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ANNUAL CYCLE OF CHANGES IN MITOTIC ACTIVITY OF THE GASTRIC MUCOSAL EPITHELIUM IN HIBERNATING RODENTS

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UDC 612.32.014.2:612.6]-019:591.543.42"45"

KEY WORDS: gastric mucosa; mitotic activity; hibernation.

The digestive system undergoes marked seasonal changes in hibernating animals. However, the mitotic activity of the epithelium of the gastrointestinal tract of these animals has not been adequately studied. There are reports [3, 7, 8, 10, 12, 14, 15] of absence of mitoses during hibernation or a considerable reduction in their number. Under these circumstances it is suggested that the cell cycle is blocked in the G_0-, G_1-, and G_2-periods or in the course of mitoses.

The object of this investigation was to study mitotic activity and DNA syntheses in the mucosa of different parts of the stomach of the red-cheeked suslik *Citellus erythrogenus* Brandt in different seasons of the year.

EXPERIMENTAL METHOD

Active animals (May-June), animals in a state of deep hibernation (December-January), before the spring awakening (March-April), and at different times after awakening (1-2, 3-4, 5-7, and 14 days), and also animals kept during winter in a warm room (active in winter) were investigated. Each group contained 5-6 susliks. All the active animals were deprived of food for 24 h before sacrifice, which was always carried out in the morning. Material for investigation was taken from the fundus, body, and pylorus of the stomach. Paraffin sections were stained by the PAS reaction and counterstained with hematoxylin. Mitoses were counted at the level of the terminal portions (in the fundal glands in 3000 epithelial cells, in the pyloric glands in 6000 cells) and at the level of the pits and isthmus (in 3000 epithelial cells). A third level of counting also was used for glands of the body of the stomach, namely the lower portions of the neck of the glands (in 2000 epithelial cells). Diurnal fluctuations of mitotic activity were studied in summer in susliks with free access to food. The animals were killed in groups of six every 3 h. Mitoses were counted in the gastric glands at three levels: pits and isthmus, upper portions of the neck, and terminal portions (in 3000 epithelial cells in each case). Mitoses were always counted by one investigator in numbered preparations with a magnification of 1500 ×. The results were analyzed on the BÉSM-6 computer. To investigate DNA synthesis, autoradiography with [^{3}H]thymidine was used (injected intraperitoneally in a dose of 1.0 μCi/g, in 1, 2, 4, or even 16 injections). Pieces of mucosa also were incubated in Eagle's medium with 100 μCi/ml of [^{3}H]thymidine (30 min at 37°C, and in the case of animals killed during hibernation, at their body temperature). Paraffin sections were stained by the PAS reaction and coated with type M or type PR-2* emulsion. The sections were counterstained 4 weeks later with 1% toluidine blue in borate buffer, pH 9.2.

*The author is grateful to V. L Zakharov, on the staff of the Khlopin Radium Institute, for providing the PR-2 emulsion.

Department of Physiology, Novosibirsk University. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 91-93, May, 1982. Original article submitted September 12, 1981.

 0007-4888/82/9305-0658$07.50

TABLE 1. Changes in MI in Epithelium of Gastric Mucosa of Red-Cheeked Suslik in Different Seasons

State of animals	Fundus of stomach		Body of stomach		Pylorus	
	n	MI, %	*n*	MI, %	*n*	MI, %
Active in summer	7	0,56±0,29	5	0,18±0,05	6	3,45±1,28
Deep hibernation	8	0,77±0,22	5	0,35±0,18	5	1,27±0,82
Hibernation before awakening	5	1,58±0,49	5	1,18±0,52	5	3,32±0,93
Days after awakening						
1–2	5	3,76±1,42	5	2,07±1,2	5	9,28±2,66
3–4	5	5,84±2,0	5	5,24±1,7	5	14,44±2,7
5–7	6	3,13±1,45	5	4,0±1,4	5	11,52±2,65
14	5	3,1±1,3	5	0,2±0,07	6	4,35±1,87
Active in winter	6	4,75±1,63	5	3,94±0,84	5	6,32±0,55

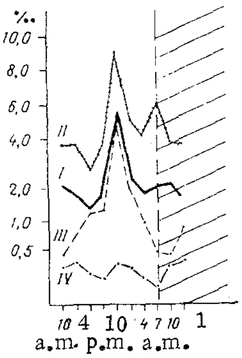

Fig. 1

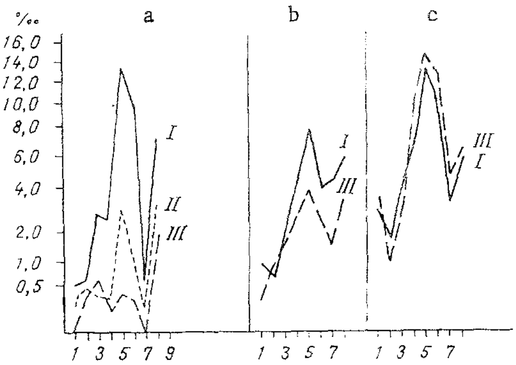

Fig. 2

Fig. 1. Diurnal changes in MI in gastric epithelium of a suslik in summer. I) All cells of gland, II) cells of pits and isthmus, III) cells of upper portions of neck, IV) cells of terminal portions. Abscissa, clock time; ordinate, MI (in %).

Fig. 2. Annual changes in MI in epithelium of body (a), fundus (b) and pylorus (c) of suslik stomach. Abscissa: 1) active in summer, 2) deep hibernation, 3) hibernation before awakening, 4) 1-2 days after awakening, 5) 3-4 days, 6) 5-7 days, 7) 14 days after awakening, 8) active in winter; ordinate, MI (in %). Curves represent: I) cells of pits and isthmus, II) cells of lower portions of neck, III) cells of terminal portions.

EXPERIMENTAL RESULTS

In all seasons of the year (Table 1) highest mitotic activity was found in the antral portion. This is evidently the usual state of affairs [5, 11]. Much lower activity was found in the fundus, lower still in the body of the stomach. Very weak mitotic activity was observed in the summer, especially in glands of the fundus and body, which disagrees with the observations of all authors cited above. Admittedly they do not indicate what they took to be the level of the active period: If it was values of the mitotic index (MI) during the first days after awakening, our differences are easily explained, for after hibernation MI was at its highest level. During the recording of mitosis the rate at which it takes place is important, and in summer its course is shortest. Diurnal changes in MI of all epithelial cells in the body of the stomach were characterized by a monomodal curve with a maximum at 10 p.m., and with a hardly perceptible rise at 10 a.m. (Fig. 1).

During hibernation (Table 1) mitoses were found in all parts of the stomach, and many of them were changed. (The chromosomes formed clumped masses, and pro- and metaphases were difficult to differentiate.) All the functional parameters accompanying the state of hibernation ought to contribute to reducing the ability of the epithelium to divide by mitosis [4]. However, contrary to expectations, no significant fall in MI was found in winter. This was evidently due to the blocking of mitosis and its preservation (often in modified form) in a state of suspended activity. The data given in Table 1 on MI during hibernation thus do not reflect the true values of mitotic activity at the time of sacrifice of the animal, but they correspond to the number of blocked mitoses remaining since the time of spontaneous awakening.

After awakening MI rose sharply to reach a maximum after 3-4 days, when it began to fall again. After 2 weeks MI in the body of the stomach was at the same level as in the summer active state, in the pylorus it was close to the summer level, but in the epithelium of the fundus it was still very high. Not only was the highest mitotic activity observed in this period but also, as our previous investigations showed [1], reactivation of the cells and renewal of their organoids take place and intensive functioning of the endocrine apparatus of the gastric mucosa is observed [2].

In susliks active in winter, kept under unusual conditions for this species, very high mitotic activity was observed. Their cells evidently functioned intensively and the large number of mitoses reflected their more rapid physiological regeneration. Changes in the diurnal mitotic rhythm possibly took place in these animals, with smoothing of the maximal values of MI or a shift of the peak toward the morning hours [6].

In the fundal glands a zone of predominant location of mitoses at the level of the pits and isthmus of the glands was clearly demonstrated (Fig. 2), but they were found throughout the length of the glands in all seasons, except in the terminal portions in summer and 2 weeks after awakening.

It is remarkable that MI in the terminal portions of the pyloric glands was rather higher than in the pits and isthmus. This supports the view of other workers [5, 13] that the glandular cells in this situation can renew themselves by true division.

Mitoses found in virtually all parts of the glands were located in young specialized cells and in undifferentiated cells. Both types are widely represented in susliks along the length of the glands, far beyond the boundaries of their classically restricted zones. The writer showed previously that the number of these cells increases in the last stages of hibernation and remains high on the first days after awakening. However, two weeks after hibernation and in the active state they are rarely seen.

An attempt to obtain additional information on cell renewal by studying DNA synthesis proved unsuccessful. Many experiments with injection of [^{3}H]thymidine *in vivo* confirmed observations [9, 10] that DNA precursors are not taken up into the tissues in susliks, or are taken up only to an extremely small degree. Very weak labeling of nuclei in individual parts of the mucosa was found in 5 of the 99 animals studied. On incubation of specimens of mucosa with labeled thymidine *in vitro*, DNA-synthesizing cells were found in all cases in the pits and isthmus of the glands. Cells were labeled even in the mucosa of sleeping animals, if incubation was carried out at 37°C. The absence of labeling of cells in the lower portions of the glands was probably due to the experimental technique used.

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