DEPENDENCE OF SISTER CHROMATID EXCHANGES ON DURATION OF EXPOSURE OF HUMAN CELLS TO ETHYLENIMINE DERIVATIVES

G. G. Listopad and A. N. Chebotarev

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There are many references in the literature to the effect of many chemicals on the frequency of sister chromatid exchanges (SCE) [6, 7], but no data can be found on dependence of induced SCE on exposure. Lengthening of contact between mutagen and cells is known to increase the frequency of chromosomal aberrations [5]. Since the mechanism of formation of aberrations evidently differs from the mechanism of SCE, exposure dependences of the frequencies of chromosomal aberrations cannot be extrapolated to SCE.

The aim of this investigation was to study dependence of the number of SCE on exposure of cells to mutagens in various concentrations.

EXPERIMENTAL METHOD

Human peripheral blood lymphocytes were used. The cultures were fixed after 96 h, colchicine having been added 2 h before fixation. For differential staining of sister chromatids, 10 μg/ml of bromodeoxyuridine (BUdR) was added 40 h before fixation. Cultures were treated with different concentrations of mutagens 20 h before fixation for different exposures at 37°C. For rapid inactivation of the mutagens in the culture medium, 1 ml of a 10% solution of sodium hyposulfite was added for every 10 ml [4]. The cells were then washed twice with cold and warm Hanks' solution. The medium was then changed for fresh with 10 μg/ml BUdR.

Two series of experiments were carried out, with two repetitions of each for two mutagens — thiophosphamide (thio-TEPA) and dipin. In series I, to determine dependence on concentration, an exposure of 1 h to nine different concentrations for each mutagen was used. In series II the cells were treated with 4.23×10^{-5} M thiophosphamide and 1.16×10^{-4} M dipin for 10, 20, 30, 40, 50, and 60 min, and also with 2.11×10^{-5} M thiophosphamide and 5.78×10^{-5} M dipin for 30, 60, 90, 120, and 150 min. Exposure and concentration were chosen so that mitotic activity and the frequency of mitoses with differential staining of sister chromatids were not significantly depressed.

The specimens were prepared and stained by the method described previously [3].

In each version of the experiments 25 cells were analyzed.

EXPERIMENTAL RESULTS

Dependence of the number of SCE induced by thiophosphamide and dipin on concentration are shown in Fig. 1. Within the range of chosen concentrations the number of SCE was a linear function of the concentration of the substance within the range of chosen concentrations for both mutagens (inadequacy not significant, $P > 0.05$; regression significant, $P < 0.001$). Extrapolation of these regression lines to zero concentration gives an SCE level indistinguishable from the control. The angular coefficient of regression (an index which characterizes the angle of slope of the regression line and indicates how many SCE could be induced by 1 mole of the substance) was 5 times higher than the corresponding coefficient for dipin $6.26925 \cdot 10^5 \pm 5.6405 \cdot 10^4$ and $1.2103 \times 10^5 \pm 8.6414 \cdot 10^3$ respectively). Consequently, the effect of isomolar concentrations of thiophosphamide was 5 times stronger than that of dipin. Accordingly, when exposure dependences were studied, dipin was used in a concentration 5 times higher than that of thiophosphamide for the same length of exposure.

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TABLE 1. Number of SCE per Cell Induced by Thiophosphamide and Dipin, Depending on Duration of Exposure and Dose (M ± m)

Exposure, min	Thiophosphamide		Dipin		Exposure, min	Thiophosphamide		Dipin	
	dose, min·M·10^{-3}	no. of SCE per cell	dose, min·M·10^{-3}	no. of SCE per cell		dose, min·M·10^{-3}	no. of SCE per cell	dose, min·M·10^{-3}	no. of SCE per cell
10	0,43	20,08±1,10 16,88±0,67	1,16	21,40±1,15 29,63±1,02	30	0,63	17,84±0,68 27,53±1,04	1,73	30,96±1,48 32,44±0,83
20	0,85	25,32±0,99 25,15±1,08	2,31	28,88±1,31 32,50±1,38	60	1,27	25,80±0,99 35,28±1,37	3,47	30,40±1,28 40,12±1,20
30	1,27	28,04±1,01 34,56±1,03	3,47	39,72±1,98 33,67±1,12	90	1,90	31,88±1,07 41,04±0,98	5,20	40,56±2,07 45,67±2,15
40	1,69	31,92±1,29 41,60±1,64	4,62	46,60±3,74 44,52±1,72	120	2,54	41,64±1,82 50,24±2,31	6,93	45,89±3,87 53,46±1,74
50	2.11	33,24±1,72 47,70±2,20	5,78	48,20±3,32 42,12±2,40	150	3,17	45,76±1,93 54,24±1,81	8,66	45,96±2,13 60,00±2,17
60	2,54	41,28±1,23 43,80±2,57	6,93	57,08±2,41 47,13±3,63					
0 Control		5,24±0,46 6,08±0,53							

Legend. Dose equal to product of exposure and concentration.

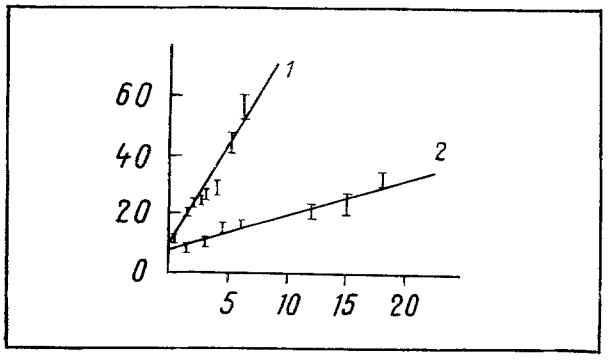

Fig. 1. Dependence of number of SCE on concentration of thiophosphamide (1) and dipin (2). Abscissa, concentration of mutagens (in M × 10^{-5}); ordinate, number of SCE per cell.

The results of the experiments of series II are given in Table 1. The number of SCE increased with lengthening of exposure to thiophosphamide and dipin; just as with dependence on concentration, the number of SCE was a linear function of exposure to the mutagens within the range of time intervals studied. The efficacy of thiophosphamide, just as in the case of dependence on concentration, likewise was higher than that of dipin. Regression was linear: Inadequacy was not significant for thiophosphamide (P = 0.57) or for dipin (P = 0.14). For regression in all cases P < 0.001.

During irradiation a constant level of aberrations is observed within a certain dose range, irrespective of dose rate, but provided that the product of dose rate and exposure remains constant [2]. It will be clear from Table 1 that the number of SCE increased with an increase in dose, which in the case of chemical mutagenesis can be taken to be the product of concentration and exposure. This dose produced the same number of SCE regardless of whether a high concentration acted for a short time or a low concentration for a long time. Induction of SCE increased as a linear function with an increase in dose (P of inadequacy < 0.005; P for regression < 0.001). Extrapolation of these regression lines to zero dose predicted a heightened control SCE level per cell (15.72 for thiophosphamide and 23.45 for dipin), whereas the number of SCE in the control was 5-6 exchanges per cell. A jump in the number of SCE was thus observed between the control and minimal exposure dose. This jump was unconnected with the possible equal increase in the number of SCE for variants treated with the mutagen, for in that case it must also have occurred in the tests of dependence

on concentration, but this was not observed in the experiments of series I. It might be supposed that with an increase in exposure, any subsequent increase in the number of SCE on account of decomposition of the mutagen in the medium would be delayed. However, during the chosen exposures no significant decomposition of the substances is observed [1]. This jump appeared only when the duration of exposure was varied. This effect is evidently connected with the aftereffect of the compounds in the cell after their removal from the culture medium. However, it is manifested essentially only during short exposures (under 10 min), whereas during longer exposures this aftereffect is not manifested and is not reflected in the linearity of the dose–effect relationships.

LITERATURE CITED

1. O. P. Kirichenko, A. N. Chebotarev, and K. N. Yakovenko, Byull. Éksp. Biol. Med., No. 5, 552 (1976).
2. D. E. Li, The Action of Radiation on Living Cells [in Russian], Moscow (1963).
3. A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova, Byull. Éksp. Biol. Med., No. 2, 242 (1977).
4. E. Allen and W. Seaman, Anal. Chem., 27, 540 (1955).
5. I. G. Breven and N. F. Christie, Exp. Cell Res., 46, 276 (1967).
6. A. P. Craig-Holmes and M. W. Shaw, Mutat. Res., 46, 375 (1977).
7. P. Perry and H. J. Evans, Nature, 258, 121 (1975).