Regularities of Spatial and Time Organization of the Proliferative System of Small Intestinal Cryptic Epithelium in Intact Mice in the Circahoralian Biological Rhythm of Its Cells Multiplication

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The proliferative system of the small intestinal cryptic epithelium in intact mice in circahoralian rhythm of mitotic activity of its cells (with a 80-140-min period) is characterized by spatial and time organization. The characteristics of the studied proliferative system do not dub it in individual parts, but present new properties intrinsic of this system in general. The features are greatly similar to the features of spatial and time organization of this proliferative system in circadian rhythm of cell division.

Key Words: *circahoralian biological rhythm; proliferative system; small intestinal cryptic epithelium; spatial and time organization*

Among the biological rhythms with different periods, type 3 time organization of the proliferative system (PS) of the small intestinal cryptic epithelium is distinguished; this organization consists of rhythms of the same function with different periods [4,5]. In addition to circadian and ultradian rhythms, great attention is paid to circahoralian rhythms [1,2]. The presence of these rhythms in the work of tissue PS is established [3,7-9]. Biological significance of circahoralian rhythms is not quite clear, but they extend our notions on time organization of biological systems. Along with this organization, biological systems are characterized by spatial organization, which creates their universal spatial and time organization (STO) [4,5]. However, this latter was virtually never studied in type 3 time organization of a biosystem. We studied the regularities of PS STO in the small intestinal cryptic epithelium of intact mice in the circahoralian rhythm of mitotic activity.

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MATERIALS AND METHODS

The study was carried out on 100 outbred male mice (21-23 g) kept at 23°C and 12:12 day:night regimen (light from 6.00 till 18.00, illumination 250-300 lux). The object of the study was small intestinal cryptic epithelium of 20 longitudinally dissected crypts in their proximal portions, the wall consisting of 25 epithelial cells.

In order to detect circahoralian fluctuations in the number of dividing cells, the mitotic indexes (MI) were estimated every 20 min from 6.20 to 12.00. Common mitotic index (CMI) in the small intestinal cryptic epithelium was estimated (in percent) per 1000 epithelial cells in examined crypts. The parameters of circahoralian biological rhythm of proliferation were determined using graphic parametrical method for analysis of biological rhythms [10,11]: mesor, acrophase, active and passive phases, their lengths, rhythm period, absolute and relative amplitudes, circahoralian pool of mitoses, pool of mitoses in the active phase of the rhythms, relative pool of mitoses in the active phase of the rhythm.

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Chronotopobiological method using coefficient of correlations was also used for the study of PS STO in the small intestinal cryptic epithelium [6]. Using this method, spatial gradients of MI distribution in cryptic walls were determined, these gradients being determined for each point of the study over the period of circahoralian rhythm of number of mitoses. Paired analysis of correlations between circahoralian rhythmic changes in MI in subpopulations of small intestinal cryptic epithelium was carried out in order to clear out the relationship between time changes in MI and topography of cryptic cell subpopulations and between spatial changes in MI in small intestinal cryptic epithelial subpopulations in different time of the study in order to detect the relationship between MI changes and time fluctuations of this index. The significance of differences between the values was evaluated by Student's time test. The results were considered significant at $p \leq 0.05$.

RESULTS

Three cycles of time changes in CMI are distinguished for the studied period of the day: cycle 1 (6.20-8.40), cycle 2 (8.40-10.00), and cycle 3 (10.00-12.00). The period of these cycles is 80-140 min,

TABLE 1. Parameters of CMI Circahoralian Rhythm (Mean MIsubp, $MIsubp_{bas}$, $MIsubp_{max}$) in Intact Mouse Small Intestinal Cryptic Epithelium in Different Cycles of Its Changes

Parameter		Cycle I	Cycle 2	Cycle 3
CMI	Acrph, hours	6:20	8:40	10:00
	AA, %	1.6	1.5	3.2
	RA	1.3	1.3	1.9
	Mesor, %	6.5±0.2	6.3±0.3	5.0±0.3
	LAP, min	102	27	60
	LPP, min	38	53	60
	Rhythm period, min	140	80	120
	Pm, %	32.3±0.1	18.6±0.2	21.4±0.3
	AP Pm, %	26.5±0.1	9.0±0.2	15.1±0.3
	AP Pm/Pm, %	82	48	71
$MIsubp_{bas}$	Acrph, hours	7:20	8:40	10:00
	AA, %	1.1	2.1	2.9
	RA	1.2	1.5	1.9
	Mesor, %	5.7±0.3	5.5±0.3	4.2±0.3
	LAP, min	95	30	55
	LPP, min	47	50	65
	Rhythm period, min	140	80	120
	Pm, %	27.5±0.2	15.5±0.3	17.9±0.3
	AP Pm, %	22.9±0.1	9.8±0.3	11.3±0.3
	AP PM/Pm, %	83	64	63
$Misubp_{max}$	Acrph, hours	6:20	9:20	10:00
	AA, %	4.3	2.1	4.5
	RA	1.6	1.3	1.7
	Mesor, %	9.8±0.3	9.7±0.3	8.1±0.3
	LAP, min	90	45	55
	LPP, min	50	35	65
	Rhythm period, min	140	80	120
	Pm, %	48.8±0.3	29.0±0.3	34.3±0.3
	AP Pm, %	34.8±0.3	20.2±0.3	21.4±0.3
	AP Pm/Pm, %	71	70	63

Note. Here and in Tables 2-4: Acrph: acrophase; AA: absolute amplitude; RA: relative amplitude; LAP: length of active phase; LPP: length of passive phase; Pm: circahoralian pool of mitoses.

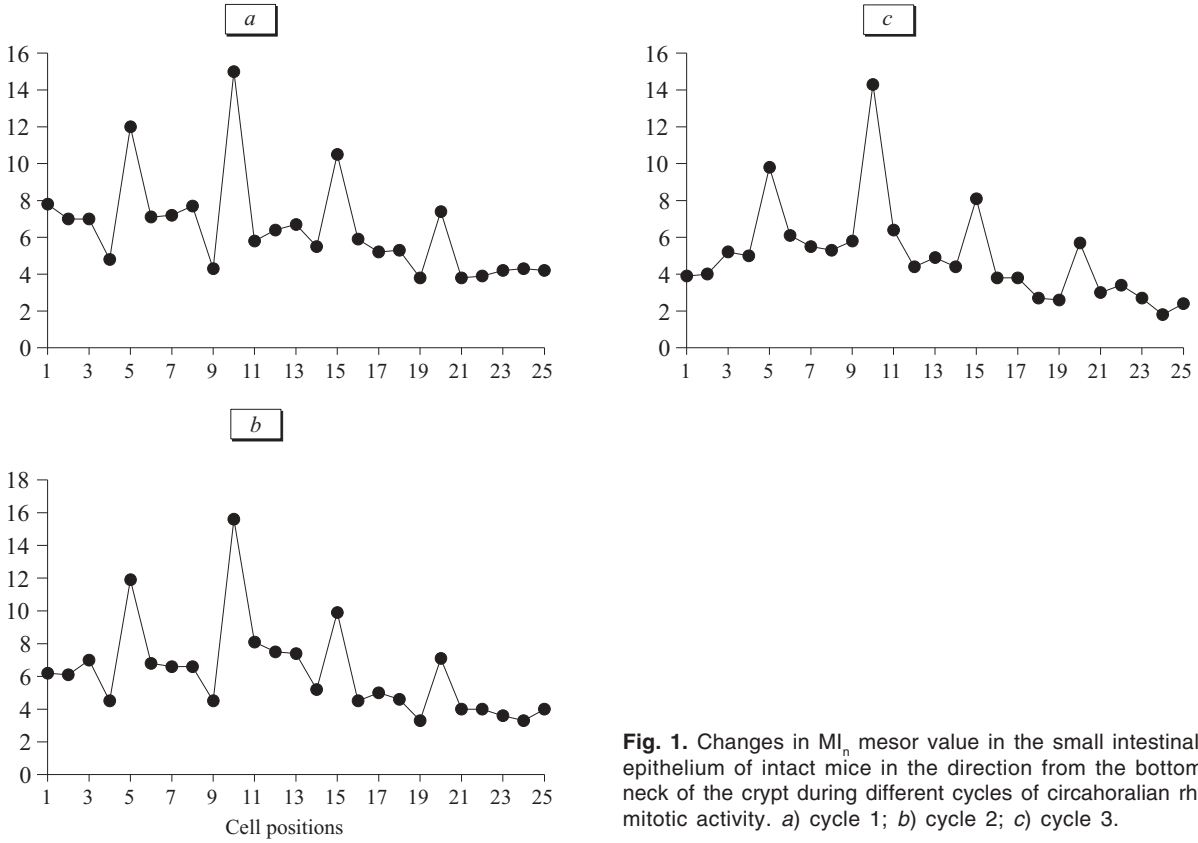

Fig. 1. Changes in MI_n mesor value in the small intestinal cryptic epithelium of intact mice in the direction from the bottom to the neck of the crypt during different cycles of circahoralian rhythm of mitotic activity. *a*) cycle 1; *b*) cycle 2; *c*) cycle 3.

that is, the rhythm of mitotic activity of small intestinal cryptic epithelium PS is circahoralian, with different periods for different cycles.

Monophase changes in CMI with an acrophase at 6.20 and a 140-min period were observed during cycle 1 (Table 1). The absolute and relative amplitudes differed little from each other, while active phase was almost 3-fold longer than passive phase of the rhythm. About one-third of small intestinal cryptic epithelial cells, capable of proliferation, divided during this cycle, while the greater part of mitoses took place during the active phase of the rhythm. Cycle 2 was also characterized by a monophase circahoralian rhythm of CMI with an acrophase at 8.40 and period of 80 min. The length of active phase in this cycle was 2-fold shorter than of the passive phase, while the values of absolute and relative amplitudes were almost the same. Only one-fifth of cells capable of division entered mitosis during this cycle. About one-half of dividing cells were in the active phase of the rhythm. Circahoralian changes in CMI during cycle 3 were characterized by monophase rhythm with acrophase at 10.00 and period of 120 min. The lengths of active and passive phases were the same, the absolute amplitude being 1.7 times greater than the relative one. The number of dividing cells in this cycle was about one-fifth of all cells capable of mitosis. About $^3/_5$ mitoses were observed during the active phase of the rhythm.

Mitotic index was evaluated for each of 25 cell positions in the cryptic wall (MI_n). Spatial changes in cryptic cells were characterized by a pulsatile pattern during all cycles of circahoralian rhythm with the maximum values of MI_n mesor after every 5 cells (nuclear positions 5, 10, 15, and 20; Fig. 1). For all cycles of the rhythm the highest MI_n value was observed in cell position 10, which was 1.3, 1.3, and 1.5 times higher than in position 5 during

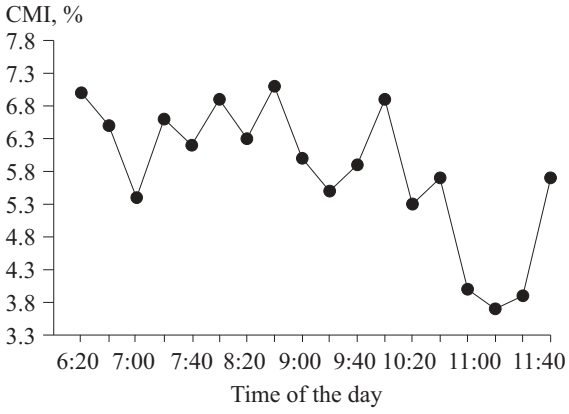

Fig. 2. Circahoralian changes in the mean MIsubp, $MIsubp_{bas}$, and $MIsubp_{max}$ in the small intestinal cryptic epithelium of intact mice during 340 min of one day.

cycles 1, 2, and 3, respectively. The closer to the neck of the crypt, the lesser became periodical elevations of MI_n. MI_n in cell positions 15 and 20 was 1.4 and 2 times, 1.5 and 2.3 times, and 1.8 and 2.7 times lower than in position 10 during cycles 1, 2, and 3, respectively.

This pattern of spatial distribution of mitoses along the cryptic wall enabled us to distinguish discrete units of mitotic activity changes in the small intestinal cryptic epithelium: 5 cell subpopulations, each consisting of 5 cells. All subpopulations included cells with high and low (basal) mitotic activity, for which $MIsubp_{max}$ and $MIsubp_{bas}$, respectively, were determined (Fig. 1). During the studied period of the day circahoralian changes were characteristic of not only mean MIsubp, but also $MIsubp_{bas}$ and $MIsubp_{max}$, which also formed three cycles of the same duration as the fluctuations in the mean MIsubp during this time (Fig. 2). The following conclusions can be made on the basis of the analysis of circahoralian rhythm parameters: 1) acrophases of these rhythms during the same cycle do not coincide in time and can differ from the mean MIsubp rhythm; 2) if the absolute amplitude

TABLE 2. Parameters of MIsubp Circahoralian Rhythm in Various Subpopulations of Small Intestinal Cryptic Epithelium of Intact Mice during Cycles 1, 2, and 3 of Its Changes

Parameter		Subpopulation				
		1	2	3	4	5
Cycle 1	Acrph, hours	8:20	8:00, 8:40	6:40, 8:40	6:20	6:40, 7:20, 8:00
	AA, %	2.4	2.6	2.5	3.7	1.5
	RA	1.4	1.4	1.5	1.8	1.5
	Mesor, %	7.7±0.2	8.3±0.2	7.0±0.2	5.5±0.2	4.1±0.2
	LAP, min	45	47	106	30	64
	LPP, min	95	93	34	110	76
	Rhythm period, min	140	140	140	140	140
	Pm, %	52.0±0.3	57.6±0.3	34.4±0.3	27.1±0.3	19.0±0.2
	AP Pm, %	14.0±0.2	18.6±0.2	26.5±0.3	8.9±0.2	11.9±0.2
	AP Pm/Pm, %	27	31	77	33	64
Cycle 2	Acrph, hours	9:40	10:00	No rhytm	8:40	No rhytm
	AA, %	2.9	3.8	—	2.5	—
	RA	1.6	1.6	—	1.7	—
	Mesor, %	7.1±0.2	8.0±0.2	7.6±0.2	4.9±0.1	3.8±0.1
	LAP, min	50	27	—	23	—
	LPP, min	30	53	—	57	—
	Rhythm period, min	80	80	—	80	—
	Pm, %	23.7±0.3	24.7±0.3	22.7±0.3	14.4±0.2	13.7±0.2
	AP Pm, %	17.5±0.2	12.1±0.2	—	7.4±0.1	—
	AP Pm/Pm, %	74	49	—	51	—
Cycle 3	Acrph, hours	12:00	10:00	10:00, 10:40	10:00	10:20, 11:20
	AA, %	2.2	5.8	3.6	4.2	3.3
	RA	1.7	2.3	2.0	3.3	5.8
	Mesor, %	5.6±0.1	7.4±0.1	5.6±0.1	3.7±0.1	2.7±0.1
	LAP, min	45	55	52	60	60
	LPP, min	75	65	68	60	60
	Rhythm period, min	120	120	120	120	120
	Pm, %	23.9±0.3	31.5±0.3	23.7±0.3	15.9±0.2	11.7±0.2
	AP Pm, %	12.6±0.2	19.9±0.2	15.4±0.2	10.8±0.2	8.5±0.1
	AP Pm/Pm, %	53	63	65	68	73

Note. Here and in Table 3: "—": parameters were not calculated.

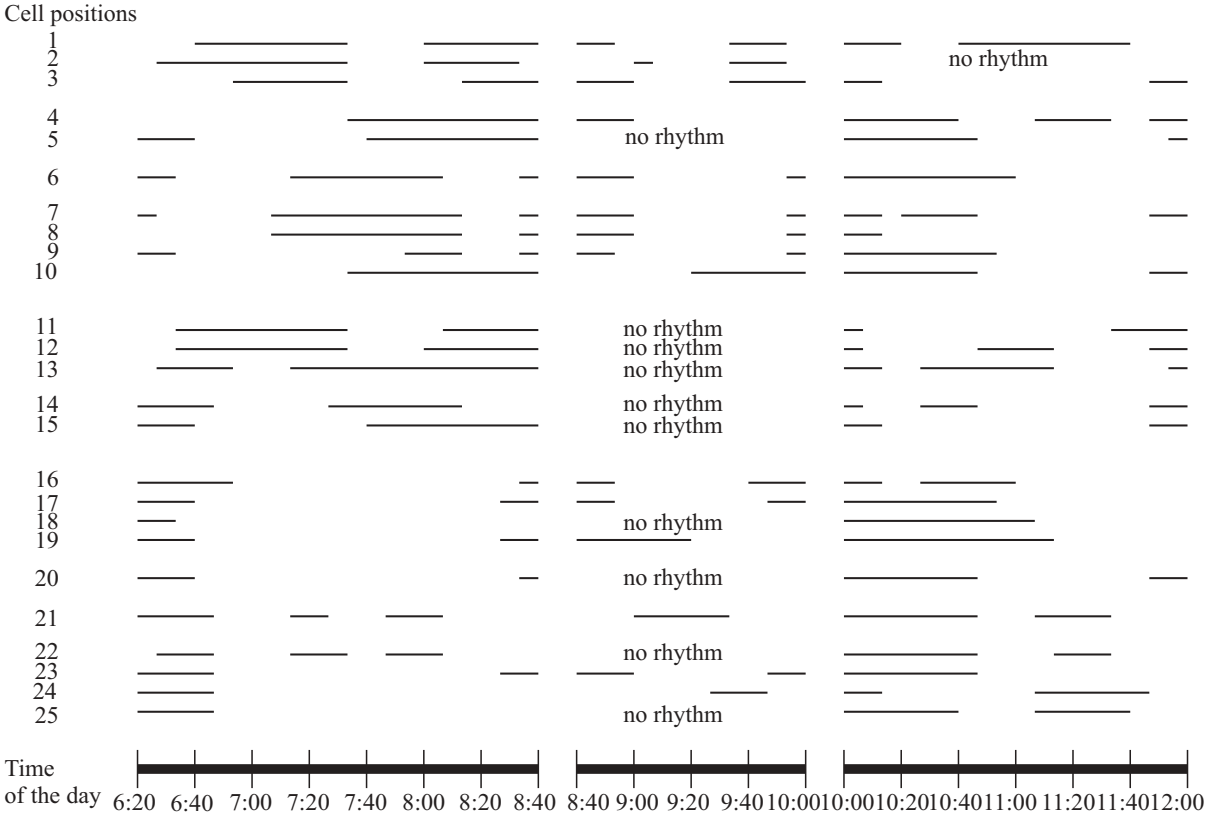

Fig. 3. Phasograms of MI_n circahoralian rhythms in the small intestinal cryptic epithelium for different cycles of mitotic activity changes. Lines represent active phases of rhythms.

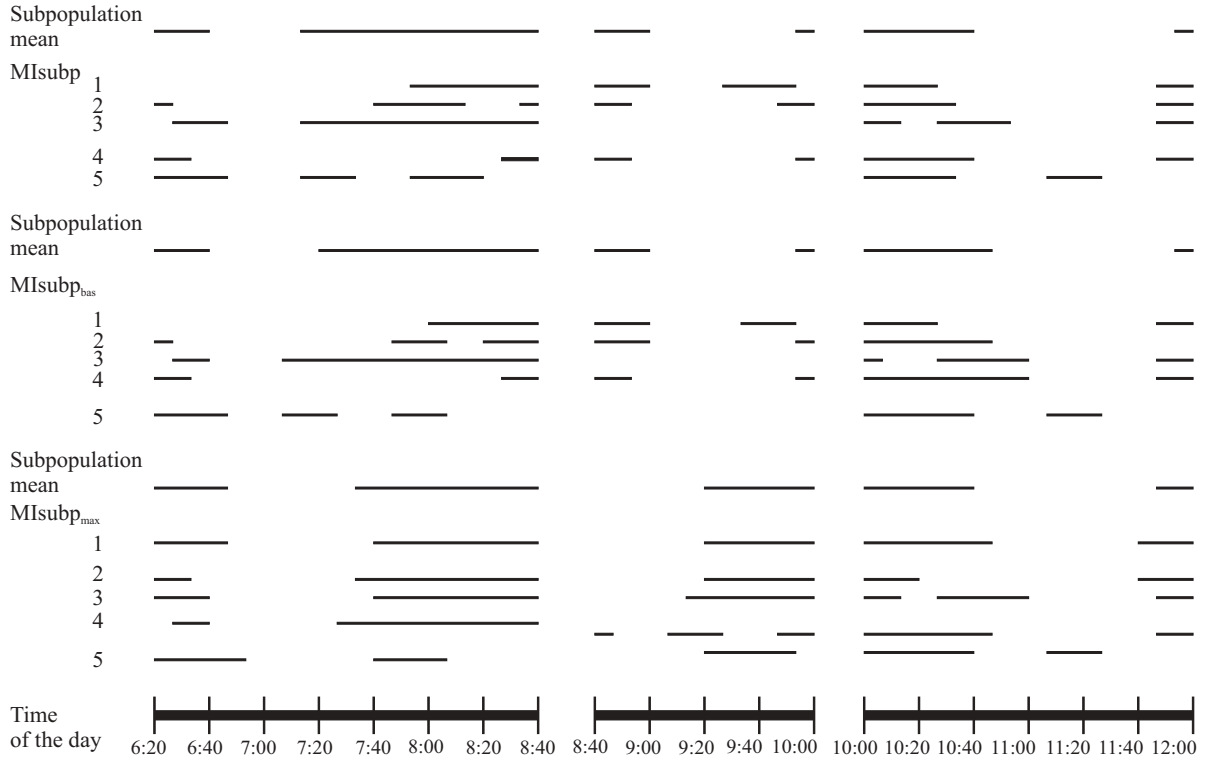

Fig. 4. Phasograms of MIsubp, $MIsubp_{bas}$, and $MIsubp_{max}$ circahoralian rhythms in the small intestinal cryptic epithelium of intact mice for different cycles of mitotic activity changes.

value coincides with the relative amplitude or is higher (1.4-1.5 times) during cycles 1 and 2 of $MIsubp_{bas}$ rhythm, during cycles 2 and 3 of $MIsubp_{max}$ rhythm the absolute amplitude is significantly (2.7 times) greater than the relative; the lengths of these rhythms' active phases differ in different cycles, but the ratio of active to the passive phase length is different for $MIsubp_{bas}$ and $MIsubp_{max}$ rhythms. If the active phase during cycle 1 is longer than passive phase for rhythms 1 and 2 (2.0 and 1.8 times, respectively), then during cycle 2 the active phase is 1.7 times shorter than passive phase for rhythm 1, while for rhythm 2 their lengths are almost the same; during cycle 3 these parameters are almost the same for both rhythms; 3) $MIsubp_{max}$ and $MIsubp_{bas}$ rhythms differ by the number of dividing cells during the active phase of the rhythm. If the greater part of these cells in $MIsubp_{bas}$ rhythm is observed during cycle 1, for $MIsubp_{max}$ rhythm this parameter is almost the same for all three cycles; 4) similarly as in the circahoralian rhythm of the mean MIsubp, the mesor and circahoralian pool of mitoses in $MIsubp_{bas}$ and $MIsubp_{max}$ rhythms differed for different cycles. They were the highest in cycle 1 and the lowest in cycle 2; 5) $MIsubp_{max}$ circahoralian rhythm mesor was 1.8-1.9 times higher than $MIsubp_{bas}$ rhythm mesor.

TABLE 3. Parameters of $MIsubp_{bas}$ Circahoralian Rhythm for Various Subpopulations of Small Intestinal Cryptic Epithelium of Intact Mice in Cycles 1, 2, and 3 of Its Changes

Parameter		Subpopulation				
		1	2	3	4	5
Cycle 1	Acrph, hours	8:20	8:00, 8:40	6:40, 8:40	6:20	6:40, 7:20, 8:40
	AA, %	2.8	2.9	3.0	3.9	1.6
	RA	1.5	1.6	1.7	2.0	1.4
	Mesor, %	6.7±0.1	6.6±0.1	6.1±0.1	5.1±0.1	4.1±0.1
	LAP, min	40	40	104	25	62
	LPP, min	100	100	36	115	78
	Rhythm period, min	140	140	140	140	140
	Pm, %	33.4±0.3	32.6±0.3	30.6±0.3	24.8±0.2	20.2±0.2
	AP Pm, %	12.5±0.2	10.1±0.2	23.8±0.2	8.2±0.1	11.9±0.1
	AP Pm/Pm, %	37	31	78	33	59
Cycle 2	Acrph, hours	9:40	10:00	No rhytm	8:40	No rhytm
	AA, %	3.5	5.0	—	2.5	—
	OA	1.6	2.4	—	1.8	—
	Mesor, %	6.0±0.1	6.1±0.1	7.1±0.1	4.4±0.1	3.7±0.1
	LAP, min	48	30	—	23	—
	LPP, min	32	50	—	57	—
	Rhythm period, min	80	80	—	80	—
	Pm, %	17.7±0.2	17.5±0.2	7.1±0.1	12.8±0.2	3.7±0.1
	AP Pm, %	12.9±0.2	11.8±0.2	—	6.8±0.1	—
	AP Pm/Pm, %	74	67	—	53	—
Cycle 3	Acrph, hours	12:00	10:00	10:00, 10:40	10:00	10:20, 11:20
	AA, %	2.2	5.8	4.7	4.4	3.4
	OA	1.3	7.0	2.2	5.0	6.8
	Mesor, %	4.6±0.1	5.7±0.1	5.0±0.1	3.2±0.1	2.7±0.1
	LAP, min	38	47	52	66	73
	LPP, min	82	73	68	54	67
	Rhythm period, min	120	120	120	120	120
	Pm, %	19.6±0.2	22.9±0.2	21.1±0.2	12.5±0.1	12.1±0.1
	AP Pm, %	8.5±0.1	12.1±0.1	13.8±0.1	8.6±0.1	9.6±0.1
	AP Pm/Pm, %	43	53	66	69	80

Hence, many parameters of $MIsubp_{bas}$ and $MIsubp_{max}$ circahoralian rhythms differ from each other and from the parameters of the mean MIsubp in different cycles of their changes. However, the intensity of small intestinal cryptic epithelium proliferation was higher during active (*vs.* passive) phases of $MIsubp_{bas}$ and $MIsubp_{max}$ for all cycles of their changes.

Phase structure was different for different cell positions and for different cycles (Fig. 3). In cycle 1 twelve of 25 rhythms in cell positions were monophase, 11 biphase, and 2 rhythms were three-phase. In cycle 2 there were 13 monophase rhythms, 2 biphase rhythms, and rather many cell positions [10] without MI_n rhythms. In cycle 3 there were 12 monophase rhythms, 12 biphase rhythms, and no rhythm in one cell position. Phase structure of MI_n cicahoralian rhythm in different cycles was in the majority of cases different for the same cell positions of the small intestinal cryptic epithelium.

Many parameters of MIsubp, $MIsubp_{bas}$, and $MIsubp_{max}$ circahoralian rhythms differ significantly from each other in different subpopulations of the small intestinal cryptic epithelium and in different cycles of these rhythms (Tables 2-4). This indicates variability of the time organization in spatial gra-

TABLE 4. Parameters of $MIsubp_{max}$ Circahoralian Rhythm for Various Subpopulations of Small Intestinal Cryptic Epithelium of Intact Mice in Cycles 1, 2, and 3 of Its Changes

Parameter		Subpopulation				
		1	2	3	4	5
Cycle 1	Acrph, hours	6:20	8:00	6:20	6:20	6:40, 6:40, 8:00
	AA, %	4.8	8.4	5.0	4.6	1.6
	RA	1.5	1.8	1.7	1.9	1.5
	Mesor, %	12.0±0.1	15.0±0.2	10.5±0.1	7.4±0.1	4.2±0.1
	LAP, min	85	77	71	88	46
	LPP, min	55	63	69	52	94
	Rhythm period, min	140	140	140	140	140
	Pm, %	59.6±0.3	74.7±0.4	52.2±0.3	36.5±0.3	21.0±0.2
	AP Pm, %	41.8±0.3	30.0±0.3	32.0±0.3	26.2±0.2	9.7±0.1
	AP Pm/Pm, %	70	60	61	72	46
Cycle 2	Acrph, hours	9:40	9:40	10:00	9:20, 9:40, 10:00	9:40
	AA, %	2.0	5.2	2.0	1.9	2.2
	RA	1.2	1.4	1.2	1.3	1.6
	Mesor, %	11.9±0.1	15.6±0.2	9.9±0.1	7.1±0.1	4.0±0.1
	LAP, min	45	42	48	36	33
	LPP, min	35	38	52	44	47
	Rhythm period, min	80	80	80	80	80
	Pm, %	35.6±0.3	43.4±0.3	33.0±0.3	21.2±0.2	12.1±0.2
	AP Pm, %	24.5±0.2	24.9±0.2	23.9±0.2	10.3±0.1	6.1±0.1
	AP Pm/Pm, %	70	56	72	49	50
Cycle 3	Acrph, hours	12:00	12:00	12:00	12:00	10:20, 11:20
	AA, %	6.3	7.4	6.7	4.9	3.4
	RA	2.0	1.7	2.2	2.3	3.5
	Mesor, %	9.8±0.1	14.3±0.2	8.1±0.1	5.7±0.1	2.4±0.1
	LAP, min	55	37	26	57	59
	LPP, min	85	83	94	63	61
	Rhythm period, min	120	120	120	120	120
	Pm, %	41.5±0.3	61.2±0.4	34.2±0.3	23.7±0.2	10.5±0.1
	AP Pm, %	23.7±0.2	21.9±0.2	13.6±0.1	15.5±0.2	7.7±0.1
	AP Pm/Pm, %	57	36	40	65	73

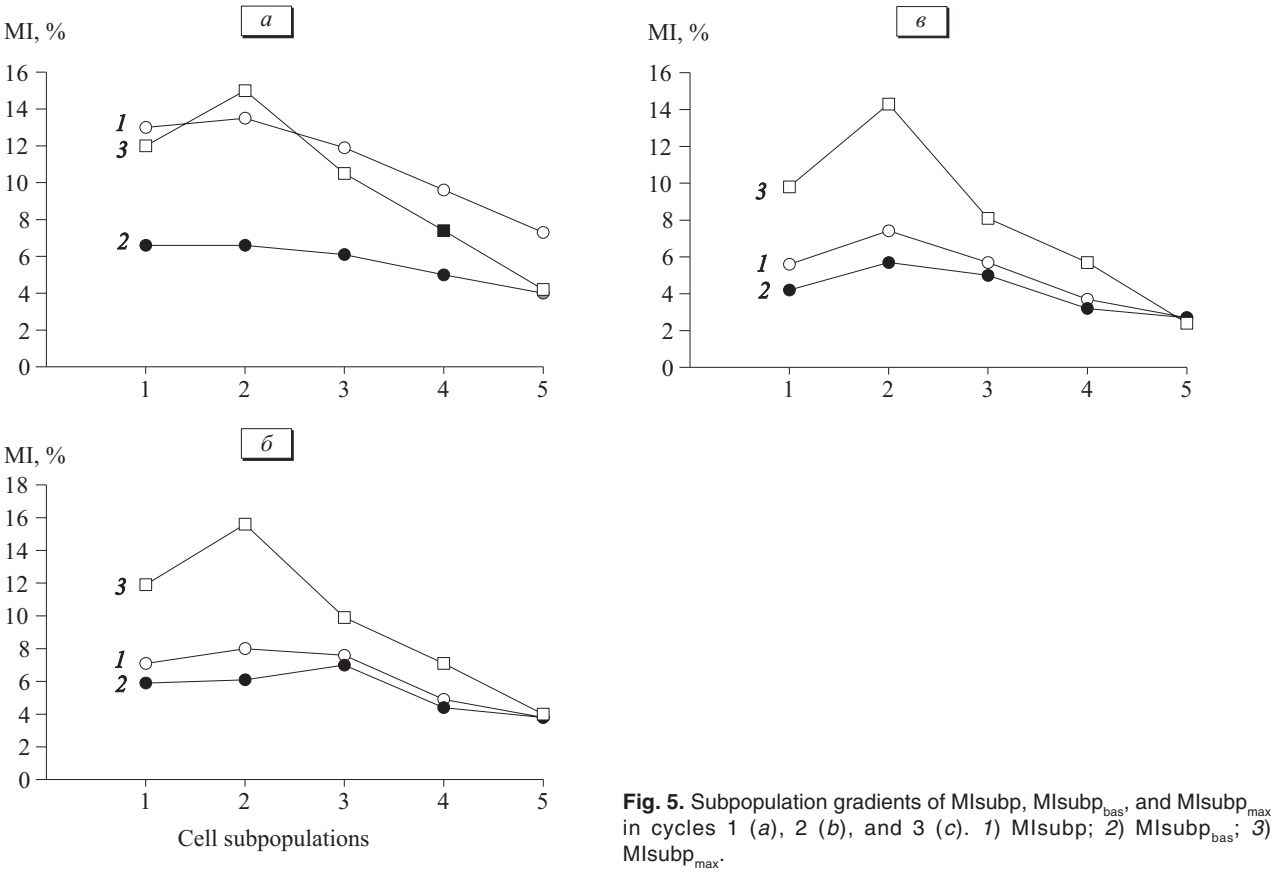

Fig. 5. Subpopulation gradients of MIsubp, $MIsubp_{bas}$, and $MIsubp_{max}$ in cycles 1 (*a*), 2 (*b*), and 3 (*c*). *1*) MIsubp; *2*) $MIsubp_{bas}$; *3*) $MIsubp_{max}$.

dient of proliferation level in the small intestinal cryptic epithelium and in the course of its cyclic changes in the studied period of the day. Time and spatial changes in the ratio of the active and passive phase lengths and percentage of dividing cells in the active phase of circahoralian rhythm are particularly important for the general PS of small intestinal cryptic epithelium. The latter value drops below 50% in some cases, but generally it is higher, reaching 70-80%, this indicating an important role of the active phase of the rhythm for maintenance of the common level of cell proliferation.

Phase structure of the rhythm of the mean MIsubp in all cycles of circahoralian MI rhythms was largely determined by the phase structure of $MIsubp_{bas}$ rhythm in subpopulations (Fig. 4). Phase structure of $MIsubp_{max}$ rhythm in subpopulations during cycles 1 and 2 differed significantly from that of $MIsubp_{bas}$ rhythms in the subpopulations and of the mean MIsubp. Hence, though multiplication of the small intestinal cryptic epithelium is an integral PS, circahoralian rhythms of proliferation in its individual parts (subpopulations, parts of subpopulations with basal and maximum levels of proliferation, cell positions) differ from the rhythms in the entire PS. We conclude that the characteristics of the small intestinal cryptic epithelial PS do not represent the sum of characteristics of its fragments.

The subpopulation changes in MIsubp, $MIsubp_{bas}$, and $MIsubp_{max}$ during different cycles of their circahoralian changes, presented in this report, are almost identical (Fig. 5). The highest MIsubp values in cycles 1, 2, and 3 were observed in subpopulation 2, in which they were 8, 13, and 32% higher, respectively, than the index in subpopulation 1. $MIsubp_{bas}$ was virtually the same in subpopulations 1 and 2. $MIsubp_{max}$ in cycles 1, 2, and 3 was higher in subpopulation 2 in comparison with subpopula-

TABLE 5. Mean Coefficient of Correlations between Circahoralian Rhythmic Fluctuations of MIsubp in Small Intestinal Cryptic Epithelial Subpopulations of Different Location in Different Cycles of Its Changes

Subpopulation	Coefficient of correlation		
	cycle 1	cycle 2	cycle 3
Neighboring subpopulations	0.68	0.87	0.73
Every other subpopulation	0.79	0.67	0.47
Every 2th subpopulation	0.61	0.75	0.27
Every 3th subpopulation	0.08	0.51	0.37
Mean	0.54	0.70	0.46

TABLE 6. Mean Coefficient of Correlations between Gradient Changes in MIsubp after Different Periods of Time during Different Cycles of Its Circahoralian Changes

Periods, min	Coefficient of correlation		
	cycle 1	cycle 2	cycle 3
20	0.61	0.66	0.50
40	0.66	0.65	0.53
60	0.55	0.63	0.57
80	0.71	0.78	0.61
100	0.71	—	0.57
120	0.75	—	0.55
140	0.55	—	—
Mean	0.65	0.68	0.56

Note. "—": no correlation.

tion 1 by 25, 31, and 46%, respectively. In subpopulations 3-5 MI decreased significantly in comparison with subpopulation 2, its values in subpopulation 5 *vs.* subpopulation 2 being: MIsubp 49, 48, and 37%, $MIsubp_{bas}$ 62, 61, and 47%, and $MIsubp_{max}$ 28, 26, and 19% in cycles 1, 2, and 3, respectively. Hence, the level of mitotic activity first increases in the direction from cryptic bottom to the neck, and then drops. In general, the course of spatial changes in cell division in the small intestinal cryptic epithelial subpopulations is determined by $MIsubp_{bas}$ changes along the cryptic axis. The degree of increase and subsequent drop in the number of dividing cells are more pronounced for the spatial changes in $MIsubp_{max}$.

Analysis of correlations (Table 5) showed that *r* coefficient of correlation between time changes in subpopulations situated at different distance from each other decreased with longer distance for all cycles of MIsubp circahoralian fluctuations. It was the most pronounced for cycle 1 (88% decrease of *r*) and the least for cycle 2 (41% decrease of *r*). These data indicate a significant relationship between the pattern of circahoralian changes in MIsubp and subpopulation location in the small intestinal cryptic epithelium, that is, on its spatial changes.

The value of coefficient of correlation between MIsubp gradient changes during all cycles did not depend on the periods of time at which MIsubp was determined (Table 6). Hence, the pattern of spatial changes in MIsubp is similar in different points of the study over the course of the cycle of its circahoralian changes, which confirms the assumption on higher stability of spatial organization of STO in comparison with time organization [4,5].

Hence, STO in the circahoralian rhythm of mitotic activity is intrinsic of the small intestinal cryptic epithelial PS; basically it is similar to that in the circadian rhythm of proliferation in mouse small intestinal cryptic epithelium [6].

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