LITERATURE CITED

1. V. S. Baranov, A. L. Shvartsman, V. N. Gorbunova, et al., Genetika, No. 10, 1584 (1984).
2. V. S. Baranov, A. L. Shvartsman, V. N. Gorbunova, et al., Genetika, No. 3, 409 (1985).
3. V. S. Baranov, A. L. Shvartsman, V. N. Gorbunova, et al., Genetika, No. 1, 23 (1985).
4. A. N. Klimov and N. G. Nikulicheva, Lipoproteins, Dyslipoproteinemias, and Atherosclerosis [in Russian], Leningrad (1984).
5. S. A. Neifakh and M. M. Shavlovskii, Progress in Medical Genetics [in Russian], Moscow (1978), p. 106.
6. J. Z. Breslaw, Annu. Rev. Biochem., 54, 699 (1985).
7. M. T. Davisson and T. H. Roderick, Manipulating the Mouse Embryo, Cold Spring Harbor (1986), p. 285.
8. D. S. Gerhard, E. S. Kawasaki, F. C. Bankroff, and P. Szabo, Proc. Natl. Acad. Sci. USA, 78, 3755 (1981).
9. A. C. Henderson, Int. Rev. Cytol., 76, 1 (1982).
10. S. Naylor and A. Y. Sakaguchi, Protides of the Biological Fluids, New York (1985), p. 71.
11. P. Yang, S. L. Naylor, J. B. Lum, et al., Proc. Natl. Acad. Sci. USA, 83, 3257 (1986).

SIMULTANEOUS ANALYSIS OF SISTER CHROMATID EXCHANGES AND CELL CYCLE DELAY

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Analysis of sister chromatid exchanges (SCE) is a highly sensitive method of evaluating the mutagenic action of environmental factors [8]. The degree of indeterminacy associated with this method is due to uncertainty about the mechanism of SCE formation. One approach to the elucidation of the mechanisms of SCE formation is to use various chemical modifiers of DNA synthesis, replication, and repair. However, the use of such substances as a rule causes cells to be held up in a particular phase of the cell cycle. Meanwhile, we know that the level of inducible SCE depends strongly on the phase of the cell cycle and on the number of cell cycles elapsing between application of the agent and fixation [6]. This may perhaps explain why data on the effect of modifiers on the level of induced SCE at the end of the S and G_2 stage differ [7]. To avoid mistaken conclusions on the action of modifiers of DNA synthesis, replication, and repair, affecting changes in the duration of the cell cycle, during SCE analysis in these particular variants of the experiment delay of the cell cycle must be estimated. An appropriate technique for this may be the connection found previously between the phase of the cell cycle at which the cells are treated with 5-bromodeoxyuridine (5-BUdR) and the type of differential staining of the sister chromosomes in mitosis [5].

The aim of this investigation was to develop a method of simultaneous analysis of the frequency of SCE and evaluation of cell cycle delay under the influence of the mutagen and of modifiers of DNA synthesis, replication, and repair.

EXPERIMENTAL METHOD

Experiments were carried out on a transplantable culture of Chinese hamster cells (clone 237). Culture medium (Eagle's medium containing 10% bovine serum and 0.03% glutamic acid) in a volume of 1 ml, containing 200,000 cells, was introduced into each well of 24-well plastic dishes (Nune, Denmark). The cells were incubated in an atmosphere of CO_2 (5%). 5-BUdR in a dose of 10 μg/ml was added to the culture 24 h after seeding and 24 h before fixation. To induce SCE the cells were treated with $1.32 \cdot 10^{-6}$ M thiophosphamide (TP), which was added 24 h before fixation and allowed to remain until the end of culture. To evalu-

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TABLE 1. Effect of TP and DNA Replication and Repair Modifiers on Changes in Duration of Cell Cycle and Frequency of SCE

Substance and concentration	Phase of mitotic cycle, number of cells					β	T_{cc}	SCE per cell ± m
	late SI	middle SI	$G_{1_I}+G_{2_{II}}$	late SII	middle SII			
Control	0	0	10	6	84	0,9490	14,75	5,05±0,53
TP(1,32·10⁻⁶ M)	0	0	11	16	73	0,9236	15,16	26,16±1,13
Caffeine								
10^{-5} M	0	0	11	14	75	0,9281	15,08	6,20±0,61
10^{-4} M	0	0	19	9	72	0,9259	15,12	6,28±0,64
10^{-3} M	0	0	28	4	68	0,9213	15,20	6,12∓0,57
Caffeine + TP								
10^{-5} M+1,32·10⁻⁶ M	0	0	20	22	58	0,8952	15,64	24,64±0,87
10^{-4} M+1,32·10⁻⁶ M	0	0	3	23	64	0,9091	15,40	22,92±0,94
10^{-3} M+1,32·10⁻⁶ M	0	0	22	25	53	0,8821	15,87	23,84±1,04
CA								
10^{-8}	0	0	6	4	90	0,9617	14,56	6,28±0-43
10^{-7} M	0	1	1	6	92	0,9611	14,57	4,76±0,37
10^{-6} M	0	0	7	10	83	0,9438	14,83	4,84±0,47
10^{-5} M	0	14	86	0	0	0,5768	23,27	—
CA + TP								
10^{-8}M+1,32·10⁻⁶ M	0	1	10	17	72	0,9218	15,18	24,84±1,10
10^{-7}M+1,32·10⁻⁶ M	0	0	18	11	81	0,9392	14,91	26,84±1,22
10^{-6}M+1,32·10⁻⁶ M	1	0	21	44	34	0,8527	16,42	28,72±0,91
10^{-5}M+1,32·10⁻⁶ M	6	47	47	0	0	0,4569	30,64	—
HU								
10^{-5} M	1	0	12	34	53	0,8857	15,81	6,59±0,71
10^{-4} M	8	79	13	0	0	0,3410	41,06	—
10^{-3}	0	0	94	2	4	0,6543	21,40	—
HU + TP								
10^{-5}M+1,32·10⁻⁶ M	2	0	22	45	31	0,8496	16,48	25,04±1,17
10^{-4}M+1,32·10⁻⁶ M	29	62	9	0	0	0,2331	60,06	—
10^{-3}M+1,32·10⁻⁶ M	4	4	72	4	16	0,7365	19,01	—

Legend. β) Coefficient of passage from one stage into next stage; T_{cc}) mean duration of cell cycle; m) standard error.

ate the modifying action of caffeine, cytosine arabinoside (CA), and hydroxyurea (HU), the cells were treated with these substances simultaneously with the mutagen and without it. To accumulate mitoses 0.1 μg/ml of colcemid was added 2 h before fixation. The cells were removed from the dishes with a solution of versine and fixed, after which chromosome preparations were obtained and stained by the method described previously [4]. Types of differential staining of the chromosomes were analyzed in 100 metaphases for each variant. To estimate the mean number of SCE 25 cells were analyzed: 50 cells each were analyzed in the control and after treatment with TP. To esimate cell cycle delay quantitatively a mathematical model of division of a cell population was used [1]. The parameters of the model were found by stochastic optimization by computer, based on a criterion of minimization of the sum of the squares of deviations.

EXPERIMENTAL RESULTS

Depending on the types of differential staining of the chromosomes it could be judged in what phase of the cell cycle the cell had been when 5-BUdR was added and what chemical compounds had been used. Data on the distribution of cells by phases of the cell cycles are given in Table 1. These data were used to find parameters of the model of deletion of the cell population. In accordance with the model described previously [1] the cell cycle was divided into time intervals of 1 h. As a result of optimization by computer values of the coefficient β, given in Table 1, were obtained for all variants. This coefficient defines the probability that the cells will pass from one time interval into another, and it may have values of between 0 and 1. When β = 1, the cell population divides absolutely synchronously, but when β = 0 the cells do not pass through the phases of the cell cycle. The model of cell division based on the formula $T_{cc} = 1/\beta$ can be used to determine the duration of the cell cycle of a population (Table 1). For the control variant, T_{cc} has the least value, namely 14.70 h. The frequency of SCE in the control was 5.05 ± 0.53 exchanges per cell. Treatment of the cells with TP alone did not significantly increase the duration of the cell cycle (T_{cc} = 15.16 h), although the frequency of SCE was increased fivefold (26.16 ± 1.13).

Under the influence of different concentrations of caffeine, which inhibits postreplicative DNA repair, and also of a combination of caffeine with TP, no increase was found in the mean duration of the cell cycle and no change in the frequency of SCE compared with variants in which caffeine was not added.

Treatment with CA, an inhibitor of initiation of DNA replication, in concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M, and CA in a combination with TP likewise did not increase the duration of the cell cycle. Comparison of the frequency of SCE in the control and after treatment with TP with the frequency of SCE in the presence of CA showed that the latter had no modifying action. The highest concentration of CA used (10^{-5} M) affected the duration of the cell cycle of the population, which it increased by 1.6 times (T_{cc} = 24.27 h). Thus it was this concentration which led to delay of DNA replication. The frequency of SCE in this variant could not be determined because of the marked delay of the cell cycle and the absence of cells which had passed through two replication cycles with 5-BUdR. Under the influence of the same concentration of CA in combination with TP, even greater lengthening of the cell cycle was found: twice as long as in the control and twice as long as under the influence of TP alone. This additional delay may have been associated not only with depression of initiation of replication, but also with the need to repair injuries caused by TP. Since additional time to repair injuries caused by TP was required only in the presence of CA, this may be evidence that the repair process in this case takes place at the time of initiation of DNA synthesis.

Treatment of the cells with HU, which inhibits replicative DNA synthesis, in a concentration of 10^{-5} M did not increase the duration of the cell cycle, evidence that HU does not have any significant inhibitory effect on replicative DNA synthesis. The frequency of SCE under the influence of HU in the same concentration, and also of HU together with TP, did not differ from the control values. HU in a concentration of 10^{-4} M considerably increased the duration of the cell cycle (by 2.8 times), and in combination with TP it caused particular marked delay of passage through the cell cycle by the cells (a fourfold increase in duration), i.e., the replication process for the same course as under the influence of CA in a concentration of 10^{-5} M in combination with TP. The frequency of SCE under these circumstances and the higher HU concentration could not be determined because, as has already been mentioned, of delay of the cell cycle. The action of 10^{-3} M HU will be noted, when there was much less delay of the cell cycle than when a lower concentration (10^{-4} M) was used (T_{cc} = 41.06 h). Thus at first glance paradoxical action of a higher concentration of HU than of lower concentrations is connected not only with the formation of single DNA breaks [2], but also with the fact that high concentrations of HU, besides inhibiting replicative DNA synthesis, also inhibit repair [2, 3]. It can be tentatively suggested that the quite short delay of the cell cycle caused by a high concentration of HU is connected with replication as a means of circumventing injury without any preliminary repair of SOS type [9]. If that is so, with an increase in the number of injuries, under the influence of the mutagen, for example, the probability of DNA replication in circumvention of injuries ought to be even higher in the presence of high HU concentrations, and this ought to lead to shortening of delay of the cell cycle. This did in fact take place, for when cells were treated with a high concentration of HU in combination with TP, the duration of the cell cycle was 19.01 h compared with 21.4 h in the absence of TP.

To conclude, the method of simultaneous analysis of the frequency of SCE and of delay of the cell cycle under the influence of various substances, used in this investigation, can be applied when experiments are planned for the study of the effect of cell cycle delay on the frequency of SCE. Depending on the value of the coefficient β found by a model of cell division, it is possible to predict at what moment of time before fixation the largest number of cells will be in a given phase of the cell cycle, and of which cell cycle included in the analysis.

LITERATURE CITED

1. A. K. Dondua and G. K. Dondua, Investigation of Cell Cycles and of Nuclei Acid Metabolism during Cell Differentiation [in Russian], Moscow–Leningrad (1964), pp. 1-163.
2. I. I. Pelevina, A. S. Saenko, V. Ya. Gotlib, and B. I. Synzynys, Survival Rate of Irradiated Mammalian Cells and DNA Repair [in Russian], Moscow (1985).
3. N. V. Titenko, Tsitol. Genet., No. 2, 58 (1983).
4. A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova, Byull. Éksp. Biol. Med., No. 2, 242 (1978).
5. A. N. Chebotarev and T. G. Selezneva, Tsitol. Genet., No. 3, 163 (1979).
6. A. N. Chebotarev and T. G. Selezneva, Tsitol. Genet., No. 4, 21 (1982).
7. F. Palitti, F. Degrassi, R. de Salvia, et al., Sister Chromatid Exchanges, Part A, New York (1984), pp. 313-318.
8. R. R. Tice and A. Hollaender (ed.), Sister Chromatid Exchanges, Part A, New York (1984).
9. D. A. Shafer, Hum. Genet., 39, No. 2, 177 (1977).