conclusion: atropine injections (0.2-1.0 mg/kg), switching off the descending inhibitory effects, induced tachycardia [8]. Atropine injection (1 mg/kg in the pericardial cavity) in an anesthesized crucian carp increased the heart rate 2.1 times on the average and eliminated the variability of the diastolic intervals [10].

Emotional stress, as was shown in another series of our experiments, may also cause a significant reduction of vagus tone; this is demonstrated by the comparatively low increment of the heart rate in 8 alert pigeons after they were injected with atropine in a state of some agitation: from 242±14 to 340±28 beats/min (41%, $p<0.001$) as against the marked increase of the heart rate in 7 pigeons at rest (from 161±9 to 374±33 beats/min, 133%, $p<0.001$).

Thus, previous notions on vagus nerve tone and its role in heart work regulation are valid. At rest the sympathetic nervous system tone is virtually not manifested. Inadequate conditions for the detection of vagus tone have been responsible for variable outcomes of experiments carried out by different researchers. Different interpretations of the same facts have also contributed to the formation of erroneous conclusions.

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Serotonin-Producing Cells in Eu- and Hypothermia

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Hibernation is of interest to biologists in various fields, but the majority of investigations have been devoted to neurochemical aspects of this phenomenon. Studies of the structural mechanisms providing restructuring of the hibernating organism in critical periods of the year were started in the 60s. Some scientists studied the reactivity of brain structures and endocrine glands of hibernating animals [10,11,13], while others researched the morphological characteristics of a number of organs and systems [5,6,12,14].

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It has been revealed that despite a drastic drop of the body temperature (as low as 4-5°C) and of the level of metabolism (by 50-100 times), many systems and organs do not stop their functioning, but just reduce it. The gastrointestinal tract in many hibernants almost completely ceases its main function, digestion, during the long winter and functions as an endocrine organ, producing hormones and bioactive substances necessary for the body. One of these substances is serotonin, a biogenic amine (5-hydroxytryptamine, 5-HT) that is widespread in nature. It is characterized by a wide spectrum of biologic action. It is hardly possible to name another substance comparable to serotonin in the breadth of its spectrum and the complexity of its physiological effects. It is involved in the sleep mechanisms and in the formation of behavioral reactions, it is related to sexual maturation, inhibits the proliferative processes, takes part in digestion, etc. Serotonin is characterized by one more property: it suppresses thermogenesis, and therefore is conducive to the maintenance of hypothermia in hibernants. Numerous investigations of the possible role of this amine in thermoregulation have been aimed mainly at elucidating the effect of central serotonin originating from the brain cellular elements. It is known, however, that about 90% of the total content of 5-HT in the body is produced by gastrointestinal enterochromaffin cells (EC) [7]. Gastric EC cells of red-cheeked sousliks were previously studied in our laboratory [2-4].

In the present study our purpose is to reveal the structural arrangement and functioning of the duodenal serotonin-producing cells of a hibernant in summer and winter in a torpid state and during spontaneous arousals.

MATERIALS AND METHODS

The hibernating rodents red-cheeked sousliks (*Citellus erythrogenys* Brandt) were used in the study. Their hibernation is characterized by alternating periods of prolonged hypothermia lasting 12-18 days and short euthermia lasting 18-24 h. The animals were decapitated in summer (body temperature 37°C), in a state of deep torpor (5-6°C), and during spontaneous arousals (winter euthermia, 34-36°C). For electron microscopic examination duodenal fragments were fixed in 3% glutaraldehyde and then in 1% osmic acid and embedded in an araldite-epon mixture. For orientation on a block semithin sections were used, stained with 1% methylene blue in 1% borax solution. Sections of 60 nm were made with a Tesla ultramicrotome and contrasted in uranyl acetate alcohol solution and lead citrate aqueous solution and examined under a JEM-100 CX electron microscope.

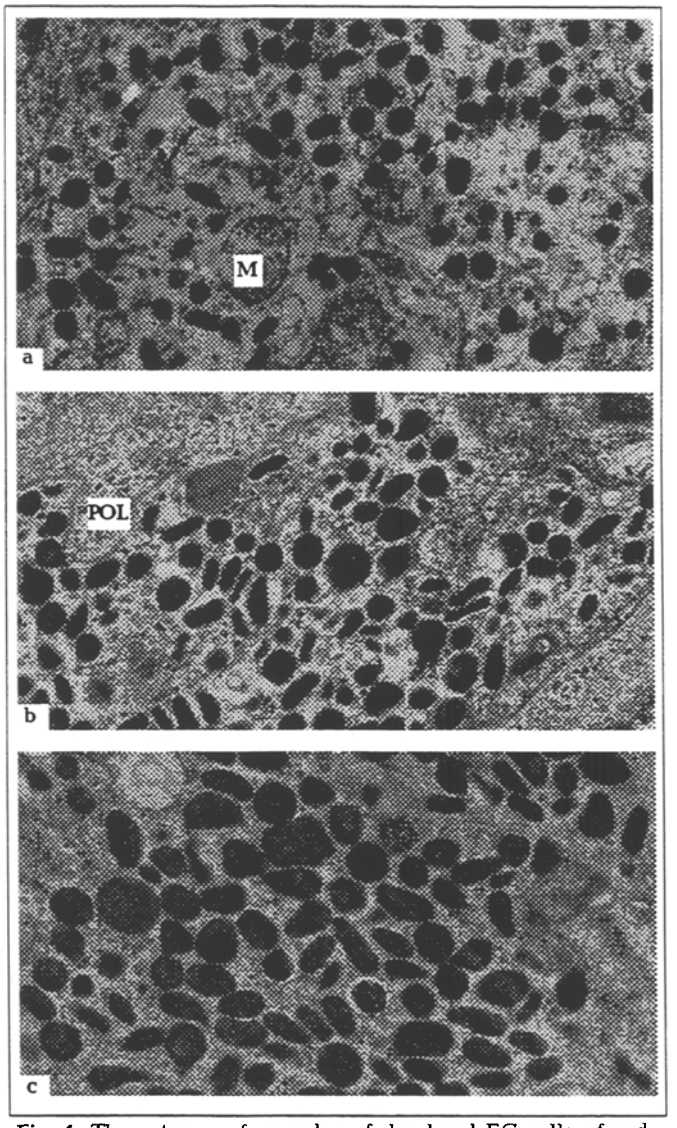

Fig. 1. Three types of granules of duodenal EC cells of red-cheeked souslik. Summer period. ×16,000. *a*) EC cell fragment with small granules (arrow). Mean granule diameter 200 nm; *b*) EC cell fragment with medium-sized granules (300 nm); *c*) EC cell fragment with large granules (350 nm).

Morphometric analysis was carried out in an open test system with a short step d=0.36 μ and final amplification 55,000. The cells were counted on paraffin sections stained with Strong Garnet (the diazonium method) and the nuclei were stained with Ehrlich hematoxylin.

RESULTS

Enterochromaffin cells are particularly numerous among duodenal enterocytes of *Citellus erythrogenys*. They are found mainly near the crypts, this agreeing with previous reports of other authors who investigated the intestine of other rodents [1,8]. These cells may be found in small numbers on the villi, and

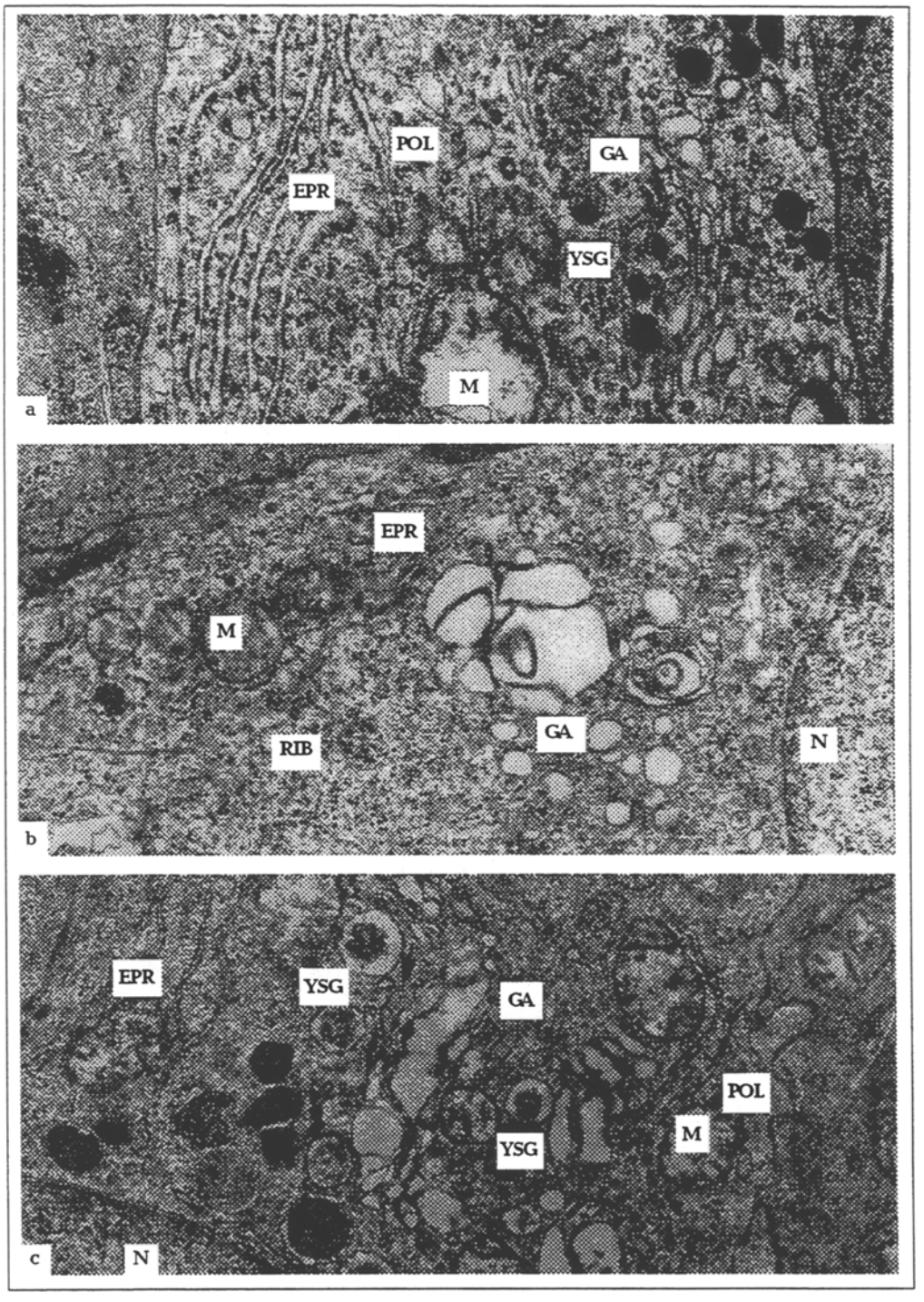

Fig. 2. Golgi apparatus of duodenal EC cells of red-cheeked souslik. *a*) summer period (×28,600); *b*) days 5–6 of torpidity period (×20,000); *c*) winter euthermia (×22,000); EPR – endoplasmic reticulum; M – mitochondria; GA – Golgi apparatus; pol – polysomes; rib – free ribosomes; N – nucleus; ysg – young secretory granules; (arrow) – granule formation.

solitary cells are present in Brunner's glands. Estimation of histochemically detectable EC cells near the crypts has shown that in summer 30.6±2.9 EC cells are detectable per 1000 epithelial cells. Serotonin-producing duodenal cells of red-cheeked sousliks are "open-type" cells, whose apical part with a few microvilli comes in contact with the intestinal lumen, their base lying on the basal membrane. In contrast to the neighboring epitheliocytes, the enterochromaffin cells have a light cytoplasm and basally located heteromorphous electron-dense secretory granules. Long cytoplasmic processes may often be seen, filled

with granules and drawn out along the basal membrane. This gives grounds for assuming that the hibernant's serotonin-producing cells are characterized not only by endocrine but by paracrine effects as well. A large nucleus is located in the basal part of the cell and contains heterochromatin at the wall and one or two large nucleoli. Polymorphous granules lie in the basal part; they are characterized by a high electron density and vary in size from 150 to 400 nm (Fig. 1). This is in line with data [9] on the heterogeneity of the EC cell population of the intestine. The synthetic apparatus of serotonin-producing cells works hard in summer. Endoplasmic reticulum is formed by long narrow profiles with ribosomes densely packed on their membranes. The Golgi complex consists of several groups of flattened and widened cisternae with contents of medium electron density. Granules are actively formed. Numerous "downy" and smooth-walled vesicles may be found near the Golgi complex. The cytoplasm contains many polysomes and free ribosomes (Fig. 2, *a*). Secretory granules pinched off from the Golgi complex are accumulated in the basal part of the cell. Dissolution of granules occasionally occurs.

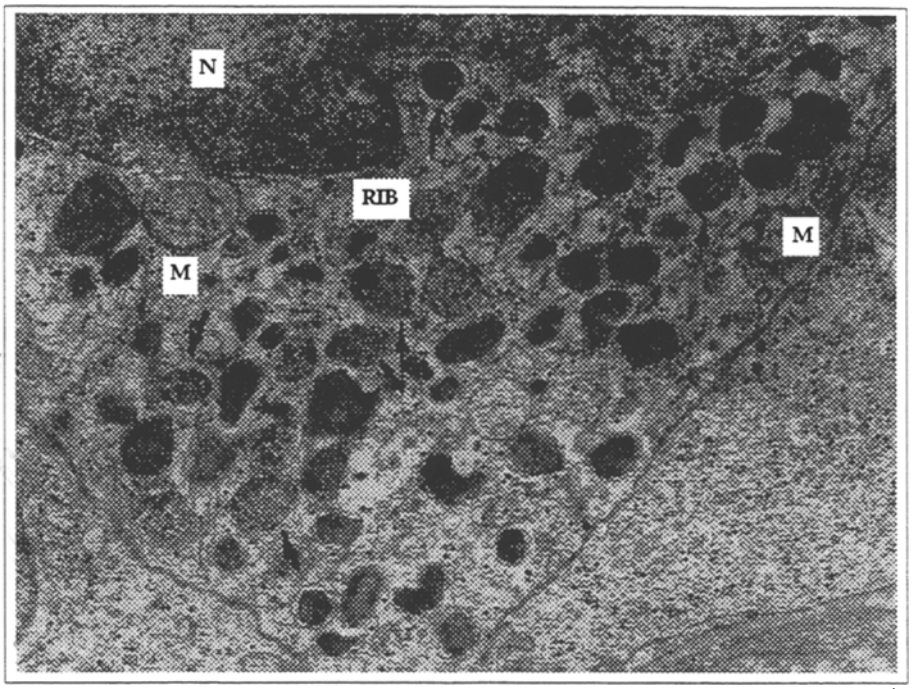

Fig. 3. Basal part of a duodenal EC cell of a red-cheeked souslik with multiple granule disintegration (arrow). Days 5-6 of torpidity period (×20,000).

During deep hibernation the count of histochemically detectable EC cells is significantly reduced, constituting 21.7±3.3 per 1000 epitheliocytes. Significant changes are found in the cells. The endoplasmic reticulum becomes fragmented. The number of attached ribosomes decreases and that of free ribosomes grows; polysomal formations seldom seen. The lamellar complex cisternae become shorter, acquiring a vacuolar structure (Fig. 2, *b*). All these changes indicate an inhibition of the synthetic processes. Numerous processes of disintegration of the serotonin-containing secretory granules run parallel with these changes (Fig. 3). The enterochromaffin cells even in a state of deep torpor seem to continue to function, releasing the granule contents. This confirms the existing concept on the important role played by peripheral serotonin in the maintenance of winter hypothermia in hibernants [7].

Studies of EC cells during spontaneous awakening of animals (winter euthermia, body temperature 34-36°C) have shown the typical summer active state with intensively working protein-synthesizing system in the majority of the cell population. The cytoplasm contains many polysomes and ribosomes. The granular endoplasmic reticulum most frequently appears as long profiles whose membranes are densely set with ribosomes. Ring-shaped structures of endoplasmic

TABLE 1. Morphometric Characteristics of the Protein-Synthesizing System of a Hibernant's EC Cells in Eu- and Hypothermia *(M±m)*

Animal status	Morphometric parameters				
	superficial density, Sv, μ^{-1}		numerical density of ribosomes, μ^{-3}		
	Golgi complex	EPR	free	polysomes	attached
Summer activity (37°C)	1.95±0.16	2.52±0.41	260±29	312±40	409±64
Torpid state (5-6°C)	1.11±0.22**	2.01±0.24	994±7**	90±15**	194±28**
Winter euthermia (34-36°C)	1.33±0.08**	1.94±0.15	262±39	583±64*	459±55

Note: One asterisk – $p<0.05$, two asterisks – $p<0.01$ (the values were compared to those during an active state).

reticulum may be found in the cells. The Golgi apparatus usually appears active in spontaneous awakening. The lamellar complex consists of 1-3 groups consisting of flattened cisternae, in places widened, with contents of medium electron density. Granule formation may be observed (Fig. 2, *c*). Mature polymorphous granules are found in the basal and basal-lateral sections. Granule disintegration occurs along with this.

The ultrastructural findings permit the hypothesis that the serotonin-producing cells of a spontaneously awakening animal are actively functioning in the majority of cases, aiming at the accumulation of the secretory material that is needed to maintain the torpid state for a long time. The data of a morphometric analysis of the EC cellular synthetic system, presented in Table 1, are indicative of this.

The functioning of any secretory cell may be represented as an aggregate of two basic processes, synthetic and secretory. Our findings provide evidence of different relationships between the secretory and synthetic activities of EC cells in different physiological states of the hibernant. Synthetic processes predominate in summer and winter euthermia. In winter, when a hibernant is in a torpid state, the picture is different. Cellular function is shifted toward secretion of the accumulated material. The fact that in winter the number of soluble granules sharply increases as against periods of euthermia (57.6 vs. 29.6% in summer and 34.2% in spontaneous arousal) seems to confirm this.

Thus, a similar ultrastructure and direction of functioning of serotonin-producing cells of euthermic animals were observed during summer activity and winter spontaneous awakening. The morphology and function of EC cells of torpid animals are characterized by a reduced synthetic apparatus and marked activation of the secretory processes. All these facts permit us to conclude that the duodenal EC cells of red-cheeked sousliks are among the most important structural components in the intricate mechanism of hibernation regulation in mammals.

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