MITOTIC RHYTHM IN CROCKER SARCOMA OF MICE

(UDC 616-006.3-092.9-018.825 "414.21/22")

S. M. Kolomina

Laboratory of Experimental Cytology and Cytochemistry of Cancer Cells
(Head-Prof. G. I. Roskin [deceased]), Biology-Pedology Faculty, M. V. Lomonosov,
Moscow State University
(Presented by Active Member AMN SSSR V.V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 7,
pp. 83-86, July, 1964
Original article submitted May 25, 1963

Little attention has been devoted in the literature to an analysis of the diurnal periodicity of mitoses in malignant tumors. There are available only individual observations on clinical and experimental material. According to the assumption of a number of investigators, a study of the mitotic rhythm in malignant tumors has little importance for biotherapy of neoplasma, since it is assumed that many mitotic inhibitors used during maximal mitotic activity, if it could be detected in tumors, will more efficaciously inhibit the growth of malignant cells than with their administration at the period of decline of mitotic activity [10].

The data on the presence of a diurnal rhythm in the frequency of mitoses in malignant tumors are contradictory: some investigators have not detected variations in the mitotic activity in tumors at different times of the day [3, 8, 12, 11], whereas others indicate a diurnal mitotic rhythm [6, 7, 13, 14]. In our opinion, the most interesting in this respect is work [13] in which the authors on a large number of animals (102 mice) established a monophasic diurnal rhythm of mitoses with a maximum of cell division during the 8-12 P.M. period and a minimum at 8 A.M. in ascites sarcoma S_2 and Ehrlich's ascites carcinoma in white male mice. Experiments with the effect of colchicine confirmed the existence of a diurnal variation of mitosis in these tumors.

According to the data in the literature [3, 6, 7, 8, 13], the diurnal periodicity of mitoses was investigated in a quite limited number of experimental tumors: induced cancer of the skin of rats and mice, the ascites form of sarcoma S_2 of mice, ascites and solid forms of Ehrlich carcinoma of mice, carcinoma M-1 of rats, wherein males were used primarily as the experimental animals.

In the present work we attempted to establish whether there existed variations of mitotic activity during the morning and evening of the day in Crocker sarcoma of mice, males and females.

EXPERIMENTAL METHOD

The observations were carried out on random-bred white male and female mice weighing 18-20 g which were inoculated with Crocker sarcoma under the skin on the right side. In the experiment we used 122 mice (66 males and 56 females). The mice were maintained under normal light conditions and on the usual food ration. On the tenth day after inoculation a certain number of the animals were killed at definite hours of the day and the extracted tumors were weighed and fixed in Carnoy's fluid. From the paraffin-embedded material we prepared sections 5 μ thick which were stained with methyl green and pyronin after Unna. The count of mitoses was carried out in 100 fields of the microscope at a magnification of 945. In each individual case we counted not less than 6000 tumor cells. In all we counted about 894,000 cells. The count of cells which were in the stage of mitosis was carried out in the tumor growth zones. Early prophases, late telophases, and pathological forms of mitosis in degenerating cells were not taken into account. To estimate the mitotic activity we calculated the mitotic coefficient (the ratio of the number of dividing cells to the total number of cells for each definite time of day) and established the difference between the values of the mitotic coefficients. The data obtained were statistically processed. The significant difference was determined from Student's tables.

TABLE 1. Mean Values of Mitotic Coefficients During Morning and Evening H and the Difference Between Them in Crocker Sarcoma of Mice

Experimental series	Tumor weight (mg)	Mitotic coefficient	Tumor weight (mg)	Mitotic coefficient	Difference between values of mitotic coefficients	Significance
	8 A. M.		8 A. M.			
I	1,365	17.3	1,411	43.7	26.4	0.999
II	1,751	17.7	1,657	26.6	8.9	0.988

TABLE 2. Diurnal Variations of the Number of Mitoses in Crocker Sarcoma of Mice

Sex of mice	Mean value of mitotic coefficients					Between 8 A. M. and 3 P.M.	Between 8 A.M. and midnight	Between 8 A.M. and 3 P.M.	Between 3 P.M. and midnight
	8 AM	3 PM	8 AM	midnight					
♂	9.6	11.4	16.8	14.2	Difference between values of mitotic coefficients	7.2	4.6	1.8	2.8
					Significance	0.999	0.995	0.791	0.923
♀	14.2	15.4	19.1	16.4	Difference between values of mitotic coefficients	4.9	2.2	1.2	1.0
					Significance	0.999	0.770	0.516	0.452

EXPERIMENTAL RESULTS

In the I (test) series of experiments we used 12 males; 6 mice were killed at 8 A.M. and 6 at 8 P.M. A count of the number of dividing cells showed that at 8 P.M. the number of mitoses was appreciably greater than at 8 A.M.: the mitotic coefficients of the tumors extracted at 8 P.M. varied within 36.6-51.3, whereas in the tumors extracted at 8 A.M. it varied within 13.3-20.3.

It follows from the data of the I series in Table 1 that the number of mitoses at 8 P.M. in Crocker sarcoma of male mice is 2.5-fold greater than that at 8 A.M. The difference between the values of mitotic activity established for the morning and evening h is statistically significant.

In the II series of experiments set up on 16 males we attempted to confirm the results of I experimental series. Half of the animals were killed at 8 A.M. and the other half at 8 P.M. The indexes of the mitotic coefficients for tumors extracted in the morning were within the limits of 10.5-21.5, and for tumors extracted during the evening, within 17.3-41.8.

The data of Table 1 (II experimental series) also indicates a statistically significant difference of the mean values of mitotic activity of Crocker sarcoma of male mice killed at different times of the day. In the II series of experiments the difference in the number of mitoses during the morning and evening was less; the number of mitotically dividing cells as 8 P.M. was 1.5-fold greater than that at 8 A.M.

In the III series of experiments performed on 38 males, we studied the dirunal variations of mitoses: the mitotic activity was determined at 8 A.M., 3, 8 P.M. and at midnight. An analysis of the obtained data (Table 2) indicates that the number of mitoses varies during different times of the day: at 3 P.M. the level of mitotic activity slightly exceeds that recorded at 8 A.M. and is appreciably higher at 8 P.M., and again drops by midnight. The highest mitotic coefficient was established for 8 P.M. and the lowest for 8 A.M. The maximal value was 1.7 times greater than the minimal, the difference being statistically reliable. The variations of mitotic activity between 8 A.M., 3 P.M., and midnight were less appreciable and only the difference between the magnitudes obtained at 8 A.M. and midnight was statistically significant; the difference between the magnitudes obtained at 8 A.M. and 3 P.M. and between the magnitudes obtained at 3 P.M. and midnight was not significant.

Our data obtained for Crocker sarcoma of male and female mice coincide with the results of the above indicated investigations [13] obtained for the ascites form of sarcoma S_2 and Ehrlich carcinoma of male mice. Other

authors [7] found a mitotic peak in Ehrlich ascites carcinoma of mice in the late evening and early morning. The observations of Yu. N. Mol'kov [6] also testify to a difference in mitotic activity between the night and day h in the solid form of Ehrlich carcinoma of mice.

The opinion was expressed in the literature concerning the existence of a diphasic cycle of cell division in certain tumors [7, 14]. A number of investigators deny the presence of periodicity in the division of cancer cells [3, 8, 10-12].

The discrepancy in the results obtained by different investigators is in all probability due to a certain extent to the fact that they did not all, as indicated by Pohle and cohorts [13], take into account certain essential features when setting up the experiments. For example, taking samples during the course of the day from the tumor of one and the same carrier [12, 14] naturally could lead to masking the true mitotic activity, since the effect of the biopsy cannot be ignored. In other cases conclusions were based on analysis of an insufficient quantity of experimental data and also on an inadequate number of counted cells [7]. When setting up the experiments we endeavored to avoid these features. However, completely convincing data are cited in the literature concerning the absence of mitotic periodicity, for example, in transplanted sarcoma M-1 [3]. Such a contradiction of results suggests that possibly a diurnal rhythm of mitoses does not take place in all tumors.

Our data indicate that in Crocker sarcoma of mice the mitotic activity is different at different times of the day. The greatest divergence in the number of mitoses was between 8 A.M. and 8 P.M. During the evening the mitotic activity of sarcoma cells is greater than during the morning, this difference being more evident in males than in females. Our data indicate that within the investigated time period the curve of the change in the number of mitoses in Crocker sarcoma cells has a single peak with this maximum of mitoses at 8 P.M.

It is interesting to note that the rhythm of mitoses in Crocker sarcoma of mice was opposite to the rhythm characteristics of most normal tissues of mice whose mitotic peak, according to the data in the literature [1, 2, 4, 5, 9, 15], is during the morning h and the minimum during the evening h.

LITERATURE CITED

1. I. A. Alov and N. V. Krasil'nikova, AN SSSR, 142, 4, p. 933 (1962).
2. G. A. Vinogradova, Byull. éksper. biol., 11, p. 105 (1960).
3. M. T. Gololobova, éksper. biol., 9, p. 118 (1958).
4. M. T. Gololobova, In: Restorative Processes in Vertebrates [in Russian], Moscow, p. 266 (1959).
5. L. P. Kosichenko, Byull. éksper. biol., 6, p. 98 (1960).
6. Yu. N. Mol'kov, On Certain Regularities of Cell Division, Growth, and Metastasis of Tumors in Connection with Their Trauma (Biopsy). Author's Abstract of Candidate's Dissertation, Moscow (1953).
7. I. P. S. Agrell and E. Welin-Berger, Nature, Vol. 180, p. 705 (1957).
8. C. M. Blumenfeld, Arch. Path., Vol. 35, p. 667 (1943).
9. W. S. Bullough, Proc. Roy. Soc. B., Vol. 135, p. 212 (1948).
10. E. Cowdry, Cancer Cells [Russian translation], Moscow, p. 140 (1958).
11. W. B. Dublin, R. O. Gregg, and A. C. Broders, Arch. Path., Vol. 30, p. 893 (1940).
12. W. B. Dublin, Northw. Med. (Seattle), Vol. 43, p. 232 (1944).
13. K. Pohle, K. Meng, and E . Matthies, Z. Krebsforsch., Bd. 64, S. 208 (1961).
14. A. Voutilainen, Acta path. microbiol., scand., Suppl. 99, p. 1 (1953).
15. J. W. Wilson, Anat. Rec., Vol. 101, p. 672 (1948).