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# Role of Lipids in the Assembly of Endoplasmic Reticulum and Dictyosomes in Neuronal Cells from the Cerebral Cortex of Yakutian Ground Squirrel (*Citellus undulatus*) during Hibernation

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**Abstract**—Phospholipids and cholesterol were assayed in homogenates and microsomal fractions from the cerebral cortex of summer-active, winter-torpid, and winter-active Yakutian ground squirrels (*Citellus undulatus*). Ultrastructural analysis of both microsomal fraction and intact neurons was performed by serial ultramicrotomy. The levels of sphingomyelin (SM), phosphatidylserine (PS), and phosphatidylethanolamine (PEA) were decreased in homogenates from the cerebral cortex of winter ground squirrels compared with the summer-active animals, while the levels of phosphatidylcholine (PC) and cardiolipin (CL) were increased. The level of cholesterol was decreased in the cerebral cortex of winter-torpid animals compared with both winter-active and summer-active animals, and the level of total phospholipids was decreased in comparison to the summer-active animals. Three-dimensional reconstruction of serial membrane profiles displayed the microsomal fraction to be an interconnected system of cisterns and vesicles, which corresponds to endoplasmic reticulum and dictyosomes (Golgi stacks) of intact neurons. In winter the content of PC was increased in the microsomal fraction, while the contents of lysophosphatidylcholine (LPC), PS, phosphatidylinositol (PI), and SM were decreased. In winter-torpid animals compared with the winter-active ones the contents of total phospholipids, PEA, LPC, and cholesterol were decreased. As for the winter-active ground squirrels, their lipid contents did not differ from those in the summer-active animals, but LPC content was decreased. The changes in microsomal lipid contents in intact pyramidal neurons throughout the hibernation were accompanied by disassembly of dictyosomes and endoplasmic reticulum (ER), including the decomposition of polyribosomes to monosomes. The ultrastructural analysis of nucleoli, ER, and dictyosomes of both winter-active and torpid ground squirrels showed a direct correlation between the increasing contents of both cholesterol and total phospholipids (mainly PEA and LPC) in microsomes and the structural recovery of endoplasmic reticulum, Golgi stacks, and nucleoli in intact pyramidal neurons. A role of seasonal variations in lipid contents of brain cells in their adaptation to low temperature is discussed. We also propose an involvement of cholesterol in the activation of protein-synthesizing function of endoplasmic reticulum and Golgi stacks in intact neurons.

*Key words*: hibernation, ground squirrel, cerebral cortex, phospholipids, cholesterol, microsomes, pyramidal neurons, endoplasmic reticulum, dictyosomes

Mammalian hibernation is a cyclic process consisting of hibernation bouts: entrance into cold lethargy – torpor – arousal to euthermia. As a rule, prolonged torpor bouts are interrupted by short (one or two days) arousal periods of spontaneous normothermia. The mammalian hibernation as an adaptive process allowing survival under extreme conditions is characterized by dramatic declines in body temperature, heart rate, respiration, and metabolic rate [1]. In mammals, the brain plays the leading role in mechanisms of winter torpor and adaptation to the cold [2, 3]. Numerous active substances responsible for certain steps of the hibernation process are found in brain and other tissues of hibernating animals [4]. Membrane lipids seem to play an important role in cold-resistance of mammals. Unsaturated fatty acids were found to increase in level among phospholipids in various organs and tissues of hibernating mammals, although this increase may be insignificant [5-7].

*Abbreviations*: SM) sphingomyelin; CL) cardiolipin; PI) phosphatidylinositol; PS) phosphatidylserine; PC) phosphatidylcholine; PEA) phosphatidylethanolamine; LPC) lysophosphatidylcholine; ER) endoplasmic reticulum.

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Membrane fluidity is a function of several parameters, and the level of cholesterol is one of the most important [8]. Mammalian brain is enriched with cholesterol, whereas the level of cholesterol in parenchymal organs is about 3-5% of total lipids. In human brain the level of cholesterol is 30% of total lipids. PC and PEA are the great bulk of phospholipids in cerebral cortical cells of rats and humans [9]. Data on the distribution of cholesterol and phospholipids in membranes of various organelles of cortical pyramidal neurons in brains of hibernating animals are of great interest. However, such studies are very complex because neurons and glial cells comprising about 50% of the total brain cells are hard to separate. The meager data on brain lipid changes in various physiological states in mammals require further investigations of organelles from certain brain areas. A zone of particular interest is the microsomal fraction of cerebral cortex, which is enriched with ribosomes and associated with protein-synthesizing activity of brain. Quantification of cholesterol and phospholipids in brain and brain microsomes of hibernators depending on their functional state (summer-active, torpid, or winter-active) could elucidate the role of membrane lipids in adaptation of mammals to low body temperatures. The Yakutian ground squirrel *Citellus undulatus* can survive at body temperature −2°C [10]. So we thought the determination of cholesterol and phospholipids at the sites of lipid synthesis, in microsomal fraction of brain cortex [11] to be an important task. A drastic inhibition of protein synthesis due to the decomposition of polyribosomes and disassembly of endoplasmic reticulum and dictyosomes were shown previously to occur in gustatory cells of Yakutian ground squirrel during hibernation [12]. So we studied ultrastructural features of cell organelles and native pyramidal neurons of brain cortex and hippocampus simultaneously with quantification of lipid contents in microsomal fraction of brain cortex.

## MATERIALS AND METHODS

Adult Yakutian ground squirrels *C. undulatus*, both females and males with body weight of 400-800 g, were trapped in the vicinity of Yakutsk and held in individual cages in a vivarium with natural light cycle. The animals had free access to food (sunflower seeds and fresh carrot) and supplied *ad libitum* with nesting material. In November, the animals were placed into special individual wooden cages (20 × 20 × 25 cm) and transferred into a dark room with the temperature of 1-3°C. Monitoring of the functional state of the animals was conducted using a sensor inserted into the nest bottom as described previously [12]. The nest temperature was 1-4°C throughout hibernation of squirrels and increased to 14-20°C during their arousal. The mean duration of torpor bout was one week in December and 1.5-2 weeks in January through February. Torpid squirrels were taken in the mid-bout (brain temperature ($T_b$) ≈ 2-6°C), and inter-bout (winter-active) squirrels were taken in 3 h and 2 days after provoked arousal from the mid-bout ($T_b$ ≈ 34-35°C). All winter squirrels were taken in January through March. The third group consisted of summer-active ground squirrels taken in July through August ($T_b$ ≈ 36-37°C). Brain temperature was determined directly after decapitation using a pinpoint thermometer graduated at 0.2°C intervals.

To produce homogenate and isolate microsomal fraction, the brain cortex was homogenized in 0.25 M sucrose solution in the cold, and aliquots were used for protein determination, lipid extraction, and isolation of microsomal fraction by a standard differential centrifugation technique [13].

Lipids were extracted with 10 volumes of the chloroform–methanol mixture (2 : 1 v/v) by the method of Folch et al. [14]. Phospholipids were separated by TLC (60 × 0.2 mm plates, Merck, Germany) in the solvent system methylacetate–*n*-propanol–chloroform–methanol–0.25% KCl (25 : 25 : 25 : 10 : 9 v/v) [15]; neutral lipids were separated on Silica gel L (5/40 μm, Chemapol, Czech Republic) in the solvent system hexane–ethyl ether–acetic acid (73 : 25 : 2 v/v) [16].

Cholesterol was determined by the technique of Liberman and Burchard [17], and phospholipids (after separation of neutral lipids) were determined following Gerlach and Deuticke [18].

The contamination of microsomal fraction with cytoplasmic membranes was determined from the 5′-nucleotidase activity and with mitochondrial fraction from the succinate dehydrogenase activity. The contamination levels were 14 and 30%, respectively. Protein was determined by Lowry et al. [19].

Isolated cortical microsomal fraction and native tissues (sensomotor brain area and hippocampus) were used for electron microscopic studies. Fixation was carried out in 2.5% glutaraldehyde at room temperature overnight with subsequent post-fixation of both microsomal pellets and brain blocks in 1% osmium tetroxide at room temperature for 2-3 h. All fixatives were prepared in 0.1 M sodium cacodylate buffer, pH 7.2-7.4. The fixed samples were dehydrated through ethanols (40, 60, 70, 80, and 96%, 10 min each) and then in 100% acetone (three changes, 10 min each). The samples were embedded in Epon 812/Araldite M epoxy-resin and polymerized at 60°C for 2-3 days. Serial ~70 nm thick sections were sliced using an ultramicrotome (LKB, Sweden) with a diamond knife. Serial sections in the form of a band were taken from the surface of 5% ethanol solution in a knife tray onto special blends covered with Pioloform film. The sections were stained for 5-10 min in 4-5% uranyl acetate dissolved in 70% ethanol, as well as in Reinold's lead citrate for 10-20 min. The sections were examined using a JEM 100B electron microscope (JEOL, Japan) at accel-

erating voltage of 80 kV. Magnification was calibrated using a special replica (2160 lines per 1 mm) (Electron Microscopy Sciences Inc., Fort Washington, PA, USA).

The software recommended by Drs. J. Fiala and K. Harris (http://synapses.bu.edu) was used for the 3-D-reconstruction. Serial images scanned were aligned using the Alignment program and after the contouring of membrane profiles by the Trace program the 3-D-image was generated in vmrl/wrl format. The images were converted by the Crossroads utility into RAW format, and the final three-dimensional images were produced using 3-D View software (Actify, Inc.).

Data were processed by Student's *t*-test. The mean ± SD values are given in the tables.

## RESULTS

**Lipid amounts in homogenate and microsomal fraction of brain cortex.** Amounts of protein, cholesterol, total phospholipids, PC, PS, PI, PEA, SM, LPC, and CL were determined in brain cortex and microsomal fraction in summer-active, winter-active, and torpid ground squirrels.

As evident from Tables 1 and 2, the protein amount in cortex and microsomal membranes as calculated per 1 g of wet brain cortex tissue did not depend on the season or functional state of the animals.

The data on the amounts of phospholipids and cholesterol in homogenate of brain cortex per 1 mg of protein are given in Table 1. The amount of total phospholipids in brain cortex is clearly decreased in torpid animals. Both the elevation of PC and CL quantities and decrease in PS, PEA, and SM are seasonal, because these alterations take place in both winter-active and torpid ground squirrels. PC and PEA constitute the bulk of phospholipids in winter squirrels. PEA and SM are the most abundant phospholipids in brain cortex of summer squirrels. The amount of cholesterol in homogenate drastically decreases in torpid animals compared with both winter- and summer-active squirrels. The amount of cholesterol in brain tissue in winter-active squirrels is undistinguished from that in summer-active squirrels.

The cholesterol-to-phospholipid ratio was found to grow in homogenate of brain cortex of winter-active ground squirrels. The cholesterol-to-sphingomyelin ratio is drastically increased in winter compared with the summer period (Table 1).

**Table 1.** Lipid composition in homogenates of ground squirrel brain cortex

| Lipids, μg/mg protein | Winter squirrels | | Summer-active squirrels |
|---|---|---|---|
| | torpid | active | |
| Total phospholipids | 425.0 ± 46.0 (8) *$p < 0.05$ | 444.4 ± 108.2 (6) | 509.1 ± 47.6 (3) |
| Phosphatidylcholine | 109.7 ± 20.8 (7) *$p < 0.002$ | 118.4 ± 27.8 (6) *$p < 0.006$ | 47.3 ± 18.6 (3) |
| Phosphatidylserine | 57.3 ± 12.9 (6) *$p < 0.008$ | 64.0 ± 6.7 (6) *$p < 0.002$ | 87.6 ± 7.2 (3) |
| Phosphatidylinositol | 13.0 ± 2.0 (5) | 11.8 ± 2.3 (5) | 14.7 ± 4.2 (3) |
| Phosphatidylethanolamine | 134.7 ± 30.5 (7) *$p < 0.028$ | 144.6 ± 26.0 (6) *$p < 0.044$ | 187.4 ± 21.3 (3) |
| Sphingomyelin | 24.5 ± 2.8 (7) *$p < 0.0001$ | 37.2 ± 6.3 (4) *$p < 0.05$ | 158.2 ± 2.0 (4) |
| Lysophosphatidylcholine | 22.5 ± 13.2 (3) | 23.6 ± 10.0 (4) | 42.9 ± 8.0 (3) |
| Cardiolipin | 11.1 ± 1.1 (5) * | 11.8 ± 2.0 (6)* | trace |
| Cholesterol | 90.0 ± 9.0 (6) *$p < 0.01$ | 129.0 ± 29.0 (6) ** $p < 0.003$ | 161.0 ± 16.0 (3) |
| Protein, mg/g wet tissue | 113.0 ± 8.0 (3) | 99.0 ± 18.0 (3) | 81.0 ± 6.7 (3) |
| Cholesterol/phospholipids molar ratio | 0.56 ± 0.08 (6) | 0.74 ± 0.16 (4) ** $p < 0.05$ | 0.62 ± 0.40 (3) |
| Cholesterol/sphingomyelin molar ratio | 6.0 ± 0.8 (7) *$p < 0.01$ | 7.4 ± 1.6 (6) * $p < 0.05$ | 2.5 ± 0.4 (4) |

Note: The number of animals is given in parentheses.
* Significant difference from summer-active animals.
** Significant difference from torpid animals.

**Table 2.** Lipid composition of the microsomal fraction of ground squirrel brain tissue

| Lipids, μg/mg protein | Winter squirrels | | Summer-active squirrels |
|---|---|---|---|
| | torpid | active | |
| Total phospholipids | 358.8 ± 52.0 (3)* $p < 0.019$ | 566.8 ± 78.7 (3)** $p < 0.016$ | 608.0 ± 93.6 (3) |
| Phosphatidylcholine | 159.8 ± 17.5 (3)* $p < 0.023$ | 202.0 ± 39.6 (3)* $p < 0.041$ | 88.8 ± 37.4 (3) |
| Phosphatidylserine | 55.4 ± 4.5 (3)* $p < 0.005$ | 60.1 ± 5.4 (3)* $p < 0.013$ | 84.9 ± 7.8 (3) |
| Phosphatidylinositol | 16.4 ± 2.4 (3)* $p < 0.005$ | 13.6 ± 6.6 (3)* $p < 0.047$ | 24.4 ± 1 (3) |
| Phosphatidylethanolamine | 114.4 ± 9.3 (3)* $p < 0.002$ | 159.2 ± 12.1 (3)** $p < 0.007$ | 177.9 ± 12.8 (3) |
| Sphingomyelin | 27.0 ± 1.3 (3)* $p < 0.0001$ | 29.4 ± 1.6 (2)* $p < 0.0001$ | 127 ± 3 (3) |
| Lysophosphatidylcholine | trace | 34.6 ± 3 (2)** | 42.5 ± 2.2 (3)** |
| Cholesterol | 57 ± 6 (3)* $p < 0.003$ | 177 ± 26 (2)** | 142 ± 9 (3) |
| Protein, mg/g wet tissue | 7.3 ± 0.8 (3) | 8.5 ± 1.7 (2) | 6.0 ± 1.8 (3) |
| Cholesterol/phospholipids molar ratio | 0.32 ± 0.2 (3)* $p < 0.001$ | 0.58 ± 0.04 (2)** | 0.48 ± 0.02 (3) |
| Cholesterol/sphingomyelin molar ratio | 4.2 ± 0.4 (3)* $p < 0.01$ | 14.6 ± 1.8 (2)* ** $p < 0.01$ | 2.2 ± 1.2 (3) |

Note: The number of animals is given within parentheses.
* Significant difference from summer-active animals.
** Significant difference from torpid animals.

A decrease in amount of phospholipids and cholesterol was observed in microsomal fraction of torpid ground squirrels compared both with winter-active and summer animals (Table 2). Seasonal changes common for winter-active and torpid squirrels consist in the growth of PC and decrease of LPC, PS, PI, and SM contents. Winter-active squirrels differ from torpid ones in increase in total phospholipids, PEA, and cholesterol up to the levels found in summer-active animals. LPC level increases as well, although remains lower than in summer animals. The cholesterol-to-phospholipids molar ratio is decreased in microsomal fraction of brain cortex of torpid ground squirrels compared with summer squirrels and grows up to the level found in summer-active animals. The cholesterol-to-sphingomyelin ratio is increased in torpid ground squirrels compared with summer animals. This ratio further increases in winter-active ground squirrels.

**Ultrastructural analysis of neuronal cytoplasm.** The ultrastructure of pyramidal neurons from the hippocampal CA1 area was analyzed simultaneously with the homogenization and isolation of microsomal fraction from brain cortex (based on the differences in cellular structure, hippocampus is divided into four main areas including CA1). In Fig. 1 the ultrastructure of the nucleolus is presented for the torpid ground squirrels (a and b) and for the winter-active animals 3 h (c) and 2 days (d) after provoked arousal from the mid-bout. Like the nucleolus in gustatory receptor cells [12], the nucleolus in pyramidal neurons in torpid ground squirrels possesses a large vacuole-like compartment with filamentous material, so-called fibrillar center (Fig. 1, a and b), whereas in active ground squirrels those centers disappear, and a granular material prevails (Fig. 1, c and d). Concentrated heterochromatin is revealed around "vacuoles" in the nucleolus in torpid ground squirrels (Fig. 1, a and b). Most free and membrane-associated (rough endoplasmic reticulum) polyribosomes dissociate to monosomes in neuronal cytoplasm (Fig. 2, a-d). The polyribosome dissociation is accompanied by disassembly of membranes comprising rough endoplasmic reticulum and Golgi apparatus that forms numerous dictyosomes in pyramidal neurons. The dictyosomes comprise a system of interrelated vesicles, and cisterns are barely visible on single sections, as reported for gustatory receptor cells [12]. Numerous lipofuscin granules are revealed in neuronal bodies of torpid ground squirrels (Fig. 2, a and d). The assembly of both free and membrane-associated polyribosomes occurs already 3 h after the arousal from mid-bout (Fig. 2, e and g). Coincidentally with the assembly of rough endoplasmic reticulum, the assembly of dictyosomes takes place with the formation of the system of

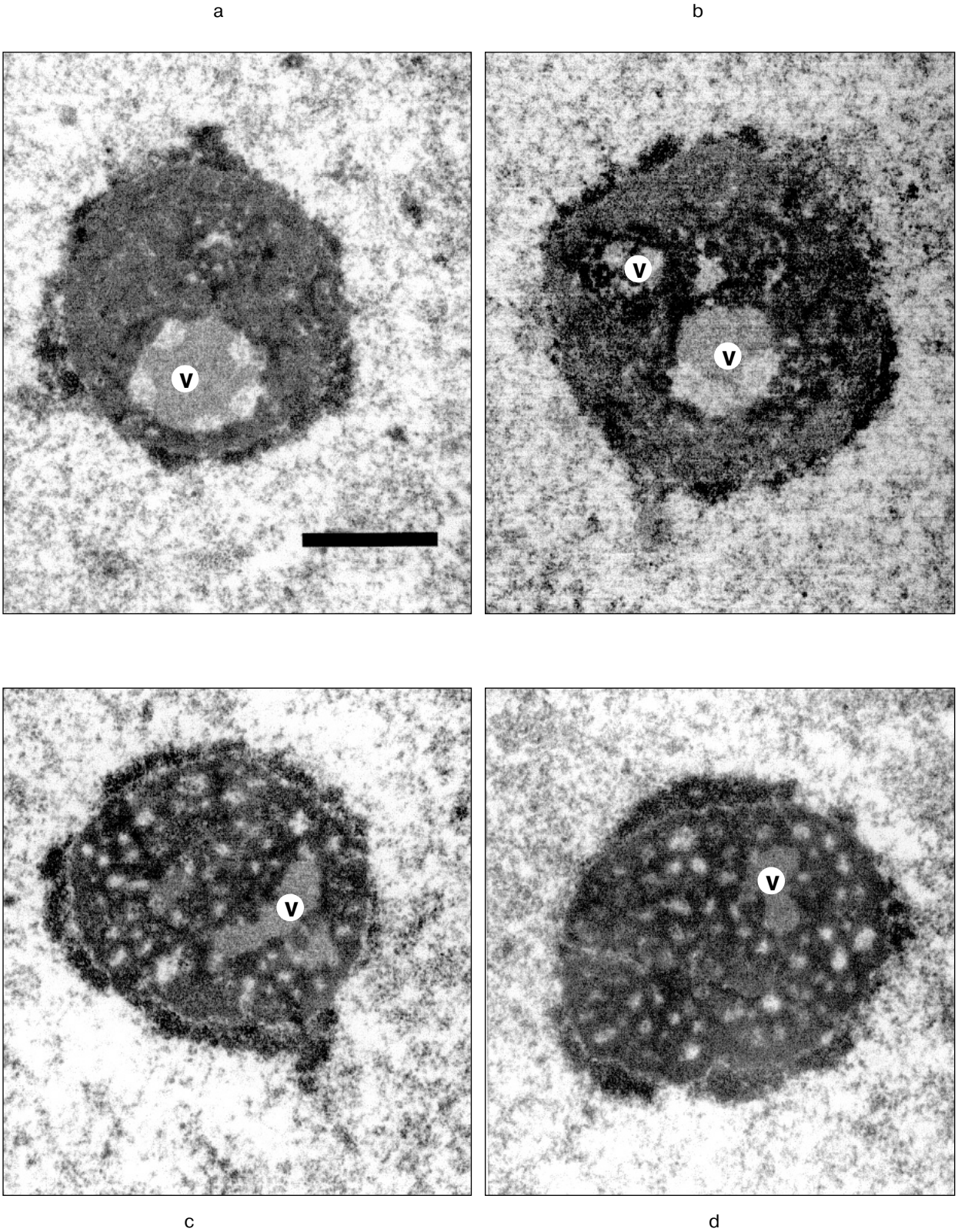


**Fig. 1.** Ultrastructure of nucleoli in pyramidal neurons from hippocampal CA1 area of ground squirrels: in the hibernational mid-bout, $T_b$ = 4°C (a) and 6°C (b); three hours ($T_b$ = 34°C) (c) and two days ($T_b$ = 36°C) (d) after a provoked arousal from the mid-bout. The scale is 1 μm for all of the pictures. V, fibrillar center.

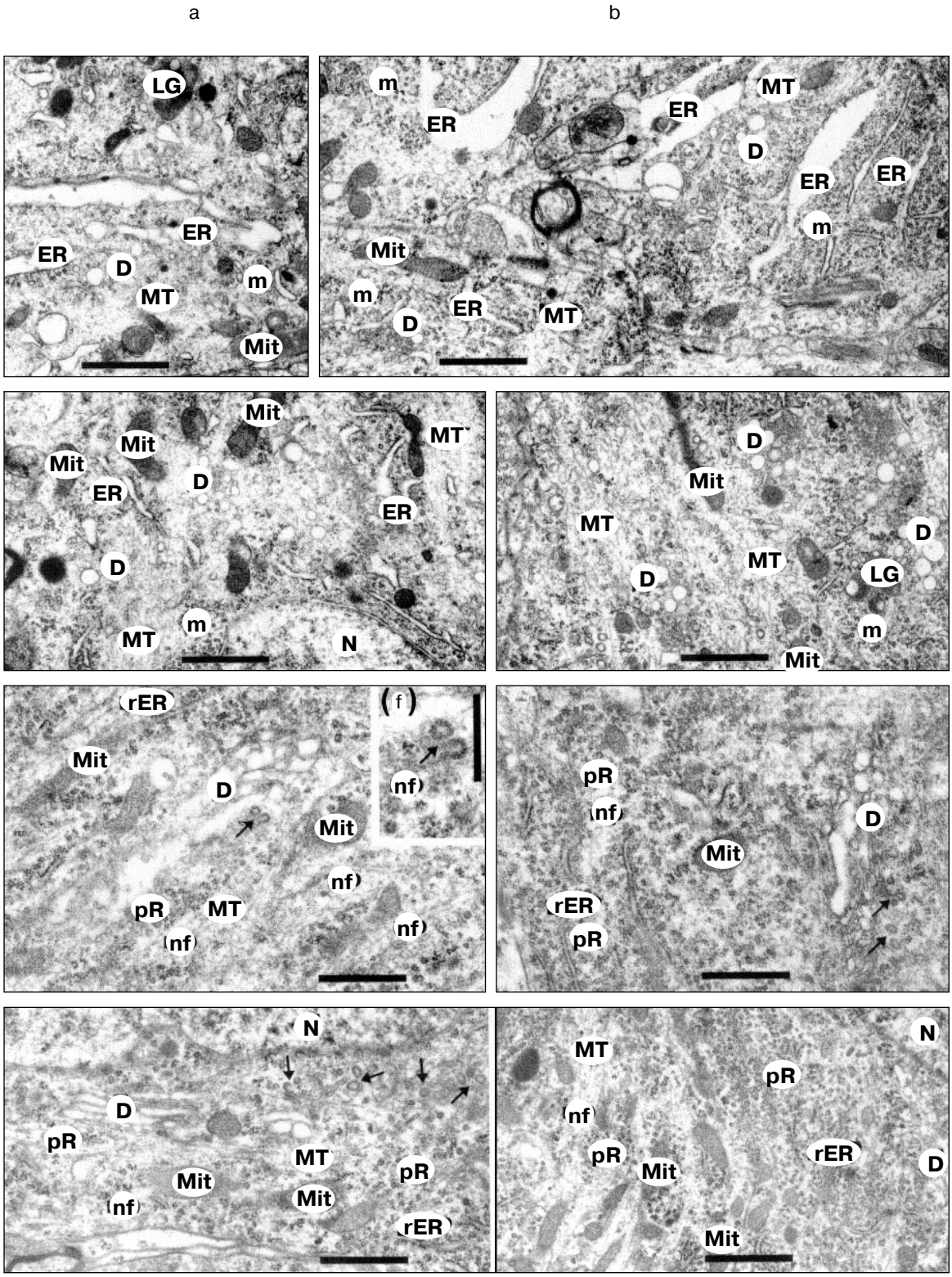


**Fig. 2.** Ultrastructure of cytoplasm in pyramidal neurons from hippocampal CA1 area of ground squirrels in the hibernation mid-bout ($T_b$ = 4°C) (a-d) and 3 h ($T_b$ = 34°C) (e, g) and 2 days ($T_b$ = 36°C) (h, i) after a provoked arousal from the mid-bout. The scale is 1 μm for all of the pictures but (e), for which the scale is 0.25 μm. Symbols: V, fibrillar center; D, dictyosomes; LG, lipofuscin granules; m, monosomes; MT, microtubules; Mit, mitochondria; nf, neurofilaments; pR, polyribosomes; ER, endoplasmic reticulum; rER, rough endoplasmic reticulum; N, nucleus.

interrelated cisterns and vesicles (Fig. 2, e and g). Numerous cisterns and coated vesicles comprising dictyosomes are not found in torpid ground squirrels (in the mid-bout). The ultrastructure of neuronal cytoplasm 3 h after the arousal from mid-bout (Fig. 2, e and g) does not differ from that in both winter-active (Fig. 2, h and i) and summer-active (data not shown) ground squirrels. The number of lipofuscin granules is drastically decreased in cytoplasm in all active (winter and summer) ground squirrels. Thus, the entrance into hibernation is accompanied by disassembly of both polyribosomes and membranes of smooth and rough reticulum and dictyosomes with the disappearance of coated vesicles. All these changes are reversible throughout the hibernation. No significant differences between winter- and summer-active ground squirrels were found in neuronal cytoplasm.

**Ultrastructural analysis of microsomal fraction.** We found no morphological differences between the torpid and active ground squirrels in the structure of the microsomal fraction from their brain cortexes. The typical morphology of microsomal fractions on eight serial sections (the series consisted of 20 sections) is displayed in Fig. 3. Most membranes are present as vesicles, but rod-shaped membrane structures are found as well (Fig. 3, arrows). The 3-D reconstruction of microsomal fraction fragments is shown in Fig. 4. Figure 4b gives 3-D recon-

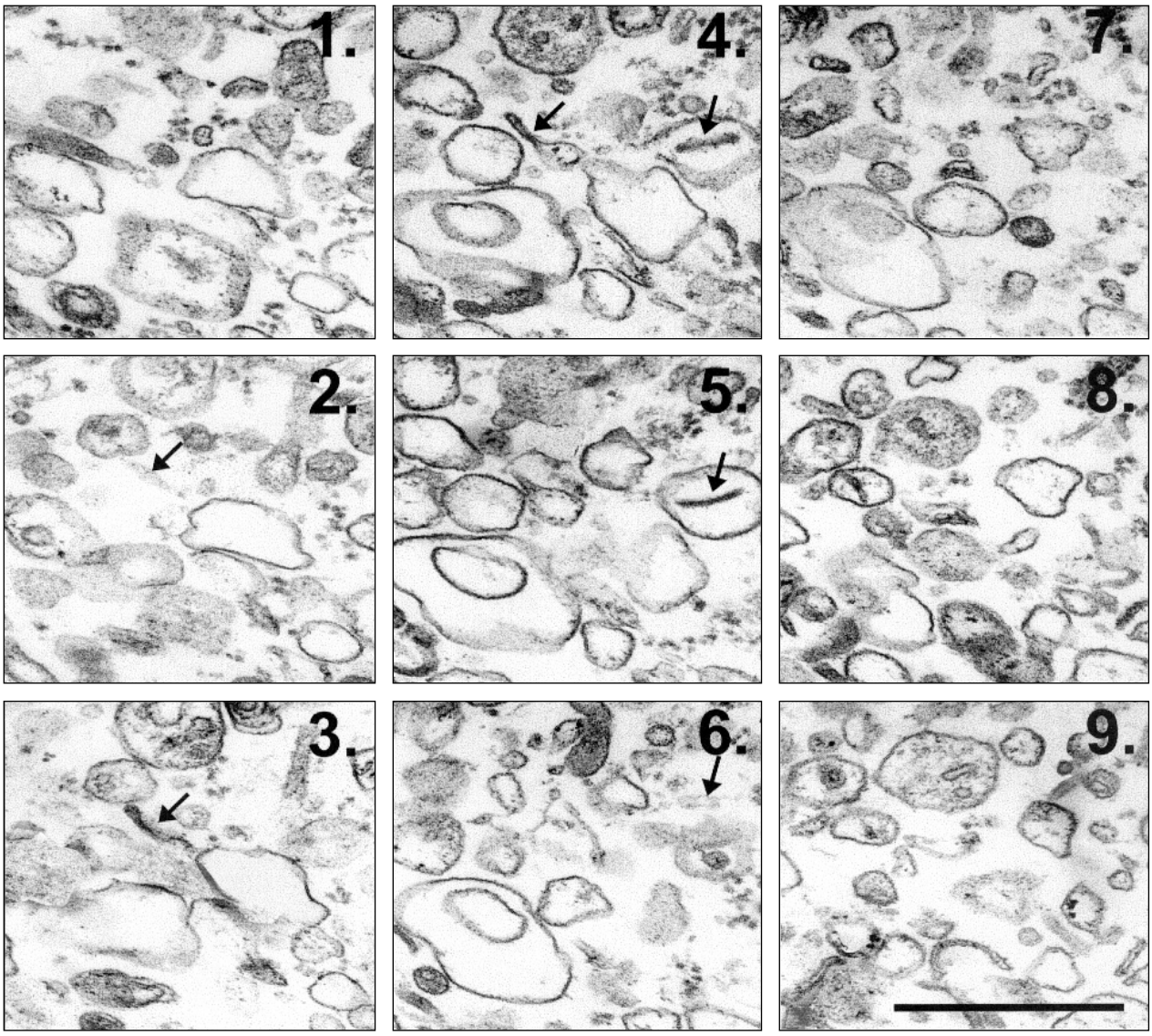


**Fig. 3.** Ultrastructural characteristics of the microsomal fraction from the cerebral cortex of hibernating ground squirrel. Sequential membrane profiles on nine sections after the "alignment" of a series consisting of 20 serial sections are shown. Microsomal fraction is represented mainly by vesicles and tubular structures (arrows). The scale is 0.5 μm.

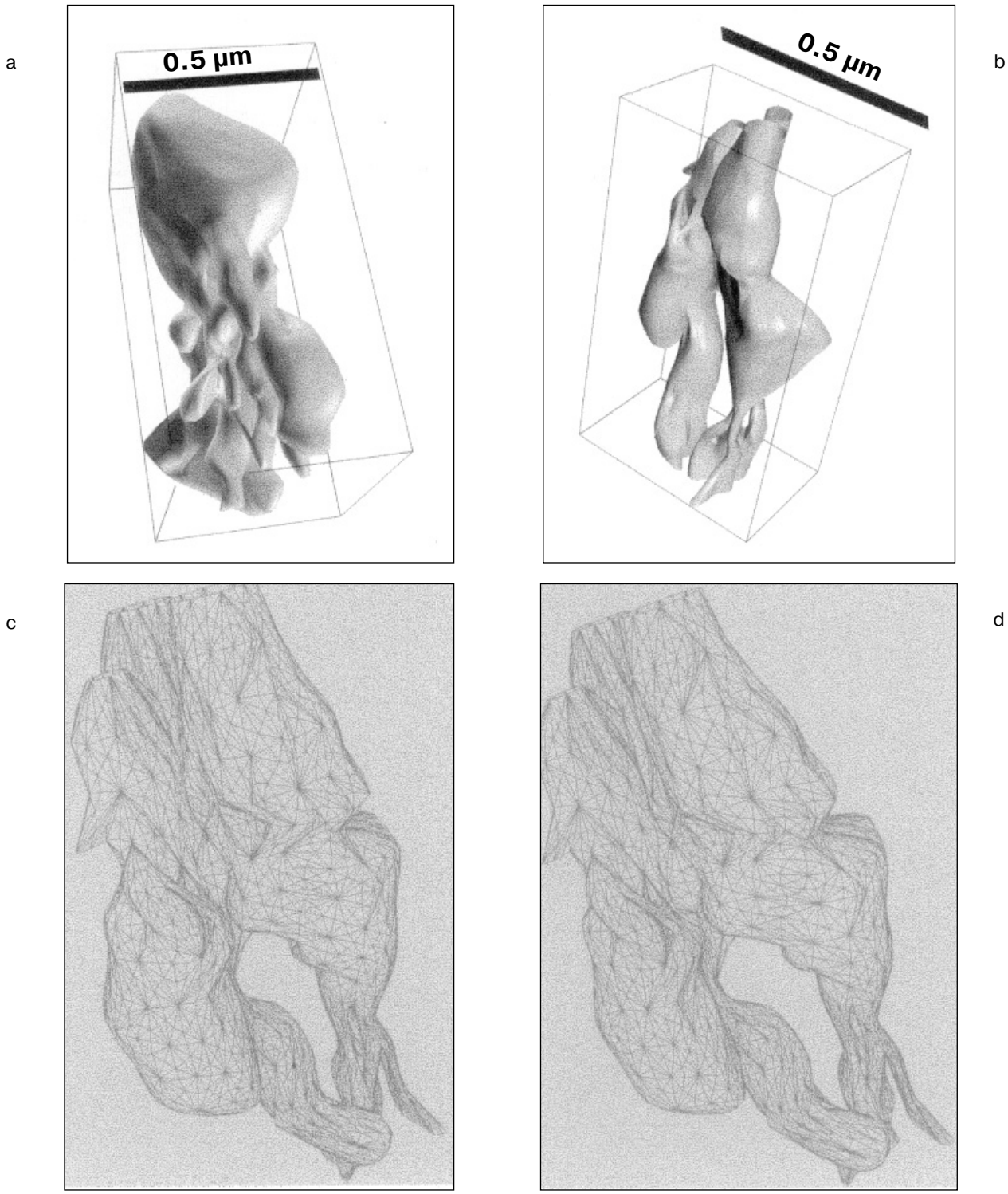


**Fig. 4.** Three-dimensional structure of membrane profiles in microsomal fraction (a, b) reconstituted the using IBM PC program utilities Alignment (alignment of sections) of Trace (the respective contour is drawn on each serial image using the mouse with subsequent generation of three-dimensional image formatted as wrl(vmrl) or raw file; three-dimensional image is surveyed using the programs for three-dimensional graphics, such as 3-D View (Actify Inc.) or VRweb). The halftone images (a, b) are produced using 3-D View (Actify Inc.). The pictures (c, d) display the stereo pair created by VRweb in the "hidden lines" mode allowing visualization of the surface relief that cannot be seen on the halftone 3-D image (b).

struction, and Fig. 4 (c, d) demonstrates stereo pair of membrane profiles partially presented in Fig. 3. Unlike Fig. 4b, the stereo pair shows a hidden surface geometry revealing the intricate shape of membranous structures. As follows from the analysis of 3-D reconstruction, microsomal fractions are represented by a system of interconnected cisterns corresponding to the morphology of endoplasmic reticulum and dictyosomes in intact brain cells of either neuronal or glial origin.

## DISCUSSION

The protein content in brain cortex of ground squirrels is about 100 mg per 1 g of wet tissue, which agrees with data reported for rat brain [20]. This amount is significantly lower than the protein level in parenchymal organs, e.g., 250-290 mg per 1 g of wet tissue for hepatic cells [21]. The cholesterol-to-phospholipids molar ratio in brain cortex of summer-active and torpid ground squirrels is equal to the values determined for the rat brain cortex [9]. Yet the phospholipid composition of brain cortex in summer ground squirrels differs from that of rats in lower PC content and elevated contents of LPC, PS, and especially SM. Decrease in cholesterol and phospholipids, but elevated level of PC with simultaneous decrease in SM, PEA, and PS are found in brain cortex of Yakutian ground squirrels in the state of winter hibernation. The lipid composition of brain cortex of winter-active ground squirrels differs from that of torpid animals preferably in the increase in cholesterol amount (Table 1). Thus, certain circaannual changes in phospholipid composition are found in brain cortex: the levels of PC and CL are increased and the levels of PS, PEA, and SM are decreased in winter. The cholesterol-to-phospholipids ratio is elevated in winter-active ground squirrels compared with the torpid animals. The change in membrane fluidity in brain cortex during the arousal from hibernation may play an important role in synaptic transmission. However, no significant seasonal changes in cholesterol or phospholipid amounts were found in brain of European hamster *Cricetus cricetus*, and cholesterol-to-phospholipids molar ratio was even slightly increased in winter [22]. The absence of changes in amounts of cholesterol and phospholipids in brain of hamsters during the entrance into hibernation was demonstrated in a number of studies [6, 7]. The difference may be due to that the Yakutian ground squirrel is an obligate hibernator, whereas hamsters are facultative hibernators. Hibernation bouts in hamsters are shorter, and their brain and body temperatures are supported at higher level. The rapid seasonal changes in lipid composition we observed in brain cortex of Yakutian ground squirrel may be due to the longer hibernation bouts and/or to the adaptation of these animals to low environmental temperature [1, 6, 10]. A decrease in the portion of lipids (such as cholesterol, SM, and PEA) increasing the viscosity of phospholipid bilayers and modifying their structures and the increase in amount of phospholipids decreasing the membrane viscosity (PC, CL) in cellular membranes result in increase of lateral mobility of membrane proteins, for example, channel subunits and receptors. The role of decreased membrane fluidity due to the changes in fatty-acid composition of phospholipids as a way of membrane adaptation to low surrounding temperature in mammals has been discussed in literature [5-7]. Earlier, we found similar seasonal changes in phospholipid composition of synaptic membranes in brain cortex of ground squirrels [23].

In winter-torpid ground squirrels, the levels of cholesterol and LPC in microsomal fraction (Table 2) were lower than in cell homogenate. As a rule, the level of cholesterol in microsomal membranes of parenchymal organs is known to be significantly higher than that in the tissue homogenate [21]. In our experiments, relatively higher cholesterol concentration in brain homogenates than in microsomal fraction may be due to the presence of cholesterol-enriched myelin in the homogenates.

The analysis of cholesterol-to-sphingomyelin ratio in microsomal fraction is of special interest. The concentrations of sphingomyelin and other sphingolipids are important for the intracellular distribution of cholesterol among membrane compartments [24, 25]. The digesting of SM by exogenous sphingomyelinase in cytoplasmic membrane is known to induce cholesterol leakage into other cellular compartments, whereas the PC-dependent phospholipase does not induce such an effect [26]. Sphingomyelin concentration in cytoplasmic membrane regulates cholesterol synthesis via the genetic apparatus [27]. SM participates in cholesterol transport from Golgi apparatus into cytoplasmic membrane. Cholesterol and SM were found to be involved in the formation of "rafts", specific membrane domains, in cytoplasmic membranes of mammalian cells [28]. These rafts are supposed to play an important role in membrane protein sorting, intercellular signal transduction, and other processes [28]. It has been shown for a number of mammalian cells that cholesterol and SM are localized almost completely in cytoplasmic membrane (up to 96%) [29]. Evidences exist that SM in the cell line MDCK is localized virtually completely (up to 96% of total cellular SM) in detergent-insoluble membrane vesicles with tight association with cholesterol, and the cholesterol-to-SM molar ratio in those vesicles is about three [28]. The cholesterol-to-SM ratio is $2.2 \pm 1.2$ in microsomal fraction from the brain cortex of summer ground squirrels. It is probable that in brain cortical cells of summer ground squirrels both endoplasmic reticulum and membranes of dictyosomes are enriched with SM. Hibernation is accompanied by the growth of the cholesterol-to-SM ratio due to the more prominent drop of SM amount in comparison with the decrease in cholesterol. The arousal from hibernation is also accompanied by the elevation of this ratio, but due

to the more acute increase in cholesterol level in microsomal fraction (Table 2). In our opinion, the drastic increase in cholesterol-to-SM ratio in endoplasmic reticulum of brain cortex in winter-active ground squirrels suggests the decrease in the portion of cholesterol transported from endoplasmic reticulum membranes, as compared with summer ground squirrels. One may suppose that cholesterol is utilized for the rapid and acute enhancement of protein synthesis. The activation of specific functions in organelles of hepatic cells is accompanied by the elevation of cholesterol amount in these organelles [30]. The activation of protein and lipid syntheses in mammalian liver under ionizing irradiation was accompanied by a significant increase in cholesterol level in microsomal fraction [21]. The wintertime increase in cholesterol amount was previously reported in microsomal fraction of brain cortex of active animals belonging to other hibernating species. In particular, in winter the cholesterol amount elevated by 11% with slightly decreased PC and PEA levels found in microsomal fraction of brain cortex in active hamster, *Mesocricetus auratus* [31]. The change in cholesterol amount during hibernation is apparently typical for the brain. In other organs, such as liver and kidney, the cholesterol amount in winter was unchanged [6].

Possible activation of synthesis *de novo* as well as the enhanced influx from blood plasma may provide the basis for the increase in cholesterol level in neurons and astroglia. Normally, the synthesis of cholesterol in neuronal cells and its influx with plasma lipoproteins are known to be the main sources of neuronal cholesterol [11].

Seasonal increase in PC level reflects the profound changes in metabolism of choline-containing phospholipids, which is consistent with the decrease in SM, for which PC serves as a source of choline. PC enhances the interaction between catalytic and regulatory sites of adenylate cyclase [32] and is a source of eicosanoids in a protein kinase C-dependent path of phospholipase $A_2$ activation [33].

The analysis of changes in amounts of LPC, the central metabolite in the synthesis of choline-containing phospholipids and acetylcholine in the brain, is of particular interest. LPC plays the role of second messenger influencing transmembrane signal transduction through various receptors coupled with G-proteins [34]. LPC activates protein kinase C in the presence of phospholipids containing unsaturated fatty acids [35]. Insignificant decrease in LPC content was found in brain cortex homogenates in winter-torpid and winter-active ground squirrels (Table 1) compared with summer ones, whereas LPC was not found at all in microsomal fraction from brain of torpid squirrels (Table 2). These data suggest a substantial rearrangement within the system of signal transduction in brain cells during the entrance into torpor.

Moreover, torpor is accompanied by a decrease in PEA level in microsomal fraction. PEA prominently activates the protein kinase C family and C1 domain in protein kinases C is concerned as a sensor of the lipid bilayer [36]. In brain of torpid bats *Myotis lucifugus* compared with the normothermal bats, the protein kinase C (gamma) activity decreased to 63%, whereas the level of membrane-associated protein kinase C remained unchanged [37]. Hence, the decrease in protein kinase C activity in torpid animals compared with normothermal ones might result from a decrease in the PEA level.

Phosphatidylserine (PS) plays an important role in neuronal apoptosis [38]. The decrease in PS amount in brain cortex may be involved in the mechanism of resistance to low temperatures. Similarly, one can speculate on the role of seasonal decrease in levels of other signaling phospholipids, such as PI and SM. Thus, the changes in lipid composition during hibernation of ground squirrels are connected with not only reversible structural, but also with functional changes in brain cells.

Ultrastructural analysis of intact pyramidal neurons in the CA1 hippocampal area has been conducted in parallel with the analysis of lipid composition in microsomal fraction of cerebral cortex of the same brain.

The hippocampus is the archeocortex and possibly triggers hibernation [3, 39, 40]. The pyramidal neurons in CA1 hippocampal area are virtually identical with those in cerebral cortex by their morphological features. So, we can map the changes in ultrastructural features of hippocampal pyramidal neurons into the changes in lipid composition of the microsomal fraction of cerebral cortex. We have found no correlation between the circaannual (winter- and summer-active animals) changes in the lipid composition (PC elevation and PS, PI, and SM drop) of microsomal fraction and the ultrastructures of endoplasmic reticulum and nucleus of neurons.

The drastic drop in amounts of cholesterol and total phospholipids, the decrease in cholesterol-to-phospholipids molar ratio, and the decrease in PEA and LPC amounts are characteristic for the state of torpor (cold lethargy) (Table 2). These changes in lipid composition of microsomal fraction are accompanied by the appearance of lipofuscin granules in neuronal bodies (Fig. 2, a, c, and d), decomposition of membranes comprising endoplasmic reticulum and dictyosomes (Fig. 2, a-d), and disappearance of "coated" vesicles. The decomposition of membranes comprising endoplasmic reticulum and dictyosomes in gustatory receptor cells of winter-torpid ground squirrels has been observed earlier [12]. Thus, the decrease in the level of total phospholipids by 37%, and PEA by 28% as well as drastic decrease in the levels of LPC and cholesterol are associated with the structural rearrangement of membrane compartments in neuronal cells of the cerebral cortex. Modification of membrane lipid composition is apparently associated with changes in protein composition of neuronal endo-

plasmic structures in winter-torpid ground squirrels as well.

The winter-torpor state of Yakutian ground squirrel is accompanied by a profound decrease in rRNA synthesis and processing and by the decrease in protein synthesis in neurons of brain cortex [41]. The ribosome lifetime also increases [42]. These observations are completely consistent with ultrastructural characteristics of nucleoli (Fig. 1) and cytoplasm of pyramidal neurons (Fig. 2) from the CA1 hippocampal area of the animals studied. Virtually complete cessation of neuronal protein synthesis occurs at least in the mid-bout, which manifests as changes in nucleoli in which fibrillar centers appear surrounded by condensed heterochromatin. The presence of these fibrillar centers corresponds to the inactive nucleolus. Three areas are visible within the nucleolus, which correspond to different structural states of chromosomal DNA–protein complex, such as euchromatin, heterochromatin, and centromere domains (Fig. 1, a and b). In the state of normothermy, this picture is retouched by the components of RNA-synthesizing complex (rRNA and RNA-polymerase) with higher electron density. Against their background, the basic structure of nucleolar DNA is practically not visible (Fig. 1, c and d). In Fig. 1, one can see a great amount of identical ring structures (apparently, the enzyme plus RNA). The borders of chromosomal domains in the form of thin curves are also visible. One can anticipate that the cessation of rRNA synthesis takes place in nucleoli during torpor [12]. The disassembly of free and membrane-associated polyribosomes (rough endoplasmic reticulum) and the decomposition of smooth reticulum and dictyosomes occur herein. All these processes are reversible, since the morphology of these organelles is restored already 3 h after the provoked arousal from the mid-bout and it negligibly differs from that in ground squirrels 2 days after arousal or in summer ground squirrels.

In spite of the observation that a distinct disassembly of smooth and rough endoplasmic reticulum membranes and dictyosomes takes place during the torpor, the protein yield of microsomal fraction and its electron-microscopic characteristics are virtually independent of the seasons or functional state of ground squirrels (Table 1, Fig. 1). The entrance into torpor is apparently accompanied by the decrease in cholesterol, LPC, and PEA levels, as well as by prominent quantitative and qualitative changes in other membrane components, particularly proteins. Activation of phosphorylation of membrane proteins in brain as well as the appearance of a specific tyrosine-phosphorylated membrane-bound protein in brain of torpid mammals have been demonstrated [43, 44].

Areas consisting exclusively of lipids have been found in endoplasmic reticulum membranes of brain neurons and other tissues of hibernating 13-lined ground squirrels *Spermophilus tridecemlineatus* with body temperature of 4°C [45]. One can anticipate that the decrease in cholesterol, PEA, and LPC levels as well as qualitative and quantitative changes in proteins during the entrance into torpor result in formation of membrane areas consisting exclusively of lipids. Possibly, the reversible formation of the lipid areas is important for maintenance of cell viability under the low-temperature conditions.

We were interested in determining 3-D structures of the studied microsomal fractions using serial ultrathin sections. The Fig. 3 shows only nine serial sections with the microsomal membranes represented mainly as vesicles and, rarely, as membrane tubules. However, two-dimensional profiles of microsomes after three-dimensional reconstruction really form a complex anastomosing system of cisterns (Fig. 4). This 3-D reconstruction is similar to the interrelated cistern system of endoplasmic reticulum as well as to dictyosomes in intact cells. The data obtained here suggest that microsomal fraction is represented mainly by the membrane fragments of Golgi apparatus and endoplasmic reticulum. Hence, there is the reason to suppose that the changes in lipid composition found in microsomal fractions may be more probably assigned to the membranes composing endoplasmic reticulum and dictyosomes in neuronal and glial cells from brain cortex.

The changes in viscosity of cell membranes during the entrance into torpor and the arousal are probably important for the previously revealed processes, such as reversible retraction of dendrite spinule [39, 40]. Changes in lipid composition of cell membranes might involve pre- and postsynaptic membranes of axons and dendrites and finally result in modification of already existing synapses. In this connection, the data obtained here on the change in lipid composition of cell membranes may reflect morphological changes of dendrite spinule [39, 40] connected with reversible synaptic plasticity [46].

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