on individual animals; each point was the mean of the measurements made on 3 different individual samples or pools. Lead was estimated in whole blood using a graphite furnace atomic absorption spectrophotometer (Spectr AA 30 40 Zeeman, Varian Techton, Australia). The absorption wavelength was 283.3 nm and the detection limit, 4.1 μg/dl. The measurements were made by the staff of the Laboratory of Toxicology at the Fernand Widal Hospital (Paris, France).

On post-natal day 22, 13 males pups in group 1, 15 in group 2 and 9 group 3 controls were randomly chosen, weighed and killed under pentobarbital anesthesia. Blood was collected by cardiac puncture in heparinized tubes for determination of the lead in whole blood (see above) and for assay of plasma follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. The plasma, obtained by centrifugation, was stored at -20°C until measurement. Testes and epididymides were dissected out and weighed. The left testes were processed for histology and the right testes frozen at -80°C for testosterone assay.

On post-natal day 90, when the reproductive function of the male rats had reached maturity, 14 males in group 1, 15 in group 2 and 13 group 3 controls were each allowed to mate with 2 virgin females of the same group for 4 days. All the pregnant females were killed on day 20 of gestation; the number of fetuses, sex ratio, and numbers of implantation sites, resorption sites and corpora lutea were determined, as well as the weight of the ovaries. On post-natal day 97, the males were weighed and killed under pentobarbital anesthesia; blood was collected as described above. The ventral prostate, seminal vesicles, testes and epididymides were dissected out and weighed; the right epididymes were used to assess sperm motility and for morphological studies and the left epididymes were immediately frozen and stored at -80°C until determination of sperm reserves; the left testes were processed for histology or used to study the *ex vivo* production of testosterone, and the right testes were frozen for testosterone assay as previously described (Pinon-Lataillade et al. 1993). Data are expressed as means ± SEM, except for the weights of the fetuses after the fertility test which were expressed as means ± SD. The effects of exposure to

lead were estimated by Student's t test and the Mann Whitney U test. A significance level of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Prenatal exposure to 0.7% lead acetate in the drinking water of group 1 dams led to a high blood lead level in the fetuses of this group (136±10 μg/dl). After birth, this blood lead content rapidly decreased to 44 μg/dl in five days, and was undetectable after 40 days (Fig 1). On post-natal day 22, prepubertal rats exposed via maternal milk (group 2), had a blood lead level which reached 242±32 μg/dl, but 4 days later it had dropped to 48±9 μg/dl and remained very low until maturity (97 days, Fig 1). These rapid decreases are consistent with the short half-life of lead in the blood of rats (Grobler et al. 1988). In the control rats (group 3), blood lead levels always remained under the detection limit.

On post partum day 22, the body weight of the rats exposed to lead either prenatally (group1) or during suckling (group 2) were significantly reduced ($p<0.05$ and $p<0.001$ respectively, Table 1). The testis weight of the animals in both groups dropped significantly ($p<0.05$ in group 1 and $p<0.001$ in group 2) compared to control weight. The epididymis weight of the rats in group 2 was also significantly less than that of the controls ($p<0.001$, Table 1). Histological evaluation of the testis showed no disturbance in the spermatogenic process, but a significant reduction of the diameter of the seminiferous tubules was noted in both the exposed groups (122± 4 μm, $p< 0.001$ for group 1 and 129 ± 4 μm, $p< 0.01$ for group 2 as compared to 134 ± 4 μm for the control group). A non-significant reduction of 4% in diameter was previously observed by Wiebe et al. (1982) at 13 and 21 days post-partum, but without any decrease in testis weight. This difference may be due to the level of intoxication in their study (blood lead level under 10 μg/dl) which was much lower than in the present study.

With regard to pituitary hormones, the levels of plasma FSH and LH tended to increase in group 1 pups (8.8 ± 0.7 ng FSH/ml and 0.50 ± 0.01 ng LH /ml vs 7.0 ± 0.7 and 0.38 ± 0.01 for the controls) but not in group 2 pups (7.7 ± 0.6 ng FSH/ml and 0.44 ± 0.06 ng LH/ml). In rats exposed to lead neonatally (corresponding to our group 2), Petrusz et al. (1979) did not find any change in the serum FSH level, but reported a rise in pituitary FSH. They concluded that this rise resulted from increased FSH production rather than from a decrease in its release. Furthermore, in 13 and 21 day-old rats, a decrease in the steroid metabolism of testes homogenates and of Sertoli cell preparations from prenatally plus neonatally lead-exposed pups was previously observed; these decreases were accompanied by a reduction in FSH binding to Sertoli cells (Wiebe et al. 1982) and occurred during the period when the capacity of Sertoli cells to convert progesterone into C-21 and C-19 steroids is maximal (Tilbe and Wiebe 1981). Here, the decrease in seminiferous tubule diameter observed in both treated groups, combined with the rises that we and Petrusz et al. (1979) found in pituitary hormone levels, and the abnormalities of steroid metabolism and FSH binding observed in Sertoli cells by Wiebe et al. (1982), enable us to conclude that lead might alter Sertoli cell function in rats when administered during the prenatal period and the early post-natal life. The level

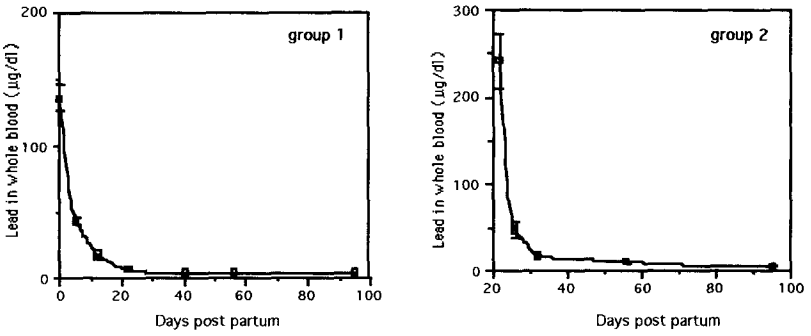

Figure 1. Post-natal evolution of the blood lead level in rats after exposure to lead during prenatal life (group 1) or the suckling period (group 2). Each value is the mean ± SEM of 3 individual samples or pools.

Table 1. Body and sex organ weights of prepubertal 22 day-old male rats exposed to lead prenatally (group 1) or during suckling (group 2).

	Controls (a)	Group 1(b)	Group2 (c)
Body weight (g)	60 ± 1	53 ± 2*	48 ± 1***
Testis (mg)	158 ± 4	119 ± 12$	116 ± 5***
2 epididymes (mg)	35.9 ± 1.1	33.7 ± 1.6	30.6 ± 0.6***

values are means ± SEM; a= 9 rats , b= 13 rats, c= 15 rats.*p<0.05, ***p<0.001 compared to controls, Student's t test. $ p< 0.05 Mann Whitney test.

of plasma testosterone was similar to the control level in both lead-exposed groups, which was also the case for testicular testosterone (data not shown), indicating that there was no alteration in Leydig cell function.
As regards body weight at maturity (97 days of age), this was not significantly reduced in either of the lead-exposed groups (Table 2), despite the significant reduction at 22 days (Table 1). The weights of both the testes and epididymes dropped significantly in group 1 and group 2 rats as compared to the controls, whereas the weights of seminal vesicles, ventral prostate and pituitary did not change (Table 2). With respect to epididymal parameters, a non-significant decrease in the sperm reserves of the cauda epididymis was observed in both exposed groups (130±11 million for group 1 and 133±16 million for group 2 vs 160±13 million for the controls) and this reduction might explain the reduction in the weight of the testes and epididymes. In a different study dealing with the effects of lead-oxide inhalation by the dams during gestation (blood lead level at birth: 83µg/dl), male offspring did not exhibit these modifications at maturity (Coffigny et al. 1994). We assume that the modifications observed

Table 2. Body weight and organ weights of 97 day-old adult male rats exposed to lead prenatally (group 1) or during suckling (group 2)

	Control (a)	Group 1 (b)	Group 2 (c)
Body weight (g)	444 ± 8	439 ± 7	470 ± 10
Testis (g)	2.04 ± 0.03	1.89 ± 0.06*	1.89 ± 0.02***
Epididymis (g)	0.59 ± 0.01	0.54 ± 0.02$*	0.55 ± 0.01**
Ventral prostate (g)	0.67 ± 0.05	0.61 ± 0.03	0.69 ± 0.04
Seminal vesicles (g)	1.54 ± 0.05	1.42 ± 0.06	1.65 ± 0.10
Pituitary (mg)	12.4 ± 0.4	11.6 ± 0.4	13.0 ± 0.5

Results are means ± SEM; (a) 13 rats, (b) 14 rats, (c) 15 rats; *p<0.05, $ * p< 0.02, ***p< 0.001 compared to controls, Student's t test.

here in mature male rats after early lead exposure were due to the higher level of lead exposure in the present study.

With regard to the decrease in sperm production, our results are in agreement with those of McGivern et al. (1991), who observed a decrease in the intratesticular sperm count at 60 and 160 days in rats prenatally exposed to lead. This decrease might be attributable to the dysfunction of the Sertoli cells. Because these cells are responsible for the environment of germ cell proliferation and maturation (For review, see Jegou 1992), we may justifiably assume that the damage to these cells by prenatal or neonatal exposure to lead is responsible for the slight impairment of spermatogenesis observed here in mature rats, although no alterations in the histologic appearance of the seminiferous epithelium were noted. The motility and morphology of the cauda epididymis spermatozoa were not modified (data not shown), suggesting that no alteration occurred in epididymal sperm maturation.

Plasma FSH and LH levels returned to normal at maturity in both lead-exposed groups. Plasma and intratesticular testosterone and the *ex vivo* production of testosterone were also normal (Table 3). This normal testosterone production together with the normal weights of ventral prostate and seminal vesicles, whose growth and maintenance are androgen-dependent (Mooradian et al. 1987), suggest that lead exposure during prenatal life or the early stages of post-natal life did not lead to abnormal testosterone synthesis by Leydig cells.

Lead intoxication in our two experimental groups did not impair the reproductive ability of either males or females. Neither was the day of fertilization delayed compared to the controls (data not shown). The number and weight of fetuses did not change significantly (14.8 ± 0.6 fetuses/dam in group 1 and 14.6 ± 0.6 in group 2 vs 15.7 ± 0.5 in the control group, and 4.00 ± 0.42 g/fetus in group 1 and 3.86 ± 0.46 in group 2 vs 4.22 ± 0.41 in the control group). The sex ratio (number of male fetuses/total number of fetuses) was not modified either by exposure to lead prenatally or during the suckling period as compared to the controls (48 ± 4% and 51±2% vs 52 ± 3%). The results of the fertility test were comparable to those of a previous study (Coffigny et al. 1994), and revealed that the probable alteration in Sertoli cell function at weaning did not impair the reproductive ability of the mature male rats, despite the diminution in the number of spermatozoa. This absence of

Table 3. Hormonal levels in 97 day-old adult male rats exposed to lead prenatally (group 1) or during suckling (group 2)

	Control	Group1	Group 2
Plasma FSH, ng/ml (a)	5.6 ± 0.5	5.6 ±0.8	5.9 ± 0.5
Plasma LH, ng/ml (a)	0.28 ± 0.03	0.23 ±0.01	0.26 ±0.03
Plasma testosterone, ng/ml(a)	3.1 ± 0.8	3.4 ±0.7	3.5 ± 0.6
Intratesticular testosterone (b)			
ng/g	88 ± 15	83 ±16	108 ± 15
ng/testis	171 ± 31	147 ±31	187 ± 26
Ex vivo testosterone production (c) (ng/g testis/ 4h)			
basal	101 ± 19	115 ±20	118 ± 24
hCG stimulated	241 ± 56	317 ±73	412 ± 69

(a) 8 rats, (b) 7 rats and (c) 6 rats per group.

impairment can be explained by the large daily sperm production and reserves observed in the rat, in which epididymal sperm counts were sometimes reduced by as much as 90% without significantly affecting fertility (Working 1988). Here, the number of corpora lutea in the ovaries of pregnant females (17.5 ± 0.4 in group 1 and 16.6 ± 0. 4 in group 2 vs 17.2 ± 0.4 in the controls) and weight of these ovaries (60 ± 2 mg in group 1 and 60 ± 2 mg in group 2 vs 59 ± 2 mg in the controls) were not modified by lead exposure, suggesting that when this exposure was confined to prenatal life or the suckling period, it did not affect reproduction in the mature female rat, despite the previously observed delay in female puberty (McGivern et al. 1991).

In conclusion, the present observations made at 22 and 97 days of age in rats, suggest that Sertoli cell function might be impaired by prenatal or lactational exposure to lead, leading to the slight decrease in sperm number we observed in adulthood but this decrease did not affect the male rat fertility. Nevertheless, these results are difficult to extrapolate to humans as no epidemiological data are available on the fertility of human males which were lead-exposed prenatally or through mother's milk.

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