Long-term Effects of dietary Lead Acetate on Survival, Body Weight and Seminal Cytology in Mice

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It is well known that lead compounds exert toxic effects on renal, haemopoietic and central nervous systems, and the embryo (GOYER 1971; CHISOLM 1971; GOYER and RHYNE 1973; JACQUET et al. 1975). These effects are of increasing importance to man and the animals and plants with which he comes into contact, in view of the high contemporary levels of heavy metal pollutants in the environment (KEHOE 1976). Much of our knowledge of lead-effects comes from clinical observations of acutely intoxicated individuals (CHISOLM 1971; GOYER 1971) while less is known of the chronic effects which may be expected as a result of long-term exposure to lead from polluted environments.

In order to extend our knowledge of the chronic effects of lead intoxication, we have investigated the long-term administration of dietary lead acetate in laboratory mice. In this paper, some preliminary observations on long-term survival, weight, and seminal cytology are presented.

Materials and Methods

Balb/c^{+} male mice, 3 months old at the start of lead acetate exposure, were used throughout. They were permitted food and water ad libitum, caged in twos, and maintained at a constant ambient temperature of 23°. Treated animals received lead acetate at levels of 0.1% to 4.0% in identical food to that of controls.

In the study of seminal cytology, spermatozoa from the cauda epididymidis in each of 5 mice were examined per experimental point, except where this was precluded by lack of survival (as in one case - 1.0% at 11 weeks). Animals were sacrificed at monthly intervals after the start of treatment, by cervical dislocation. The cauda was removed into 30-35° phosphate-buffered saline and minced with fine scissors to release the sperms. The resulting suspension was mixed with an equal volume of 1.5% glutaraldehyde in 0.1M pH 7.2 cacodylate buffer. In each animal 1000 spermatozoa were observed for abnormality in wet films by phase contrast optics in a Zeiss photomicroscope. The criteria of abnormality used conform to those described by EYDEN and MAISIN (1977, in press) for this animal.

Bulletin of Environmental Contamination & Toxicology,
0007-4861/78/0019-0266 $1.40

Results

Mean survival time

Table 1 shows the relation between mean survival time and concentration of DLA. The control mean survival time is 745±17 days. At levels between 4% and 0.5% the mean survival time is reduced and the reduction is dose-dependent. Data for lead acetate at 0.1% are as yet incomplete. After 257 days 86% of males were still alive, as against 98% in the control survival curve, while after 541 days 57% of females were still living compared with 83% in the control group.

TABLE 1

Relation between dietary lead acetate dose and mean survival time

Dose of lead acetate (%)	Mean survival Time (days)	Sex of Animal	Number of Animals
4.0	11.3 ± 1.6	F	10
3.0	18.3 ± 3.6	F	10
2.0	43.2 ± 8.25	F	10
1.0	98.7 ± 1.6	F	21
1.0	99.9 ± 1.9	M	21
0.5	115.1 ± 1.6	F	10
0	745 ± 17.2	M	150

Body weight

Variation of body weight with time of exposure to lead acetate is given in table 2. At the start of treatment, animals weighed 28.5 ± 0.5 g (mean ± standard error) (N = 70). After 4 weeks a significant weight-reduction was noted which was maintained at 8 and 11 weeks. Mice given 0.1% lead acetate for 16 months had a mean body weight (from 5 individuals) of 28.0±0.95g, a value not significantly different from controls (26.8 ± 0.6 g).

TABLE 2

Relation between body weights and time of exposure to lead acetate at 1.0%

Time (weeks)	Mean body weight (g) ± standard error	
	Control	Treated
4	30.4 ± 0.5 (N=18)	22.0 ± 0.4 (N=18)
	$p < 0.01$	
8	31.2 ± 0.5 (N=14)	15.8 ± 0.6 (N=5)
	$p < 0.001$	
11	30.7 ± 0.5 (N=5)	15.1 ± 0.9 (N=3)
	$p < 0.001$	

N = Number of animals

Seminal cytology

Sperm abnormality counts are expressed as the number of abnormalities per 1000 counted spermatozoa. These values for lead-treated (at 1.0%) and control mice are shown in table 3 for different times after the start of treatment. After 4 weeks there was no significant difference, as detected by t-test, between the mean value for treated individuals and controls. At 8 weeks, a statistically significant difference was noted, which was maintained at 11 weeks. Animals exposed to lead acetate at 0.1% showed no spermatozoan abnormality elevations during these periods. Even after 16 months of treatment at 0.1%, 5 animals were found to have a mean sperm abnormality count of 330.8±22.0, a value comparable to those for control groups at 4, 8 and 11 weeks.

TABLE 3

Relation between sperm abnormality count (number of abnormal forms per 1000 sperms in each of 5 animals) and time of exposure to lead acetate (1.0%)

Time (weeks)	Mean sperm abnormality counts ± standard error	
	Control	Treated
4	381.4 ± 31.4 (N=5)	382.0 ± 10.8 (N=5)[a]
8	315.2 ± 31.3 (N=5)	585.4 ± 51.8 (N=5)
		$p < 0.01$
11	353.6 ± 17.3 (N=5)	634.3 ± 60.3 (N=3)

[a]difference not significant $p < 0.01$

Discussion

The observations presented above concern the effects of orally administered lead acetate on long-term survival, body weight, and seminal cytology. They show that such treatment profoundly influences these biological features, but generally only at comparatively high concentrations.

Long-term survival

The present data demonstrate that with dietary lead acetate at 0.5 - 4%, Balb/c$^+$ male and female mice show reductions in mean survival time which are dependent on dose. A similar phenomenon has been recorded by VAN ESCH and KROES (1969) for Swiss mice. Although their conditions differed - they started exposure at 5 weeks of age as against 3 months for the Balb/c$^+$ animals in our procedure - they demonstrated survival reductions for male and female animals at 0.5 and 1.0% of basic lead acetate. At 0.1%, they observed no effect on survival. Data from the present paper, however, point to a slight reduction at this level, though these observations, being preliminary, must await completion before being used for a definitive comparison.
Since autopsies were not carried out in the present experimental procedure, it is necessary to consider the toxic effects described from other systems to evaluate causes of death in these animals. Hormonal and immunological disturbances (SANDSTEAD et al. 1969; KOLLER and KOVACIK 1974) have been reported for human patients and mice with clinical & experimental lead intoxication respectively, and effects due to lead on the nervous, renal and haemopoietic systems (CHISOLM 1964, 1971; GOYER 1971; GOYER and RHYNE 1973) are well documented. Death could therefore result either from internal organ malfunction as a result of enzyme interference (ULMER and VALLEE 1968) or lack of nervous or hormonal control, or alternatively through viral or bacterial infection resulting from depressed immunological competence (KOLLER and KOVACIK 1974) or indeed from an interplay of these factors.

Total Body weight

The presented data indicate a severe weight loss in Balb/c$^+$ mice on exposure to lead acetate at 1%. Corroborative observations for other strains of mice are lacking, although the effect of orally administered lead on rats and non-rodent animals has been studied. These results, however, are contradictory and not easy to compare by virtue of different experimental means of exposure and different chemical forms of lead used.
ALVARES et al. (1976) working on Sprague-Dawley Rats, found that 2% lead acetate caused a significant weight loss (about 21%) for 9-10 weeks, although at 12 weeks only a small and insignificant difference was noted. VAN ESCH et al. (1962) using Wistar rats, found a greater weight-loss (45%) after 10 weeks, for a lower level of lead acetate (1%). Also with Wistar rats, TANIGUCHI et al. (1976) found a reduced weight gain in comparison with controls for even lower doses, i.e. 0.1 and 0.5% in drinking water.

These observations may be explicable in terms of strain differences in physiology in relation to challenge by toxic substances or to differences in diet, since calcium, for example, is known to influence gastro-intestinal absorption of lead compounds (SIX and GOYER 1970). Without giving details, VAN ESCH et al. (1962) also reported a weight-loss with lead acetate at 0.1%, a concentration which in male Balb/c^{+} mice has no effect. In ruminants, Holtsein steer calves showed decreased body weights and reduced food uptake on oral administration of lead and/or cadmium (LYNCH et al. 1976), whereas these parameters were unaffected in wether lambs studied by FICK et al (1976). Differences of administration procedure - FICK et al. (1976) permitted ad libitum access to food supplemented with a lead compound, while LYNCH et al. (1976) administered a lead salt in gelatin capsules at defined intervals - and of the chemical form of administered lead - carbonate by LYNCH et al. (1976) and acetate by FICK et al. (1976) - make these data difficult to compare. The procedure of a basal diet supplemented by a lead compound, as described in the present paper, affords a method of study whose main advantage is of simulating toxicity which might result from environmental pollution through contaminated food. Several mechanisms are possible for weight-loss, one of which could be reduced food uptake

Seminal cytology.

The data presented here indicate a significant increase in spermatozoan abnormalities after dietary exposure to lead acetate. Lead under similar conditions of administration is known to disturb early embryonal development and to reduce pregnancy-incidence in female mice (JACQUET et al. 1975). Studies of the effects of lead on the mammalian reproductive system, on the other hand, are scarce.

The increased number of sperm abnormalities might be supposed to lead to infertility. Unpublished information from this laboratory indicate no consistently significant reduction in sperm number or motility - indeed, many severely malformed spermatozoa express full motility. On the other hand, VARMA et al. (1974) studied the effect of lead exposure in Swisse male mice on the pregnancy-incidence which they were able to establish in untreated females. Lead-treated males given 2% lead sub-acetate in water could establish only half the pregnancy-level of untreated males. Unpublished results from our laboratory indicate that male Balb/c^{+} mice given 0.1% lead acetate orally suffer no impairment of fertility as judged by their ability to establish pregnancies in untreated females. These studies, however, have not been extended to higher lead concentrations.

It was implied (VARMA et al. 1974) that oral administration of lead acetate produced effects similar to the oligospermia and testicular degeneration reported by GOLUBOVICH et al. (1968) in the rat after exposure to lead in several discrete doses. That oligospermia and testicular degeneration have not been found in our experimental system may indicate differential effects of

lead among strains and species, but differences in experimental design and procedure may also contribute to divergences in results.

Regarding the mechanism(s) by which lead-exposure might increase sperm abnormalities, two considerations should be borne in mind. One is that lead ions may localise in the testis and result possibly in a direct mutagenic effect. The other is that they may disturb the metabolism of the animal so grossly and systematically as to produce an indirect effect. This might be mediated by metabolites which are normally in minimal concentrations and which as a result of lead action reach elevated levels. While a direct effect cannot be excluded, the poor general state of health of mice exposed to 1.0% lead acetate (i.e. early hair-loss, lethargy - unpublished observations - weight-deficiency and reduced size), and the need for high concentrations of lead for an effect on sperm structure, point to systemic metabolic disturbances as being responsible for this morphogenetic derangement.

The high level of spermatozoan abnormalities in control animals of the Balb/c‡ strain merits comment. Comparison with abnormality-levels for other murine strains must take into account the inclusion in our figures of tail as well as head deformations. We have tested our procedure for determining abnormality-levels by enumerating sperm abnormalities in an AKR strain showing chromosomal translocations (WYROBEK et al. 1975). Our figure of 2.9% head malformations compares favourably with that of 3.1% according to WYROBEK et al. (1975).

ACKNOWLEDGEMENT

This work was supported by Grant n° 080-74-7 ENV. B from the Commission of the European Communities.

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