Archives of Environmental Health: An International Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/vzeh20

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Published online: 16 Apr 2013.

To cite this article: Alessandra Forni MD, MIAC , Giovanni Cambiaghi MD & Gian Carlo Secchi MD (1976) Initial Occupational Exposure to Lead, Archives of Environmental Health: An International Journal, 31:2, 73-78, DOI: 10.1080/00039896.1976.10667193

To link to this article: http://dx.doi.org/10.1080/00039896.1976.10667193

Initial Occupational Exposure to Lead

Chromosome and Biochemical Findings

Alessandra Forni, MD, MIAC; Giovanni Cambiaghi, MD; Gian Carlo Secchi, MD

• **Serial chromosome and biochemical studies were carried out in 11 subjects before and during initial occupational exposure to moderate quantities of lead fumes in a storage battery plant. The rate of abnormal metaphases, mostly with chromatid and one-break chromosome aberrations, was approximately doubled after one month of work; it further increased after two months of work; remained in this range up to seven months of exposure; and then tended to decrease somewhat. Blood lead levels increased progressively in the first few months, then reached a steady state. Urinary lead and coproporphyrin levels increased sharply after one month of work, while urinary delta-aminolevulinic acid (ALA) levels increased moderately. The ALA dehydratase (ALAD) activity of red blood cells (RBCs) was reduced to almost 50% of the initial values after one month, decreased further in subsequent months, and remained decreased through the remainder of the study.**

(*Arch Environ Health* 00:000-000, 1975)

In the last few years, a number of reports have appeared on the chromosomal effects of increased lead absorption in animals[1] and man.[2-8] While some of these reports have been essentially negative,[5,6,8] others conclude there is a definite increase in the number of chromatid and chromosome changes in subjects who are occupationally exposed to lead.[2-4,7]

The most extensive study was that published by two of us,[4] in which we reported results of chromosome studies of cultured blood lymphocytes from 65 male workers who were occupationally exposed to lead and from 65 unexposed controls matched for sex and age. On the basis of the clinical and laboratory findings, the workers were subdivided into three groups: group one, 15 workers with preclinical intoxication; group two, 37 workers with clinical lead poisoning; group three, 13 workers with past lead poisoning, but no longer exposed to lead for at least 18 months. The statistical evaluation of the data showed that the rates of chromatid and unstable chromosome changes (mostly of the one-break type) were significantly higher in subjects with preclinical and clinical lead poisoning than among the matched controls, while the differences were not significant for subjects with past poisoning. The observation that the highest mean values of chromatid and unstable chromosome abnormalities were found in group one, the group of workers with abnormal lead absorption in the absence of clinical signs or symptoms of lead poisoning, prompted us to undertake a prospective study on subjects during their first occupational lead exposure, in order to detect the eventual appearance of increased rates of chromosome changes and to follow up their development in time, in comparison with the biochemical measurements of lead absorption. (This kind of design provides a built-in control.) The data obtained in this investigation are reported herein.

Submitted for publication March 21, 1975; accepted June 9.

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SUBJECTS AND METHODS

Subjects

At the time of a preemployment checkup done for a storage battery plant and a lead

Table 1.—Chromosome Findings* in Cultured Lymphocytes of Workers Before and During First Occupational Lead Exposure†

Time, mo	No. of Cases	Total Cells Studied	% Cells		% Abnormal Metaphases
			B	C_u	
0	11	1,075	4.30	0.58	4.88
1	10	1,000	7.50	1.90	9.50‡
2	6	600	8.00	3.00	11.00‡
3	6	580	8.50	1.54	10.04‡
4	6	583	8.73	2.33	11.07§
5	6	600	6.00	2.50	8.67||
6	4	400	9.25	2.00	11.25‡
7	3	300	10.00	1.33	11.67‡
8	3	300	5.67	2.33	8.00||
9	4	380	5.44	2.38	7.81||
11	2	175	3.50	2.50	6.00
15	1	100	15.00	1.00	16.00
18	1	100	9.00	2.00	11.00

* For statistical analysis, see also Table 4.
† Figures indicate mean values.
‡ Significantly different at $P < .05$ from value of time 0 by Dunnett's procedure.[17]
§ Significantly different at $P < .01$ from value of time 0 by Dunnett's procedure.[17]
|| Not significant.

foundry, 29 male subjects received, besides a physical examination and a blood count that included determination of basophilic stippling of red blood cells (RBCs), a chromosome study of peripheral blood lymphocytes and biochemical investigations, including determination of levels of lead in blood and urine, delta-aminolevulinic acid dehydratase (ALAD) activity of RBCs, and levels of ALA and coproporphyrin in urine. Each subject was carefully questioned about previous occupations, consumption of drugs, medical x-ray exposure, and recent viral diseases. None of these individuals had been occupationally exposed to lead, or to other known chromosome-damaging agents including drugs, nor had they received ionizing radiations for medical purposes, except for occasional chest roentgenograms. Eighteen subjects either were not engaged in jobs involving lead risk or were dismissed within the first four weeks of work, and therefore did not enter the follow-up.

Eleven subjects (mean age, 30.5 years; age range, 21 to 42 years) who were employed in jobs involving a mild or moderate exposure to lead were serially studied at approximately monthly intervals from 1 to 11 times (in addition to the preemployment control). At each interval, all the tests previously listed were repeated and any other intervening exposure to possible chromosome-damaging agents (especially drugs, x-rays, and viral diseases) was ruled out.

The number of successful chromosome studies was as follows: ten after one month of work; six after 2, 3, 4, and 5 months; four after 6 months; three after 7 and 8 months; four after 9 months; two after 11 months; and one after 15 and 18 months. Three cultures in two subjects, one each at 1, 4, and 5 months of work, gave less than 30 scorable mitoses, and were therefore not included in the evaluation.

Four subjects resigned after one to three months of work and were therefore sampled only once or twice after the beginning of occupational exposure. Also, a monthly study could not be performed regularly in each subject due to occasional absence from work.

Occupational Exposure

The workers were employed in different departments of a storage battery plant, with different environmental hygiene conditions. Air samplings performed during, or at the end of the period of study showed lead concentrations not higher than 0.8 mg/cu m of air in the work areas of the worst departments. Personal protective equipment (masks) were generally used.

In the working environments where the subjects under study were employed, no other known chromosome-damaging agents were present. In particular, the presence of other heavy metals was excluded.

During the time of study, none of the subjects showed signs or symptoms of lead poisoning.

Methods

Chromosome studies were carried out on peripheral blood lymphocytes cultured for 68 to 70 hours with phytohemagglutinin M (Difco), according to the method of Moorhead et al,[9] partially modified. Both microcultures and macrocultures in TC Medium 199 (Difco) were set up on every occasion; both types of cultures contained autologous plasma in the proportion of 1 ml in 10-ml cultures for the micromethod, and 2 ml in 8-ml cultures for the macromethod. The culture time of 68 to 70 hours, including two hours of demecolcine (Colcemid [Britain]) treatment, was chosen, since as stated in a previous article,[10] in preliminary studies we had not found significant differences in rates of chromosomal abnormalities from those of 48- to 50-hour cultures, and a larger number of scorable mitoses were available. Whenever possible, 100 well-spread metaphases were counted and scored for chromosome changes by direct observation at ×1,250. Some metaphases of each culture were photomicrographed and karyotyped. The different types of chromatid changes (gaps, breaks, and exchanges) and chromosome aberrations (acentric fragments, dicentric, and ring chromosomes and abnormal monocentric chromosomes) were separately scored. In the evaluation of the abnormal metaphases, the classification of Buckton et al[11] was used: cells with chromatid aberrations were classified as B cells; cells with chromosome aberrations were subdivided into C_u ("unstable" chromosome changes, ie, fragments, dicentric, and ring chromosomes) and C_s ("stable" chromosome changes, ie, abnormal monocentric chromosomes in the absence of acentric fragments). When a cell contained both a chromatid and a chromosome aberration, it was classified as a cell with chromosome aberration. Scoring and counting of the chromosomes was done by one of us (A.F.), who at the time of the chromosome studies was unaware of the source of the material.

Lead levels in blood and in urine were determined by the atomic absorption spectrophotometry methods of Andreoletti et al[12] and Zurlo et al,[13] respectively.

The determination of urinary ALA levels was performed by the method of Grisler and Griffini.[14] Urinary coproporphyrin levels were determined by the method of Grisler et al.[15] The ALA dehydratase activity of RBCs was determined by the method of Nakao et al.[16]

The determination of lead levels in urine, urinary ALA, and coproporphyrin were carried out on "spot" samples, and are reported as amounts per liter.

Statistical Analysis

The analysis of variance (F test) was performed on cytogenetic and biochemical data. The percent values of abnormal metaphases were analyzed after square root transformation.[17] For measurements

Table 2.—Biochemical Findings* in Workers Before and During First Occupational Lead Exposure†

Time, mo	No. of Cases	Lead in Blood, μg/100 ml	Lead in Urine, μg/Liter	Urinary ALA, mg/Liter	Urinary Coproporphyrin, μg/Liter	ALAD, Milliunits/ml RBC
0	11	34.00 ± 12.59	37.88 ± 16.86 (8)‡	5.29 ± 1.78	35.44 ± 22.98 (9)‡	14.55 ± 6.44
1	10	45.09 ± 17.34	105.10 ± 62.76	7.60 ± 2.66	118.78 ± 121.63 (9)‡	8.25 ± 5.52§
2	6	45.50 ± 14.76	97.33 ± 53.83	6.17 ± 2.89	105.67 ± 180.72	5.65 ± 3.99‖
3	6	56.83 ± 33.17	91.00 ± 22.98	7.40 ± 3.89	82.33 ± 52.50	7.73 ± 5.69#
4	6	51.00 ± 11.98	132.29 ± 110.28	6.98 ± 3.61	105.86 ± 82.87	6.37 ± 4.85§
5	6	46.29 ± 17.02	107.71 ± 53.26	6.92 ± 2.03	59.50 ± 21.52	5.54 ± 2.75‖
6	4	42.50 ± 4.51	91.50 ± 46.52	6.63 ± 0.75	93.25 ± 75.25	4.35 ± 1.71‖
7	3	63.67 ± 31.79	121.33 ± 107.64	6.67 ± 2.08	73.67 ± 6.03	6.50 ± 3.97#
8	3	40.33 ± 3.22	106.00 ± 54.37	8.36 ± 3.81	100.33 ± 56.98	5.10 ± 1.84 (2)‡#
9	4	46.75 ± 7.63	112.50 ± 44.85	5.47 ± 0.67	not determined	6.08 ± 3.44§
11	2	52 (1)‡	87.00 ± 1.41	7.50 ± 2.12	66.00 ± 63.64	8.20 ± 1.84#
15	1	38	150	7.50	not determined	3.9#
18	1	57	60	4.00	33	5.1#

* For statistical analysis, see also Table 4.
† Figures indicate mean values ± SD.
‡ The number of determinations when less than those stated in column 2.
§ Significantly different at $P < .05$ from value of time 0 by Dunnett's procedure.[17]
‖ Significantly different at $P < .01$ from value of time 0 by Dunnett's procedure.[17]
Not significant.

Table 3.—Cells With Chromatid* and Chromosome† Aberrations in Cultured Lymphocytes of Workers Before and During First Occupational Exposure to Lead Fumes‡

Time, mo	No. of Cases	Total Cells Studied	% B cells				% C_u Cells		% C_s Cells
			Gaps	Isochromatid Gaps	Breaks	Chromatid Exchanges	Acentric Fragments	Dicentric + Rings	Abnormal Monocentric Chromosomes
0	11	1,075	1.49	0.73	2.00	0.09	0.49	0.09	0
1	10	1,000	3.10	0.90	3.40	0.10	1.80	0.10	0.10
2	6	600	2.83	1.17	3.83	0.17	3.00	0	0
3	6	580	3.21	0.50	4.79	0	1.17	0.38	0
4	6	583	3.57	0.73	4.27	0.17	1.83	0.50	0
5	6	600	2.00	1.33	2.67	0	2.33	0.17	0.17
6	4	400	2.00	1.50	5.75	0	2.00	0	0
7	3	300	3.00	1.00	5.33	0.67	0.67	0.67	0.33
8	3	300	1.00	1.00	3.33	0.33	2.00	0.33	0
9	4	380	2.13	0.25	3.06	0	1.88	0.50	0
11	2	175	1.68	0	1.83	0	2.50	0	0
15	1	100	6.00	2.00	7.00	0	1.00	0	0
18	1	100	1.00	0	8.00	0	2.00	0	0

* B cells.
† C_u and C_s cells.
‡ Figures indicate mean values.

where the analysis of variance was significant, comparisons were made between the data of groups studied at various times during occupational exposure and the data obtained at the preemployment checkup (time 0) by Dunnett's procedure.[17]

RESULTS

The results of the serial chromosome and biochemical studies carried out at the preemployment checkup (time 0) and at different times during work involving lead risk are summarized in Tables 1 and 2. The total numbers of abnormal metaphases are the sum of cells with chromatid alterations (B), plus cells with unstable chromosome aberrations (C_u), plus the extremely rare cells with stable chromsome changes (C_s), which are not reported in Table 1. The mean values of the single types of chromatid and chromosome alterations are reported in Table 3, as recently recommended.[18]

The results of the analysis of variance of cytogenetic and biochemical findings are reported in Table 4, where it is shown that significant differences exist for percentages of abnormal metaphases and, with regard to the biochemical data, only for values of ALAD activity of RBCs.

In the chromosome studies, a significant increase of percent values of abnormal metaphases is already evident after one month and persists up to seven months of exposure (see Table 1 for values of P). The data ob-

Table 4.—Analysis of Variance of Percentage of Abnormal Metaphases and Biochemical Findings in Initial Occupational Lead Exposure

Measurement Analyzed	Source of Variation	df	Sum of Squares	Mean Square	F	P
% Abnormal metaphases (square root transformation of counts made on 100 metaphases)	Among groups	9	9.489	1.054	2.908	<.01
	Within groups	46	16.680	0.363		
	Total	**55**	**26.169**			
Lead in blood	Among groups	9	3524.573	391.619	1.255	NS
	Within groups	51	15917.754	312.113		
	Total	**60**	**19442.327**			
Lead in urine	Among groups	11	41770.852	3797.350	0.998	NS
	Within groups	50	190222.632	3804.453		
	Total	**61**	**231993.484**			
Urinary ALA	Among groups	11	52.689	4.790	0.686	NS
	Within groups	52	362.877	6.978		
	Total	**63**	**415.566**			
Urinary coproporphyrin	Among groups	9	43986.097	4887.344	0.593	NS
	Within groups	45	370792.885	8239.842		
	Total	**54**	**414778.982**			
ALAD activity of RBCs	Among groups	11	682.981	62.089	2.674	<.01
	Within groups	53	1230.551	23.218		
	Total	**64**	**1913.532**			

tained for the next groups either were not significant (time 8 and 9), or were not analyzed due to the small number of observations (times 11, 15, and 18).

With respect to the biochemical data, only the values of ALAD activity of RBCs showed a significant F ($P < 0.01$). The decrease of this enzymatic activity was already evident at time 1, and was confirmed after two, four, five, six, and nine months of exposure, while, probably owing to the small number of observations and the relatively high dispersion of data, it was not significant at the other times (see Table 2 for values of P).

The actual number of aberrations observed was somewhat higher than the number of abnormal mitoses, since occasionally more than one aberration was present in a single cell. At any time of study, this was true for chromatid and isochromatid gaps, chromatid breaks, and more rarely, for acentric fragments.

Of a total of 103 cells with acentric fragments without dicentric or ring chromosomes, 38 contained centromere or near centromere breaks, most frequently in chromosomes of A and C groups. Chromosome pulverization was occasionally observed, but was not quantitated.

In each chromosome study, the number of polyploid metaphases was evaluated by scoring 500 mitoses. While at time 0 the number of polyploid metaphases in the different cases ranged from zero to two per thousand, during exposure a general tendency to increase was noted, occasionally reaching eight per thousand. The behavior of this characteristic, however, was very irregular and did not seem to correlate in the individual subjects either with biochemical parameters or with an increase of chromosome aberration rates.

As rather typical examples of the behavior of the different measurements studied, the data obtained in two of the most extensively studied cases are reported in Tables 5 and 6. Worker 3 (age 26), who was first studied at the beginning of 1973, started working in February 1973 in the lead foundry department of a storage battery plant, and was followed up until January 1974. During the period of study, there were two periods of leave: One of one month's duration, which included vacations and a work accident, occurred between times 5 and 8 of study. The second leave, which was due for a work accident, lasted two weeks, and occurred between times 9 and 11 (Table 5). Worker 5 (age 42), received the preemployment checkup in January 1973, started working at the end of January and was followed up until the end of October 1973; between times 5 and 8 of study there was a vacation period of one month's duration (Table 6).

COMMENT

The cytogenetic data obtained in this study confirm that ocupational exposure to lead is associated with a significant increase in rates of chromatid and chromosome aberrations in cultured lymphocytes, as previously reported for lead-poisoned subjects.[2-4,7] Moreover, from this follow-up investigation of initial occupational lead exposure, one can also observe that the increase in rate of abnormal metaphases was very fast at the beginning of exposure to lead levels that were higher than those in the general environment. In fact, the rate of abnormal metaphases was approximately doubled after one month of work and was further increased after two months of work, and remained in this range up to seven months of exposure. After this period, a tendency to decrease was noted at times 8, 9, and 11 (Table 1). The smaller number of cases evaluated in the period from 7 to 18 months might account for the irregular behavior of responses at these times, due to the dispersion of the base values at time 0 and to the different individual increase during exposure.

The analysis of the cytogenetic data in Tables 1 and 3 shows that the absolute increase in rates of abnormal metaphases is primarily due to chromatid changes (gaps and breaks). However the relative increase is most evident for unstable chromosome aberrations, the majority of which are represented by acentric fragments. These might actually be "derived" chromosome changes, as suggested by O'Riordan and Evans[8]; however, it is interesting to note that the values of this type of aberration remained well above that at time 0 even at times 8, 9, and 11, when the rate of cells with chromatid alterations tended to decrease sharply (Table 1).

The changes observed might actually be culture-produced aberrations, not repaired in the presence of lead or

Table 5.—Results of Serial Chromosome and Biochemical Studies in Case 3

	Chromosome Studies				Biochemical Studies				
Time, mo	Total Cells Studied	% Cells B	% Cells C_u	% Abnormal Metaphases	Lead in Blood, μg/100 ml	Lead in Urine, μg/Liter	Urinary ALA, mg/Liter	Urinary Coproporphyrin, μg/Liter	ALAD, milliunits/ml RBC
0	100	3	1	4	27	Not determined	8.24	50	23
1	100	7	1	9*	31	79	9.5	190	12
2	100	8	2	10	33	40	5.0	50	12.6
3	100	13	1	14	87	98	7.0	150	6
4	83	8.4	0	8.4	62	264	6.0	110	4.5
5	100	7	2	9	75	170	6.0	50	6.4
8	100	5	2	7	44	46	7.5	71	6.4
9	100	4	4	8	58	152	4.52	Not determined	7.8
11	75	4	4	8	Not determined	88	9.0	111	9.5

* Includes one C_s cell.

Table 6.—Results of Serial Chromosome and Biochemical Studies in Case 5

	Chromosome Studies				Biochemical Studies				
Time, mo	Total Cells Studied	% Cells B	% Cells C_u	% Abnormal Metaphases	Lead in Blood, μg/100 ml	Lead in Urine, μg/Liter	Urinary ALA, mg/Liter	Urinary Coproporphyrin, μg/Liter	ALAD, milliunits/ml RBC
0	100	2	0	2	24	42	6.88	10	7.8
1	100	11	2	13	68	114	4.84	10	1.6
2	100	12	2	14	66	141	4.52	30	2.6
3	100	7	4	11	51	96	4.20	80	2.3
4	100	7	2	9	61	311	5.86	100	1.6
5	100	1	3	5*	55	176	11.00	70	1.5
8	100	2	4	6	38	152	5.05	64	Not determined
9	100	9	1	10	42	48	5.06	Not determined	1.8

* Includes one C_s cell.

of some abnormal lead-induced metabolite, as already suggested.[4] This hypothesis is based on the fact that most alterations are chromatid in type, ie occurring in culture after DNA synthesis, whereas, rearrangements are very scanty. Moreover, in previous studies we had not detected such aberrations in uncultured chromosome preparations of bone marrow cells of lead-poisoned subjects.[4]

Different culture times and methods might partially explain the discordance of results in different laboratories; for instance, our cultures contain autologous plasma instead of the bovine serum used by O'Riordan and Evans[8] in their essentially negative report. On the other hand, increased rates of chromatid and chromosome changes in human lymphocytes cultured in the presence of lead acetate have been reported.[2,19]

The analysis of the biochemical data reported in Table 2 shows that during an initial occupational exposure to lead, there is a progressive increase in the blood lead level in the first few months, then a levelling-off of the values. Urinary lead and coproporphyrin levels increased sharply after one month of work, while the urinary ALA level did not reach high values in these moderate exposures. All these data, however, did not differ significantly in the analysis of variance.

In the first month of work, ALA activity of RBCs decreased significantly to almost 50% of the original values; it tended to decrease moderately in subsequent months, and remained in this range with some fluctuation for the duration of the period of study.

By observing both the cytogenetic and the biochemical data in our study, the suggestion can be made that some kind of adjustment mechanism might intervene after some time of exposure. The fact that the various biochemical measurements reach a steady state some time after the beginning of new lead exposure has already been reported by Tola et al.[20] However, one must keep in mind that the real situation, as far as lead poisoning is concerned, is better reflected by the evaluation of the total lead body-burden than by the determination of the single indexes.

One more observation that can be drawn from the follow-up of the single cases is that, at least in these slight or moderate occupational lead exposures, short periods (two to three

weeks) of absence from work permit a partial recovery, which is reflected by improvement of the values in samples collected shortly after readmission to work. This fact might partly explain some fluctuation of the values of the different determinations.

Nothing can be said about the significance of the cytogenetic findings in cultured lymphocytes of lead-exposed subjects. In view of the presently available data, the increased rates of chromatid and chromosome changes seem to reflect more a biological effect revealed under conditions of culture, than a real in vivo situation.

This investigation was supported by US Public Health Service research grant OH 0039-03 of the National Institute for Occupational Safety and Health.

Maria Rosa Castoldi, Elvira Ortisi, and Costanza Forti provided technical assistance. Roberto Stabilini, MD, of the University of Milan assisted with statistical analysis.

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