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Lead Toxicity and the Hypothalamic-Pituitary-Testicular Axis

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ABSTRACT

Environmental exposure to toxic levels of lead occurs in a number of industries with potential adverse effects on the reproductive capacity of exposed men. Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic lead exposure, but the pathogenetic mechanisms involved have not been identified. In order to ascertain what reproductive abnormalities occur in experimental animals when exposed to low levels of lead, 52-day-old animals were treated with water containing 0.0% (control), 0.1%, or 0.3% lead acetate for 30 days prior to killing. Whole blood serum lead levels were below detection (<7 μg/dl) in the control animals, 34 ± 3 μg/dl in the 0.1% group, and 60 ± 4 μg/dl in the 0.3% group ($P<0.001$). Significant negative correlations between whole blood lead levels and serum and intratesticular testosterone values were found ($r=0.64$, $P<0.001$ and $r=0.6$, $P<0.001$, respectively). As the level of lead exposure increased, intratesticular sperm counts significantly decreased ($r=0.81$, $P<0.001$). No significant changes in serum luteinizing hormone (LH) values were found, but sperm follicle-stimulating hormone (FSH) values were significantly suppressed ($P<0.05$) after lead treatment. There was a significant decrease in ventral prostate weight ($P<0.05$), but no differences in testicular or seminal vesicle weights. Our data indicate that dietary exposure to lead resulting in whole blood serum lead values considered acceptable in the workplace (≤40 μg/dl) causes inhibition of testicular function. The failure to demonstrate elevated LH and FSH values in the face of markedly decreased serum testosterone and ventral prostate weight values suggests either a predominant mechanism of action of lead toxicity at the level of the hypothalamic-pituitary axis or a combined defect involving the gonad and hypothalamic-pituitary sites.

INTRODUCTION

Environmental exposure to toxic levels of lead occurs in a number of industries with potential adverse effects on the reproductive capacity of exposed men (Lancranjan et al., 1975; Cullen et al., 1983). Hand-to-mouth transfer of dust may be a major source of excessive lead exposure, especially in children (Landrigan et al., 1975). The children of men working in lead-related industries are at special risk because their parents may bring significant amounts of lead dust home on their clothing or shoes (Hammond, 1982). Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic lead exposure (Hilderbrand et al., 1973; Lancranjan et al., 1975; Der et al., 1976; Wyrobek and Bruce, 1978; Petrusz et al., 1979; Eyden et al., 1981), but the pathogenetic mechanisms involved have not been identified. This study evaluates the effects of lead exposure on reproductive hormones and spermatogenesis.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased from Simonson Breeding Farms in California. They were housed in the rodent vivarium in a separate room having a 12-h light-dark cycle and controlled temperature. Animals were housed in plastic cages and given free access to laboratory chow. Animals were allowed to acclimate to their new environment for 7 days prior to initiation of lead treatment. Each treatment group consisted of eight rats.

Diet

On arrival, the animals were transferred to plastic cages containing sawdust bedding and roofed with stainless wire covers. The animals were given free access to laboratory chow. Control animals received deionized distilled water containing no lead acetate. Treatment groups received either 0.1% or 0.3% lead acetate in distilled deionized water. The 0.1% lead acetate solution was prepared by dissolving 2.5 g of

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lead acetate in 2.5 liters of distilled deionized water. The 0.3% lead acetate solution was similarly prepared using 7.5 g lead acetate. One milliliter of 5 N HCl was added to all bottles (including control) to preclude the precipitation of insoluble lead salts. Water bottles were weighed each morning and the volume consumed during the previous 24 h was recorded. Animals were weighed every other day. The length of the study was 30 days of treatment.

Blood Lead Analyses

Animals were lightly anesthetized with diethyl ether and the abdomen was opened. The animals were exsanguinated via aortic puncture using Vacutainer needles and collection into Vacutainer tubes that had been acid-washed and heparinized for lead analysis. Lead analysis was done by the staff of the Air and Industrial Hygiene Laboratory of the California Department of Health Services, as previously described (Del Rosaro et al., 1982). Blood samples were diluted 1:50 with distilled deionized water and analyzed by graphite furnace atomic absorption spectrophometry (Instrumentation Laboratory Model 257). Physiologically bound lead standards obtained from lead acetate-fed cows and aqueous reference standards (Fischer Scientific Company, Springfield, NJ) were used.

Serum Hormone Determination

After collection of whole blood into the heparinized Vacutainer tubes for lead analysis, the remaining sample was collected into a standard test tube and allowed to clot. Serum luteinizing hormone (LH), follicle-stimulating hormone, and testosterone were assayed using previously described radioimmunoassays (Odell et al., 1967, 1968, 1974).

Tissue Collection

After killing, the testes, seminal vesicles, and ventral prostate were removed and weighed.

Intratesticular Testosterone Measurements and Sperm Counts

The capsule of the right testis from each animal was dissected. Each testis was homogenized in a glass homogenizer using 20 strokes in 2 ml of 0.5 M phosphate buffer. One-tenth milliliter of the homogenate was removed for the determination of the intratesticular sperm count (Roble et al., 1978). One-tenth milliliter of the homogenate was diluted with 0.9 ml of buffer and 0.1 ml was counted using a phase-contrast microscope with 400X magnification and a bright-light hemacytometer. The results are reported as number of sperm per testis. The remainder of the testicular homogenate was extracted with diethyl ether and assayed for intratesticular testosterone (Odell et al., 1974).

Statistical Analyses

Serum and tissue hormonal variables, sperm counts, and whole serum blood lead levels are reported as mean ± SEM for each group. One-way and two-way analyses of variance were performed as well as multiple regression analysis.

RESULTS

Body and Tissue Weights (Table 1)

Control and the 0.1% treatment animal groups consumed comparable volumes of water. Both groups gained weight at essentially comparable rates and mean group weights were not significantly different at the end of the 30-day treatment period. Although the 0.3%-treated rats lost weight, no significant correlations were found between body weight and serum testosterone (r=0.30) or body weight and intratesticular testosterone (r=0.29). A significant correla-

TABLE 1. Body and tissue weights and water consumption in 52-day-old rats treated with lead acetate for 30 days.

Lead treatment		Body weight (g)	Tissue weights: L testis (g)	R testis (g)	Ventral prostate (mg)	Seminal vesicles (mg)	Water consumed [ml/(day•rat)]
Control	Mean	365.63	1.49	1.46	40.00	58.25	145.0
	SEM	6.71	0.03	0.04	2.23	4.51	–
	SD	18.98	0.08	1.10	6.30	12.76	–
0.1%	Mean	371.88	1.45	1.42	33.13	68.38	135.0
	SEM	6.12	0.04	0.03	2.30	3.88	–
	SD	17.31	0.11	0.09	6.51	10.98	–
0.3%	Mean	331.88	1.42	1.40	31.50	61.13	103.0
	SEM	6.5	0.05	0.04	1.31	5.00	–
	SD	18.310	0.13	0.12	3.70	14.15	–
Analysis of variance		P< 0.0005	NS	NS	P<0.05	NS	

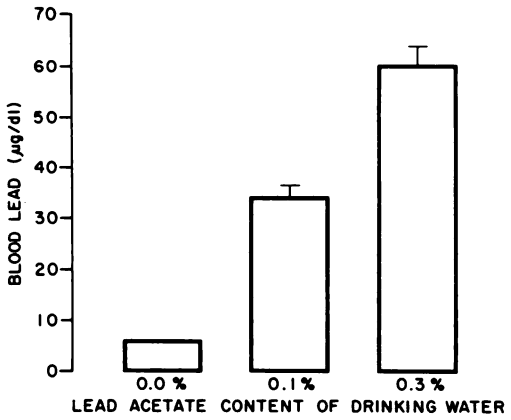

FIG. 1. Blood lead levels after 30 days of lead exposure in 3 groups of male rats treated with water containing lead acetate.

tion was noted between body weight and sperm count (r=0.52, P<0.05), but the effect of dose remained highly significant ($F_{2,20}$=21.4) even after adjustment of body weight as a potential covariant. A significant decrease in ventral prostate weight persisted after correction for total body weight (P<0.05).

Blood Lead Concentrations

The changes in blood lead concentrations as a function of increasing lead concentration in drinking water are shown in Fig. 1. The blood lead levels of the control animals were consistently less than 7 μg/dl (lower limits of detectability). Blood samples from the control animals were therefore not contaminated by the collection procedure. Both treatment groups showed a dose-related elevation in whole blood lead levels (P<0.001).

Effects of Lead on Serum Gonadotropins

There were no significant changes in serum LH values before and after lead exposure. Follicle-stimulating hormone values were significantly suppressed (P<0.05) during the treatment period (Fig. 2).

Effect of Lead Treatment on Intratesticular Testosterone, Serum Testosterone, and Intratesticular Sperm Count

A significant negative correlation between increasing blood lead levels and serum testosterone values was found (r=0.64,

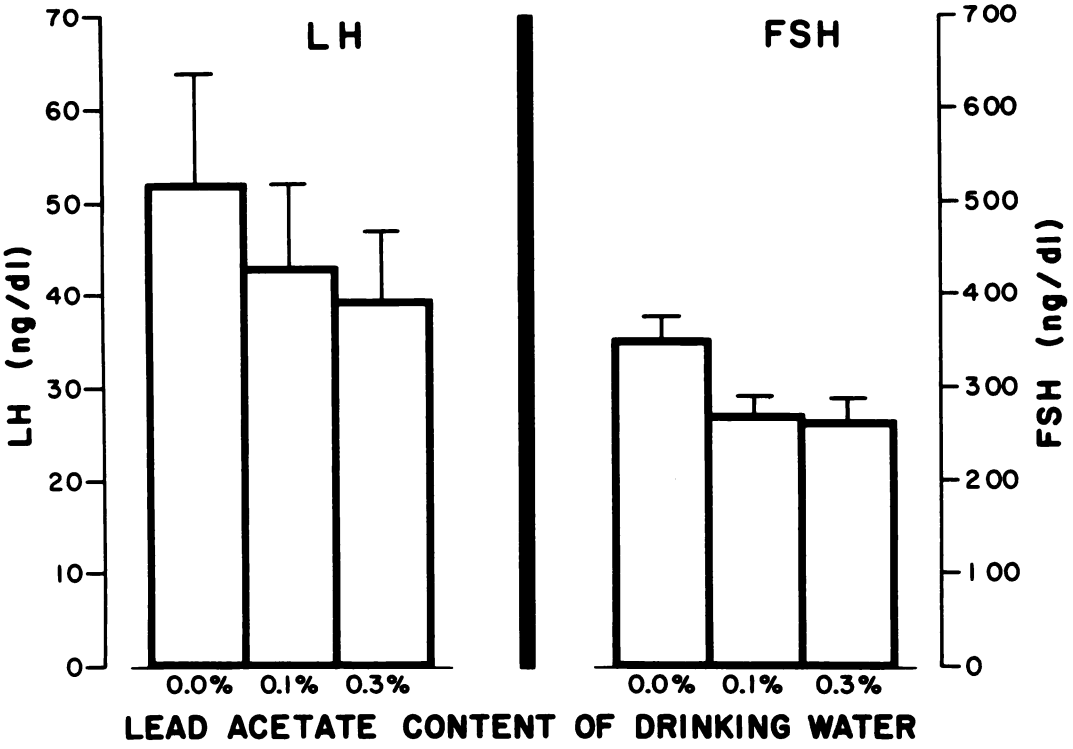

FIG. 2. Serum LH and FSH levels after 30 days of lead exposure.

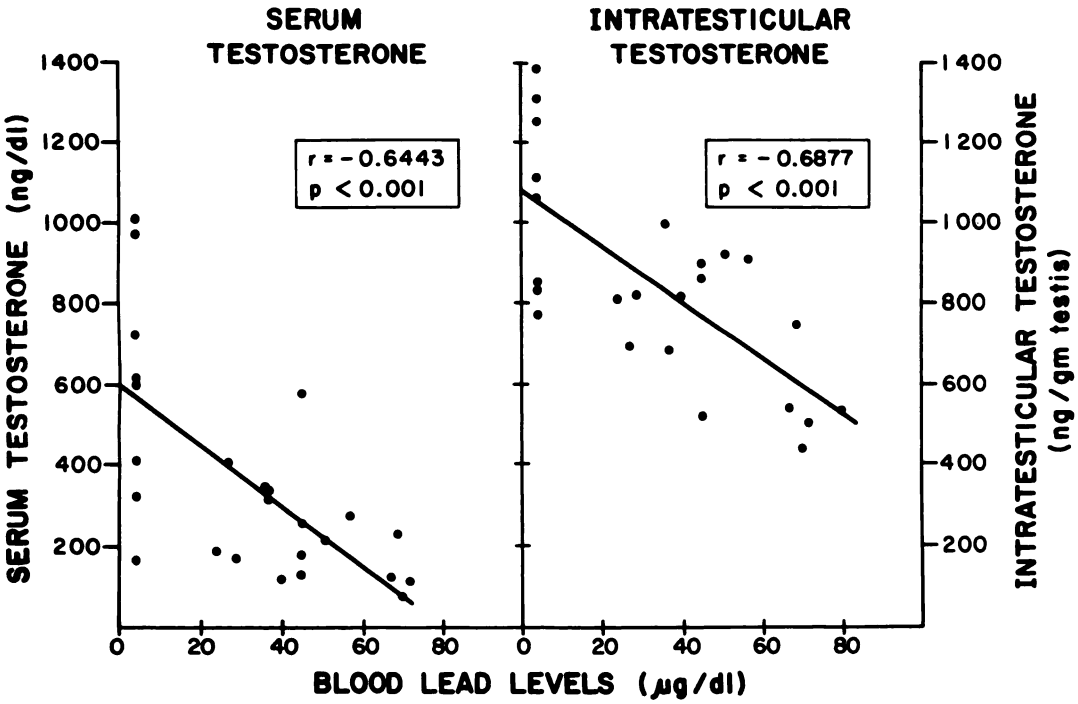

FIG. 3. Relationship of blood lead levels to serum testosterone and intratesticular testosterone.

P<0.001). A similar significant negative correlation was found between blood lead levels and intratesticular testosterone levels (r=—0.69, P<0.001) (Fig. 3). Intratesticular sperm counts (expressed as counts x 10^6/testis) significantly decreased as the level of lead exposure increased (r=—0.81, P<0.0001) (Fig. 4).

DISCUSSION

Our data indicate that exposure to lead results in the inhibition of testicular function as manifested by suppressed spermatogenesis and decreased serum and intratesticular testosterone levels. It is important to note that the serum blood lead levels achieved in the 0.1%-treated animals are below the level that is permitted in a worker exposed to lead as established by the National Institute for Occupational Safety and Health (NIOSH) (NIOSH Recommendations for Occupational Health Standards, 1983). The mean blood level in the 0.3%-treated animals is the same as that at which a man should be removed from his worksite (NIOSH Recommendations for Occupational Health Standards, 1983).

Our findings of decreased spermatogenesis in exposed animals are in agreement with some published studies and in contrast to others. Hilderbrand et al. (1973) reported that exposure to lead concentrations of 50 μg/dl resulted in histologic evidence of testicular damage and inhibited spermatogenesis. Eyden et al. (1981) reported similar histologic abnormalities in the testes of adult mice exposed to 3—6 mo of 0.1% or 4.0% lead acetate water solution. Others observed an increased frequency of abnormal sperm morphology in mouse semen samples collected 30 days after an acute exposure to lead acetate by peritoneal injection (Wyrobeck and Bruce, 1978). In contrast, Valor and coworkers (1980) did not observe abnormalities of spermatogenesis in pubertal rats exposed to low-dose lead in utero and throughout suckling. Studies of men exposed to lead in their workplace also suggest abnormalities of spermatogenesis (Lancranjan et al., 1975; Cullen et al., 1983).

In our study, a decrease in mean ventral prostate weight after exposure to lead was noted. These data correspond to the decrease in serum testosterone concentrations. No changes

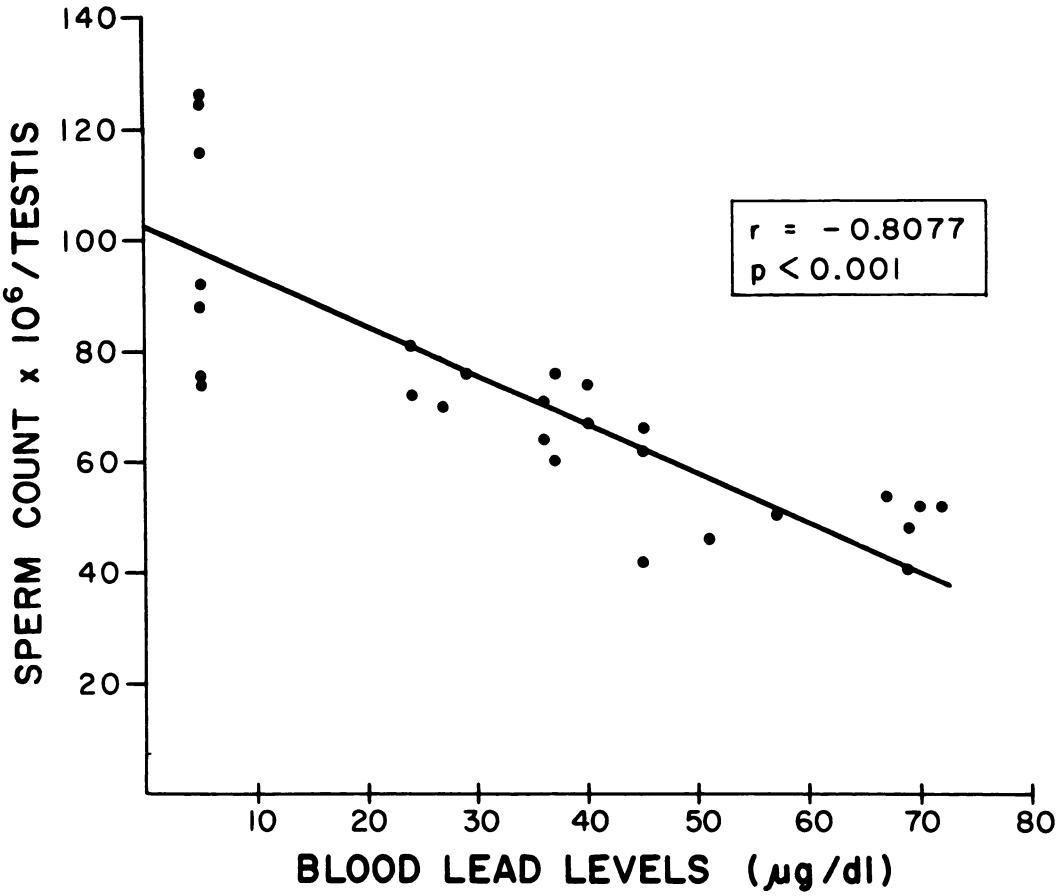

FIG. 4. Relationship of blood lead levels to sperm count.

in testicular or seminal vesicle weights were found. This discrepancy in prostate and seminal vesicle weights after lead exposure may be explained by published data indicating that the prostate is more sensitive to the effects of androgens and antiandrogens than are the seminal vesicles (Sufrin and Coffey, 1974). Our data on ventral prostate weight contrast with those of Hilderbrand et al. (1973), who reported prostatic hyperplasia and increased prostate weights, and Der et al. (1976), who found no changes in prostatic weight after lead exposure. As in our study, neither of these investigators reported any changes in testicular or seminal vesicle weights in treated animals.

Another method of studying the effects of lead toxicity is to evaluate the fertility rate of exposed male animals. Varma et al. (1974) studied the fertility of 14 Swiss male mice fed 2% lead acetate for 4 wk and found that, when these rats were mated with untreated females, the overall incidence of pregnancy was 52.7% in the control group as compared to 27.6% in the treated group. Wyrobek and Bruce (1978) reported an increased incidence of sperm abnormalities in the male progeny of male mice exposed acutely to lead prior to mating.

In none of the above experiments were serum gonadotropins and testosterone values measured. Our finding of normal serum LH and suppressed FSH values in the face of markedly decreased serum testosterone values indicates that the predominant mechanism of action of lead toxicity appears to be the suppression of the hypothalamic-pituitary axis. Although no other similar studies have been performed on adult animals, Petrusz et al. (1979) investigated the effects of neonatal lead poisoning on gonadotropins. They reported that serum FSH levels and pituitary LH content remained un-

changed during lead treatment, whereas pituitary FSH content was significantly increased in male rats treated with 25 mg lead/kg or 200 mg lead/kg ($P<0.01$). No serum LH or testosterone values were reported.

Investigations into the anatomic and neurochemical changes in brains of animals chronically treated with lead compounds suggest the hypothalamus as a primary site of the neurotoxic action of lead. Autoradiographic studies have localized ^{210}Pb to the median eminence of the hypothalamus (Stumpf et al., 1980). Lead is also known to affect the release of a number of brain neurotransmitters, including norepinephrine and dopamine (Silbergeld, 1983). Unfortunately, scarce and conflicting data have been reported on the effects of lead on hypothalamic neurotransmitter concentration and turnover (Hrdina et al., 1980). Finally, in a clinical study, Braunstein et al. (1978) reported blunted clomiphene and gonadotropin-releasing hormone responses in lead-poisoned men. These data further support the concept that lead toxicity impairs hypothalamic-pituitary function.

Although our data are more consistent with toxicity directed at the hypothalamic-pituitary axis, it is possible that in addition to this effect a direct testicular toxicity may also occur. A number of enzymes are altered by lead exposure. Such exposure decreases the biosynthesis of heme and reduces the hepatic content of heme-containing cytochromes, which are involved in steroid hormone metabolism (Alvares, 1979). Preliminary data published by Wiebe and coworkers (1983) suggest that in vitro and in vivo neonatal exposure to lead may act on testicular cells by inhibiting the activity of steroidogenic enzymes and the synthesis of enzymes and hormone receptor proteins. It is unclear whether the inhibition in sperm count seen with lead exposure is secondary to the decreased concentrations of serum FSH and intratesticular testosterone or whether it also represents direct lead toxicity on spermatogenesis. Further studies are required to clearly delineate the biochemical events that alter spermatogenesis in the rat.

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