Alkaline phosphatase is known to be the marker enzyme of osteoblasts. There is evidence that on retransplantation of colonies of stromal fibroblasts into syngeneic animals bone tissue is formed [5]. We also have observed on more than one occasion deposition of calcium in individual clones in human bone marrow cultures. Consequently, the presence of alkaline phosphatase in cells of the colonies indicates that the precursors forming them are osteogenic precursor cells.

The fact that cells containing alkaline phosphatase and not possessing enzyme activity are present in the same colony indicates that cells at different stages of differentiation are present in the composition of the colonies.

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CIRCADIAN RHYTHM OF SENSITIVITY OF PROLIFERATING LINGUAL AND ESOPHAGEAL EPITHELIAL CELLS TO ADRENALIN

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KEY WORDS: adrenalin; dose; mitotic index; circadian rhythm.

Adrenalin, the hormone of the adrenal medulla, is known to cause considerable changes in cell proliferation. Most frequently it has an antimitotic action by blocking the end of the G_2 phase of the mitotic cycle [1, 2, 4, 5]. However, there is little information in the literature on the effect of adrenalin on cell proliferation if administered at different times of day or on dependence of the antimitotic effect of the hormone on its dose [3, 5, 6]. The object of this investigation was to study these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 198 noninbred male albino mice weighing 25 g and kept in the animal house with alternation of 12 h daylight and 12 h darkness (daylight from 8 a.m. to 8 p.m.). Some of the mice received a single intraperitoneal injection of adrenalin solution in a dose of 0.5, 1, or 2 μg/g body weight at noon, 4 and 8 p.m., midnight, or 4 or 8 a.m. Control animals were injected with physiological saline at the same times. Experimental animals were killed 40 and 60 min and control animals 50 min after injection of the solutions. The tongue and esophagus were removed from the mice (the esophagus was taken twice — at 8 a.m. and 8 p.m.). After ordinary histological treatment, from 5000 to 25,000 cells in paraffin sections were analyzed in each case and the mitotic index (MI) was calculated in promille. Epithelium from the dorsal surface of the tongue was studied. The numerical results were subjected to statistical analysis by Student's test. Differences were considered to be significant at the $P \leqslant 0.05$ level.

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TABLE 1. Changes in MI (M ± m) in Lingual and Esophageal Epithelium 40 and 60 min after Injection of Various Doses of Adrenalin into Mice

Test object	Time of sacrifice	Control	Time of sacrifice	Dose of adrenalin, μg/g		
				0,5	1	2
Lingual epithelium	12:50 p.m.	8,4±0,9	12:40 p.m.	7,3±0,7 (—13)	7,0±0,6 (—17)	5,5±0,5* (—35)
			1 p.m.	7,0±0,6 (—17)	6,3±0,3* (—25)	4,4±0,4* (—48)
	4.50 p.m.	4,9±0,4	4:40 p.m.	4,6±0,7 (—6)	4,4±0,5 (—10)	3,1±0,4* (—37)
			5 p.m.	4,3±0,5 (—12)	3,9±0,3 (—20,5)	3,0±0,4* (—39)
	8:50 p.m.	3,0±0,3	8:40 p.m.	3,2±0,3 (+6)	2,5±0,3 (—17)	2,2±0,2* (—27)
			9 p.m.	2,6±0,2 (—13)	2,2±0,2* (—27)	2,0±0,3* (—33)
	12:50 a.m.	6,2±0,5	12:40 a.m.	5,5±0,7 (—11)	4,6±0,4* (—26)	3,5±0,5* (—44)
			1 a.m.	5,3±0,8 (—14,5)	4,6±0,5* (—26)	3,6±0,6* (—40)
	4:50 a.m.	12,8±1,2	4:40 am	10,4±0,9 (—19)	7,2±1,0* (—44)	5,1±1,0* (—60)
			5 a.m.	9,6±0,8* (—25)	7,0±0,8* (—45)	4,5±0,8* (—65)
	8:50 a.m.	26,3±2,0	8:40 p.m.	18,8±1,7* (—28,5)	11,7±1,5* (—56)	4,9±0,7* (—81)
			9 a.m.	17,4±2,0* (—34)	12,4±1,4* (—53)	7,2±0,8* (—73)
Esophageal epithelium	8:50 p.m.	4,3±0,5	8:40 a.m.	3,8±0,7 (—11,5)	3,3±0,6 (—23)	2,9±0,3* (—32,5)
			9 p.m.	3,8±0,5 (—11,5)	3,2±0,4 (—25,5)	2,9±0,4* (—32,5)
	8:40 a.m.	23,8±2,1	8:40 a.m.	17,5±1,5* (—26,5)	11,0±1,1* (—54)	3,2±0,5* (—87)
			9 a.m.	17,0±1,6* (—29)	8,1±1,0* (—66)	1,5±0,2* (—94)

Legend. Changes in MI compared with control (in %) shown in parentheses. Asterisk indicates values of MI differing significantly from control.

EXPERIMENTAL RESULTS

A monophasic circadian rhythm of MI with a maximum at 8:50 a.m. and a minimum at 8:50 p.m. was found in the lingual and esophageal epithelium of the control animals ($P \leqslant 0.001$; Table 1).

Injection of adrenalin into the animals as a rule caused a decrease in MI in the lingual and esophageal epithelium, but the degree of inhibition of mitosis depended both on the time of injection of the hormone (more exactly, on the phase of the circadian rhythm of MI) and on the dose of adrenalin used (Table 1).

Analysis of the effects of different doses of the hormone on proliferating cells shows that at all times of testing the antimitotic action of adrenalin was directly dependent on its dose. Meanwhile the degree of depression of MI depended directly on the phase of the circadian rhythm of cell division and on the initial level of the number of mitoses in the lingual and esophageal epithelium. For instance, in the lingual epithelium, after injection of adrenalin in the mice during the period of the maximum of MI in the circadian rhythm (8:50 a.m.), it fell after 40 min by 28.5-81% (with different doses), and after 60 min by 34-73%. Meanwhile injection of adrenalin during the minimum of the number of mitoses (8:50 p.m.) was accompanied by a much smaller decrease in MI (the maximal effect of a dose of 2 μg was 27-33% and there was virtually no effect from a dose of 0.5 μg). Similar results also were obtained for the esophageal epithelium (Table 1) and also by other workers for the mouse corneal epithelium [5]. Dependence of the effect of adrenalin on cell proliferating in the tongue on the initial MI level also was observed at other times of the 24-hour period.

To what can this dependence of the action of adrenalin be attributed? The results show that the number of cells in the G_2 phase of the mitotic cycle and the number of cells sensitive to adrenalin in that phase change rhythmically during the 24-hour period, and these rhythms coincide in time. In other words, the fewer the number of cells in the G_2 phase of the cycle during the 24-hour period, the fewer the cells in the G_2 population responding to adrenalin. With an increase in size of the G_2 population (during the maximum of MI in the circadian rhythm) the number of cells sensitive to adrenalin increases and this increase is

not directly proportional to changes in MI during the 24-hour period. It can accordingly be concluded that the cell composition of the G_2 population differs in the course of the 24-hour period, as reflected in the response to adrenalin. Considering the important role of adrenalin in the control of cell proliferation, the fact that the number of cells sensitive to adrenalin increases during the 24-hour period in a proportion different from that of mitotic activity during the circadian rhythm can be interpreted as evidence that mechanisms regulating the rhythms of mitosis exist at the cell population level.

The results of this investigation show that the degree of inhibition of mitosis depends directly on the dose of adrenalin. The size of the cell population reacting to adrenalin is thus related to the strength of action of the hormone. This is evidence that cells in the G_2 phase which respond to adrenalin differ from one another in their sensitivity to the hormone. Nevertheless, the general rule expressed in the words that significant saturation of the G_2 population by cells sensitive to adrenalin takes place during an increase in MI, is observed with all doses of the hormone.

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ANDROGENIC FUNCTION OF STEROID-PRODUCING GLANDS DURING THE METOPIRONE TEST ON BABOONS

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KEY WORDS: baboon (*Papio hamadryas*); metopirone; hormonal reaction.

Substances with an oriented effect on the level of function of particular endocrine glands are nowadays finding increasing application in clinical and experimental endocrinology. One such substance, used to study the functional reserves of the pituitary gland, is metopirone [2-methyl-1,2-bis-(pyridyl)-propan-1-one] (metyrapone), which has a selective inhibitory action mainly on 11β-hydroxylase [5]. The result of this is to block synthesis of key corticosteroids, mainly hydrocortisone. A fall in the blood hydrocortisone level leads to marked activation of the adrenocorticotrophic function of the pituitary, the degree of which is estimated by the rise in the level of steroid precursors in the blood or urine.

However, despite much research into the effect of metopirone on pituitary and adrenal function, there is practically no reference to the study of the endocrine function of the gonads during the metopirone test in the world literature. Yet this is a most important aspect, particularly in the study of functional reserves of the adenohypophysis in children. According to the most widely approved scheme, within a short time interval the child receives several grams of metopirone, and this may have untoward consequences.

Baboons were chosen as the model in which to study the effect of metopirone on gonadal function because, as previous investigations showed [2], of all the lower monkeys baboons are closest to man in thier endocrine parameters.

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