In the course of 6 h after injection of thiophosphamide in this investigation a logarithmic increase in the frequencies of SCE and CA with time after injection of the mutagen was thus observed. The dynamics of the change in frequencies of the cytogenetic effect depends on the initial quantity of thiophosphamide injected, but depends only a little on the elimination constant of the compound, for with a change in the dose injected, the values of the constant change only negligibly.

Equations for determining maximal cytogenetic effects of treatment with mutagens (8, 9, and 10) can be used to determine the maximal cytogenetic effects of small doses of mutagens, such as are used in chemotherapy and which do not induce an appreciable cytogenetic effect when about 100-200 cells are counted in the usual way [3]. In fact, if it is necessary to estimate the degree of cytogenetic damage which can be induced by administration of chemotherapeutic agents, this can be done before treatment. To do this it is necessary to know the spontaneous levels of the number of SCE and frequency of CA for a given individual or to use these parameters determined for the population. The efficacy constant of the given preparation, determined *in vitro*, and the illumination constant of the substance, determined by a study of its pharmacodynamics, and also the quantity of the substance injected must also be known.

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COMPARISON OF LEVEL OF SISTER CHROMATID EXCHANGES AND CHROMOSOMAL ABERRATIONS INDUCED BY CHEMICAL MUTAGENS *IN VITRO*

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The use of analysis of sister chromatid exchanges (SCE) as a quantitative test for mutagenicity of chemicals has several advantages, the most important of which is the high sensitivity of the method [4, 5]. However, a great handicap to the wide use of this method is still uncertainty regarding the mechanisms of their appearance [12] and the connection with the formation of chromosomal aberrations, despite the many investigations devoted to this question [6, 12].

One approach to the solution of this problem may be a simultaneous quantitative study of dose dependences for the appearance of SCE and chromosomal aberrations induced by different chemical compounds, and the investigation described below was undertaken for that purpose.

EXPERIMENTAL METHOD

Dose dependences of five compounds with different numbers and types of alkylating groups were studied: mitomycin C — a bifunctional alkylating antibiotic, E39 — a quinone derivative with two ethylenimine groups, thiophosphamide and dipin — derivatives of aminophosphoric acid

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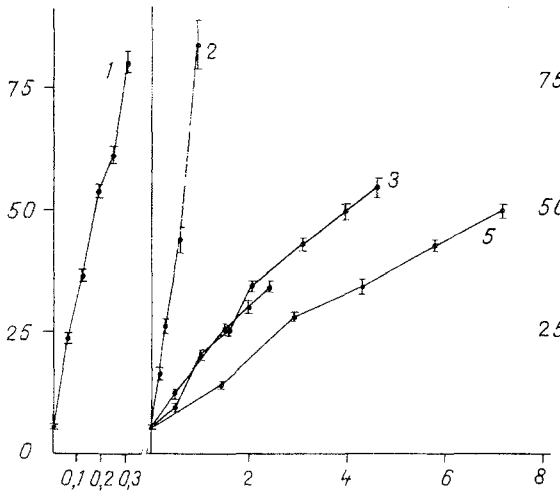

Fig. 1

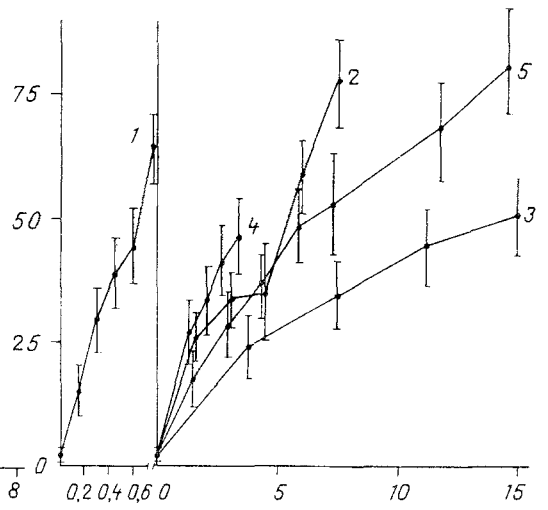

Fig. 2

Fig. 1. Change in number of SCE depending on concentration of substance. Abscissa, concentration of substance (10^{-5} M); ordinate, number of SCE per cell. 1) Mitomycin C, 2) E_{39}, 3) thiophosphamide, 4) degranol, 5) dipin.

Fig. 2. Dependence of fraction of aberrant metaphases on concentration of substance. Ordinate, fraction of aberrant metaphases (in %). Remainder of legend as to Fig. 1.

TABLE 1. Comparative Effectiveness of Chemical Compounds in Inducing Chromosomal Aberrations and SCE *In Vitro*

Substance	SCE ($B_1 \pm m$)·10^6, mole^{-1}	Chromosomal aberrations ($K \pm m$)·10^4, mole^{-1}	B_1:K
Mitomycin C	20,90±3,01	16,25±1,53	129
E_{39}	6,70±1,19	2,27±0,22	295
Thiophosphamide	1,47±0,06	0,78±0,06	144
Degranol	1,11±0,06	3,45±0,38	32
Dipin	0,60±0,04	1,70±0,07	35

Legend. m) Standard error.

with three and four ethylenimine groups, respectively, and degranol — a derivative of di-β-chloroethylamide with two chloroethyl groups. Blood from one donor was used in the experiments, peripheral blood lymphocytes were cultured by the usual method [9], and two to four repetitions were provided. After 48 h in culture the mutagen was added for 1 h, after which the culture was washed three times with Hanks' solution. To study SCE, 5-bromodeoxyuridine was added in the dose of 10 μg/ml at the 24th hour of culture, after which it was washed off together with the mutagen at the 49th hour, and was not added to the fresh medium. Cells were fixed at the 72nd hour. To analyze chromosomal aberrations preparations were stained with azure-eosin, 100 metaphases were studied in each repetition, and the fraction of aberrant metaphases, the total number of breaks, the fraction of chromatid breaks, and the number of exchanges per 100 cells were calculated. For counting SCE the preparations were stained by the method described previously [3]; the mean number of SCE per cell was counted in 25 metaphases.

EXPERIMENTAL RESULTS

Dose dependences for SCE and chromosomal aberrations are given in Figs. 1 and 2. For all substances, larger doses were taken for induction of chromosomal aberrations than for induction of SCE. In addition, it will be clear from Figs. 1 and 2 that the range of 95% confidence limits, given for each point, is less for recording of SCE than for chromosomal aberrations, although the number of metaphases analyzed was greater for recording the latter. This is evidence of the greater sensitivity of the method of counting SCE than chromosomal aberrations for assessing mutagenic action *in vitro*.

A quantitative expression for differential sensitivity of the two types of effects during the action of mutagens, and also of the comparative effectiveness of the substances tested can be obtained by analysis of the dose dependences. The number of SCE rose in a straight line with an increase in dose for all substances, since the dose dependences satisfy linear regression (inadequacy not significant, $P > 0.05$). The change in the number of SCE with dose can therefore be described by the equation $S = B_0 + B_1 \cdot C$, where S denotes the number of SCE per cell, C the concentration, and B_0 and B_1 are coefficients. The fraction of aberrant metaphases (ρ), depending on dose, changes in a nonlinear manner, and can be satisfactorily described for all substances by the equation $\rho = (1 - e^{(-\alpha+KC)})^2$, where C stands for concentration, and α and K are coefficients [2].

In the equations given above the relationship of SCE and chromosomal aberrations to concentration is reflected by the coefficients B_1 and K, respectively: the higher their values, the more effective the substance as a mutagen. Values of these coefficients are given in Table 1, in which the substances are arranged in descending order of the coefficient B_1, i.e., in their effectiveness of inducing SCE. It will also be clear from Table 1 that the value of K was highest for mitomycin C, and for the remaining substances the effectiveness of induction of chromosomal aberrations changed in a different order from SCE formation. For example, degranol was penultimate in its ability to induce SCE, but its effectiveness in inducing chromosomal aberrations was in second place after mitomycin C. This shows that the effectiveness of these substances in inducing SCE and in inducing chromosomal aberrations differs. Their different relative effectiveness of inducing the two types of injuries is reflected in the value of the ratio B_1/K, which shows by how many times SCE is a more sensitive test than the chromosomal aberrations.

The results showed that mitomycin C is the most effective substance for inducing both SCE and chromosomal aberrations, but first place for the ratio B_1/K is occupied by E_{39}. It will also be noted that the relative effectiveness of induction of SCE compared with chromosomal aberrations varies quite strongly for the different substances (from 300 to 30). The substances tested can also be subdivided into two groups on the basis of the value of B_1/K: group 1 includes mitomycin C, E_{39}, and thiophosphamide, for which its value is much higher than 100, whereas group 2 includes degranol and dipin, for which B_1/K is much less than 100. It was also found that the first three substances induce relatively few aberrations of chromosomal type (5-10%), but degranol and dipin induce this type of chromosomal aberrations with high frequency (23 and 20%, respectively). These results agree with the subdivision of alkylating mutagens suggested previously into monocentric and polycentric [1], which presupposes a high probability of injury to both DNA strands for the latter. Comparing these results with those obtained for assessment of the relative effectiveness of induction of SCE compared with chromosomal aberrations, it can be concluded that compounds inducing mainly the chromosomal type of aberrations through action on the S_1 phase of the cell cycle induce SCE less effectively. This contradicts the widely held view that SCE arise as a result of injury to both DNA strands simultaneously in one locus, as a result of cross-linkages, for example [11]. Nevertheless, our hypothesis is supported by data showing the low effectiveness of agents such as x-rays and bleomycin in induction of SCE [10], although they are effective as inducers of double-stranded DNA breaks [7, 8].

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