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PROLIFERATIVE ACTIVITY OF LYMPHOCYTES IN CULTURE AFTER EXPOSURE TO THE MUTAGENIC ACTION OF THIOPHOSPHAMIDE *IN VIVO* AND *IN VITRO*

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The effect of chemicals with mutagenic activity on cell proliferation has been studied as a rule incidentally to the main investigation of cytogenetic effects of mutagens. The research workers concerned were interested chiefly in the degree of correlation between frequencies of cytogenetic effects and changes in cell proliferation [4-6]. No work has been done on the study of dependence of proliferative activity of cells on the mutagenic dose *in vivo* or *in vitro*. Such investigations are important for choosing the conditions under which the results of cytogenetical analysis of mutagenic action *in vitro* can be extrapolated directly to the living organism.

In this investigation the proliferative activity of lymphocytes was studied after exposure to the mutagenic action of thiophosphamide *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

The effect of dose of mutagenic action on proliferative activity of the cells was studied on rabbit blood lymphocytes treated with thiophosphamide *in vivo* and *in vitro* [1]. The term "exposure dose" of thiophosphamide *in vitro* was taken to mean the product of concentration and duration of exposure (60 min), whereas *in vivo* it was taken to be the integral of the change in blood thiophosphamide concentration from the time of injection of the compound to the time of taking the blood sample. The method of culture of the lymphocytes in experiments *in vivo* and *in vitro* was the same [1]; the cells were fixed after 56-60 h. To distinguish between the 1st, 2nd, and 3rd mitoses, 5-BUdR was added to the cultures and preparations were stained by the modified method of differential staining of sister chromatids [3].

Dependence of the change in proliferative activity was analyzed by the use of the parameter T, standing for the mean number of divisions through which the cells passed after

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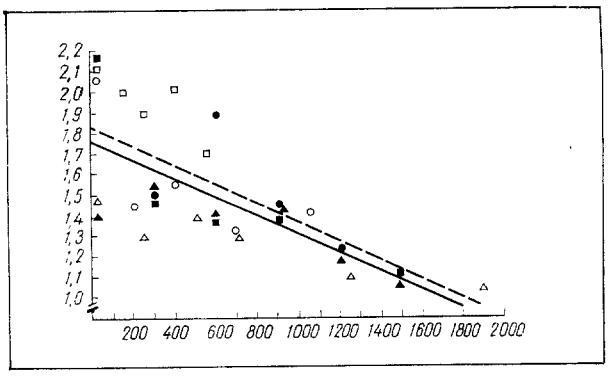

Fig. 1. Dependence of mean number of divisions in culture on changes in mutagenic exposure dose. Broken line and empty symbols — *in vivo*: continuous line and filled symbols — *in vitro*. Abscissa, dose (in μg/ml/min); ordinate, mean number of divisions (T).

exposure to mutagenic action and until fixation. This parameter was calculated by the equation:

$$T = \frac{\ln\left(\sum_{i=1}^{3} 2^i \cdot fi\right)}{\ln 2},$$

where i denotes the serial number of the mitosis; fi the frequency of the i-th mitosis among all the metaphases analyzed. By using this parameter the degree of delay of cell division after exposure to mutagenic action can be adequately estimated.

EXPERIMENTAL RESULTS

Data are given to show changes in the mean number of divisions (T) with a change in mutagenic exposure dose (D) *in vivo* and *in vitro*. As Table 1* shows, the mean number of divisions falls with an increase in exposure dose. To analyze dependence of the decrease in value of T with an increase in dose, data for three rabbits after treatment of their blood lymphocytes *in vivo* and *in vitro* were subjected to regression analysis [2]. This analysis shows that the data in Table 1 are satisfactorily described by the linear regression equation $T = T_0 + kD$, where k is a parameter of the equation, D the dose, and T_0 the number of divisions in the absence of exposure to the mutagen.

Inadequacy of the linear model for the experiments *in vitro* is not significant ($P = 0.84$, $r^2 = 0.58$), regression is highly significant ($P < 0.001$), and the regression equation is as follows:

$$T = 1{,}7553(\pm 0{,}1777) - 0{,}0004453(\pm 0{,}0002128) \cdot D.$$

Regression for the results of the experiments *in vivo* also is significant ($P = 0.004$, $r^2 = 0.45$) and the regression equation is as follows:

$$T = 1{,}8290(\pm 0{,}2083) - 0{,}0004590(\pm 0{,}0002888) \cdot D,$$

where D is the mutagenic exposure dose and the 95% confidence limits are given in parentheses.

Regression lines for dependence of the mean number of divisions on dose of mutagen for the experiments *in vivo* and *in vitro* are given in Fig. 1.

It will be clear from the regression equation and from Fig. 1 that the mean number of divisions fell by about the same amount after treatment with the mutagen *in vivo* and *in vitro*. These data indicate that the cytotoxic action of thiophosphamide on the cells was the same as a result of their treatment with the mutagen *in vivo* and *in vitro*.

*Table missing Russian original — Publisher.

When the effect of chemicals on proliferative activity of cells is studied, most frequently the ratio (in percentages or fractions of unity) of the 1st, 2nd, and 3rd mitotic divisions is used for analysis of the data, but this is inconvenient. The writers suggest that the use of the mean number of divisions, which can be calculated by a simple method, be used as the parameter for these purposes, and the parameter itself gives an adequate estimate of the degree of inhibition of cell proliferation with an increase in the mutagenic exposure dose *in vivo* and *in vitro*.

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