EXPERIMENTAL BIOLOGY

Effect of *Salmonella Typhi* Infection on the Mitotic Activity of Esophageal Epithelium in Mice Infected at Different Times of the Day

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After infection in the morning, the mitotic index at first rises and then drops, followed by normalization by the end of the experiment. Infection in the evening is at first attended by a rise of the mitotic index followed by its fall, after which the mitotic index again increases and then normalizes, by the end of the experiment being lower than in the control.

Key Words: *mitotic activity; typhoid fever; biological rhythms*

Intestinal infections, specifically typhoid fever, are still of clinical interest [1,2]. However, experimental and clinical chronopathology of typhoid fever has been virtually unstudied; in particular, little is known about the effect of the infectious process on the proliferation system of the tropic and nontropic (with respect to infection) organs. It has been experimentally found that bombesin-stimulated synthesis of DNA is reduced in a stationary Swiss 3T3 cell culture exposed to *Pertussis* toxin [14].

The present study was aimed at performing a chronobiological investigation of the effect of *Salmonella typhi* infection on mitotic activity in the nontropic organ.

MATERIALS AND METHODS

Stratified squamous epithelium of the esophagus served as the object of investigation. A clearly pronounced diurnal rhythm of proliferation is characteristic of this fast-regenerating tissue [3,7,8, 10-12].

One hundred fifty male albino nonpedigree mice weighing 15-18 g were involved in the experiments. The animals were maintained under a 12:12 light-dark cycle (light from 8:00 to 20:00 h). Food and water were given ad libitum. The experiments comprised three series. The animals of series I served as the control (a morning and an evening group). The animals of series II were infected with a *Salmonella typhi* culture (strain 4446) at 10:00 h, and the animals of series III at 22:00 h on the same day. The mice were intraperitoneally injected with an infectious dose of 1×10^6 CFU/ml of low-nutrient agar. The mice of series I were sacrificed every 4 h over 40 h, from 10:00 h on day 1 to 2:00 on day 2. The mice of series II were sacrificed every 4 h over 24 h, from 14:00 h of day 1 to 14:00 h of day 2, and the mice of series III every 4 h over 24 h, from 2:00 h on day 1 to 2:00 h on day 2. Each experimental point comprised 6 animals. The esophagus was

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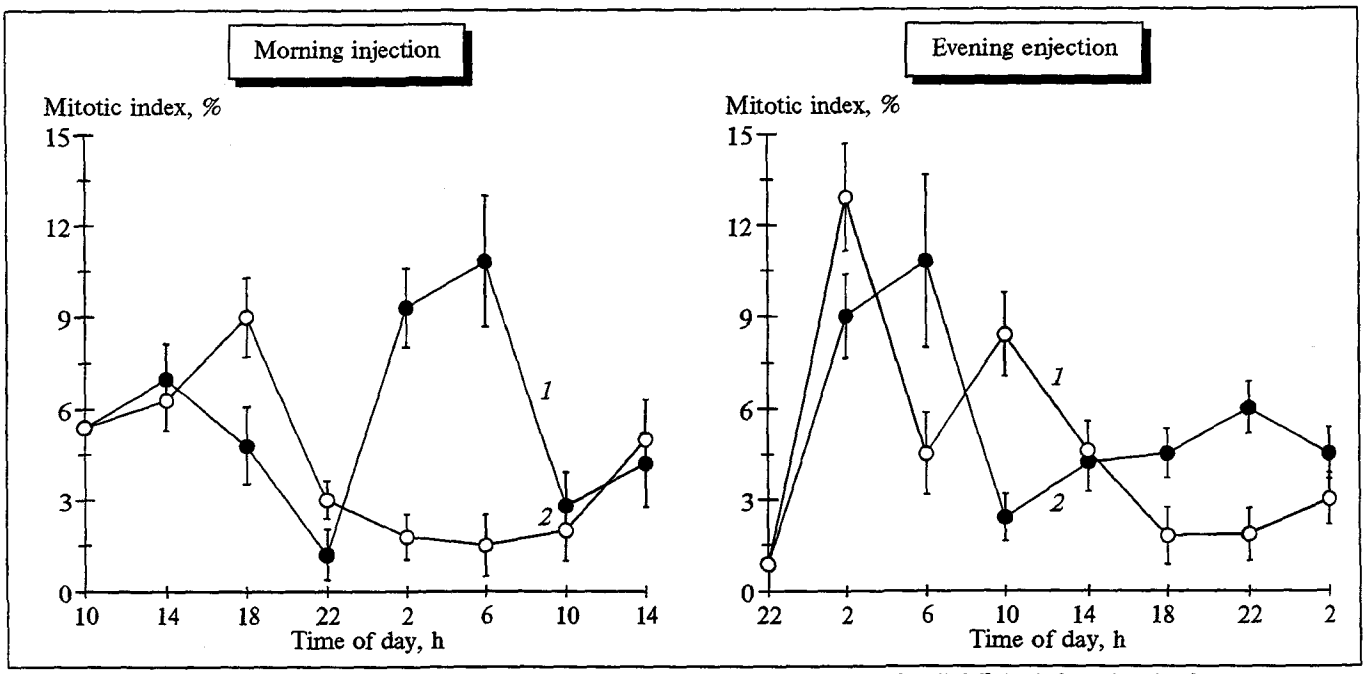

Fig. 1. Variation of MI of esophageal epithelium in mice. *1*) MI in control animals; *2*) MI in infected animals.

excised for examination. After standard histological procedure, sections 5 μ thick were prepared and stained with hematoxylin after Meyer. In sections derived from each animal the mitotic index (MI) was calculated as the fraction of dividing cells among the examined 10,000 cells of the basal layer of epithelium and expressed in pro mille. The results were statistically processed using the Fisher-Student test; the differences were regarded as reliable at $p<0.05$. The plotted curves of changes of MI over 24 h were used in graphic-parametric analysis [9] of the diurnal biological rhythms of cell proliferation. The curves were analyzed by taking segments 24 h long (between 10:00 on day 1 and 10:00 on day 2 for infection in the morning and between 22:00 h on day 1 and 22:00 h on day 2 for infection in the evening). The values of MI at 10:00 and 22:00 h on day 1 were taken as the MI values for the same hours on day 2.

RESULTS

As is seen from Fig. 1, *a*, a monophasic diurnal rhythm of MI with the maximum of dividing cells at 6:00 h and the minimum at 22:00 h ($p<0.001$) is observed in the esophageal epithelium of the control mice of the morning group. The same pattern of changes is characteristic of rhythmic fluctuations of MI in the esophageal epithelium of the control animals in the evening group (Fig. 1, *b*), in which the maximum MI is also observed at 6:00 h and the minimum at 10-18 h ($p<0.002$). The parameters of the diurnal rhythm of MI of epithelial cells of the esophagus of the control animals from both groups are presented in Table 1. As is seen from the table, the majority of parameters differ little between the two groups. Meanwhile, in the animals of the evening group their values (the mesor, the duration of the active phase of the rhythm (AP), the pool of dividing cells (P_m), the pool of dividing cells over the AP (P_{map}), and the P_{map}/P_m ratio) are reduced (by 17%, 43%, 17%, 35%, and 21%, respectively) as compared to the animals of the morning group. The reduced values of P_m (possibly, of the mesor), P_{map}, and P_{map}/P_m, along with the unchanged absolute amplitude (AA), relative amplitude (RA), and synchronization coefficient (SC), in the animals of the evening group can be attributed to a shorter AP of the diurnal rhythm of MI. It should be mentioned that the temporal correlation between the dark-light cycle and the phasic structure of the diurnal rhythm of MI of the esophageal epithelial cells in the control mice of the two groups corroborates the published data [3,7,12]. An increased mitotic activity in the morning (when the dark gives way to the light) is typical of this rhythm.

When the mice were injected with *Salmonella* in the morning, after 8 h (at 18:00) this resulted in an increase of MI in the esophageal epithelium as compared to the control (by 83%, $p<0.05$). After 12 hours (at 22:00 h) MI normalized, but after 16 and 20 hours (at 2:00 and 6:00 h) it dropped sharply (82 and 85%, respectively, $p<0.001$) vs. the control. After 24 and 28 hours of the experiment, MI was unchanged from the

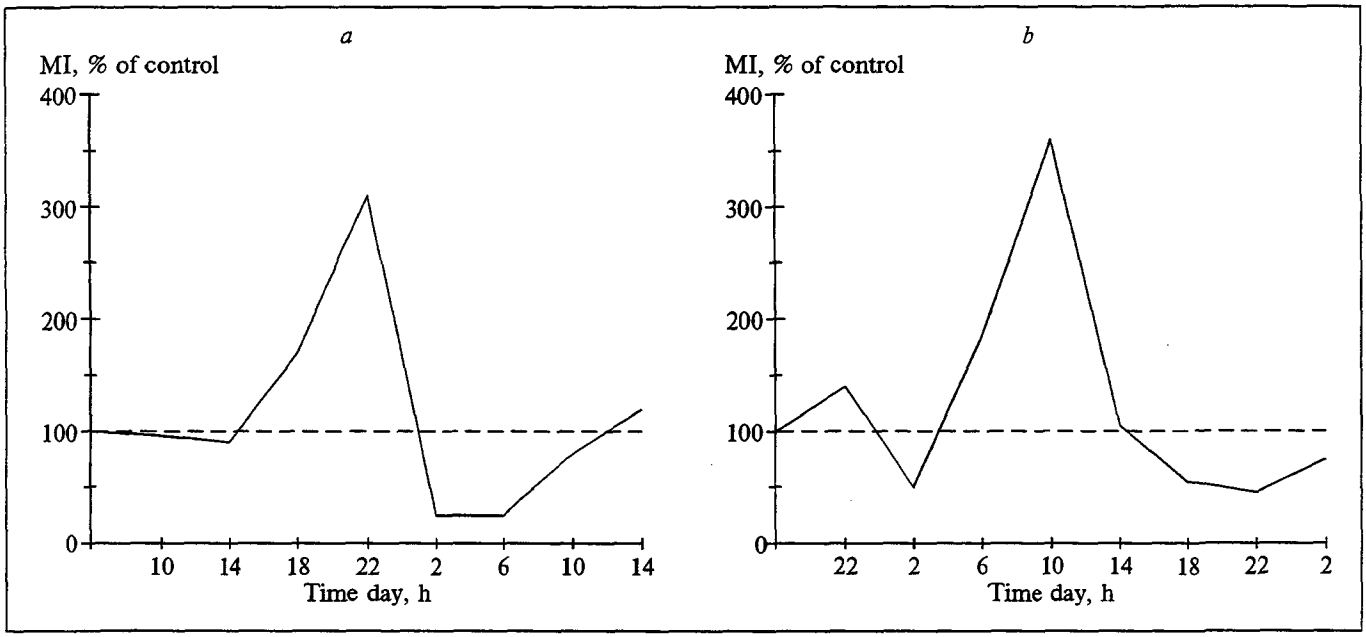

Fig. 2. Variation of MI of esophageal epithelium in mice infected in the morning (*a*) and in the evening (*b*) with *S. typhi*.

control (Figs. 1, *a* and 2, *a*). Thus, morning infection of mice with *S. typhi* caused changes in the proliferation system of the esophageal epithelium, which exhibited a phasic character (first, an increase, next, a decrease, and then normalization of MI) of their time course over the follow-up period (28 hours). It is worthy of note that the drop of MI lasted longer than its rise. This resulted in a reduced P_{mt} (the pool of dividing cells over the follow-up period) in infected animals vs. the control (by 28%).

On the whole, the nonuniform (phasic) changes of MI of the esophageal epithelium of animals infected in the morning exhibit the kind of pattern that can be regarded as a monophasic diurnal rhythm of MI (over the period from 10:00 to 6:00 h) with the maximum at 18:00 h and the minimum at 2:00-6:00 h ($p<0.001$) (Fig. 1, *a*). This assumption implies that the virtual inversion of the diurnal rhythm of MI observed in this case results from its phasic shift. The acrophase, the onset of AP, and the end of AP were shifted 12,

TABLE 1. Results of Graphic–Parametric Analysis of Diurnal Rhythms of Cell Division in the Esophageal Epithelium of the Control Mice and Mice Infected with *S. typhi*

Parameter	Morning		Evening		
	control	infection	control	infection	
Acrophase, time of day	6.00	18.00	6.00	2.00	10.00
AP, %	10.1	7.2	10.1	11.9	3.9
RA	12.2	5.5	12.2	14.2	1.8
Mesor, %	6.4	4.5	5.3		5.5
AP, time of day	1.00–15.00	9.00–21.00	24.00–8.00	23.30–5.30	7.00–13.00
Duration of AP, h	14	12	8	6	6
Midpoint of AP, time of day	8.00	15.00	4.00	2.30	10.00
SC, 1/h	1.5	0.5	1.5	3.6	0.5
$P_{m'}$ %	153.6	107.6	127.2		132.4
$P_{map'}$ %	120.2	83.6	78.2		103
$P_{map}/P_{m'}$ %	78.2	77.7	61.5		77.8
$P_{mt'}$ %	164.8	119.2	160.7		146.3

Note. P_{mt}: pool of dividing cells over the follow-up period; other notation mentioned in the text (AA: absolute amplitude, RA: relative amplitude; AP: active phase of rhythm; SC: synchronization coefficient of MI over rhythm; P_m: pool of dividing cells over one day; P_{map}: pool of dividing cells over AP). For calculations of $P_{m'}$, $P_{map'}$, and P_{mt} the duration of mitosis was taken as 1 h [8].

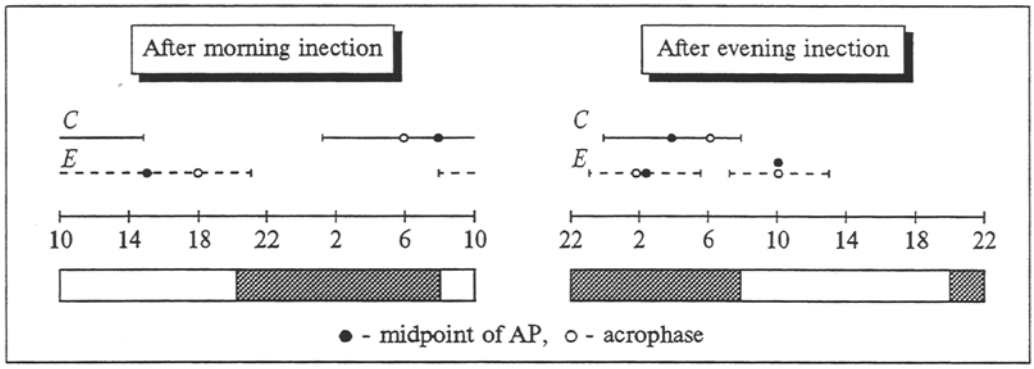

Fig. 3. Phasogram of biological rhythms of cell division in esophageal epithelium of mice in the control (*C*) and after infection (*E*).

8, and 6 hours, respectively, to the right (Table 1, Figs. 1, *a* and 3, *a*). Some changes were observed in the parameters of the diurnal rhythm of MI as compared to the control. Morning infection caused a decrease of the mesor, AA, RA, AP, SC, P_m, and P_{map} by 30, 29, 55, 14, 67, 30, and 30%, respectively (Table 1). Therefore, the mesor and the 24-h proliferative pool were markedly reduced for morning infection of mice, i.e., the overall performance of the proliferation system of the esophagus was reduced. In addition, the rhythmic changes of MI were leveled (a reduction of AA and RA), and their synchronization was weakened (a drop of SC). Minor deviations in the duration of the AP of MI in infected animals did not lead to any changes (versus the control) of the relative number of dividing cells over the AP of the rhythm. As in the control, in mice infected in the morning about 3/4 of the daily cell proliferation is accounted for by cell multiplication during the AP of the diurnal rhythm of MI.

As soon as after 4 hours (at 2:00 h), evening infection of mice with *S. typhi* resulted in a 39% increase of MI of the esophageal epithelium vs. the control ($p<0.05$) (Figs. 1, *b* and 2, *b*), followed by its sharp drop (58%, $p<0.02$) after 8 hours (at 6:00 h). Twelve hours after infection, (10:00 h) MI again rose (by 254%, $p<0.001$), 16 hours later (at 14:00 h) it normalized, and after 20-28 hours (at 18:00-2:00 h) it fell (by 54%, $p<0.025$), as compared to the control. It is worthy of note that in the case of evening infection of mice with *Salmonella*, the drop of P_{mt} below the control level was smaller (only 9%) than in the case of morning infection. The wide variability of MI in the case of evening infection resulted in a biphasic pattern of fluctuations over the day with the maximum MI at 2:00 and 10:00 h and the minimum at 22:00 and 6:00 h ($p<0.001$ and $p<0.025$, respectively). Thus, in this case the phasic pattern of the diurnal rhythm of MI in the esophageal epithelium is transformed (from a monophasic rhythm into a biphasic one) (Fig. 3, *b*). On the other hand, this is not attended by marked changes of the mesor of the rhythm or of Pm (Table 1). Moreover, P_{map} and P_{map}/P_m even exceeded the control values (by 32 and 27%, respectively). This was evidently due to a 50% prolonged overall duration of AP of the MI rhythm in infected mice. The values of AA, RA, and SC over the first, more pronounced cycle of diurnal fluctuations of MI, were even higher than the control indexes, although, on average, they were slightly lower than the control values or equal to them (Table 1).

Thus, both morning and evening infection of mice with *Salmonella typhi* causes definite changes in the proliferative system of the esophageal epithelium over the first 24 h of the experiment. The two experimental variants are similar with regard to an increased mitotic activity over the early period; however, the rate of increase is higher in the case of evening infection. In addition, in either case the MI in infected animals drops when its values in the control are high and rises when it is reduced in the control animals. On the other hand, various changes are observed in the rhythmic pattern of diurnal fluctuations of MI and their parameters, depending on the time of infection. However, it is worthy of note that after morning infection the result of rhythmic performance of the proliferation system of the esophageal epithelium in mice proves to be markedly lower than in the control, whereas after evening infection it differs little from the control. This implies that the effect of evening infection of mice with *Salmonella* is more favorable for the proliferation system of the esophageal epithelium, and the changes in this case are of an adaptive nature.

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MORPHOLOGY AND PATHOMORPHOLOGY

Proliferative Activity of Cardiomyocytes and Specific Features in the Course of Acute Experimental Myocardial Infarction in Rats with Chronic Alcohol Poisoning

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Vascular mesenchymal changes are less expressed in rats preexposed to alcoholization, and the fibrillogenesis associated with these changes occurs later and is less severe. In rats suffering myocardial infarction in the presence of chronic alcohol poisoning the proliferative activity of cardiomyocytes is reduced.

Key Words: *experimental myocardial infarction; chronic alcohol poisoning; cardiomyocytes; proliferation*

Previously we revealed a depression of manifestations of a regeneratory nature on the part of muscular elements of the heart in both humans and rats with chronic alcoholism [3]. We thought it interesting to study the course of myocardial infarction in rats with chronic alcohol poisoning.

Pathoanatomy Department, I. N. Ul'yanov Chuvash State University . (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Forty-five outbred adult male rats weighing 255-320 g were subjected to semiforced alcoholization with a 15% ethanol solution with a 0.08% tasty saccharine additive [4]. After 20 weeks of alcoholization the thorax was opened under ether mask anesthesia and after removal of the pericardial leaflet the descending branch of the left coronary artery was ligated at the level of the middle third.

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