CHAPTER VII: PAPER AND ABSTRACTS PUBLISHED
Cytogenetic Studies in Human Populations Exposed to Gas Leak at Bhopal, India

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Frequencies of chromosomal abnormalities, sister chromatid exchanges, and replicative index were assessed following peripheral lymphocyte culture in 129 individuals from Bhopal, India. Of these, 83 persons (40 male and 43 female) had been exposed directly to the methyl isocyanate (MIC) gas after the accident at the Union Carbide plant on December 2 and 3, 1984. The remaining 46 samples were taken from age-matched unexposed persons in the same city. Chromosome aberrations were recorded at first cycle metaphase (M1) and sister chromatid exchanges, at second cycle metaphase (M2), following standard schedule. The frequency of chromosomal aberrations was, in general, higher in individuals from the exposed populations, with the females showing a higher incidence. Nondisjunction of chromosomes or laggard was rare. The frequencies of sister chromatid exchanges and depression in mitotic and replicative indices could not be related to exposure or sex. The persistence of chromosomal abnormalities in the form of replicating minutes and exchange configurations, even 1114 days after exposure to the gas, may indicate a residual effect on T-cell precursors.

Introduction

The disaster in the Union Carbide plant at Bhopal, India, between December 2 midnight and the early morning of December 3, 1984, affected more than 14,000 individuals (1). The inhabitants in the township of Bhopal were exposed in different degrees, depending on their proximity to the plant and atmospheric factors. A major lacuna in treating the exposed persons was the lack of adequate information on the toxicological effects of MIC (2). The unprecedented mortality and morbidity rates initiated detailed studies in experimental animals (3) and in some exposed individuals (4). The resultant publications show the wide spectrum covered by the toxic effects of MIC (1-4). Individual publications are now available on the chemistry of the reaction (5) as well as its genotoxic and clastogenic effects on laboratory test systems, ranging from Salmonella, Drosophila, and mice in vitro to Chinese hamster ovary cells in vitro (6-8,11,12). Sister chromatid exchanges (SCEs) have been reported in some exposed persons after the accident (4,5,10,13).

In the present case, chromosomal analysis was carried out in cases randomly selected in the exposed area up to 1114 days after exposure to the gas in order to study the residual effects, if any, on the chromosomes.

Material and Methods

The 129 individuals studied were categorized into those persons directly exposed with history of exposure and those who were not exposed, that is, residents in neighboring areas where the gas did not spread. The group of exposed persons was chosen through a random computer-based selection of listed survivors. The control group was taken from the unexposed population and was matched by age and sex.

Peripheral venous blood was collected in heparinized vials and plasma was separated out by gravity sedimentation. After an hour, leukocytes were inoculated to RPMI-1640 medium (GIBCO, Grand Island, NY), supplemented with 20% heat-inactivated fetal calf serum (Sera Lab., England) and phytohemagglutinin (M Form, GIBCO) at 0.2 mL/5 mL of culture.

In the set for the study of sister chromatid exchanges, 6.0 μg of 5-bromo-2-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) was added per 1.0 mL of the culture medium in the dark to prevent photocinactivation.

Four cultures were maintained for each subject in two
replicate sets in the dark at 37°C. The period of incubation was 48 hr for the study of chromosome aberrations and 72 hr for SCEs; 2 hr before termination of the culture, colchicine (40 μg/mL) was added to each culture. After further incubation for 2 hr, cells were centrifuged into pellets and treated successively in hypotonic solution (0.09% NaCl in deionized water) and fixative (methanol acetic acid 3:1) for 25 to 30 min at 37°C. The cells were centrifuged and resuspended in fixative three times. Air-dried slides were prepared and stained in Giemsa according to the standard procedure (14–16) with slight modifications as required.

The slides were coded and scored blind by two observers. The parameters recorded in each subject were (17–22): (a) chromosomal abnormalities (including damaged cells; total aberrations with and without gaps, breaks per damaged cell) recorded in 100 first-cycle scattered metaphases (M₁) per subject; (b) sister chromatid exchanges in 50 scattered complete second-cycle metaphases (M₂) per subject; and (c) cell cycle kinetics in 200 metaphases per subject. Parameters included replicative index (RI) and cell cycle metaphases in first (M₁), second (M₂), and third (M₃) cycles (2.1). The aberrations for the first three sets of parameters were compared by Student's t test between exposed and unexposed populations of both sexes.

**Results and Discussion**

Chromosomal aberrations were found to occur in statistically higher frequencies in the exposed group as compared to the control, especially in female subjects (Table 1). The types of abnormalities recorded were chromosome breaks, gaps, dicentrics, rings, and triradial and quadriradial configurations. Persistent, replicating minute chromosomes and quadriradial configurations were seen even after 1114 days (Figs. 1—4). In general, the number of breaks per cell was higher in exposed females as compared to unexposed ones. In males the difference was not significant. These observations are contradictory to the record of the higher incidence of micronuclei in male mice subjected to MIC in vivo (7,8).

| Sex     | Exposed type | Number of subjects | Cells scored | Total damaged cells* | Breaks/cell* | Aberrations damaged cell* |
|---------|--------------|--------------------|--------------|----------------------|--------------|----------------------------|
| Male    | Exposed      | 39                 | 4141         | 5.77 ± 1.99          | 0.072 ± 0.024 | 0.064 ± 0.023 | 1.27 ± 0.32 |
|         | Unexposed    | 20                 | 1884         | 5.49 ± 1.83          | 0.068 ± 0.031 | 0.056 ± 0.025 | 1.22 ± 0.24 |
| Female  | Exposed      | 43                 | 3963         | 6.74 ± 2.42          | 0.094 ± 0.027 | 0.077 ± 0.025 | 1.44 ± 0.37 |
|         | Unexposed    | 27                 | 2536         | 5.46 ± 2.16*         | 0.063 ± 0.032*| 0.050 ± 0.021*| 1.15 ± 0.36*|
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| Female  | Unexposed    | 27                 | 2536         | 5.46 ± 2.16          | 0.063 ± 0.032 | 0.050 ± 0.021 | 1.15 ± 0.36 |

*Figures are expressed as mean ± SD significant in Student's t test.
**p<0.05.
***p<0.01.
****p<0.001.

**Figure 1.** Replicating minutes.

**Figure 2.** Quadriradial and dicentric configurations.
The frequencies of SCEs did not differ markedly between exposed and unexposed populations. In general, the range was above the baseline ranges of 4 to 14 per cell reported earlier (24). The range of SCEs, number of breaks per cell, and percentage of cells with SCE could not be related to exposure or the sex of the person (Table 2). These observations do not support an earlier isolated report of increased frequency of SCEs in individuals exposed to MIC during the Bhopal accident (10). In another communication, no relation has been observed to factors such as smoking, alcohol intake, and pregnancy (13). The replicative index was not altered significantly between the exposed and unexposed populations.

The study indicates an apparent increase in the frequency of chromosomal aberrations in exposed females 1114 days after exposure to the gas. Since the selection was made as double blind in order to facilitate independent checks by two observers, the history and clinical features (if any) of these cases have not yet been fully related to the observations. The incidence of chromosomal alterations like dicentrics, rings, and quadriradial configurations even after 1114 days may indicate persistent clastogenic effects. Because the lesions induced by chemicals are mostly S dependent for expression in the subsequent divisional cycle, the damaged T-lymphocytes may remain circulating for long periods, and these aberrations can be observed only if the cells are stimulated to divide in vitro (25). The results are comparable to those obtained following exposure to p-dioxane (26).

Some of the females with persistent chromosomal aberrations have a history of fetal loss, which may have been the consequence of exposure to MIC. None of them has been exposed to any other known clastogenic agent except a single chest X-ray. The majority of the exposed females studied were housewives and the males were day laborers.

At present the observations are not complete and more needs to be done. In assessing the effects of exposure to toxic chemicals such as MIC, the sex of the subject and the physiological and nutritional status as well as other compounding factors such as genetic composition have to be taken into account (27,28). The persistence of aberrations even after 1114 days indicates the possibility of a higher susceptibility to chromosome damage of persons exposed to MIC.

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30. Chromosome Number in Two Species of Indian Hypsidae Moths (Lepidoptera).

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Cytological investigations on the gonial chromosomes of two species of moths Hypsaolephron and Hypsa ficus revealed a diploid count of 62 chromosomes at spermatogonial metaphase stages and a haploid chromosome number 31 at primary and secondary spermatocytic metaphase stages. Spermatogonial chromosomes were homomorphic, isodiametric and holocentric, being circularly arranged with only a few peripherally placed. The primary spermatocytic metaphase chromosomes were oval-dumbbell shaped, while metaphase II chromosomes were dot-shaped. The chromosomes were not differentiable into autosomes and sex-chromosomes as none of them exhibited heteropycnotic behaviour. Possible chromosome evolution in these species have been discussed.

31. Cytological Effects of Metallic Arsenic in Mice:

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Sodium arsenate (Na₃H₂AsO₄·7H₂O) in both chronic and acute doses was orally administered to male albino mice. The effect of the chemical was studied at doses ranging from 10 mg/kg body weight to 100 mg/kg body weight, and that included spindle disturbances as well as direct effect on chromosomes.

32. Absence of N-O-R in No. 13. Chromosome Pair of Black Rats from Quillon.

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Nucleolar organizer regions have been detected on No. 3, 8 and 13 chromosomes of black rat, Rattus norvegicus genome with
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and quantitative traits in treated plants were attributed to change in chromosome number.

55: CYTOTOXIC EFFECTS OF ARSENIC SALTS ON BONE MARROW CELLS OF MICE

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Sodium Arsenate and Sodium Arsenite in both chronic and acute doses were orally administered to male albino mice at doses ranging from 10 mg/kg to 100 mg/kg b. w. The effects induced, included spindle disturbances as well as direct action on chromosomes. The overall result indicated that both these salts are clastogenic to mice.

56: CYTOTOXIC EFFECTS OF LANTHANUM ON THE ANIMAL SYSTEM

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Lanthanum belonging to the group of elements known as "Lanthanoids" is used widely in various industries. The effects of this element and its compounds on cellular systems are of considerable interest due to their increasing use in industry and as a substitute or antagonist for calcium in a variety of chemical reactions. Chronic treatment to mice Mus musculus with various doses of LaCl₃ ranging from 43.73 to 6.3 mg/100 g b. w. induced spindle disturbances as well as gross chromosomal abnormalities.

57: ANTIMUTAGENIC EFFECT OF SOME FRUIT JUICES IN AMES' SALMONELLA REVERSION ASSAY

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Several studies have revealed the presence of mutagenic substances in the environment, which are thought to be involved in the deadly diseases like cancer and in the process of ageing too. It is, therefore, important
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EFFECT OF 3 - AMINO BENZAMIDE ON TRANSCRIPTION IN POLYTENE CHROMOSOMES
OF Drosophila melanogaster

Sushmita Maitra

Amino Benzamide (3AB) prevents ligation of replication intermediates leading to the accumulations of short DNA sequences. It also prevents poly-ADP-ribosylation. Benzamide is known to specifically induce a heat shock puff - 93D in Drosophila melanogaster and homologous region in other Drosophila species. Since 3AB is an amino derivative of Benzamide and it has a significant role in chain elongation, it would be interesting to understand the relation of its effect in poly-ADP-ribosylation to the replicative and transcriptive activity.

Salivary glands were treated with 3AB at different concentrations. RNA synthetic activity was monitored by autoradiography using H-uridine after the treatment. Appropriate controls were maintained whereby glands (either sister glands or glands from developmentally synchronized larvae) were incubated in Ringer. The following puff sites are induced by the treatment at the concentration range - 10 mM to 100 mM, 1A-2E on the X chromosome; 50CD, 57DEF on the 2R. These sites show a 2-fold higher H-uridine grain incorporation. The results indicate the possible implication of poly-ADP-ribosylation is not only controlling replication but also in vivo transcription.

METALS AS CLASTOGENS IN EUKARYOTIC TEST SYSTEMS

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A systematic analysis of the clastogenic potential of inorganic salts of individual metals, both cations and anions, on multiple test systems in vivo following both acute and chronic exposure, has shown that: i) most salts are effective mitotic poisons (Turbagens) at particular concentrations, due to their known affinity for thiol groups and induce different types of spindle disturbances. The clastogenic effects are S-dependent. In mammalian systems, the degree of clastogenicity is directly proportional to the concentration of the chemical and duration of exposure within a limited range. In plant systems in vivo, the degree of dissocation and the availability of cations affect the frequency of aberrations induced quantitatively.