[CANCER RESEARCH 44, 938–941, March 1984]

Sodium Fluoride-induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges, and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells[1]

Takeki Tsutsui,[2] Nobuko Suzuki, and Manabu Ohmori

Department of Pharmacology, Nippon Dental University, Tokyo Facility, 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102, Japan

ABSTRACT

The effects of exposure of early-passage Syrian hamster embryo cells in culture to sodium fluoride have been studied with respect to induction of morphological and neoplastic transformation, chromosome aberrations, sister chromatid exchanges, and unscheduled DNA synthesis. Exposure of Syrian hamster embryo cells to NaF concentrations between 75 and 125 μg/ml for 24 hr caused approximately 90 to 40% cell survival and resulted in a dose-dependent increase in the frequency of morphological transformation of the cells. Mass cultures of cells treated with NaF (75 or 100 μg/ml) for 24 hr, followed by continuous cultivation for 35 to 50 passages, developed the ability to grow in soft agar and to produce anaplastic fibrosarcomas when injected into newborn hamsters. In contrast, no morphological and neoplastic transformation was observed in untreated cells. Furthermore, a significant increase in chromosome aberrations at the chromatid level, sister chromatid exchanges, and unscheduled DNA synthesis was induced by NaF in a dose- and time-dependent manner. These results indicate that NaF is genotoxic and capable of inducing neoplastic transformation of Syrian hamster embryo cells in culture. A potential for carcinogenicity of this chemical, which is widely used by humans, is suggested. However, the carcinogenic risk of this chemical to humans may be reduced by factors regulating *in vivo* dose levels.

INTRODUCTION

Fluoride is an ubiquitous substance found naturally in food and water and utilized extensively for industrial purposes. Furthermore, fluoride is widely recommended for prevention of dental caries through various methods, such as fluoridation of communal water supplies; mouth rinsing with fluoride solutions; toothbrushing with fluoride solutions, gels, and prophylactic pastes; and the application of fluoride gels in mouthpieces (7).

It is generally assumed that sodium fluoride is safe to use as a preventative of dental caries. Epidemiological examination shows no relationship between fluoridation of water supplies and cancer mortality in people (10). However, the cytogenetic effect of fluoride on mammalian cells *in vivo* and *in vitro* remains unclear. Jachimczak and Skotarczak (8) showed that sodium fluoride induced chromosome aberrations in cultured human leukocytes. Jagiello and Lin (9) also reported that NaF caused chromosome aberrations in cultured ovarian oocytes from mice, ewes, and cows. On the other hand, there have been negative results on the induction of chromosome aberrations in cultured human leukocytes by NaF (14). In addition, Martin *et al.* (11, 13) presented evidence that NaF was negative in the Ames *Salmonella* test, with and without microsomal activation, as well as in *in vivo* assay for chromosome aberrations and SCEs[3] in bone marrow cells of mice fed a fluoride-supplemented diet.

Previous studies (20, 23) showed that treatment of cultured human diploid fibroblasts from newborn foreskin tissue and human keratinocytes from adult oral mucosa with NaF resulted in the induction of chromosome aberrations and UDS, which is repair synthesis of damaged DNA. Since many chemical carcinogens cause chromosome aberrations and DNA damage, we tried to examine whether NaF could induce neoplastic transformation of early-passage SHE cells which are used widely for the study of *in vitro* carcinogenesis (1–4, 16, 18, 21, 22). The assay system used in this report, which has been developed by Berwald and Sachs (3) and DiPaolo *et al.* (4), is rapid, is quantitative, and can distinguish carcinogens from noncarcinogens with a high degree of accuracy (16).

The present results provide evidence that NaF induces morphological and neoplastic transformation of SHE cells in culture as well as chromosome aberrations, SCEs, and UDS in the same cells.

MATERIALS AND METHODS

Cells, Growth Medium, and Chemicals. SHE cell cultures were established from 13-day-gestation fetuses collected aseptically by cesarean section from inbred Syrian hamsters, strain LSH/ss LAK (Lakeview Hamster Colony, Newfield, NJ). Pools of primary cultures from littermates were stored at liquid nitrogen temperature (1, 2, 18, 21, 22). Secondary cultures were initiated from the frozen stocks, and all experiments were performed with tertiary cultures in a humidified atmosphere with 10% CO_2 in air at 37°. All cultures were routinely tested by Microbiological Associates (Bethesda, MD) and were found to be free of *Mycoplasma* contamination. The cell culture medium (complete medium) used was IBR Dulbecco's modified Eagle's reinforced medium (Biolab, Northbrook, IL) supplemented with 0.37% $NaHCO_3$ and 10% FBS (GIBCO, Grand Island, NY). Cells were transferred by gentle trypsinization with 0.1% trypsin solution (1:250; GIBCO) for 5 min at 37°. NaF (greater than 99% pure) from Koso Chemical Co. (Tokyo, Japan) was dissolved in complete medium at 1 mg/ml, filter sterilized, and diluted with complete medium to the desired concentrations immediately before use. Hydroxyurea and 5-bromodeoxyuridine were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]dThd (specific activity, 49 Ci/mmol) was purchased from Amersham-Searle Corp., Arlington Heights, IL.

Transformation Assay. For morphological transformation experiments, 5000 cells were seeded into 100-mm plastic dishes (Falcon Labware, Oxnard, CA) and, after overnight incubation, treated with NaF (75 to 125 μg/ml) for 24 hr. After treatment, the cultures were washed

[1] This work was supported in part by a grant for cancer research from the Ministry of Education, Science, and Culture of Japan.

[2] To whom requests for reprints should be addressed.

Received August 29, 1983; accepted November 21, 1983.

[3] The abbreviations used are: SCE, sister chromatid exchange; FBS, fetal bovine serum; UDS, unscheduled DNA synthesis; [^{3}H]dThd, [^{3}H]thymidine; SHE cells, Syrian hamster embryo cells; GIBCO, Grand Island Biological Co.

Downloaded from cancerres.aacrjournals.org on February 6, 2014. © 1984 American Association for Cancer Research.

twice with 5 ml of complete medium and incubated for 7 days for colony formation. The cells were fixed with absolute methanol and stained with 3% aqueous Giemsa solution, and the number of surviving colonies and morphologically transformed colonies was scored by previously established criteria (1, 2, 4, 18). The percentage of survival was calculated relative to the survival of the untreated controls. The percentage of morphological transformation was determined from the number of colonies with an altered morphology (1, 2, 4, 18) relative to the total surviving colonies × 100.

For neoplastic transformation studies, cells (5×10^5) were seeded into 75-sq cm flasks (Falcon) and, after an overnight incubation, treated with NaF (75 or 100 μg/ml) for 24 hr. After treatment, the cells were continually subcultured once or twice a week at an inoculum concentration of 1 to 5×10^5 cells/75-sq cm flask. At various times following treatment, the cells were tested for the ability to grow in semisolid agar and for tumorigenicity in newborn Syrian hamsters by s.c. injections of 1×10^6 cells/animal (2, 21). All animals were checked weekly for the appearance of palpable tumors for up to 1 year after inoculation. When tumors were approximately 2 cm in diameter, animals were sacrificed, and a sample of tumor was fixed in 10% neutral buffered formalin. Fixed tissues were processed according to routine histological procedures, and sections were stained with hematoxylin and eosin.

Chromosome Aberrations. SHE cells (5×10^5) were seeded in 75-sq cm flasks. After an overnight incubation, NaF was added, and the cultures were incubated for an additional 16 and 28 hr. Three hr before the end of treatment time, Colcemid (GIBCO) was administered at 0.2 μg/ml, and metaphase chromosomes were prepared as described previously (18, 22). After trypsinization, the cells were treated with 0.9% sodium citrate at room temperature for 13 min, fixed in Carnoy's solution (methanol:acetic acid, 3:1), and spread on glass slides by the air-drying method. The specimens were stained with 3% Giemsa in 0.07 M phosphate buffer (pH 6.8) for 10 min. One hundred metaphases were analyzed for chromosome aberrations.

SCEs. Cells (5×10^5) were plated overnight in 75-sq cm flasks and treated with various concentrations of NaF for 24 hr in the presence of 5-bromodeoxyuridine (10 μg/ml) under dark conditions. Three hr before the end of treatment, Colcemid was added to give the final concentration of 0.2 μg/ml, and metaphase chromosomes were prepared as described above. The differential staining of sister chromatids was performed according to a modification of the fluorescence-plus-Giemsa technique (15). The slides were stained for 15 min in a solution of Hoechst 33258 (50 μg/ml in water), washed, dipped in 0.14 M NaCl:3 mM KCl:8 mM Na_2HPO_4:1 mM KH_2PO_4 (pH 7.4), and exposed to near UV from a row of 6 F15T8/BLB fluorescent bulbs (33 J/sq m/sec; Sylvania, Inc., Springfield, VA) for 1 hr at 55°. The slides were then stained with 3% Giemsa solution for 10 min. Thirty second-division metaphases with the diploid number of chromosomes were analyzed for SCE frequency.

UDS. The procedure for detection of UDS has been described recently (19).[4] Briefly, 1×10^5 cells in logarithmic growth were plated in triplicate on 15-mm-diameter Thermanox coverslips (Lux, Miles Laboratories, Inc., Naperville, IL) in 16-mm tissue culture dishes (Costar, Cambridge, MA) in complete medium. After overnight incubation, the medium was replaced with medium containing 1% FBS (1% FBS medium), and the cultures were incubated for 2 days. The cells were then treated with various doses of NaF for appropriate time in 1% FBS medium containing 10 mM hydroxyurea and [^{3}H]dThd (1.0 μCi/ml). The uptake of [^{3}H]dThd was stopped by rinsing the coverslips with cold 0.15 M NaCl and immersing them 3 times in 5% cold trichloroacetic acid for 15 min. The coverslips were placed in scintillation vials to determine the radioactivity in the samples. The background levels of [^{3}H]dThd incorporation per coverslip in untreated cultures were <1000 cpm, and these were subtracted from each determination. The number of cells on the coverslips was counted using the culture cluster dishes treated under the same conditions as in the experiments for detection of UDS with [^{3}H]dThd. No significant difference in the number of cells was found between the control and experimental groups.

[4] T. Tsutsui, N. Suzuki, H. Maizumi, and J. C. Barrett. Development of an unscheduled DNA synthesis assay with Syrian hamster embryo cells, submitted for publication.

RESULTS

Cytotoxicity and Morphological Transformation Induced by NaF. Treatment with NaF was cytotoxic to early-passage SHE cells grown as described in Table 1. Treatment of cells with NaF (100 μg/ml) for 24 hr resulted in about 50% lethality, and the relative cell survival was reduced with increasing concentrations of NaF. NaF treatment caused morphological transformation which was indistinguishable from morphologically altered colonies induced by benzo(a)pyrene and other chemical carcinogens (2, 4, 18, 21, 22). The frequency of transformation increased with increasing dose of NaF. No morphological transformation of SHE cells was observed in untreated cultures.

Neoplastic Transformation Induced by NaF. NaF also induced neoplastic transformation of SHE cells. Mass cultures of cells were treated with NaF (75 or 100 μg/ml) for 24 hr and passaged continually. They were tested for growth in soft agar and tumorigenicity at various times following treatment; the results are shown in Chart 1. Since each culture grew at a different rate, the biological test results during continuous cultivation were plotted against the number of population doublings after treatment. Six of 7 independent mass cultures of untreated control cells senesced by 50 doublings and did not produce cells capable of growth in soft agar. These cells were nontumorigenic when up to 10^7 cells at 10 to 20 doublings were injected s.c. into newborn hamsters. One of 7 untreated cultures escaped senescence. However, the cells at the 330th doubling (320 days and 75 passages after treatment) were negative for growth in soft agar and for tumorigenicity. Cells from all treated cultures exhibited colony formation in soft agar between 50 to 155 doublings after treatment (0.2 to 14.0% efficiency of colony formation). When NaF-treated cells at 120 to 270 doublings or 35 to 50 passages after treatment were injected into newborn hamsters, one of 2 cultures (I-75 and II-75) treated with NaF (75 μg/ml) and 2 of 2 cultures (I-100 and II-100) treated with NaF (100 μg/ml) formed progressively growing tumors at the site of injection (Table 2). The latent period for tumor appearance was 28 to 39 days. Histological examination of the tumors formed *in vivo* revealed that the tumors were anaplastic fibrosarcomas. No tumors were produced by injection of untreated cells.

Chromosome Aberrations and SCEs Induced by NaF. The effect of NaF treatment on chromosome aberrations and SCEs

Table 1

Relative cell survival and frequency of morphologically transformed colonies by treatment of cells with sodium fluoride for 24 hr

NaF (μg/ml)	% of survival[a]	No. of colonies scored[b]	No. of transformed colonies	% of transformation[c]
0	100	3142	0	0
75	90.4 ± 1.8[d]	2840	3	0.11
100	52.9 ± 0.8	1860	9	0.48
125	39.4 ± 0.5	1519	14	0.92

[a] Calculated by using untreated cultures as a control.
[b] Total number of colonies per ten 100-mm dishes.
[c] $\frac{\text{Total no. of morphologically transformed colonies}}{\text{Total no. of colonies scored}} \times 100$
[d] Means ± S.D.

Downloaded from cancerres.aacrjournals.org on February 6, 2014. © 1984 American Association for Cancer Research.

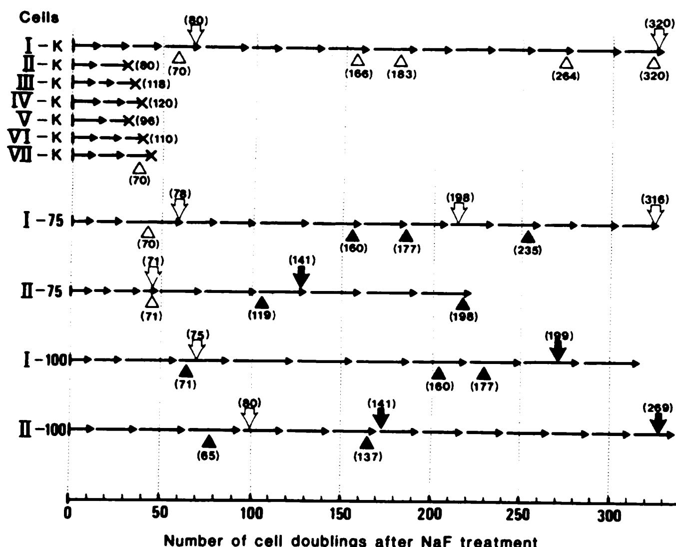

Chart 1. Acquisition of anchorage independency and tumorigenicity of SHE cells treated with NaF following successive cultivation after treatment. SHE cells were treated with NaF as described in "Materials and Methods." At various times following treatment, the cells were tested for the ability to grow in soft agar and for tumorigenicity in newborn Syrian hamsters. The number of population doublings was calculated from the cell number increase in each passage. This is based on the assumption that all surviving cells divided equally. Each *arrow* represents 5 passages, and its length indicates the growth rate of cells during every 5 passages. The cells (I-75 and II-75) were subjected to treatment with NaF (75 μg/ml). I-100 and II-100 cells were exposed to NaF (100 μg/ml). I-K to VII-K groups are untreated cells. △, test for soft agar, negative; ▲, test for soft agar, positive; ▽, test for tumorigenicity, negative; ▼, test for tumorigenicity, positive; →, 5 passages; ×, senescence; *numbers* in *parentheses*, culture days after treatment.

Table 2

Results of injection of cells from NaF-treated and untreated cultures into newborn hamsters

Cells[a]	No. of passages	Time (days) in culture	Time (days) of observation after inoculation	No. of tumor-bearing animals/ no. of animals tested
I-K	74	320	360	0/8
I-75	74	316	360	0/10
II-75	34	141	28	3/3
I-100	48	199	35	3/8
II-100	36	141	39	8/8

[a] I-K cells, untreated cells. I-75 and II-75 cells were treated with NaF (75 μg/ml). I-100 and II-100 cells were exposed to NaF (100 μg/ml).

was examined. In control cultures, 2% of metaphases contained chromatid gaps. However, a significant increase in chromosome aberrations was observed in NaF-treated cells (Table 3). Chromosome aberrations were induced at the chromatid level, and the incidence increased in a dose-dependent manner. Treatment of cells with NaF for 24 hr also caused a dose-dependent increase in SCE frequency, which was elevated approximately 2-fold by NaF (80 μg/ml) (Table 4).

UDS Induced by NaF. We also examined whether NaF treatment could induce DNA repair synthesis detected by UDS. When SHE cells were treated for 4 or 8 hr with NaF at 10 to 40 μg/ml, UDS was not detected. In contrast, treatment of the cells with NaF at the same dose range for over 12 hr resulted in a dose-dependent increase in UDS. Furthermore, the inducibility was dependent on the treatment time examined (Chart 2). No detectable UDS was observed even when the cells were exposed for 4 or 8 hr to NaF at concentrations up to 400 μg/ml (data not shown).

DISCUSSION

We have shown that NaF induces morphological and neoplastic transformation of SHE cells in culture as well as chromosome aberrations, SCEs, and UDS in the same cells. Treatment with NaF at concentrations between 75 and 125 μg/ml resulted in approximately 90 to 40% cell survival and a dose-dependent increase in morphological transformation. The transformed colonies were morphologically similar to those which have been described previously as a result of treatment with chemical and physical carcinogens (2, 4, 18, 21, 22). Pienta *et al.* (16) demonstrated a very high positive correlation between morphological transformation and the reported carcinogenic activity of several chemicals. In further experiments, we have observed that 3 of 4 mass cultures treated with NaF transformed to the tumorigenic state following successive passage, indicating that the treatment induced neoplastic transformation. Approximately 50 to 100

Table 3

Chromosome aberrations in SHE cells induced by sodium fluoride

Concentration (μg/ml)	Treatment time (hr)	Type of aberration (%)						Aberrant metaphases (%)
		G[a]	B	E	D	O	F	
0	16	2	0	0	0	0	0	2
50	16	18	0	0	0	0	0	18
100	16	46	8	0	0	0	0	48
0	28	2	0	0	0	0	0	2
100	28	36	4	0	0	0	0	38
200	28	59	5	0	0	0	0	59

[a] G, gap; B, break; E, exchange; D, dicentric; O, ring; F, fragmentation.

Table 4

SCEs in SHE cells treated with sodium fluoride

Concentration (μg/ml)	SCEs/cell
0	6.17 ± 2.25[a]
20	8.63 ± 2.88
40	9.97 ± 2.28
80	12.87 ± 3.00

[a] Means ± S.D.

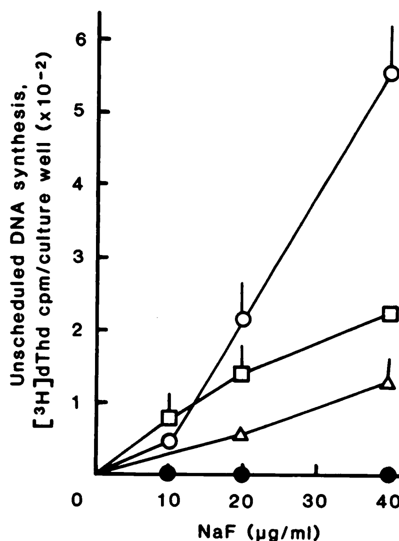

Chart 2. UDS induced by sodium fluoride in SHE cells. The cells were treated with NaF for 4 or 8 hr (●), 12 hr (△), 24 hr (□) and 33 hr (○) in medium containing 1% FBS, 10 mM hydroxyurea, and [^{3}H]dThd (1.0 μCi/ml). *Bars*, S.D.; when not indicated, S.D. are within the *symbols*.

Downloaded from cancerres.aacrjournals.org on February 6, 2014. © 1984 American Association for Cancer Research.

population doublings after NaF treatment were required for the development of anchorage-independent growth. Anchorage-independent growth was not observed in untreated cultures even after 330 population doublings. In benzo(*a*)pyrene-treated cultures with the similar experimental conditions, SHE cells acquired the ability to grow in soft agar after 32 to 75 population doublings (1). We do not know the precise number of population doublings at the time when NaF-treated cells acquired the anchorage independency, because the cells were tested only at every 5 passages.

The mechanisms by which NaF induces transformation of SHE cells are unknown. It is conceivable that, since NaF induced chromosome aberrations, SCEs, and UDS in the cells, NaF treatment results in DNA damage which is involved in the initiation of transformation. Emsley *et al.* (6) have proposed that F^- could play a disruptive role towards DNA through a N—H ··· F^- ··· H—N hydrogen bond in the base pair of thymidine-adenine base pairing.

UDS induced by NaF in SHE cells (Chart 2) was not detected when the cells were treated for 4 or 8 hr. Significant levels of UDS were detected in the cells treated for over 12 hr. Two possible explanations may be considered as to the delay in the response of DNA repair in NaF-treated cells: (*a*) the DNA-damaging activity of fluoride may be weak or insufficient to induce detectable DNA damage during the short treatment time of our conditions; (*b*) inhibition of protein synthesis by fluoride may retard the progression of DNA repair following DNA damage. It is well known that fluoride inhibits a number of metalloproteins (25), including DNA polymerase of *Escherichia coli* (12). Further experiments are necessary to elucidate these possibilities.

There have been conflicting results on the induction of chromosome aberrations in cultured human leukocytes by NaF (8, 14). The different response of cultured human leukocytes could be due either to individual variations in susceptibility to NaF or to the use of different culture conditions. Genotoxicity of NaF has been found in many studies with *in vitro* experimental system (Refs. 8, 9, 20, and 23; this report) but negative in *in vivo* system, in which NaF was administered p.o. or i.p. The intracellular concentration of fluoride in HeLa cells and L-cells was 30 to 40% of extracellular fluoride concentration (5). In contrast, the concentration of fluoride in blood of animals is held constant when the amount of fluoride consumed varies widely. This was shown in rats given up to 50 ppm in their drinking water while the serum fluoride concentration remained constant at about 0.25 ppm (17).

We have previously demonstrated that treatment of cultured human diploid fibroblasts from newborn foreskin tissue and human keratinocytes from adult oral mucosa with NaF caused the induction of chromosome aberrations and UDS (20, 23). The present studies are directly relevant to the carcinogenic potential of NaF, since they provide evidence that NaF induces morphological and neoplastic transformation of cells in culture. Therefore, NaF could be potentially dangerous to humans. However, factors regulating *in vivo* dose levels, *e.g.*, rapid excretion via urine (24), may reduce the carcinogenic risk of this chemical.

ACKNOWLEDGMENTS

The authors are indebted to Dr. J. Carl Barrett for his critical reading of the manuscript and to Dr. M. Miyaki for her helpful discussions.

REFERENCES

1. Barrett, J. C., and Ts'o, P. O. P. Evidence for the progressive nature of neoplastic transformation *in vitro*. Proc. Natl. Acad. Sci. U. S. A., *75:* 3761–3765, 1978.
2. Barrett, J. C., Tsutsui, T., and Ts'o, P. O. P. Neoplastic transformation induced by a direct perturbation of DNA. Nature (Lond.), *274:* 229–232, 1978.
3. Berwald, Y., and Sachs, L. *In vitro* transformation of normal cells to tumor cells by carcinogenic hydrocarbons. J. Natl. Cancer Inst., *35:* 641–661, 1965.
4. DiPaolo, J. A., Donovan, P., and Nelson, R. Quantitative studies of *in vitro* transformation by chemical carcinogens. J. Natl. Cancer. Inst., *42:* 867–874, 1969.
5. Drescher, M., and Suttie, J. W. Intracellular fluoride in cultured mammalian cells. Proc. Soc. Exp. Biol. Med., *139:* 228–230, 1972.
6. Emsley, J., Jones, D. J., and Overill, R. E. The uracil-fluoride interaction: *ab initio* calculations including solvation. J. Chem. Soc. Chem. Commun., 476–478, 1982.
7. Horowitz, S. H. A review of systemic and topical fluorides for the prevention of dental caries. Community Dent. Oral Epidemiol., *1:* 104–114, 1973.
8. Jachimczak, D., and Skotarczak, B. The effect of fluoride and lead ions on the chromosomes of human leukocytes *in vitro*. Genet. Pol., *19:* 353–357, 1978.
9. Jagiello, G., and Lin, J. Sodium fluoride as potential mutagen in mammalian eggs. Arch. Environ. Health, *29:* 230–235, 1974.
10. Kinlen, L., and Doll, R. Fluoridation of water supplies and cancer mortality III: a re-examination of mortality in cities in the USA. J. Epidemiol. Community Health, *35:* 239–244, 1981.
11. Kram, D., Schneider, E. L., Singer, L., and Martin, G. R. The effects of high and low fluoride diets on the frequencies of sister chromatid exchanges. Mutat. Res., *57:* 51–55, 1978.
12. Lehman, I. R. DNA synthesis (bacteria). Methods Enzymol., *6:* 34–39, 1963.
13. Martin, G. R., Brown, K. S., Matheson, D. W., Lebowitz, H., Singer, L., and Ophaug, R. Lack of cytogenetic effects in mice or mutations in *Salmonella* receiving sodium fluoride. Mutat. Res., *66:* 159–167, 1979.
14. Obe, G., and Slacik-Erben, R. Suppressive activity by fluoride on the induction of chromosome aberrations in human cells with alkylating agents *in vitro*. Mutat. Res., *19:* 369–371, 1973.
15. Perry, P., and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature (Lond.), *251:* 156–158, 1974.
16. Pienta, R. J., Poiley, J. A., and Lebherz, W. B. III. 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, 1977.
17. Singer, L., and Armstrong, W. D. Regulation of plasma fluoride in rats. Proc. Soc. Exp. Biol. Med., *117:* 686–689, 1964.
18. Tsutsui, T., Barrett, J. C., and Ts'o, P. O. P. Morphological transformation, DNA damage, and chromosomal aberrations induced by a direct DNA perturbation of synchronized Syrian hamster embryo cells. Cancer Res., *39:* 2356–2365, 1979.
19. Tsutsui, T., Degen, G. H., Schiffmann, D., Wong, A., Maizumi, H., McLachlan, J. A., and Barrett, J. C. Dependence on exogenous metabolic activation for induction of unscheduled DNA synthesis in Syrian hamster embryo cells by diethylstilbestrol and related compounds. Cancer Res., *44:* 184–189, 1984.
20. Tsutsui, T., Ide, K., and Maizumi, H. Induction of unscheduled DNA synthesis in cultured human oral keratinocytes (abstract). J. Dent. Res., *61:* 203, 1982.
21. Tsutsui, T., Maizumi, H., and Barrett, J. C. Colcemid-induced neoplastic transformation and aneuploidy in Syrian hamster embryo cells. Carcinogenesis (Lond.), in press, 1984.
22. Tsutsui, T., Maizumi, H., McLachlan, J. A., and Barrett, J. C. Aneuploidy induction and cell transformation by diethylstilbestrol: a possible chromosomal mechanism in carcinogenesis. Cancer Res., *43:* 3814–3821, 1983.
23. Tsutsui, T., Suzuki, N., and Ohmori, M. Cytotoxicity, chromosome aberrations and unscheduled DNA synthesis in cultured human diploid fibroblasts induced by sodium fluoride. Mutat. Res., in press, 1984.
24. Volker, J. F., Sognnaes, R. F., and Bibby, B. G. Studies on the distribution of radioactive fluoride in the bones and teeth of experimental animals. Am. J. Physiol., *132:* 707–712, 1941.
25. Wiseman, A. Effect of inorganic fluoride on enzymes. *In:* F. A. Smith (ed.), Handbook of Experimental Pharmacology, Vol. 22/2, pp. 48–97. Berlin: Springer-Verlag, 1970.

Downloaded from cancerres.aacrjournals.org on February 6, 2014. © 1984 American Association for Cancer Research.