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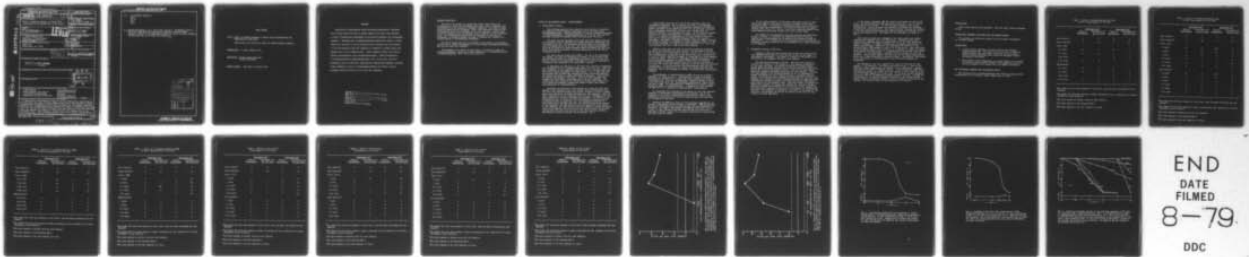
BRIGHAM YOUNG UNIV PROVO UTAH DEPT OF MICROBIOLOGY F/G 6/13  
EFFECT OF CHEMICAL MUTAGENS ON HERPES VIRUS TRANSFORMATION AND --ETC(U)  
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AFOSR-TR-79-0829

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19 REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER  
**AFOSR TR-79-0829**

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)  
**Effect of Chemical Mutagens on Herpes Virus Transformation and Backmutation of THO Cells**

5. TYPE OF REPORT & PERIOD COVERED  
**Final Report  
1 May 78 - 30 April 79**

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)  
**F. Brent Johnson Ph.D.**

8. CONTRACT OR GRANT NUMBER(s)  
**AFOSR-78-3686**

9. PERFORMING ORGANIZATION NAME AND ADDRESS  
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10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS  
**61102F  
2312/D9**

11. CONTROLLING OFFICE NAME AND ADDRESS  
**AFOSR (NL)  
Bolling AFB, D.C. 20332**

12. REPORT DATE  
**June 1979**

13. NUMBER OF PAGES  
**23**

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)  
**UNCLASSIFIED**

16. DISTRIBUTION STATEMENT (of this Report)  
**Approved for public release;  
distribution unlimited.**

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

**DDC  
RECEIVED  
JUL 9 1979  
RESOLVED  
A**

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

- transformation
- herpes simplex virus type II
- transformation enhancement
- mutagenesis testing in THO cells
- ethylmethanesulfonate
- 3-methylcholanthrene
- Hydrazine
- monomethyl hydrazine
- 1,1-dimethylhydrazine (over)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Quantitative transformation assays detecting morphological transformation of Swiss mouse 3T3 cells by herpes simplex virus type 2 were employed to detect transformation enhancement by potentially mutagenic and carcinogenic chemicals. Hydrazine and 1,2-dimethylhydrazine enhanced the number of transformed foci from two to two and one-half fold in cultures that were exposed to both UV-irradiated virus and chemicals as compared to cultures which were exposed only to the irradiated virus. Other chemicals tested, that did not enhance transformation, were ethylmethanesulfonate, 3-methylcholanthrene, 1,1-dimethylhydrazine.

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19. • 1,2-dimethyl hydrazine  
• JP-5  
• JP-10  
• RJ-4  
• RJ-5

20. → monomethylhydrazine, JP-5, JP-10, RJ-4, and RJ-5. Mutagenesis tests in THO cells, employing the nonactivated compounds, detected slight mutagenic activity of ethylmethanesulfonate but failed to detect mutagenic activity with any of the other test compounds.

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FINAL REPORT

TITLE: Effect of Chemical Mutagens on Herpes Virus Transformation and Backmutation of THO Cells

(This project was carried out under the AFOSR minigrant program.)

INVESTIGATOR: F. Brent Johnson, Ph.D.

INSTITUTION: Brigham Young University  
Provo, Utah 84602

PROJECT PERIOD: 1 May 1978 to 30 April 1979



## ABSTRACT

Quantitative transformation assays detecting morphological transformation of Swiss mouse 3T3 cells by herpes simplex virus type 2 were employed to detect transformation enhancement by potentially mutagenic and carcinogenic chemicals. Hydrazine and 1,2-dimethylhydrazine enhanced the number of transformed foci from two to two and one-half fold in cultures that were exposed to both UV-irradiated virus and chemicals as compared to cultures which were exposed only to the irradiated virus. Other chemicals tested, that did not enhance transformation, were ethylmethanesulfonate, 3-methylcholanthrene, 1,1-dimethylhydrazine, monomethylhydrazine, JP-5, JP-10, RJ-4, and RJ-5. Mutagenesis tests in THO cells, employing the nonactivated compounds, detected slight mutagenic activity of ethylmethanesulfonate but failed to detect mutagenic activity with any of the other test compounds.

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RESEARCH OBJECTIVES:

The work to be carried out during this project will involve the utilization of a Swiss 3T3 cell-herpes virus type 2 model to measure viral transformation in the presence of mutagenic chemicals. Two types of transformation assays will be performed. First, cells previously infected with UV-irradiated virus will be exposed to the test chemicals. Second, cells previously exposed to the test chemical will be infected with UV-irradiated virus. The transformation levels will be determined in both types of experiments to ascertain whether the order of exposure may influence the ultimate degree of transformation enhancement.

The second aspect that will be examined in this project is the ability of THO cells to register mutagenesis of a known mutagen (Ethylmethanesulfonate, EMS) and the other test chemicals.

The compounds to be tested in this project, in addition to EMS, are 3-methylcholanthrene, hydrazine, monomethylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, JP-5, JP-10, RJ-4, and RJ-5.

STATUS OF THE RESEARCH EFFORT: ACCOMPLISHMENTS.

A. Transformation Assays.

The following chemicals were tested for their activity in enhancing the formation of HSV-2-induced transformed foci in 3T3 mouse cells: ethylmethanesulfonate, 3-methylcholanthrene, hydrazine, monomethylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, JP-5, JP-10, RJ-4 and RJ-5. The assay utilized UV-irradiated HSV-2 and Swiss mouse 3T3 cells.

The cells utilized throughout this study were Swiss albino mouse 3T3 cells obtained from the American Type Culture Collection (Rockville, MD) at passage level 118. All experiments described here were performed with cells at passage levels 124 to 128. The culture medium used throughout the study was Dulbecco's Modified Eagles medium containing 10% fetal calf serum, 0.1% sodium bicarbonate, 100 units penicillin per ml and 100  $\mu$ g streptomycin per ml.

The virus used was strain 333 of HSV-2. For use in the transformation assays, the virus was irradiated as follows: 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/mm<sup>2</sup> of UV light for 2 minutes, agitated for 30 seconds, then irradiated for an additional 2 minutes.

In experiment one (protocol 1), 10<sup>6</sup> cells were infected in suspension with irradiated virus at 5 plaque-forming units (pfu) per cell. After one hour incubation at room temperature the cells were distributed among four 60 mm petri dishes containing 4 ml normal media. After 6 hours the cells had attached and the media were renewed with media containing chemicals (at various dilutions), or media containing no chemicals. Each dilution of chemical was exposed to 10<sup>6</sup> cells. After four days the media were changed with fresh media, containing none of the test chemicals. The cultures were then incubated for 28 days with weekly media changes, the cells fixed with formalin, stained with Wright's stain, then counted microscopically for transformed foci.

Experiment two (protocol 2) was carried out so that the cells were exposed to the chemicals first, then infected with UV-inactivated virus. 10<sup>6</sup> cells were distributed among four plates, allowed to attach in normal media, then exposed to the media containing the test chemicals. After 24 hours' exposure to the test chemicals the cells were challenged with virus, then incubated for 28 days with weekly media changes. At the end of the incubation period, the cells were fixed in formalin, stained and scored as previously done in experiment one. All experiments included (1) some dishes which contained cells that were neither infected nor exposed to test chemicals, (2) some cultures that were exposed to virus and not to the chemicals, (3) some cultures that were exposed to various concentrations of the chemical, and not to the virus, and of course, (4) the test systems where the cells were exposed to both virus and test chemicals. The results are shown in Tables 1-10 and Figures 1 and 2.



These results show that 8 of the 10 test chemicals, under the experimental conditions, did not enhance the level of virus-mediated cellular transformation. Two of the chemicals, hydrazine and SDMH, did enhance the number of viral-mediated foci (2-2 1/2 fold) and it appears that a synergistic effect does, in fact, occur between these two chemicals and HSV-2 in transforming 3T3 cells. In the tables the blanks indicate that those concentrations of chemical were not tested. The dashes ("-") indicate the appearance of 4+ viral cytopathic effects so that the cell sheet was completely destroyed and no foci could be determined. The degree of cytopathic effects (viral or chemical induced) was judged as follows: 0 indicates no visible cytopathic effect, + = 25% of the cells affected, ++ = 50% of the cells affected, +++ = 75% of the cells affected, and ++++ = 100% of the cells affected.

Figures 1 and 2 show the plots of the data obtained in experiment two on the two chemicals that were positive. The mean number of foci per  $10^6$  cells in the virus-infected controls was  $16 \pm 2.8$ . It was found that concentrations of hydrazine at and above  $0.005 \mu\text{l/ml}$  (equivalent to  $1 \mu\text{l}$  per  $10^6$  cells) enhanced the number of transformed foci which appeared on the cell sheets. The level of enhancement reached approximately two fold and diminished somewhat at higher concentrations of hydrazine. Concentrations of hydrazine greater than  $0.05 \mu\text{l}$  per ml (equivalent to  $1.0 \mu\text{l}$  per  $10^6$  cells) were overtly cytotoxic to the 3T3 cells and destroyed the monolayer. It can be seen from this dose-response curve that low concentrations of hydrazine did not enhance the virally-mediated transformation, that is the synergistic activity of hydrazine was concentration dependent.

The enhancement of transformation by SDMH, also in the protocol 2 experiment, is shown in Fig. 2. Concentrations of SDMH at and above  $100 \text{ ng per ml}$  (equivalent to  $2 \mu\text{g}$  per  $10^6$  cells) enhanced the number of transformed foci. The level of enhancement reached a maximum of about 2 1/2 fold at  $500 \text{ ng per ml}$ , then gradually diminished at higher concentrations of SDMH. Low concentrations of SDMH failed to enhance significantly the number of transformed foci. It appears that both hydrazine and SDMH can act as enhancers in promoting HSV-2 transformation.

No transformed foci developed in the absence of virus and chemicals; therefore, no spontaneous transformation was detected. All of the chemicals at all of the concentrations tested failed to induce transformation by themselves, as no foci were detected where the cells were not co-exposed to the virus.

Hydrazine and SDMH were tested in three different experiments: two protocol 1 experiments and one protocol 2 experiment. SDMH was positive in all three experiments and its peak activity has been consistently at  $500 \text{ ng/ml}$ . The range of enhancement with SDMH has been from 2 1/2 to 5 fold. To date, hydrazine has been positive only in the protocol 2 experiment, perhaps, because of its greater cytotoxicity to non-attached 3T3 cells (data not shown).



All the other chemicals (listed above) which were tested in these assays were negative, and did not increase the number of foci above the control level. If anything, ethylmethanesulfonate and RJ-4 may have slightly inhibited transformation. It should be noted that the assays in which JP-5, JP-10, RJ-4, and RJ-5 were tested, detected primarily only those components which were soluble in the cell culture media.

The results of the experiments with both hydrazine and SDMH (shown in Fig. 1 and Fig. 2) illustrate the diminishing activity that was observed as the concentration of the chemical was increased beyond its peak activity. It is likely that as the concentration of the chemical is increased, a greater proportion of the susceptible cells become inactivated by the chemical.

#### B. Mutagenesis Testing in THO Cells.

Cultures of THO cells were treated with various dose levels of the test chemicals and scored for overt toxicity as well as the number of cell mutants (revertants). The number of colonies in HAT medium is an indication of the mutagenic activity of the chemical.

The test was carried out as follows: THO cells were planted in either 25 cm<sup>2</sup> flasks or 75 cm<sup>2</sup> flasks at a cell density of 500,000 to 2 x 10<sup>6</sup> cells per flask. The medium was Dulbecco's modified Eagle's Medium (DME) containing 10% fetal calf serum and 100 units of penicillin per ml and 100 µg of streptomycin per ml. After 24 hrs, the initial medium was replaced with medium containing the test compound. The cells were thus exposed to the potential mutagen for 24 hrs, whereupon the mutagen was washed out with a sterile solution of phosphate buffered saline. These cultures were incubated in fresh media for two days, then replated at a dilution of 1:10 and incubated further for eight more days. Following this expression period, the cells were plated in HAT medium which selects for the revertant cells. After four weeks' incubation with weekly media changes, the flasks were examined for revertant colonies. Replicate cultures were produced for each dilution of test chemical and were plated after exposure of the cells to the chemical to determine the toxicity of the chemical as reflected by its effect on the number of cell survivors: the efficiency of plating (or ability of the cells to form colonies in normal DME media).

In an initial experiment, EMS was tested for mutagenic activity in THO cells. The concentrations of EMS tested were 0.001, 0.01, 0.1, 1.0 and 10 microliters per ml of media.  $1 \times 10^6$  cells were exposed to EMS at each concentration. Control cultures were included to determine the efficiency of plating of non-treated cells. The "cell survivors" represent the fraction of the cell population which survived chemical treatment as compared to the control non-treated cells. The results of this experiment are shown in Fig. 3.

These results showed that a maximum of only three mutants per million original cells appeared (at 1.0  $\mu$ l EMS per ml). Ordinarily these cells should yield a maximum number of mutants when 50% of the cells survive the chemical treatment. At higher concentrations of mutagen, many mutants, in theory, are destroyed. Therefore, this experiment was modified to test concentrations of EMS between 0.1 and 1.0  $\mu$ l of EMS per ml. Also, more cells in each original cultures were treated so as to enhance the number of mutants. These results are shown in Fig. 4. Disappointingly, more mutant cells failed to appear. In this experiment, a maximum of only one mutant per  $10^6$  cells appeared. These results suggest that THO cells are not highly mutagenized by EMS so that a high percentage of revertants appear. Nevertheless, some mutant cells were found so it was determined to test the other compounds for mutagenesis.

The remainder of the test compounds were tested as described for EMS against  $1 \times 10^6$  THO cells. The results are shown in Fig. 5. None of the compounds yielded demonstrable cell mutants at any concentration. However, there was a wide range of chemical toxicity. Of the hydrazines, UDMH, MMH and hydrazine itself, as a group, were very similar in their toxicity to THO cells while SDMH appeared to be less toxic. Of the water soluble components of the jet fuels, JP-10 appeared to exhibit greatest toxicity. Although the significance of the negative mutagenic responses of the compounds tested remains somewhat in question because of the low response in the positive EMS controls, the toxicity levels of the compounds are of interest.

**PUBLICATIONS:**

None of this work has been published. This will await further experimentation.

**PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH EFFORT:**

This project was carried out entirely by the principal investigator, Dr. F. Brent Johnson.

**INTERACTIONS:**

1. A visit with Dr. Ken Back on 26 April 1978 at Toxic Hazards, Wright-Patterson AFB. The nature of the visit was to obtain the chemicals which were tested under this grant and we discussed various problems regarding their testing for mutagenic activity and metabolic activation.
2. Participation in and presentation of a paper (report) at the Review of Air Force Sponsored Basic Research in Environmental Protection and Toxic Hazards, 16-17 January 1979, Los Angeles, California.

**NEW DISCOVERIES STEMMING FROM THE RESEARCH EFFORT:**

That hydrazine and 1,2-dimethylhydrazine will enhance in vitro cellular transformation by the oncogenic herpes simplex type II virus.



TABLE 1. Effect of Ethylmethansulfonate (EMS)  
on HSV-2 Transformation of 3T3 Cells

	Experiment One <sup>a</sup>		Experiment Two <sup>b</sup>	
	Chemical Cytotoxicity	Transformed foci per 10 <sup>6</sup> cells	Chemical Cytotoxicity	Transformed foci per 10 <sup>6</sup> cells
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + EMS				
0.5 µl/ml	++	-	++	5
0.1 µl/ml	0	21	+	4
0.05 µl/ml			0	6
0.01 µl/ml	0	8	0	4
EMS Controls <sup>e</sup>				
0.5 µl/ml	++	0	++	0
0.1 µl/ml	0	0	+	0
0.05 µl/ml			0	0
0.01 µl/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.



TABLE 2. Effect of 3-methylcholanthrene (MCA) on HSV-2 Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + MCA				
50 µg/ml	++	12	++++	0
5 µg/ml	0	16	++	0
1 µg/ml	0	14	0	6
0.5 µg/ml	0	8	0	13
0.1 µg/ml	0	5	0	12
0.01 µg/ml	0	6	0	9
MCA Controls <sup>e</sup>				
50 µg/ml	++	0	++++	0
5 µg/ml	0	0	++	0
1 µg/ml	0	0	0	0
0.5 µg/ml	0	0	0	0
0.1 µg/ml	0	0	0	0
0.01 µg/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

TABLE 3. Effect of Hydrazine on HSV-2 Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + hydrazine				
0.05 $\mu$ l/ml			0	25
0.01 $\mu$ l/ml	0	-	0	27
0.005 $\mu$ l/ml	0	6	0	29
0.001 $\mu$ l/ml	0	4	0	13
Hydrazine Controls <sup>e</sup>				
0.05 $\mu$ l/ml			0	0
0.01 $\mu$ l/ml	0	0	0	0
0.005 $\mu$ l/ml	0	0	0	0
0.001 $\mu$ l/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

TABLE 4. Effect of Mono-Methylhydrazine (MMH)  
on HSV-2 Transformation of 3T3 cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + MMH				
0.05 $\mu$ l/ml			++	1
0.01 $\mu$ l/ml	0	-	0	14
0.005 $\mu$ l/ml	0	-	0	14
0.001 $\mu$ l/ml	0	-	0	11
MMH Controls <sup>e</sup>				
0.05 $\mu$ l/ml			+	0
0.01 $\mu$ l/ml	0	0	0	0
0.005 $\mu$ l/ml	0	0	0	0
0.001 $\mu$ l/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

TABLE 5. Effect of 1,1-dimethylhydrazine (UDMH)  
on HSV-2 Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + UDMH				
0.05 $\mu$ l/ml	0	19	0	14
0.01 $\mu$ l/ml	0	22	0	16
0.005 $\mu$ l/ml	0	12	0	9
0.001 $\mu$ l/ml	0	22	0	10
UDMH Controls <sup>e</sup>				
0.05 $\mu$ l/ml	0	0	0	0
0.01 $\mu$ l/ml	0	0	0	0
0.005 $\mu$ l/ml	0	0	0	0
0.001 $\mu$ l/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.



TABLE 6. Effect of 1,2-dimethylhydrazine (SDMH)  
on HSV-2 Transformation of 3T3 Cells

	Experiment One <sup>a</sup>		Experiment Two <sup>b</sup>	
	Chemical Cytotoxicity	Transformed foci per 10 <sup>6</sup> cells	Chemical Cytotoxicity	Transformed foci per 10 <sup>6</sup> cells
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + SDMH				
5 µg/ml	0	-	0	33
1 µg/ml	0	-	0	35
0.5 µg/ml	0	100	0	39
0.1 µg/ml	0	32	0	31
0.05 µg/ml	0	-	0	20
SDMH Controls <sup>e</sup>				
5 µg/ml	0	0	0	0
1 µg/ml	0	0	0	0
0.5 µg/ml	0	0	0	0
0.1 µg/ml	0	0	0	0
0.05 µg/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

TABLE 7. Effect of JP-5 on HSV-2  
Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + JP-5				
5 µl/ml	0	-	0	11
1 µl/ml	0	-	0	11
0.5 µl/ml	0	-	0	11
0.1 µl/ml	0	-	0	13
0.01 µl/ml	0	-	0	16
JP-5 Controls <sup>e</sup>				
5 µl/ml	0	0	0	0
1 µl/ml	0	0	0	0
0.5 µl/ml	0	0	0	0
0.1 µl/ml	0	0	0	0
0.01 µl/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

TABLE 8. Effect of JP-10 on HSV-2  
Transformation of 3T3 Cells

	<u>Experiment One</u> <sup>a</sup>		<u>Experiment Two</u> <sup>b</sup>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + JP-10				
5 µl/ml	0	12	0	10
1 µl/ml	0	-	0	6
0.5 µl/ml	0	-	0	12
0.1 µl/ml	0	4	0	8
0.01 µl/ml	0	-	0	12
JP-10 Controls <sup>e</sup>				
5 µl/ml	0	0	0	0
1 µl/ml	0	0	0	0
0.5 µl/ml	0	0	0	0
0.1 µl/ml	0	0	0	0
0.01 µl/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.



TABLE 9. Effect of RJ-4 on HSV-2  
Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>e</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + RJ-4				
5 µl/ml	0	14	+	2
1 µl/ml	0	-	+	3
0.5 µl/ml	0	-	0	8
0.1 µl/ml	0	-	0	11
0.01 µl/ml	0	-	0	18
RJ-4 Controls <sup>e</sup>				
5 µl/ml	0	0	0	0
1 µl/ml	0	0	0	0
0.5 µl/ml	0	0	0	0
0.1 µl/ml	0	0	0	0
0.01 µl/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.



TABLE 10. Effect of RJ-5 on HSV-2  
Transformation of 3T3 Cells

	<u>Experiment One<sup>a</sup></u>		<u>Experiment Two<sup>b</sup></u>	
	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>	<u>Chemical Cytotoxicity</u>	<u>Transformed foci per 10<sup>6</sup> cells</u>
Cell Controls <sup>c</sup>	0	0	0	0
Virus Controls <sup>d</sup>	0	18	0	16
Virus + RJ-5				
5 $\mu$ l/ml	0	-	0	10
1 $\mu$ l/ml	0	4	0	10
0.5 $\mu$ l/ml	0	4	0	9
0.1 $\mu$ l/ml	0	8	0	8
0.01 $\mu$ l/ml	0	-	0	19
RJ-5 Controls <sup>e</sup>				
5 $\mu$ l/ml	0	0	0	0
1 $\mu$ l/ml	0	0	0	0
0.5 $\mu$ l/ml	0	0	0	0
0.1 $\mu$ l/ml	0	0	0	0
0.01 $\mu$ l/ml	0	0	0	0

<sup>a</sup>The target 3T3 cells were exposed to virus first, then the media containing the test chemical.

<sup>b</sup>The target 3T3 cells were exposed to media containing the test chemical for 24 hours, followed by virus infection.

<sup>c</sup>3T3 cells exposed to neither virus nor test chemical.

<sup>d</sup>3T3 cells exposed to UV-irradiated HSV-2.

<sup>e</sup>3T3 cells exposed to the test chemical; no virus.

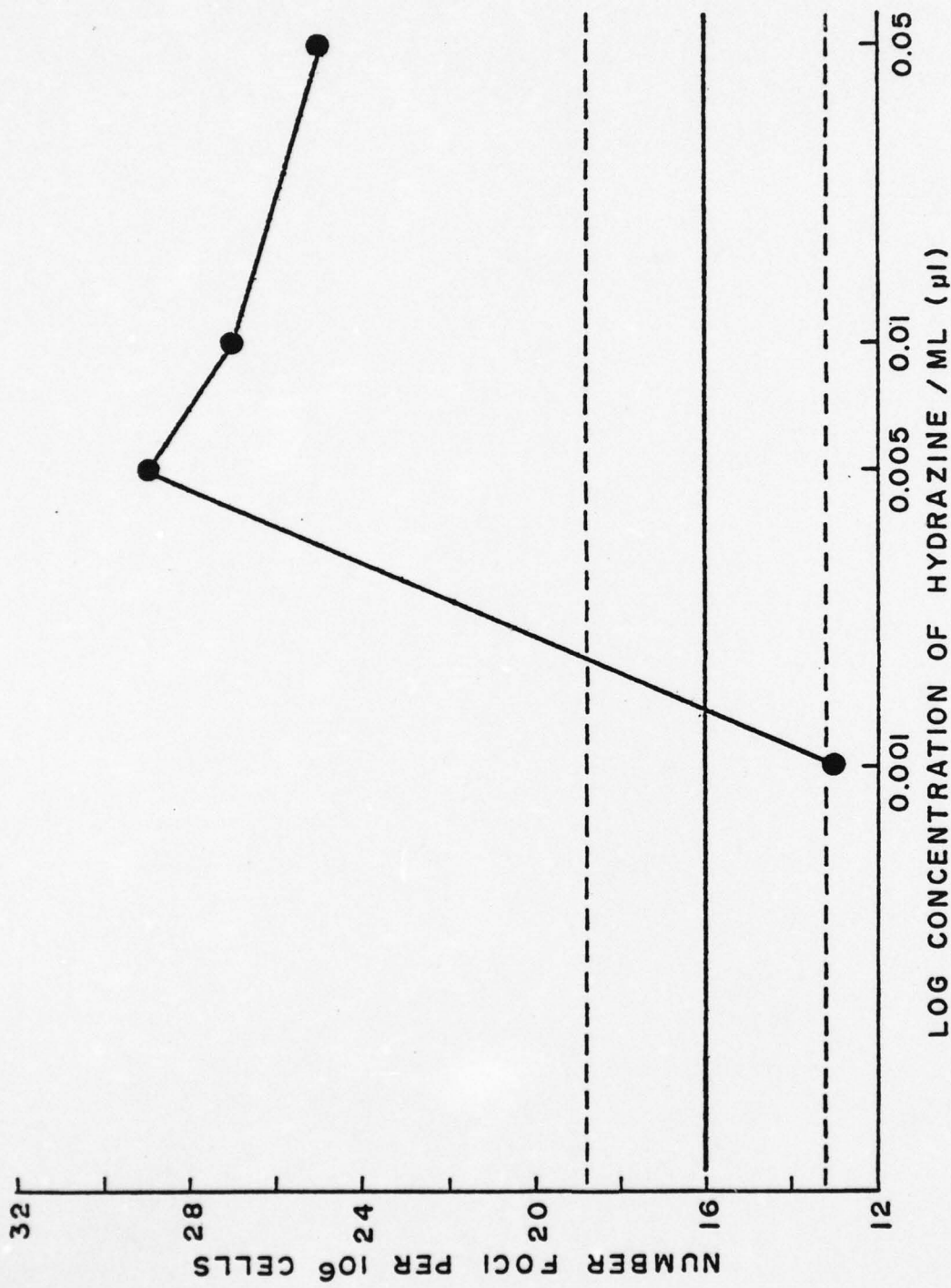


Fig. 1. Enhancement of HSV-2 transformation by hydrazine. The mean number of foci produced in the virus controls without chemicals was 16 foci per 10<sup>6</sup> cells (solid line). The standard deviation in the controls was ±2.8 (dashed lines). Concentrations of hydrazine at and above 0.005 μl per ml enhanced the number of transformed foci up to a maximum of about two fold. Concentrations of hydrazine greater than 0.05 μl were toxic to the 3T3 cells.

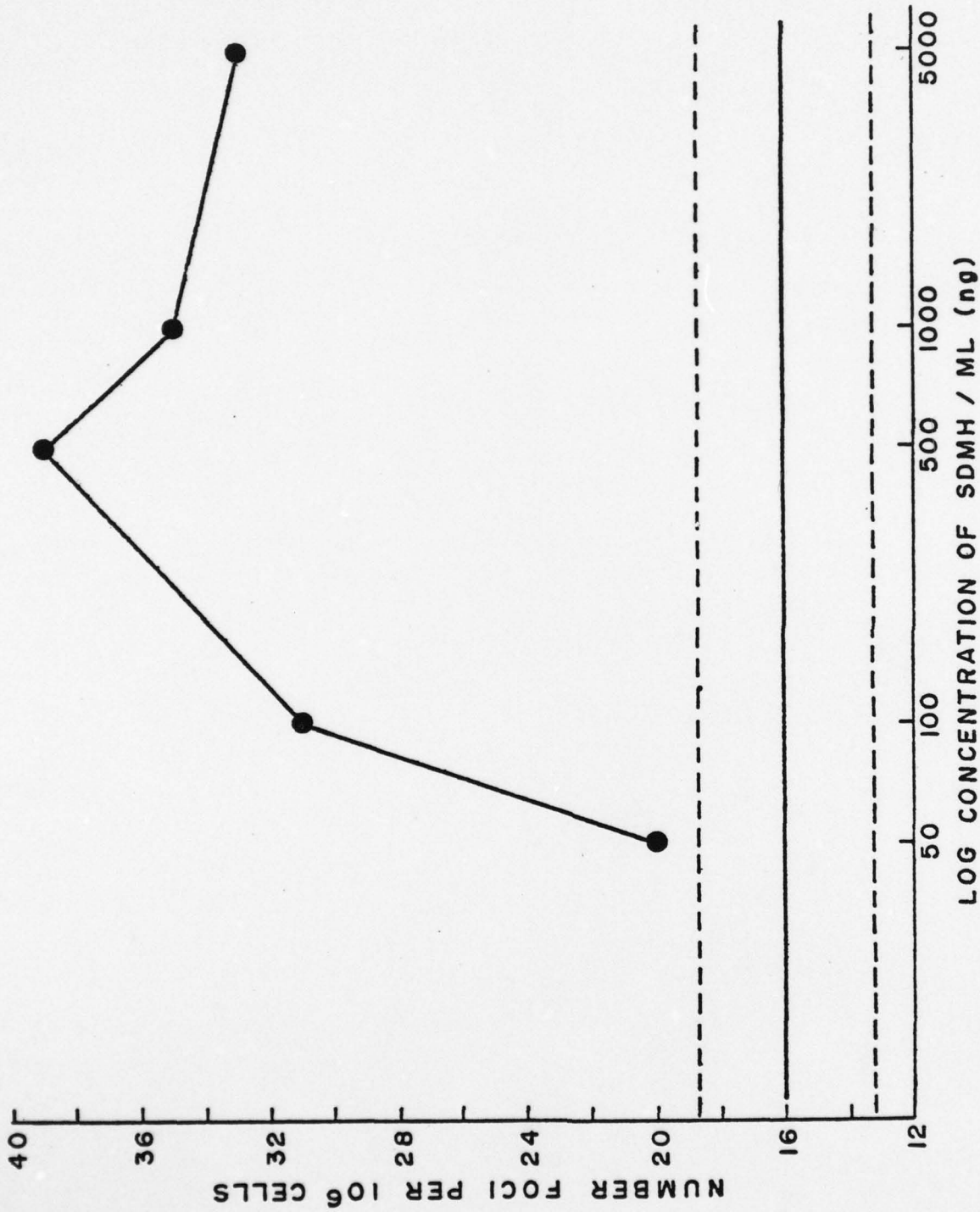


Fig. 2. Enhancement of HSV-2 transformation by 1,2-dimethylhydrazine (SDMH). The virus controls produced  $16 \pm 2.8$  foci per  $10^6$  cells (solid and dashed lines). SDMH enhanced the number of transformed foci formed up to a maximum of about 2 1/2 fold at a concentration of 500 ng per ml.



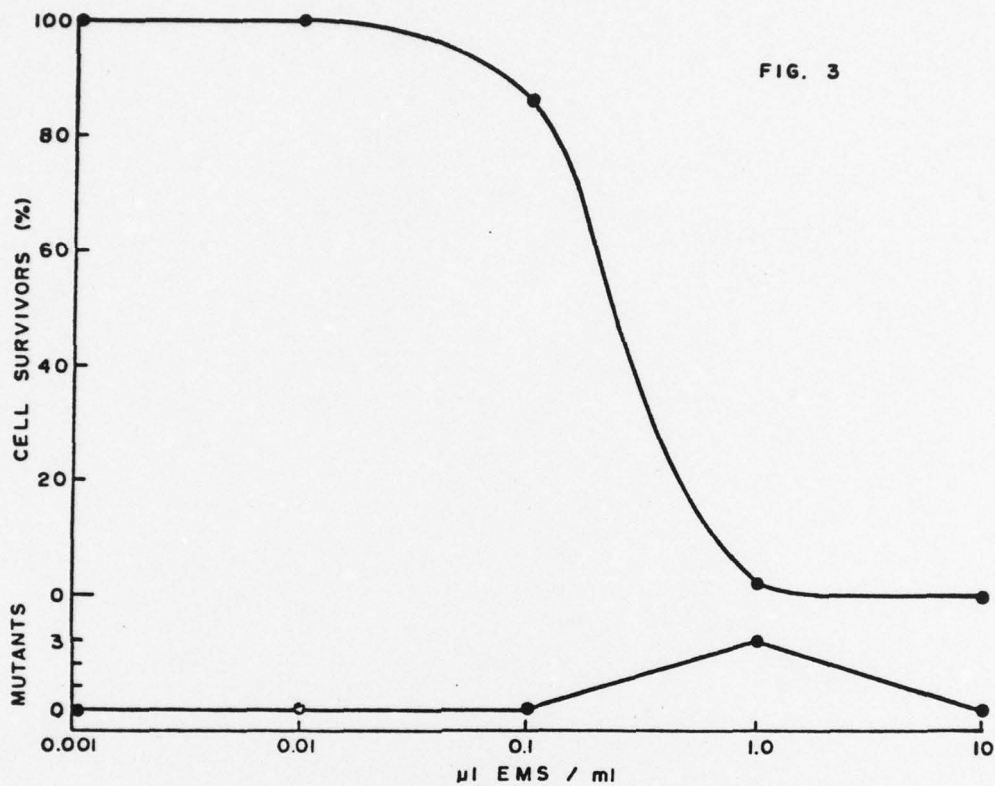


FIG. 3

Fig. 3. The survival of THO cells in the presence of various concentrations of ethylmethanesulfonate (EMS) and the generation of cell mutants. At each concentration of EMS,  $1 \times 10^6$  cells were treated for 24 hrs. Samples of the cultures were plated for cell survivors and the remainder were plated in HAT medium to select for mutants.

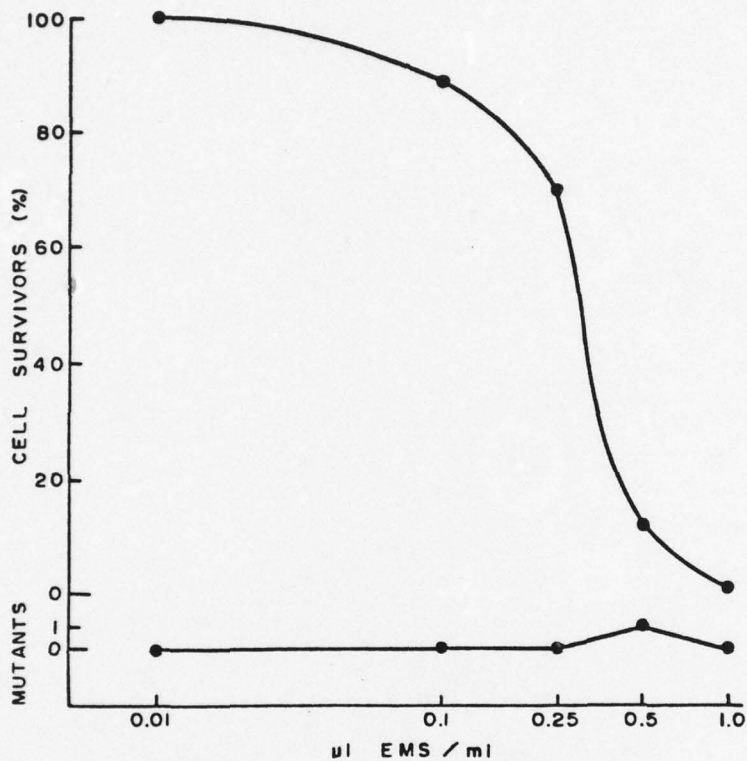


Fig. 4. Treatment of THO cells with concentrations of EMS intermediate between 0.1 and 1.0  $\mu$ l per ml. At each concentration of EMS,  $2 \times 10^6$  cells were treated for 24 hrs. Replicate cultures were prepared. One set was plated for cell survivors and the other set, after the 10 day expression time, was plated in HAT medium to select for mutants.

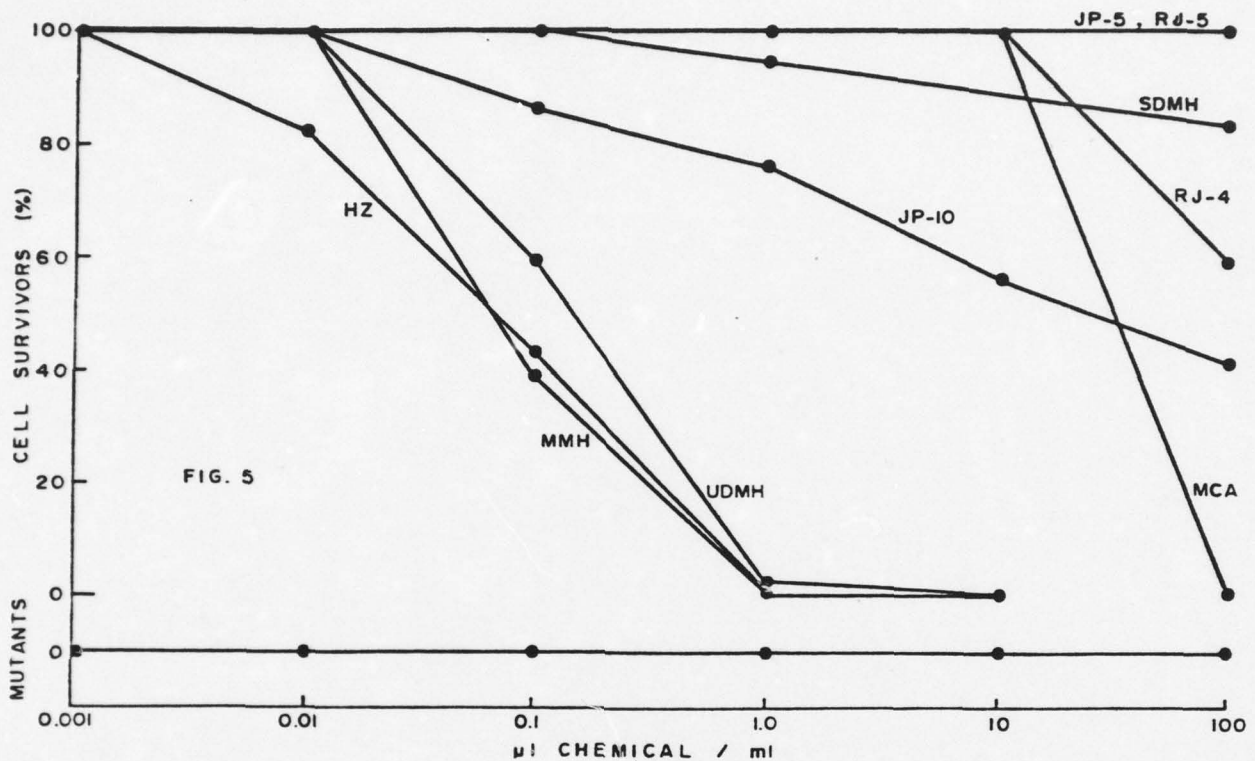


Fig. 5. Testing for mutagenic activity of the following compounds in THO cells: Hz = hydrazine, MMH = monomethylhydrazine, UDMH = 1,1-dimethylhydrazine, SDMH = 1,2-dimethylhydrazine, MCA= 3-methylcholanthrene, JP-5, JP-10, RJ-4, and RJ-5.  $1 \times 10^6$  THO cells were exposed to each concentration of each compound for 24 hrs. After 9 days' expression time, the cells were plated in HAT medium. Samples of the cultures were plated for cell survivors. For SDMH and MCA the figures on the abscissa are in  $\mu\text{g}$  per ml.