ACTION OF THYROXINE ON CELL PROLIFERATION IN A HYPOTETRAPLOID STRAIN OF EHRLICH'S ASCITES TUMOR: CHRONOBIOLOGICAL ANALYSIS

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The concept of autonomy of tumors in the body is currently under critical appraisal. It has been shown that factors regulating cell division in normal tissues also act on tumor tissue [1-3, 4-6]. However, there is insufficient evidence of this kind at present for definite conclusions to be drawn on the mechanisms regulating cell division in tumors. Previously [3] the writers reported similar chronobiological characteristics of the response of dividing cells of a hyperdiploid strain of Ehrlich's ascites tumor (EAT) with cells of normal tissues, as shown by the presence of rhythms of the dose effect of sensitivity to this hormone.

The aim of this investigation was to study the action of thyroxine on proliferative activity of cells of a hypotetraploid strain of EAT during growth for 48 h.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 18-20 g were used for the experiments. A hypotetraploid strain of EAT was used to inoculate the animals intraperitoneally (0.2 ml of undilute ascites fluid containing $8\text{-}10 \times 10^6$ tumor cells per animal). The mice were kept at a temperature of 18°C, with 12 h of daylight daily (from 4 a.m. to 4 p. m.) and were given food ad libitum. Starting from the 1st day of tumor development, the animals received intraperitoneal injections of the sodium salt of thyroxine at 11 a.m. daily for 6 days in a dose of 10 μg/100 g body weight. Thyroxine was dissolved in 1.0 ml of 0.1 M NaOH solution immediately before injection, and was diluted to the required concentration with physiological saline. On the 5th-6th day after transplantation of the tumor cells the animals were killed, five mice at each time, every 3 h. Each mouse was given an injection of ^{3}H-thymidine 1 h before sacrifice in a dose of 0.5 μCi/g body weight (specific activity 4.1 Ci/mmole). Animals receiving the solvent alone instead of thyroxine, by a similar schedule, served as the control. The method of obtaining preparations of tumor cells was described previously [1]. The number of dividing and DNA-synthesizing cells was determined on autoradiographs obtained from each animal, with examination of 3000-5000 cells. The mitotic (MI) and radioactive (RI) indices were expressed in $^0/_{00}$. Parameters of MI rhythms were determined by a graphic-parametric method [7], the duration of mitosis of EAT cells being taken as 1.5 h [2]. The numerical results were subjected to statistical analysis by the Fisher–Student test of significance. The accepted level of significance was $p < 0.05$.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that after injection of the solvent of thyroxine into the animals maximal values of MI were observed at 9 p.m. on the 5th day ($p < 0.05$) and at 6 p.m. on the 6th day ($p < 0.05$). Minimal values were observed at noon on the 5th day and at noon and 9. a.m. on the 6 th day. The rhythm of MI was monophasic with a period of about 21 h. The

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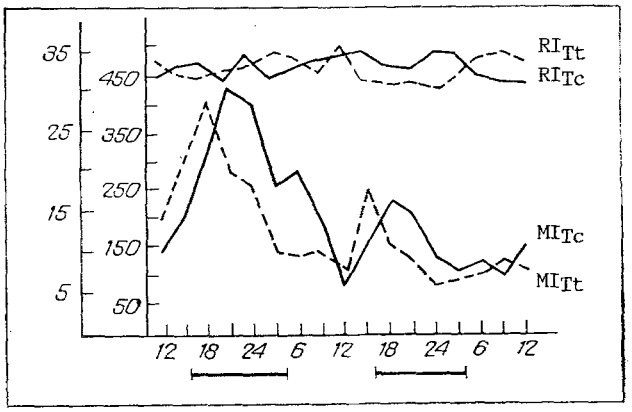

Fig. 1. Changes in number of dividing and DNA-synthesizing cells in hypotetraploid strain of EAT on 5th-6th days of its growth in control (MI_{Tc}, RI_{Tc}) and during prolonged administration of thyroxine (MI_{Tt}, RI_{Tt}). L/D = 12:12. Abscissa, clock time; ordinate, RI and MI, $^0/_{00}$.

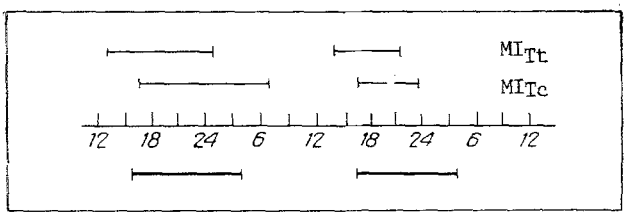

Fig. 2. Phase Analysis of biological rhythms of cell division (MI) in hypotetraploid strain of EAT on 5th-6th days of its growth in control (MI_{Tc}) and during prolonged administration of thyroxine (MI_{Tt}). L/D = 12:12. Abscissa, clock time.

duration of the active phase (AP) was 13.5 h on the 5th day and 7 h on the 6th day. AP on the 6th day occurred 0.5 h sooner than on the 5 th day (Fig. 2). The absolute amplitude (AA) of the MI waves was $23^0/_{00}$ and $10^0/_{00}$ for two consecutive peaks of MI, and the relative amplitude (RA) was 3.3 and 2.3; the coefficient of synchronization (CS) was 0.4 and 0.4; the mean daily MI was $19.1^0/_{00}$ on the 5th day and $11.2^0/_{00}$ on the 6th day, whereas the mean periodic MI was $20.1^0/_{00}$ and $12.1^0/_{00}$ respectively. The pool of dividing cells on the 6th day ($181.5^0/_{00}$) was lower than on the 5th day ($307^0/_{00}$), and the fraction of mitoses in AP of the rhythm was 71.2% on the 5th day and 42.1% on the 6 th day.

Thus as in previous investigations on animals with a hyperdiploid strain of EAT [3], in animals with a hypotetraploid strain of EAT we observed a monophasic rhythm of mitotic activity (MA) on the 5th-6th days of tumor growth.

RI in the tumors in the control animals thus showed no significant change during the 2 days of the experiment. AA did not exceed $64^0/_{00}$. The mean daily values of RI were $457^0/_{00}$ on the 5th day and $483^0/_{00}$ on the 6th day. Thus the change in number of DNA-synthesizing cells in hypotetraploid strain of EAT, in contrast to the change in number of dividing cells, had no mechanism of rhythmic fluctuation. Accordingly, such a mechanism was recorded for the hyperdiploid strain of this tumor.

After injection of thyroxine (T_4) the acrophase of MI was shifted to the left by 3 h compared with the control on both the 5 th and 6th days of growth of EAT. It was located at 6 and 3 p.m. respectively on the 5th and 6th days. The rhythm of MI was monophasic with a period, just as in the control, of 21 h. Minimal values of MI were observed at noon on the 5th day and at noon and midnight of the 6th day of tumor growth. The length of AP on the 5th day was 11.5 h and on the 6th day 8.5 h. The beginning of AP on the 6th day occurred 0.5 h sooner than on the 5th day (Fig. 2). On both days of the experiment the position of AP was shifted to the left compared with the control. A significant increase in MI during administration of thyroxine was observed at noon to 6 p. m. on the 5th day and at 3 p.m. on the 6th day ($p < 0.05$), i.e., at a time when an increase in the value of this parameter was

observed in the control. On the 5th day MI during administration of T_4 was significantly lower at 9 p.m. and 3 and 6 a.m. than the control values ($p < 0.05$). It was also lower than the control on the 6th day of the experiment, when the value of MI in the control animals fell after the peak in the rhythm. These changes in MI were reflected in the mean daily and mean periodic values of MI on the 5th and 6th days of the experiment ($16^0/_{00}$, $10.3^0/_{00}$ and $16.5^0/_{00}$, $10.8^0/_{00}$). AA was $15^0/_{00}$ and $11^0/_{00}$, and RA was 2.2 and 2.4. Clearly the values of these parameters were lower on the 5th day of the experiment than in the control, and were equal to the control values on the 6th day. CS on the 5th day was the same as in the control (0.4) but on the 6th day it was higher than in the control (0.8). The pool of dividing cells was smaller than in the control: $251.5^0/_{00}$ and $162^0/_{00}$ respectively on the 5th and 6 th days of tumor growth. The fraction of mitoses in AP was lower on the 5th day (61.8%) than in the control, but on the 6th day it was about equal to it (44.4%).

During administration of T_4 RI did not differ significantly from the control values at any point of the experiment. Just as in the control, a rhythm of RI was not found during the course of 5 or 6 days. AA was $80^0/_{00}$, a little higher than in the control. The mean daily values were close to the control mean daily values of RI ($480^0/_{00}$ and $479^0/_{00}$ respectively).

During prolonged administration of thyroxine a response of similar character of the tumor cells was thus observed in hyperdiploid [3] and hypotetraploid strains of EAT, evidence that rhythms of sensitivity of the cells to the action of thyroxine are present in both strains. An increase in MI was observed in both strains at the beginning of the periods of rhythmic increase of MI in the control animals. In animals receiving T_4 the rhythm of MI was preserved but the acrophase was observed 3 h earlier than in the control. T_4 evidently has a similar synchronizing action on rhythmic entry of the cells into mitosis in both hypotetraploid and hyperdiploid strains of EAT on the 5th and 6th days of tumor growth due to shortening of the G_2-phase of the cycle.

During administration of the hormone a tendency for the number of proliferating cells to fall in the course of the 24-h period was observed with both strains, whereas the number of DNA-synthesizing cells remained unchanged compared with the control. No rhythm of RI was observed with either strain of EAT.

It can accordingly be concluded that the ploidy of EAT cells does not affect the character of rhythms of cell division in this tumor on the 5th-6th days of its growth or the changes in them arising under the influence of thyroxine. On the whole these changes are similar to those induced by thyroxine in circadian rhythms of the number of mitoses in normal tissues.

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