Cytogenetic analysis of bone marrow cells and spermatogonia of male mice after in vivo treatment with arsenic

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Summary. Male Swiss Albino mice were injected i.p. with an As_2O_3 solution (0; 4; 8 and 12 mg As/kg) and sacrificed 12, 24, 36 and 48 h after injection. Neither chromatid nor chromosome aberrations were observed in bone marrow cells and spermatogonia.

Arsenic is known as a thiol inhibiting substance[2]. It may also replace the phosphorus in the phosphate group of the DNA and form a weak bond in the DNA[3].

In vitro studies showed that arsenic may inhibit the DNA synthesis in human lymphocytes and the DNA repair in human epidermal cells[4]. Moreover, chromosomal aberrations were observed in human lymphocyte cultures and in human fibroblast cultures after administration of different arsenic compounds[4].

In *Drosophila melanogaster*, no increase of recessive lethal mutations with respect to the control could be demonstrated. Negative results were also observed in a dominant

Table 1. Frequency of different aberrations in mouse bone marrow cells 12, 24, 36 and 48 h after an acute i.p. injection of different arsenic concentrations

Treatment	Mice No.	Metaphase No.	Type of aberrations			
			Gaps (%)	Breaks (%)	Chromatid exchanges (%)	Chromosomal exchanges (%)
0 mg As/kg						
12 h	8	1000	0.50	0.00	0.00	0.00
24 h	8	730	0.55	0.14	0.00	0.00
36 h	7	790	0.25	0.00	0.00	0.13
48 h	7	742	0.14	0.14	0.00	0.14
4 mg As/kg						
12 h	8	885	0.90	0.11	0.00	0.00
24 h	5	535	0.56	0.19	0.00	0.00
36 h	5	580	0.69	0.00	0.00	0.00
48 h	4	425	2.36 ($p < 0.01$)	0.47	0.00	0.71
8 mg As/kg						
12 h	8	1000	0.70	0.00	0.00	0.10
24 h	8	1000	1.00	0.00	0.00	0.00
36 h	7	875	1.14 ($p < 0.05$)	0.11	0.00	0.00
48 h	8	785	0.64	0.13	0.00	0.00
12 mg As/kg						
12 h	8	972	0.21	0.00	0.00	0.10
24 h	8	766	0.52	0.00	0.00	0.00
36 h	8	875	0.57	0.11	0.00	0.11
48 h	8	830	0.72	0.00	0.00	0.00

Table 2. Frequency of different aberrations in mouse spermatogonia cells 12, 24, 36 and 48 h after an acute i.p. injection of different arsenic concentrations

Treatment	Mice No.	Metaphase No.	Type of aberrations			
			Gaps (%)	Breaks (%)	Chromatid exchanges (%)	Chromosomal exchanges (%)
0 mg As/kg						
12 h	8	250	0.00	0.00	0.00	0.00
24 h	8	185	0.00	0.00	0.00	0.00
36 h	7	167	0.00	0.00	0.00	0.00
48 h	7	130	0.00	0.00	0.00	0.00
4 mg As/kg						
12 h	8	240	0.84	0.00	0.00	0.00
24 h	8	179	0.00	0.00	0.00	0.00
36 h	8	191	0.00	0.00	0.00	0.00
48 h	7	142	0.70	0.00	0.00	0.00
8 mg As/kg						
12 h	8	262	0.00	0.00	0.00	0.00
24 h	8	260	0.38	0.00	0.00	0.00
36 h	7	215	0.00	0.00	0.00	0.00
48 h	8	250	1.60	0.00	0.00	0.00
12 mg As/kg						
12 h	8	255	0.39	0.00	0.00	0.00
24 h	8	230	0.43	0.00	0.00	0.00
36 h	8	189	1.06	0.00	0.00	0.00
48 h	8	204	0.49	0.00	0.00	0.00

lethal test in mice[5,6]. A tendency toward an increased frequency of chromosome aberrations after arsenic in vivo treatment was found in marrow cells of Chinese hamsters[5]. Considering these contradictory data, an in vivo test was performed to estimate the mutagenicity of arsenic in bone marrow cells and in spermatogonial cells of mice.

Material and methods. Arsenic, as As_2O_3 compound was dissolved in distilled water by adding NAOH and this solution was neutralized. Male Swiss Albino OF1 mice, 12 weeks old, were given a single i.p. injection of 0.5 ml of the arsenic solution with a concentration of 0; 4; 8 and 12 mg As/kg b.wt. Control animals were injected with 0.5 ml distilled water. Animals were sacrificed after recovery periods of 12, 24, 36 and 48 h. Microscopic slides were prepared using the 'blowing' technique[7,8]. Cytogenetic damage was evaluated by determining the frequencies of gaps, breaks and exchanges in bone marrow cells and in spermatogonia.

Results and discussion. In the bone marrow cells, no dose-effect relationship was found either for gaps, or for chromatid and for chromosome type aberrations (table 1). No significantly increased frequency of breaks and exchanges was observed. However, a concentration of 4 mg As/kg b.wt and a concentration of 8 mg As/kg b.wt induce significantly more gaps in animals killed after 48 h ($p < 0.01$) and 36 h ($p < 0.05$) respectively than in nontreated mice.

Since gaps are rather unreliable measures, it may be considered that arsenic does not induce chromatid or chromosome aberrations in bone marrow cells. The spermatogonial cells also show no significant increase of gaps, chromatid or chromosome type aberrations after arsenic administration (table 2).

Our data confirm that parameters of mutagenicity such as chromatid and chromosome aberrations, are not increased by arsenic in vivo in mammals. This does not exclude mutagenic action of arsenic through other mechanisms, such as repair inhibition, DNA polymerase inhibition.

Taking into account the hypothesis of an inhibiting effect of arsenic on the repair mechanism, a combined exposure to arsenic and a known mutagenic agent may help to elucidate the action of arsenic in vivo.

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Isocitrate dehydrogenase gene duplication and fixed heterophenotype in the cultivated soybean *Glycine max*

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Summary. Isocitrate dehydrogenase (E.C. 1.1.1.42) gene duplication was demonstrated in the self-pollinated soybean *(Glycine max)* by means of starch-gel electrophoresis. This finding explains the heterogeneity and/or fixed heterophenotype observed in some soybean cultivars.

The cultivated soybean *(Glycine max)* is normally self-pollinated; natural cross-pollination is usually considerably less than 1%. Like other self-pollinated plants, cultivars (or varieties) of soybean are expected to have attained homozygosity in their genetic constitution. This appears to be so for seed proteins, INT-oxidase, peroxidase, urease, esterase activity, acid phosphatase, alcohol dehydrogenase, amylase and tetrazolium oxidase, which have been demonstrated to be varietyspecific[1-6]. In our survey for inter-varietal differences employing biochemical characters, a number of soybean cultivars of diploid origin were found to be heterogeneous with respect to isocitrate dehydrogenase (E.C. 1.1.1.42). This is rather unexpected and we report here our finding of isocitrate dehydrogenase gene duplication and fixed heterophenotype in the cultivated soybean.

Conventional horizontal starch-gel electrophoresis employing the citrate-phosphate buffer system at pH 6.8 reveals that isocitrate dehydrogenase in soybean is represented by at least 4 electrophoretic patterns at the faster anodal zone (figure). There is also a distinctly slower anodal zone *(Idh-S)* which is represented by a single band and appears to be variety-specific.

The electrophoretic patterns observed in 12 varieties of soybean are summarized in the table. Of these varieties, 7 are homogeneous and 5 heterogeneous. The homogeneous varieties are of 3 distinct types (IDH-B, IDH-AB and IDH-BC respectively), while the heterogeneous varieties fall into 2 groups (with IDH-B and IDH-AB or IDH-BC and IDH-AC respectively).

Electrophoretic studies of seeds produced by individual plants reveal that their IDH-phenotype is the same, and is identical to that of the parental plant; e.g., an IDH-AC plant produces only seeds with the IDH-AC phenotype. On

Distribution of IDH-F electrophoretic patterns in 12 varieties of cultivated soybean

Variety	IDH-F phenotypes			
	B	AB	BC	AC
BM 10	39	–	–	–
Clark 63	51	–	–	–
KS-437	–	31	–	–
Kahala	–	30	–	–
GC-30094-1-32	–	30	–	–
McNair 800	–	–	30	–
L 114	–	–	30	–
G.BM 11	6	24	–	–
66d-20	15	15	–	–
G No.2043	13	17	–	–
Palmetto	–	–	16	31
GC-30279-15-8	–	–	9	21