

COMBINED ACTION OF ADRENALIN AND GROWTH-  
INHIBITING EXTRACT OF EHRLICH'S ASCITES  
CARCINOMA, ADMINISTERED AT DIFFERENT  
TIMES OF DAY, ON CELL DIVISION IN THIS  
TUMOR

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Interaction between chalone and stress hormones in tumor systems has not been adequately studied. Experiments in this direction have been carried out mainly *in vitro* [8, 9]. The influence of adrenalin on manifestation of the biological effects of chalones *in vivo* still remains unexplained, especially with respect to the role of the chalone system in formation of the circadian rhythms of cell division. Only indirect evidence has been obtained to show that interaction is possible at tissue and hormonal levels of the cell proliferation *in vivo* in a situation of experimental stress [7].

The object of this investigation was to study the action of adrenalin and of a tissue-specific extract of Ehrlich's ascites carcinoma (EAC) cells on division of the cells of this tumor when administered together and separately to animals with a 5-day-old tumor at different times of the 24-hour period.

#### EXPERIMENTAL METHOD

Experiments were carried out on 830 noninbred male albino mice weighing 18-20 g and aged 1.5-2 months. A diploid strain of EAC was transplanted into animals by intraperitoneal injection of ascites fluid containing 3-4 million tumor cells every 7 days. An aqueous extract containing EAC chalone was isolated by the method described in [2]. The animals were adapted to a schedule of 12 h each of light and darkness: light from 6 a.m. to 6 p.m. The test substances were injected intraperitoneally once at noon, 6 p.m., and midnight on the first day and at 6 a.m. and noon on the second day into animals of five corresponding groups. Each group included the following subgroups: 1) intact control, the mice were killed every 2 h from 10 a.m. on the first day to 8 p.m. on the second day of the investigation; 2) injection of physiological saline (control); 3) injection of adrenalin 1.5  $\mu\text{g/g}$  body weight; 4) injection of 1 ml of extract; 5) injection of adrenalin + extract. The preparations were injected in equal volumes. The animals in subgroups 2, 3, 4, and 5 were killed after 2, 4, 6, and 8 h respectively. Films of the tumor were fixed with methanol and stained with methylene blue. The proliferative activity of the EAC was estimated from the mitotic index (MI), expressed in pro mille. The chronobiological study included the use of a graphic-parameteric method to study the rhythm of cell division [3].

#### EXPERIMENTAL RESULTS

Investigation of reproduction of EAC cells in the intact control revealed a biphasic rhythm of mitotic activity in which the active phases of longest duration occurred at the times of transition from light to darkness and in the middle of the daily period of darkness. The maximal values of MI occurred at 6 p.m. and midnight on the first day and 4 p.m. on the second day, the minimal values at 10 p.m. on the first day and at 10 a.m. and 6 p.m. on the second day of the experiment (Fig. 1).

Injection of physiological saline at different times of the 24-h period caused changes in cell proliferation in the tumor, manifested as either an increase, a decrease, or no change in MI at different periods of the experiment (Fig. 2A).

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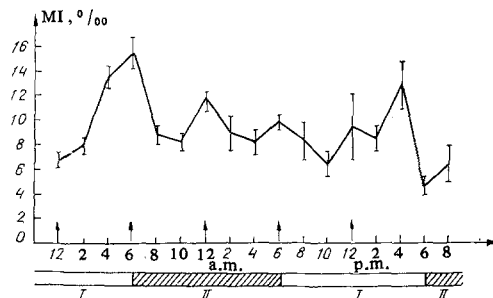


Fig. 1. MI in EAC cells during 36 h of investigation (intact control). Arrows indicate times of injection of test preparations in experimental groups. Unshaded areas (I) indicate period of daylight, shaded areas (II) — period of darkness. Abscissa, clock time; ordinate, MI (in ‰).

Injection of adrenalin in the middle of the daylight period (at noon on the first and second days) and also at the end of the daylight period (at 6 p.m.) showed a clear inhibitory effect on mitosis (Fig. 2B), which lasted on average 4 h of the experiment and amounted to 65.7% ( $P < 0.01$ ). Later MI increased significantly compared with the control. Administration of the hormone in the middle of the dark period (midnight) was accompanied by a significant decrease in the number of mitoses only after 2 h of the experiment. Adrenalin injected at the end of the dark period (at 6 a.m.) caused significant depression of MI only after 4 h; after 8 h the number of mitoses was increased by 80% ( $P < 0.01$ ).

Administration of the extract (Fig. 2C) in the middle of the period of daylight (at noon on the first and second days) caused a decrease in MI for 4 h, on average by 70.1% ( $P < 0.001$ ).

Later MI did not differ significantly from the control values. When the extract was injected at another time of the 24-h period inhibition of MI also was observed for 4 h: by 48.1% when the injection was at 6 p.m. ( $P < 0.01$ ), on average by 65.9% at midnight ( $P < 0.001$ ), and on average by 65.9% ( $P < 0.001$ ) at 6 a.m. An increase in MI after 8 h was observed, by 56.3% when the extract was injected at 6 p.m., by 42.1% at midnight, and by 173% at 6 a.m. ( $P < 0.05$ ).

In response to combined administration of adrenalin and extract (Fig. 2D) in the middle of the period of daylight (at noon) and also at the end of the daylight period (at 6 p.m.) significant prolongation of inhibition of MI by 2.4 h was observed compared with this parameter when the preparations were injected separately, and the increase in mitotic activity was prevented. Combined use of adrenalin and the extract in the middle of the period of darkness (at midnight) did not change MI significantly for 4 h, but a significant decrease in MI was observed after 6 h. Injection of the preparations at the end of the period of darkness (at 6 a.m.) caused little change in MI compared with that when the extract alone was used.

The results of this investigation confirm data in the literature on the existence of a biphasic rhythm of mitotic activity in EAC [5, 6]. Under these conditions, adrenalin characteristically had a marked effect on inhibition of mitosis when administered during the period of daylight, in agreement with results obtained by other workers [4]. Synchronization of cell division as a result of the use of the hormone also was observed to a greater degree at this time during the 24-h period. The effect of adrenalin on mitotic activity was not definitely found to be dependent on the phases of the rhythm of cell division in EAC. On the contrary, the action of the extract was determined to a greater degree by circadian variations in the sensitivity of the cell population to it in the rhythm of proliferation, and it depended very little on the phases of the diurnal cycle of light and darkness, in agreement with the results of studies of the effect of EAC extract on cell division in this tumor [1]. Combined administration of the preparations led to longer inhibition of cell division and also prevented the increase in mitotic activity. This effect was complex in character and depended both on the phases of the diurnal cycle of light and darkness and on the rhythmic fluctuations in sensitivity of the cell population in the course of the 24-h period. The phenomenon of prolonged inhibition of cell division was observed only when the preparations were given together during the period of daylight, and it was thus due mainly to adrenalin. Phases of the daily cycle of light and darkness and phases of the rhythm of mitotic activity of the tumor cells also played an important role in the effect of physiological saline on cell division in EAC. However, as Fig. 2 shows, the

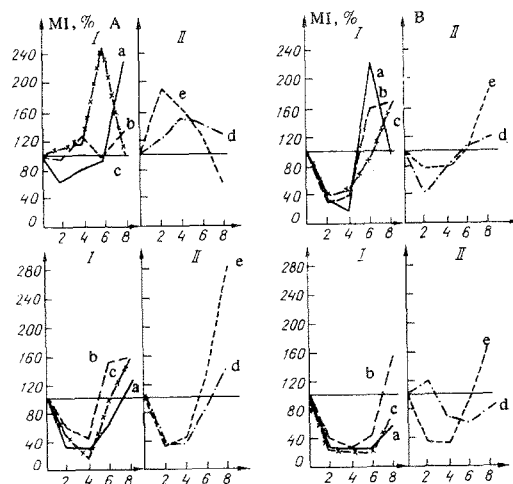


Fig. 2. Changes in MI in EAC cells after injection of physiological saline (A), adrenalin (B), tumor extract (C), and extract + adrenalin (D) in period of daylight (I) and darkness (II). Abscissa, time after beginning of experiment (in h); ordinate, MI (in % of control). a) At noon on first day, b) at 6 p.m. on first day, c) at noon on second day, d) at midnight on second day, e) at 6 p.m. on second day.

temporary character of the action of physiological saline was different from that of adrenalin, of the extract, and of the extract and adrenalin together. Accordingly changes in MI reflected the influence of the test preparations and not that of the solvent or of painful stimulation of the animals during injection.

Cell division in Ehrlich's tumor thus obeys two levels of regulation, namely tissue and hormonal, which exert their controlling influences unequally at different times of the 24-h period, and may interact.

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