of shivering heat production is situated in the same region. Nevertheless this does not rule out the participation of the brown fat in the overall heat production during the awakening of hibernating animals.

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EFFECT OF CYCLIC AMP ON MITOSIS IN THE ESOPHAGEAL EPITHELIUM OF MICE WITH TUMORS

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Mitotic activity and the number of DNA-synthesizing cells were studied in the epithelium of the esophageal mucosa of albino mice with tumors during the 24 h after administration of dibutyryl-cyclic 3,5-AMP. Injection of the compound leads to delay of the cells in the G_2 phase of the mitotic cycle and to an increase in the duration of mitosis during the first few hours of the experiments, without changing the total number of cells passing through the mitotic cycle in the 24-h period.

KEY WORDS: esophagus; mitosis; mitotic cycle; dibutyryl-cyclic 3,5-AMP.

Investigations both in vitro and in vivo have shown that cyclic 3,5-AMP and its derivatives have an antimitotic action in many tissues of different origin [5, 6, 11]. The most sensitive period of the mitotic cycle (MC) is the G_2 phase [11, 12]. It has also been shown that injection of cyclic AMP into animals with tumors inhibits further growth of malignant tumors [7]. There are insufficient data from comparative studies of the response of normal and tumor tissues to cyclic AMP. The writer showed previously that a single injection of dibutyryl-cyclic AMP (DBCAMP) modifies the proliferative regime in Ehrlich's ascites carcinoma by producing definite synchronization of mitoses through the appearance of a preprophase block and, probably, of stimulation of the entry of the cells into the S period of MC [3]. The diurnal dynamics of cell division and of the number of DNA-synthesizing cells in the esophageal epithelium of animals with tumors was studied in the experiments described below following injection of DBCAMP.

EXPERIMENTAL METHOD

Experiments were carried out on 140 sexually mature male albino mice weighing 20-25 g inoculated 4 days before the beginning of the experiments with Ehrlich's ascites carcinoma [3]. The animals were divided into two groups: The mice of group 1 received an injection of DBCAMP at 10 a.m. in a dose of 20 μg/g body weight; group 2 consisted of intact control mice.

The mice were killed in groups at intervals of 2 h during the 24 h period. [^{3}H]Thymidine was injected into the animals 1 h before sacrifice in a dose of 0.5 μCi/g (specific activity 4.6 Ci/mmole). Pieces of the lower third of the esophagus were fixed in Carnoy's fluid and histological sections 5-6 μ thick were coated

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TABLE 1. Diurnal Changes in MI during Phases of Mitosis and in ILN in Esophageal Epithelium of Control Mice and Mice with Tumors after Injection of DBCAMP at 10 a.m.

Time of day	Control animals							Experimental animals						
	MI, ‰	P	PI	Mtl	TI	ILN, %	P	MI, %	P	PI	Mtl	TI	ILN, %	P
$10^1/_2$	—	—	—	—	—	—	—	3,0	0,5	0,5	1,9	0,6		
11	5,4		0,4	3,8	1,2	7,1		4,5		0,1	3,4	1,0	7,5	
12		0,01					—	4,3	—	0,1	3,3	0,9	8,2	—
14	2,2		0,2	1,3	0,7	5,7		4,8	—	0,1	3,9	0,8	5,6	—
		0,012					0,07		0,32					—
16	0,6	—	0,0	0,5	0,1	2,3	—	3,3	0,02	0,0	2,7	0,6	5,1	—
18	0,7	—	0,1	0,5	0,1	3,4	0,001	1,2	0,22	0,0	1,1	0,1	4,7	0,012
20	0,4	0,3	0,0	0,3	0,1	8,0	0,032	2,5	—	0,0	2,2	0,3	1,5	—
22	0,8	—	0,1	0,6	0,2	4,4	0,061	1,7	—	0,0	1,4	0,3	2,8	—
24	1,1	—	0,1	0,7	0,2	6,7	—	1,0	—	0,0	1,0	0,0	3,1	0,01
2	1,7	—	0,2	1,2	0,3	7,9	—	1,1	0,0001	0,1	0,9	0,1	6,6	0,02
4	1,4	0,14	0,1	1,1	0,2	8,8	—	5,1	—	0,7	3,3	1,1	12,4	—
6	2,2	0,21	0,2	1,6	0,4	8,5	—	5,2	—	0,6	4,2	0,4	10,1	—
8	3,7	0,23	0,2	2,6	0,9	9,0	—	6,1		0,7	4,1	1,3	10,1	
Mean diurnal MI=1,8±0,26‰				Mean diurnal ILN=6,5±0,44%				Mean diurnal MI=3,2±0,3‰			Mean diurnal ILN =6,47±0,45%			

Notes. 11 a.m.-4 p.m. P=0,0001; 8 p.m.-2 a.m. P=0,01; 4-8 a.m. P=0,0001; in interval 11 a.m.-4 p.m. P=0,005; 4 p.m.-4 a.m. P=0,002; 10 p.m.-4 a.m. P=0,006; 4 p.m.-8 a.m. P=0,002; 2 p.m.-6 p.m. P=0,0001; 8 p.m.- midnight P=0,19; 8 p.m.-midnight P=0,002; midnight-4 a.m. P=0,0001

PI) prophase index, Mtl) metaphase index, TI) telophase index.

with type M liquid photographic emulsion. After exposure of the autoradiographs for 60 days and development, the sections were stained with hematoxylin. The overall mitotic induces (MI, in promille) and the indices of the individual phases of mitosis and indices of labeled nuclei (ILN, in %) were counted in 7000-9000 cells of the stratum basale of the esophageal epithelium in each case. The results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

A diurnal rhythm of mitosis with a maximum in the morning (6-11 a.m.) and a minimum during the afternoon and evening (4-10 p.m.; Table 1) was found in the esophageal epithelium of the control mice. The changes in the relative proportions of each of the phases of mitosis determining the overall MI during the 24-h period will be noted. They indicated that the diurnal rhythm of mitosis in the esophagus reflects variation in the rate of entry of the cells into mitosis rather than delay of cell division in individual phases. The mean diurnal MI in the esophagus of the control animals was 1.8 ± 0.26 ‰. Such a low mean diurnal value of MI when the general character of the diurnal rhythm of mitosis in this tissue remains unchanged, whether described in healthy mice or in animals with tumors [2], points to a decrease in the general level of proliferation in tissues not affected by tumor growth and also the stability of function of the regulatory system of physiological regeneration in the body.

The general character of the diurnal changes in MI in the esophageal epithelium of the experimental animals was similar to the changes in MI in the control group of mice, but almost throughout the period of the experiment the values of MI in the mice after receiving DBCAMP were lower than in the intact animals (Table 1). During the first 8 h the higher values of MI in the experimental mice were due entirely to the discovery of a large number of metaphases, whereas the values of the prophase indices as early as 1 h after injection of DBCAMP had fallen to 0.1‰ and were much lower than in the control animals (0.4 ‰; P = 0.001).

These results are evidence that the number of cells starting on mitosis was sharply reduced in the experimental animals, at least during the first 6 h of the experiments. The high values of the metaphase indices at this time can be explained by delay of the cells in mitosis. This conclusion is in agreement with previous reports of the ability of cyclic AMP to cause disaggregation of microtubules [8], which could naturally lead to a hold up of the dividing cells in the late phases of division. Similar data indicating an increase in the duration of mitosis in the young animals under the influence of adrenalin, a specific β-adrenergic mediator, have been obtained by other workers also [1]. The higher value of the mean diurnal MI in the esophagus of the experimental animals (3.2 ±0.3 ‰) cannot therefore be evidence of increased mitotic activity but simply reflects the greater chance of finding mitoses with a longer course.

The diurnal rhythm of the number of DNA-synthesizing cells in the intact mice with maximal values of ILN at night (midnight-8 a.m.) and minimal values during the afternoon (2-6 p.m.; Table 1) corresponds to that described by other workers in this tissue [4, 10].

Diurnal changes in the values of ILN in the esophagus of the experimental animals can be represented by a unimodal curve with a maximum in the early morning (4-8 a.m.). The minimal number of DNA-synthesizing cells was observed in the experimental group at 8 p.m., i.e., 4 h later than in the control animals. At 4 p.m., when the values of ILN in the control group reached a minimum, the number of cells in the S phase still remained considerable in the experimental animals ($P = 0.001$).

The longer period of discovery of high values of ILN (from 11 a.m. to 6 p.m.) in the esophagus of the experimental animals could indicate that a larger number of cells passed through the S phase during the first 10 h after injection of the preparation in these animals than in the mice of the control group. The more synchronized entry of the cells into the S phase of MC at night after injection of DBCAMP in these experiments, as reflected in the rapid rise in the values of ILN toward a maximum at 2-4 a.m. also was interesting.

The absence of differences in the mean diurnal values of ILN in the control ($6.55 \pm 0.44\%$) and experimental ($6.47 \pm 0.45\%$) groups is an indication that the same number of esophageal cells passes through the S phase during the 24-h period in both groups.

Injection of DBCAMP thus led to redistribution of the esophageal cells with respect to the times of the phases of MC without affecting the total number of cells passing through MC in the 24 hours. The period of minimal values of MI, which in the esophagus lasts from 6 p.m. to 2 a.m., coincided with the time of maximal synchronization of cell division in tumors in these animals [3], so that the relative selectivity of action of antimitotic preparations on the tumor in this section of the 24-h period can be postulated.

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