Arh. hig. rada, *29* (1978) 305.

CHROMOSOMAL INVESTIGATIONS IN HUMANS AFTER LONG-TERM EXPOSURE TO LEAD

J. Sušnik[1], D. Blatnik[2], Mirka Fugaš[3] and Danica Prpić-Majić[3]

[1]*Health Centre, Ravne na Koroškem,* [2]*Institute of Public Health of Slovenia, Ljubljana, and* [3]*Institute for Medical Research and Occupational Health, Zagreb*

(Received for publication July 20, 1977)

Chromosomal damage in the lymphocytes from the peripheral blood were studied in two groups of inhabitants living for generations near a lead smelting plant and in a control group. In the exposed groups the concentration of lead in blood was increased. These groups showed in a certain number of analysed mitoses structural abnormalities compared with none in the control group. A relationship between chromosomal aberrations and the variable »residence time« was not found to indicate that chromosomal damage was induced by a direct effect of lead.

The interest in elevated lead exposure has increased with the increasing air pollution by this pollutant owing to the wide use of leaded gasoline. *Bove* and co-workers reported about 0.01 μg lead/m^3 air in Thule (Greenland) compared with 7.5 μg lead/m^3 air in New York City (4). One is therefore confronted with a worldwide problem in which the potential damage to human health should be emphasized.

The population of the Meža valley, located in the Slovene Alpine area, has been exposed for generations to high levels of lead owing to aerosol emissions from a lead smelting plant. It is therefore assumed to represent a good model of the health risks caused by long-term exposure and for this reason has been subjected to many investigations (8, 9, 10, 12, 16, 17).

The aim of the present study was to find out whether lead has produced chromosomal abnormalities in the inhabitants of the valley. By

This research was supported by Raziskovalna skupnost Slovenije, (312/379—73 and 346/170—75)

inhibiting the SH-group of enzymes lead affects numerous anabolic processes such as hem or DNA synthesis. Therefore, chromosomal damage due to lead can also be expected.

EXPOSED SUBJECTS AND METHODS

The investigation was carried out in approximately 30 families living in the area near a lead smelting plant and in 30 families living in the control area. Each family consisted of a father, mother and child attending the elementary school. The families were chosen at random from the total population in the area. The condition for selection was residency in the area, and father's occupation other than farmer's. Subjects were divided into three groups: Group I with an occupationally exposed father, and a nonoccupationally exposed mother and child, Group II with a nonoccupationally exposed father, mother and child, living in the smeltery area, and Group III which was the non-exposed group. As it will be seen later in the text, not all of the subjects were taken in the study.

The control group originated from another Alpine valley, separated from the Meža valley by the mountains 1500 m high, practically without communication and with a poor migration flow. From the ethnographic and anthropologic point of view the population could be regarded as the same.

Lead exposure of inhabitants from the smeltery area was evaluated in an earlier paper (8). The monthly mean of 15 μg lead/m^3 air exceeded many times the permissible limits, while in the control area it was below them. In the same study the lead content in drinking water and diet was discussed. *Graovac-Leposavić* and co-workers showed an increase in the total lead body burden of the population, which they had determined by means of lead mobilization using CaEDTA injections (10). All the data can be referred to the investigated Groups I and II.

Health examinations were carried out in all families. Data were collected by means of a questionnaire. Clinical examinations and anthropometric measurements were performed and samples were collected for laboratory analysis (8). Lead in blood was determined by flameless atomic absorption spectrophotometry (21) in a Perkin-Elmer HGA-72 apparatus. Chromosome studies were carried out on peripheral blood lymphocytes cultured approximately for 72 hours according to the method of *Moorhead* and co-workers (14), but partially modified by *Blatnik* (3). The chromosomes were classified according to the Denver classification (Colorado, 1960). The number of counted mitoses was usually between 15 to 20, which is rather low for this type of investigation.

RESULTS AND DISCUSSION

The number and age of subjects are given in Table 1.

Table 1

Number and age of examined subjects

Group		N	Age	
			$\bar{x}$	SD
I	Men	16	42.56	6.95
	Women	17	40.18	7.37
	Children	16	13.19	2.90
II	Men	8	41.88	5.38
	Women	8	35.25	5.01
	Children	8	12.88	1.46
I+II	Men	24	42.33	6.36
	Women	25	38.60	7.01
	Children	24	13.08	2.48
III	Men	26	42.90	8.19
	Women	25	38.56	12.69
	Children	30	11.17	2.39

Groups I and II exposed to lead, Group III control.

There was no significant difference in age between the groups except for a difference between children of Group I and II as compared with those of Group III.

The lead content in blood was determined in all subjects. According to *Lehnert* the amount of lead in the peripheral blood represents a valuable parameter of the total lead burden in the organism (13). *Baloh* (1) is more precise and in his opinion the blood lead gives a measure of the dynamic body lead pool. Findings are listed in Table 2.

It is evident that the concentration of lead in blood of the exposed population exceeded the accepted limit, i. e. 40 μg lead per 100 ml blood in subjects without occupational exposure, and 80 μg lead per 100 ml blood in occupationally exposed subjects (5). The differences between the occupationally and nonoccupationally exposed groups, as well as the differences between the exposed and the non-exposed group, are significant. It would appear that in all other aspects both groups, the one exposed to lead and the other non-exposed, could be regarded as being rather homogeneous.

Table 2

Significance of the difference in blood lead concentrations as tested by t-test

Concentration of lead in μg/100 ml blood

		Group I	Group II		Group III
Men	N	16	8		24
	$\bar{x}$	96.45	61.94		34.86
	SD	9.99	10.30		11.50
	t-test	I/II		4.229	$p \ll 0.05$
		I/III		17.459	$p \ll 0.05$
		II/III		5.906	$p \ll 0.05$
Women	N	17	8		23
	$\bar{x}$	47.54	51.71		23.33
	SD	12.51	6.81		6.18
	t-test	I/II		0.877	not sign.
		I/III		8.070	$p \ll 0.05$
		II/III		3.357	$p \ll 0.05$
Children	N	15	8		25
	$\bar{x}$	53.73	53.44		26.06
	SD	13.66	10.53		4.98
	t-test	I/II		0.052	not sign.
		I/III		9.222	$p \ll 0.05$
		II/III		10.136	$p \ll 0.05$

Muro and *Goyer* reported on chromosomal damage in mice experimentally caused by the application of lead acetate. They assumed an activation of lysosomal DNA-ases after a lysosomal damage induced by lead (15). *Forni* and *Secchi* consider as more probable the inhibition of enzyme activity which impairs the restoration of chromosomes (6). In another report they suggest that chromatid changes might be culture produced aberrations, which are not repaired in the presence of lead or of some abnormal lead induced metabolite (7). In 1970 *Schwanitz, Lehnert* and *Gebhart* found a positive correlation between chromosomal aberrations and δ-ALA in urine. They report having found chromosomal damage induced by lead in occupationally exposed population (19). In

a later investigation however they could not confirm this statement (20). The lead content in the blood and the ALA excretion in urine did not show any significant correlation with cytogenetic findings. The lead exposure was low. *Bauchinger, Schmid* and co-workers also deny chromosome damage which can be casually related to lead (2, 18). *Graovac-Leposavić* and co-workers found that out of 15 randomly selected subjects from the lead smeltery area 13 had some kind of chromosomal aberrations in the blood lymphocytes which seems to be associated with lead effects (10). Our results are listed in Tables 3—8.

In 52 per cent of the subjects from Group I and 39 per cent from Group II a certain number of analysed mitoses showed some chromosomal damage compared with none in Group III. Though the number of mitoses with chromosomal aberrations is not high, the difference between the exposed and non-exposed groups is considered unexpected. Structural abnormalities of this type may also be spontaneous, particularly in elderly people, but *Brown* and co-workers report only 0.42 per cent, or *Kahn* and *Abe* 0.38 per cent of chromatide gaps (11).

Table 3

Number and type of chromosomal damage in occupationally exposed men (Group I)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage*
I. L.	18	1,1	CG,IG
L. F.	17	7	CG
Š. E.	14	ϕ	ϕ
Z. J.	23	ϕ	ϕ
Ž. H.	16	1	IG
G. L.	11	1	B
J. S.	18	2	CG
O. S.	18	ϕ	ϕ
K. P.	10	ϕ	ϕ
S. P.	17	1	CG
P. J.	12	1,1	CG,F
Č. A.	20	ϕ	ϕ
A. J.	17	1	CG
K. J.	19	2,1,2	CG,IG,F
R. I.	12	2	B
G. B.	13	1	CG

* CG = chromatid gaps,
IG = isochromatid gaps
B = breaks
F = fragments
T = tetraradius (quadriradius)

Table 4

Number and type of chromosomal damage in exposed women (Group I)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage
I. T.	15	1	CG
Č. M.	3	ϕ	ϕ
L. A.	2	1	B
K. L.	21	1	T
Š. D.	17	3	CG
Z. M.	8	ϕ	ϕ
Ž. Z.	9	1	CG
G. M.	20	1	B
J. M.	6	1	CG
O. F.	20	1	CG
K. M.	16	2,1	CG,F
S. T.	13	1	CG
A. H.	16	2	CG
P. M.	1	1	CG
B. A.	11	1	F
R. I.	19	1	IG
G. M.	19	ϕ	ϕ

Table 5

Number and type of chromosomal damage in exposed children (Group I)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage
I. T.	15	1	CG
L. E.	5	ϕ	ϕ
K. A.	21	1	CG
Š. D.	14	ϕ	ϕ
Z. V.	7	ϕ	ϕ
Ž. H.	18	1	IG
G. V.	15	1	F
O. M.	23	1	CG
K. M.	22	1,12	B,IG
Č. A.	16	1	CG
A. D.	18	1	CG
P. R.	19	2,1	B,CG
G. B.	19	ϕ	ϕ
B. I.	22	ϕ	ϕ
R. C.	16	2	CG
S. P.		1	CG

Table 6

Number and type of chromosomal damage in exposed men (Group II)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage
S. M.	17	1	CG
B. A.	20	ϕ	ϕ
J. M.	12	ϕ	ϕ
S. A.	16	2	CG
Š. F.	20	3	CG
K. E.	16	ϕ	ϕ

Table 7

Number and type of chromosomal damage in exposed vomen (Group II)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage
S. H.	6	ϕ	ϕ
Z. M.	22	1	CG
J. M.	16	1,1,1	F,CG,F
S. M.	14	ϕ	ϕ
P. H.	17	1	F
S. M.	23	ϕ	ϕ
Š. V.	10	ϕ	ϕ
K. O.	22	1,4	T,B

Table 8

Number and type of chromosomal damage in exposed children (Group II)

Initials	No. of counted mitoses	No. of mitoses with chromosomal damage	Type of damage
S. D.	23	ϕ	ϕ
Z. Z.	23	2,1	CG,IG
J. B.	16	ϕ	ϕ
S. N.	12	ϕ	ϕ
P. M.	17	1	CG
S. D.	14	ϕ	ϕ
Š. C.	16	1	CG
K. D.	12	ϕ	ϕ

Two typical metaphase spreads are demonstrated in Figures 1 and 2.

Fig. 1. *1 = chromatid gap; 2 = isochromatid break*

The total absence of chromosomal changes in the control group consisting of 26 men, 25 women and 30 children may be interpreted as a consequence of the relatively small number of counted mitoses. On the other hand it should be underlined that the same method was applied, with 15—20 metaphases counted and scored for chromosome changes in each subject, and performed in the same laboratory with the same expected error as in the exposed groups.

Chromosomal damages are associated with lead concentrations in the blood as tested by means of the chi-coefficient. Subjects with more than 30 μg lead/100 ml blood were regarded as exposed to lead. In the first stage when Groups I and II were tested against Group III, χ^2 was 13.08 ($P < 0.1$). In the second stage we left out men from Group I (occupationally exposed) and selected at random an equal number of men from Group III (non-exposed). The χ^2 obtained was 24.76 ($P < 0.1$) suggesting that the difference was not caused by occupational exposure.

Some other possible influences on chromosomes, such as alcoholism, antibiotics, contraceptive drugs, smoking and exposure to x-rays were

also tested. Only a slight association with smoking ($\chi^2 = 6.42$) and x-rays ($\chi^2 = 3.80$) was found. The total number of subjects, however, was relatively low.

The previously set condition to examine only residents living in the area for many generations could not be entirely fulfilled: about one third of the total number were not born in the Meža valley or in the control area though they had lived there for many years. However, when the association of chromosomal aberrations with the variable »residence time« was tested no relationship could be found ($\chi^2 = 0.04$). It is assumed, therefore, that chromosomal damage may be induced by a direct effect of lead.

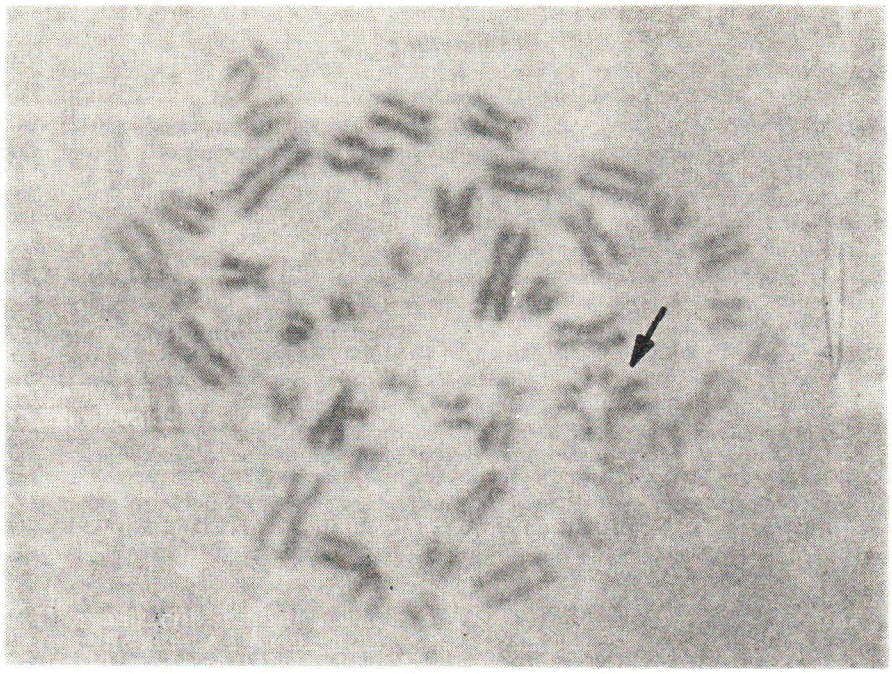

Fig. 2. *Quadriradius*

CONCLUSIONS

Almost one half of the exposed population showed in a certain number of analysed mitoses structural abnormalities of chromosomes, such as chromatid breaks, isochromatid breaks, gaps, fragments, minutes and quadriradius. Most damages were neither numerous nor severe. On the other hand no structural damage could be ascertained in control su-

bjects. The small number of counted mitoses could be regarded as a possible reason for the unexpected absence of chromosomal changes. It should be stressed, however, that the same method was applied in both groups and therefore a difference between Groups I and II and Group III may be regarded as an indication of the association of lead effect and chromosomal abberations, but this should be further investigated.

The relationship between chromosomal aberrations and the variable »residence time« was also tested, but no indication that chromosomal damage is induced by a long term effect of lead was found. The damage is likely to be reparable. It is maintained that periodical investigations of chromosomes in the same person are necessary to clarify this problem.

Literatura

1. *Baloh, R. W.:* Arch. Environ. Health, *28* (1974) 198.
2. *Bauchinger, M., Schmid, E., Schmid, D.:* Mutation Research, *16* (1972) 407.
3. *Blatnik, D.:* Kromozomske anomalije pri razvojnih, formacijskih in funkcijskih anomalijah s povdarkom na vpeljavo metode raziskovanja, disertacija, Univerza v Ljubljani, (1965).
4. *Bove J., Siebenberg, S.:* Science, *167* (1970) 986.
5. Anonimous: Brit. Med. J., *4* (1968) 699.
6. *Forni, A., Secchi, G.:* Chromosome changes in preclinical and clinical lead poisoning and correlation with biochemical findings, Int. Symp. Environmental health aspects, Amsterdam, (1972).
7. *Forni, A., Cambiaghi, G., Secchi, G. C.:* Arch. Environ. Health, *31* (1976) 73.
8. *Fugaš, M., Markićević, A., Prpić-Majić, D., Rudan, P., Seničar, Lj., Sušnik, J., Šarić, M.:* Arh. hig. rada, *26* (1976) 119.
9. *Graovac-Leposavić, Lj., Djurić, D., Seničar, Lj., Milić, S., Delić, V., Seničar, H., Sušnik, J.:* Kontaminacija olovom Mežiške doline, Zbornik III. jugoslovanskega kongresa medicine dela, Ljubljana, (1971).
10. *Graovac-Leposavić, Lj., Djurić, D., Valjarević, V., Seničar, H., Seničar, Lj. Milić, S., Delić, V.:* Environmental lead contamination of Meža Valley — study of lead exposure of population, Int. Symp. Environmental Health Aspects of Lead, Amsterdam, (1972).
11. *Hienz, H. A.:* Chromosomenfibel, G. Thieme Verlag, Stuttgart, (1971) page 392.
12. *Kerin, Ž.:* Ekološki parametri kontaminacije biosfere z industrijskimi ekshalacijami aerosolov svinca v Mežiški dolini, disertacija, Univerza v Ljubljani, (1974).
13. *Lehnert, G.:* Der Blutbleispiegel und seine Bedeutung für die Pathogenese und Prävention der beruflich bedingten Bleivergiftung, A. W. Gentner Verlag, Stuttgart, (1968).
14. *Moorhead, P. S., Nowell, P. C., Mellman, W. J., Pattips, D. M., Hungerford, D. A.:* Exptl. Cell. Res., *20* (1960) 613.
15. *Muro, L. A., Goyer, R. A.:* Arch. Pathol., *87* (1969) 660.
16. *Prpić-Majić, D., Fugaš, M., Sušnik, J.:* The value of biological parameters in the assessment of occupational and non-occupational lead exposure, Int. Symp. Environmental Health Aspects of Lead, Amsterdam, (1972).
17. *Prpić-Majić, D., Sušnik, J., Fugaš, M.:* Arh. hig. rada, *25* (1974) 39.

18. *Schmid, E., Bauchinger, M., Pietruck, S., Hall, G.:* Mutation Research, *16* (1972) 401.
19. *Schwanitz, G., Lehnert, G., Gebhart, E.:* Dtsch. Med. Wschr., *95* (1970) 1636.
20. *Schwanitz, G., Gebhart, E., Rott, H. D., Schaller, K. H., Essing, H. G., Lauer, O., Prestele, H.:* Dtsch. Med. Wschr., *100* (1975) 1007.
21. *Fernandez, T. T.:* Clin. Chem., *21* (1975) 558.

Povzetek

RAZISKAVA KROMOSOMOV PRI LJUDEH, DOLGO IZPOSTAVLJENIH UČINKOM SVINCA

V študiji poročamo o raziskavi vplica svinca na kromosome ljudi, ki so svincu — razen majhne podskupine — nepoklicno izpostavljeni. Za primerjavo smo izbrali enako veliko kontrolno skupino s področja, ki s svincem ni onečiščeno. Pri izpostavljeni skupini smo potrdili povečano ekspozicijo svincu z raziskavo okolja, našli pa smo tudi zvišano koncentracijo svinca v krvi, ki je dober korelat njegove celokupne količine v telesu. Pri približno polovici ljudi iz te skupine smo našli strukturne spremembe kromosomov v limfocitih periferne krvi kakršne so kromatidni in izokromatidni lomi, špranje, fragmenti ter kvadriradius. Spremembe vsekako niso bile niti zelo številne, niti posebej hude. V primerjalni skupini nasprotno nismo našli niti ene take spremembe. Ta nepričakovani rezultat smo razložili z relativno majhnim številom preštetnih mitoz. Pri eni osebi smo prešteli le 15 do 20 vzorcev. Seveda pa je hkrati treba povdariti, da so bili metoda, kot raziskovalci in laboratorij v vseh primerih isti. Sklepamo, da obstoja zveza med povečano resorbcijo svinca ter kromozomskimi aberacijami v perifernih limfocitih. Raziskovali smo tudi povezanost variable »avtohtonost prebivalca« ter kromosomskih okvar, vendar nismo našli nobene zveze. Svinec po vsej verjetnosti na nek način neposredno deluje na kromosome, okvare pa so videti reparabilne.

Zdravstveni centar, Ravne na Koroškem,
Inštitut za zdravstveno varstvo Slovenije,
Ljubljana in Inštitut za medicinske
raziskave in medicino dela, Zagreb

Zaprimljeno 20. VII 1977.