DIURNAL CHANGES IN MITOTIC ACTIVITY AND DNA SYNTHESIS IN HYPERDIPLOID AND HYPOTETRAPLOID STRAINS OF EHRLICH'S ASCITES TUMOR

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Hyperdiploid or hypotetraploid strains of Ehrlich's ascites tumor were transplanted into noninbred male albino mice. The results observed on the 4th, 5th, and 6th days of development of the Ehrlich's tumor show fluctuations in the mitotic index both in the hypotetraploid strain, under natural lighting conditions, and in the hyperdiploid strain with a regular 12-h photoperiod. Maximal values of the mitotic index were observed in the morning and afternoon. The results indicate that fluctuations in the mitotic index and labeling index during the 24-h period are not synchronized in different strains and under different conditions of illumination. It is concluded that the temporal organization of cell reproduction in Ehrlich's ascites tumor cells differs from that in normal tissue cells.

KEY WORDS: Ehrlich's ascites tumor; diurnal rhythm; mitotic index; radioactive index.

Among the parameters characterizing cell division, the diurnal rhythm of cell multiplication occupies an important position. However, data in the literature on diurnal rhythms of mitosis in tumors are extremely contradictory [1, 2, 7, 10]. The presence of rhythms of DNA synthesis in tumors and its connection with the rhythm of mitosis have been inadequately studied [5, 6, 11]. The object of this investigation was to study diurnal rhythms of mitotic activity and DNA synthesis in cells of hyperdiploid and hypotetraploid strains of Ehrlich's ascites tumor.

EXPERIMENTAL METHOD

Experiments were carried out on 170 noninbred male albino mice weighing 18-20 g. The animals inoculated with a hypotetraploid strain of Ehrlich's ascites tumor (series I) were kept at 18°C under natural conditions of lighting and were fed ad libitum. Animals inoculated with the hyperdiploid strain (series II) were kept in the laboratory at 18°C and with a 12-hourly photoperiod, and were fed ad libitum for 30 days. Mice of series I were killed starting from 72 h, whereas animals of series II were killed starting 96 h after inoculation of the tumor, every 3 h for 48 h, 5 animals at each time. Thymidine-^{3}H was injected into the animals 1 h before sacrifice in a dose of 0.5 μCi/g body weight (specific activity 4.1 Ci/mmole). The ascites fluid was removed and treated with hypotonic solution (0.56% KCl, T = 37°C), fixed in a mixture of ethyl alcohol and glacial acetic acid (3 : 1), and cytological and autoradiographic preparations were made from it by the usual method. In preparations from each animal, after examination of 3000-5000 cells the mitotic index (MI) and index of DNA-synthesizing cells (RI) were calculated. MI and RI were expressed in promille. The numerical results were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

The experimental results showed that during 4-5 days of development of the hypotetraploid strain of Ehrlich's ascites tumor MI did not remain constant. Maxima of MI for the hypotetraploid strain were observed at noon on the 4th day of tumor development, at 6 a.m.-3 p.m. on the 4th-5th days, and at 9 a.m. on the 5th day after transplantation of the tumor ($P < 0.05$, $P < 0.03$, and $P < 0.001$ respectively). The minima of MI occurred at 3 a.m. on the 4th day and at 6 p.m. and noon on the 5th day of tumor growth (Fig. 1). The mean diurnal values

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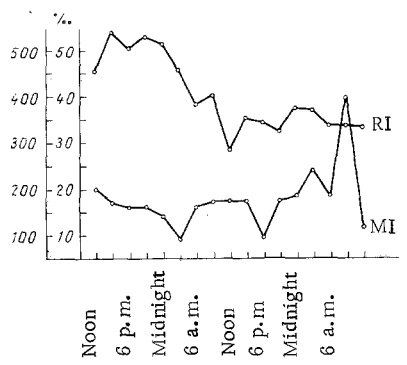

Fig. 1

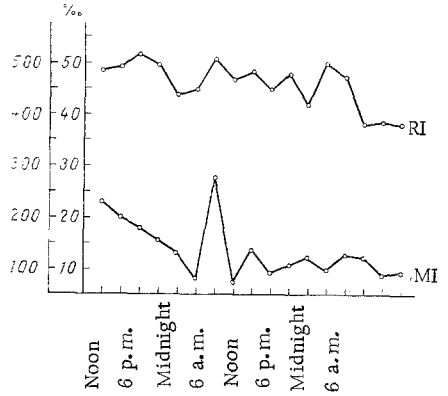

Fig. 2

Fig. 1. Changes in mitotic activity and number of DNA-synthesizing cells in hypotetraploid strain of Ehrlich's ascites tumor on 4th-5th days of tumor growth. Abscissa, time of day; ordinate, RI and MI (in $^0/_{00}$).

Fig. 2. Changes in mitotic activity and number of DNA-synthesizing cells in hyperdiploid strain of Ehrlich's ascites tumor on 5th-6th days of tumor growth. Legend as in Fig. 1.

of MI on the 4th and 5th days of tumor development were 19 and $16^0/_{00}$ respectively. The relative amplitude of fluctuations in MI on the 4th and 5th days of tumor growth differed (2.2 and 3.5), indicating that the rhythm of cell division varies in the exponential phase of tumor development. At the same time it must be pointed out that all the observed rises of mitotic activity in the tumor took place in the morning and afternoon. RI also changed during the 48 h of the experiment (Fig. 1). The mean diurnal values of RI fell significantly on the 5th day of tumor growth compared with those on the 4th day (474 and $339^0/_{00}$ respectively; $P < 0.01$). A gradual fall in RI was observed during the 4th day of tumor development (maximum at 3 p.m.-midnight, minimum at noon; $P < 0.017$). During the 5th day of tumor growth RI remained practically constant. It can be concluded from these results that changes in the number of dividing and DNA-synthesizing cells in the hypotetraploid strain of Ehrlich's ascites tumor during the 24-h period were not synchronized.

Investigation of changes in MI in the hyperdiploid strain of Ehrlich's ascites tumor in animals kept under artificial conditions of illumination showed that maxima of MI were observed at noon and 6 a.m. on the 5th day of tumor growth and minima at 3 and 9 a.m. ($P < 001$; Fig. 2). The increase in mitotic activity in the tumor of this strain, just as in the hypotetraploid strain, on the 5th day of tumor development took place in the morning and afternoon. On the 6th day of tumor development no significant changes were found in MI. During the 5th and 6th days after transplantation of the tumor RI fluctuated only slightly and its changes were not significant (Fig. 2). In the hyperdiploid strain of Ehrlich's ascites tumor also, the changes in MI and RI were not synchronized.

The diurnal rhythm in the number of DNA-synthesizing cells is regarded by some workers [3] as one mechanism of the diurnal rhythm of mitosis. The dissimilarity of the changes in MI and RI during the 24-h period in Ehrlich's ascites tumor suggests disturbances of normal mechanisms of diurnal rhythms of cell reproduction in this tumor. Other workers [11] also have noted the absence of synchronization of rhythms of RI and MI in rapidly growing undifferentiated tumors. Most probably in these cases also synchronization of division of tumor cells takes place immediately before starting mitosis (in the G_2-phase).

The results of the present investigation also indicate variation in the rhythm of mitotic activity in the course of development of Ehrlich's ascites tumor. This is shown by the absence of rhythmic fluctuations in MI on the 6th day of growth of the hyperdiploid strain of the tumor. Significant differences may thus be found in the temporal organization of cell reproduction in Ehrlich's ascites tumor compared with normal tissues.

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EFFECT OF ONE AND TWO INJECTIONS OF CHALONE ISOLATED FROM MOUSE EHRLICH'S ASCITES TUMOR ON ITS MITOTIC ACTIVITY

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Chalone from Ehrlich's ascites tumor has a short and reversible inhibitory action on proliferation of cells of this tumor when administered as both one and two injections. A considerable increase in mitotic activity in the tumor cells compared with the control is observed 10 h after one or two injections (the second injection being given at 6 p.m.) of the chalone, indicating that it acts on the G_2-cell population in the mitotic cycle and that it synchronizes cell division. If the second of two injections of chalone is given at 9 p.m. it leads to more prolonged inhibition of the cells and to a more marked synchronization wave of the G_2-cell population. The duration of inhibition of cells in the G_2-phase of the mitotic cycle after two injections of the chalone thus depends on the state of the cell population on which the chalone acts.

KEY WORDS: chalone; Ehrlich's ascites tumor; mitotic cycle, mitotic index.

During the last two decades, in the problem of regulation of cell proliferation tissue-specific inhibitors of cell division (chalones) have received intensive study, for some workers ascribe to them the role of physiological regulators of cell reproduction [7, 9, 10]. In some investigations chalones have been found to synchronize the cell population in the mitotic cycle, but the data so far obtained are few and further investigation is required. The use of chalones as natural synchronizers, with no toxic action on living organisms, can play an important role in improving the efficacy of tumor chemotherapy. It was accordingly decided to study the synchronizing action of chalone on a tumor cell population by testing its administration by different schemes.

The object of this investigation was to compare the mitotic activity of Ehrlich's ascites tumor (EAT) cells after one or two injections of chalone from that tumor into animals.

EXPERIMENTAL METHOD

Experiments were carried out on 150 noninbred male albino mice (from the Central Nursery, Academy of Medical Sciences of the USSR) aged 1.5 months (weight 18-20 g). The animals were kept under standard conditions: temperature 18°C, food ad libitum, 12 h of daylight and 12 h of darkness (daylight from 6 a.m. to 6 p.m.)

A diploid strain of EAT (Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR) was transplanted by intraperitoneal injection of 0.2 ml ascites fluid, containing 10^7 cells (with this dose of tumor cells a 100% take was observed with this strain). The EAT chalone was obtained by the method of Hondius and Laurence [8] and of Okulov and Chekulaev [2], modified by S. G. Mamontov and V. B. Zakharov. The stages of obtaining the chalone-containing preparation were as follows. The ascites fluid was isolated from a 13-day tumor on an icebath, after which the cell mass was separated by centrifugation of the ascites fluid at 3000 rpm for 10 min at 4°C. Later the residue was resuspended 3 times in 20 volumes of acetone at 4°C, for 30 min each time, and centrifuged after each treatment for 10 min at 3000 rpm. The resulting acetone powder was dried and homogenized in an agate mortar. An aqueous extract was obtained by adding 20 volumes

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