DIURNAL CHANGES IN MITOTIC ACTIVITY AND DNA SYNTHESIS IN NORMAL AND TUMOR TISSUES OF RATS

L. V. Sokolova, A. G. Mustafin,
V. N. Dobrokhotov, and S. I. Baluev

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Diurnal changes in mitotic activity and in the number of cells synthesizing DNA in a spontaneous sarcoma of rats and in the esophageal and corneal epithelium were studied in noninbred albino rats weighing 190 g by autoradiography. The animals were killed in groups every 3 h for 24 h, 1 h after receiving an injection of thymidine-H^3. The experiments showed a bimodal diurnal rhythm of mitotic activity in spontaneous rat sarcoma with a maximal number of mitoses at 10 p.m. and 7 a.m. and a bimodal diurnal rhythm of the index of labeled nuclei, with maxima at 10 a.m. to 1 p.m. and at 1 a.m. The increase in the index of labeled nuclei preceded the increase in mitotic activity by 9 and 6 h. The presence of a tumor in the animal did not change the character of the curve of diurnal rhythm of mitosis and DNA synthesis in the corneal and esophageal epithelium, although these indices were rather lower than in intact animals.

To evaluate the rates of cell division in the tissues correctly the data for the mean diurnal number of mitoses must be studied [5, 9]. Irradiation and antitumor preparations, if used at the period of the maximum or minimum of the number of mitoses, may differ in their action on cell reproduction in normal tissues and in tumors, with consequent differences in their therapeutic effect [4, 7, 11, 12, 15].

The character of the diurnal rhythm of mitosis and DNA synthesis in normal tissues has been studied in detail [1, 5, 6, 9]; it has received less investigation in tumors, and the results obtained have been contradictory [3, 4, 8, 11-15]. The effect of a malignant tumor on cell division in normal tissues not directly adjacent to the tumor nodule has not been made clear [1, 3, 4].

The object of the present investigation was to study diurnal changes in the number of cells synthesizing DNA and in the number of dividing cells in a transplantable tumor (spontaneous rat sarcoma, SRS) and in normal tissues (corneal and esophageal epithelium).

EXPERIMENTAL METHOD

Noninbred male albino rats with a mean weight of 190 g were used. Each animal received a subcutaneous injection of 170,000 cells of a suspension of the SRS tumor; 61 rats with a subcutaneous tumor measuring 2 × 2.5 cm, were given an intraperitoneal injection of thymidine-H^3 (USSR) with a specific activity of 4.5 Ci/mmole, in a dose of 0.3 μCi/g body weight, 1 h before sacrifice on the 19th-20th day after innoculation of the suspension. At each period of the investigation 7 or 8 rats were sacrificed (Table 1). Pieces of the tumor, esophagus, and cornea were fixed in Carnoy's solution. Sections 5 μ in thickness were coated with type M emulsion. Preparations of the tumor were exposed for 45 days, esophagus for 28 days, and cornea for 35 days. The autoradiographs were stained with Mayer's hematoxylin. Cells were regarded as labeled if they contained more than three grains of silver. The mitotic index (MI) and index of labeled nuclei

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TABLE 1. Diurnal Changes in Number of Cells Synthesizing DNA, Mitoses, and Number of Grains of Silver in SRS and Esophageal and Corneal Epithelium (in ‰)

Time of day	SRS			Esophageal epithelium			Corneal epithelium					
							stratum basale			stratum spinosum		
	ILN	MI	Mean No. of grains of silver	ILN	MI	Mean No. of grains of silver	ILN	MI	Mean No. of grains of silver	ILN	MI	Mean No. of grains of silver
10 a.m	405,1	29,7	17,5	24,9	3,4	16,5	44,5	9,8	8,8	14,1	2,1	9,1
1 p.m.	414,0	30,9	19,0	22,2	3,4	10,8	52,4	7,7	7,5	17,2	1,9	7,9
4 p.m.	327,8	31,3	13,6	20,7	2,3	10,8	68,2	5,9	8,6	22,9	1,3	8,7
7 p.m	279,9	40,1	12,3	44,9	1,5	8,1	57,4	6,2	6,1	17,2	2,1	6,5
10 p.m.	332,2	45,4	14,0	33,8	1,8	9,1	43,5	4,3	6,5	14,0	1,3	6,4
1 a.m	351,2	37,0	17,0	45,4	2,1	8,4	52,2	11,8	6,5	17,2	2,6	6,5
4 a.m.	231,1	26,7	14,6	51,4	4,2	7,9	40,9	10,8	6,9	14,3	2,7	6,8
7 a.m.	363,6	41,1	19,0	36,9	6,3	6,6	29,0	10,9	6,0	9,6	2,8	6,3
Mean diurnal value	338,1	35,4	16,0	36,0	3,1	9,8	48,5	8,4	7,1	15,8	2,1	7,3
Mean diurnal value of ratio ILN/MI	8,5				11,6			5,8			7,5	

(ILN) were determined per 1000 cells, by counting 2500-3000 cells in the tumor, 4000 cells in the esophagus, and 6000-8000 cells in the cornea of each animal. The intensity of DNA synthesis was estimated from the number of grains of silver determined in 50 labeled cells. The statistical analysis of the material was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that the diurnal rhythm of the number of tumor cells synthesizing DNA was bimodal in character with a maximum of ILN at 10 a.m.-1p.m. and at 1 a.m. The diurnal changes in MI in the tumor cells also followed a bimodal curve with a maximum of the number of mitoses at 10 p.m. and 7 a.m.

A maximum of ILN occurring at 10 a.m.-1 p.m. was 9 h earlier than the maximum at MI at 10 p.m., whereas the second increase in MI at 7 p.m. preceded the increase in ILN at 1 a.m.; i.e., the difference was 6 h. The number of grains of silver in the tumor cells varied throughout the 24-h period in accordance with the changes in the number of cells synthesizing DNA.

The diurnal changes in ILN and MI in the esophageal epithelium followed a unimodal curve. The maximal value of ILN preceded that of MI by 3 h, but the increase in ILN began much sooner, in fact from 7 p.m. or 9-12 h sooner. The intensity of DNA synthesis varied during the 24-h period, reaching a maximum at 10 a.m. and a minimum at 7 a.m.

The character of the diurnal changes in ILN and MI in the stratum basale and stratum spinosum of the cornea was similar. The mean-diurnal ILN of cells of the stratum basale was about 4 times higher than ILN of the more differentiated cells of the stratum spinosum. The maximal value of ILN preceded that of MI by 9 h in the stratum basale and by 15 h in the stratum spinosum. The intensity of labeling of the two layers of the cornea also fluctuated during the 24 h period and was similar in character.

A clear bimodal rhythm of mitotic activity was thus found in SRS. Similar results have been obtained for carcinoma of the mouse mammary gland and for a tumor induced by DMBA [8]. A definite bimodal diurnal rhythm of DNA synthesis was observed in SRS. Diurnal changes in DNA synthesis have also been demonstrated in a slowly growing differentiated hepatoma SSIH in mice with a maximum of the number of labeled cells at 4 a.m. [14].

The high mean diurnal values of MI(35.4 ‰) and ILN (338.1‰) and also the short mitotic cycle (18 h) [10] are evidence of the high proliferative activity of the SRS cells.

These results showing the character of the mitotic rhythm in the esophageal and corneal epithelium are similar to (or identical with) those found by most workers who have investigated these tissues in intact animals [1, 4, 6, 7]. However, the mean diurnal values of MI in the esophageal epithelium were somewhat lower than in intact animals, as other workers have noted previously [1, 2, 6]. The presence of a tumor in an animal thus was not significantly reflected in the character of the curve of the diurnal rhythm of mitosis and DNA synthesis in the normal tissues investigated, in agreement with data in the literature [1, 3, 4]. The amplitude of the diurnal changes in MI and ILN was less in SRS than in normal tissues. In the esophageal epithelium the maximum of the number of mitoses was more than four times greater than the minimum, in the stratum basale it was almost three times greater, and in the stratum spinosum it was more than twice greater, whereas in the tumor it was only 1.7 times greater. Similar relationships have been observed by other workers [1, 3].

The mean diurnal ILN exceeded MI in the tumor by 8.5 times, in the esophagus by 11.6 times, in the stratum basale of the cornea by 5.8 times, and in the stratum spinosum by 7.5 times. The ratios between the values of ILN and MI obtained in the tumor and the normal tissues are evidence that the proportion of cells synthesizing DNA and the proportion of cells taking part in mitosis was normal, in agreement with data in the literature [9]. However, the number of proliferating cells in the tumor was considerably greater than in the normal tissues studied, although the intensity of DNA synthesis by the tumor cell was many times greater than its intensity in the cells of the normal tissues.

It can be concluded from these results that the greatest effect of suppression of proliferation of SRS cells can be obtained by irradiation or by the action of cytostatic agents in the period of the maximum of the number of mitoses or of DNA synthesis; this conclusion is supported by the results of investigations on other tumors [3, 4, 7, 8, 11].

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