DIURNAL RHYTHM OF MITOTIC ACTIVITY AND OF NUMBER OF DNA-SYNTHESIZING NUCLEI IN SARCOMA 37 OF ALBINO MICE

V. I. Demskii

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The diurnal rhythm of mitotic activity in mouse sarcoma 37 is made up of rhythms of labeled and unlabeled mitoses, probably characterizing two cell populations differing in the duration of their G_2 phase. The duration of the G_2 phase in one group is about 1 h and in the other group about 4 h or more.

KEY WORDS: index of labeled nuclei; labeled and unlabeled mitoses; G_2 phase; diurnal rhythm.

Several workers have demonstrated a diurnal rhythm of mitosis in tumors. However, the character of that rhythm at the level of mitotic activity in different tumors was found to vary [1, 2, 4]. It has also been shown that cytostatic substances, if administered at different times of day or night, had different effects on cell division in both tumor and normal tissues [1-3].

The further study of this problem requires data on the principles governing not only the diurnal rhythm of mitosis, but also of the other phases of the mitotic cycle, for different factors acting on cell division act on particular phases of the cycle.

The object of this investigation was to study the character of the diurnal rhythm of mitotic activity and the rhythm of the number of nuclei synthesizing DNA in sarcoma 37 of albino mice.

EXPERIMENTAL METHOD

Male albino mice weighing 30 g were used. The animals were kept under natural conditions of illumination and with food ad lib. Ascites tumor cells of sarcoma 37 were grafted subcutaneously in the axillary region in a dose of 0.1 ml ascites fluid, diluted twofold with physiological saline. The animals were killed in groups of 16 at each period of the investigation at intervals of 3 h for 24 h on the 9th day after inoculation of the tumor, and each time thymidine-H^3 (specific activity 16 Ci/g) was injected 1-1.5 h beforehand in a dose of 30 μCi per mouse. Pieces of tumor were fixed in Carnoy's fluid. Histological sections 5 μ in thickness were coated with type M photographic emulsion. The preparations were exposed for 10-11 days and stained with Mayer's hematoxylin. Mitoses and labeled nuclei were counted in 10,000-12,000 cells.

The mitotic index (MI) and the index of labeled nuclei (ILN) were expressed per 1000 cells. Statistical analysis of the results was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

The results given in Table 1 show that the value of MI in the tumor was maximal at 10 a.m. and minimal at 1 a.m. ($P = 0.006$). The changes in MI were made up of diurnal fluctuations in the indices of unlabeled mitoses (IUM) and labeled mitoses (ILM), each of which varies differently during the 24-h period.

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TABLE 1. Diurnal Changes in MI, IUM, ILM, and ILN in Sarcoma 37

Time of day	MI	P	IUM	P	ILM	P	ILN	P
10 a.m.	17,5	0,04	9,1	—	8,4	0,01	361,0	—
1 p.m.	13,2	—	9,5	0,017	3,7	0,01	346,0	0,02
4 p.m.	14,2	—	13,2	—	0,9	—	144,0	0,23
7 p.m.	14,0	—	13,4	—	0,5	0,01	198,0	0,14
10 p.m.	13,9	0,12	13,9	0,14	0,03	—	88,0	0,08
1 a.m.	11,2	0,04	11,2	0,08	0,01	—	115,0	—
4 a.m.	14,4	0,06	14,4	0,11	0,03	—	120,0	—
7 a.m.	11,5		11,5		0,03		117,0	
Mean diurnal values	13,7		12,0		1,72		186,0	

The diurnal rhythm of IMM was characterized by a well-defined maximum at 10 a.m. IMM then fell gradually to reach a minimum at between 10 p.m. and 1 a.m. ($P_{10-1} = 0.001$).

Diurnal changes in IUM were less sharply defined and were characterized by higher values between 4 and 10 p.m. Maximal values of ILN were observed between 10 a.m. and 1 p.m. By 4 p.m. the decrease in ILN was significant ($P_{10-4} = 0.0001$). Later in the day differences in the values of ILN were not significant. However, the increase in ILN from 7 to 10 a.m. was significant ($P = 0.0001$).

The coincidence between the times of maximal ILN and maximal MI in the experimental group can be explained as follows. The presence of the ILN peak indicates some synchronization of the entry of the cells into the S phase. As the cells pass through the S phase and new cells enter it, the values of ILN will increase. The maximal number of cells will be labeled if thymidine-H^3 is injected at a time when most of them have reached the end of the S phase. Since the animals were killed 1 h after the injection of thymidine, most of the labeled cells would also have passed through 1 h of the G_2 phase, and during this time another batch of cells would also be recorded as having entered the S phase.

As a result, in the period of the maximum of ILN most of the labeled cells characterized by that maximum would have been in the G_2 phase for about 1 h. If the G_2 phase for these cells lasts about 1 h, naturally the times of the maxima of ILN and MI would coincide.

The duration of the S phase is equal to the period of rise of ILN to the maximum. The duration of the G_2 phase will be equal to the period between the maximum of ILN and the maximum of MI plus 1 h.

Since the duration of the G_2 phase for cells characterized by ILM is about 1 h, as can be judged from the interval between injection of thymidine and the appearance of labeled mitoses, the maxima of ILN at 10 a.m. must evidently have been due to these cells. In that case, the possibility cannot be ruled out that the high value of ILN at 1 p.m. was connected with cells characterized by unlabeled mitoses, and the G_2 phase of these cells will be 4 h or more, for a significant increase in IUM to the maximal level was observed between 1 and 4 p.m. To this it can be added that the appearance of unlabeled mitoses 1-1.5 h after the injection of thymidine points directly to a longer duration of the G_2 phase of these cells than of the G_2 phase of cells giving labeled mitoses.

Because of the differences in the diurnal rhythms of ILM and IUM and in the durations of the G_2 phase of the cells characterized by these indices, it can be postulated that this strain of sarcoma 37 contains two different cell populations.

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