# GENETICS

# Relationship between the Duration and Number of Cell Cycles after Mutagenic Exposure and the Incidence of Sister Chromatid Exchanges

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The incidence of sister chromatid exchanges during the second and third mitoses in Jungar hamster bone marrow cells and human lymphocytes is assessed at different times after mutagenic exposure to thiophosphamide. In the control, the mean number of sister chromatid exchanges in bone marrow cells did not change during three consecutive cycles irrespective of the rate of cell proliferation. Thiophosphamide exposure resulted in replicative or nonreplicative repair of injuries inducing sister chromatid exchanges. About 50-70% of injuries are eliminated during a replicative (mitotic) cycle. Nonreplicative repair is intensive in proliferating bone marrow cells and weakly expressed in blood lymphocytes.

**Key Words:** *sister chromatid exchanges; lymphocytes; bone marrow cells; thiophosphamide*

Sister chromatid exchanges (SCE) are formed during DNA replication as realization of primary chromosome injuries. The number of such injuries and, therefore, SCE after mutagenic exposure gradually decreases due to reparative processes and death of cells damaged worst of all [3,6,9]. SCE-inducing injuries can be repaired in proliferating cells during DNA replication and other phases of mitotic cycle and in nonproliferating cells [1,7,9]. Published data about the contribution of each of these processes to the decrease in the SCE level after mutagenic exposure are contradictory. However, it is the crucial point determining the conditions of SCE test for analysis of mutagenic loading in genetic monitoring.

Our purpose was to assess the incidence of SCE during the second and third mitoses at different times after mutagenic exposure of Jungar hamster bone marrow cells *in vivo* and human lymphocytes *in vitro*.

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## MATERIALS AND METHODS

*In vivo* experiments were carried out on 36 Jungar hamsters weighing 35 g. A 50-mg tablet of 5-bromo-2'deoxyuridine (BDU, Sigma), 80% of which was coated with an MK-6 biological glue for decelerating the drug absorption and prolonging its effect, was subcutaneously implanted into each animal. In parallel with this, experimental hamsters were intraperitoneally injected with thiophosphamide (TP) in a dose of 1.5 μg/g dissolved in normal saline. Controls were injected with saline alone. The animals were sacrificed by cervical dislocation after 13-37 h at 6-h intervals. Two hours before sacrifice the animals were injected colchicine in a dose of 2 μg/g intraperitoneally. Bone marrow was collected from femoral bones.

For *in vitro* experiments, human donor blood was incubated for 96 h in the presence of BDU. Blood specimens were treated with TP for 1 h (10

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μg/ml) immediately or after 24-h incubation, after which they were washed 3 times in a 10-fold volume of Eagle's medium. Cytogenetic preparations of hamster bone marrow and human lymphocytes were differentially stained as described previously [4]. The number of SCE in the second mitosis and the number of symmetrical and nonsymmetrical SCE in the third mitosis were counted. Results were statistically processed using Student's test.

## RESULTS

Table 1 shows the results of *in vivo* experiments with bone marrow cells. The number of SCE in the second mitosis and the number of nonsymetrical SCE in the third mitosis reflects all exchanges, which occurred in the cells during the first and second mitotic cycles. In the control this value was about 4 SCE/cell, *i. e.*, 2 SCE/cell/cycle. Symmetrical SCE in the third mitosis show only half of exchanges in the third mitotic cycle, because only half of the third mitosis chromosomes are characterized by "harlequin" color. Therefore, for assessing the total number of SCE during the third mitotic cycle, the number of symmetrical SCE is doubled. In the control, the number of SCE formed during the third cycle is about 2 per cell (Table 1). Hence, in the control the mean number of SCE/cell/cycle remains stable during three cycles after incorporation of BDU irrespective of the time of fixation, i. e., does not depend on the rate of cell proliferation.

After mutagenic exposure, 24 more SCE per cell are formed during the first and second mitotic cycles. The number of induced SCE decreases with prolongation of the interval between mutagenic exposure and fixation (the decrease from 19th to 37th h is statistically significant, $p$=0.003). The number of SCE formed during the third cycle after TP exposure (symmetrical SCE in the third mitosis) is two times higher than in the control ($p$=0.001).

Similar data were obtained after *in vitro* exposure of human cells to TP (Table 2). The number of SCE induced in the third cycle drops in comparison with the first and second cycles but remains 2-3 times higher than in the control ($p$=0.001). The number of exchanges formed during the first and second cycles after exposure to TP negligibly increases if the period between exposure and fixation is decreased (during the 24th h of exposure in comparison with the pre-exposure values).

Thus, our data demonstrate that multiplication of cells after mutagenic exposure is associated with active repair of chromosome damage inducing SCE. 50-70% of injuries are eliminated during a replicative (mitotic) cycle, which is in line with published data [7]. The decrease in the number of SCE during the

**TABLE 1. Number of SCE per Bone Marrow Cell in Jungar Hamsters fefore and after Exposure to TM (*M±m*)**

| Time after exposure, h | Control | | | | Experiment | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2nd mitosis | | 3rd mitosis | | 2nd mitosis | | 3rd mitosis | | | |
| | SCE | cell number | nonsymmetrical SCE | symmetrical SCE | cell number | SCE | cell number | nonsymmetrical SCE | symmetrical SCE | cell number |
| 13 | 4.06±0.11 | 62 | | | | 30.76±0.58 | 63 | | | |
| 19 | 4.88±0.46 | 66 | | | | 29.17±0.51 | 97 | | | |
| 25 | 4.23±0.18 | 136 | 4.18±0.51 | 0.87±0.14 | 31 | 26.66±0.62 | 209 | 26.62±0.82 | 2.13±0.06 | 36 |
| 31 | 4.32±0.34 | 119 | 3.75±0.31 | 1.06±0.10 | 95 | 26.58±1.17 | 126 | 27.57±1.14 | 2.12±0.13 | 149 |
| 37 | | | | | | 28.04±1.18 | 495 | 27.17±0.71 | 2.12±0.07 | 185 |
| Mean... | 4.35±0.18 | 383 | 3.89±0.25 | 0.99±0.08 | 126 | | | | | |

Note. Absence of data means that by this time the cells did not pass through the second or third mitosis. In control, SCE were not counted at 37th h.

TABLE 2. Number of SCE per Cell in Human Lymphocyte Culture Exposed to TP (*M±m*)

| Experiment conditions | SCE in 2nd mitosis | SCE in 3rd mitosis | |
|---|---|---|---|
| | | nonsymmetrical | symmetrical |
| Control | 17.94±0.68 | 9.27±0.64 | 2.04±0.36 |
| Exposure before culturing | 40.50±1.40 | 42.00±1.18 | 4.70±0.41 |
| Exposure during 24th h of culturing | 41.85±1.07 | 43.25±1.23 | 6.64±0.54 |

Note. Each value was derived in analysis of 25 metaphases.

second mitosis with prolongation of the interval between exposure and fixation suggests that repair is possible without DNA replication.

From our results it can be concluded that decreased SCE level in lymphocytes after exposure to mutagens [2,3,6] is due predominantly to substitution of lymphocytes by cells formed in hemopoietic tissue after exposure. Our data provide no unambiguous answer about the mechanisms and intensity of nonreplicative repair of injuries inducing SCE in blood lymphocytes. Published reports on this problem are contradictory [5,8,10].

Further studies of regularities of SCE repair in blood lymphocytes are needed for adequate use of this test and proper interpretation of results of genetic monitoring of man.

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