Specific Morphofunctional Features of the Parietal Cells of Hibernating Rodents in Different Physiological States

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Low pH values of the stomach contents and activation of the parietal cells are revealed during the spontaneous awakening-associated normothermia of red-cheek sousliks. These factors help maintain the acid-base balance in the organism.

Key Words: *hibernation; spontaneous awakening; parietal cells; ultrastructure; stereology*

Multiple aspects of the morphological rearrangements of cells during hibernation are still unclarified. The study of the digestive tract is apparently of prime significance in this context, since most hibernating animals abstain from all exogenous nourishment during the whole winter. Earlier, inhibition was observed in the functional activity of the epithelial cells of the gastrointestinal mucosa during hibernation, this being expressed both in depressed function and in structural reorganization of the cells [1,4,9]. The reaction of the stomach contents against the background of the low body temperature of torpid animals is of a nearly neutral pH. However, during the periods of spontaneous awakening that invariably punctuate the long winter sleep [2] the gastric pH rapidly shifts in the acid direction, reaching the level in the summer (active) period (pH 1.5-2.0).

Our goal was to study the HCl-producing parietal cells of the hibernant's gastric glands during the period of spontaneous awakening. Parietal cells obtained during the periods of summer activity and winter torpor served as controls.

MATERIALS AND METHODS

Pubertal red-cheek sousliks (*Citellus erythrogenys Brandt*) weighing 300-340 g were examined in summer (June - July), during the winter hibernation (December - January), and during periods of spontaneous awakening in winter (December - January). Each group consisted of 5 animals.

The methods of electron microscopy and stereological analysis were used. The specimens were fixed with 3% glutaraldehyde, postfixed with 1% osmium tetroxide, and embedded in Epon-Araldite. The orientation of the blocks was judged by the analysis of semithin sections. Ultrathin sections were impregnated with uranyl acetate and lead citrate. The sections were examined under a JEM-100C electron microscope.

The stereological analysis was performed using a square test grid with 1-cm spacing [3]. The final magnification was 20,000. The following parameters were evaluated: area of cells and cytoplasm, volume density of mitochondria, surface density of secretory membranes (of apical plasmalemma, secretory canals, and tubulovesicles) and of the membranes of the lateral and basal plasmalemma. The results were statistically evaluated using the Student *t* test.

RESULTS

During the summer period the parietal cells of the hibernating rodents are large (Table 1). Cells of oval and pyramidal shape predominate, arranged in a single row with other cellular elements of the gastric glands.

When different functional states are compared, the most striking differences observed are con-

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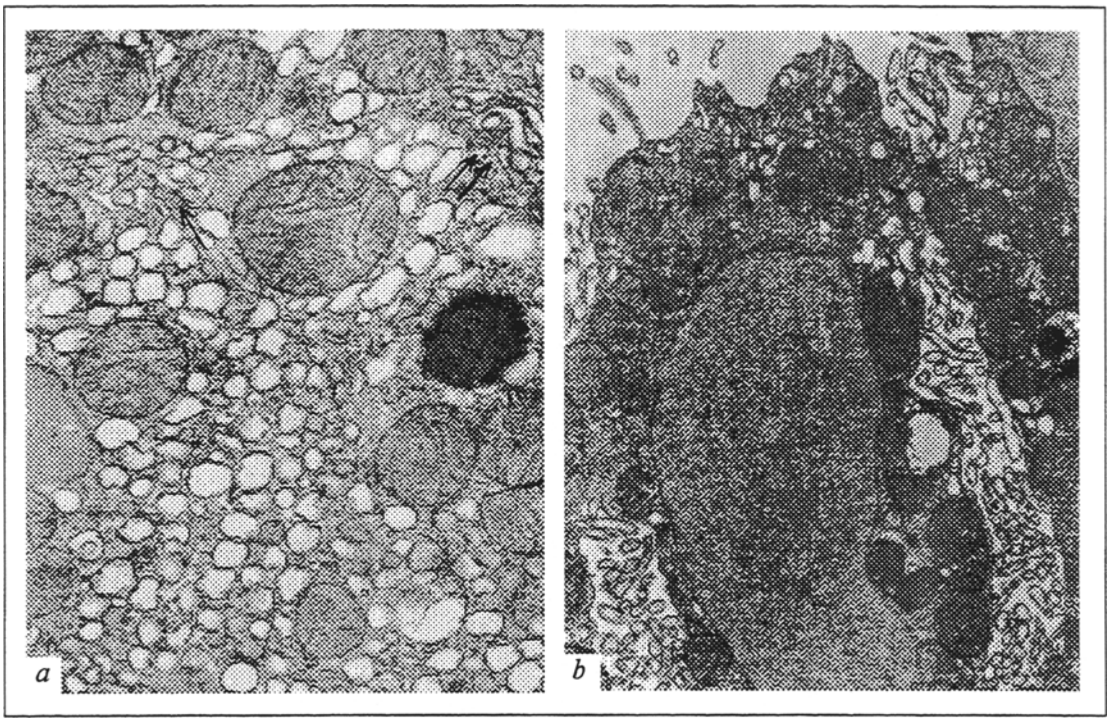

Fig. 1. Parietal cells of souslik taken in different seasons. *a*) subnuclear part of parietal cell in the summer; multiple tubulovesicles. ×17,000. One arrow: secretory canals; two arrows: Golgi apparatus; *b*) parietal cell of middle part of gastric gland in the period of hibernation; dilated secretory canals. ×12,000.

nected with the system of the secretory membranes of parietal cells. After a 24-hour fast in the summer, when the basal HCl secretion is in progress, the secretory membranes are represented mainly by multiple tubulovesicles that are absent only in the basal part of the cells. They have a smooth-membrane profile of round or oval shape, vary in size, and are filled with electron-transparent contents. In the summer period secretory canals on longitudinal sections are encountered mostly in the basal part of the cells. The canals are of irregular shape, and their lumen is filled with microvilli (Fig. 1, *a*).

In the torpid period the structure of the parietal cells is considerably altered. The area of the cells is cut by one-half, owing to the shrinking of the area of the cytoplasm (Table 1). Acid secretion is nil. The number of tubulovesicles in the cells is drastically reduced, and their secretory membranes are incorporated in the microvilli membranes of the dilated secretory canals that intrude deep into the cytoplasm. Cytoplasmic electron density is increased due to the appearance of free ribosomes. The nuclei of the majority of cells have become homogenous (Fig. 1, *b*).

During the winter normothermia (associated with spontaneous awakening) the parietal cells are polymorphic. At this time morphometry makes it possible to distinguish 3 groups of parietal cells according to the parameters of the secretory membranes: 1) cells structurally resembling those after fasting in the summer; 2) the largest population with an intermediate structure, in the cells of which the surface density of secretory canals and apical plasmalemma membranes is lower when compared to cells in torpid state; however, the surface density of the tubulovesicle membranes does not reach the level of the summer period; 3) cells that are similar to those in the torpid state (Table 1).

The cells of the 1st group are morphologically almost indistinguishable from those in fasting animals in the summer; they are large, of oval and pyramidal shape. The tubulovesicles are characterized by an even distribution within the cytoplasm and are absent only in the region adjacent to the basal membrane. The secretory canals are narrow and shallow and are filled with microvesicles (Fig. 2, *a*).

Within the 2nd (intermediate) group the cells have a nonuniform structure. A variability can be seen in the structure of the secretory canals and in the number of tubulovesicles (Fig. 2, *b*). A cell may contain canals of various shape: some represent markedly dilated cavities with a few microvilli on the surface, while others are narrow, intruding into the cytoplasm, and tightly packed with microvilli. At the same time, activation of the protein-synthesizing apparatus takes place; the cytosol contains an abundance of polysomes and short profiles of rough endoplasmic reticulum; the Golgi apparatus undergoes a significant increase in size (Fig. 2, *c*).

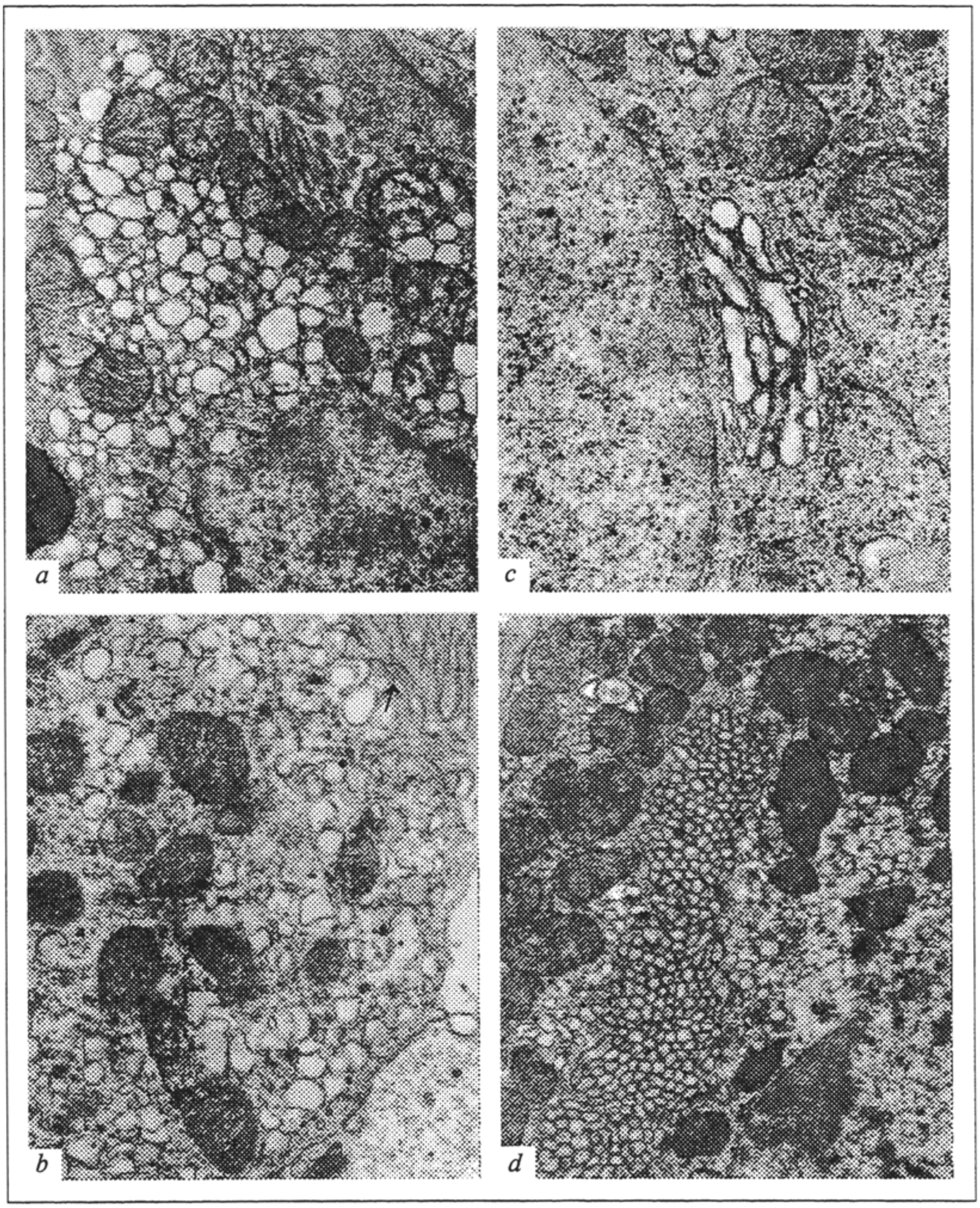

Fig. 2. Fragments of parietal cells during spontaneous awakening. Normothermia. *a*) region of parietal cell from the 1st group. ×14,500; *b*) apical region of parietal cell from the 2nd group; tubulovesicles and a secretory canal (arrow). ×13,500; *c*) cell from the 2nd group; Golgi apparatus and polysomes. ×21,000; *d*) region of parietal cell from the 3rd group. ×10,000.

The cells of the 3rd group differ morphologically from the corresponding cells in the torpid phase, despite a similarity in secretory membrane distribution and size (Fig. 2, *d*). Changes in nuclear structure are observed: the karyoplasm has a moderate electron density; heterochromatin is well-defined, forming small lumps around the karyolemma. In the cytoplasmic matrix both polysomes and free ribosomes can be found. Narrow, short cisternae of the rough endoplasmic reticulum are occasionally revealed. This last feature is characteristic for the active state of the animal.

The ultrastructural changes of parietal cells during spontaneous awakening point to enhanced functioning of the synthesizing systems in the cells and to processes of membrane structure renewal. An analogous activation of cells is observed during the springtime awakening, when the animals come out of hibernation completely [4].

Earlier [5] the participation of parietal cells in the regulation of the acid-base balance in the

TABLE 1. Morphometric Characteristics of Parietal Cells from the Middle Region of the Neck of the Stomach of the Red-Cheek Souslik in Different States

Parameter	Active summer state (n=25)	Deep hibernation (n=30)	Winter normothermia, spontaneous awakening		
			1st group (n=8)	2nd group (n=16)	3rd group (n=14)
Area of cell, m^2	155.2±2.68	77.5±2.08***	143.0±4.44*,++	117.6±4.0	79.4±2.84++
Area of cytoplasm, m^2	128.9±2.33	57.2±2.14***	115.0±4.24*,++	87.9±4.1	58.8±3.16++
Volume density of mitochondria, %	20.9±0.80	39.6±1.53***	24.5±2.21+	31.5±1.21	37.8±2.28+
Surface density of lateral and basal plasmalemma, m^2/m^3	0.53±0.02	1.28±0.05**	0.65±0.09++	1.05±0.08	1.34±0.07+
Surface density of tubulovesicle membranes, m^2/m^3	1.44±0.08	0.47±0.04**	1.11±0.15*,+	0.71±0.09	0.51±0.06+
Surface density of secretory canal and apical plasmalemma membranes, m^2/m^3	0.41±0.02	1.23±0.07**	0.56±0.68*,+	0.77±0.07	1.30±0.08++

Note. *$p<0.05$; **$p<0.01$; ***$p<0.001$ in comparison with the active summer state; $^+p<0.05$; $^{++}p<0.01$ in comparison with the 2nd winter normothermic group.

blood under the conditions of hibernation-associated metabolic acidosis [8] was discussed. It was shown that in the course of the stepwise drop of the body temperature that occurs with the entry into hibernation, the bicarbonate that is released into the blood against the background of acid secretion makes a certain contribution to the rise of the blood alkaline reserve. During hibernation the organism is especially sensitive to changes in the pH of the blood and interstitial fluid due to the inhibition of renal function [7], the kidneys being the key organs maintaining a constant pH in the organism [6]. As no digestion takes place during the whole period of hibernation, the secretion of acid in the stomach at the time of spontaneous awakening is probably directed at maintaining the acid-base balance in the blood.

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