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Initiation of *in Vitro* Cell Transformation by Formaldehyde and Acetaldehyde as Measured by Attachment-Independent Survival of Cells in Aggregates*

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Abstract—*The ability of formaldehyde and acetaldehyde to initiate transformation of a rat kidney cell line has been studied using a newly developed two-stage* in vitro *cell transformation assay. The assay is based on measurements of attachment-independent survival of cells in aggregates. Short treatment with non-cytotoxic doses of formaldehyde and acetaldehyde did not affect survival of the cells in the aggregate assay system. However, when the aldehyde treatment was followed by exposure of the cells to the tumor promoters TPA and PDD, a considerable increase in the number of viable cells was observed. On a molar basis, formaldehyde was about* 100 *times more potent than acetaldehyde in initiation of cell transformation. The data showed that cells derived from aggregates of cultures treated with formaldehyde or acetaldehyde followed by exposure to TPA possessed a considerably higher ability to form colonies in soft agar than untreated control cells.*

INTRODUCTION

ALDEHYDES have received increasing attention as potential carcinogens. Formaldehyde and acetaldehyde are two common chemicals in the human environment. The widespread use of these substances in industry has led to great interest concerning their possible health hazards.

Formaldehyde is genotoxic in a variety of test systems. It causes DNA single strand breaks [1–3], DNA-protein cross links [1–5], inhibition of DNA repair [6], mutations [7–13], sister chromatide exchanges [14, 15] and chromosome aberrations [16] as well as transformation of cells in culture [7, 8, 14, 17–21]. Formaldehyde gas has been shown to induce nasal tumors in rats [22, 23] and data has recently been reported suggesting that formaldehyde may also cause nasal cancer in man [24]. Furthermore, the increased frequency of brain tumors and leukemia in embalmers and pathologists has been related to formaldehyde exposure [25–28].

Acetaldehyde causes DNA damage *in vitro* [29] and is reported to cause mutations [30–33] and to induce sister chromatid exchanges [31, 34–38] and chromosomal aberrations [36, 37, 39, 40]. No reports have been presented on the ability of acetaldehyde to induce cell transformation *in vitro*. Recently, it has been shown that acetaldehyde vapors induce respiratory tract tumors in hamster [41] and rat [42]. Increased prevalence of malignant tumors has been reported among workers in a factory producing acetaldehyde [43] and the possibility is considered that the increased cancer frequency among alcoholics may in part be caused by acetaldehyde [44].

The purpose of the present communication has been to compare the ability of formaldehyde and acetaldehyde to induce cell transformation. In this study, a new *in vitro* cell transformation assay [45] for evaluation of initiators and promoters of carcinogenesis has been used. The assay uses a rat kidney cell line ($HRRT^4$) established in our laboratory [46] and is based on attachment-independent survival of cells in aggregates.

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The abbreviations used are: HRRT, hereditary renal rat tumor; TPA, 12-0-tetradecanyl-phorbol-13-acetate; PDD, phorbol-12, 13-didecanoate; 4α-PDD, 4α-phorbol-12,13-didecanoate; NCS, newborn calf serum; FCS, fetal calf serum.

MATERIALS AND METHODS

Chemicals

TPA, PDD and 4α-PDD were purchased from C.C.R. Inc. Eden Prairie, Minnesota, NCS, FCS and Waymouth's medium were obtained from Gibco Biocult, Paisley, U.K. Analytical grade 35% formaldehyde solution and acetaldehyde were purchased from Merck, Darmstadt, FRG. Stock solutions of the aldehydes in water were kept ice-cold until added to the culture medium.

Cells

The HRRT cell line has been established from a hereditary renal tumor of a 13-month-old female rat. Electron microscopic studies indicate that the cells are fibroblasts [46]. The cell line has been maintained in monolayer culture for about 5 yr and multiplies with a population doubling time of about 24 hr. The cells were grown in Waymouth's medium (MAD 82/3) supplemented with 10% NCS, penicillin (100 μg/ml) and streptomycin (50 μg/ml). The cells are free of mycoplasma contaminations as tested by the method of Chen [47].

Cell treatment and aggregation assay

3×10^6 cells in 5 ml of medium were transferred to 25 cm^2 culture flasks and incubated for 37°C. After 24 hr the aldehydes were added to the medium to the specified concentrations. After 3 hr of incubation the cells were harvested by trypsinization and suspended in medium (2.5×10^5 cells/ml) containing 0.1 μg/ml of the phorbol ester. Control cultures were treated in the same way, except that only the solvents were present in the medium during the 3-hr incubation period. The ability of the cells to survive in aggregates was assayed using a modification of the method of Steuer *et al.* [48] as described in a previous report [45]. Two millilitres of cell suspension (5×10^5 cells) were seeded in 35-mm Petri dishes having a solid bottom layer of agar (1% Difco Noble agar in 2 ml of growth medium containing 10% NCS). Dishes were incubated undisturbed at 37°C in a humidified atmosphere of 5% CO_2 in air. Viable cell counts (trypan blue exclusion) were performed in triplicate on trypsinized aggregates after 3 or 6 days incubation. The relative S.D. of each experimental point has on the basis of all experiments been calculated to 9.2%.

Colony formation in soft agar

1×10^5 cells were suspended in 1.5 ml of medium containing 10% calf serum and 0.3% Difco Noble agar and laid over 7 ml of a 0.5% agar-medium basal layer in 50 mm Petri dishes. After 14 days of incubation at 37°C in an atmosphere of 5% CO_2 in air, colonies (> 0.2 mm) were scored in an inverted phase microscope.

RESULTS

The cytotoxicity of formaldehyde and acetaldehyde were established in preliminary experiments. It was found that the growth of HRRT cells was not affected by 3 hr treatment with concentrations of formaldehyde and acetaldehyde up to 10^{-4} M and 3×10^{-3} M, respectively. In the transformation assay, the cells were treated with the aldehydes for 3 hr in monolayer culture. After removal of the aldehydes, the cells were incubated in the aggregation assay system in the presence of the promoter substances for up to 6 days. From Fig. 1A it is seen that treatment with formaldehyde alone or 6 days exposure to 0.1 μg/ml of TPA did not affect the ability of the cells to survive in the aggregate form. On the other hand, if the formaldehyde treatment was followed by subsequent exposure of the cells to TPA, a marked increase in the survival was observed. Thus, after 6 days of incubation in the presence of the promoter, the number of viable cells derived from trypsinized aggregates was nearly two times higher in the formaldehyde – promoter treated cultures than in the controls. It appears from Fig. 1B that treatment with formaldehyde at concentrations as low as 10^{-6} M induced a significant increase in cell viability. No further increase in survival occurred when the formaldehyde concentration was raised above 10^{-5} M.

In separate experiments (data not presented) it was found that up to 6 days exposure of the cells to TPA (0.1 μg/ml) prior to treatment with the aldehydes in the aggregation assay system did not induce any enhancement of cell viability. Thus, only addition of the promoter after treatment with the aldehydes induces cell transformation as judged by increased cell survival in the aggregate form.

In Fig. 2A is shown the effect of acetaldehyde and TPA treatment on viability of HRRT cells in the aggregate assay system. Exposure to the highest non-cytotoxic concentration of the aldehyde, 3×10^{-3} M, had no effect on cell survival. Similar to that found with formaldehyde, acetaldehyde treatment followed by exposure to the tumor promoter TPA induced a considerable increase in the number of viable cells. The results in Fig. 2B show the effect of various concentrations of acetaldehyde on cell survival in the aggregation assay system. A significant increase was obtained with 10^{-5} M acetaldehyde.

The effects of different phorbol esters on cells initiated by formaldehyde or acetaldehyde have been compared. After treatment of the cultures with formaldehyde or acetaldehyde for 3 hr, the carcinogen was removed and the cells exposed to the phorbol esters for 6 days. The number of viable cells was then counted and compared to that found with cells not exposed to aldehydes or phorbol

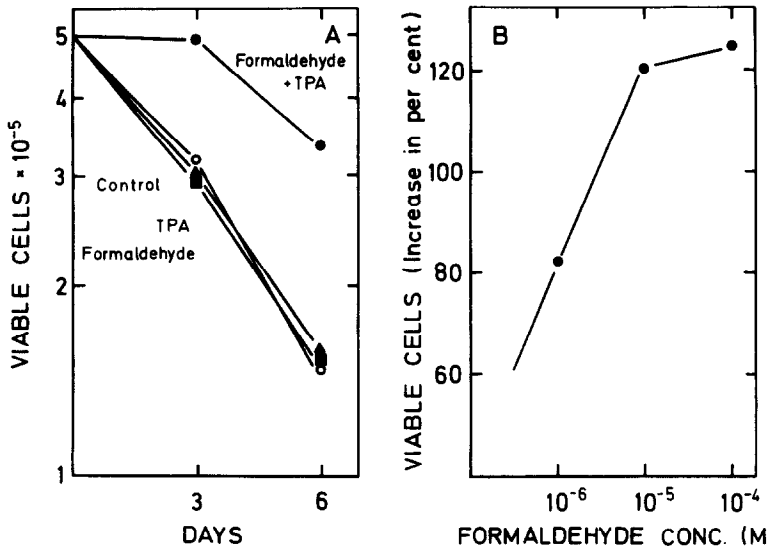

Fig. 1. Effect of formaldehyde and TPA on viability of HRRT cells in the aggregation assay system. Monolayer cultures were exposed to various concentrations of formaldehyde for 3 hr, *followed by a continuous treatment with TPA as described in Materials and Methods. The number of viable cells was determined after* 3 *and* 6 *days. A. Viability after treatment of the cells with formaldehyde* (0.1 mM) *for* 3 hr *and TPA* (0.1 μg/ml) *for* 3 *and* 6 days *or combined formaldehyde and TPA treatment. B. Increase in cell survival compared to the control after combined treatment with indicated concentrations of formaldehyde for* 3 hr *and TPA* (0.1 μg/ml) *for* 6 *days.*

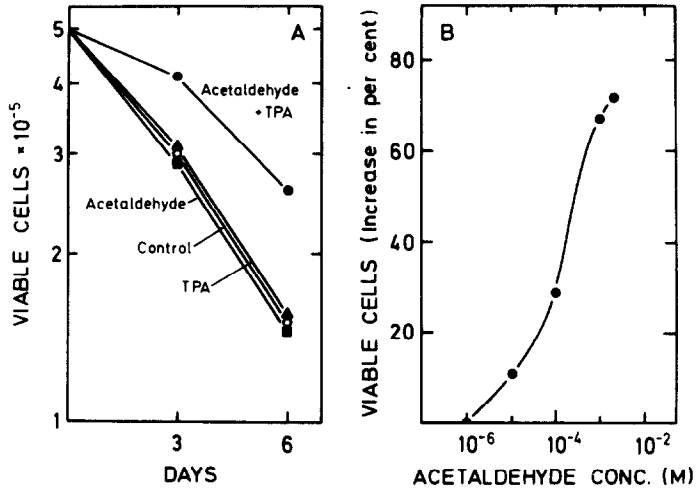

Fig. 2. Effect of acetaldehyde and TPA on viability of HRRT cells in the aggregation assay system. Monolayer cultures were exposed to various concentrations of acetaldehyde for 3 hr, *followed by a continuous treatment with TPA as described in Materials and Methods. The number of viable cells was determined after* 3 *and* 6 *days. A. Viability after treatment of the cells with acetaldehyde* (3.0 mM) *for* 3 hr *and TPA* (0.1 μg/ml)) for 3 *and* 6 *days or combined acetaldehyde and TPA treatment. B. Increase in cell survival compared to the control after combined treatment with indicated concentrations of acetaldehyde for* 3 hr *and TPA* (0.1 μg/ml) *for* 6 *days.*

esters (Table 1). It is seen that the promoter PDD was nearly as effective as TPA in enhancing the viability of aldehyde-treated cells, whereas the nonpromoting phorbol analogue 4α-PDD had no effect.

Experiments were carried out to establish whether a correlation exists between cell survival in the aggregate form and growth in soft agar. The ability of cells from trypsinized aggregates to grow in soft agar was tested at a total serum concentration of 10% in experiments where the relative amounts of NCS and FCS were varied as shown in Fig. 3. With only NCS present in the medium, very few cells were able to form colonies in soft agar but with increasing concentrations of FCS, the number of colonies increased gradually. Cells derived from aggregates of cultures treated with formaldehyde or acetaldehyde followed by exposure to TPA possessed a considerably higher ability to form colonies in soft agar than untreated cells from

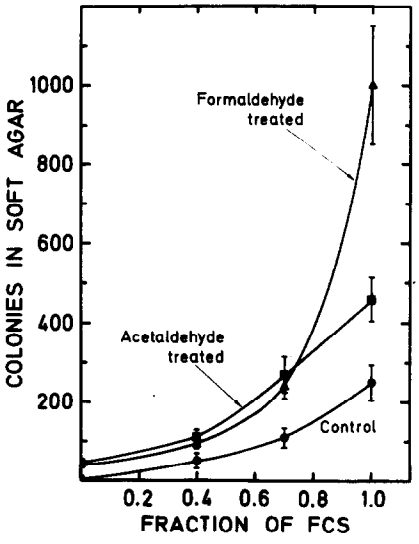

Fig. 3. Effect of FCS on colony formation in soft agar of untreated and aldehyde-treated HRRT cells. Cell cultures were treated with formaldehyde (0.1 mM) *or acetaldehyde* (3.0 mM) *as described in Materials and Methods and incubated in the aggregation assay system in the presence of TPA* (0.1 μg/ml). *Control cultures were treated with solvent only. After* 6 days *of incubation, the aggregates were collected by centrifugation and trypsinized* (0.05% *trypsin and* 0.02% *EDTA) for* 20 min. *The single cell suspension* (1×10^5 cells) *were seeded in soft agar as described in Materials and Methods.*

control cultures. At 10% FCS, the ability of formaldehyde-treated cells to form colonies was about 4 times higher than for untreated cells. With acetaldehyde-treated cells the number of colonies in soft agar was twice that found with the untreated cells. These data suggest that a correlation exists between cell survival in the aggregation assay system and colony forming ability in soft agar.

DISCUSSION

It has previously been shown that the ability of mammalian cells to survive in aggregates is closely correlated with their tumorigenic potential (48–52). Recently, we have shown that the aggregation assay system using the HRRT cell line may be a useful model system for studies on the initiation and promotion phases of transformation [45]. It was shown that with the carcinogens urethane and *N*-methyl-*N*-nitrosourea as initiators followed by subsequent exposure to the tumor promoters TPA or PDD an increased cell survival occurred. In the present work it was found that formaldehyde and acetaldehyde acted as initiators when TPA and PDD were used as promoters. 4α-PDD did not function as a promoter. Addition of TPA prior to aldehyde treatment had no effect on cell viability in the aggregation assay. This is one of the classic criteria of initiation-promotion experiments.

The mechanisms involved in cell transformation assays may differ with the end-point used and on whether primary cells or established cell lines are used. It can be argued that the cells of established lines are not normal. The HRRT cell line used in the present study was originally derived from a hereditary renal rat adenoma. However, the HRRT cells are not able to form colonies in soft agar, do not multiply at low serum concentrations or form tumors in nude mice [46]. Thus, the HRRT cell line lacks properties generally considered to be typical of transformed cells and behave as an untransformed cell line.

The ability of cells seeded in soft agar to produce colonies is one of the criteria commonly used to determine malignant transformation. Therefore, in order to establish whether the aggregation assay is actually measuring neoplastic transformation, the ability of the aldehyde- and TPA-treated HRRT-cells to form colonies in soft agar was tested with single cell suspensions from trypsinized aggregates. It was shown (Fig. 3) that increased survival of formaldehyde or acetaldehyde treated HRRT cells in the aggregate form was correlated with an enhancement of the ability to form colonies in soft agar. This finding indicates that measurements of cell viability in the aggregation assay system used in the present work is a reliable endpoint for *in vitro* studies of cell transformation. Since the aggregation properties seem to occur before the appearance of other conventional criteria of cell transformation, the assay may prove to be a valuable short-term test system for carcinogen screening *in vitro*. Moreover, the results so far obtained with the HRRT cell line suggest that it may be a valuable assay for studies on initiation and promotion-like effects *in vitro*.

The results in the present report show that

Table 1. Effect of various phorbol esters on the viability of formaldehyde and acetaldehyde treated HRRT cells in the aggregation assay system. The cultures were treated with formaldehyde (0.1 mM) *or acetaldehyde* (3.0 mM) *for* 3 hr. *The carcinogen was subsequently removed and the cells were exposed to the phorbol esters* (0.1 μg/ml) *as described in Materials and Methods. The number of viable cells was determined after* 6 days *of incubation*

Treatment	No. of viable cells ($\times 10^{-4}$)	Enhancement of viable cells (% above control)
None	12.0 ± 0.7*	0
Formaldehyde + TPA	25.0 ± 1.7	108
+ PDD	20.0 ± 0.3	67
+ 4α-PDD	11.7 ± 0.3	−3
Acetaldehyde + TPA	20.7 ± 1.5	73
+ PDD	18.7 ± 2.9	56
+ 4α-PDD	12.0 ± 1.1	0

* Standard deviation.

formaldehyde and acetaldehyde act like an initiator in the assay of attachment independent survival of HRRT cells in aggregates. Previous data have shown that formaldehyde acts as a complete carcinogen in Styles' cell transformation assay [19] and as initiator [20] and a weak promoter [17] of the C3H/10T1/2 cells and as an initiator in the Syrian hamster embryo cell transformation assay [21]. To our knowledge, no data of cell transformation by acetaldehyde has previously been published. The present results indicate that acetaldehyde and formaldehyde are both able to initiate cell transformation. However, on a molar basis formaldehyde was more than 100 times more potent than acetaldehyde. The difference in potency between formaldehyde and acetaldehyde is similar to that found for cytotoxic effects in human bronchial epithelial cells as measured by inhibition of clonal growth rate [53] and for induction of tumors in rats [22, 23, 42]. The present results strengthen the view that formaldehyde and acetaldehyde may pose a carcinogenic risk and suggest that these substances have potential to act as initiators of cell transformation.

REFERENCES

1. Bedford P, Fox BW. The role of formaldehyde in methylene dimethanesulphonate-induced DNA cross-links and its relevance to cytotoxicity. *Chem Biol Interact* 1981, **38**, 119–126.
2. Fornace AJ. Detection of DNA single-strand breaks produced during the repair of damage by DNA-protein cross-linking agents. *Cancer Res* 1982, **42**, 145–149.
3. Ross WE, Shipley N. Relationship between DNA damage and survival in formaldehyde-treated mouse cells. *Mutat Res* 1980, **79**, 277–283.
4. Fornace AJ, Lechner JF, Grafstrom RC, Harris CC. DNA repair in human bronchial epithelial cells. *Carcinogen* 1982, **3**, 1373–1377.
5. Ross WE, McMillan DR, Ross CF. Comparison of DNA damage by methylmelamines and formaldehyde. *JNCI* 1981, **67**, 217–221.
6. Grafstrom RC, Fornace AJ, Autrup H, Lechner JF, Harris CC. Formaldehyde damage to DNA and inhibition of DNA repair in human bronchial cells. *Science* 1983, **220**, 216–218.
7. Ashby J, Lefevre P. Genetic toxicology studies with formaldehyde and closely related chemicals including hexamethylphosphoramide (HMPA). In: Gibson JE, ed. *Formaldehyde Toxicity*. New York, 1983, 85–97.
8. Boreiko CJ, Couch DB, Swenberg JA. Mutagenic and carcinogenic effects of formaldehyde. *Environ Sci Res* 1982, **25**, 353–367.
9. Connor TH, Barrie MD, Theiss JC, Matney TS, Ward JB. Mutagenicity of formalin in the Ames assay. *Mutat Res* 1983, **119**, 145–149.
10. Goldmacher VS, Thilly WG. Formaldehyde is mutagenic for cultured human cells. *Mutat Res* 1983, **116**, 417–422.
11. Nishioka H. Lethal and mutagenic action of formaldehyde in HCR+ and HCR- strains in *Escherichia coli*. *Mutat Res* 1980, **17**, 261–265.
12. Szabad J, Soos I, Polgar G, Heffa G. Testing the mutagenicity of malondialdehyde and formaldehyde by the Drosophila mosaic and the sex-linked recessive lethal tests. *Mutat Res* 1983, **113**, 117–133.
13. Temcharoen P, Thilly WG. Toxic and mutagenic effects of formaldehyde in *Salmonella typhimurium*. *Mutat Res* 1983, **119**, 89–93.
14. Brusick D. Genetic and transforming activity of formaldehyde. In: Gibson JE, ed. *Formaldehyde Toxicity*. New York, Hemisphere, 1983, 73–84.
15. Obe G, Beek B. Mutagenic activity of formaldehyde. *Drug Alcohol Depend* 1979, **4**, 91–94.
16. Levy S, Nocentini S, Billardon C. Induction of cytogenetic effects in human fibroblast cultures after exposure to formaldehyde or X-rays. *Mutat Res* 1983, **119**, 309–317.
17. Frazelle JH, Abernethy DJ, Boreiko CJ. Weak promotion of C3H/10T1/2 cell transformation by repeated treatments with formaldehyde. *Cancer Res* 1983, **43**, 3236–3239.
18. Hatch GG, Conklin PM, Christensen CC, Casto BC, Nesnow S. Synergism in the transformation of hamster embryo cells treated with formaldehyde and adenovirus. *Environ Mutagen* 1983, **5**, 49–57.
19. Plesner BH, Hansen K. Formaldehyde and hexamethylenetetramine in Styles' cell transformation assay. *Carcinogen* 1983, **4**, 457–459.
20. Ragan DL, Boreiko CJ. Initiation of C3H/10T1/2 cell transformation by formaldehyde. *Cancer Lett* 1981, **13**, 325–331.
21. Sanner T, Eker P, Rivedal E. Initiation of cell transformation by formaldehyde. *Mutat Res* 1983, **113**, 303.
22. Albert RE, Sellakumar AR, Laskin S, Kuschner M, Nelson N, Snyder CA. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *JNCI* 1982, **68**, 597–603.
23. Kerns WN, Pavkov KL, Donofrio DJ, Grulla EJ, Swenberg JA. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res* 1983, **43**, 4382–4392.

24. Olsen JH, Plough Jensen S, Hink M, Faurbo K, Breum NO, Möller Jensen O. Occupational formaldehyde exposure and increased nasal cancer risk in man. *Int J Cancer* 1984, **34**, 639–644.
25. Harrington JM, Oakes D. Mortality study of British pathologists 1974–80. *Br J Ind Med* 1984, **41**, 188–191.
26. Levine RJ, Andjelkovich DA, Shaw LK. Mortality of Ontario undertakers: a first report. In: Clary JJ, Gibson JE, Waritz RS, eds. *Formaldehyde: Toxicology–Epidemiology–Mechanisms.* New York, Marcel Dekker, 1983, 127–140.
27. Walrath J, Fraumeni JF. Mortality patterns among embalmers. *Int J Cancer* 1983, **31**, 407–411.
28. Walrath J. Mortality among embalmers. *Am J Epidemiol* 1983, **118**, 432.
29. Ristow HJ, Obe G. Acetaldehyde induces cross-links in DNA and causes sister-chromatid exchanges in human cells. *Mutat Res* 1978, **58**, 115–119.
30. Bandas EL. Studies on the role of metabolites and contaminants in the mutagenic action of ethanol in the yeast mithochondria. *Genetica* 1982, **18**, 1056–1061.
31. Igall S, Gazso L. Mutagenic effect of alcohol and acetaldehyde on *Escherichia coli*. *Mutat Res* 1980, **74**, 209–210.
32. Osztovics M, Igali S, Antal A, Veghelyi P. Alcohol is not mutagenic. *Mutat Res* 1980, **74**, 274.
33. Rappoport IA. Mutations under the effect of unsaturated aldehydes. *Dokl Akkad Nauk SSSR* 1978, **61**, 713–715.
34. De Raat WK, Davies PB, Bakker GL. Induction of sister-chromatide exchanges by alcohol and alcoholic beverages after metabolic activation by rat-liver homogenate. *Mutat Res* 1983, **124**, 85–90.
35. Jansson T. The frequency of sister chromatid exchanges in human lymphocytes treated with ethanol and acetaldehyde. *Heredit* 1982, **97**, 301–303.
36. Korte A, Obe G. Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. *Mutat Res* 1980, **88**, 389–395.
37. Obe G, Natarajan A, Meyers M, den Hertog A. Induction of chromosomal aberrations in peripheral lymphocytes of human blood *in vitro*, and of SCEs in bone-marrow cells of mice *in vivo* by ethanol and its metabolite acetaldehyde. *Mutat Res* 1979, **68**, 291–294.
38. Obe G, Ristow H. Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells *in vitro*. *Mutat Res* 1977, **56**, 211–213.
39. Au W, Badr FM. Does ethanol induce chromosome damage? *In Vitro* 1979, **15**, 221.
40. Bird RP, Draper HH, Basrur PK. Effect of malonaldehyde and acetaldehyde on cultured mammalian cells. Production of micronuclei and chromosomal aberrations. *Mutat Res* 1982, **101**, 237–246.
41. Feron VJ, Kruysse A, Woutersen RA. Respiratory tract tumors in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. *Eur J Cancer Clin Oncol* 1982, **18**, 13–31.
42. Woutersen RA, Appelman LM, Feron VJ, van der Heijden CA. Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: Interim results after 15 months. *Toxicology* 1984, **31**, 123–133.
43. Biffersohl G. Epidemiological research on cancer risk by aldol and aliphatic aldehydes. *Environ Qual Saf* 1975, **4**, 235–238.
44. Obe G. Acetaldehyde not ethanol is mutagenic. *Progr. Mutat Res* 1981, **2**, 19–23.
45. Eker P, Sanner T. An assay for initiators and promoters of carcinogenesis based on attachment-independent survival of cells in aggregates. *Cancer Res* 1983, **43**, 320–323.
46. Eker P, Eker R, Johannessen JV. Establishment and characterization of a new cell line from a hereditary renal rat tumor. *In Vitro* 1983, **19**, 495–503.
47. Chen TR. *In situ* detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 3358 stain. *Exp Cell Res* 1977, **104**, 255–262.
48. Steuer AF, Rhim JS, Hentosh PM, Ting RC. Survival of human cells in the aggregate form: Potential index of *in vitro* cell transformation. *JNCI* 1977, **58**, 917–921.
49. Putman DL, Park DK, Rhim JS, Steuer AF, Ting RC. Correlation of cellular aggregation of transformed cells with their growth in soft agar and tumorigenic potential. *Proc Soc Exp Biol Med* 1977, **155**, 487–494.
50. Rhim JS, Koh KS, Chen A, Essex M. Characterization of clones of tumorigenic feline cells transformed by a chemical carcinogen. *Int J Cancer* 1981, **28**, 51–57.
51. Rhim JS, Park DK, Arnstein P, Nelson-Rees WA. Neoplastic transformation of canine embryo cells *in vitro* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Int J Cancer* 1978, **22**, 441–446.
52. Traut KA, Kachevsky V, Wolff JS. A rapid *in vitro* assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independence endpoint. *Int J Cancer* 1979, **23**, 193–196.
53. Saladino AJ, Willey JC, Lechner JF, Grafstrom RC, LaVeck M, Harris CC. Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. *Cancer Res* 1985, **45**, 2522–2526.