DIURNAL RHYTHM OF CELL DIVISION IN TUMORS

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The dynamics of mitotic activity was studied in cells of transplantable sarcomas. A well-defined diurnal rhythm of cell division was found in sarcomas IMR-1 and T-1 transplanted into August rats. In sarcoma IMR-1 mitotic activity reached a maximum at between 4 and 7 A.M. and a minimum at 1 P.M. In sarcoma T-1 mitotic activity reached a maximum at 1 P.M. and a minimum at 7 A.M. and 7 P.M.

Considerable attention has been paid to the question of whether there is a diurnal rhythm of cell division in malignant tumors. Evidence is constantly being published to the effect that many tumors have a diurnal rhythm of cell division [3, 5, 9, 10, 14, 16]. Investigations by other workers [2, 11, 15], however, have failed to reveal any diurnal rhythm of division in tumor cells.

The existing contradictions are not always easily explained. To begin with, tumors obtained by different methods and from different tissues develop along different lines and at the time of investigation they may vary in their degree of differentiation, not to mention their sensitivity to exogenous and endogenous factors concerned in the mechanism of diurnal cell division [12, 13]. Another fact of considerable importance is that the investigations described were carried out on different strains of transplantable tumors, many of which were maintained for long periods of time in noninbred animals. An inevitable result of this has been that, besides loosing their tissue specificity, the tumors have also lost their characteristic rhythm of mitotic activity [10].

Without going into detail on these contradictions it is worth pointing out that the study of diurnal rhythms of cell division in general, and in tumors in particular, is of great practical as well as theoretical importance. The different stages and phases of the mitotic cycle are known to differ considerably in their sensitivity to the same antitumor agent [1, 4, 6, 7, 9, 10]. The facts described above suggest that determination of the phases of cell division most sensitive to a particular cytostatic agent could greatly increase the effectiveness of treatment of malignant neoplasms. For such experiments to be carried out it is necessary to have a biological model of a tumor with a stable diurnal rhythm of cell division. Investigations lasting several years have shown that line-specific sarcomas IMR-1 and T-1, obtained in the writers' laboratory [9-10], could be tumors with this property.

The object of the present investigation was to study the diurnal rhythm of mitotic activity in the cells of these sarcomas.

EXPERIMENTAL METHOD

Sexually mature August rats weighing 100-120 g, into which sarcomas IMR-1 and T-1 were transplanted, were used in the experiments. On the 20th day after transplantation of the IMR-1 cells and on the 11th day after transplantation of the T-1 cells the animals were sacrificed and the tumor nodules removed were fixed in Carnoy's mixture at intervals of 3 h during the 24-h period. The diurnal rhythm of division of the tumor cells was investigated at different times of year and in different generations. Altogether 205 August rats were used in the investigation. Histological sections of the tumors were stained with Ehrlich's

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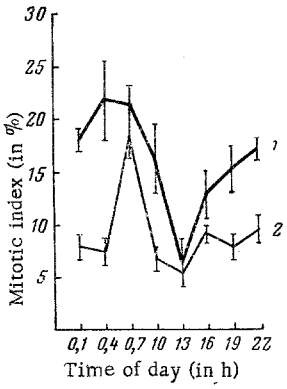

Fig. 1

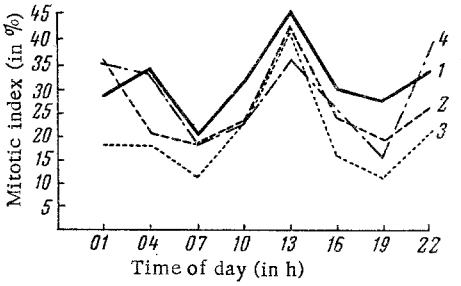

Fig. 2

Fig. 1. Change in mitotic activity of sarcoma IMR-1 cells during the 24-h period: 1) 15th generation; 2) 122nd generation of the tumor.

Fig. 2. Diurnal changes in mitotic activity of sarcoma T-1 cells at different times of year: 1) May (9th generation); 2) October (40th generation); 3) February (52nd generation); 4) June (60th generation.

hematoxylin. The phases of mitosis were counted in 3000-4000 cells. Student's t criterion was used in the statistical analysis of the results.

EXPERIMENTAL RESULTS

The diurnal activity of the sarcoma IMR-1 cells was studied when the tumor had just been transformed into a strain and when it had passed through 15 generations (1967), and also when it had passed through 122 generations (1970). The results of this investigation are shown in Fig. 1. Their analysis demonstrates that sarcoma IMR-1 (15th generation) has a diurnal rhythm of cell division with a maximum of mitotic activity at 4-7 A.M. (mitotic indices 21.6 ± 3.0 and 21.4 ± 2 ‰ respectively) and a minumum at 1 P.M. (mitotic index 6.9 ± 2 ‰; $P < 0.01$). The same pattern of diurnal rhythm of cell division was preserved in the same tumor, but in the 122nd generation (1970), with a maximum at 7 A.M. (18.2 ± 0.1 ‰) and a minimum at 1 P.M. (6 ± 0.1 ‰). Fluctuations in mitotic activity of the cells of this tumor took place as the result of fluctuations in the number of cells taking part in mitosis, but not as the result of lengthening or shortening of any of the phases of mitosis. This is shown by the fact that values reflecting fluctuations in the early phases of mitosis repeated the fluctuations of mitotic activity of the cells of this tumor during the 24-h period.

The study of the mitotic activity of the sarcoma T-1 cells showed that this tumor has a clearly defined diurnal rhythm of division with a maximum of mitotic activity at 1 P.M. (mitotic index 46 ± 4 ‰) and a minimum at 7 A.M. and 7 P.M. (mitotic indices 21.2 ‰ and 18 ± 2 ‰ respectively; 9th generation, May; Fig. 2). The probability of random differences in mitotic activity at 1 and 7 P.M. and 7 A.M. is statistically significant ($P < 0.001$). Investigation of the mitotic activity of the sarcoma T-1 cells during the 24-h period in the 40th, 52nd, and 60th generations showed that the diurnal rhythm of cell division is stable for this tumor with a maximum of mitotic activity at 1 P.M. and a minimum at 7 A.M. and 7 P.M.

Analysis of these results thus suggests that both sarcomas IMR-1 and T-1 have a well-marked and stable diurnal rhythm of cell division, which is independent of seasonal fluctuations during the year or of the number of generations of the tumor.

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