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INDUCTION AND ELIMINATION OF CYTOGENETIC DISTURBANCES IN LYMPHOCYTES OF MONKEYS EXPOSED TO THIOPHOSPHAMIDE

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To evaluate correctly the results of testing for mutagenicity and to predict the mutagenic affects of external environmental factors it is necessary to describe the dose–time–effect relationships, i.e., the dynamics of the mutation process, which has two components: induction of mutations and elimination of mutations (or of primary injuries to the genetic apparatus). The rules governing induction of mutations have been studied sufficiently well, the principles of calculation of the increase in frequencies of cytogenetic disturbances and of determination of the maximal effect after exposure to chemical mutagens have been suggested [5], but the principles and methods of elimination of mutations in the living organism have not been adequately studied.

The aim of this investigation was to study induction and elimination of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in monkeys over a long period after administration of thiophosphamide, and to create a mathematical model describing the time course of these processes.

EXPERIMENTAL METHOD

Experiments were carried out on four mature male rhesus monkeys given thiophosphamide by intravenous injection in a dose of 3 mg/kg body weight. For 6 months after injection of the compound, blood samples were taken at various time intervals for lymphocyte culture in the presence of 10 μg/ml of 5-bromodeoxyuridine, followed by analysis of frequencies of SCE in the second, and of CA in the first mitoses. The concentration of thiophosphamide was determined in blood samples obtained during the first 4 h after injection, by the nitrobenzylpyridine test [2].

To construct a mathematical model the approach suggested previously [1] was used. In accordance with this approach, induction and elimination of cytogenetic disturbances are described by curves of probability functions. In this work we used probability functions of a Weibull distribution. When the parameters of the model were defined, the time, expressed in days, was plotted on a logarithmic scale.

EXPERIMENTAL RESULTS

The results are evidence that the blood thiophosphamide concentration in monkeys falls exponentially after intravenous injection. Calculations show that during 10 h the thiophosphamide concentration fell approximately by 90%, and its elimination was virtually complete

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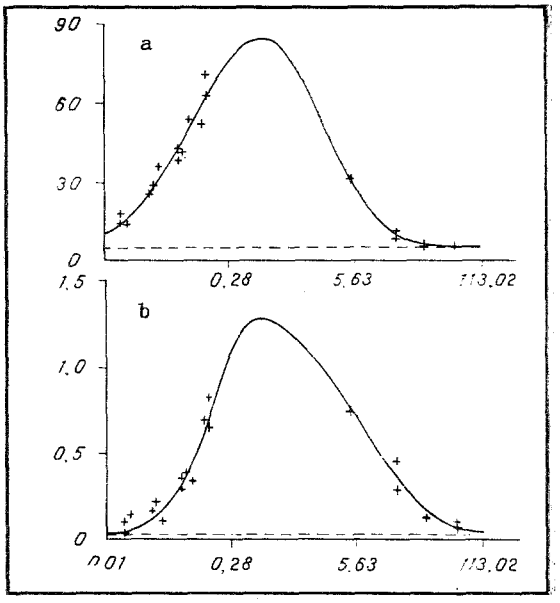

Fig. 1. Time course of number of SCE per cell (a) and of CA (chromosomal breaks) per cell in metaphase (b). Abscissa, time after injection of thiophosphamide (in days, logarithmic scale); broken line indicates control level of SCE and CA.

after the 1st day. The presence of the mutagen in the animals' blood at this time determined its action on the lymphocytes and the increase in the number of SCE and CA in them.

A short time after injection of the mutagen, elimination of mutations began to predominate over induction and a decrease was observed in the number of SCE and CA. The change in the frequencies of SCE and CA was described by the following equation:

$$E = SU + k \cdot \left\{ exp\left[-\left(\frac{t}{b_2}\right)^{C_2} \right] - exp\left[-\left(\frac{t}{b_1}\right)^{C_1} \right] \right\},$$

where SU denotes the spontaneous frequency of cytogenetic disturbances, t the time, k, b_1, and b_2 are scale coefficients, and C_1 and C_2 are shape coefficients.

The model proposed, which reflects the biphasic character of the time course of the mutations, consists of two components describing processes of induction and elimination of SCE and CA. Table 1 gives the results of determination of the parameters of the model, for which the method of least squares was used. Analysis of the residues proved that the suggested model is adequate.

By using the parameters of the equation thus found, we plotted the functions of the change in number of SCE and CA against time. The experimental data and the calculation regression dependences are illustrated in Fig. 1.

Calculations show that the maximal number of both SCE and CA is reached 14 h after injection of thiophosphamide, after which these parameters begin to fall. As the data show, the half-elimination time of SCE is only half as long as that of CA. As early as after 1 month the number of SCE regains the control level, whereas the frequency of CA remains high for 6 months after exposure.

Differences in the time course of SCE and CA are evidently due to differences in the mechanisms of their elimination. SCE, which can be recorded in a cytogenetic preparation, are formed when the cells pass through two replication periods during culture in the presence of 5-bromodeoxyuridine, on account of primary chromosomal injuries that are preserved until the time of collection of the blood sample. The number of these primary injuries after mutagenic action decreases steadily due to death of the most severely damaged cells, and also of repair processes taking place both in the lymphocytes themselves and in their precursor cells during proliferation in the hematopoietic tissue. Under these circumstances, after

TABLE 1. Results of Description of Experimental Data by Model of Time Course of Number of SCE and CA

Parameter	k	b_1	b_2	C_1	C_2	Maximal effect	Half-elimination time, days
SCE	147,6	6,01	8,12	4,58	6,06	88,7**	3,26
CA	1,37	5,66	9,46	8,20	7,26	1,30***	6,66

Legend. *) Time during which maximal cytogenic effect reduced by half, **) number of SCE per cell, ***) number of chromosomal breaks per cell in metaphase.

each replication cycle between 50% [8] and 100% [6, 7] of injuries that are the source of SCE are lost. Our data show that complete repair of such injuries in the monkeys' lymphocytes takes place in the course of 1 month after exposure to the mutagen.

By contrast with SCE, it is possible to record both those CA which arise during culture of the cells on account of unrepaired chromosomal injuries and those formed previously at any moment after exposure to the mutagen. A definite proportion of them (up to 50% or more) is preserved as the cells pass through the mitotic cycle [3, 4]. All this leads to long preservation of a raised CA level.

Precise determination of the rate of repair, the coefficients of passage of CA through the mitotic cycle, and the kinetics of the cells after exposure to the mutagen enables the principles discovered to be used to calculate the parameters of the suggested mathematical model, and thus to transform it from descriptive into prognostic. In this way the mutagenic effect can be predicted and the genetic consequences of exposure to a mutagen can be assessed.

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