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CELL DIVISION AND CHANGES IN THE DURATION OF MITOSIS IN EHRLICH'S ASCITES MOUSE CARCINOMA AFTER SINGLE EXPOSURE TO CHALONE-CONTAINING EXTRACT FROM THIS TUMOR

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Despite many investigations of the action of chalones on cell reproduction [2, 7, 11], the time course of the changes in mitotic activity of the cells under the influence of these substances has received little study. Yet their study is essential for a deeper understanding of the action of chalones on cell division.

The object of this investigation was to study the time course of cell division and the duration of mitosis in an Ehrlich's ascites carcinoma of mice after a single injection of chalone-containing extract from this tumor.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred albino mice weighing 18-20 g aged 1.5-2 months. A diploid strain of Ehrlich's ascites carcinoma (EAC), obtained at the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, was inoculated by intraperitoneal injection of ascites fluid containing 10^7 cells every 7 days. Animals with a 5-day-old tumor were used in the experiments. The chalone-containing extract (CCE) from EAC was obtained from mice with a 13-day-old tumor by the method of Savchenko et al. [5] The dose of CCE injected was 10 mg per mouse. Colchicine was injected into the animals in a dose of 1 μg/g body weight. Altogether there were four experiments. The CCE was always injected at 1 p.m. Control animals were given an injection of physiological saline.

In the first two experiments the time course of mitotic activity (MA) of the EAC cells was studied after a single injection of CCE into the mice. In the experiments of series I the control and experimental animals were killed 5, 9, 13, 17, 21, and 25 h, and in series II 5, 8, 10, 12, 15, 17, 20, 22, and 24 h after injection of CCE. In the experiments of series III the animals were given an injection of CCE or physiological saline, and, before each animal was sacrificed, 5, 10, 15, 19, and 23 h after the injection, it was given an injection of colchicine in order to study the dynamics of colchicine (C) mitoses. In the experiments of series IV changes in the duration of mitosis were studied in the tumor cells after injection of CCE. The animals were divided into four groups: Groups 1 and 2 were controls, groups 3 and 4 consisted of experimental mice. The animals of groups 1 and 3 were given an injection of 0.2 ml of colchicine solution and the mice of groups 2 and 4 received an injection of the same volume of physiological saline 4 h before sacrifice. The mice were killed 5, 9, and 13 h after the beginning of the experiment. Films were made from the ascites fluid taken from the mice, fixed twice with methyl alcohol, and then stained with methylene blue. To estimate the inhibitory action of CCE on mitosis, the mitotic index (MI) and index of C mitoses were calculated per 5000 cells in each preparation and expressed in promille. The duration of mitosis was determined by the equation:

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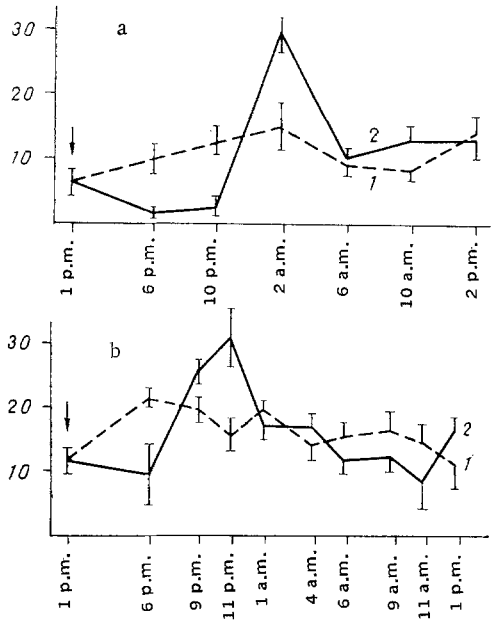


Fig. 1. Time course of MA in cells of EAC during 24-h period after a single injection of CCE. a, b) Preparation injected 13 and 5 h respectively before maximum recorded in MA rhythm in control. 1) Control, 2) experiment. Mean data shown (M ± m). Abscissa, clock time; ordinate, MI (in %).

$$t_m \frac{MI \times t}{MI_{colch}},$$

where MI_{colch} is the index of C mitoses, and t the duration of exposure to colchicine. At each experimental period 7 to 10 mice were used. The significance of differences between the parameters was determined by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a (experiments of series I) that a marked fall in MA by 86.4% was observed 5 h after injection of CCE ($P < 0.01$). At the next stage of the experiment the degree of inhibition of mitosis was virtually unchanged (percentage of inhibition 82.5; $P < 0.01$). After 13 h MI rose sharply, and was 1.6 times higher than the control level ($P < 0.01$). It will be clear from Fig. 1b (series II) that 5 h after injection of CCE MI was below the control level, but inhibition of cell division in the experiments of this series was less than in the previous series, amounting to 57.1% ($P < 0.01$). A sharp rise in MI was then observed, to 1.3 times the control level, although the difference from it was not significant. After 10 h of the experiment MI was twice as high as in the control ($P < 0.01$). At all subsequent periods of the experiment there was no difference between MI in the control and experimental animals.

The results indicate that after administration of a single dose of CCE three phases are observed in the time course of MA of the EAC cells: a phase of inhibition of mitosis, a phase of stimulation (synchronization) of cell division, and a phase of normalization of MA. The onset of the first phase is connected with the holding up of the cells in the G_2 period of the mitotic cycle [1, 4, 6]. The second phase, which has been investigated less than the first, evidently reflects the relatively synchronized entry of cells into mitosis after previously being held up in the G_2 period of the cycle by CCE. The existence of a third phase is in harmony with views on chalones as physiological regulators of cell division whose action is reversible.

In the experiments of series II a less marked and shorter (5 h, rather than 9 h) inhibitory effect of CCE on mitosis was observed than in series I. These differences can probably be explained by the different state of the cell reproduction system at the time of injection of CCE. Injection of the preparation in the experiments of series II occurred at a higher

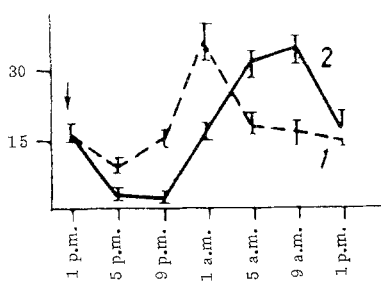


Fig. 2

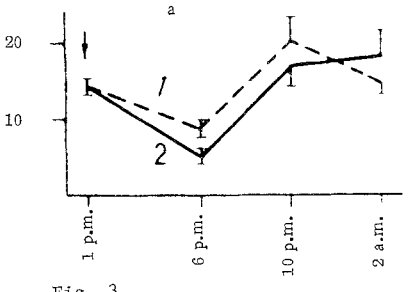
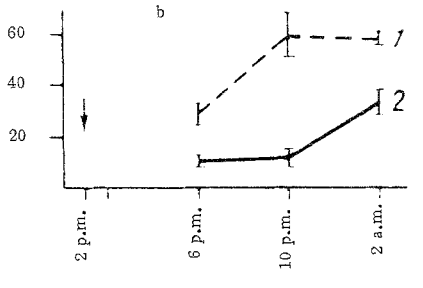


Fig. 3



t_M — Control	1.21	1.19	1.0
t_M — expt	1.86	5.3	2.1

Fig. 2. Time course of number of C mitoses in EAC cells during 24-h period after a single injection of CCE. Ordinate, MI_{colch} (in %). Remainder of legend as to Fig. 1.

Fig. 3. Time course of MI (a) and MI_{colch} (b). Duration of mitosis (t_m, in h) in EAC cells after a single injection of CCE also is shown. Remainder of legend the same as to Fig. 1.

initial level of MA, and the acrophase of the MA rhythm in the control occurred after 5 h ($P < 0.01$), and not after 13 h ($P < 0.01$) as in series I.

It must pointed out that by no means all workers who have studied changes in MA after a single exposure to chalones have obtained synchronization of cell division. Although this phenomenon has been described in a number of publications [3, 9], it still requires further study. Accordingly, in the experiments of series III the time course of the number of C mitoses in EAC after injection of CCE was studied.

It will be clear from Fig. 2 that the index of C mitoses was reduced compared with the control 4, 8, and 12 h after the beginning of the experiment (inhibition amounted to 73, 86, and 54% respectively; $P < 0.01$). After 16 h this parameter was 1.7 times more than the control level ($P < 0.01$). MI_{colch} later continued high, at 223% of the control ($P < 0.01$), but by the end of the experiment it no longer differed significantly from the control. These findings confirm the existence of three phases in the changes in cell division after the action of a single dose of CCE, including a phase of relative synchronization of cell division.

It will be clear from Fig. 3 that the duration of mitosis of the EAC cells 5, 9, and 13 h after injection of CCE was 1.5, 4.5, and 2.1 times longer respectively than in the control. The phase of inhibition of MA by the chalone-containing preparation was thus accompanied by an increase in the duration of mitosis, evidence that CCE acts not only on the G_2 period of the mitotic cycle, but also on the processes of mitosis proper. These results are in agreement with those obtained by other workers [8, 10] who found lengthening of mitosis in epidermal cells under the influence of epidermal chalones.

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