

# **ENVIRONMENTAL MUTAGENESIS**

**Official Journal of the  
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Mutagen  
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**Seymour Abrahamson**

EDITOR-IN-CHIEF

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# ENVIRONMENTAL MUTAGENESIS

Official Journal of the Environmental Mutagen Society

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# Contents

Volume 2, Number 1

1980

## EDITORIAL

### Editor's Prologue

Seymour Abrahamson ..... 1

## INVITED REVIEW

### Bacterial Mutagenesis: Review of New Insights

Philip E. Hartman ..... 3

## RESEARCH PAPERS

### Tissue-Specific Induction of Sister Chromatid Exchanges by Ethyl Carbamate in Mice

Gladwin T. Roberts and James W. Allen ..... 17

### Effects of Epoxide Hydratase Inhibitors in Forward and Reversion Bacterial Mutagenesis Assay Systems

Derek Guest and John G. Dent ..... 27

### Sister Chromatid Exchange In Vivo in Mjce: I. The Influence of Increasing Doses of Bromodeoxyuridine

James L. Wilmer and E.R. Soares ..... 35

### Sex-Related Differences in Cytogenetic Effects of Benzene in the Bone Marrow of Swiss Mice

Julianne Meyne and M.S. Legator ..... 43

### Regulation of Dimethylnitrosamine Metabolism by Androgenic Hormones

K. Bakshi, D. Brusick, L.P. Bullock, and C.W. Bardin ..... 51

### Mutagenicity of 2- and 3-Carbon Halogenated Compounds in the Salmonella/Mammalian-Microsome Test

S.J. Stolzenberg and C.H. Hine ..... 59

### Mitotic Arrest by Benzimidazole Analogs in Human Lymphocyte Cultures

Henry E. Holden, Paula A. Crider, and Margitta G. Wahrenburg ..... 67

### I. Bacterial Mutagenicity of Particulates From Houston Air

Barbara Lee Borns Preidecker ..... 75

### II. Comparative Extraction of Houston Air Particulates With Cyclohexane or a Mixture of Benzene, Methanol, and Dichloromethane

Barbara Lee Borns Preidecker ..... 85

### Mutagenic Effects of Bleomycin in *Drosophila melanogaster*

H. Traut ..... 89

## MEETING REPORT

### Second European Workshop on Bacterial In Vitro Mutagenicity Test Systems

J.P. Seiler, I.E. Mattern, M.H.L. Green, and D. Anderson ..... 97

## RESEARCH PAPERS

|  |     |
|--|-----|
| <b>Mutagenicity of Effluents From an Experimental Fluidized Bed Coal Combustor</b><br>Charles R. Clark and Charles H. Hobbs . . . . .  | 101 |
| <b>Lack of an Indication of Mutagenic Effects of Dinitrotoluenes and Diaminotoluenes in Mice</b><br>E.R. Soares and L.F. Lock . . . . .  | 111 |
| <b>Increases in Morphologically Abnormal Sperm in Rats Exposed to Co<sup>60</sup> Irradiation</b><br>L.F. Lock and E.R. Soares . . . . .   | 125 |
| <b>The Mutagenic Effect of Platinum Compounds in <i>Drosophila melanogaster</i></b><br>R.C. Woodruff, R. Valencia, R.F. Lyman, B.A. Earle, and J.T. Boyce . . . . .  | 133 |
| <b>In Vitro Induction of Segregational Errors of Chromosomes by Natural Cannabinoids in Normal Human Lymphocytes</b><br>Richard T. Henrich, Takayuki Nogawa, and Akira Morishima . . . . .   | 139 |
| <b>X Irradiation and Sister Chromatid Exchange in Cultured Human Lymphocytes</b><br>William F. Morgan and Peter E. Crossen . . . . .   | 149 |
| <b>Sister Chromatid Exchange Studies in Human Fibroblast—Rat Hepatocyte Co-Cultures: A New In Vitro System to Study SCEs</b><br>Andrew D. Kligerman, Stephen C. Strom, and George Michalopoulos . . . . .  | 157 |
| <b>Enzyme Mutants Induced by Low-Dose-Rate <math>\gamma</math>-Irradiation in <i>Drosophila</i>: Frequency and Characterization</b><br>Robert R. Racine, Charles H. Langley, and Robert A. Voelker . . . . .   | 167 |
| <b>Genetic Effects of Strong Magnetic Fields in <i>Drosophila melanogaster</i>: II. Lack of Interaction Between Homogeneous Fields and Fission Neutron-Plus-Gamma Radiation</b><br>P.G. Kale and J.W. Baum . . . . .   | 179 |
| <b>Evidence That the Repair Deficient <i>mei-9<sup>d</sup></i> Female in <i>Drosophila melanogaster</i> Is a Strong Potentiator of Chromosome Loss Induced in the Paternal Genome by Dimethylnitrosamine</b><br>S. Zimmering, A.W. Hartmann, and S.F. Cooper . . . . . | 187 |
| <b>The Action of Three Anticlastogens on the Induction of Sister Chromatid Exchange by Trenimon and 8-Hydroxyquinoline Sulfate in Human Lymphocyte Cultures</b><br>E. Gebhart and H. Kappauf . . . . .   | 191 |
| <b>BOOK AND ARTICLE REVIEWS</b>  |     |
| B.P. Sonnenblick . . . . .   | 201 |
| <b>MEETING REPORT</b>  |     |
| <b>Eleventh Annual Meeting of the Environmental Mutagen Society</b>  |     |
| Officers of the Society . . . . .  | 203 |
| Councilors . . . . .   | 203 |
| Program Committee . . . . .  | 204 |
| Registration Fees and Hours . . . . .  | 204 |
| General Information . . . . .  | 204 |
| Program . . . . .  | 206 |
| Abstracts . . . . .  | 230 |
| Author Index to Abstracts . . . . .  | 317 |
| Announcement . . . . .   | 321 |

**EDITORIAL**

- Laboratory Safety and Handling Procedures for Chemical Mutagens**  
David J. Brusick . . . . . 323

**RESEARCH PAPERS**

- Variation in the Baseline Sister Chromatid Exchange Frequency in Human Lymphocytes**  
A.V. Carrano, J.L. Minkler, D.G. Stetka, and D.H. Moore II . . . . . 325
- Induction of Chromosome Shattering and Micronuclei by Ultraviolet Light and Caffeine. I. Temporal Relationship and Antagonistic Effects of the Four Deoxyribonucleosides**  
C. Cremer, T. Cremer, and M. Simickova . . . . . 339
- The Anaerobe-Mediated Mutagenicity of 2-Nitrofluorene and 2-Aminofluorene for Salmonella typhimurium**  
George E. Karpinsky and Herbert S. Rosenkranz . . . . . 353
- Mutagenicity of Pesticides Evaluated by Means of Gene-Conversion in Saccharomyces cerevisiae and in Aspergillus nidulans**  
M. de Bertoldi, M. Griselli, M. Giovannetti, and R. Barale . . . . . 359
- Absence of Arsenite Mutagenicity in E coli and Chinese Hamster Cells**  
T.G. Rossman, D. Stone, M. Molina, and W. Troll . . . . . 371
- Use of Hydroxyurea in the Measurement of DNA Repair by the BND Cellulose Method**  
James Irwin and Bernard Strauss . . . . . 381
- Sunlight-Induced Mutagenesis and Toxicity in L5178Y Mouse Cells: Determination and Comparison With Other Light Sources**  
Kenneth Krell and Elizabeth D. Jacobson . . . . . 389
- Effect of N-Alkyl Chain Length on the Mutagenicity of N-Nitrosated 1-Naphthyl N-Alkylcarbamates**  
Bryan K. Eya and Ronald E. Talcott . . . . . 395
- Mutagenicity Tests of Fabric-Finishing Agents in Salmonella typhimurium: Fiber-Reactive Wool Dyes and Cotton Flame Retardants**  
James T. MacGregor, Martin J. Diamond, Laurence W. Mazzeno, Jr., and Mendel Friedman . . . . . 405
- Estimation of the Weight-Dependent Probability of Detecting a Mutagen With the Ames Assay**  
James B. Johnston and Philip K. Hopke . . . . . 419

**BRIEF COMMUNICATION**

- Genes Controlling Sensitivity to Alkylation and X-Ray Damage on Chromosome 3 of Drosophila melanogaster**  
A.K. Beck, R.R. Racine, and F.E. Würzler . . . . . 425

**MEETING REPORT**

- Meeting Report on the Second EMS Workshop**  
David J. Brusick . . . . . 431
- Program Announcement** . . . . . 433

## RESEARCH PAPERS

- Differential Induction of Sister Chromatid Exchanges by Indirect-Acting Mutagen-Carcinogens at Early and Late Stages of Embryonic Development**  
Lori A. Todd and Stephen E. Bloom ..... 435
- How Many Loci on the X-Chromosome of *Drosophila melanogaster* Can Mutate to Recessive Lethals?**  
S. Abrahamson, F.E. Würzler, C. DeJongh, and H. Unger Meyer ..... 447
- Short-Term Cytogenetic Assays of Nine Cancer Chemotherapeutic Drugs With Metabolic Activation**  
William W. Au, Dennis A. Johnston, Cheryl Collie-Bruyere, and T.C. Hsu ..... 455
- Hyperthermia Induced Dissociation of the X-Y Bivalent in Mice**  
M.L. Garriott and C.L. Chrisman ..... 465
- The Relative Contributions of B and T Lymphocytes in the Human Peripheral Blood Mutagen Test System as Determined by Cell Survival, Mitogenic Stimulation, and Induction of Chromosome Aberrations by Radiation**  
Jeffrey L. Schwartz and Mary Esther Gauden ..... 473
- A Comparison of the Ability of Frog and Rat S-9 to Activate Promutagens in the Ames Test**  
Albert M. Cheh, Alan B. Hooper, Jill Skochdopole, Craig A. Henke, and Robert G. McKinnell ..... 487
- Clastogen-Induced Micronuclei in Peripheral Blood Erythrocytes: The Basis of an Improved Micronucleus Test**  
James T. MacGregor, Carol M. Wehr, and Daniel H. Gould ..... 509
- Potential of Chromosome Loss Induced in the Paternal Genome by Methyl Methanesulfonate and Procarbazine Following Matings With Repair Deficient *mei-9<sup>a</sup>* Females of *Drosophila***  
S. Zimmering and K.L. Kammermeyer ..... 515
- The Evaluation of the Epoxide Diluent, n-Butylglycidyl Ether, in a Series of Mutagenicity Assays**  
T.H. Connor, J.B. Ward, Jr., J. Meyne, T.G. Pullin, and M.S. Legator ..... 521
- Genotoxic Activity in Microorganisms of Tetryl, 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene**  
Douglas B. McGregor, Colin G. Riach, Rowan M. Hastwell, and Jack C. Dacre ..... 531

## BRIEF COMMUNICATION

- A Maternal Effect in Homozygous *mei-9<sup>a</sup> mei-4<sup>D5</sup>* Repair Deficient *Drosophila melanogaster* Females Influencing the Recovery Rate of Progeny Bearing a Y Chromosome**  
S. Zimmering and S.F. Cooper ..... 543

## BOOK AND ARTICLE REVIEWS

- B.P. Sonnenblick ..... 547
- Author Index** ..... 549
- Subject Index** ..... 551

# Induction of Chromosome Shattering and Micronuclei by Ultraviolet Light and Caffeine. I. Temporal Relationship and Antagonistic Effects of the Four Deoxyribonucleosides

C. Cremer, T. Cremer, and M. Simickova

*Institute of Human Genetics, University of Freiburg, Albertstr. 11, D-7800 Freiburg i. Br. (C.C., M.S.), and Institute of Anthropology and Human Genetics, University of Heidelberg, Im Neuenheimer Feld 328, D-6900 Heidelberg (T.C.), Federal Republic of Germany*

It is known that nucleosides may have antimutagenic and anticlastogenic effects. Here, we have investigated the influence of nucleosides on the induction of shattered chromosomes (fragmentation and/or pulverization of chromosomes of a mitotic cell) and of micronuclei by ultraviolet (UV) light and caffeine. Asynchronous cell cultures of a V79 subline of the Chinese hamster were irradiated at wavelength 254 nm using fluences up to 5.2 joules/m<sup>2</sup>. Following irradiation, the cells were postincubated either with 1.0 mM or 2.0 mM caffeine alone or with caffeine plus the four deoxyribonucleosides (dXs) (concentration 0.1 mM each). After different incubation times (three to 24 hours), chromosome preparations were performed. In other experiments, synchronized cells were used. The percentage of metaphase spreads with shattered chromosomes and the percentage of cells with micronuclei were determined. Post-treatment with caffeine alone resulted in shattered chromosomes in a high percentage of cells at the first post-irradiation mitosis as described previously. Formation of cells with micronuclei was observed only after the appearance of mitotic cells with shattered chromosomes, the maximum percentage of cells with micronuclei being smaller than the maximum percentage of cells with shattered chromosomes. The strong potentiating effect of UV-light plus caffeine was significantly reduced, however, if the post-treatment was performed with caffeine plus nucleosides. A significant reduction was also observed in the percentage of micronuclei. An evaluation of the mitotic indices and of cell-cycle parameters indicates that the effect of nucleosides was not due to enhanced interphase death.

**Key words:** chromosome shattering, ultraviolet (UV) light, caffeine, nucleosides, antimutagens, micronuclei

## INTRODUCTION

In a number of cell strains, especially in rodent cells, caffeine is known to potentiate the chromosome damaging effects of ultraviolet (UV) light and a number of chemical mutagens [Kihlman, 1974; Nilsson and Lehmann, 1975; Hartley-Asp, 1976; Kihlman, 1977;

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Parts of this investigation will be presented in the doctoral dissertation of M. Simickova to be submitted to the Faculty of Medicine, University of Freiburg i. Br.



Roberts, 1978]. A striking phenomenon observed after UV irradiation ( $\lambda = 254$  nm) and caffeine post-treatment is the frequent occurrence of cells with generalized chromosome shattering (GCS) (fragmentation and/or pulverization of all chromosomes of a mitotic cell) [Nilsson and Lehmann, 1975; T. Cremer et al, 1979].

In the present investigation, we have studied the influence of nucleosides on this phenomenon. These substances are known to have antimutagenic and anticlastogenic effects [Novick and Szilard, 1952; Kihlman, 1977; Gebhart, 1977]. Here, it is shown that the addition of deoxyribonucleosides to the postirradiation medium exerts a strong antagonistic effect on the induction of GCS. In addition, we examined the influence of nucleosides on the production of cells with micronuclei [Boller and Schmid, 1970; Countryman and Heddle, 1976]. The presence of nucleosides also reduced the percentage of micronuclei produced by UV light and caffeine.

## MATERIAL AND METHODS

### Cell Material and Culture Conditions

Cells of a subline of V79 Chinese hamster cells [Cremer et al, 1976] were used. This cell line has a modal chromosome number of 21 and a mean generation time of 13–14 hours. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), nonessential amino acids, and antibiotics (100 units/ml penicillin and 100  $\mu$ g/ml streptomycin) in a humidified atmosphere with 5% CO<sub>2</sub>. MEM (Flow Laboratories, Germany) was free from deoxyribonucleosides.

For experiments, cells were grown in 6-cm plastic petri dishes (Nunc/Denmark). Asynchronous cultures were inoculated after trypsination and grown two days before use. Synchronous populations were obtained by the mitotic shake-off procedure which resulted in at least 90% mitotic cells as revealed by direct chromosome preparations. The percentage of S-phase cells in these populations was small ( $\leq 2\%$ ) as shown by pulse labeling with <sup>3</sup>H-thymidine prior to detachment.

### UV Irradiation and Post-treatment

Asynchronous cells grown in petri dishes were labeled with <sup>3</sup>H-thymidine (0.1  $\mu$ Ci/ml, 5  $\mu$ Ci/mmol; Amersham Buchler) for 30 minutes prior to irradiation. Synchronized cells were used without prelabeling. Medium was removed before irradiation and the cells were washed twice with Hanks' solution (without phenol red). Thereafter, cells were irradiated from above, while covered with a 1 mm layer of Hanks' solution. A germicidal lamp (Sterisol 5143, Original Hanau) was used emitting predominantly at 254 nm wavelength. The fluence rate was determined to be 3.5 W/m<sup>2</sup> by means of a calibrated photodiode (United Detector Technology, Santa Monica, California). The duration of irradiation (0.75 seconds, 1.5 seconds) was controlled by openings in rotating masks above the cells which were moved by the wheel of a record player. This procedure allowed a precise adjustment of the irradiation time. Immediately after the irradiation, the cells were postincubated at 37°C in MEM with 10% FCS, either with caffeine (1–2 mM) alone or with caffeine plus the four deoxyribonucleosides (dXs) (deoxyadenosine, deoxycytidine, deoxyguanosine, and thymidine; concentration 0.1 mM each). Except for the addition of dXs, all treatments were identical and made in duplicate. After different incubation times (3–27 hours), in situ chromosome preparation was performed [Zorn et al, 1976]. Colchicine

(2  $\mu\text{g/ml}$ ) was added 3 hours before preparation. The dishes were air-dried and stained with aceto-orcein. The scoring of dishes was performed "blind," ie, in the absence of information about the treatment regimen. To examine chromosome damage and micronucleus formation, at least 50 metaphases and 500 interphase cells, respectively, were analyzed per dish; ie, at least 100 metaphase figures and 1,000 interphase cells were scored for each treatment and fixation time.

For statistical evaluation the binomial assumption was made, and the confidence limits  $p_1 \leq p \leq p_2$  for proportions were determined [Beyer, 1974]. The observed frequency is  $p$  and  $p_1$  and  $p_2$  are the lower and upper limits of the 95% confidence interval, respectively. Throughout the text, the 95% confidence intervals are given as decimals. The actual data are expressed as a percent. The term "nonsignificant difference" between two observed frequencies  $p_I$ ,  $p_{II}$  means that the 95% confidence intervals of  $p_I$  and  $p_{II}$  overlapped; if the term "significant difference" is used, the 95% confidence intervals of  $p_I$  and  $p_{II}$  were clearly separated from each other.

### Autoradiography

After microscopic evaluation as described above, pulse-labeled cells were covered with Ilford nuclear emulsion K2 and processed following standard procedures [Zorn, 1978]. Exposure time at 4°C was two weeks. In autoradiographs, the percentage of labeled metaphases was determined.

## RESULTS

### 1. Classification of Metaphase Figures

The metaphase figures obtained following whole-cell irradiation ( $\lambda = 254 \text{ nm}$ ) of V79 cells and post-treatment with caffeine in the presence or absence of deoxyribonucleosides were classified in the following way [Zorn et al, 1977; Zorn, 1978; T. Cremer et al, 1980a]: class A: No recognizable alterations of chromosome morphology; class B: Metaphase plates with one, occasionally two alterations. In most cases, these alterations were achromatic lesions ("gaps") or chromatid breaks. Occasionally, chromatid exchange figures were found. Deviations from the modal chromosome number were not classified as aberrations; class C: All metaphase spreads containing more than two aberrations with the majority of chromosomes (11 and more) remaining intact, were classified as class C. In the whole-cell irradiation experiments presented here, class C figures were a very rare event ( $< 1\%$ ), in contrast to experiments in which only a small part of the cell nucleus was UV- microirradiated [Zorn et al, 1977; Zorn, 1978]; class D: This class contains metaphase spreads in which the majority of chromosomes (11 and more) showed an aberrant morphology, only one or several chromosomes remaining intact (Fig. 1a); class E: In metaphase spreads of class E, all chromosomes were affected (GCS), appearing fragmented and/or pulverized (Fig. 1b, c). In the majority, figures as shown in Figure 1c were observed. The term GCS is introduced as a descriptive one to avoid any prejudice concerning the continuity or discontinuity of the DNA strand. Following UV- irradiation alone (up to 7 joules/m<sup>2</sup>) or caffeine post-treatment alone (1–2 mM), metaphase figures of classes C-E were rare events ( $< 1\%$  each) [Zorn, 1978]. UV-irradiation plus caffeine post-treatment, however, induced GCS in a synergistic (potentiating) way up to almost 100% of all metaphase figures obtained.

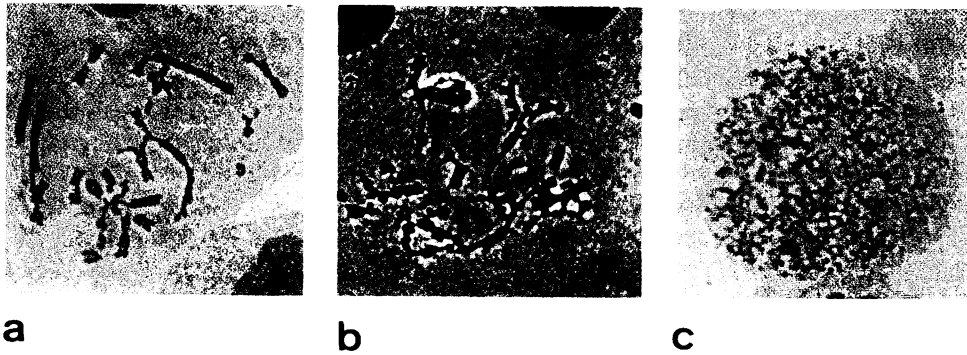


Fig. 1. Chromosome shattering following UV irradiation ( $\lambda = 254$  nm) of V79 cells and postincubation with caffeine. a) Aberrant morphology in the majority of chromosomes, only one or several chromosomes remaining intact: Class D; b, c) All chromosomes affected (generalized chromosome shattering, GCS): Class E; fragmentation of all chromosomes (b); pulverization of all chromosomes (c).

## 2. Percentage of Cells with GCS

To investigate the influence of the addition of the dXs on the amount of GCS, cells were treated under identical conditions except addition of dXs.

Figure 2 shows the result for asynchronous cells irradiated with  $2.6 \text{ joules/m}^2$  or  $5.2 \text{ joules/m}^2$  and post-treated with 1 mM caffeine with or without dXs. At  $2.6 \text{ joules/m}^2$ , the percentage of cells with GCS was low ( $\leq 3\%$ ) in both cases (Fig. 2a, b), and no significant difference was observed.

Irradiation with  $5.2 \text{ joules/m}^2$  and postincubation with 1 mM caffeine alone induced a maximum of 33% ( $0.23 \leq p \leq 0.42$ ) of cells with GCS (Fig. 2c). This percentage was significantly reduced, however, if dXs were added. In this case, a maximum of 12% ( $0.06 \leq p \leq 0.20$ ) cells with GCS was obtained (Fig. 2d).

In Figure 3, the frequencies of metaphase figures classes A through E are shown for cells post-treated with 2 mM caffeine. At this concentration, irradiation with  $2.6 \text{ joules/m}^2$  and post-treatment with caffeine alone had a considerable effect: Up to 40% ( $0.30 \leq p \leq 0.50$ ) of cells with GCS were found (Fig. 3a). Again, the percentage of cells with GCS was significantly reduced if dXs were added (Fig. 3b), the maximum being 12% ( $0.06 \leq p \leq 0.20$ ). The antagonistic effect of the addition of dXs was also observed following irradiation with  $5.2 \text{ joules/m}^2$ . While a maximum of 97% ( $0.92 \leq p \leq 0.99$ ) of cells with GCS was observed without addition of dXs (Fig. 3c), the maximum was 78% ( $0.69 \leq p \leq 0.86$ ) in the presence of dXs.

In Figure 4, the results of experiments with synchronized cells are shown. These cells were irradiated 3–5 hours after mitotic detachment and post-treated with 1 mM caffeine in the presence or absence of dXs. Again, a significant reduction of cells with GCS was obtained if nucleosides were added (Fig. 4a, b). After 15 hours incubation with 1 mM caffeine in the absence of nucleosides, the amount of cells with GCS was 15% ( $0.08 \leq p \leq 0.24$ ) after irradiation with  $2.6 \text{ joules/m}^2$  and 50% ( $0.4 \leq p \leq 0.8$ ) after  $5.2 \text{ joules/m}^2$ . In the presence of dXs, the maximum percentages were 1.5% ( $0 \leq p \leq 0.07$ ) and 3% ( $0 \leq p \leq 0.08$ ) for the two doses, respectively. It is interesting to note that, in all cases with a significant reduction of the percentage of cells with GCS, the presence of nucleosides also significantly reduced the total aberration frequency (sum of percentages of cells with aberrations classes B through E, achromatic lesions excluded).

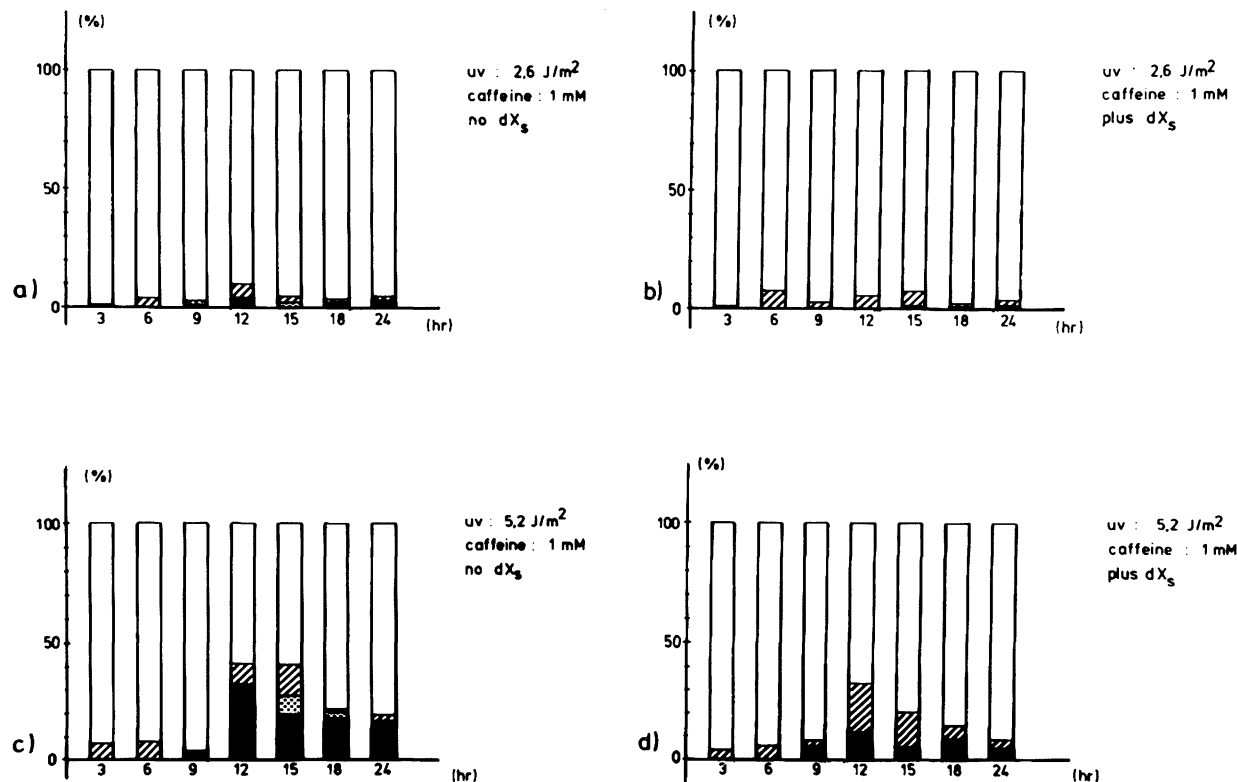


Fig. 2. UV irradiation ( $\lambda = 254 \text{ nm}$ ) of asynchronous V79 cells and caffeine post-treatment (1 mM) with or without the four deoxyribonucleosides ( $dX_s$ ; concentration 0.1 mM each): Induction of chromosome alterations. Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of metaphase figures class A–E –  $\square$ , no chromosomal alterations: Class A;  $\text{▨}$ , single defects (one, occasionally two aberrations): Class B;  $\text{▩}$ , aberrant morphology in the majority of chromosomes, only one or several chromosomes remaining intact: Class D;  $\blacksquare$ , all chromosomes affected (fragmentation and/or pulverization): generalized chromosome shattering (GCS): Class E. (a) 2.6 joules/ $m^2$ , no  $dX_s$ ; (b) 2.6 joules/ $m^2$ , plus  $dX_s$ . (c) 5.2 joules/ $m^2$ , no  $dX_s$ , (d) 5.2 joules/ $m^2$ , plus  $dX_s$ . For each value, at least 100 mitotic cells were scored.

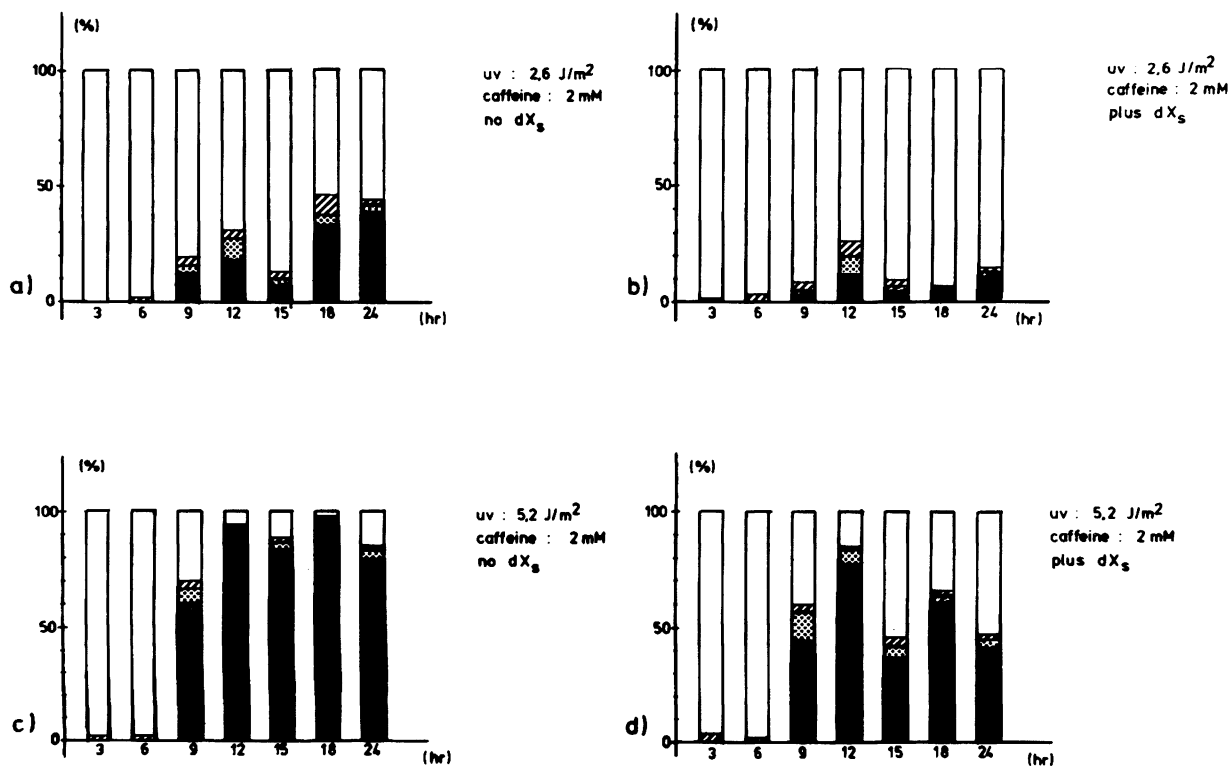


Fig. 3. UV-irradiation ( $\lambda = 254$  nm) of asynchronous V79 cells and caffeine post-treatment (2 mM) with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of chromosome alterations. Abscissa: Incubation time following irradiation (hr); Ordinate: Percentage of metaphase figures classes A–E (see legend to Fig. 2) —□, class A; ▨, class B; ▩, class D; ■, class E. (a) 2.6 joules/m<sup>2</sup>, no dXs; (b) 2.6 joules/m<sup>2</sup>, plus dXs; (c) 5.2 joules/m<sup>2</sup>, no dXs; (d) 5.2 joules/m<sup>2</sup>, plus dXs. For each value, at least 100 mitotic cells were scored.

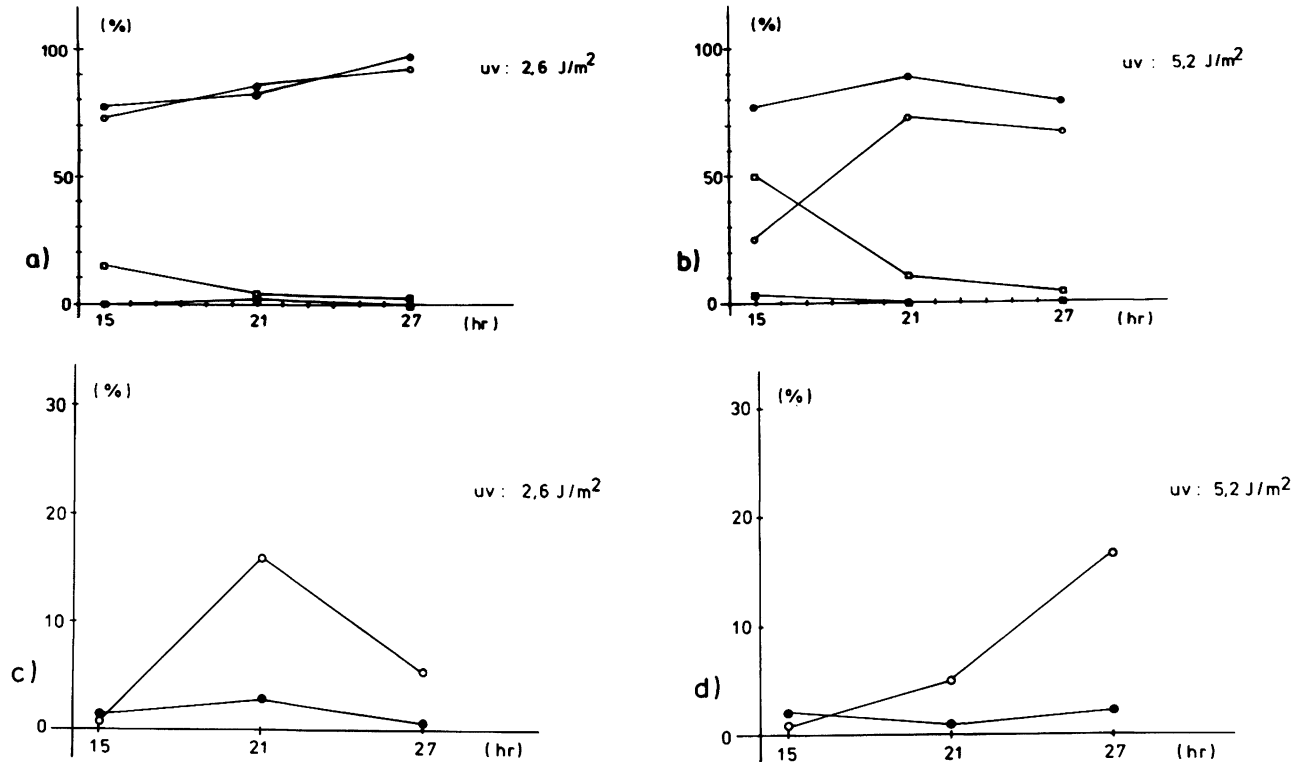


Fig. 4. UV-irradiation ( $\lambda = 254$  nm) of synchronized V79 cells (G1 and early S) and caffeine post-treatment (1 mM) with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of generalized chromosome shattering and formation of micronucleated cells. a,b: Induction of generalized chromosome shattering (GCS). Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of metaphase figures; classes A–E – ○, no chromosomal alterations (class A), caffeine post-treatment without dXs; ●, no chromosomal alterations (class A), caffeine post-treatment in the presence of dXs; □, all chromosomes affected (GCS), caffeine post-treatment without dXs; ■, all chromosomes affected (GCS), caffeine post-treatment in the presence of dXs. (a) 2.6 joules/m<sup>2</sup>; (b) 5.2 joules/m<sup>2</sup>. For each value, at least 100 mitoses were scored. (c, d) Induction of cells with micronuclei: Abscissa: Incubation time following irradiation (hr); Ordinate: Percentage of cells with micronuclei; ○, no dXs; ●, plus dXs. (c) 2.6 joules/m<sup>2</sup>; (d) 5.2 joules/m<sup>2</sup>. For each value, at least 1000 cells were scored.

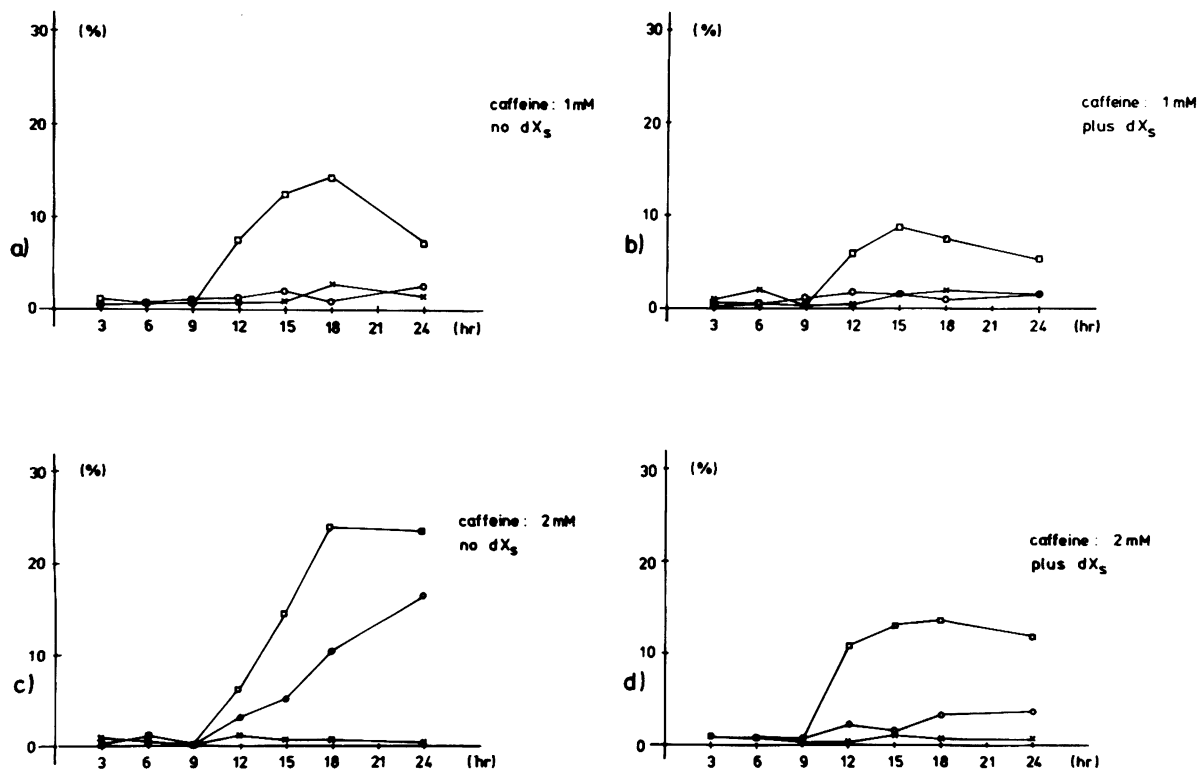


Fig. 5. UV-irradiation ( $\lambda = 254$  nm) of asynchronous V79 cells and caffeine post-treatment with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of cells with micronuclei. Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of cells with micronuclei; x, O joules/m<sup>2</sup>; o, 2.6 joules/m<sup>2</sup>; □, 5.2 joules/m<sup>2</sup>. (a) 1 mM caffeine, no dXs; (b) 1 mM caffeine, plus dXs; (c) 2 mM caffeine, no dXs; (d) 2 mM caffeine, plus dXs. For each value, at least 1000 cells were scored. In the majority of cases (70%), micronucleated cells had one to three micronuclei.

### 3. Formation of Micronuclei

The percentage of cells with micronuclei obtained after UV-irradiation (2.6 joules/m<sup>2</sup> and 5.2 joules/m<sup>2</sup>) without caffeine post-treatment or after caffeine treatment of un-irradiated cultures was low ( $\leq 2\%$ ,  $0.01 \leq p \leq 0.03$ ), the differences being not significant. A significant increase (up to 14%,  $0.11 \leq p \leq 0.16$ ), however, was observed following the combined treatment with 5.2 joules/m<sup>2</sup> and 1 mM caffeine (Fig. 5a). While 2.6 joules/m<sup>2</sup> and 1 mM caffeine post-treatment did not result in a significant increase of cells with micronuclei (Fig. 5a), 2 mM caffeine post-treatment produced a considerable effect (maximum 16%,  $0.14 \leq p \leq 0.18$ ) even at this lower UV-dose (Fig. 5c). Addition of dXs significantly reduced the percentage of cells with micronuclei (Fig. 5b, d). The maxima of the percentage of micronucleated cells obtained in the presence of dXs were 8% ( $0.06 \leq p \leq 0.10$ ) for 5.2 joules/m<sup>2</sup> plus 1 mM caffeine; 3.5% ( $0.02 \leq p \leq 0.045$ ) for 2.6 joules/m<sup>2</sup> plus 2 mM caffeine; 13% ( $0.10 \leq p \leq 0.15$ ) for 5.2 joules/m<sup>2</sup> plus 2 mM caffeine (without dXs – 24% ( $0.21 \leq p \leq 0.26$ )).

A comparison of the data presented for the induction of GCS (Figs. 2 and 3) and the results obtained for the production of micronuclei (Fig. 5) clearly shows that cells with micronuclei appeared only after a significant increase of cells with GCS was observed. For example, irradiation with 5.2 joules/m<sup>2</sup> and 2 mM caffeine post-treatment resulted in 60% mitotic cells with GCS after 9 hours incubation (Fig. 3c). At this time, the percentage of cells with micronuclei did not exceed the level of unirradiated controls.

A significant reduction of the percentage of cells with micronuclei by the addition of dXs was observed also in case of synchronized cultures (Fig. 4c, d). A comparison with the data presented in Fig. 4a, b shows that the formation of cells with GCS again preceded the appearance of cells with micronuclei.

### 4. Mitotic Index and Cell-Cycle Parameters

For all incubation times, the mitotic indices were determined. It was found that the addition of nucleosides did not significantly reduce the mitotic indices if compared with cells treated in the absence of dXs. In some cases, the mitotic index was even slightly higher when nucleosides were added. Furthermore, at 5.2 joules/m<sup>2</sup> plus caffeine post-treatment a reduction of the mitotic delay of approximately 3 hours was observed in the presence of dXs.

The duration of S-phase and of G<sub>2</sub> + prophase was estimated from the metaphase labeling index (MLI) curves according to the method of Evans and Scott [1964]. The results of these estimates are presented in Table I. Up to UV fluences of 2.6 joules/m<sup>2</sup>, the differences in the duration of S-phase and G<sub>2</sub> + prophase were small to nonexistent whether nucleosides were added or not. At 5.2 joules/m<sup>2</sup> and postincubation with 1 mM and 2 mM caffeine, the duration of S-phase was observed to be reduced by approximately three hours in the presence of dXs. This fits well to the reduction of the mitotic delay derived from the mitotic index curves. No influence of dXs on the duration of G<sub>2</sub> + prophase was found at this UV fluence. It should be emphasized, however, that these measurements of cell-cycle parameters are only rough estimates since sampling times of 3 hours with colchicine were used to collect cells in metaphase. In any case, the mitotic indices and the cell-cycle estimates obtained from the MLI curves indicate that the reduction of the percentage of GCS and of micronucleated cells by the addition of the four deoxyribonucleosides cannot be explained by enhanced interphase death.



TABLE I. Duration of S-phase and G<sub>2</sub> + Prophase as Estimated from MLI Curves\*

| UV fluence                     | 0 joule/m <sup>2</sup> |                                | 2.6 joule/m <sup>2</sup> |                                | 5.2 joule/m <sup>2</sup> |                                |
|--------------------------------|------------------------|--------------------------------|--------------------------|--------------------------------|--------------------------|--------------------------------|
|                                | S-phase (hr)           | G <sub>2</sub> + prophase (hr) | S-phase (hr)             | G <sub>2</sub> + prophase (hr) | S-phase (hr)             | G <sub>2</sub> + prophase (hr) |
| Post-treatment                 |                        |                                |                          |                                |                          |                                |
| 0 mM Caffeine,<br>0 mM dXs     | 7.0                    | 3.8                            | nd                       | nd                             | nd                       | nd                             |
| 0 mM Caffeine,<br>0.1 mM dXs   | 6.0                    | 3.8                            | nd                       | nd                             | nd                       | nd                             |
| 1.0 mM Caffeine,<br>0 mM dXs   | 7.0                    | 3.5                            | 8.0                      | 3.0                            | 9.5                      | 4.1                            |
| 1.0 mM Caffeine,<br>0.1 mM dXs | 6.5                    | 4.2                            | 6.5                      | 4.2                            | 6.8                      | 4.3                            |
| 2.0 mM Caffeine,<br>0 mM dXs   | 6.3                    | 4.5                            | 6.7                      | 4.5                            | 10.0                     | 5.0                            |
| 2.0 mM Caffeine,<br>0.1 mM dXs | 7.0                    | 4.5                            | 6.1                      | 4.7                            | 7.0                      | 5.0                            |

\*The cell-cycle parameters given were calculated from metaphase labeling index (MLI) curves according to the method of Evans and Scott [1964].

S-phase (hr): Estimated from the time interval between half of the maximum labeling index on the ascending limb to half the maximum labeling index on the descending limb of the first peak, minus the duration of the <sup>3</sup>H-thymidine treatment (30 minutes).

G<sub>2</sub> + prophase: Estimated from the time interval between the beginning of <sup>3</sup>H-thymidine treatment to the time when the labeling index reached half of the maximum value.

dXs: deoxyadenosine + deoxycytidine + deoxyguanosine + thymidine; concentration 0.1 mM each.

nd = not determined.

From growth curves of exponentially growing cells, a generation time of 13–14 hours was estimated.

## DISCUSSION

### 1. GCS and Premature Chromosome Condensation

GCS (class E) was the most frequently observed class of altered chromosome morphology in the present experiments using whole-cell irradiation ( $\lambda = 254$  nm) and post-treatment with caffeine. Two types of GCS were obtained: 1) Metaphase figures with chromatid breaks and/or gaps in all chromosomes (Fig. 1b); 2) metaphase figures with "pulverization" [Zorn et al, 1976; Vogel and Bauknecht, 1978] of the chromosomes (Fig. 1c). We have seen many examples of GCS where chromosomes with numerous chromatid breaks were still present besides pulverized chromosomes. This suggests that types 1 and 2 are closely related to each other. Type 2 was obtained in the large majority of metaphase figures with GCS. These figures resemble prematurely condensed chromosomes (PCC) during S-phase (S-PCC) [Johnson and Rao, 1970; Sperling and Rao, 1974] or G1-PCC following UV irradiation [Schor et al, 1975]. Therefore, we have considered the possibility that type 2 figures might be due to micronucleus-derived premature chromosome condensation [Obe and Beek, 1975]. The temporal relationship between the observation of shattered chromosomes and the production of micronuclei observed in our experiments, however, rules out such a possibility: Enhanced formation of micronuclei was observed after mitotic cells with shattered chromosomes appeared in the cultures (compare Figs. 2, 3, and 5).

Different mechanisms for the induction of GCS and PCC, however, do not exclude the possibility that pulverized chromosomes in metaphase plates with GCS indicate a failure of chromosome condensation. Whether the pulverized appearance of chromosomes in GCS figures is mainly due to a large number of DNA breaks or to a failure of chromosome condensation, remains to be investigated. A model developed by us previously to explain the induction of GCS by UV light and caffeine [Zorn et al, 1977; T. Cremer et al, 1980a, b] is compatible with both possibilities.

### 2. Antagonistic Effects of Nucleosides

The antimutagenic and anticlastogenic action of deoxyribonucleosides is well known [Novick and Szilard, 1952; Kihlman, 1977; Gebhart, 1977]. In the present investigation, it is shown that the synergistic effect of UV light plus caffeine on the induction of GCS and of micronucleated cells is significantly reduced by the addition of the four dXs to the post-irradiation medium. The evaluation of mitotic indices and cell-cycle parameters indicates that the effect is not due to enhanced interphase death.

A possible explanation of the antagonistic effects of nucleosides on the induction of chromosome shattering by UV light and caffeine may be obtained by findings of Collins and Johnson [1979] that the addition of nucleosides may enhance DNA repair synthesis in UV-irradiated *Microtus agrestis* cells. Unscheduled DNA synthesis following UV irradiation was observed also in the V79 line used in the present investigation [Zorn, 1978]. This suggests the following line of reasoning: First, it seems to be generally assumed that the synergistic action of UV light plus caffeine on the induction of chromosome aberrations is due to the inhibition of daughter strand repair (postreplication repair) of DNA photolesions [Nilsson and Lehmann, 1975; Kihlman, 1977; Roberts, 1978]. Here it is assumed that the inhibition of daughter strand repair by caffeine plays a decisive role also in the induction of generalized chromosome shattering: GCS is induced if the number of daughter strand repair sites exceeds a certain threshold which may vary from cell to cell [T.

Cremer et al, 1980a, b). Second, addition of nucleosides to the postirradiation medium may enhance the excision of UV-induced pyrimidine dimers.

If so, the number of remaining DNA photolesions and, hence, the number of daughter-strand-repair sites becomes smaller in nucleoside-treated cells than in untreated ones. If this number falls below the threshold value characteristic for a given cell as first described, no GCS is induced. Although the model outlined above is far from proven, it offers a plausible mechanism for the antagonistic effect of nucleosides on GCS and fits well with the data available. It is also consistent with evidence obtained by Nakano and co-workers [1979] that the adverse effect of caffeine on the survival of UV-irradiated V79 cells is strongly diminished by an enhancement of the period of time available for excision repair. While the anticlastogenic effect of nucleosides may be due to their effect on excision-repair capacity, possible effects of an improved supply of cells with nucleosides on daughter-strand-repair capacity may also be considered.

Caffeine may have an inhibiting effect on the uptake and on the metabolism of DNA-precursors [Lehmann and Kirk-Bell, 1974] (C.A. Waldren, personal communication, 1980). The addition of nucleosides might overcome the adverse effect of a reduced supply of nucleosides. Furthermore, some interference of nucleosides with uptake and/or metabolism of caffeine, thus reducing the effective intracellular concentration of caffeine, should not be omitted from consideration.

## CONCLUSIONS

Deoxyribonucleosides exert an antagonistic effect on the induction of GCS and micronucleus production by UV light and caffeine in V79 cells. It is suggested that by the addition of nucleosides, the excision repair capacity may be promoted, thus reducing the number of caffeine-sensitive sites of daughter strand repair (postreplication repair).

## ACKNOWLEDGMENTS

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# Author Index

- Abrahamson, Seymour, 1, 447  
Allen, James W., 17  
Anderson, D., 97  
Au, William W., 455
- Bakshi, K., 51  
Barale, R., 359  
Bardin, C.W., 51  
Baum, J.W., 179  
Beck, A.K., 425  
Bloom, Stephen E., 435  
Boyce, J.T., 133  
Brusick, David J., 51, 323, 431  
Bullock, L.P., 51
- Carrano, A.V., 325  
Cheh, Albert M., 487  
Chrisman, C.L., 465  
Clark, Charles R., 101  
Collie-Bruyere, Cheryl, 455  
Connor, T.H., 521  
Cooper, S.F., 187, 543  
Cremer, C., 339  
Cremer, T., 339  
Crider, Paula A., 67  
Crossen, Peter E., 149
- Dacre, Jack C., 531  
de Bertoldi, M., 359  
DeJongh, C., 447  
Dent, John G., 27  
Diamond, Martin J., 405
- Earle, B.A., 133  
Eya, Bryan K., 395
- Friedman, Mendel, 405
- Garriott, M.L., 465  
Gaulden, Mary Esther, 473  
Gebhart, E., 191  
Giovannetti, M., 359  
Gould, Daniel H., 509  
Green, M.H.L., 97  
Griselli, M., 359  
Guest, Derek, 27
- Hartman, Philip E., 3  
Hartmann, A.W., 187  
Hastwell, Rowan M., 531  
Henke, Craig A., 487  
Henrich, Richard T., 139  
Hine, C.H., 59  
Hobbs, Charles H., 101  
Holden, Henry E., 67  
Hooper, Alan B., 487  
Hopke, Philip K., 419  
Hsu, T.C., 455
- Irwin, James, 381
- Jacobson, Elizabeth D., 389  
Johnston, Dennis A., 455  
Johnston, James B., 419
- Kale, P.G., 179  
Kammermeyer, K.L., 515  
Kappauf, H., 191  
Karpinsky, George E., 353  
Kligerman, Andrew D., 157  
Krell, Kenneth, 389
- Langley, Charles H., 167  
Legator, M.S., 43, 521  
Lock, L.F., 111, 125  
Lyman, R.F., 133
- MacGregor, James T., 405, 509  
Mattern, I.P., 97  
Mazzeno, Laurence W., Jr., 405  
McGregor, Douglas B., 531  
McKinnell, Robert G., 487  
Meyer, H. Unger, 447  
Meyne, Julianne, 43, 521  
Michalopoulos, George, 157  
Minkler, J.L., 325  
Molina, M., 371  
Moore, D.H., II, 325  
Morgan, William F., 149  
Morishima, Akira, 139
- Nogawa, Takayuki, 139
- Pullin, T.G., 521

Preidecker, Barbara Lee Borns,  
75, 85

Racine, Robert R., 167, 425

Riach, Colin G., 531

Roberts, Gladwin T., 17

Rosencranz, Herbert S., 353

Rossman, T.G., 371

Schwartz, Jeffrey L., 473

Seiler, J.P., 97

Simickova, M., 339

Skochdopole, Jill, 487

Soares, E.R., 35, 111, 125

Sonnenblick, B.P., 201, 547

Stetka, D.G., 325

Stolzenberg, S.J., 59

Stone, D., 371

Strauss, Bernard, 381

Strom, Stephen C., 157

Talcott, Ronald E., 395

Todd, Lori A., 435

Traut, H., 89

Troll, W., 371

Valencia, R., 133

Voelker, Robert A., 167

Wahrenburg, Margitta G., 67

Ward, J.B., Jr., 521

Wehr, Carol M., 509

Wilmer, James L., 35

Woodruff, R.C., 133

Würgler, F.E., 425, 447

Zimmering, S., 187, 515, 543

# Subject Index

- Abstracts to the Eleventh Annual Meeting of the Environmental Mutagen Society, 230
  - Author index to, 317
- Activation, 353
- Aflatoxin B<sub>1</sub> (AF-B<sub>1</sub>), 435
- Air particulates, 75
- Allozyme deficiencies, 167
- Anaerobes, 353
- Anaphase preparation of chromosomes, 139
- Aneuploidy, 89
- Anticlastogens, 191
- Antimutagens, 339
- Arsenic, 371
- Aspergillus, 359
  
- Bacterial mutagenesis, 27
- Baseline frequency, 325
- Benzene, 43
- Benzimidazole, 67
- Bleomycin, 89
- Bromodeoxyuridine, 35, 325
  
- Caffeine, 339
- Cancer chemotherapeutic drugs, 455
- Cannabidiol, 139
- Cannabinol, 139
- Cascade impactors, 101
- Cell survival, 473
- Chemical mutagens, 381
- Chick embryo, 435
- Chinese hamster cells, 371
- Chromosome
  - aberrations, 473
  - breakage, 455
  - loss, 187, 515
  - shattering, 339
- Chronic irradiation, 167
- Clastogens, 509
- Coal fly ash, 101
- Co-culture, 157
- Cyclohexene oxide, 27
- Cyclophosphamide, 157
- Cytogenetics, 43, 465
  - assays, 455
  - screening, 509
  
- Delta-9-tetrahydrocannabinol, 139
- Development, 435
- Diaminotoluene, 111
- Dimethylnitrosamine, 51
- Dinitrotoluene, 111
- DMN, 187
- DNA repair, 75, 371, 381, 531
- Dominant lethal, 111
- Drosophila
  - females, 543
  - melanogaster, 89, 133, 179, 187
- Dyes, 405
  
- E coli, 371, 531
- 8-hydroxyquinoline sulfate, 191
- Enzyme inhibition, 27
- Epoxide hydratase, 27
- Ethyl carbamate, 17
  
- 5-Bromodeoxyuridine, 149
- Flame retardants, 405
- Fluidized bed combustion, 101
- Fluorescent lamp, 389
- 4-(*p*-nitrobenzyl) pyridine, 395
- Fractionation, 75
  
- Gamma radiation, 473
- Gene-conversion, 359
- Genetic
  - effects, 179
  - factors, 51
  
- Halogenated hydrocarbons, 59
- Hepatocytes, 157
- Human, 325
  - B lymphocytes, 473
  - lymphocyte cultures, 139
  - T lymphocytes, 473
- Hydroxyurea, 381
- Hyperthermia, 465
  
- In vitro mutagenesis assay, 51
- In vivo, 17, 35
- Ionizing radiation, 179
  
- Lethals, 133
- L5178Y cells, 389

- Loci number, 447  
Lymphocyte, 325  
    chromosomes, 149  
    cultures, 191
- Magnetic fields, 179  
Maternal effect, 543  
Medroxyprogesterone acetate, 51  
*mei-9<sup>a</sup>*, 515  
Metabolic activation, 435  
Metaphase, 43  
Mice, 35, 111, 465  
Micronucleus, 43, 339, 509  
Mitotic  
    arrest, 67  
    index, 473  
    recombination, 531  
MMS, 515  
Mutagenesis, 59, 371, 395  
Mutagenicity, 75, 353, 405  
Mutations, 133, 179, 389, 531
- Nitrosocarbamates, 395  
N-methyl-N'-nitro-N-nitroguanidine, 381  
NO-carbaryl, 395  
Nucleosides, 339  
Nulls, 167
- 1,3-dinitrobenzene, 531  
1,3,5-trinitrobenzene, 531
- Pellet implantation, 35  
Peripheral blood, 509  
Pesticides, 359  
Platinum, 133  
Potentiation, 515  
Procarbazine, 515
- Radiation, 125  
Rats, 125  
Recessive sex-linked lethals, 89  
Repair deficiency, 187, 543
- Replicative synthesis, 381  
RF/J mice, 51
- Saccharomyces cerevisiae*, 359,  
    395, 531  
*Salmonella typhimurium*, 353,  
    395, 405, 531  
    mutagenicity test, 101  
Segregational errors of  
    chromosomes, 139  
Sister chromatid exchange, 17,  
    35, 149, 157, 191, 325, 435  
Size-dependent mutagenicity, 101  
Spermatogenesis, 465  
Sperm morphology, 111, 125  
Spindle disruption, 67  
Spontaneous mutation rates for  
    lethals and specific loci, 447  
Spot test, 111  
Sterility, 133  
Structure-activity relationship, 59  
Sun lamp, 389  
Sunlight, 389
- Testosterone, 51  
Tetryl, 531  
Tissue specific, 17  
Toxicity, 133, 389  
Translocations, 89  
Trenimon, 191  
Trichloropropene oxide, 27  
2-acetylaminofluorene (2-AAF),  
    435  
2-aminofluorene, 353  
2-nitrofluorene, 353
- Ultraviolet light, 339
- Vapor phase hydrocarbons, 101
- X-linked recessive lethals, 447  
X irradiation, 149