could lead to inhibition of DNA synthesis and, consequently, could reduce the rate of multiplication of the tumor cells. The question arises whether concentration changes in the composition of the elements in the blood can be found after local x-ray irradiation of animals in a comparatively small dose (500-1000 R). The experimental results confirmed that this was possible. A steady accumulation of Ca in the blood serum was discovered 1, 4, and 24 h after the end of irradiation (Fig. 2). The change in the concentrations of Mg and Cu did not differ by a statistically significant degree from the control.

Even by investigation of the blood serum differences could thus be found in the concentration of certain elements after local x-ray irradiation of tumor tissue in experimental animals.

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COMPARISON OF THE LEVEL OF MITOTIC ACTIVITY AND DURATION OF MITOSIS IN NORMAL AND NEOPLASTIC MOUSE TISSUES DURING THE 24-HOUR PERIOD

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Diurnal rhythms of cell division in the epithelium of the forestomach and in a transplantable carcinoma of the forestomach were found to be largely similar and the duration of mitosis in both these tissues varied during the course of the 24-hour period. The mean diurnal mitotic activity in the tumor was twice as high as in the normal forestomach. By contrast, in the course of 24 h colchamine (colcemid) led to the accumulation of 121.1 °/₀₀ of mitoses, compared with only 83.8 °/₀₀ in the carcinoma. The larger number of mitoses in the tumor when counted in the ordinary way can be explained by the 2.7 times greater mean diurnal duration of mitosis in carcinoma of the forestomach than in the normal epithelium of the forestomach.

KEY WORDS: *Duration of mitosis; carcinoma of the forestomach; epithelium of the forestomach; colcemid; mitotic index.*

The comparative study of the level of cell proliferation in tumors and in the normal tissues from which these tumors arise is of great importance in the study of the principles governing malignant growth. According to some workers [6-8, 10] the intensity of cell division in tumors is lower than in healthy tissues, and the high mitotic activity can be explained by the slow course of mitosis itself; other workers [4, 5, 9], on the other hand, consider that tumor cells proliferate faster than normal. However, in the study of this problem it is essential to remember that the duration of mitosis in both healthy and tumor tissues varies during the 24-hour period within quite wide limits [1-3].

Changes in mitotic activity and in the duration of mitosis during the 24-h period were investigated in a transplantable carcinoma of the mouse forestomach (strain OZh-5) and, at the same time, in the stratified squamous epithelium of the forestomach of these mice.

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TABLE 1. Diurnal Changes in Number of Mitoses, Number of C-Mitoses, and Duration of Mitosis in Carcinoma and Normal Epithelium of Mouse Forestomach

Time of day or night	Group 1 (control)		Group 2 (colchamine)		Duration of mitosis (t_m), h	
	forestomach	carcinoma	forestomach	carcinoma	forestomach	carcinoma
	MI	MI	MI_c	MI_c		
10—14 (I)	6,3	11,1	17,8	17,7	1,4	2,5
14—18 (II)	5,7	13,1	12,0	18,6	1,9	2,8
18—22 (III)	2,2	10,8	10,4	10,0	0,8	4,3
22—02 (IV)	3,4	7,6	14,6	10,4	0,9	2,9
2—6 (V)	5,8	9,7	40,2	12,4	0,6	3,1
6—10 (VI)	6,7	10,0	26,1	14,7	1,0	2,7
Mean	5,0	10,4	20,2	14,0	1,1	3,0
	$P_{I-III}=0,003$ $P_{III-VI}=0,002$	$P_{II-IV}=0,002$ $P_{IV-VI}=0,048$	$P_{I-III}=0,04$ $P_{III-V}=0,0001$ $P_{V-VI}=0,007$	$P_{II-III}=0,0001$ $P_{III-VI}=0,009$	$P_{I-V}=0,007$ $P_{II-V}=0,028$ $P_{V-VI}=0,01$	$P_{I-III}=0,0001$ $P_{II-III}=0,007$ $P_{III-IV}=0,01$ $P_{III-VI}=0,004$

EXPERIMENTAL METHOD

Sexually mature male C3HA mice into which a suspension of minced tumor cells was injected into the thigh muscles were used. Experiments were carried out on the ninth day after transplantation of the carcinoma. The animals were divided into two groups. The mice of group 1 acted as the control and they were killed at intervals of 2 h throughout the 24-h period starting from 10 a.m., two to four mice at each time. The mice of group 2 received an intraperitoneal injection of colchamine (colcemid) in a dose of 5 mg/kg 4 h before sacrifice; they were killed at 10 a.m., 2, 6, and 10 p.m., and 2 and 6 a.m., five or six animals at each time. Pieces of tumor and of normal forestomach were fixed in Carnoy's fluid. The number of mitoses was counted in histological sections in areas of tumor free from necrosis, and also in the epithelium of the normal forestomach; in each case about 10,000 cells were examined. The mitotic index (MI) in the control animals and in animals receiving colchamine (MI_c) was calculated in promille. The duration of mitosis was determined by the equation

$$t_m = \frac{MI \cdot t}{MI_c},$$

where t_m is the duration of mitosis (in h); t the time during which the colchamine acted (4 h); MI the mean of the individual values of MI during a 4-h interval; MI_c the same for mice receiving colchamine. The results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

As Table 1 shows, a clear diurnal rhythm of MI and MI_c was present in the epithelium and carcinoma of the mouse forestomach. The maximal values of MI and MI_c in cells of the normal forestomach occurred between 2 a.m. and 2 p.m. and minimal values between 6 and 10 p.m. A largely similar pattern also was observed in the tumor cells in which the highest values of MI and MI_c were found between 10 a.m. and 6 p.m. and the lowest values between 6 p.m. and 2 a.m.

The parallel between the changes in the number of mitoses and the number of C-mitoses in the tissues studied is evidence that more cells start to undergo mitosis in the morning and afternoon than in the evening and night. Characteristically the degree of synchronization of the commencement of cell division in periods of increased mitotic activity was higher in the normal forestomach than in the carcinoma. For instance, between 2 and 10 a.m. the number of mitoses accumulating in the epithelium of the forestomach following administration of colchamine was 54.7% of the total number for the 24-h period, whereas in the carcinoma 43.3% of mitoses accumulated between 10 a.m. and 6 p.m.

Meanwhile analysis of mitosis activity (expressed in MI values) at certain times of day and night shows that MI did not always reflect the true number of cells starting to divide. For instance, between 2 and 6 p.m. mitotic activity in the forestomach was the same (MI = 5.7 °/₀₀) as between 2 and 6 a.m. (MI = 5.8 °/₀₀), but during the period from 2 to 6 a.m. colchamine caused the accumulation of nearly 3.5 times more mitoses than between 2 and 6 p.m. The leveling of the MI values took place because the duration of mitosis (Table 1) in the interval from 2 to 6 p.m. was 3 times longer, as a result of which the number of mitotic

figures recorded was greater. A similar example can also be given for carcinoma of the stomach: In the intervals 6-10 p.m. and 10 p.m.-2 a.m. colchamine caused the accumulation of the same number of mitoses, but MI between 6 and 10 p.m. was greater than between 10 p.m. and 2 a.m., a fact that can also be explained by the longer duration of mitosis during this period.

Consequently, although the general character of the diurnal rhythm of mitotic activity in the forestomach and in the tumor was very similar whether investigated with or without colchamine, at certain periods MI did not reflect the true number of cells starting mitosis at these times.

Comparison of the values of MI for the control animals in the forestomach and carcinoma showed that during all 4-h intervals studied MI was greater for the tumor than for the normal forestomach; even the highest value of MI (6.7 °/₀₀) in the forestomach did not exceed the minimal value (7.6 °/₀₀) in the tumor. The important point is that the mean diurnal MI in the carcinoma was twice as high as in the normal forestomach ($P = 0.0001$). On this basis it might be concluded that the level of mitotic activity in the tumor was much higher than in the normal tissue. However, investigations by the colchamine method showed that this was not so. Only between 2 and 6 p.m. was the number of mitoses accumulating after colchamine greater in the carcinoma than in the epithelium of the forestomach. At all other times MI_c in the tumor was less than MI_c in the forestomach.

After adding together the number of mitoses blocked during the 24-h period in the normal forestomach (121.1 °/₀₀) and in the carcinoma (83.8 °/₀₀), the next step was to calculate the time of cell renewal in the epithelium of the forestomach, which was 8 days, and the time for the number of tumor cells to double itself, which was 12 days; in other words, the level of cell multiplication in the tumor was lower than in the normal forestomach.

These results show that the level of cell proliferation in carcinoma of the forestomach is not higher than in the epithelium of the normal forestomach, as might be concluded simply from counting the number of mitoses in the control group. At all times of the investigation the time of mitosis in the carcinoma was greater than in the forestomach; whereas in the tumor it varied during the 24-h period from 2.5 to 4.3 h, in normal tissue it varied from 0.6 to 1.9 h. The mean diurnal value of the duration of mitosis in the carcinoma (3 h) was 2.7 times greater than that (1.1 h) in the epithelium of the forestomach ($P = 0.0001$), as a result of which the number of mitotic figures recorded by counting in the ordinary way was increased.

No significant difference was found when mean diurnal prophase coefficients in the forestomach were compared in the control (1.9 °/₀₀) and experimental (1.8 °/₀₀) mice, as also was the case in carcinoma in the animals of groups 1 (0.4 °/₀₀) and 2 (0.3 °/₀₀).

The results of this experiment can be applied only to the strain of carcinoma actually studied and in the precise stage of growth and development when the investigation was carried out, although on the general plane the results may be evidence that cell proliferation in tumors is not at a higher level than in normal tissues but that the course of mitosis itself is very much slower.

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