TIME COURSE OF FREQUENCY OF SISTER CHROMATID EXCHANGES AFTER EXPOSURE TO THIOPHOSPHAMIDE IN VIVO

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A strategy for studying dependence of induction of sister chromatid exchanges (SCE) in mammalian blood lymphocytes on the dose of mutagenic exposure in vivo has recently been developed [1, 2]. However, on total excretion or inactivation of the mutagen, the process of induction of mutations is followed by their elimination [3, 4, 6]. Knowledge of the principles governing elimination of mutations is essential for the correct testing of chemicals for mutagenicity and for adequate interpretation of the results of cytogenetic studies of groups of the population in contact with active chemical agents. Elimination of mutations (in particular, lesions leading to SCE) in vivo can take place through death of the cells, repair of the lesions, and selective multiplication of cells.

The aim of this investigation was to analyze the time course of the frequency of SCE, taking all these processes into consideration.

EXPERIMENTAL METHOD

An aqueous solution of thiophosphamide was injected intravenously into a rabbit in a dose of 4 mg/kg and venous blood samples were taken after 6 and 9 h, and 1, 2, 4, 7, 14, and 21 days. Blood lymphocytes were cultured for 65 h with bromodeoxyuridine (BUdR) [4]. After preparations of metaphase chromosomes had been obtained, the cells were analyzed 100 at a time to determine the mean frequency of SCE, and the distribution of the cells by number of SCE at each time also was examined.

EXPERIMENTAL RESULTS

The average number of SCE in lymphocytes of the rabbit's blood began to decrease as early as on the first day, immediately after elimination of the compound and in the course of the first 21 days it fell to values close to the control. Experimental points obtained during analysis of the frequency of SCE after administration of thiophosphamide are shown in Fig. 1. The fastest decrease in the frequency of SCE, namely from 60 to 20 exchanges, was observed during the first 4 days, and this was followed by a steadier fall to 9 SCE per cell (broken line) on the following days. That the time course of the frequency of SCE could be described by an exponential function was tested during analysis of the residues. This analysis showed the presence of two phases of decline on the frequency of SCE: a fast phase initially followed by a slow phase later.

Data on the distribution of the cells by number of SCE at different times after exposure to the mutagen are given in Fig. 2. The average value of SCE, it is apparent, decreased not because of the appearance of cells with a number of exchanges that varied within the control limit, but because of disappearance of cells with a large number of exchanges and the appearance of cells with an intermediate number of SCE.

The rapid decrease in the frequency of SCE during the first 4 days was connected with mass death of the most severely affected cells, due to the combined mutagenic and cytotoxic action of thiophosphamide [4]. Restoration of the blood cell population began on the 4th day, and was attributable to proliferation and release of less severely affected cells into the blood stream from the hematopoietic organs, and together with repair of the lesions, this led to a steady decrease in the frequency of SCE.

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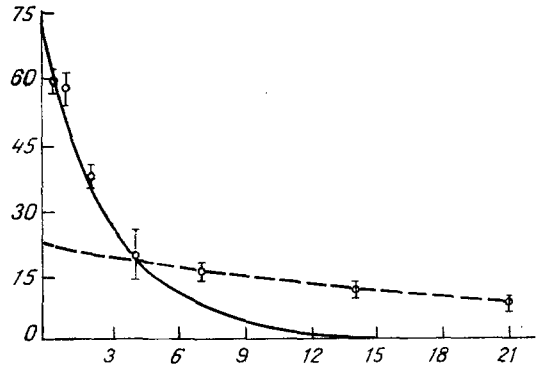

Fig. 1

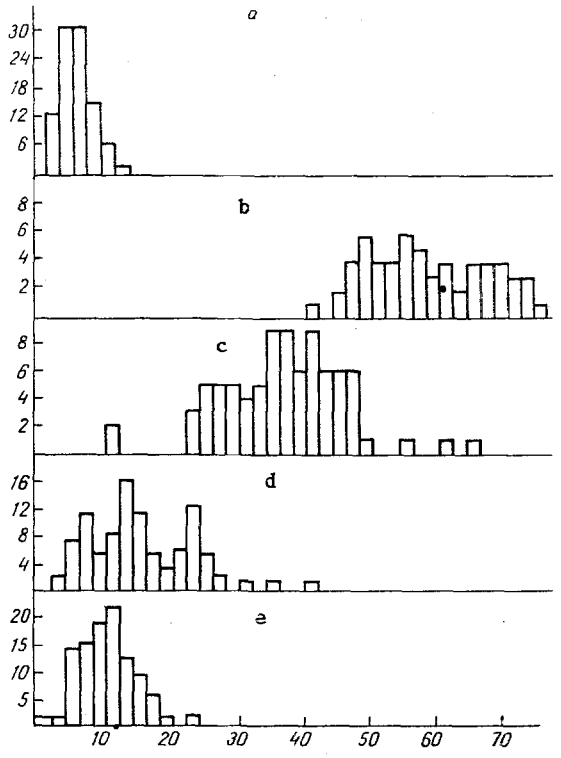

Fig. 2

Fig. 1. Time course of frequency of SCE in rabbit blood cells after exposure to thiophosphamide. Explanation in text. Abscissa, days after injection of thiophosphamide; ordinate, number of SCE per cell.

Fig. 2. Distribution of cells by number of SCE at different times after injection of thiophosphamide. a) Before injection of thiophosphamide; b-e) 9 h, and 2, 7, and 21 days respectively after injection of thiophosphamide. Horizontal axis, number of SCE per cell; vertical axis, number of cells.

For a correct estimation of the mutagenic activity of thiophosphamide, it is essential to determine the exact maximal level of frequency of the cytogenetic effects induced with the given dose. This level can be determined on the basis of species-specific sensitivity to this compound and its pharmacodynamics in vivo, by analysis of dose dependence of induction of its effects in vivo or in vitro. However, with large doses of the mutagen, because of mass death of the cells the observed effect will be less than the expected effect. The maximal effect can be estimated by analysis of the time course of elimination of mutations.

On extrapolation of the line describing the fast phase of decline of the frequency of SCE in the present investigation, to the origin of the time axis the line intersects the axis at a value of SCE of 71.7. With the same dose the expected value of the maximum was 88.05 ± 17.24 (confidence interval ±95%), quite close to the value obtained by extrapolation. The maximal value of the average in the experiment was 59.31 ± 17.97, with variation of individual values from 41 to 81 exchanges.

Extrapolation of the exponential curve represented by a broken line to the origin of the time axis gives the value of SCE (22 exchanges per cell) that probably corresponds to the level of lesions at which cells of the hematopoietic organs survive and, through repair, can be rid of some of their injuries [5, 7]. Cells of this class appear in small numbers on the 2nd day and form a separate peak on the 7th day, when a process of restoration of the normal number of blood cells is actively taking place (Fig. 2d).

Analysis of the time course of the frequency of SCE after a single exposure to thiophosphamide leads to the conclusion that the time course of the fall of frequency of SCE has two phases: an initial fast phase and a later slow phase; the fall of the average number of SCE is due to the appearance of classes of cells with an intermediate number of SCE and disappearance of cells with a large number of exchanges.

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