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Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture

Te-Chang Lee[1], Mitsuo Oshimura and J.Carl Barrett[2]

Environmental Carcinogenesis Group, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC 27709, USA

[1]Present address: Institute of Zoology, Academic Sinica, Nankang, Taipei, Taiwan 115, Republic of China

[2]To whom reprint requests should be sent

Sodium arsenite and sodium arsenate were observed to induce morphological transformation of Syrian hamster embryo cells in a dose-dependent manner. A linear dose-dependence with a slope of ~1 was observed with both compounds when the data were plotted on a log-log graph. The trivalent sodium arsenite was >10-fold more potent than the pentavalent sodium arsenate. The compounds also exhibited toxicity; however, transformation was observed at non-toxic as well as toxic doses. At low doses, enhanced colony-forming efficiency of the cells was observed. To understand the mechanism of arsenic-induced transformation, the genetic effects of the two arsenicals were examined over the same doses that induced transformation. No arsenic-induced gene mutations were detected at two genetic loci. However, cell transformation and cytogenetic effects, including endoreduplication, chromosome aberrations, and sister chromatid exchanges were induced by the arsenicals with similar dose-responses. These results support a possible role for chromosomal changes in arsenic-induced transformation.

Introduction

Arsenic is a very interesting environmental carcinogen because it is one of the few, possibly the only, chemical which has sufficient evidence for carcinogenicity in humans but inadequate evidence in animals (1,2,3). In the absence of an animal model to study the mechanism of arsenic-induced carcinogenicity, *in vitro* studies have been conducted to determine the cellular effects of arsenic and arsenical compounds. DiPaolo and Casto (4) reported that sodium arsenate induced morphological transformation of Syrian hamster embryo cells. Since cell transformation assays *in vitro* have been shown to measure one step in neoplastic development (5,6), the findings of DiPaolo and Casto indicate that the Syrian hamster embryo cell transformation system may be useful to study the mechanism of arsenic carcinogenicity (4). However, only a limited dose range of sodium arsenate was examined and the trivalent form of arsenic, sodium arsenite, which is more toxic than the pentavalent arsenate (7), was not studied (4).

The other predominant activities of arsenic compounds that have been studied with *in vitro* systems include toxicity (8), gene mutations (9–11), sister chromatid exchanges (SCEs)* and chromosome aberrations (12–16). Mutagenic activity of arsenicals in prokaryotic systems is generally lacking (2). Sodium arsenite and sodium arsenate are inactive in inducing mutations at two genetic loci (Na^+/K^+ ATPase and hypoxanthine phosphoribosyl transferase) in V-79 cells (11) and weakly active at the thymidine kinase locus in L5178Y mouse lymphoma cells, yielding 2- to 3-fold increases in mutation frequencies above solvent controls at >10% survival (10). Sodium arsenate is inactive in L5178Y cells (9,10), except in the presence of rat liver microsomes, which may reduce the arsenate to arsenite (10). In contrast to the negative or weak activity in gene mutation assays, arsenic compounds are very active inducers of SCEs and chromosome aberrations in human and rodent cells in culture (12–18) and *in vivo* (19–23). The trivalent arsenite is more active than the pentavalent arsenate in these assays (13,16), which is consistent with its greater activity in other assays. Arsenic also enhances viral transformation (24).

In this report we have confirmed and extended the initial observations of DiPaolo and Casto (4) that arsenic can induce morphological transformation of Syrian hamster embryo cells in culture. We have compared the dose-response of sodium arsenite and sodium arsenate and shown that the trivalent form is >10-fold more potent than the pentavalent form. In addition, we have compared the dose-responses for cell transformation with cytotoxicity, induction of gene mutations at two genetic loci, and cytogenetic effects, including SCEs, chromosome aberrations and numerical chromosome changes. Our results indicate that arsenic can induce cell transformation at non-toxic and toxic doses in the absence of any detectable gene mutations. However, cell transformation and chromosome abnormalities are induced by arsenicals with similar dose-responses which may indicate that these effects are related.

Materials and methods

Cells, culture conditions and chemicals

Syrian hamster embryo cell cultures were established from 13-day gestation fetuses collected aseptically by Caesarean section from inbred Syrian hamsters, strain LSH/ss LAK (Lakeview Hamster Colony, Newfield, NJ). Pools of primary cultures from littermates were cryopreserved in liquid nitrogen (6). Secondary cultures were initiated from the frozen stocks and all experiments were performed with tertiary cultures. The culture medium was IBR-modified Dulbecco's Eagle's reinforced medium (GIBCO, Grand Island, NY) supplemented with 3.7 g sodium bicarbonate per liter, 20% (v/v) Hy-Clone fetal bovine serum (FBS) (Sterile Systems Inc., Logan, UT), 100 units penicillin per ml, and 200 μg streptomycin (GIBCO) per ml. Cells were gently trypsinized with 0.1% trypsin solution (1:250; GIBCO) in PBS for 5 min at 37°C. Cultures were routinely tested and found to be free of mycoplasma. Sodium arsenite and sodium arsenate (>99% pure) were obtained from J.T.Baker Chemical Company (Phillipsburg, NJ) and benzo[a]pyrene (B[a]P) was obtained from the Chemical Repository of the National Cancer Institute (Bethesda, MD).

Quantitation of cytotoxicity and morphological transformation

The *in vitro* transformation procedure was a modification of the earlier work of Berwald and Sachs (15), DiPaolo *et al.* (4,26) and Pienta *et al.* (27). Target cells (250) were seeded on a layer of 2×10^4 lethally irradiated (5000 R) homologous feeder cells in 60-mm dishes (Falcon Plastics, Oxnard, CA) in complete medium. After 18 h the cells were exposed to various concentrations of arsenic compounds in fresh medium and the cultures were then incubated at 37°C in a humidified atmosphere of 12% CO_2 in air for 7 days. The dishes were then fixed in ab-

*Abbreviations: SCEs, sister chromatid exchanges; PBS, phosphate buffered saline; B[a]P, benzo[a]pyrene; TG^r, thioguanine resistant; Oua^R, ouabain resistant; BrdU, 5-bromo-2′-deoxyuridine; CFE, colony forming efficiency.

solute methanol (Fisher Scientific Co.) and stained with 10% Giemsa (Fisher Scientific Co.). The colony-forming efficiency (CFE) (%) was determined by dividing of the number of target cells plated (250) by the number of colonies formed at 7 days after treatment and multiplying by 100. The cytotoxic response of the treated cultures was expressed as the percent relative survival, i.e., the CFE of the treated cells divided by the CFE of the untreated cells (controls) × 100. Colonies that were morphologically transformed were scored according to criteria that has been described previously (6,25,26). The transformation frequency (%) was calculated as (the total number of transformed colonies divided by the total number of colonies surviving treatment) × 100. The data presented represent the combined results of four experiments.

Gene mutation assays

Cells ($2.5-5 \times 10^5$) were seeded into 75-cm² flasks and after overnight incubation, treated with sodium arsenite, sodium arsenate, or B[a]P in complete medium for 48 h. After treatment the cultures were washed twice with 10 ml of medium and replenished with fresh medium. After 24 h, the cells were sub-cultured at a split ratio of 1 – 10. Following 3 days cultivation (4 days after treatment), 5000 cells were plated on 100-mm dishes and incubated for 7 days for colony formation. For mutation experiments, 10^5 cells were plated on each of 10 – 20 dishes (100 mm in diameter) with medium containing 3 μg/ml 6-thioguanine or 1.2 mM ouabain (28) and incubated 7 days for colony formation. Cells were also plated in new flasks at 5×10^5 cells/75-cm² flask and grown for an additional 3 days expression time and then assayed for colony formation and thioguanine-, or ouabain-resistant (TG^r or Oua^R) mutants as above. The mutation frequency was calculated as described previously (28). The experiments were repeated three times.

Cytogenetic assays

Tertiary-passage cells were inoculated into 75-cm² flasks at $5-10 \times 10^5$ cells/flask. After overnight incubation, the culture medium was removed, 10 ml of either culture medium or medium containing 0.8, 3.0, 6.2 or 10 μM of sodium arsenite, or 10, 32, 64 or 96 μM of sodium arsenate were added to the flasks, and the cultures were then incubated. Chromosome preparations were made following colcemid (0.4 μg/ml) addition for the last 3 h of culture. The cells were trypsinized, collected by centrifugation, treated with 0.075 M KCl for 10 min, and fixed in methanol:acetic acid (3:1). The cells were dropped onto slides and stained wth Giemsa (15% in 0.05 M phosphate buffer, pH 6.8, for 15 min). At least 100 metaphases at each dose were scored for numerical and structural changes. The experiments were repeated twice.

SCEs were also examined 24 – 27 h after the initiation of 5-bromo-2'-deoxyuridine (BrdU) (Sigma Co., St. Louis, MO) treatment; BrdU (10 μg/ml) was added at the same time as the chemical treatment. Slides were stained by FPG treatment (29). The experiment was repeated three times.

Results

The cytotoxic effects of sodium arsenite and sodium arsenate treatments of Syrian hamster embryo cells were assessed using a colony-forming assay with the cells grown on a homologous feeder layer of irradiated cells. The CFE of the untreated cells was between 12 and 20%. When treated with either arsenical, the CFE of the cells was actually enhanced at low doses and then inhibited at higher doses. A small but reproducible increase in CFE was observed with 0.7 – 3 μM sodium arsenite and 10 – 50 μM sodium arsenate (Figure 1). This increase was also observed with other cell types, for example V-79 and 3T6 cells (data not shown). The extent of the increase varied between 10 and 70% in four different experiments, but an increase was consistently observed. At higher doses (3 – 10 μM sodium arsenite and 50 – 200 μM sodium arsenate), a logarithmic decrease in survival was observed with a linear increase in dose.

The dose-dependence of arsenic-induced morphological transformation of Syrian hamster embryo cells was examined. Both sodium arsenite and sodium arsenate were effective inducers of transformation (Figure 2). The trivalent form was >10-fold more potent than pentavalent arsenic. The shapes of the dose-response curves were similar (Figure 2); a linear dose-dependence with a slope of ~1 was observed when the data were plotted on a log-log graph (slope = 1.19 ± 0.20 for sodium arsenite and 1.20 ± 0.13 for sodium arsenate). Morphological transformation was observed at both non-toxic and toxic doses of arsenic.

The mutagenic activity of arsenic was also examined in the

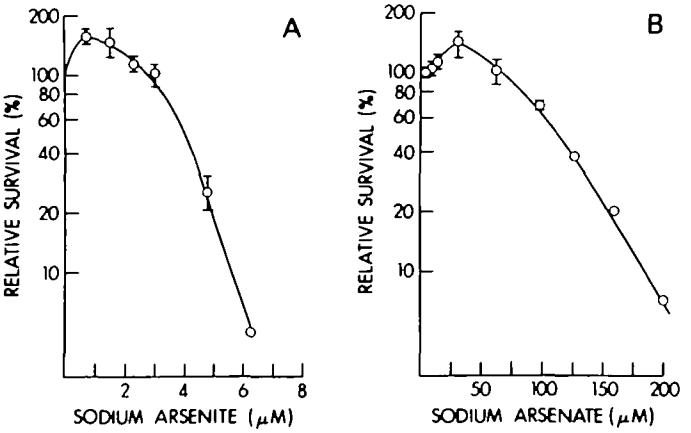

Fig. 1. Cytotoxicity of sodium arsenite and arsenate. Syrian hamster embryo cells were exposed to various concentrations of arsenicals and incubated for 7 days. The relative survival was determined from (the number of colonies in the treated cultures divided by the number of colonies in the untreated culture) × 100. **A**, sodium arsenite; **B**, sodium arsenate. The results are the averages ± S.D. of four experiments except for some of the high doses which were single experiments (no error bars).

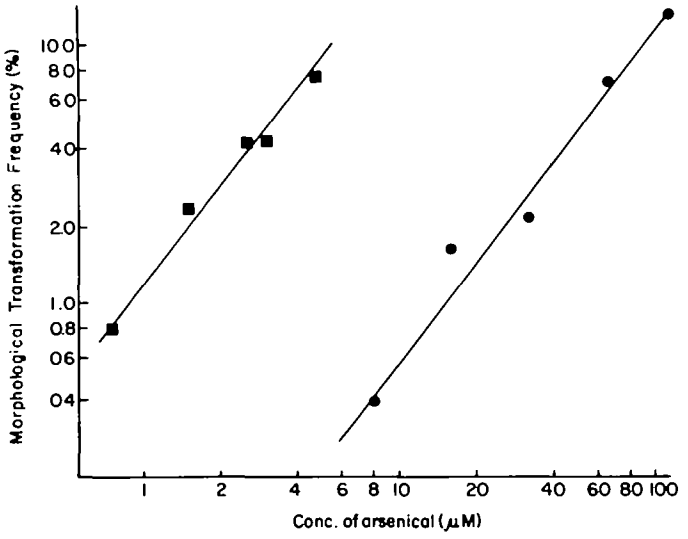

Fig. 2. Dose-dependence of morphological transformation of Syrian hamster embryo cells by sodium arsenite (■) or sodium arsenate (●). Cells were treated with the indicated dose of arsenical and grown for 7 days to allow colony formation. The number of morphologically transformed colonies were determined by the criteria described (6). The percentage of morphological transformation is (the number of transformed colonies divided by the total number of colonies) × 100.

Syrian hamster embryo cells at the doses which induced morphological transformation. Neither sodium arsenite nor sodium arsenate induced any detectable gene mutations at the Na^+/K^+ ATPase (Oua^R) or hypoxanthine phosphoribosyl transferase (TG^r) loci (Table I). B[a]P, used as a positive control in the experiments, was active in these experiments.

The cytogenetic effects of sodium arsenite and sodium arsenate were studied. Cells treated with either chemical did not have increased chromosome losses or gains; the near diploid chromosome number was unaltered by treatment for 48 h (Table II). However, a large increase in polyploidy cells (i.e., cells with a tetraploid or near tetraploid chromosome number) was observed at 48 h. These cells may have arisen due to endoreduplication which was observed in cells treated for 24 h (Figure 3A; Table II). The percentage of metaphases with endoreduplicated

Table I. Lack of mutagenicity of arsenic at two genetic loci

Chemical	Dose	Relative survival (%)	Specific locus mutation frequency	
			Oua^R	TG^r
Control	0	100	$<10^{-6}$	$<10^{-6}$
Sodium arsenate	3.1 μg/ml (10 μM)	106 ± 7	$<10^{-6}$	$<10^{-6}$
	10.0 μg/ml (32 μM)	117 ± 20	$<10^{-6}$	$<10^{-6}$
	20.0 μg/ml (64 μM)	73 ± 8	$<10^{-6}$	$<10^{-6}$
	31.0 μg/ml (100 μM)	42 ± 11	$<10^{-6}$	$<10^{-6}$
Sodium arsenite	0.31μg/ml (1 μM)	136 ± 22	$<10^{-6}$	$<10^{-6}$
	0.4 μg/ml (3.1 μM)	82 ± 19	$<10^{-6}$	$<10^{-6}$
	0.8 μg/ml (6.2 μM)	9 ± 8	$<10^{-6}$	$<10^{-6}$
	1.3 μg/ml (10 μM)	<1	$<10^{-6}$	$<10^{-6}$
Benzo[a]pyrene	1.0 μg/ml (4 μM)	95 ± 4	7.5×10^{-5}	2.0×10^{-5}

The cells were treated for 48 h with the indicated dose of chemicals, grown for 4 and 7 days, and then plated in selective media (1.1 mm ouabain or 3 μg/ml 6-thioguanine). Mutant colonies were selected in this media for 7–10 days and then the cells were fixed and stained and surviving TG^r or Oua^R colonies enumerated. The mutation frequency was calculated as described (28) and corrected for toxicity and recovery efficiency of the mutant cells. Relative survival was measured in parallel cultures treated with the indicated concentration of chemicals and allowed to form colonies for 7 days. No mutants were observed in any of the arsenate or arsenite treated cultures at either of the mutant expression times. The experiment was repeated three times.

Table II. Cytogenetic effects of sodium arsenate or sodium arsenite treatment of Syrian hamster embryo cells[a]

Treatment	Chromosome number distribution				Polyploid cells (%)	Endoreduplication (%)	Aberrations[b]					Metaphases with aberrations (%)
	42	43	44	45			G	B	F	Ex	Dic	
Control		2	98		5	0	3					2.0
Sodium arsenite (μM)												
0.8		2	98		9	0	2					1.3
3.0		1	99		16*	7*	2	1		2		3.3
6.2		2	98		62*	31*	5	10	2	4		14.0*
10		5	95		58*	13*	6	6	1	6	1	13.3*
Sodium arsenate (μM)												
10		4	94		8	0	1					0.6
32		2	98		14*	1	2	2				2.7
64	1	3	96		33*	12*	5	1		5		7.3*
96		1	15		32*	6*	6	1	1	6		9.3*

[a]Cells in metaphase were harvested from cultures treated with the indicated dose of either chemical for 24 h (endoreduplication and chromosome aberrations) or 48 h (chromosome number and polyploid cells) and then fixed and scored for the indicated abnormalities. Different times of treatment were examined and the reported treatment times were chosen because maximum induction of the specific cytogenetic effects were observed.
[b]G, gaps; B, breaks; F, chromosome fragments; Ex, chromatid exchanges; Dic, dicentric chromosomes. 150 cells per treatment were scored.
*Significantly different from control, ($p < 0.05$) by the Fisher exact test.

chromosomes generally decreased at 48 h after treatment (data not shown) which corresponded with the increase in polyploid cells. Both sodium arsenite and sodium arsenate induced endoreduplication and polyploidy over the dose ranges that induced transformation. Arsenite was active at 10-fold lower concentrations than arsenate.

Chromosome aberrations were also induced by these arsenicals in a dose-dependent manner over the same concentration range required for transformation induction and again the trivalent form was more active than the pentavalent arsenate (Table II). Chromatid type aberrations were primarily induced (chromatid gaps, breaks and exchanges) and a few chromosome type aberrations (fragments and dicentrics) were observed (Figure 3B and 3C). The latter may have arisen from the chromatid aberrations following cell replication. SCEs were also induced by both chemicals (Figure 3D; Table III).

Discussion

In this study we have determined the dose-dependence for arsenic-induced effects in the Syrian hamster embryo cell system in which a number of end-points can be compared including toxicity, cell transformation, gene mutations and chromosome mutations. We observed that both sodium arsenite and sodium arsenate were toxic to these cells, with the trivalent form being >10-fold more toxic. This finding is consistent with results of others (7,8,13). This may relate to differences in cellular uptake (30,31) of the two forms of arsenic possibly due to differences in ionization at physiological pH (30). One interesting finding of ours was that low doses of arsenic actually enhanced the colony forming ability of the cells. We are not aware of any other *in vitro* study showing this effect, although this may not be surprising since this effect is small and observed only over a narrow dose range; hence, it may have been overlooked previously. Whether this effect has any relevance to the well-known growth promoting effects of arsenicals *in vivo* (7) is an interesting question for future research.

We have confirmed the studies of DiPaolo and Casto (4), which showed that arsenic induces cell transformation. Both arsenical compounds were effective inducers of morphological transformation of Syrian hamster embryo cells; arsenite was >10-fold more potent than arsenate. The dose-response curves for both

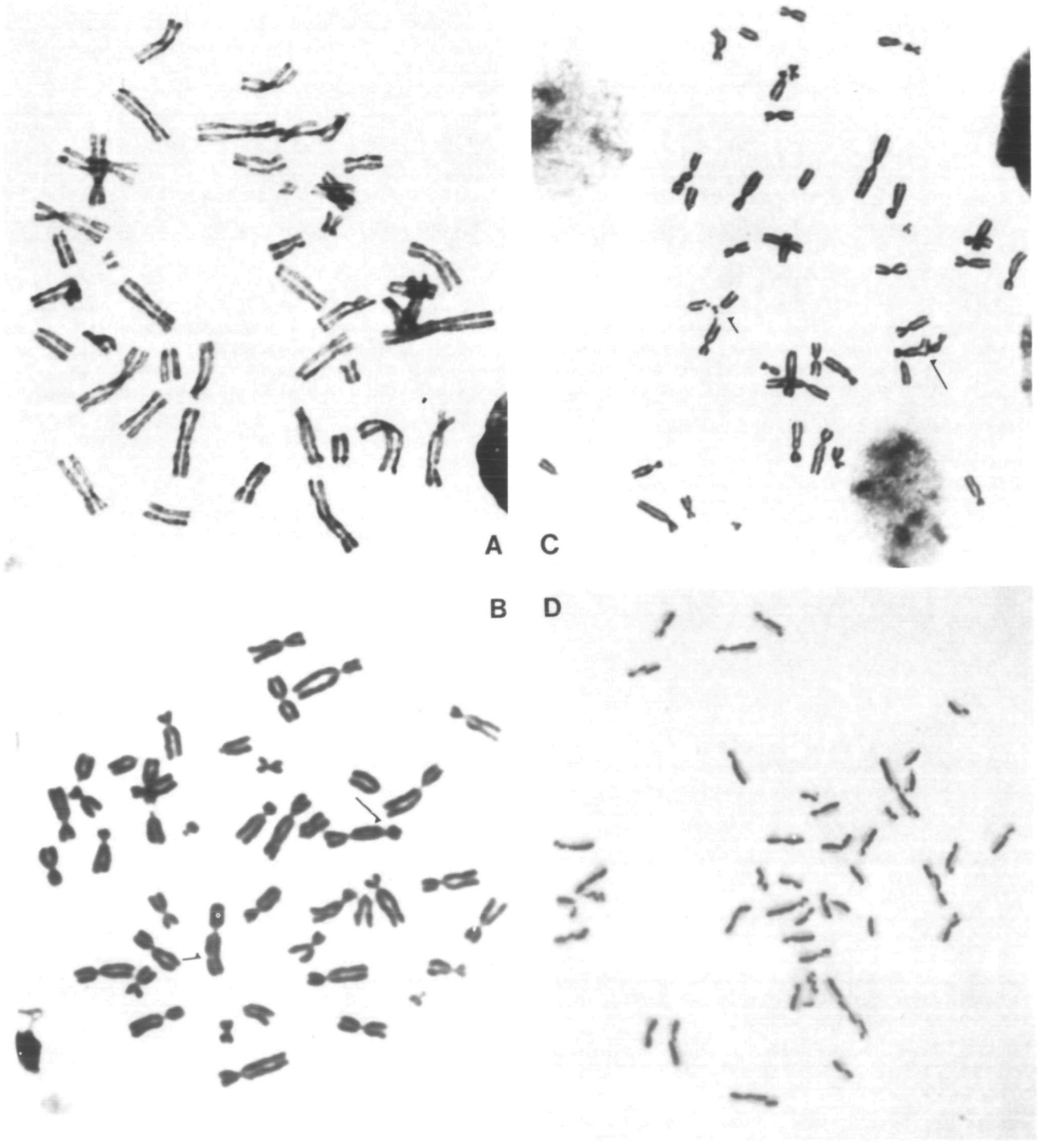

Fig. 3. Photomicrographs of metaphases with various types of arsenic-induced cytogenetic changes: (A) endoreduplication, (B) chromatid break (long arrow) and gap (short arrow), (C) chromatid exchanges of simple complete type (long arrow) and incomplete, complex type (short arrow), and (D) metaphase chromosomes with a high incidence of sister chromatid exchanges.

chemicals were linear with a slope of ~ 1 on a log-log plot. This is similar to the results with other chemical carcinogens (32 – 35). Having established the dose-response curves for transformation, we determined which genetic changes were also induced by the chemicals with the same dose-dependence.

Neither sodium arsenite nor arsenate induced any detectable gene mutations at the Na^+/K^+ ATPase or hypoxanthine phosphoribosyl transferase loci which is consistent with the results of Rossman *et al.* (11) with V-79 cells. Oberly *et al.* (10) reported that sodium arsenite was a weak mutagen in L5178Y mouse cells at the thymidine kinase locus. It is possible that this weak activity (2- to 3-fold increase over background at low survival) could not be detected in our assay, which employs early passage cells with a lower cloning efficiency, or that the thymidine kinase locus is more sensitive to arsenic-induced mutations than the HPRT or Na^+/K^+ ATPase loci. The clastogenic activity of arsenic in-

Table III. Effects of sodium arsenite and sodium arsenate on SCEs of Syrian hamster embryo cells

Treatment	Number of metaphases scored	Number of SCE per cell ± SE
Control	50	10.4 ± 0.5
B[a]P 10 μM	25	44.0 ± 5.5*
Sodium arsenite (μM)		
0.8	50	12.0 ± 0.5*
3.0	50	15.4 ± 0.7*
6.2	50	15.2 ± 0.6*
10	No metaphases in culture	–
Sodium arsenate (μM)		
10	50	13.0 ± 0.6*
32	50	15.7 ± 0.6*
64	7	25.3 ± 4.2*
96	No metaphases in culture	–

*Significantly different from control ($p < 0.05$) by t-test.

dicates a potential mechanism for deletion mutations which may account for the weak mutational activity at specific genetic loci. Why thymidine kinase mutants are induced, but not HPRT mutants, is unclear as both can be induced by deletion mutations (36,37). However, the results from all the mutational assays suggest that arsenic is at best a weak mutagen and thus, its transforming activity is unlikely to result from point mutations. Arsenic is co-mutagenic in bacteria (38), but the relevance of this finding to our results is unclear, as arsenic alone is a very effective transforming agent.

In contrast to the equivocal results with gene mutation assays, arsenic is clearly a very active chromosome mutagen. In Syrian hamster embryo cells, sodium arsenite and sodium arsenate induced polyploidy (possibly from endoreduplication), chromosome aberrations, and increased SCEs with similar dose-dependences to induction of cell transformation. These cytogenetic results are similar to findings of others, who have reported arsenic-induced endoreduplication (39,40), chromosome aberrations (12 – 16) and SCEs (12,16).

The mechanism by which arsenic induces chromosome aberrations is unknown. Evidence for direct DNA damage by arsenicals, as measured by gene mutation or unscheduled DNA synthesis, is limited (2,41). In contrast, chromosome damage (aberrations and SCEs) is reproducibly induced by arsenic in a variety of cell types (*vide supra*). Arsenicals are known to affect a number of enzymes through interactions with sulfhydryl groups as well as other interactions (8). This raises the interesting possibility that the target for arsenic-induced transformation is not DNA but proteins, possibly those involved in DNA replication (42); the consequence of this putative interaction, however, may be DNA damage.

We have previously reported that other chemicals including diethylstilbestrol (43), colcemid (44) and asbestos fibers (34,45), induce cell transformation in the absence of detectable gene mutations. Therefore, these three chemicals plus arsenic provide useful probes for studying mechanisms of carcinogenesis (46). Presumably none of them act by causing point mutations; however, all of them cause chromosome mutations. Interesting differences exist in the specificity of observed chromosome changes. Diethylstilbestrol and colcemid induce primarily aneuploidy, via non-disjunction (43,44). Asbestos induces tetraploidy, anaphase abnormalities (resulting in aneuploidy), and to a lesser extent, chromosome aberrations (45). In comparison, arsenic apparently does not induce non-disjunction or chromosome loss since we did not observe any significant changes in the chromosome number in the near diploid range. It is possible that arsenic-induced transformation is caused by numerical chromosome changes resulting from endoreduplication. Asbestos and other mineral dusts induce tetraploidy, possibly by inhibition of cytokinesis (45). However, we have observed with a variety of mineral dusts that tetraploidy correlates with toxicity, while aneuploidy and chromosome aberrations induction correlate with transformation (45). Furthermore, early passage asbestos-transformed cells have primarily non-random chromosome changes, trisomy of chromosome 11 (46 and unpublished data). Arsenic may be acting by inducing segmental aneuploidy or chromosome rearrangements. Further karyotypic analysis of early passage cells transformed by these different chemicals will hopefully identify the critical changes involved. It will be interesting to compare cells transformed by chemicals with different patterns of induced chromosome alterations.

In summary, we have shown that arsenic induces chromosomes, but not gene mutations, over the dose-range that cell transformation is induced. However, the pattern of chromosome aberrations induced by arsenic differs from other chemicals which induce transformation but not gene mutations. Arsenic may be a useful carcinogen for understanding the cytogenetic basis for cell transformation and possibly for identifying non-DNA targets for carcinogens.

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References

1. IARC (1980), Carcinogenesis of arsenic and arsenic compounds, *IARC Monograph on Evaluation of Carcinogenic Risk to Humans*, **23**, 37-141.
2. IARC (1982), *Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. **4**, 50-51.
3. Léonard,A. and Lauwerys,R.R. (1980), Carcinogenicity, teratogenicity and mutagenicity of arsenic, *Mutat. Res.*, **75**, 49-62.
4. DiPaolo,J.A. and Casto,B.C. (1979), Quantitative studies of *in vitro* morphological transformation of Syrian hamster cells by inorganic metal salts, *Cancer Res.*, **39**, 1008-1013.
5. Barrett,J.C., Hesterberg,T.W. and Thomassen,D.G. (1984), Use of cell transformation systems for carcinogenicity testing and mechanistic studies of carcinogenesis, *Pharmacol. Rev.*, **36**, 535-705.
6. Barrett,J.C. and Ts'o,P.O.P. (1978), Evidence for the progressive nature of neoplastic transformation *in vitro*, Proc. Natl. Acad. Sci. USA, **75**, 3761-3765.
7. Fowler,B.A. (1977), Toxicity of environmental arsenic, in Goyer,R.A. and Mehlman,M.A. (eds.), *Advances in Modern Toxicology*, Vol. **2**, *Toxicology of Trace Elements*, Hemisphere Publishing, Washington, DC, pp. 79-122.
8. Squibb,K.S. and Fowler,B.A. (1983), The toxicity of arsenic and its compounds, in Fowler,B.A. (ed.), *Biological and Environmental Effects of Arsenic*, Elsevier, Amsterdam, pp. 233-269.
9. Amacher,D.E. and Paillet,S.C. (1980), Induction of trifluorothymidine-resistant mutants by metal ions in L5178Y/TK$^+$-cells, *Mutat. Res.*, **78**, 279-288.
10. Oberly,T.J., Piper,C.E. and McDonald,D.S. (1982), Mutagenicity of metal salts in the L5178Y mouse lymphoma assay, *J. Toxicol. Environ. Health*, **9**, 367-376.
11. Rossman,T.G., Stone,D., Molina,M. and Troll,W. (1980), Absence of arsenite mutagenicity in *E. coli* and Chinese hamster cells, *Environ. Mutagenesis*, **2**, 371-379.
12. Larramendy,M.L., Popescu,N.C. and DiPaolo,J.A. (1981), Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberrations in human Syrian hamster cell strains, *Environ. Mutagenesis*, **3**, 597-606.
13. Nakamuro,K. and Sayato,Y. (1981), Comparative studies of chromosomal aberrations induced by trivalent and pentavalent arsenic, *Mutat. Res.*, **88**, 73-80.

14. Paton,G.R. and Allison,A.C. (1972), Chromosome damage in human cell cultures induced by metal salts, *Mutat. Res.*, **16**, 332-336.
15. Sweins,A. (1983), Protective effect of selenium against arsenic-induced chromosomal damage in cultured human lymphocytes, *Hereditas*, **98**, 249-252.
16. Wan,B., Christian,R.T. and Soukup,S.W. (1982), Studies of cytogenetic effects of sodium arsenicals on mammalian cells *in vitro*, *Environ. Mutagenesis*, **4**, 493-498.
17. Oppenheim,J.J. and Fishbein,W.N. (1965), Induction of chromosome breaks in cultured normal human leukocytes by potassium arsenite, hydroxyurea and relative compounds, *Cancer Res.*, **25**, 980-985.
18. Zanzoni,F. and Jung,E.G. (1980), Arsenic elevates the sister chromatid exchange (SCE) rate in human lymphocytes *in vitro*, *Arch. Dermatol. Res.*, **267**, 91-95.
19. Beckman,G., Beckman,L. and Nordensen,I. (1977), Chromosome aberrations in workers exposed to arsenic, *Environ. Health Perspect.*, **19**, 145-146.
20. Burgdorf,W., Kurvink,K. and Cervenka,J. (1977), Elevated sister chromatid exchange rate in lymphocytes of subjects treated with arsenic, *Hum. Genet.*, **36**, 69-72.
21. Nordenson,I., Beckman,G., Beckman,L. and Nordström,S. (1978), Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic, *Hereditas*, **88**, 47-50.
22. Nordenson,I., Salmonsson,S., Brun,E. and Beckman,G. (1979), Chromosome aberations in psoriatic patients treated with arsenic, *Hum. Genet.*, **48**, 1-6.
23. Srám,R.J. (1976), Relationship between acute and chronic exposures in mutagenicity studies in mice, *Mutat. Res.*, **41**, 25-42.
24. Casto,B.C., Meyer,J. and DiPaolo,J.A. (1979), Enhancement of viral transformation for evaluation of the carcinogenic and mutagenic potential of inorganic metal salts, *Cancer Res.*, **39**, 193-198.
25. Berwald,Y. and Sachs,L. (1965), *In vitro* transformation of normal cells to tumor cells by carcinogenic hydrocarbons, *J. Natl. Cancer Inst.*, **35**, 641-661.
26. DiPaolo,J.A., Donovan,P. and Nelson,R. (1969), Quantitative studies of *in vitro* transformation by chemical carcinogens, *J. Natl. Cancer Inst.*, **42**, 867-874.
27. Pienta,R.J., Poiley,J.A. and Lebherz,W.B. (1977), Morphological transformation of early passage golden Syrian hamster embryo cells derived from cryopreserved primary cultures as a reliable *in vitro* bioassay for identifying diverse carcinogens, *Int. J. Cancer*, **19**, 642-655.
28. Barrett,J.C., Bias,N.E. and Ts'o,P.O.P. (1978), A mammalian cellular system for the concomitant study of neoplastic transformation and somatic mutation, *Mutat. Res.*, **50**, 121-136.
29. Perry,P. and Evans,H.J. (1975), Cytological detection of mutagen-carcinogen exposure by sister chromatid exchanges, *Nature*, **258**, 121-125.
30. Lerman,S.A., Clarkson,T.W. and Gerson,R.J. (1983), Arsenic uptake and metabolism by liver cells is dependent on arsenic oxidation state, *Chem.-Biol. Interactions*, **45**, 401-406.
31. Vahter,M. and Marafante,E. (1983), Intracellular interaction and metabolic fate of arsenite and arsenate in mice and rabbits, *Chem.-Biol. Interactions*, **47**, 29-44.
32. DiPaolo,J.A., Donovan,P.J. and Nelson,R.L. (1971), Transformation of hamster cells *in vitro* by polycyclic hydrocarbons without cytotoxicity, *Proc. Natl. Acad. Sci. USA*, **68**, 2958-2961.
33. Gart,J.J., DiPaolo,J.A. and Donovan,P.J. (1979), Mathematical models and the statistical analyses of cell transformation experiments, *Cancer Res.*, **39**, 6069-6075.
34. Hesterberg,T.W. and Barrett,J.C. (1984), Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension, *Cancer Res.*, **44**, 2170-2180.
35. Huberman,E. and Sachs,L. (1966), Cell susceptibility to transformation and cytotoxicity by the carcinogenic hydrocarbon benzo[a]pyrene, *Proc. Natl. Acad. Sci. USA*, **56**, 1123-1129.
36. Cox,R. (1982), Mechanisms of mutagenesis in cultured mammalian cells, in Sugimura,T., Kondo,S. and Takebe,H. (eds.), *Environmental Mutagens and Carcinogens*, Alan R.Liss Inc., New York, pp. 157-166.
37. Hozier,J., Sawyer,J., Moore,M., Howard,B. and Clive,D. (1981), Cytogenetic analysis of L5178Y/TK^{+}/$^{-}$ → TK^{-}/$^{-}$ mouse lymphoma mutagenesis assay system, *Mutat. Res.*, **84**, 169-181.
38. Rossman,T.G. (1981), Enhancement of UV-mutagenesis by low concentrations of arsenite in *E. coli*, *Mutat. Res.*, **91**, 207-211.
39. Deaven,L.L. and Nock,A.E. (1979), Induction of endoreduplication in mammalian cells by sodium arsenite, *J. Cell Biol.*, **83**, 159a.
40. Petres,J., Baron,D. and Hagedon,M. (1977), Effects of arsenic cell metabolism and cell proliferation: cytogenetic and biochemical studies, *Environ. Health Perspect.*, **19**, 223-227.
41. Waters,M.D., Garrett,N.E., Covone-de Serres,C.M., Howard,B.E. and Stack,H.F. (1984), Genetic toxicology of some known or suspected human carcinogens, in de Serres,F.J. (ed.), *Chemical Mutagens*, Vol. **8**, Plenum Press, NY, pp. 261-341.
42. Tkeshelashvilli,L.K., Shearman,C.W., Zakour,R.A., Koplitz,R.M. and Loeb,L.A. (1980), Effects of arsenic, selenium and chromium on the fidelity of DNA synthesis, *Cancer Res.*, **40**, 2455-2466.
43. Tsutsui,T., Maizumi,H., McLachlan,J.A. and Barrett,J.C. (1983), Aneuploidy induction and cell transformation by diethylstilbestrol: a possible chromosome mechanism in carcinogenesis, *Cancer Res.*, **43**, 3814-3821.
44. Tsutsui,T., Maizumi,H. and Barrett,J.C. (1984), Colcemid-induced neoplastic transformation and aneuploidy in Syrian hamster embro cells, *Carcinogenesis*, **5**, 89-93.
45. Oshimura,M., Hesterberg,T.W., Tsutsui,T. and Barrett,J.C. (1984), Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture, *Cancer Res.*, **44**, 5017-5022.
46. Barrett,J.C., Hesterberg,T.W., Oshimura,M. and Tsutsui,T. (1985), Role of chemically induced mutagenic events in neoplastic transformation of Syrian hamster embryo cells, in Barrett,J.C. and Tennant,R.W. (eds.), *Carcinogenesis: A Comprehensive Survey – Mammalian Cell Transformation Assays*, Vol. **9**, Raven Press, NY, in press.

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