*Bull. Inst. Zool., Academia Sinica* 27(2): 105-110

# INDUCTION OF SISTER CHROMATID EXCHANGES BY ARSENIC IN PRIMARY RAT TRACHEAL EPITHELIAL CELLS[1]

TE-CHANG LEE[2], K. Y. JAN and TSING-CHENG WANG

*Institute of Zoology, Academia Sinica*
*Taipei, Taiwan 11529,*
*Republic of China*

(Accepted December 17, 1987)

**Te-Chang Lee, K. Y. Jan and Tsing-Cheng Wang** (1988) Induction of sister chromatid exchanges by arsenic in primary rat tracheal epithelial cells. *Bull. Inst. Zool., Academia Sinica* 27(2): 105-110. Arsenic is known to induce sister chromatid exchanges (SCEs) in various mammalian fibroblasts and lymphocytes, but there is no report in an epithelial cell system. Since airway epithelium is an important toxicological target during the exposure to environmental arsenic, we studied the effect of arsenic on primary rat tracheal epithelial (RTE) cells. We report that both sodium arsenite (trivalent) and sodium arsenate (pentavalent) significantly elevate the frequency of SCEs in primary RTE cells *in vitro*. The trivalent sodium arsenite is >10-fold more potent than the pentavalent sodium arsenate. The apparent doubling time for RTE cells isolated from a Sprague Dawley strain is 16 hours. Poisson model is found applicable to describe the behaviour of SCE rates in RTE cells.

**Key words:** Arsenic, Sister chromatid exchange, Tracheal epithelial cells.

Arsenic is a widely distributed environmental carcinogen (Leonard and Lauwerys, 1980; Pershagen, 1981). Syrian hamsters which are given intratracheal instillations of arsenic develop adenomas and carcinoms of the lung (Ishinish, *et al.*, 1983; Pershagen, *et al.*, 1984; Pershagen and Bjorklund, 1985). These results indicate that airway epithelium is an important toxicological target during the exposure to arsenic. Recently, primary rat tracheal epithelial (RTE) cells have been used to investigate the chemical carcinogenesis *in vitro* (Thomassen, *et al.*, 1983: Pai, *et al.*, 1983: Mass, *et al.*, 1984a, b: Thomassen, *et al.*, 1985), because these cells have several unique advantages for study of the genotoxic effects of environmental mutagens and/or carcinogens (Nettesheim and Barrett, 1984). Sister chromatid exchange (SCE) is a popular and sensitive indicator for detecting DNA-damaging agents (Latt, 1981: Takehisa, 1982). Arsenicals have been reported to induce SCEs in mammalian fibroblasts and lymphocytes (Zanzoni and Jung, 1980; Larramendy, *et al.*, 1981; Nakamuro and Sayato, 1981; Wen, *et al.*, 1981; Wan, *et al.*, 1982), but there is still no report in epithelial cells, which are more relevant in association with carcinogenesis *in vivo*. Thus, in this communication, we report that both trivalent and pentavalent arsenicals can induce SCEs in primary epithelial cells.

1. Paper No. 306 of the Journal Series of the Institute of Zoology, Academia Sinica.
2. To whom reprint request should be sent. The present address is Institute of Biomedical Sciences, Academia Sinica, Taipei, Tiwan 11529, R. O. C.

## MATERIALS AND METHODS

### Cell culture

Culture method for primary RTE cells has been published previously (Wang *et al.*, 1987). Briefly, tracheas were surgically isolated from 8-week-old male Sprague Dawley rats (purchased from Animal Center, College of Medicine, National Taiwan University), and the epithelial cells were dissociated from the trachea by treating with protease (5 mg/ml, Type XIV, Sigma) overnight at 4°C. Single RTE cell suspension was seeded onto a monolayer of 3T3 cells ($7\times10^3$ cells per $cm^2$) pretreated with mitomycin C (2 $\mu$g/ml for 2 h), and incubated at 37°C in a humidified air with 5% $CO_2$. The culture medium for RTE cells was Ham's fl2 (GIBCO) supplemented with 5% fetal calf serum (GIBCO), 10 $\mu$g/ml insulin (Sigma), $5\times10^{-6}$ M hydrocortisone (Sigma), 25 ng/ml epidermal growth factor (Collaborative Research Inc.), 100 units/ml penicillin (GIBCO) and 100 $\mu$g/ml streptomycin (GIBCO).

### Cytotoxicity assay

In each treatment, 2,000 primary RTE cells were plated in a 35-mm petri dish in triplicate and incubated overnight. The cultures were then treated with various concentrations of sodium arseenite (Merck) or sodium arsenate (Merck) in complete medium for 72 h. The media were replaced with fresh media without drugs and the incubation was continued for another 4 days. The colonies were fixed with absolute methanol and stained with 10% Giemsa solution. The relative survival was calculated as follow: (number of colony in treated culture/number of colony in control culture) X 100.

### SCE analysis

The primary RTE cells ($5\times10^5$) were seeded in a 60-mm petri dish and incubated overnight. The cultures were treated with various concentrations of sodium arsenite or sodium arsenate for 72 h. The media were then replaced with fresh media containing 20 $\mu$M bromodeoxyuridine (BrdU, Sigma), and the cultures were further incubated in the dark for another 32 h. Colcemid (final 0.2 $\mu$g/ml, Sigma) was added 3 h before metaphase cells were harvested. Metaphase cells were harvested by trypsinization and and air-dried techniques. Sister chromatid differential stain of chromosomes was performed by a modified fluorescence plus Giemsa method (Jan, *et al.*, 1982). At least 30 second-division cells were randomly sampled from each treatment in coded slides for scoring of SCE frequency.

## RESULTS AND DISCUSSION

The cytotoxic effects of sodium arsenite (trivalent) and sodium arsenate (pentavalent) in primary RTE cells are shown in Fig. 1. The doses of arsenite and arsenate that kill 50% of cells are 3 and 45 $\mu$M, respectively. These values are consistent with those reported in fibroblasts and lymphocytes. Furthermore, these results, that trivalent arsenite is >10-fold more potent than pentavalent arsenate, is consistant with the findings in Syrian hamster fibroblasts (Lee, *et al.*, 1985) and in human lymphocytes (Wan, *et al.*, 1982). The reason why trivalent arsenic is more toxic than the pentavalent form is not clear. It is probably due to differences in cellular uptake of the two forms of arsenic (Leoman, *et al.*, 1983; Vahter and Marafante, 1983).

The doubling time of RTE cells in culture was assessed by the BrdU differential chromatid labelling technique described by Schneider, *et al.* (1981). Briefly, the metaphase cells were harvested for a series of time points after the addition of BrdU. After the differential staining of sister chromatids, the cell doubling time was then calculated from the percentages of first-, second- and third-division metaphases. The apparent doubling time of RTE cells under our culture condition was determined to be 16 h (Fig. 2).

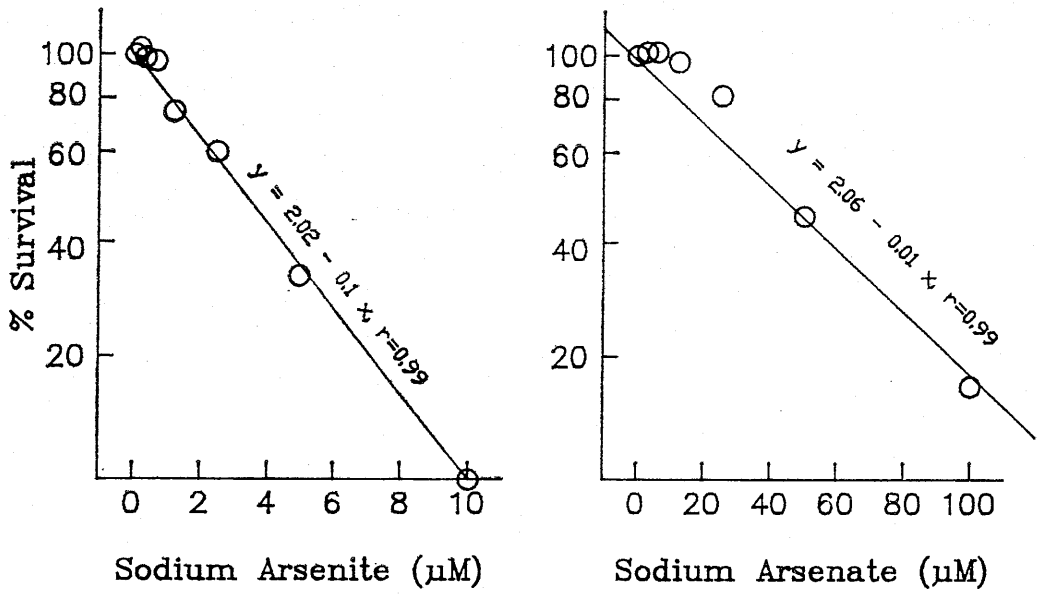


Fig. 1. Cytotoxic effect of sodium arsenite and sodium arsenate on rat tracheal epithelial cells. Primary rat tracheal epithelial cells were exposed to various concentrations of arsenicals for 72 h and then incubated in normal medium for another 4 days. The results were the averages of two experiments. The colony-forming efficiency of untreated culture was 7.5%.

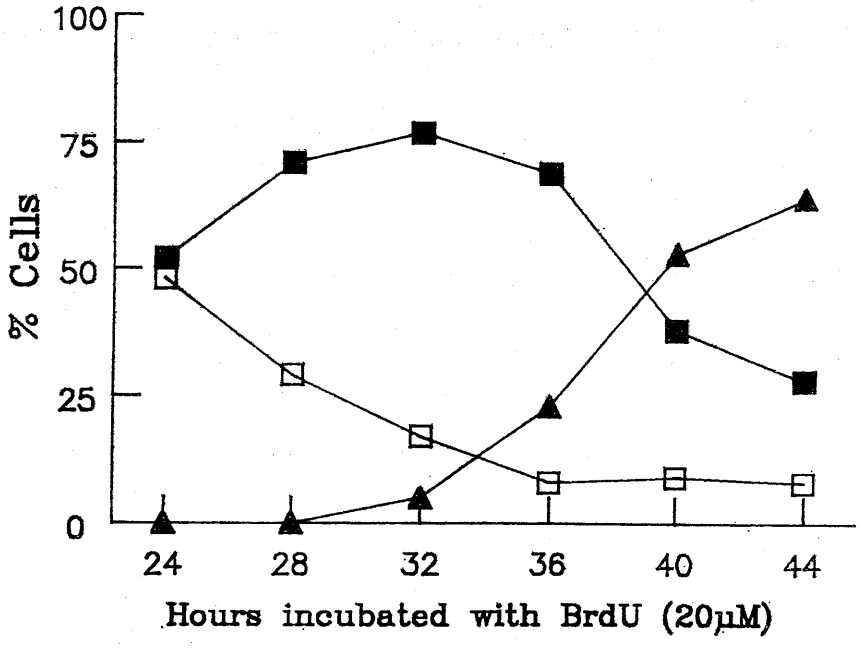


Fig. 2. Percentages of 1st, 2nd and 3rd division cells in experiments with different time intervals for BrdU incubation. □, 1st division metaphase; ■, 2nd division metaphase; ▲, 3rd division metaphase.

Thus the metaphase cells for SCE analysis were harvested after incubating in the presence of BrdU for 32 h, since SCE examination should be done in second-division metaphases.

Margolin *et al.* (1986) have demonstrated that the induction of SCE in Chinese hamster ovary (CHO) cells *in vitro* is a Poisson distribution. Based on that, Galloway *et al.* (1985) have analyzed the significance of SCE induction in CHO cells using trend probability and the numbers of dose treatment with significant SCEs. A dose is significant for the induction if the SCEs raised are more than 20% over the concurrent control in their study. Probability values in SCE frequency profile based on 10 independent control data (Table 1) are significantly higher than 0.01. This indicates that the Poisson model is also applicable to describe the behaviour of SCEs rate in RTE cells. Statistical method proposed by Galloway *et al.* (1985) is therefore adapted in our report. Since dimethyl sulfoxide (DMSO) is frequently used as a solvent for various chemicals, SCE rate of DMSO is also considered as the control data.

Arsenite at dose levels higher than 1 $\mu$M and arsenate at 12.5 $\mu$M induces SCE significantly in RTE cells (Fig. 3). Trend probabilities in treatments with both arsenicals are smaller than 0.005 indicating the inductions of SCE are dose dependent (Galloway *et al.*, 1985; Margolin *et al.*, 1985). Sodium arsenite and sodium arsenate therefore unequvocally induce positive SCEs in primary RTE cells. Only the doses needed are more than 10 times higher for sodium arsenate. Furthermore, the doses used in this report did not cause remarkable cell cycle delay (data not shown). Most metaphase cells harvested (more than 87%) have completed two cell cycles. Therefore, the elevation of SCE frequency was not due to the effects of arsenicals on cytokinetics.

SCE induction may not directly correlate to carcinogenesis *in vivo*. However, we have previously demonstrated that arsenic induces morphological transformation, chromosome aberrations and SCEs over the same dose range in Syrian hamster embryo cells (Lee, *et al.*, 1985). Therefore, the results that arsenicals can induce SCEs seem more relevant in RTE cells than in fibroblasts and lymphocytes, in the carcinogenic effect of arsenic in respiratory epithelium. Thus, further investigation on whether arsenic could induce neoplastic transformation in RTE cells is necessary.

TABLE 1
SCE frequency profile of control data in RTE cells[a]

| Experiment | Solvent | Frequency of cells with SCE counted as indicated | | | | | | | | | | | | | Mean | Variance | H= | T= | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | (M) | (V) | V/M | (r−1) H | Value |
| M-1 | Medium | | 2 | 3 | 6 | 5 | 5 | 1 | 3 | 3 | 2 | | | | 6.67 | 5.40 | 0.81 | 23.49 | 0.75 |
| M-2 | Medium | | | | 5 | 10 | 6 | 1 | 7 | 1 | | | | | 6.93 | 2.34 | 0.34 | 9.79 | >0.99 |
| M-3 | Medium | | 2 | 3 | 5 | 5 | 5 | 2 | 2 | 2 | 3 | | 1 | | 6.93 | 6.75 | 0.97 | 28.13 | 0.51 |
| M-4 | Medium | | | 1 | 5 | 4 | 6 | 5 | 3 | 4 | 1 | 1 | | | 7.50 | 4.12 | 0.55 | 15.95 | 0.98 |
| M-5 | Medium | | | | 4 | 3 | 9 | 8 | 1 | 2 | 1 | 2 | | | 7.63 | 3.55 | 0.47 | 13.49 | 0.99 |
| D-1 | 1% DMSO | | | 1 | 3 | 2 | 3 | 8 | 7 | 3 | 1 | 1 | 1 | | 8.14 | 4.21 | 0.52 | 14.94 | 0.99 |
| D-2 | 1% DMSO | | | 2 | 13 | 10 | 3 | 1 | 1 | | | | | | 6.70 | 1.18 | 0.18 | 5.22 | >0.99 |
| D-3 | 1% DMSO | | | 5 | 9 | 10 | 2 | 3 | 1 | | | | | | 6.77 | 1.98 | 0.29 | 8.41 | >0.99 |
| D-4 | 0.5% DMSO | 2 | 5 | 7 | 7 | 5 | 4 | | | | | | | | 4.67 | 2.16 | 0.46 | 13.42 | 0.99 |
| D-5 | 0.5% DMSO | 1 | 3 | 5 | 6 | 4 | 7 | 5 | 2 | 2 | 1 | 1 | 2 | 1 | 6.88 | 8.68 | 0.26 | 7.67 | >0.99 |

a. P values in the last column are associated with a test based on 'H' of the fit of the Poisson model on each trial's SCE counts according to Margolin *et al.* (1986).

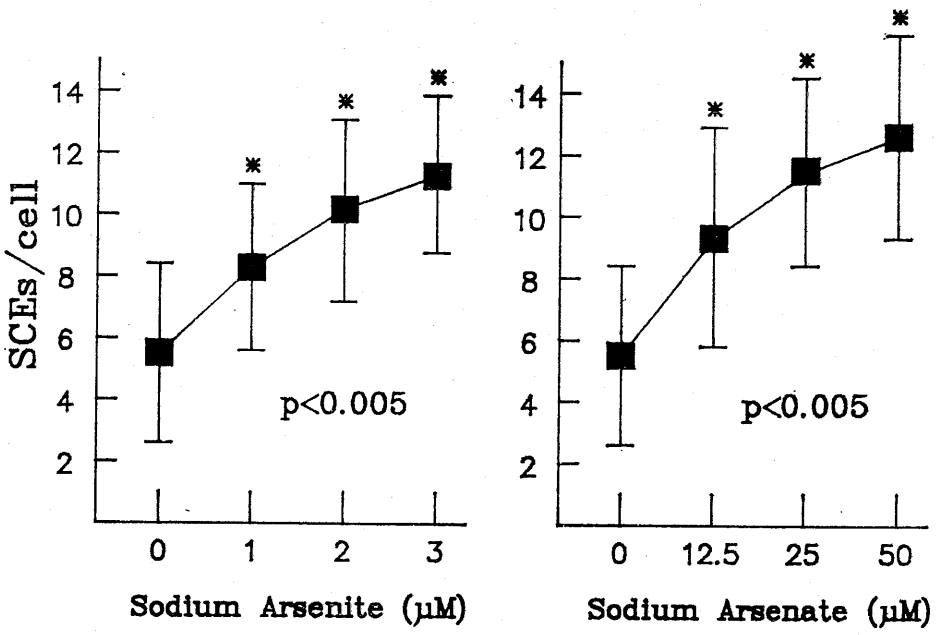


Fig. 3. Sister chromatid exchanges induced by sodium arsenite (trivalent) and sodium arsenate (pentavalent) in primary tracheal epithelial cells.

**Acknowledgements:** This study was supported by a grant from National Science Council, Republic of China.

## REFERENCES

GALLOWAY, S. M.. A. D. BLOOM, M. RESNICK, B. H. MARGOLIN, F. NAKAMURA, P. ARCHER and E. ZEIGER (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for the 22 compounds in laboratories. *Environ. Mutagen.* **7**: 1-51.

ISHINISHI, N., A. YAMAMOTO, A. HISANAGA and T. INAMASU (1983) Tumorigenicity of arsenic trioxide to the lung in Syrian golden hamsters by intermittent instillations. *Cancer Lett.* **21**: 141-147.

JAN, K. Y., S. W. WUU and W. N. WEN (1982) A simplified fluorescence plus Giemsa method for consistent differential staining of sister chromatids. *Stain Technol.* **57**: 45-46.

LARRAMENDY, M. L., N. C. POPESCU and J. A. DIPAOLO (1981) Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberrations in human and Syrian hamster cell strains. *Environ. Mutagen.* **3**: 597-606.

LATT, S. A. (1981) Sister chromatid exchange formation. *Ann. Rev. Genet.* **15**: 11-55.

LEE, T. C., M. OSHIMURA and J. C. BARRETT (1985) Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture. *Carcinogenesis* **6**: 1421-1426.

LEONARD, A. and R. R. LAUWERYS (1980) Carcinogenicity, teratogenicity and mutagenicity of arsenic. *Mutat. Res.* **75**: 49-62.

LERMAN, A. S., T. W. CLARKSON and R. T. GERSON (1983) Arsenic uptake and metabolsm by liver cells is dependent on arsenic oxidation state. *Chem.-Biol. Interactions* **45**: 401-416.

MARGOLIN, B. H., M. A. RESNICK, J. Y. LIMPO, P. ARCHER, S. M. GALLOWAY, A. D. BLOOM and E. ZEIGER (1986) Statistical analysis for in vitro cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen.* **8**: 183-204.

MASS, M. J., P. NETTESHEIM, T. E. GRAY and J. C. BARRETT (1984a) The effect of 12-O-tetradecanoylphorbol-13-acetate and other tumor promoters on the colony formation of rat tracheal epithelial cells in culture. *Carcinogenesis* **5**: 1597-1601.

MASS, M. J., P. NETTESHEIM, D. K. BEEMAN and J. C. BARRETT (1984b) Inhibition of transformation of primary rat tracheal epithelial cells by retinoic acid. *Cancer Res.* **44**: 5688-5691.

NAKAMURA, K. and Y. SAYATO (1981) Comparative studies of chromosomal aberrations induced by trivalent and pentavalent arsenic. *Mutat. Res.* **88**: 73-80.

NETTESHEIM, P. and J. C. BARRETT (1984) Tracheal epithelial cell transformation: a model system for studies on neoplastic progress. *Crit. Rev. Toxicol.* **12**: 215-239.

PAI, S. B., V. E. STEELE and P. NETTESHEIM (1983) Neoplastic transformation of primary tracheal epithelial cell cultures. *Carcinogenesis* **4**: 369-374.

PERSHAGEN, G. (1981) The carcinogenicity of arsenic. *Environ. Health Perspect* **40**: 93-100.

PERSHAGEN, G. and N.-E. BJORKLUND (1985) On the pulmonary tumorigenicity of arsenic trisulfide and calcium arsenate in hamsters. *Cancer Lett.* **27**: 99-104.

PERSHAGEN, G., G. NORDBERG and N.-E. BJORKLUND (1984) Experimental evidence of the pulmonary carcinogenicity of arsenic trioxide. *Arch. Toxicol., Supp.* **7**: 403-404.

SCHNEIDER, E. L., Y. NAKANISHI, J. LEWIS and H. STERNBERG (1981) Stimultaneous examination of sister chromatid exchanges and cell replication kinetics in tumor and normal cells in vivo. *Cancer Res.* **41**: 4973-4975.

TAKEHISA, S. (1982) Induction of sister chromatid exchanges by chemical agents. In *Sister Chromatid Exchange* (S. Wolff, ed.). John Wiley and Sons, New York. 87-147.

THOMASSEN, D. G., T. E. GRAY, M. J. MASS and J. C. BARRETT (1983) High frequency of carcinogen-induced early, preneoplastic changes in rat tracheal epithelial cells in culture. *Cancer Res.* **43**: 5956-5963.

THOMASSEN, D. G., P. NETTESHEIM, T. G. GRAY and J. C. BARRETT (1985) Quantitation of the rate of spontaneous generation and carcinogen-induced frequency of anchorage-independent variants of rat tracheal epithelial cells in culture. *Cancer Res.* **45**: 1516-1524.

VAHTER, M. and E. MARAFANTE (1983) Intracellular interation and metabolic fate of arsenite and arsenate in mice and rabbits. *Chem.-Biol. Interactions* **47**: 29-44.

WAN, B., R. T. CHRISTIAN and S. W. SOUKUP (1982) Studies of cytogenetic effects of sodium arsenicals on mammalian cells in vitro. *Environ. Mutagen.* **4**: 493-498.

WANG, T. C., T. C. LEE, M. F. LIN and S. Y. LIN (1987) Induction of sister chromatid exchanges by pesticides in primary rat tracheal epithelial cells and Chinese hamster ovary cells. *Mutat. Res.* **188**: 311-321.

WEN, W. N., L. T. LIEU, H. J. CHANG, S. W. WUU, M. L. YAU and K. Y. JAN (1981) Baseline and sodium arsenite-induced sister chromatid exchanges in cultured lymphocytes from patients with blackfoot disease and healthy persons. *Hum. Genet.* **59**: 201-203.

ZANZONI, F. and E. G. JUNG (1980) Arsenic elevates the sister chromatid exchanges (SCE) rate in human lymphocytes in vitro. *Arch. Dermatol. Res.* **267**: 91-95.

# 砷化物對大白鼠氣管表皮細胞姐妹染色分體交換之誘引

李德章　詹崑源　王清澄

在哺乳動物成纖細胞和白血球細胞中，砷化物會造成姐妹染色分體交換率之顯著增加，在表皮細胞中則未曾有類似的研究報告，有鑑於呼吸道的表皮細胞是環境中砷化物重要的致毒目標，我們乃著手探討砷化物對大白鼠氣管表皮細胞之遺傳毒性。本研究報告之結果顯示三價的亞砷酸鈉與五價之砷酸鈉均可造成該細胞活體外姐妹染色分體交換率之顯著增加。該細胞的細胞周期約略爲 16 小時。卜瓦松分佈之模式可以用爲分析本研究中姐妹染色分體交換率之顯著性。