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EFFECT OF CHEMICAL MUTAGENS ON HERPES VIRUS-INDUCED CELLULAR TR--ETC(U)
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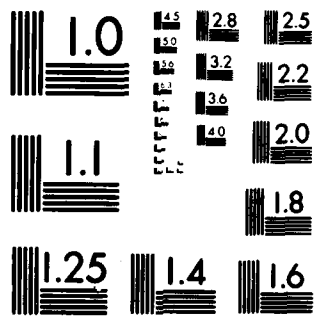
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Mutagenesis experiments using THO cells as indicator cells suggested that even after metabolic activation of the test chemicals this cell system does not provide an adequately sensitive test for mutagenesis.

In additional experiments further information was obtained which showed enhancement of transformation by hydrazine and 1,2-dimethylhydrazine of cells exposed to irradiated virus. Significant enhancement occurred in these *in vitro* tests only when the cells were exposed to the chemical 24 hours prior to virus infection.

The (ts) mutant Ag(293) of HSV-2 was employed in tests to detect chemical interactions with the virus causing increased transformation of target cells. Mouse 3T3 cells were monitored for morphological transformation. Hydrazine and 1,2-dimethylhydrazine caused an enhancement of viral transformation of between three to four fold, confirming earlier findings using ultraviolet light inactivated wild type virus.

Norharman (9H-pyrido[3,4-b]indole), a component of tobacco tars, caused a nearly three-fold enhancement of transformation. Xylene and toluene were not found to enhance viral transformation. Experiments involving host-mediated activation of the test chemicals were unsuccessful because of low target cell recovery due to inhibition of the target 3T3 cells.

Further experiments using the quantitative assay for the transformation examined the effect of chemical carcinogens and pro-carcinogens. The carcinogens tested were N-methyl-N-nitro-N-nitrosoguanidine, quinacrine mustard, N-nitrosomethyl urea, urethane, and benzene. The pro-carcinogens tested were N-nitrosodimethylamine, 3-methylcholanthrene, benzo[α]pyrene, and p-dimethylaminoazobenzene. Exposure of the cells to the chemical compound and to the virus resulted in enhancement of transformation when compared to that observed with chemical or virus alone. Enhancement of transformation occurred in cells treated with all of these compounds. In general, enhancement occurred regardless of whether the cells were pre-exposed to the carcinogen or pre-infected with virus. These results are suggestive of combined herpes virus and chemical effects on cells resulting in increased risk of oncogenic transformation.

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Final Technical Report
June 1982

**EFFECT OF CHEMICAL MUTAGENS
ON HERPES VIRUS INDUCED CELLULAR
TRANSFORMATION AND TESTING FOR
MUTAGENESIS IN MOUSE CELLS**

BRIGHAM YOUNG UNIVERSITY
PROVO, UT 84602



Dr. F. Brent Johnson

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ABSTRACT

Assays which detect quantitative increases of morphological transformation in 3T3 cells were employed to detect transformation by seven temperature sensitive (ts) mutants of herpes simplex type 2 virus (HSV-2). It was determined that all seven mutants caused transformation at the non-permissive temperature. Mutant A8 (293) caused formation of the most foci and appeared to be a more sensitive indicator of transformation in comparative experiments (31 foci per million cells compared to four foci per million cells for ultraviolet-irradiated wild type virus).

Mutagenesis experiments using TH0 cells as indicator cells suggested that even after metabolic activation of the test chemicals this cell system does not provide an adequately sensitive test for mutagenesis.

In additional experiments further information was obtained which showed enhancement of transformation by hydrazine and 1,2-dimethylhydrazine of cells exposed to irradiated virus. Significant enhancement occurred in these *in vitro* tests only when the cells were exposed to the chemical 24 hours prior to virus infection.

The ts mutant Ag(293) of HSV-2 was employed in tests to detect chemical interactions with the virus causing increased transformation of target cells. Mouse 3T3 cells were monitored for morphological transformation. Hydrazine and 1,2-dimethylhydrazine caused an enhancement of viral transformation of between three to four fold, confirming earlier findings using ultraviolet light inactivated wild type virus.

Norharman (9H-pyrido[3,4-6]indole), a component of tobacco tars, caused a nearly three-fold enhancement of transformation. Zylene and toluene were not found to enhance viral transformation. Experiments involving host-mediated activation of the test chemicals were unsuccessful because of low target cell recovery due to inhibition of the target 3T3 cells.

Further experiments using the quantitative assay for transformation examined the effect of chemical carcinogens and pro-carcinogens. The carcinogens tested were N-methyl-N'nitro-N-nitrosoguanidine, quinacrine mustard, N-nitrosomethyl urea, urethane, and benzene. The pro-carcinogens tested were N-nitrosodimethylamine, 3-methylcholanthrene, benzo[α]pyrene, and p-dimethylaminoazobenzene. Exposure of the cells to the chemical compound and to the virus resulted in enhancement of transformation when compared to that observed with chemical or virus alone. Enhancement of transformation occurred in cells treated with all of these compounds. In general, enhancement occurred regardless of whether the cells were pre-exposed to the carcinogen or pre-infected with virus. These results are suggestive of combined herpes virus and chemical effects on cells resulting in increased risk of oncogenic transformation.

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MATTHEW J. KENNER

Chief, Technical Information Division

RESEARCH OBJECTIVES:

a. Determine whether one of seven herpes simplex virus (HSV) type 2 temperature sensitive (ts) mutants will be a more sensitive indicator of viral-induced cell transformation than UV-damaged wild type virus.

b. Determine whether any of the following chemicals are mutagenic in THO cells following metabolic activation by the host mediated assay:

dimethylnitrosamine
hydrazine
monomethylhydrazine
1,2-dimethylhydrazine
1,1-dimethylhydrazine
JP-5
JP-10
RJ-4
RJ-5
Ethylmethane sulfonate (direct-acting control)

c. Determine if HSV-2-mediated transformation is enhanced following metabolic activation of the following chemicals:

dimethylnitrosamine (N-nitrosodimethylamine) (a known procarcinogen)
hydrazine
monomethylhydrazine
1,2-dimethylhydrazine
1,1-dimethylhydrazine
JP-5
JP-10
RJ-4
RJ-5

d. Determine if HSV-2-mediated transformation is enhanced when cells are simultaneously exposed to virus and the following suspected tumor promoters:

norharman
toluene
xylene

e. Determine if HSV-2 mediated transformation is enhanced when cells are exposed to both virus and the following known direct-acting carcinogens:

N-methyl-N'nitro-N-nitrosoguanidine
Quinacrine mustard
N-nitrosomethyl urea
urethane
benzene

f. Determine if HSV-2-mediated transformation is enhanced when cells are exposed to both virus and the following procarcinogens after metabolic activation:

Dimethylnitrosamine
4-dimethylaminoazobenzene
3-methylcholanthrene
benzo[α]pyrene

ACCOMPLISHMENTS:

A. Transformation of 3T3 Cells by Temperature Sensitive Mutants of Herpes Simplex Virus Type 2

A series of seven HSV-2 mutants were received for testing. Stocks of each ts mutant were prepared using standard methods in HEp-2 cells, and were titrated by plaque assay in HEp-2 cells at both permissive temperature (34°C) and restrictive temperature (38°C). The stocks were vialled in 2 ml aliquots and stored frozen at -70°C.

The transformation assays were carried out in Swiss albino mouse 3T3 cells obtained originally from the American Type Culture Collection (Rockville, MD). The culture medium was Dulbecco's Modified Eagle's medium (DME) containing 10% fetal bovine serum or 10% newborn calf serum, 0.1% sodium bicarbonate, 100 units of penicillin per ml and 100 ug of streptomycin per ml.

Two methodologies were used to determine transformation by the mutant viruses. First, cells in suspension were infected with virus, then plated. 3T3 cells (1×10^6 per 1 dram vial) were infected at a multiplicity of either 1, 5, or 10 plaque forming units (pfu) per cell. The mixtures were incubated at 38°C with shaking for one hour. The contents of each vial was distributed between four 60 mm tissue culture dishes containing 4 ml of media, so that each plate was seeded with 250,000 cells. The plates were incubated at 38°C for 4-5 weeks and the media were renewed at weekly intervals. The plates were then stained with Wright's stain and examined microscopically for transformed foci and scored for the percentage of the monolayers surviving viral CPE. Sufficient plates were included in each experiment so that from 2×10^6 to 5×10^6 cells were infected at each multiplicity, which also included the non-infected controls.

The results of these experiments are shown in Table 1. Virus Ag (293) showed promise because at $Moi=1$ 100% of the monolayers remained intact (minimum virus leak) and 31.6 foci were registered per 10^6 cells. This compares with about 4 foci per 10^6 cells for UV-inactivated wild type virus. This mutant is currently being employed in assays testing hydrazine and 1,2-dimethylhydrazine for transformation enhancement.

The second method of testing the ts mutants for transformation was to infect cells after attachment in a monolayer. 250,000 3T3 cells were seeded in each of a series of plates and the cells allowed to attach. From 12 to 20 plates were infected with each virus concentration ($Moi=1, 5, \text{ and } 10$). Upon inoculation of the virus the previous media were removed, the virus inoculum added, and the plates were incubated at 38°C with periodic shaking for one hour. Fresh media were then added, the plates incubated for 4-5 weeks, stained, and examined microscopically for monolayer survival and focus formation. The results are shown in Table 2. Again, the best results were obtained with Ag (293) which showed the greatest level of transformation with minimum leak. In comparing the results in Table 1 and Table 2, it appears that using Ag (293) at an Moi of 1 and infecting cells in suspensions provides the optimum combination and should, in fact, be a superior test system over irradiated wild type virus for detecting chemical enhancement of herpes virus-induced transformation.

B. Mutagenesis in THO Cells Following Metabolic Activation

The THO cells utilized in this study are mutant cells derived from the mouse Balb 3T3 line. They lack HPRTase function. Revertants can be selected in HAT medium as they grow to form colonies. These experiments were designed to determine if THO cells can be used as a mutagen screening test after the mutagens have been metabolically activated in the host mediated assay. In a previous report we showed that THO registered direct mutagenic activity only at a very low level.

The experiments were carried out in the following manner: 4×10^6 THO cells, contained in media (Eagle's Basal Medium supplemented with 10% newborn calf serum, 0.22% sodium bicarbonate, L-glutamine and 100 units of penicillin per ml and 100 ug of streptomycin per ml) were injected intraperitoneally in 16-18 g female Balb/c mice. Immediately following, the mice received a subcutaneous injection of the potentially mutagenic chemical at a dose of 10 ml/kg. The cells remained in the peritoneal cavity for 6 hours or until the animal died of chemical toxicity whereupon the cells were harvested by injecting 3 ml of media, then removing the cells and media from the peritoneal cavity with a syringe and perforated needle. The cells in these harvests were counted in a hemocytometer (excluding erythrocytes) and plated in media in 75 cm² flasks. The following day the media were renewed, then incubated for an expression time of 7 to 9 days. At this point, the cells were again trypsinized and seeded into HAT medium. After 7 to 10 days the colonies were stained and counted. The cell numbers were not counted at the time of seeding into HAT which accounts for the relatively large mutation frequencies shown on Table 3, but, rather, the viable recovered cells were used for this calculation.

The results of two experiments are shown on Table 3. Even though these experiments could only be regarded as preliminary, the conclusion indicated that the THO system is not a good mammalian cell test system, even when a known direct acting control (EMS), and a known mutagen requiring activation (dimethylnitrosamine) are included in the tests.

C. Further Studies on Transformation Enhancement Using UV-Irradiated Virus.

Initial studies in this laboratory suggested that hydrazine and 1,2-dimethylhydrazine (SDMH) enhanced viral transformation in a system utilizing 3T3 cells and UV-inactivated HSV-2. These results prompted further experimentation which we carried out.

Employed in these assays was the 333 strain of HSV-2. The virus was irradiated in the following manner: 1.5 ml of virus stock was distributed evenly in a 60 mm plastic petri dish and, with the lid removed, exposed to 60 ergs/s/mm² of UV light for 2 minutes, agitated for 30 seconds; then irradiated for an additional 2 minutes.

Cells (250,000 per plate) were exposed to various concentrations of hydrazine or SDMH contained in 5 ml of media. Exposure of the cells to the chemicals was for 24, 6 or 2 hours prior to addition of the virus or for 24 hours beginning at 2, 6 or 24 hours after addition of the virus. Replicate plates were included in the tests which received chemical only. These cells were trypsinized

and re-plated to determine the amount of chemical cytotoxicity. Thus, the surviving fraction could be calculated.

The plates containing cells exposed to both virus and chemical were incubated for four weeks with weekly media changes. They were then stained and examined microscopically for transformed foci. The results are shown on Tables 4 and 5.

It can be seen that both hydrazine and SDMH enhanced virus mediated transformation. Enhancement was time-dependent occurring only at -24 hours (addition of the chemical 24 hours before the virus).

D. Refinement of the Transformation Assay

We previously reported the testing of a series of ts mutants of HSV-2 for transformation activity. The mutant ts Ag(293) was selected as an indicator strain to determine transformation enhancement. This method, as it is presently being used, is shown in Fig. 1. This method avoids some of the pitfalls of the previous method, namely the sometimes erratic appearance of reactivated virus in UV-irradiated preparations.

E. Enhancement of Transformation by Hydrazine and 1,2-dimethylhydrazine Using the Mutant Virus Assay System

We previously found enhancement of virus transformation by hydrazine (Hz) and symmetrical dimethylhydrazine (SDMH) in assays which employed UV-irradiated virus. The refined assay using the mutant virus had not been tested until the second year when these tests were accomplished. (Refer to Table 6, Table 7, Fig. 2 and Fig. 3). These results show that virus interactions with Hz or SDMH (or Hz and SDMH catabolites) and the infected cell lead to greater numbers of transformed cells which grow out to recognizable foci.

F. Enhancement of Transformation by Norharman

Norharman, a compound found in tobacco tars and in tryptophan pyrolysates, was tested for enhancement. In other systems, alone, norharman has no mutagenic activity, but enhances the mutagenicity of several typical mutagens such as benzo[α]pyrene, dimethylaminoazobenzene and 2-acetyl-aminofluorene derivatives. Moreover, aniline and o-toluidine, which alone are non-mutagenic become mutagenic in the presence of norharman. The results of the present study show enhancement of herpes virus transformation in the presence of norharman when the chemical is added before the initiation of virus infection (Table 8, Fig. 4).

G. Lack of Enhancement by Xylene and Toluene

Under the conditions and at the concentrations tested reagent grade xylene or toluene did not cause enhancement of virus transformation (Table 9, Table 10).

H. Host Mediated Activation of Chemicals

The host mediated activation system of Hsie, et al. (*Mutation Res.* 51:77-84, 1978) was employed to determine whether prior activation of the chemicals would cause some of the previously negative chemicals to appear as positive virus enhancers. Balb/c mice were injected intraperitoneally with 3T3 cells and the chemical was injected subcutaneously. After two hours the cells were removed from the peritoneal cavity, counted, plated and infected with virus. After four weeks, with weekly media changes, the cells were examined for the development of transformed foci. A major technical problem was encountered, i.e., the recovery of viable 3T3 cells from the peritoneal cavity. This was a problem even in control animals which received no chemical, so the loss of viability could not simply be ascribed to chemical toxicity. No matter how many cells were inoculated (up to 16×10^6) the recovery was always low. Even in the peritoneal cavity where extensively irrigated the recovery was low. Hence, the results were based upon formation of only limited numbers of clones. In these studies none of the previously negative chemicals showed up as a positive enhancer, but the nature of the technical difficulty prevents any conclusions being drawn, except that the animals caused loss of viability of the 3T3 cells, perhaps by allogeneic inhibition.

I. Enhancement by Known Carcinogens and Procarcinogens

It was discovered in these experiments that five known chemical carcinogens and four known chemical pro-carcinogens enhanced the morphological transformation of mouse 3T3 cells by HSV-2.

The chemicals used in the study were obtained from commercial sources. N-methyl-N-nitro-N-nitrosoguanidine, 97%, and p-dimethylaminoenzyme (methyl yellow) indicator grade, were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). Quinacrine mustard (90%), N-nitrosomethyl urea (approximately 75% with 25% of a 3% acetic acid solution stabilizer), urethane, N-nitrosodimethylamine (dimethyl nitrosamine) and benzo[α]pyrene (grade I) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Reagent grade benzene was obtained from our in-house chemistry stores. The 3-methylcholanthrene was purchased from Eastman Organic Chemicals (Rochester, New York).

Infection of Swiss Mouse 3T3 cells with the transforming herpes virus ts mutant resulted in the appearance of foci of transformed cells. The foci were characterized by loss of contact inhibition and resulted in piles of spindle-shaped cells that were oriented in random fashion. Cells not exposed to virus never developed such foci. In addition, no foci were detected in extensive controls containing cells exposed only to chemical, with no virus exposure. The numbers of such foci increased over the number in virus-infected control cultures when the cells were co-exposed to virus and chemical, suggesting a synergistic relationship in the interaction between the virus and the chemical in these cells.

Experiments were performed to detect enhancement by various chemical carcinogens and pro-carcinogens. All nine chemicals tested in these studies exhibited some degree of enhancing activity. The results are shown in Tables 11 to 19 and Figures 5 to 13. The enhancement correlated in general with the toxicity of the chemical as detected in the cell survival assays. N-methyl-N-nitro-N-nitrosoguanidine and quinacrine mustard exhibited the highest level

of enhancement. Enhancement occurred with virtually identical efficiency irrespective of whether the chemical was added to the cells before or after the virus. N-nitrosomethyl urea also gave nearly identical enhancement at -24 hours and at +24 hours with only slightly less efficiency than the previous two carcinogens. In contrast, urethane and benzene showed markedly better enhancement when added to the cell system 24 hours before virus infection. Some enhancement did occur at 24 hours after virus infection, but it was less efficient than when the cells were pretreated with the chemical. Among the procarcinogens, N-nitrosodimethylamine and 3-methylcholanthrene exhibited moderate enhancement levels. Enhancement occurred with these two chemicals with virtually identical efficiency irrespective of whether the chemical was added to the cells before or after the virus. In contrast, benzo[α]pyrene appeared to enhance transformation more efficiently when added after virus infection. In opposition to this finding, p-dimethylaminoazobenzene appeared to enhance more efficiently when added before virus infection. The general patterns of enhancement in this study were repeatable in multiple experiments.

PUBLICATIONS:

Abstracts

1. Johnson, F. B. 1979. Effect of chemical mutagens on herpes virus transformation and backmutation of THO cells. (Abstract) Review of Air Force Sponsored Basic Research in Environmental Protection and Toxic Hazards.
2. Johnson, F. B. 1980. Transformation of mouse cells by temperature sensitive mutants of herpes simplex virus type 2. (Abstract) Review of Air Force Sponsored Basic Research in Environmental Protection, Toxicology, and Electromagnetic Radiation Bioeffects.
3. Johnson, F. B. and J. R. Baker. 1981. Chemical enhancement of herpes simplex type 2-induced cellular transformation. Abstr. Ann. Meet. A.S.M., p. 226.
4. Johnson, F. B. 1981. Interactions between chemicals and oncogenic herpesviruses. Review of Air Force Sponsored Basic Research in Environmental Toxicology (Abstracts).

Papers

1. Johnson, F. B. 1982. Chemical interactions with herpes simplex type 2 virus: Enhancement of transformation by hydrazine and 1,2-dimethylhydrazine. Chem.-Biological Interactions (In press)
2. Johnson, F. B. 1982. Chemical interactions with herpes simplex type 2 virus: Enhancement of transformation by norharman. Carcinogenesis (In press)
3. Johnson, F. B. (1982). Chemical interactions with herpes simplex type 2 virus: Enhancement of transformation by selected chemical carcinogens and procarcinogens. (Submitted)

PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESERACH EFFORT:

1. F. Brent Johnson, Ph.D., principal investigator.
2. Joyce R. Baker, Ph.D., research technician.
3. Lisa Bohnet Wilson, research technician.

INTERACTIONS:

Participation in and presentation of a report at the Review of Air Force Sponsored Basic Research in Environmental Protection, Toxicology, and Electromagnetic Radiation Bioeffects, 15-17 January 1980, San Antonio, Texas.

Participation in and presentation of a report at the Review of Air Force Sponsored Basic Research in Environmental Toxicology, 2-3 June 1981, Columbus, Ohio.

NEW DISCOVERIES STEMMING FROM THE RESEARCH EFFORT:

1. That HSV-2 ts A₈ (293) represents a likely candidate for further transformation-enhancement studies.
2. That THO cells are a disappointingly insensitive system for mutagen testing.
3. Further data were obtained on enhancement of HSV-2 transformation by hydrazine and 1,2-dimethylhydrazine.
4. Supportive evidence was obtained for the transformation enhancing activity of hydrazine and 1,2-dimethylhydrazine in assays involving the mutant virus.
5. That norharman, a tumor promoter, enhances the transformation potential of herpes simplex type 2 virus.
6. That xylene and toluene were negative in enhancement assays.
7. That the following known carcinogens enhance HSV-2 transformation:

N-methyl-N'-nitro-N-Nitrosoguanidine
Quinacrine mustard
N-Nitrosomethyl urea
Urethane
Benzene

8. That the following procarcinogens enhance HSV-2 transformation:

N-Nitrosodimethylamine
3-Methylcholanthrene
Benzo[α]pyrene
p-Dimethylaminoazobenzene

ENHANCEMENT ASSAY

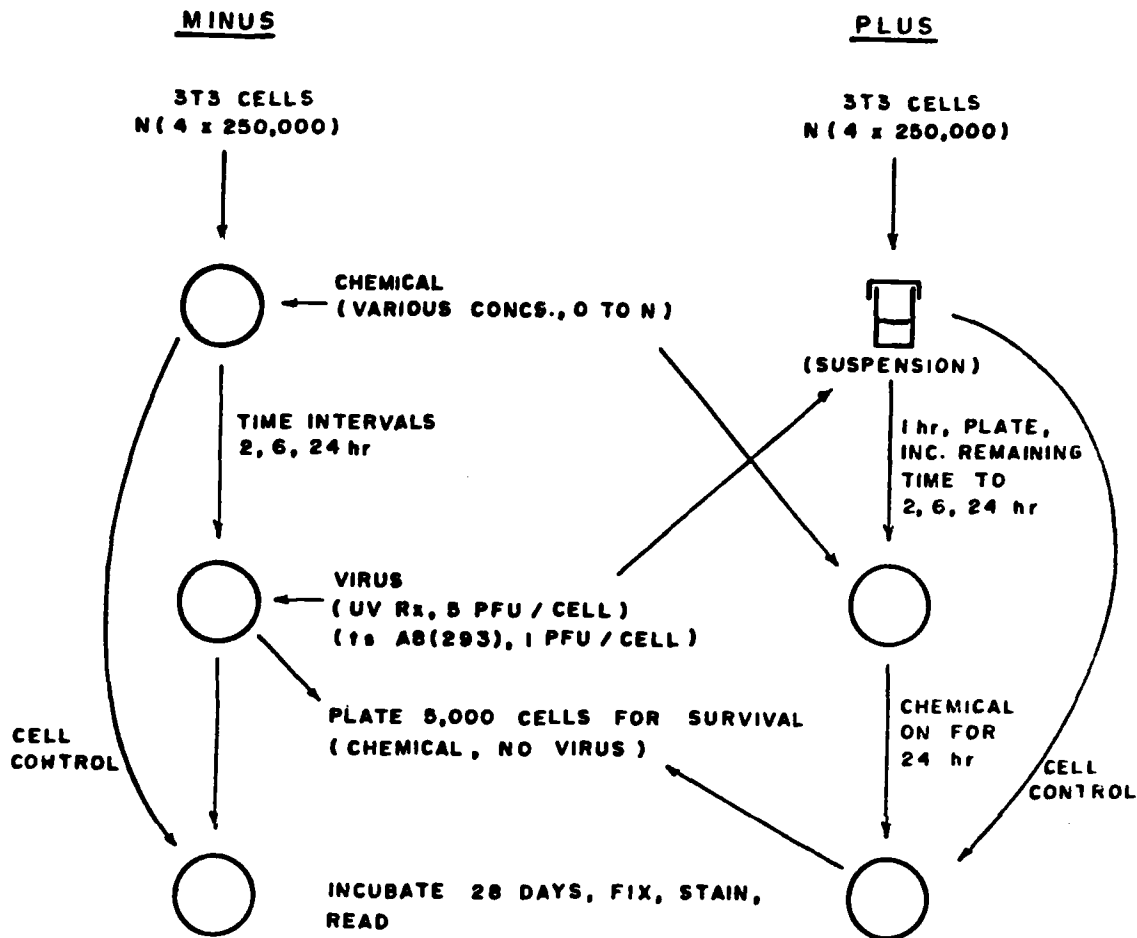


Fig. 1. Assay scheme for the determination of chemical enhancement of herpes virus transformation. The "minus" system refers to exposure of the cells to the chemical prior to virus infection. The "plus" system indicates exposure to the chemicals after virus infection. For each concentration of chemical to be tested (from 0 to N number of concentrations) four petri dishes are seeded with 250,000 cells in each dish. Some dishes are set aside as cell controls. The chemical, contained in cell culture medium, is added to the cells for the length of time to be tested. Some cells are removed and plated for survival to determine chemical toxicity. At the end of the chemical exposure time the media are removed, the cell sheets washed with PBS, the virus added and allowed to absorb for one hour then fresh media added to the cultures. In the "plus" system, the chemical is on for 24 hrs., then washed away and the cells renewed with fresh media. At the end of the incubation period the cell sheets are examined for foci of morphologically transformed cells.

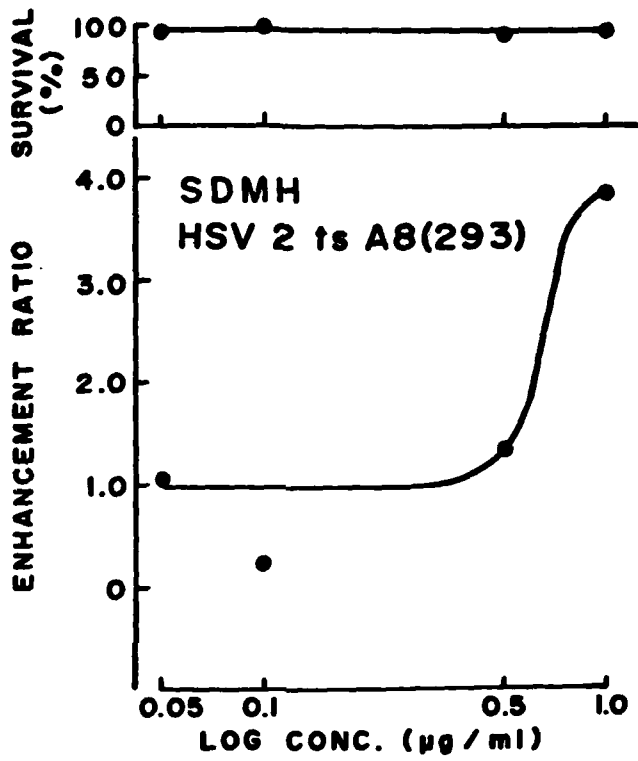


Fig. 2

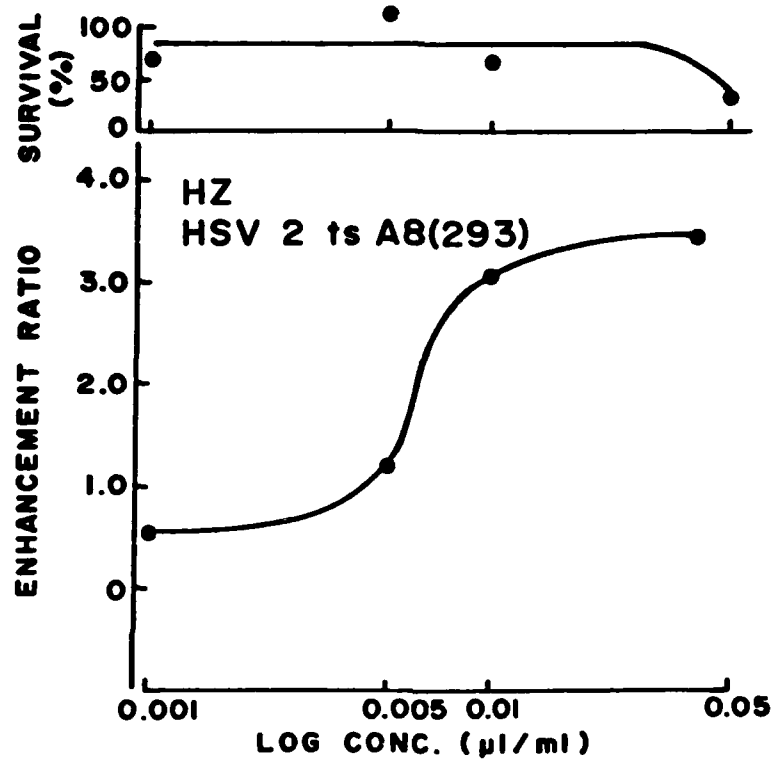


Fig. 3

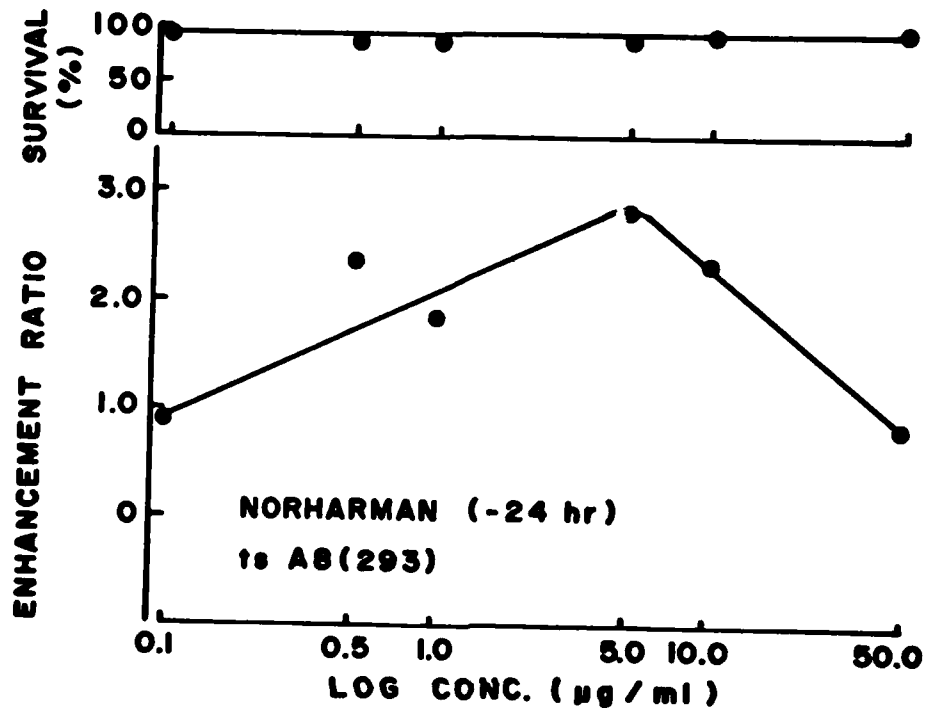


Fig. 4

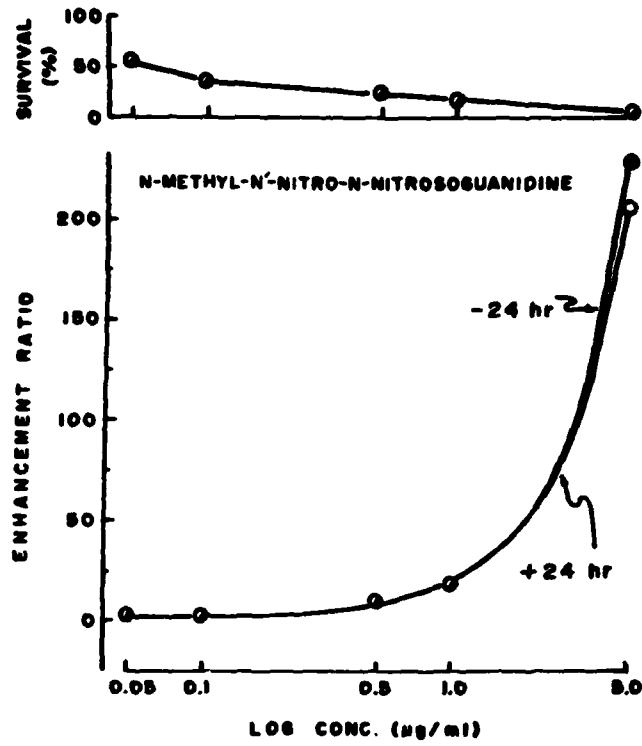


Fig. 5

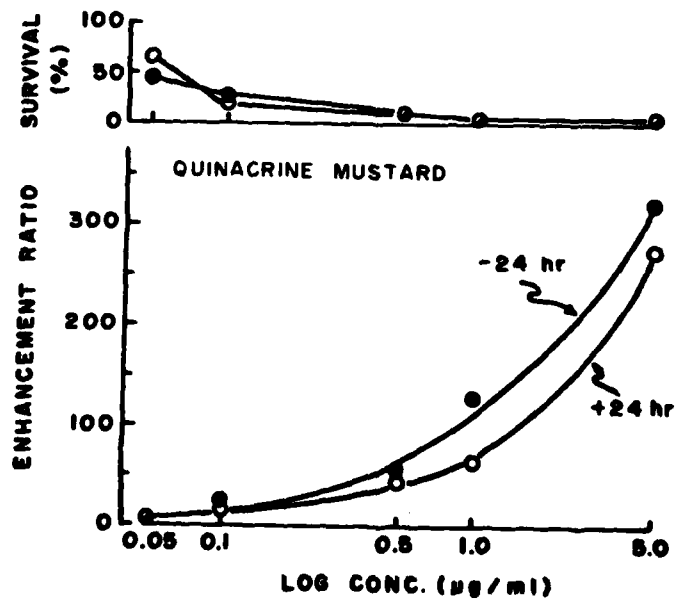


Fig. 6

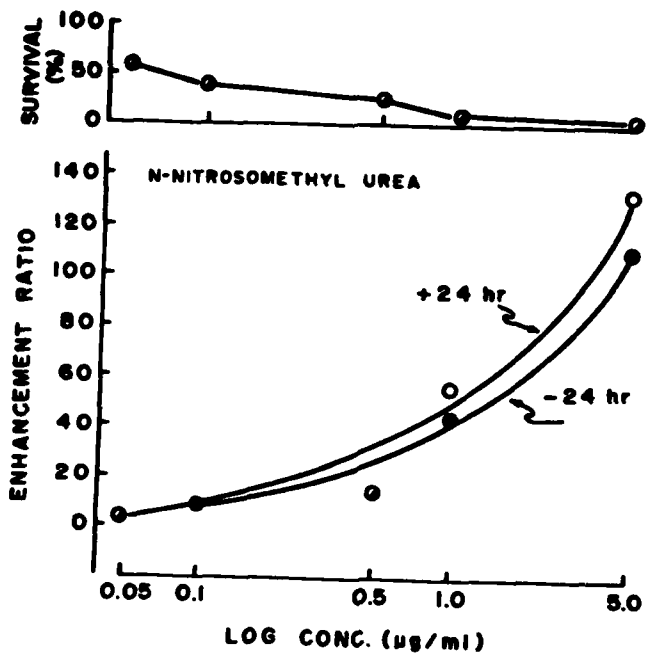


Fig. 7

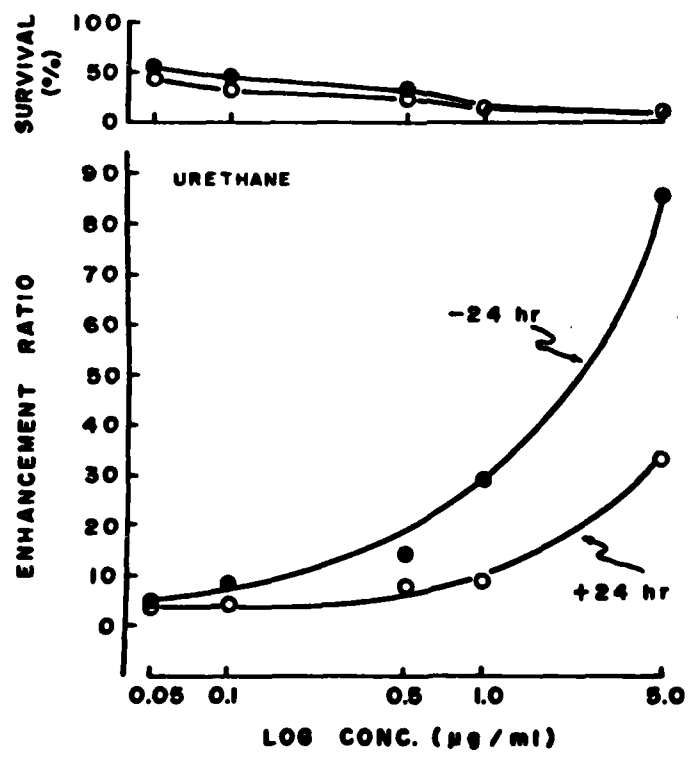


Fig. 8

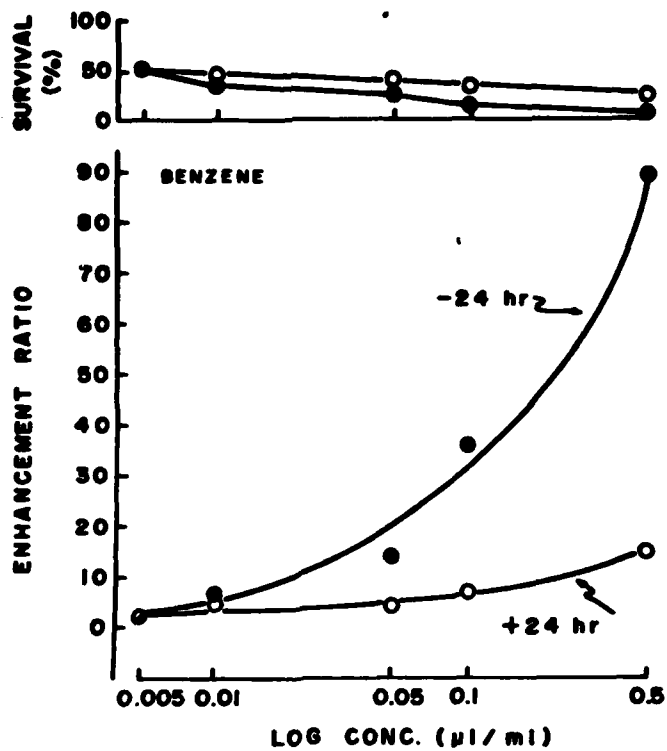


Fig. 9

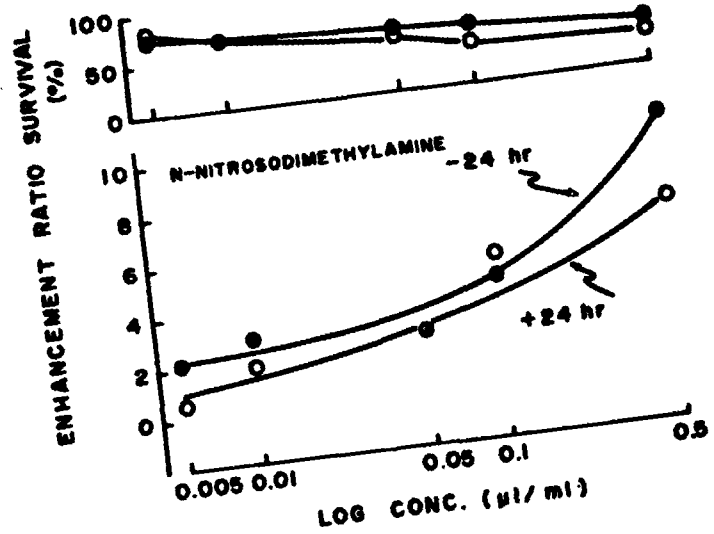


Fig. 10

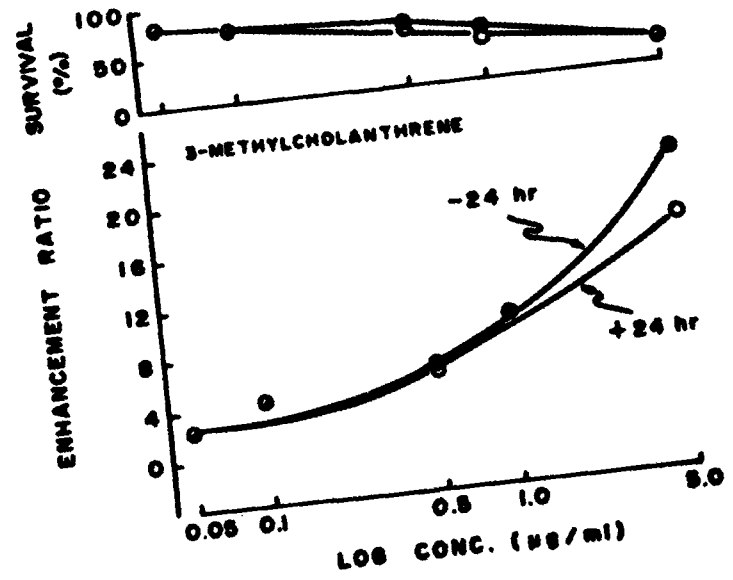


Fig. 11

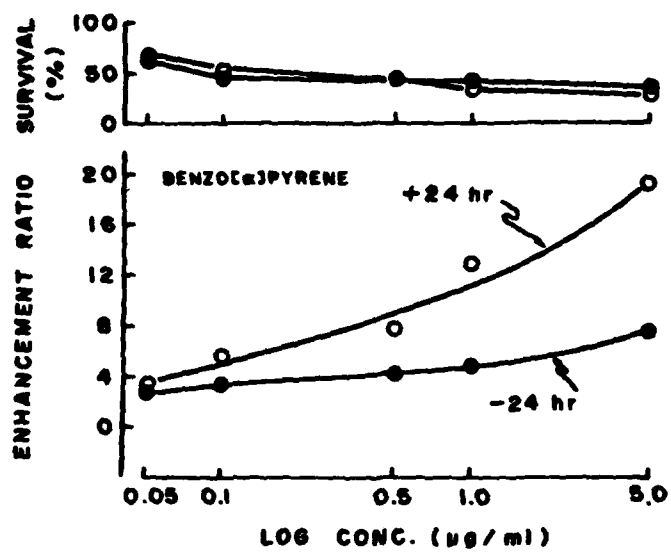


Fig. 12

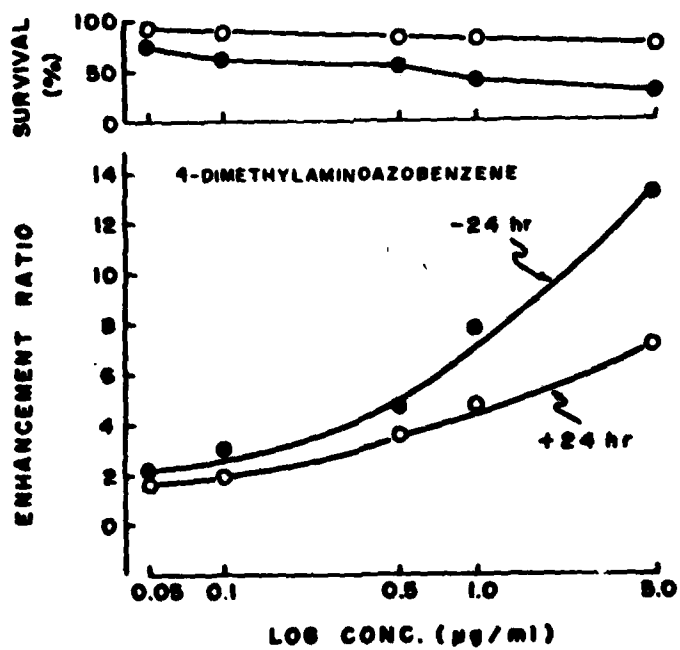


Fig. 13

Table 1

Transformation of 3T3 Cells in Suspension by HSV-2 ts Mutants

Mutant	Non-Infected Control		Moi=1		Moi=5		Moi=10	
	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells
A ₈ (293)	100%	0	100%	31.6 ± 3.8	25%	29.25 ± 12.3	<<10%	2 ± 1.4
B ₅ (155)	100%	0	100%	2.8 ± 3.3	75%	4.6 ± 2.9	50%	7 ± 2.5
C ₂ (116)	100%	0	100%	3.4 ± 1.5	25%	4.25 ± 3.0	10%	3.5 ± 0.7
E ₇ (239)	100%	0	50%	1.67 ± 1.2	25%	2.33 ± 0.6	10%	0
F ₃ (209)	100%	0	25%	6.4 ± 2.9	10%	1.75 ± 1.7	0	0
D ₆ (144)	100%	0	<10%	1.2 ± 1.6	<<10%	0.25 ± 0.5	0	0
G ₄ (148)	100%	0	10%	3.0 ± 0.7	<10%	0.5 ± 0.57	0	0

Table 2

Transformation of 3T3 Cells on Monolayers by HSV-2 ts Mutants

Mutant	Non-infected Control		Moi=1		Moi=5		Moi=10	
	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells	Surviving Monolayer	Foci/10 ⁶ cells
A ₈ (293)	100%	0	100%	8.5 ± 2.1	25%	3.5 ± 0.7	<10%	0
B ₅ (155)	100%	0	100%	1.75 ± 1.3	50%	6.0 ± 2.0	10%	5 ± 5.7
C ₂ (116)	100%	0	10%	4.5 ± 0.7	<10%	0.5 ± 0.7	0	0
E ₇ (239)	100%	0	75%	0.67 ± 0.6	50%	2 ± 1	25%	3.67 ± 2.5
F ₃ (209)	100%	0	10%	4.0 ± 1.0	0	0	0	0
D ₆ (144)	100%	0	<10%	1.0 ± 1.0	0	0	0	0
G ₄ (148)	100%	0	50%	3.0 ± 2.0	10%	0	0	0

Table 3

Mutagenicity of Test Chemicals in THO Cells Following Host-Mediated
Metabolic Activation

Chemical	Time of Activation (min.) ^a	No. Cells Recovered per ml	(Experiment 1)			Mutation Frequency ^d
			No. Cells Seeded ^b	No. Viable Cells Re-covered	No. of Revertant Cells ^c	
dimethylnitrosamine	180	230,000	345,000	37,375	207	5.5×10^{-3}
hydrazine	10	16,000	32,000	5,728	19	3.3×10^{-3}
monomethylhydrazine	10	52,000	104,000	18,754	18	1.0×10^{-3}
1,2-dimethylhydrazine	360	326,000	489,000	19,723	70	3.5×10^{-3}
1,1-dimethylhydrazine	25	100,000	150,000	26,500	123	4.6×10^{-3}
control	360	2,900,000	4,000,000	564,000	2250 est ^e	4.0×10^{-3} est.
(Experiment 2)						
ethylmethane sulfonate	40	406,000	1,015,000	36,337	394	1.1×10^{-2}
JP-5	360	61,000	12,200	3	0	<0.33
JP-10	360	986,000	197,200	39	17	4.4×10^{-1}
RJ-4	360	102,000	81,600	65	159	2.4
RJ-5	360	176,000	105,600	21 est.	34	<1.6
control	360	138,000	165,600	66	226	3.4

^a Time of in vivo exposure of the cells to activated chemical.

^b No. of cells seeded in normal media at the beginning of the expression time.

^c No. of colonies formed in HAT media.

^d Mutation Frequency = No. of mutant cells divided by the no. of viable cells.

^e Estimate

Table 4

Enhancement of HSV-2 wt Transformation by Hydrazine

<u>Time of car- cinogen addition (h)</u>	<u>Hydrazine (μl/ml)</u>	<u>Total colonies per 5000 cells</u>	<u>Surviving fraction</u>	<u>Transformed foci/10⁶ cells</u>	<u>Transform- ation frequency</u>	<u>Enhancement ratio</u>
	0.05	11	0.0045	9	2000	1093
	0.01	112	0.0460	10	217	119
-24	0.005	267	0.1097	7	64	35
	0.001	767	0.3150	7	22	12
	0.0	2435	1.00	3	1.83	1
	0.05	53	0.0222	4	180	98
	0.01	61	0.0256	3	117	64
- 6	0.005	1055	0.4423	3	7	4
	0.001	1215	0.5094	3	6	3
	0.0	2385	1.00	2	1.83	1
	0.05	607	0.2559	2	8	4
	0.01	1498	0.6315	5	8	4
- 2	0.005	1762	0.7428	1	1.3	1
	0.001	1445	0.6092	1	1.6	1
	0.0	2372	1.00	4	1.83	1
	0.05	522	0.1813	0	-	-
	0.01	759	0.2635	0	-	-
+ 2	0.005	1086	0.3771	2	5	3
	0.001	1424	0.4944	4	8	4
	0.0	2880	1.00	1	1.83	1
	0.05	1206	0.4230	6	14	8
	0.01	1336	0.4686	5	11	6
+ 6	0.005	1473	0.5167	2	4	2
	0.001	1874	0.6573	3	5	3
	0.0	2851	1.00	1	1.83	1
	0.05	920	0.3199	0	-	-
	0.01	1514	0.5264	3	6	3
+24	0.005	1774	0.6168	1	1.6	1
	0.001	2024	0.7038	3	4	2
	0.0	2876	1.00	0	1.83	1

Table 5

Enhancement of HSV-2 wt Transformation by SDMH

<u>Time of car- cinogen addition (h)</u>	<u>SDMH ($\mu\text{g/ml}$)</u>	<u>Total colonies per 5000 cells</u>	<u>Surviving fraction</u>	<u>Transformed foci</u>	<u>Transform- action frequency</u>	<u>Enhancement ratio</u>
	5.0	55	0.0195	7	359	433
	1.0	107	0.0379	1	26	31
	0.5	112	0.0396	1	25	30
-24	0.1	111	0.0393	2	51	61
	0.05	165	0.0584	1	17	20
	0	2825	1.00	1	1	1
	5.0	795	0.2689	3	11	13
	1.0	878	0.2970	1	3	4
	0.5	1902	0.6434	2	3	4
-6	0.1	1521	0.5145	3	6	7
	0.05	983	0.3325	1	3	4
	0	2956	1.00	1	1	1
	5.0	1137	0.4227	2	5	6
	1.0	878	0.3264	2	6	7
	0.5	885	0.3290	3	9	11
-2	0.1	1825	0.6784	2	3	4
	0.05	2667	0.9914	1	1	1
	0	2690	1.00	1	1	1
	5.0	1154	0.4473	1	2	2
	1.0	1336	0.5178	1	2	2
	0.5	1497	0.5802	1	2	2
+2	0.1	2180	0.8450	0	-	-
	0.05	2289	0.8872	0	-	-
	0	2580	1.00	1	1	1
	5.0	-	-	1	-	-
	1.0	1078	0.4157	2	5	6
	0.5	-	-	1	-	-
+6	0.1	1142	0.4404	1	2	2
	0.05	1278	0.4929	1	2	2
	0	2593	1.00	0	-	-

Table 5 (cont.)

Enhancement of HSV-2 wt Transformation by SDMH (cont.)

<u>Time of car- cinogen addition (h)</u>	<u>SDMH ($\mu\text{g}/\text{ml}$)</u>	<u>Total colonies per 5000 cells</u>	<u>Surviving fraction</u>	<u>Transformed foci</u>	<u>Transform- action frequency</u>	<u>Enhancement ratio</u>
	5.0	1391	0.4627	1	2	2
	1.0	1810	0.6021	1	2	2
	0.5	1997	0.6643	1	2	2
+24	0.1	2160	0.7186	3	4	5
	0.05	-	-	2	-	-
	0	3006	1.00	1	1	1

Table 6 ENHANCEMENT OF HSV-2 ts Ag(293) TRANSFORMATION OF 3T3 CELLS BY SDMH

Time of carcinogen addition (h)	SDMH ($\mu\text{g/ml}$)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency	Enhancement ratio ^d
-24 ^a	1.0	798	0.9710	41	42.22	3.84
	0.5	744	0.9050	13	14.36	1.31
	0.1	870	1.0584	2	1.89	0.17
	0.05	755	0.9180	11	11.98	1.09
	0	822	1.00	11	11.00	1.00
-6	5.0	714	0.8470	0	-	-
	1.0	614	0.7284	0	-	-
	0.5	542	0.6429	0	-	-
	0.1	476	0.5447	0	-	-
	0	410	0.4864	0	-	-
	0	843	1.00	2	2.00	0.50
-2	5.0	436	0.7415	2	2.70	0.68
	1.0	431	0.7330	2	2.73	0.68
	0.5	404	0.6871	2	2.91	0.73
	0.1	435	0.7398	4	5.41	1.35
	0	503	0.8554	0	-	-
	0	588	1.00	3	3.00	0.75
+2	5.0	566	0.9826	0	-	-
	1.0	591	1.0260	0	-	-
	0.5	725	1.2587	2	1.59	0.40
	0.1	567	0.9844	0	-	-
	0	475	0.8247	1	1.21	0.30
	0	576	1.00	9	9.0	2.25
+6	5.0	658	1.1544	0	-	-
	1.0	475	0.8333	2	2.40	0.60
	0.5	626	1.0982	1	0.91	0.23
	0.1	389	0.6825	3	4.40	1.10
	0	376	0.6596	0	-	-
	0	570	1.00	4	4.00	1.00
+24	5.0	409	0.9761	1	1.02	0.26
	1.0	348	0.8305	2	2.41	0.60
	0.5	429	1.0239	2	1.95	0.49
	0.1	448	1.0692	3	2.81	0.70
	0	459	1.0955	0	-	-
	0	419	1.00	2	2.00	0.50

^aA negative time refers to treatment of the cells with chemical before virus infection. Positive times refer to treatment of the cells after virus infection.

^bNumbers of surviving cells after chemical treatment.

^cNumber of survivors divided by number of colonies in non-treated cultures.

^dNumber of transformed foci per 10⁶ cells divided by the surviving fraction.

^eThe transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

Table 7 ENHANCEMENT OF HSV-2 ts Ag (293) Transformation of 3T3 Cells by Hydrizine

Time of carcinogen addition (h)	HZ (μz/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-24 ^a	0.05	95	0.3276	4	12.21	3.46
	0.01	187	0.6448	7	10.86	3.07
	0.005	341	1.1800	5	4.24	1.20
	0.001	198	0.6828	2	2.93	0.83
-6	0	290	1.00	4	4.0	1.13
	0.05	400	1.3600	1	2.94	0.83
	0.01	454	1.5400	3	3.90	1.10
	0.005	453	1.5400	2	0.97	0.27
-2	0.001	284	0.9659	5	5.18	1.47
	0	294	1.00	0	-	-
	0.05	585	1.2300	4	3.25	0.92
	0.01	471	0.9937	1	1.01	0.29
+2	0.005	416	0.8776	2	2.28	0.65
	0.001	414	0.8734	2	2.29	0.65
	0	474	1.00	3	3.00	0.85
	0.05	472	1.0600	4	3.77	1.06
+6	0.01	471	1.0500	7	6.60	1.87
	0.005	462	1.0400	4	3.85	1.09
	0.001	462	1.0400	0	-	-
	0	445	1.00	2	2.00	0.57
+24	0.05	405	0.8299	0	-	-
	0.01	445	0.9119	6	6.58	1.86
	0.005	522	1.07	2	1.87	0.53
	0.001	450	0.9221	5	5.42	1.53
+24	0	488	1.00	4	4.00	1.13
	0.05	485	0.6860	5	7.29	2.06
	0.01	538	0.7610	6	7.88	2.23
	0.005	555	0.7850	3	3.82	1.08
+24	0.001	525	0.7426	3	4.04	1.14
	0	707	1.00	5	5.00	1.41

^aA negative time refers to treatment of the cells with chemical before virus infection. Positive times refer to treatment of the cells after virus infection.

^bNumbers of surviving cells after chemical treatment.

^cNumber of survivors divided by number of colonies in non-treated cultures.

^dNumber of transformed foci per 10⁶ cells divided by the surviving fraction.

^eThe transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

Table 8 Enhancement of HSV-2 Transformation by Norharman

<u>Time^a</u> <u>(hr)</u>	<u>Conc.</u> <u>($\mu\text{g}/\text{ml}$)</u>	<u>Survivors per</u> <u>5000 cells</u>	<u>Surviving</u> <u>Fraction</u>	<u>Transformed</u> <u>Foci/10^6 cells</u>	<u>Transformation</u> <u>Frequency</u>	<u>Enhancement</u> <u>Ratio</u>
-24	50	535	0.9817	34	34.63	0.84
	10	518	0.9505	92	96.79	<u>2.36</u> ^b
	5	488	0.8954	106	118.38	<u>2.88</u>
	1	481	0.8826	67	75.91	1.85
	0.5	477	0.8752	85	97.12	<u>2.37</u>
	0.1	517	0.9486	35	36.90	0.90
	0	545	1.00	41	41.00	1.00
	N	485		0		
+24	50	344	0.6478	37	57.12	1.44
	10	365	0.6874	35	50.92	1.27
	5	333	0.6271	42	66.97	1.67
	1	417	0.7853	52	66.22	1.66
	0.5	476	0.8964	54	60.24	1.51
	0.1	501	0.9435	34	36.04	0.90
	0	531	1.00	40	40.00	1.00
	N	551		0		

^a Relative time of the addition of the chemical

^b (Double underline) = Significant at the 1% confidence level using Casto values

Table 9 Lack of Enhancement of HSV-2 Transformation by Xylene

<u>Time ^a</u> <u>(hr)</u>	<u>Conc.</u> <u>(μl/ml)</u>	<u>Survivors per</u> <u>5000 cells</u>	<u>Surviving</u> <u>Fraction</u>	<u>Transformed</u> <u>Foci</u>	<u>Transformation</u> <u>Frequency</u>	<u>Enhancement</u> <u>Ratio</u>
-6	0.5	498	1.0873	29	26.67	1.03
	0.1	370	0.8079	20	24.76	0.95
	0.05	484	1.0568	23	21.76	0.84
	0.01	432	0.9432	24	25.45	0.98
	0.005	422	0.9214	24	26.05	1.00
	0	458	1.00	26	26.00	1.00
	N [‡]	414		0		
+6	0.5	259	1.0319	96	93.03	1.11
	0.1	229	0.9124	100	109.60	1.30
	0.05	243	0.9681	80	82.64	0.98
	0.01	210	0.8367	88	105.18	1.25
	0.005	141	0.5618	--	--	--
	0	251	1.00	84	84.00	1.00
	N [‡]	265		0		
-24	0.5	681	0.9799	144	146.95	1.20
	0.1	612	0.8806	149	169.20	1.39
	0.05	669	0.9626	146	151.67	1.24
	0.01	573	0.8245	159	192.84	1.58
	0.005	681	0.9799	159	162.26	1.33
	0	695	1.00	122	122.00	1.00
	N <u>b</u>	736		0		
+24	0.5	365	1.1661	95	81.47	0.94
	0.1	358	1.1438	90	78.69	0.90
	0.05	311	0.9936	79	79.51	0.91
	0.01	322	1.0288	81	78.73	0.90
	0.005	304	0.9712	77	79.28	0.91
	0	313	1.00	87	87.00	1.00
	N <u>b</u>	351		0		

^a Time of addition of xylene

b N = Cell Control (cells exposed to neither chemical nor virus)

Table 10 Lack of Enhancement of HSV-2 Transformation by Toluene

<u>Time ^a</u> <u>(hr)</u>	<u>Conc.</u> <u>(μl/ml)</u>	<u>Survivors per</u> <u>5000 cells</u>	<u>Surviving</u> <u>Fraction</u>	<u>Transformed</u> <u>Foci</u>	<u>Transformation</u> <u>Frequency</u>	<u>Enhancement</u> <u>Ratio</u>
-6	0.5	788	0.9575	13	13.58	1.04
	0.1	866	1.0522	15	14.26	1.10
	0.05	772	0.9380	13	13.86	1.07
	0.01	926	1.1252	13	11.55	0.89
	0	823	1.00	13	13.00	1.00
	<u>N ^b</u>	712			0	
-24	0.5	417	0.4528	7	15.46	0.97
	0.1	438	0.4756	6	12.62	0.79
	0.05	689	0.7481	8	10.69	0.67
	0.01	846	0.9186	9	9.80	0.61
	0	921	1.00	16	16.00	1.00
	<u>N ^b</u>	911			0	

^a Time of addition of toluene

^b N = Cell control (cells exposed to neither chemical, nor virus)

Table 11

Enhancement of HSV-2 Transformation of 3T3 Cells by N-Methyl-N'-Nitro-N-Nitrosoguanidine

Time of addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	5	50	0.0169	3	177.5148	88.76
	1	700	0.2366	2	8.4531	4.23
	0.5	1015	0.3430	4	11.6618	5.83
	0.1	1631	0.5512	2	3.6284	1.82
	0.05	2268	0.7665	4	5.2185	2.61
	0	2959	1.0000	2	2.00	1.00
	N	2997		0		
-6	5	73	0.0283	13	459.3640	91.87
	1	419	0.1623	8	49.2914	9.86
	0.5	491	0.1902	9	47.3186	9.46
	0.1	726	0.2812	7	24.8933	4.98
	0.05	1391	0.5387	5	9.2816	1.86
	0	2582	1.0000	5	5.00	1.00
	N	2549		0		
-24	5	50	0.0175	16	914.2857	228.57 ^f
	1	546	0.1912	13	67.9916	17.00 ^g
	0.5	633	0.2217	9	40.5954	10.15
	0.1	1059	0.3709	5	13.4807	3.37
	0.05	1595	0.5587	7	12.5291	3.13
	0	2855	1.0000	4	4.00	1.00
	N	2850		0		
+2	5	40	0.0136	6	441.1765	147.06
	1	670	0.2280	5	21.9298	7.31
	0.5	991	0.3372	5	14.8280	4.94
	0.1	1339	0.4556	4	8.7796	2.93
	0.05	2251	0.7659	4	5.2226	1.74
	0	2939	1.0000	3	3.00	1.00
	N	2951		0		
+6	5	45	0.0172	19	1104.6512	276.16 ^f
	1	141	0.0539	15	278.2931	69.57 ^g
	0.5	456	0.1742	14	80.3674	20.09 ^g
	0.1	645	0.2465	11	44.6247	11.16
	0.05	1181	0.4513	6	13.2949	3.32
	0	2617	1.0000	4	4.00	1.00
	N	2565		0		
+24	5	52	0.0185	19	1027.0271	205.41 ^f
	1	530	0.1885	18	95.4907	19.10 ^g
	0.5	677	0.2408	14	58.1395	11.63
	0.1	1059	0.3767	7	18.5824	3.72
	0.05	1547	0.5503	5	9.0660	1.82
	0	2811	1.0000	5	5.00	1.00
	N	2839		0		

^a A negative time refers to pre-treatment of the cells with N-methyl-N'-nitro-N-nitrosoguanidine with reference to virus infection. A positive time refers to N-methyl-N'-nitro-N-nitrosoguanidine exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10⁶ cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973).

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 12

Enhancement of MSV-2 Transformation of 3T3 Cells by Quinacrine Mustard

Time of addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving fractions ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	5	365	0.1858	21	113.0248	28.26 ^f
	1	524	0.2667	14	52.4934	13.12 ^g
	0.5	624	0.3176	10	31.4861	7.87
	0.1	893	0.4545	8	17.6018	4.40
	0.05	1254	0.6382	4	6.2676	1.57
	0	1965	1.0000	4	4.00	1.00
	N	2112		0		
-6	5	109	0.0572	14	244.7552	61.19 ^g
	1	364	0.1912	12	62.7615	15.69
	0.5	499	0.2621	10	38.1534	9.54
	0.1	762	0.4002	7	17.4913	4.37
	0.05	983	0.5163	4	7.7474	1.94
	0	1904	1.0000	4	4.00	1.00
	N	1934		0		
-24	5	81	0.0341	22	645.1613	322.58 ^f
	1	165	0.0695	18	258.9928	129.50 ^g
	0.5	288	0.1213	14	115.5163	57.71 ^g
	0.1	689	0.2901	14	48.2592	24.13 ^g
	0.05	1081	0.4552	7	15.3779	7.69
	0	2375	1.0000	2	2.00	1.00
	N	2449		0		
+2	5	125	0.0556	20	359.7122	179.86 ^f
	1	387	0.1722	14	81.3008	40.65 ^g
	0.5	518	0.2305	8	34.7072	17.35
	0.1	943	0.4197	8	19.0612	9.53
	0.05	1704	0.7583	5	6.5937	3.30
	0	2247	1.0000	2	2.00	1.00
	N	2233		0		
+6	5	149	0.0673	16	237.7415	59.44 ^g
	1	424	0.1914	12	62.6959	15.67
	0.5	528	0.2384	12	50.3356	12.58
	0.1	963	0.4348	10	22.9991	5.75
	0.05	1496	0.6754	6	8.8836	2.22
	0	2215	1.0000	4	4.00	1.00
	N	2151		0		
+24	5	44	0.0235	26	1106.3830	276.60 ^f
	1	123	0.0657	18	273.9726	68.49 ^g
	0.5	193	0.1031	19	184.2871	46.07 ^g
	0.1	353	0.1886	11	58.3245	14.58
	0.05	1226	0.6549	7	10.6887	2.67
	0	1872	1.0000	4	4.00	1.00
	N	1757		0		

^a A negative time refers to pre-treatment of the cells with quinacrine mustard with reference to virus infection. A positive time refers to quinacrine mustard exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10⁶ cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973).

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 13

Enhancement of HSV-2 Transformation of 3T3 Cells by N-Nitrosomethyl Urea

Time of addition (h) ^a	Concentration (ug/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells ^d	Transformation frequency ^e	Enhancement ratio ^f
-2	5	419	0.1444	13	90.0277	45.01 ^g
	1	579	0.1995	10	50.1253	25.06
	0.5	741	0.2553	6	23.5018	11.75
	0.1	1162	0.4004	-	-	-
	0.05	1918	0.6609	2	3.0262	1.51
	0	2902	1.0000	2	2.00	1.00
N	2900		0			
-6	5	225	0.0754	10	132.6260	53.16
	1	346	0.1160	10	86.2069	21.55
	0.5	575	0.1928	8	41.4938	10.37
	0.1	821	0.2752	4	14.5349	3.63
	0.05	1917	0.6426	4	6.2247	1.56
	0	2983	1.0000	4	4.00	1.00
N	2925		0			
-24	5	145	0.0520	23	442.3077	110.58 ^f
	1	305	0.1095	20	182.6484	45.66 ^f
	0.5	783	0.2810	16	56.9395	14.23 ^g
	0.1	1061	0.3808	14	36.7647	9.19 ^g
	0.05	1648	0.5915	10	16.9062	4.23
	0	2786	1.0000	4	4.00	1.00
N	2815		0			
+2	5	216	0.0740	14	189.1892	37.84 ^g
	1	346	0.1186	10	84.3170	16.86
	0.5	766	0.2626	4	15.2323	3.05
	0.1	1114	0.3819	2	5.2370	1.05
	0.05	1900	0.6514	3	4.6055	0.92
	0	2917	1.0000	5	5.00	1.00
N	2966		0			
+6	5	143	0.0523	10	191.2046	95.60 ^g
	1	229	0.0837	6	71.6846	35.84
	0.5	389	0.1422	6	42.1941	21.10
	0.1	687	0.2511	4	15.9299	7.96
	0.05	1653	0.6042	2	3.3102	1.66
	0	2736	1.0000	2	2.00	1.00
N	2771		0			
+24	5	151	0.0542	22	405.9041	135.30 ^f
	1	281	0.1009	17	168.4837	56.16 ^g
	0.5	761	0.2732	12	43.9239	14.64 ^g
	0.1	1078	0.3859	12	31.0158	10.39 ^g
	0.05	1559	0.5596	4	7.1480	2.38
	0	2786	1.0000	3	3.00	1.00
N	2807		0			

^a A negative time refers to pre-treatment of the cells with N-nitrosomethyl urea with reference to virus infection. A positive time refers to N-nitrosomethyl urea exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10⁶ cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973).

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 14

Enhancement of MSV-2 Transformation of 3T3 Cells by Urethane

Time of addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2		416	0.1713	12	70.0525	17.51
	5	534	0.2198	8	36.3967	9.10
	1	900	0.3705	6	16.1943	4.05
	0.5	1133	0.4664	4	8.5763	2.14
	0.1	1573	0.6476	6	9.2649	2.32
	0.05	2429	1.0000	4	4.00	1.00
	0	2405		0		
-6	N	353	0.1509	16	106.0305	26.51 ^f
	5	523	0.2257	13	57.5986	14.40 ^g
	1	782	0.3343	10	29.9133	7.48
	0.5	1070	0.4575	6	13.1148	3.28
	0.1	1465	0.6263	6	9.5801	2.40
	0.05	2339	1.0000	4	4.00	1.00
	0	2395		0		
-24	N	254	0.1052	18	171.1027	85.55 ^f
	5	486	0.2013	12	59.6125	29.81 ^g
	1	821	0.3401	10	29.4031	14.70 ^g
	0.5	1145	0.4743	8	16.8670	8.43
	0.1	1291	0.5348	6	11.2191	5.61
	0.05	2414	1.0000	2	2.00	1.00
	0	2393		0		
+2	N	191	0.0846	17	200.9456	50.24 ^g
	5	474	0.2099	14	66.6984	16.67 ^g
	1	616	0.2728	7	25.6598	6.41
	0.5	711	0.3149	8	25.4049	6.35
	0.1	1191	0.5275	4	7.5829	1.90
	0.05	2258	1.0000	4	4.00	1.00
	0	2126		0		
+6	N	210	0.1016	9	88.5827	44.29
	5	496	0.2400	12	50.0000	25.00
	1	729	0.3527	6	17.0116	8.51
	0.5	835	0.4040	3	7.4257	3.71
	0.1	967	0.4678	2	4.2753	2.14
	0.05	2067	1.0000	2	2.00	1.00
	0	2209		0		
+24	N	223	0.1208	16	132.4503	33.11 ^g
	5	416	0.2254	8	35.4925	8.87
	1	457	0.2476	8	32.3102	8.08
	0.5	590	0.3196	6	18.7735	4.69
	0.1	788	0.4269	6	14.0548	3.51
	0.05	1846	1.0000	4	4.00	1.00
	0	1962		0		

^a A negative time refers to pre-treatment of the cells with urethane with reference to virus infection. A positive time refers to urethane exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10⁶ cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973)

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 15

Enhancement of HSV-2 Transformation of 3T3 Cells by Benzene

Time of addition (h) ^a	Concentration (μ l/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/ 10^6 cells ^d	Transformation frequency ^e	Enhancement ratio ^f
-2	0.5	384	0.1488	6	40.3226	20.16
	0.1	523	0.2026	6	29.6150	14.81
	0.05	913	0.3537	3	8.4818	4.24
	0.01	1286	0.4983	1	2.0068	1.00
	0.005	1629	0.6312	3	4.7529	2.38
	0	2581	1.0000	2	2.00	1.00
N		2621		0		
-6	0.5	532	0.1804	5	27.7162	6.93
	0.1	765	0.2594	7	26.9854	6.75
	0.05	990	0.3357	6	17.8731	4.47
	0.01	1443	0.4893	3	6.1312	1.53
	0.005	1606	0.5446	2	3.6724	0.92
	0	2949	1.0000	4	4.00	1.00
N		2965		0		
-24	0.5	218	0.0785	14	178.3440	89.17 ^g
	0.1	460	0.1657	12	72.4200	36.21 ^g
	0.05	746	0.2687	8	29.7730	14.87
	0.01	979	0.3527	5	14.1764	7.09
	0.005	1398	0.5036	2	3.9714	1.99
	0	2776	1.0000	2	2.00	1.00
N		2878		0		
+2	0.5	1085	0.2665	16	60.0375	12.01 ^g
	0.1	1514	0.3718	13	34.9650	6.99
	0.05	1781	0.4374	14	32.0073	6.40
	0.01	1951	0.4791	6	12.5235	2.50
	0.005	2203	0.5410	8	14.7874	2.96
	0	4072	1.0000	5	5.00	1.00
N		4139		0		
+6	0.5	1099	0.2643	22	83.2387	10.40 ^g
	0.1	1510	0.3632	19	52.3128	6.54
	0.05	1724	0.4146	13	31.3555	3.92
	0.01	2000	0.4810	13	27.0270	3.38
	0.005	2194	0.5277	12	22.7402	2.84
	0	4158	1.0000	8	8.00	1.00
N		4082		0		
+24	0.5	1103	0.2546	19	74.6269	14.93 ^g
	0.1	1501	0.3465	13	37.5180	7.50
	0.05	1756	0.4054	8	19.7336	3.95
	0.01	1958	0.4520	10	22.1235	4.42
	0.005	2169	0.5007	5	9.9860	2.00
	0	4332	1.0000	5	5.00	1.00
N		4106		0		

^a A negative time refers to pre-treatment of the cells with benzene with reference to virus infection. A positive time refers to benzene exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10^6 cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973)

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 16

Enhancement of HSV-2 Transformation of 3T3 Cells by N-Nitrosodimethylamine

Time of Addition (h) ^a	Concentration (μl/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	0.5	2042	0.8318	4	4.8088	1.60
	0.1	2112	0.8603	4	4.6495	1.55
	0.05	2172	0.8847	3	3.3909	1.13
	0.01	2243	0.9136	6	6.5674	2.19
	0.005	2382	0.9703	-	-	-
	0	2455	1.0000	3	3.00	1.00
	N	2473		0		
-6	0.5	1000	0.4355	12	27.5545	9.16 ^g
	0.1	1235	0.5379	10	18.5908	6.20
	0.05	1315	0.5727	6	10.4767	3.49
	0.01	1418	0.6176	5	8.0959	2.70
	0.005	1539	0.6703	6	8.9512	2.98
	0	2296	1.0000	3	3.00	1.00
	N	2358		0		
-24	0.5	970	0.4628	23	49.6975	9.94 ^f
	0.1	1225	0.5844	13	22.2450	4.45
	0.05	1369	0.6531	8	12.2493	2.45
	0.01	1425	0.6799	10	14.7080	2.94
	0.005	1546	0.7376	8	10.8460	2.17
	0	2096	1.0000	5	5.00	1.00
	N	2107		0		
+2	0.5	1289	0.5206	12	23.0503	7.68 ^g
	0.1	1460	0.5897	6	10.1747	3.39
	0.05	1581	0.6385	6	9.3970	3.13
	0.01	1745	0.7048	7	9.9319	3.31
	0.005	2087	0.8429	6	7.1183	2.37
	0	2476	1.0000	3	3.00	1.00
	N	2467		0		
+6	0.5	596	0.2935	16	54.5145	18.12 ^g
	0.1	785	0.3865	13	33.6352	11.21 ^g
	0.05	1211	0.5963	6	10.0620	3.35
	0.01	1419	0.6987	4	5.7249	1.91
	0.005	1614	0.7947	3	3.7750	1.26
	0	2031	1.0000	3	3.00	1.00
	N	1966		0		
+24	0.5	555	0.3231	13	40.2352	6.71
	0.1	691	0.4022	13	32.3222	5.39
	0.05	921	0.5361	8	14.9226	2.49
	0.01	1200	0.6985	8	11.4531	1.91
	0.005	1424	0.8289	3	3.6193	0.60
	0	1718	1.0000	6	6.00	1.00
	N	1712		0		

^a A negative time refers to pre-treatment of the cells with N-nitrosodimethylamine with reference to virus infection. A positive time refers to N-nitrosodimethylamine exposure after virus infection.

^b Numbers of surviving cells after chemical treatment.

^c Numbers of cell survivors divided by the number of colonies in non-treated cultures.

^d Number of transformed foci per 10⁶ cells divided by the surviving fraction.

^e The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^f Significant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973)

^g Significant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 17

Enhancement of HSV-2 Transformation of 3T3 Cells by 3-Methylcholanthrene

Time of Addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	5	1740	0.6954	12	17.2563	4.31
	1	1804	0.7210	9	12.4827	3.12
	0.5	1863	0.7446	10	13.4300	3.36
	0.1	1917	0.7662	6	7.8309	1.96
	0.05	2019	0.8070	6	7.4349	1.86
	0	2502	1.0000	4	4.00	1.00
	N	2438		0		
-6	5	28	0.0511	20	391.3894	130.46 ^f
	1	44	0.0803	20	249.0660	83.02 ^f
	0.5	49	0.0894	12	134.2281	44.74 ^g
	0.1	70	0.1277	13	101.8011	33.93 ^g
	0.05	121	0.2208	6	27.1739	9.06
	0	548	1.0000	3	3.00	1.00
	N	447		0		
-24	5	533	0.2838	24	84.5666	21.14 ^f
	1	985	0.5245	20	38.1316	9.53 ^f
	0.5	1220	0.6496	16	24.6305	6.16 ^g
	0.1	1417	0.7545	13	17.2210	4.31
	0.05	1513	0.8056	8	9.9305	2.48
	0	1878	1.0000	4	4.00	1.00
	N	1888		0		
+2	5	1416	0.5717	11	19.2409	4.81
	1	1510	0.6096	12	19.6850	4.92
	0.5	1675	0.6762	8	11.8303	2.96
	0.1	1814	0.7323	11	15.0217	3.76
	0.05	1961	0.7917	8	10.1048	2.53
	0	2477	1.0000	4	4.00	1.00
	N	2562		0		
+6	5	813	0.4087	20	48.9357	8.16 ^g
	1	1012	0.5088	14	27.5157	4.59
	0.5	1098	0.5520	12	21.7391	3.62
	0.1	1195	0.6008	12	19.9700	3.33
	0.05	1492	0.7501	8	10.6652	1.78
	0	1989	1.0000	6	6.00	1.00
	N	1998		0		
+24	5	880	0.3419	22	64.3463	16.09 ^f
	1	1046	0.4064	16	39.3701	9.84 ^g
	0.5	1469	0.5707	12	21.0268	5.26
	0.1	1862	0.7234	12	16.5883	4.15
	0.05	2012	0.7817	8	10.2341	2.56
	0	2574	1.0000	4	4.00	1.00
	N	2545		0		

^aA negative time refers to pre-treatment of the cells with 3-methylcholanthrene with reference to virus infection. A positive time refers to 3-Methylcholanthrene exposure after virus infection.

^bNumbers of surviving cells after chemical treatment.

^cNumbers of cell survivors divided by the number of colonies in non-treated cultures.

^dNumber of transformed foci per 10⁶ cells divided by the surviving fraction.

^eThe transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^fSignificant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973).

^gSignificant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 18

Enhancement of HSV-2 Transformation of 3T3 Cells by Benzo[*a*]pyrene

Time of Addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	5	1227	0.4982	12	24.0867	12.04 ^g
	1	1265	0.5136	6	11.6822	5.84
	0.5	1380	0.5603	6	10.7106	5.36
	0.1	1492	0.6056	4	6.6050	3.30
	0.05	1824	0.7406	3	4.0508	2.03
	0	2463	1.0000	2	2.00	1.00
	N	2421		0		
-6	5	923	0.3877	16	41.2690	13.76 ^g
	1	1004	0.4217	10	23.7135	7.90
	0.5	992	0.4166	9	21.6035	7.20
	0.1	1263	0.5304	9	16.9683	5.66
	0.05	1538	0.6459	6	9.2894	3.10
	0	2381	1.0000	3	3.00	1.00
	N	2356		0		
-24	5	975	0.3978	24	60.3318	7.54 ^f
	1	1057	0.4313	17	39.4157	4.93
	0.5	1166	0.4757	16	33.6346	4.20
	0.1	1210	0.4937	13	26.3318	3.29
	0.05	1531	0.6246	12	19.2123	2.40
	0	2451	1.0000	8	8.00	1.00
	N	2401		0		
+2	5	902	0.3655	12	32.8317	10.94 ^g
	1	1083	0.4388	10	22.7894	7.60
	0.5	1305	0.5288	9	17.0197	5.67
	0.1	1365	0.5531	4	7.2320	2.41
	0.05	1626	0.6588	4	6.0716	2.02
	0	2468	1.0000	3	3.00	1.00
	N	2466		0		
+6	5	690	0.3103	13	41.8949	10.47 ^g
	1	1157	0.5202	12	23.0681	5.77
	0.5	1196	0.5378	9	16.7348	4.18
	0.1	1350	0.6070	8	13.1796	3.29
	0.05	1503	0.6758	8	11.8378	2.96
	0	2224	1.0000	1	4.00	1.00
	N	2244		0		
+24	5	513	0.2751	21	76.3359	19.08 ^f
	1	630	0.3378	18	53.2860	13.32 ^f
	0.5	779	0.4177	13	31.1228	7.78 ^g
	0.1	1020	0.5469	12	21.9419	5.49
	0.05	1319	0.7072	9	12.7262	3.18
	0	1865	1.0000	4	4.00	1.00
	N	1911		0		

^aA negative time refers to pre-treatment of the cells with benzo[*a*]pyrene with reference to virus infection. A positive time refers to benzo[*a*]pyrene exposure after virus infection.

^bNumbers of surviving cells after chemical treatment.

^cNumbers of cell survivors divided by the number of colonies in non-treated cultures.

^dNumber of transformed foci per 10⁶ cells divided by the surviving fraction.

^eThe transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^fSignificant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973)

^gSignificant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

Table 19

Enhancement of HSV-2 Transformation of 3T3 Cells by p-Dimethylaminoazobenzene

Time of Addition (h) ^a	Concentration (µg/ml)	Total colonies per 5000 cells ^b	Surviving Fraction ^c	Transformed foci/10 ⁶ cells	Transformation frequency ^d	Enhancement ratio ^e
-2	5	526	0.4586	12	26.1666	6.54
	1	670	0.5841	8	13.6963	3.42
	0.5	749	0.6530	8	12.2511	3.06
	0.1	841	0.7332	7	9.5472	2.39
	0.05	959	0.8361	5	5.9801	1.50
	0	1147	1.0000	4	4.00	1.00
N	1157			0		
-6	5	59	0.0465	23	494.6237	123.66 ^f
	1	167	0.1315	17	129.2776	32.32 ^g
	0.5	433	0.3409	15	44.0012	11.00 ^g
	0.1	662	0.5213	10	19.1828	4.80
	0.05	897	0.7063	6	8.4950	2.12
	0	1270	1.0000	4	4.00	1.00
N	1247			0		
-24	5	177	0.3031	24	79.1818	13.20 ^f
	1	247	0.4229	20	47.2925	7.88 ^g
	0.5	331	0.5668	16	28.2287	4.70 ^g
	0.1	367	0.6284	12	19.0961	3.1 ^e
	0.05	427	0.7312	10	13.6761	2.28
	0	584	1.0000	6	6.00	1.00
N	622			0		
+2	5	255	0.2727	12	44.0044	11.00
	1	459	0.4909	10	20.3707	5.09
	0.5	558	0.5968	8	13.4048	3.35
	0.1	634	0.6781	8	11.7977	2.95
	0.05	757	0.8096	5	6.1759	1.54
	0	935	1.0000	4	4.00	1.00
N	1011			0		
+6	5	357	0.3225	16	49.6124	12.40 ^g
	1	544	0.4914	14	28.4900	7.12 ^g
	0.5	642	0.5799	10	17.2444	4.31
	0.1	754	0.6811	8	11.7457	2.94
	0.05	863	0.7796	4	5.1308	1.28
	0	1107	1.0000	4	4.00	1.00
N	1069			0		
+24	5	1977	0.7681	22	28.6421	7.16 ^f
	1	2140	0.8314	16	19.2446	4.81 ^g
	0.5	2170	0.8430	12	14.2349	3.56
	0.1	2313	0.8986	7	7.7899	1.95
	0.05	2390	0.9285	6	6.4620	1.62
	0	2574	1.0000	4	4.00	1.00
N	2545			0		

^aA negative time refers to pre-treatment of the cells with 4-dimethylaminoazobenzene with reference to virus infection. A positive time refers to 4-dimethylaminoazobenzene exposure after virus infection.

^bNumbers of surviving cells after chemical treatment.

^cNumbers of cell survivors divided by the number of colonies in non-treated cultures.

^dNumber of transformed foci per 10⁶ cells divided by the surviving fraction.

^eThe transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

^fSignificant enhancement at the 1% confidence level using Casto values (Casto *et al.*, 1973).

^gSignificant enhancement at the 5% confidence level using Casto values.

N = Cell control, cells exposed to neither virus nor chemical.

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