Chapter 4

Effect of Lead Acetate on the Bone Marrow Chromosomes of the Frog *Rana tigerina*

Toxicological effects of various heavy metals on several species of amphibians have been reviewed by Power *et al.* (1989). Lead was highly toxic to *Gastrophryne carolinensis* eggs between fertilization and 4 days post-hatching in continuous flow bioassay (Birge *et al.*, 1979). Frogs exposed to lead had a permanent loss of semi-erect posture and also sloughing of the integument, muscular twitching, salivation, sluggishness and death. Lead salts have a severe inhibitory effect on the development of frogs causing a slow down in growth and abnormal development (Dilling and Healey, 1926). Lead binds with the amphibian skin due to the affinity of melanin for metals (Ireland *et al.*, 1979). Ireland (1977) compared lead levels in *X. laevis* with *Peromyscus* and concluded that since amphibians had much higher level of residue, they may be important in concentrating lead in the food web. In *Xenopus laevis*, accumulation of lead was found more in liver, kidney and bone, when the animals ingested worms containing lead (Ireland, 1977).

Though the physiological and behavioural effects of lead salts have been documented in amphibians, the mutagenic effects of these compounds are not studied. There are a number of reports regarding the effect of lead salts in mammals exposed experimentally, occupationally or accidentally (Dekhnudt *et al.*, 1973; Bauchinger *et al.*, 1976; Sharma and Talukder, 1987; Chakraborti *et al.*, 1989). Both positive and negative effects have been recorded. However, most of the workers agree that lead does produce the micronuclei and severe chromosome aberrations in somatic or germ cells of the mouse (Leonard *et al.*, 1975; Jacquet *et al.*, 1977).

In the present study, the effect of sublethal concentrations of Lead acetate on the mitotic chromosome of the frog *Rana tigerina* was evaluated after the intraperitoneal administration of the chemical as well as exposing them to the lead solutions.

Materials and Methods

Animals: Medium sized frogs, *Rana tigerina* (Daudin) were collected from uncontaminated areas of villages of Dhaboi near Baroda. The animals were maintained in the laboratory in an aquaterraria, and were fed cockroaches thrice a week.

Two series of experiments were conducted which are described as follows:
Experiment 1 (Intraperitoneal administration of Lead acetate): In this series, the animals were treated with two different doses (1mg/kg body wt, 3mg/kg body weight) for three different treatment periods (6hrs, 24 hrs, and 48 hrs). Controls were injected with distilled water.

Experiment 2 (Exposure of frogs to Lead acetate solutions): In this series, the animals were treated with two different sublethal concentrations of Lead acetate (25 ppm and 50 ppm) for two different treatment periods (7 days and 14 days). Control animals were maintained separately in tap water.

Preparation of metaphase chromosomes: Preparation of mitotic chromosomes were made directly from the bone marrow of all the animals after *in vivo* colchicine treatment. The technique used for preparation of cell suspension, hypotonic treatment, fixation and staining were described in detail in Chapter 1. Sixty metaphase cells were analyzed from each animals. Statistical analysis of the data was done by equality proportion test and the value is considered to be significant when $Z \geq 1.96$

Mitotic index : Mitotic index was calculated using the following formula:
MI = (Number of cells in division/total number of cells counted)x 1000. The statistical analysis of data was done by Student's 't' test.

Results

Chromosomal aberrations induced in the bone marrow of the frog after the intraperitoneal administration of Lead acetate and the exposure to the sublethal concentration of Lead acetate solution are given in table 1 and table 2 respectively. Table 3 shows the mitotic indices recorded after the treatments.

Experiment 1: No significant increase in chromosome aberrations was recorded after the ip administration of Lead acetate with both doses for 6 hrs and 24 hrs. Frequency was found significant after 48 hrs with the high doses. Mitotic index increased in all the treatments, which was significant after 24 hrs and 48 hrs of both the doses of ip administration.

Experiment 2: The increase in the frequency of chromosomal aberrations after seven days of treatment with both the doses was insignificant. However, exposure to the higher dose of solution for 14 days caused an increase in aberrations. Low dose of Lead acetate exposure did not cause significant number of aberrations even after 14 days of treatment. The major type of aberrations induced by Lead acetate include centromere break C-mitosis, stickiness, aggregation of chromosomes into groups etc. (Plate 7) . Mitotic index was significantly increased in all the treatment groups except with the low dose for 6 hrs.

Discussion

The results of the present experiments indicate that sublethal concentration of Lead acetate is unable to cause severe clastogenic effects in the bone marrow of the frog *Rana tigerina.* A marginal increase in the aberrations was, however, recorded when frogs were continuously exposed to high sublethal dose of lead solution.

There are several reports regarding the clastogenic action of lead in mammalian systems. In the experiments performed by Sharma and Talukder (1987) the animals treated with Lead acetate revealed buckled cells in the bone marrow preparations. Besides, there were other abnormalities including C mitotic effect. Lead has also

Table 1. Frequency of chromosomal aberrations induced in the bone marrow cells of frogs by Lead acetate (ip)

Dose and Treatment period	No. of animals tested	No. of cells analysed	Numerical Anomalies				Structural anomalies						Total number of aberrations	Percentage Aberrations	Z Value
			Hypodiploidy	Hyperdiploidy	Ploypploidy	Total	Chromatid		Chromosome		Others @	Total			
							Gap	Break	Gap	Break					
Control	15	900	21	0	0	21	0	9	0	0	6	15	36	4.00	-
1.5 mg/kg b.wt															
06 hrs	5	300	6	0	0	6	1	3	0	1	6	11	17	5.66	1.21
24 hrs	5	300	8	0	1	9	1	4	0	1	4	10	19	6.33	1.67
48 hrs	5	300	7	0	0	7	1	5	0	2	5	13	20	6.66	1.90
3.0 mg/kg b.wt.															
06 hrs	5	300	8	0	0	8	0	4	0	0	6	10	18	6.00	1.44
24 hrs	5	300	7	0	0	7	3	3	0	1	5	12	19	6.33	1.67
48 hrs	5	300	11	0	1	12	1	4	0	1	7	13	25	8.33	2.95

Result is statistically significant when Z≥1.96

@ = Acentric fragment, dicentric chromosomes or abnormal chromosome configuration

Table 2 Frequency of chromosomal aberrations induced in the bone marrow cells of frogs by the treatment in lead acetate solution.

Dose and Treatment period	No. of animals tested	No. of cells analysed	Numerical Anomalies				Structural anomalies						Total number of aberrations	Percentage Aberrations	Z Value
			Hypodiploidy	Hyperdiploidy	Ploypploidy	Total	Chromatid		Chromosome		Others @	Total			
							Gap	Break	Gap	Break					
Control	15	900	17	0	2	19	5	4	0	4	11	24	43	4.77	-
25 ppm															
07 D	5	300	6	0	0	6	1	2	0	1	5	9	15	5.00	0.16
14 D	5	300	7	0	0	7	2	1	0	1	6	10	17	5.66	0.61
50 ppm															
07 D	5	300	6	0	0	6	2	2	1	2	5	12	18	6.00	0.84
14 D	5	300	8	0	1	9	2	3	1	2	8	16	25	8.33	2.31

Result is statistically significant when Z≥1.96

@ = Acentric fragment, dicentric chromosomes or abnormal chromosome configuration

Table 3. Mitotic index (MI) recorded in the frog bone marrow after various treatments with Lead acetate. The data presented as mean ± SE of 15 animals in control and 5 animals in treatment group.

(a) After ip administration

Dose	Control	1.5 mg/kg Bwt.			3.0 mg/kg Bwt.		
Time		6 hrs	24 hrs	48 hrs	6 hrs	24 hrs	48 hrs
MI	2.89 ± 0.08	3.10^{NS} ± 0.16	3.31* ±0.12	3.26* ± 0.11	2.53^{NS} ± 0.12	3.46* ± 0.08	4.28* ± 0.06

(b) After treatments in lead acetate solutions

Dose	Control	25 ppm		50 ppm	
Time		7 Days	14 Days	7 Days	14 Days
MI	2.75 ± 0.04	2.97^{NS} ± 0.14	3.56* ± 0.12	3.15* ±0.17	4.26* ±0.05

NS - nonsignificant; * Significant at 0.05 level

Plate 7. Chromosomal aberrations induced by Lead acetate in the bone marrow of the frog, *Rana tigerina* (all x 1075).

Figure 1. Separation of sister chromatids from the centromere.

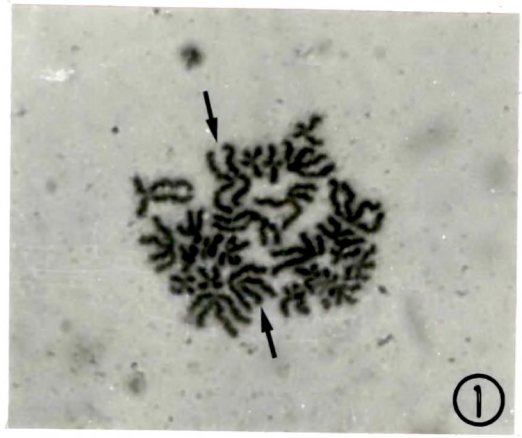
Figure 2. C-mitosis.

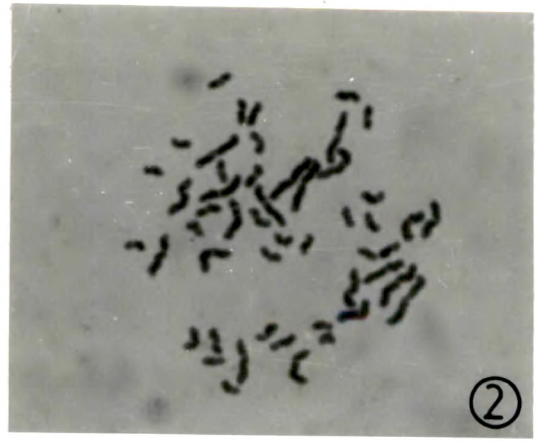
Figure 3. Stickiness of chromosomes.

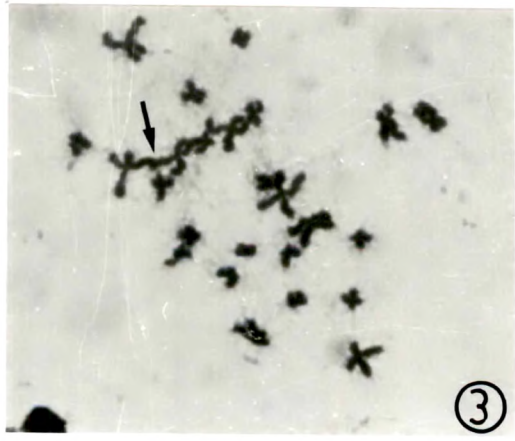
Figure 4. Aggregation of chromosomes into group.

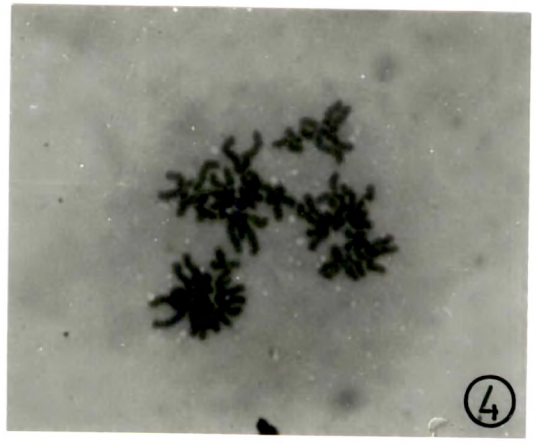
Figure 5. A break at the centromere.

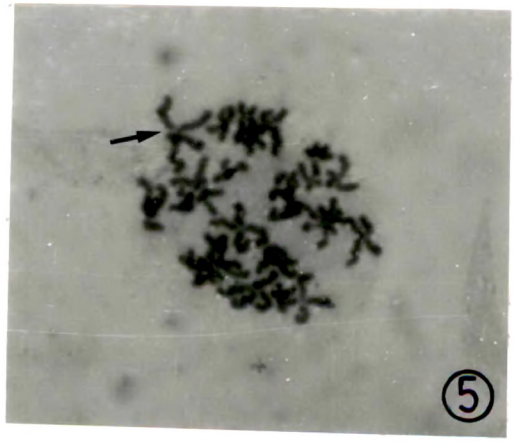
Figure 6. An exploded metaphase showing hypodiploidy.

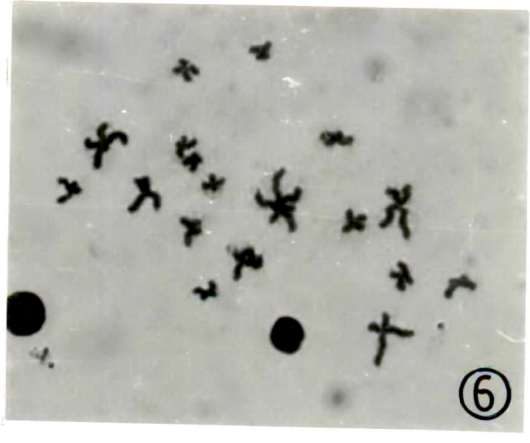

①


②


③


④


⑤


⑥

resulted in significant increase in certain aberration particularly gaps in the bone marrow cultures exposed to it (Muro and Goyer, 1969). In rats given lead acetate increased chromosomal aberrations were reported and the frequency of chromosome aberration was also dose-dependent (Sharma and Talukder, 1987). Deknudt and Gerber (1979) had however, observed negative results in the bone marrow cells of mice after exposure to lead acetate. Lead acetate inhibits the mitotic activity of PHA-stimulated human lymphocytes but subtoxic concentrations allowing cell division, do not significantly increase the yield of structural aberrations (Dekhnudt and Gerber, 1979). Chinese Hamster ovary cells showed no increase in DNA damage (Douglas *et al.*, 1980).

Adult anurans appear to be much less sensitive to lead (Kaplan *et al.*, 1967). An increase in frequency of chromosomal aberration after 14 days of treatment with the higher dose indicate that lead may have the clastogenic action if exposed to a longer duration or having a continuous exposure. The long-term exposure of *Rana nigromaculata* eggs to lead, prior to first cleavage, resulted in a reduction of the number of primordial germs cells later in development (Hah, 1978). Spindle disturbances and a distinct clastogenic action of lead were observed in bone marrow cells of female rats following daily ip. administration of Lead acetate in acute and chronic doses (Chattopadhyay, 1985)

The major type of aberrations observed in the present experiment include centromere separation and colchiploidy. The exact mechanism of action of lead on genetic material has not been fully understood (Chakrabarti *et al.*, 1989). However, it seems that the clastogenic action of lead in the bone marrow of frog is almost comparable to that observed in mammalian systems (Beck and Obe, 1974; Obe *et al.*, 1975). The induction of high frequency of single chromatids in the present study suggests that the damage occurs after the phase of DNA synthesis in the cell cycle (Muro and Goyer, 1969). The mitosis was not found inhibited in any case of the present experiment. An increase in mitotic index after the treatments points to the fact that lead induces mitosis in the normally not-so-active bone marrow of the frog. Lead acts preferentially on the chromosomes rather than on the spindle apparatus. A dissociation of sister chromatids from the centromere may reflect a secondary action

of the metal on the nuclear organizer regions (Verchaeve *et al.*, 1979b).

Since chromatid and cetromere breaks were less frequent and complex aberrations such as multiple breaks were absent, Lead acetate could be assumed to exert a weak clastogenic action on bone marrow cells of frogs, a situation that has been already found in rats (Tachi *et al.*, 1985) and mice (Savic *et al.*, 1986). However, the results of the present experiment indicate that a long-term exposure or higher dosage of this chemical may have the clastogenic effects in amphibians.