Effect of Lead Acetate on Human Leukocyte Chromosomes in vitro

The mutagenic actions of different metal ions are well known. $MnCl_2$ is a mutagen in *Escherichia coli*[1,2], a variety of metal ions induces chromosome aberrations in *Vicia faba*[3,4], *Tradescantia*[5], *Paris*[6], *Allium*[7], and *Pisum*[8].

There are many publications concerning the action of lead in physiological parameters[9], but the question of a possible mutagenic action of this metal is still an open one. MURO and GOYER[10] found an elevation of achromatic lesions and chromatid breaks in mice fed with lead acetate, but in the dominant lethal test with the mouse, lead was ineffective[11]. Two reports show an elevation of chromatid aberrations including exchange type aberrations in leukocytes of men heavily contaminated with lead[12,13], other results with this test system are negative[14–17]. In vitro studies with Chinese hamster cells and with human leukocytes showed no clearcut effects of lead on chromosomes[15,18,19]. In the latter test system, only one group reported positive results[12].

The experiments referred to concern a possible mutagenic activity of lead alone. But it is also possible that lead could be active in inhibiting metabolic repair processes by means of its deleterious effects on mitochondria and energy metabolism[20–26], and in this way potentiate a mutagenic damage otherwise induced. The enhancement of the chromosome-breaking effect of ethyl methanesulfonate by Cu and Zn ions in *Vicia faba*[27] may be an example of such an effect of metal ions.

To test this possibility with human chromosomes in vitro, we performed combination experiments with lead acetate and the bifunctional alkylating agent Chinon I with 72 h leukocyte cultures.

Another possibility of an action of lead on the genetic material is discussed by BUCHMANN and ZIMMER[28,29]. They found an enhancement of X-ray induced lethal mutations in *Drosophila melanogaster* fed with lead acetate, and speculate that this effect may be an outcome of the X-rays being better absorbed in the tissues. To test this possibility with human chromosomes, we performed experiments with 48 h leukocyte cultures, contaminated with lead acetate and irradiated with X-rays in the G_0–G_1 stage of the cell cycle.

Lead acetate and Chinon I. Leukocyte cultures were prepared as follows: 4.2 ml TC medium 199, 0.8 ml human serum, 0.1 ml phytohemagglutinin (PHA), 1.0 mg dihydrostreptomycin, 100 IU penicillin, and 0.2 ml male blood. 24 h before culture stop, watery lead acetate (2 cultures, final concentration: 10^{-5} *M*), watery Chinon I (2,5-*bis*-ethyleneimino-*p*-benzoquinone, final concentration: 0.5×10^{-6} *M*, 2 cultures), and both substances in the same concentrations mentioned (6 cultures) were added. Chromosome preparations were made 72 h after

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Table I. Chromatid aberrations induced in human 72 h leukocyte cultures by lead acetate, Chinon I and the combination of both substances. Treatment time 24 h, blood donor BB ♂

Treatment	Number of cells analyzed	Achromatic lesions (AL)		Chromatid breaks (B′)		Isochromatid breaks (B″)		Chromatid translocations (RB′)	
		% of cells	No. per cell	% of cells	No. per cell	% of cells	No. per cell	% of cells	No. per cell
Control (without treatment)	400	4.50	0.060	2.75	0.027	0.50	0.005		
Lead acetate (10^{-5} *M*)	600	20.66	0.278	13.16	0.196	5.66	0.091		
Chinon I (0.5×10^{-6} *M*)	200	37.50	0.570	45.00	0.745	28.50	0.360	17.00	0.200
Chinon I (0.5×10^{-6} *M*) Lead acetate (10^{-5} *M*)	500	39.21	0.598	56.68	1.078	29.84	0.450	15.93	0.162

Table II. Chromosome aberrations induced in human 48 h leukocyte cultures by lead acetate, X-rays and the combination of both. Treatment time 48 h and 3 h before PHA addition

Treatment	Blood donor	No. of cells analyzed	Dicentrics % of cells	Dicentrics No. per cell	Rings % of cells	Rings No. per cell	Fragments % of cells	Fragments No. per cell
Control	FW♀	600	0.10	0.001	—	—	—	—
(without treatment)	MH♂	300	0.33	0.003	—	—	—	—
Lead acetate	FW♀	400	0.75	0.007	—	—	—	—
(10^{-5} *M*)	MH♂	200	1.00	0.010	—	—	—	—
100 R	FW♀	600	12.00	0.125	7.33	0.078	6.83	0.075
100 R Lead acetate (10^{-5} *M*)	FW♀	600	16.00	0.165	6.67	0.070	7.50	0.080
200 R	MH♂	200	26.00	0.340	15.00	0.160	8.00	0.085
200 R Lead acetate (10^{-5} *M*)	MH♂	300	30.67	0.340	13.00	0.140	7.67	0.083

culture initiation, with colcemid added $4^1/_2$ h before fixation. Table I shows the results of these experiments. Lead acetate induced achromatic lesions (AL), open chromatid breaks (B′), and isochromatid breaks (B″), in frequencies well over the baseline. Exchange type aberrations were completely absent. Chinon I induced the whole spectrum of chromatid aberrations, including exchange type aberrations. The combination experiments (lead acetate + Chinon I) revealed additive effects. Lead acetate has no influence on the induction of chromatid aberrations with the alkylating agent Chinon I.

Lead acetate and X-rays. Two cultures were prepared, each containing 10 ml of culture fluid (HAM'S F-10 medium, complemented with 10% fetal bovine serum, 2.0 mg dihydrostreptomycin, 200 IU penicillin), and 0.8 ml venous blood. To one of these cultures watery lead acetate was added to a final concentration of 10^{-5} *M*. The bottles were incubated for 3 h at 37 °C. After that, 0.3 ml PHA was added and the cultures were distributed in aliquots of 2.0 ml in disposable plastic syringes, 4 with lead acetate and 4 without. From each set of 4 syringes, 2 were irradiated with 100 R immediately after the addition of PHA (100 kV, 8 mA, 2.0 mm Al filter, 58 cm FD, dose rate per minute: 30 R) and 2 were left as controls. The cultures were incubated at 37 °C and chromosome preparations were made after 48 h, with colcemid added $4^1/_2$ h before fixation. In another set of experiments with 200 R we prepared 2 cultures with lead acetate and 1 culture without. The 100 R experiment was performed with female blood and the 200 R experiment with male blood.

As can be seen from Table II, lead acetate exhibits no influence on the X-ray induced chromosome aberrations. In the cells treated with lead acetate alone, some dicentric chromosomes were found. This aberration type was also found in lower frequencies in untreated controls of the blood donors used. This may be an outcome of the fact that both blood donors received high X-ray doses for therapeutical reasons some years before. In the lead acetate treated cultures, the rate of dicentric chromosomes is slightly enhanced. Possibly this is an outcome of the treatment. This is supported by the finding that in lead acetate treated 48 h cultures of another non-irradiated blood donor (BB♂), one dicentric chromosome was found among 500 analyzed cells, in 500 cells of untreated controls of the same donor this aberration type was absent (data not presented in the table). With all 3 blood samples mentioned, the rate of chromatid aberrations in the lead acetate treated 48 h cultures was not elevated over the baseline (data not reported in Table II).

The results of our experiments show that lead acetate has no influence on the rate of chromatid aberrations induced by an alkylating agent, and on the rate of chromosome aberrations induced by X-rays. Striking differences can be seen in cultures treated with lead acetate alone. In 72 h cultures a 24-h-treatment with lead acetate induced open chromatid type aberrations in frequencies well over the baseline. Irrespective of a much longer treatment time (48 h + 3 h), the frequencies of chromatid type aberrations are not elevated in 48 h cultures. In the latter culture system we found some indications of very few dicentric chromosomes being induced by lead acetate.

Zusammenfassung. Experimente mit menschlichen Leukocytenkulturen lassen vermuten, dass die Wirkung von Bleiacetat von der Kulturmethode abhängig ist. In 72-h-Kulturen wurde eine Erhöhung von achromatischen Läsionen, Chromatidbrüchen und Isochromatidbrüchen induziert, nicht jedoch in 48-h-Kulturen. Chemisch- und strahleninduzierte Aberrationshäufigkeiten wurden durch Bleiacetat nicht beeinflusst.

B. Beek and G. Obe

Institut für Genetik der Freien Universität Berlin, Arnimallee 5–7, D–1 Berlin 33,
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Control of Tumour-Associated Peroxidases in a Genetic Tumour System in *Nicotiana*

Genetic tumours develop on individuals of specific genotypes. These have been extensively studied in the genus *Nicotiana*[1,2]. Tumours appear on hybrids between particular species of the genus *Nicotiana*, whereas the parental species involved in such hybrids are entirely non-tumourous. Näf[3] proposed that the species entering tumourous combinations may be divided into 2 groups: one arbitrarily designated the plus group, consisting of the species of the section Alatae; whereas the so-called minus group is comprized of species from the other sections of the genus *Nicotiana*. The contribution of these groups is envisaged as differing in some physiological or bio-