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REVIEW

Lipids in Mammalian Hibernation and Artificial Hypobiosis

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Abstract—Membrane lipids—phospholipids, fatty acids, and cholesterol—participate in thermal adaptation of ectotherms (bacteria, amphibians, reptiles, fishes) mainly via changes in membrane viscosity caused by the degree of fatty acids unsaturation, cholesterol/phospholipids ratio, and phospholipid composition. Studies of thermal adaptation of endotherms (mammals and birds) revealed the regulatory role of lipids in hibernation. Cholesterol and fatty acids participate in regulation of the parameters of torpor, gene expression, and activity of enzymes of lipid metabolism. Some changes in lipid metabolism during artificial and natural hypobiosis, namely, increased concentration of cholesterol and fatty acids in blood and decreased cholesterol concentration in neocortex, are analogous to those observed under stress conditions and coincide with mammalian nonspecific reactions to environmental agents. It is shown that the effects of artificial and natural hypobiosis on lipid composition of mammalian cell membranes are different. Changes in lipid composition cause changes in membrane morphology during mammalian hibernation. The effect of hypobiosis on lipid composition of membranes and cell organelles is specific and seems to be defined by the role of lipids in signaling systems. Comparative study of lipid metabolism in membranes and organelles during natural and artificial hypobiosis is promising for elucidation of adaptation of mammals to low ambient temperatures.

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The development and maintenance of structural organization by environmental exchange in substance and energy are characteristics of living organisms. If exchange intensity is governed by the rate of cell division and cell functions and is genetically determined, the rate of the genetic program depends on ambient temperature.

According to the van't-Hoff and Arrhenius rule, decreasing temperature by 10°C results in 2-2.5-fold decrease in exchange activity. This dependence is described by the temperature coefficient Q_{10}. Organisms whose temperature depends on ambient temperature are named poikilotherms or ectotherms. Homoiotherms (endotherms) are organisms (mammals and birds) capable of maintaining temperature in the range 35-42°C (within a certain range of ambient temperature). The origin of homoiothermy is likely to be due to adaptation of organisms to existence in a wide range of ambient temperature, which provided for development of specific properties of the nervous system [1].

Lipids are structural and functional components of cells because they are the main components of biological membranes and they participate in lipid-dependent signal systems. Lipids are supposed to play an important role in temperature adaptation because they have been shown to be significant in physicochemical and functional properties of biological membranes and regulation of metabolism.

Phase transitions in lipids and temperature adaptation. Phospholipid bilayers with inclusions of sterol molecules and integral proteins are characteristic of biological membranes. Two temperature-dependent physical states of phospholipid bilayer are recognized: gel (solid phase) and liquid-crystalline state (liquid phase). Various phase transitions occur on temperature change. Using model membranes, it was shown that the phase transition temperature depends on lipid composition, pH, calcium concentration, membrane voltage, and some other factors. Gel/liquid-crystal phase transitions are believed to be very important for biological functioning of membranes [2-4].

Lipid composition of bacteria appears to vary over a wide range depending on medium composition, and the fraction of unsaturated fatty acids is higher in bacteria living in northern areas. For ectotherms (fishes and helminths), the degree of unsaturation of fatty acids of phospholipids was shown to depend on ambient temper-

ature. Temperature is the main factor influencing lipid composition and spectrum of fatty acids of tissues of ectotherms. However, phospholipids, cholesterol, and polyunsaturated fatty acids were found to participate in seasonal and functional changes of vital activity. The data are considered in the context of the role of lipids in modification of membrane structure during temperature adaptation of ectotherms [5].

So, phase transitions in lipids are considered to play a very important role in adaptation of living systems to ambient temperature and the origin of homoiothermy. Mechanisms of regulation of body temperature of mammals are initiated on changes in hypothalamus temperature by several hundredths of a degree. For all participants of metabolism, the dependence on such small temperature changes is known only for the gel/liquid crystal phase transition temperature in lipid membrane [5]. It is supposed that the need for maintenance of a certain temperature range for functioning of phase transitions in lipids is responsible for the origin of homoiothermy [2, 4].

Natural hypometabolic states—the ability for reversible suppression of metabolic rates and maintenance of vitality during prolonged existence at low temperatures (down to –2°C) without water and food—are typical of some homoiothermal species and remain an intriguing fundamental phenomenon.

Biology of mammalian hibernation and artificial hypobiosis. Under unfavorable environmental conditions (cold, starvation) some mammals are able to periodically enter a natural hypometabolic state in the form of hibernation. Cyclic decrease in body temperature and decrease in energy consumption alternating with return to the normal state occur during hibernation. The persistence and depth of torpor and the duration of the active state in the course of hibernation are different in various animals and environmental conditions. The Q_{10} value is different for various mammalian tissues, e.g. for mice the Q_{10} coefficient of respiration intensity decreased in the series: brain–heart–liver–skeletal muscle [1]. Brain metabolism is the most sensitive to temperature decrease (maximal Q_{10}) and almost does not participate in heat production in hypothermy [6].

During hibernation body temperature of the ground squirrel *Spermophilus undulatus* decreases from 37-38°C (active state) to 2-4°C (torpor), heartbeat frequency decreases to 3-5 beats/min (active state value 200-300 beats/min) [7, 8], and number of breathing movements drastically decreases to 4-6 instead of 100-200 per minute [9]. For hibernating animals consumption of oxygen and energy decreases 100-fold: from 0.2 cal/(g·min) (active ground squirrel) to 0.002 cal/(g·min) (torpor) [8-10]. Economy of energy during hibernation is 80% [11, 12]. During hibernation ground squirrels are able to withstand body temperature decrease to –2°C [7]. Decoupling of oxidative phosphorylation in mitochondria by specific protein uncouplers plays an important role in thermogenesis of hibernating animals, the most part of the heat being produced by brown fat tissue [6]. Hibernation is governed by the CNS together with endocrine systems and intracellular regulation [13].

Using pharmacological media and decrease in ambient temperature, mammals that do not normally hibernate can be brought into a state of hypobiosis, so-called "chill narcosis". Brain electrical activity decreases similarly to its decrease at natural hypobiosis but with some differences. For most of these mammals, body temperature allowing spontaneous return to life on warming is in the range 15-23°C. Decrease in energy consumption (6-7-fold) in artificial hypobiosis is more than one order of magnitude lower than in hibernation [14].

According to Giaja's procedure, animals are placed in a closed chamber and cooled to 4°C under conditions of increasing hypoxia and hypercapnia, thus modeling conditions in the burrow of hibernating animals [15]. For rats, body temperature decreases to 14-23°C during 3.5-4 h (further temperature decrease leads to death). After removal from the chamber, the animal remains in hypobiosis for some time, and then its temperature spontaneously returns to the normal value without any further variations from it. Prolonged artificial hypobiosis of mammals requires special approaches described in details by Timofeev [16]. Thus, the temperature of artificial hypobiosis after cooling of mammals according to Giaja is 15-20°C higher than that during normal hibernation of hibernants. It should be noted that depression of energy metabolism under chill narcosis is one order of magnitude less than in torpor during hibernation. Comparative study of mechanisms of chill narcosis and mammalian hibernation lies in the field of fundamental physiology, astrobiology, and practical medicine.

Seasonal and functional changes of lipids in organs and tissues of hibernating mammals. All of the annual cycle of the hibernation process is under genetic control [11]. There is an annual cycle of accumulation of lipids, mainly triglycerides. For some hibernants, seasonal accumulation of lipids (in summer and in early autumn) was observed even under strictly limited access to food. Lipids, mainly fatty acids formed by lipolysis of triglycerides, are used as an energy source instead of glucose during hibernation of mammals [17]. Lipids are constituents of various structures of mammalian cells—membranes, organelles, and even chromatin [18]. The role of lipids in temperature adaptation of mammals is usually considered in the context of the effect of viscosity of the lipid phase on membrane functions.

Metabolism of lipids is governed by metabolism of proteins participating in synthesis, degradation, transfer of lipids, and specifying localization of lipids in cell compartments. Hibernation causes changes in protein metabolism at all stages beginning with gene expression, translation, phosphorylation, and conjugation of proteins [18]. In brain of hibernating horseshoe-nosed bats *Rhinolophus*

ferrumequinum, superexpression of 41 genes related with regulation of cell cycle and apoptosis, neurons, signal transfer, and neuroprotection is observed as compared with the active genes [19]. Posttranslational modification of proteins with small ubiquitin-like modifiers (SUMO) is detected during hibernation [19, 20].

Appearance of proteins and peptides able to decrease the level of metabolism in blood plasma and tissues of hibernating mammals is a specific feature of protein metabolism during hibernation [21]. It has been shown that enzymes of lipid metabolism are changed. Redistribution of enzymes of fatty acids metabolism—synthase and acyl CoA synthetase of fatty acids—between nucleus, mitochondria, and cytoplasm is observed in liver cells of a true hibernating animal—the hazel dormouse *Muscardinus avellanarius* [22]. Decrease in activity of cytosolic phospholipase A_2 in liver and muscle of the ground squirrel *Spermophilus tridecemlineatus* due to activation of dephosphorylation of the enzyme is observed in torpor [23]. Enhanced formation of acyl-transferring proteins is noted as evidence for activation of fatty acid metabolism of hibernating animals [24].

Phospholipids. Small tissue-specific changes in phospholipid composition depending on season and the stage of hibernation were detected in tissues of hibernating mammals [25]. Increased concentration of phospholipids excluding phosphatidylethanolamine (its concentration decreased) was found in blood plasma of hibernating American black bear [26]. Distinctly manifested seasonal changes, namely, 4-fold increased concentration of sphingomyelin and 2.5-fold decreased concentrations of phosphatidylcholine and cardiolipin in summer compared with those for the active winter animals are specific for lipid composition of cerebral cortex of the Yakutian ground squirrel *Citellus undulatus*. Concentrations of phosphatidylethanolamine, phosphatidylserine and lysophosphatidylcholine for the active winter animals were less than those for summer animals. The concentration of phosphatidylinositol did not change [27]. Seasonal changes of phospholipid composition in brain were not earlier detected for hamsters and other facultative hibernants [25]. Adaptation of the Yakutian ground squirrel to the severe life conditions possibly caused significant seasonal variations of phospholipid concentration in neocortex. In torpor the total phospholipid concentration in neocortex decreased by 20% compared with the summer period, and the concentration of all other phospholipids was less than that for the summer animals and did not differ from that for active winter animals [27]. Thus, tissue phospholipids take part in seasonal changes of metabolism and hibernation of hibernating mammals.

Fatty acids of organs and tissues of hibernating mammals. Fatty acids are the main energy substrate during hibernation of mammals [28, 29]. The concentration of fatty acids in blood plasma, skeletal muscles, heart, and liver of the Yakutian ground squirrel increases during the hibernation season and in the state of exit from torpor [30, 31]. While studying the role of fatty acids in hibernation it was found that prolonged dietary deficiency (lack of unsaturated fatty acids) resulted in decreased hibernation period. Lack of saturated fatty acids did not affect the length of the hibernation period [32]. A diet enriched with unsaturated fatty acids increased the length of the hibernation period and decreased body temperature in torpor. A diet enriched with saturated fatty acids resulted in decreased hibernation period [33]. While studying the effect of diet with 5% content of stearic, oleic, and linoleic acids on hibernation parameters of the chipmunk *Eutamias amoenus*, it was found that for the group supplied with stearic acid the duration of hibernation was almost two times shorter than for the two other groups. For the groups supplied with unsaturated fatty acids the critical point of temperature regulation was shifted towards to lower temperature. The concentration of unsaturated fatty acids in fatty tissue and mitochondrial phospholipids was shown to increase, hibernation parameters being correlated with their composition. Polyunsaturated fatty acids also positively influenced hibernation parameters [34]; higher concentration of these acids in the organism affected hibernation. Unsaturated fatty acids are supposed to play a functional role in metabolism of hibernants [11]. Increased concentration of fatty acids in blood and tissues during hibernation is caused by enhanced lipolysis in fatty tissue and metabolic change to lipids as the main substrates for oxidation. Blood exiting from white fat is enriched with saturated fatty acids compared with fatty acids of white fat [34]. Increased concentration of fatty acids activates the processes of non-shivering thermogenesis [31]. The data indicate that the degree of unsaturation of fatty acids of tissue phospholipids does not directly correlate with torpor. The functional role of fatty acids is brought to the fore [29].

Cholesterol in organs and tissues of hibernating mammals. Analogously to fatty acids, cholesterol influences hibernation parameters. When for two months before hibernation a chipmunk was kept on a diet enriched with cholesterol, a seven-fold increase in cholesterol concentration in blood and tissues was observed. Hibernation parameters also improved: the minimal body temperature and energy consumption (via oxygen consumption) decreased and at −1°C became more prolonged. Cholesterol is supposed to play a role in improvement of hibernation parameters either directly or via change in lipid composition of tissues [35]. Cholesterol concentration in brain of facultative hibernants usually did not change during the annual cycle and in hibernation [36]. Seasonal change was not detected in cerebral cortex of a true hibernating animal, the ground squirrel *Spermophilus undulatus*. In torpor the cholesterol concentration decreased by 30% compared with that in the active state [37]. Changes in cholesterol concentration during hiber-

nation are tissue-specific: in liver, another important organ of lipid metabolism, cholesterol concentration is increased compared with the summer period [38], the concentration of cholesterol sulfate responsible for activation of some genes being decreased [39]. Increased cholesterol concentration was detected in blood plasma of a hibernating American black bear: his body temperature decreased from 37 to 31°C, which is closer to artificial hypobiosis of mammals [26]. Thus, torpor of hibernants is accompanied by decreased cholesterol concentration in brain and its increase in blood plasma and liver. Earlier no changes in cholesterol concentration were detected in kidney and liver of hibernating animals [36].

Cholesterol in the structure of receptors. Non-membrane lipids can participate in metabolic regulation. The fact that proteins have specific cholesterol-binding sites was demonstrated by fluorescence resonance spectroscopy. The presence of cholesterol-binding sites in G-protein coupled receptors (GPCRs) is of particular interest. There is a cholesterol-sensitive domain in these proteins, which in turn is associated with biosynthesis and transport of cholesterol. A cholesterol-consensus motif with a specific amino acid sequence was found in crystalized β_2-adrenergic receptor. The receptor-bound cholesterol does not belong to the group of membrane lipids, and its interaction with protein is due to the presence of specific binding sites. Non-membrane bound cholesterol influences the structure and activity of receptors. Nicotinic acetylcholine receptor 250 kDa contains 5-10 cholesterol-binding sites per receptor. The presence of serotonin receptor$_{1A}$ was studied in biological samples containing cholesterol. Serotonin receptor$_{1A}$ appeared to be present in a wide variety of biological materials (from bacteria to humans) as evidence for its preservation in the course of evolution. Specific binding of lipid to GPCRs is also demonstrated for fatty acids. A fatty acid-binding site was found in Ca^{2+},Mg^{2+}-ATPase [40]. Changes in concentration of cholesterol and fatty acids during hibernation may also concern to non-membrane cholesterol and fatty acids.

Cholesterol and fatty acids in regulation of nuclear transcriptional activity in tissues of hibernants. Lipids of cell nuclei are participants of cell signaling systems [41, 42]. A superfamily of nuclear receptors that are transcriptional factors binding lipophilic substances as ligands has been characterized. Fatty acids, cholesterol, and its derivatives—oxysterols and cholesterol sulfate—are ligands for some nuclear membrane receptors. It is supposed that changes in transcriptional activity dependent on genes that are targets for nuclear receptors using cholesterol and fatty acids as ligands occur in tissues of hibernants [43].

Cholesterol and fatty acids influence expression of several genes regulating energy homeostasis, metabolism of carbohydrates and lipids, inflammatory reactions, and circadian rhythm via these receptors. Regulation of the stearoyl-CoA-desaturase gene by fatty acids and cholesterol via nuclear receptors is of particular interest. The membrane-bound enzyme stearoyl-CoA-desaturase (SCD) is a limiting enzyme for synthesis of monounsaturated fatty acids from saturated ones. The ratio of unsaturated and saturated fatty acids effects membrane viscosity. Activity of the SCD gene is regulated by lipophilic receptors located on the nuclear membrane. Fatty acids and cholesterol are ligands for these receptors. Polyunsaturated fatty acids and cholesterol influence transcription and stability of SCD mRNA [44]. It is supposed that increased concentrations of cholesterol and fatty acids during hibernation can activate SCD, resulting in increased concentration of polyunsaturated fatty acids in tissues during hibernation [43]. Thus, cholesterol and fatty acids influence functional pathways leading to changes in membrane viscosity.

Seasonal and functional changes in lipids of neocortex membranes of hibernants. The lipid composition of membranes and cell organelles is determined by rates of synthesis, degradation, and transport of lipids and depends on the functional roles of membranes and cell organelles. Lipids are participants of signaling systems, and participation of lipid in response reactions to stimulus is accompanied by change in its concentration. Study of intracellular localization of lipids can show specific participation of membranes and cell organelles of organs and tissues in mechanisms of hypobiosis. Earlier studies of lipids in microsomes isolated from brain of several rodent species did not reveal any regular changes in phospholipid composition [36]. The concentration of phosphatidylcholine and phosphatidylethanolamine only slightly but reliably increased in microsomes of hamster brain on hibernation [45]. The effect of hibernation on lipids of membranes and organelles is not well studied, and the specific participation of membrane lipids in seasonal and functional changes in membranes of hibernants has been studied only in membranes of endoplasmic reticulum of neocortex of the Yakutian ground squirrel. In contrast to facultative hibernants, significant seasonal changes of phospholipid composition of membranes were detected only for a true hibernating animal, the Yakutian ground squirrel, although the total concentration of phospholipids for the active summer and winter animals did not differ. The concentration of phosphatidylcholine in the microsomal fraction (endoplasmic reticulum and dictyosomes) of neocortex for winter ground squirrels is more than twofold increased compared with the summer animals, and the concentration of sphingomyelin for the active winter animals was fourfold decreased compared with the summer ground squirrels, as was already demonstrated for neocortex tissue. Seasonal changes are also typical of phosphatidylinositol of microsomes: its concentration almost twofold decreased in winter, whereas no change in phosphatidylinositol was detected in neocortex tissue.

The concentration of phospholipids in microsomes drastically (by 50%) decreased in torpor during hiberna-

tion compared with that for the summer and active winter animals. The concentration of phospholipids providing fluidity of membranes, lysophosphatidylcholine and phosphatidylethanolamine, decreased by 30-40% compared with that for the summer and active winter animals [27, 46]. Thus, contrary to the data obtained for neocortex tissue, seasonal decrease in concentration of phosphatidylinositol, the initial component of the inositol phosphate cycle, was detected in the microsomal fraction of cerebral cortex. This may be considered as an adaptation for increasing resistance [47]. For animals in torpor, decreased concentration of phosphatidylethanolamine and lysophosphatidylcholine was found. Decreased concentration of these phospholipids in torpor correlates more with functional changes in metabolism of lipids than with homeo-viscosity adaptation. Comparison of the effects of season and hibernation on cholesterol concentration in tissue and the microsomal fraction of neocortex is of great interest. For the hamster *Mesocricetus auratus*, which are true hibernants, cholesterol concentration in microsomes of neocortex was found to slightly decrease during hibernation compared with the active winter animals [45]. Season did not effect cholesterol composition in microsomes of neocortex of Yakutian ground squirrels, but in torpor cholesterol concentration decreased threefold [27]. A functional role of changes in cholesterol concentration in cells and membranes under various conditions has been studied in several works. Increased cholesterol concentration is often accompanied by activation of some functions and increased vitality. For rats, increase in cholesterol concentration in membranes of endoplasmic reticulum of liver coincides with enhanced synthesis of proteins as a response to damaging action of ionizing radiation [48]. In turn, for ground squirrels a drastic increase in cholesterol concentration in microsomes of neocortex in active state of hibernation coincides with activation of protein synthesis in neurons [49, 50]. For mice, damage of renal tubules (ischemia or other insults to the organism) was accompanied by increased resistance of tubular cells to further damage. This secondary resistance was accompanied by increased cholesterol concentration. For cells of renal tubules, it was shown that suppression of cholesterol synthesis or addition of fluidizing agents to the medium resulted in increased cell invasion [50]. Effects of cholesterol are rationalized by its ability to influence organization and temperature of phase transitions of phospholipid bilayers [51] and by the role of cholesterol in the structure of lipid rafts [52].

In neocortex of hibernants cholesterol possibly participates in development of so-called endoplasmic reticulum stress (ER stress). Violations in cell energy metabolism cause ER stress including suppression of synthesis of chaperons and accumulation of unfolded proteins. It has been shown that synthesis of resident chaperons providing maturation of unfolded proteins and their integration into ER membranes with simultaneous activation of synthesis of membrane lipids is induced via the system of unfolding protein response (UPR) [53]. Cholesterol is supposed to play an important role in ER stress [54]. A chaperon, glucose-dependent protein GRP78, was shown to be accumulated in brain and brown fat tissue of the ground squirrel *S. tridecemlineatus*. Accumulation of GRP78 was not detected in heart, kidney, liver, lungs, and skeletal muscles of hibernating ground squirrel [55]. As mentioned above, cholesterol concentration decreased during hibernation in ER of hamster brain and neocortex of ground squirrels *S. undulatus* [27, 45]. Thus, for hibernants, entering into hibernation and development of ER stress was accompanied by exit of cholesterol from ER. Studies of lipid composition of ER of functionally different tissues during hibernation compared with the effect of artificial hypobiosis elucidate the role of cholesterol in ER stress of mammals. Studies of cholesterol concentration in tissues and organelles during hibernation are important in relation with various aspects of its structural and functional roles.

Decrease in cholesterol, phosphatidylethanolamine, and lysophosphatidylcholine concentrations during hibernation was observed simultaneously with changes in morphology of ER and dictyosomes of pyramidal neurons from the CA1 field of the hippocampus: during hibernation polyribosomes decomposed to monosomes and endoplasmic reticulum membranes disassembled. The data indicate that lipids participate in changes in morphology of membrane structures in torpor of hibernation [27].

Changes in lipid composition depending on the season and functional state of another membrane structure of neocortex neurons, synaptosomal membrane, appeared to be specific. The phospholipid composition of synaptosomal membrane of neocortex of the Yakutian ground squirrel changed annually analogously to ER phospholipids. However, cholesterol concentration in synaptosomal membrane of neocortex of the Yakutian ground squirrel decreased during the hibernation season. Unlike the microsomal fraction, cholesterol concentration in synaptosomal membrane in torpor did not change compared with the active state, and the concentration of phosphatidylethanolamine increased by 30% compared with that for the active winter animals. For animals in torpor, accumulation of phosphatidylethanolamine in synaptosomal membrane during the seasonal decrease of cholesterol concentration coincided with almost twofold increase in synaptosomal area [46]. Phosphatidylethanolamines contain a wide variety of unsaturated fatty acids, easily form hexagonal structures, and fluidize membranes [56]. Seasonal decrease in cholesterol concentration and increase in phosphatidylethanolamine concentration in torpor indicate that lipids of synaptosomal membrane of neocortex participate in homeo-viscosity adaptation [46].

The data indicate that membrane lipids play an important functional role in hibernation, participate in

homeo-viscosity adaptation, and correlate with changes in morphology of membrane structures of neocortex during torpor.

Lipids of organs and tissues in artificial hypobiosis and hypothermia of mammals. Study of hypometabolic states of non-hibernating mammals is of fundamental as well as of practical interest because it touches on mechanisms of adaptive reactions important for medicine and astrobiology. The effects of artificial hypobiosis on lipid metabolism are barely studied in spite of the fact that elucidation of the role of lipids in thermoregulation is very important. It was shown that hypobiosis of rats (body temperature 20°C) was accompanied by 60% increase in concentration of saturated fatty acids in blood, unsaturated fatty acid concentration being five times decreased. Analogous changes were observed in myocardium tissue [16]. However, the state of artificial hypobiosis of rats at body temperature 15-22°C did not influence total and individual concentrations of phospholipids, fatty acids, and mono- and diglycerides of neocortex [37]. The concentration of fatty acids in blood of mammals increased under the influence of catecholamines and glucocorticoids and was observed under immobilization stress, ionizing radiation, and other physical and chemical agents [16, 57, 58].

Alpha-adrenergic reactive systems also regulate cholesterol metabolism in blood. For rats, hypothermy is accompanied by increased cholesterol concentration in blood detected after cooling of animals in water for 5 min at 20°C. Under these conditions, cholesterol concentration in rat brain decreased by 12-15% [57]. Cholesterol concentration in rat neocortex demonstrated a tendency for decrease in the state of artificial hypobiosis [37]. The data on cholesterol concentration in blood and brain of several mammals under hypo- and hyperthermia, formation of conditioned reflex, emotional stress, and under other conditions are presented in a monograph by V. N. Gurin [58]. All types of action caused increase in cholesterol concentration in blood and its decrease in brain. Decreased cholesterol concentration under stresses and hypothermia is specific for neocortex: for dogs, hypothermia was accompanied by increase in cholesterol concentration in liver [58]. Data on the effect of artificial hypobiosis on metabolism of lipids are few, but it can be concluded that the effects of artificial hypobiosis and various kinds of stress on metabolism of fatty acids in blood and cholesterol in brain are similar. Decrease in cholesterol concentration in brain and its increase in blood seem to be a stage in the systemic response of mammals to the action of environmental agents. The same direction of changes in concentration of fatty acids in blood and cholesterol in neocortex was observed during hibernation [11, 27, 31].

Lipids of membranes and nuclei of neocortex cells under artificial hypobiosis. The effect of artificial hypobiosis on membrane lipids and cell organelles is as specific as the effect of natural hypobiosis. While studying lipid composition of nuclei of neurons and glia under the same conditions as were followed while studying lipids of neocortex tissue, it was found that in nuclei of neurons and glia cholesterol/phospholipid ratio increased by 60%, and in glia nuclei of neocortex sphingomyelin and cholesterol concentrations increased by 60 and 50%, respectively [59]. These changes in lipid composition of nuclei of neurons and glia may indicate that functions of the organelle are significant for changes in lipid composition. Increased cholesterol/phospholipids ratio in nuclei of neurons and glia may be rationalized as evidence for activation of nuclear functions. Decrease in cholesterol concentration in neocortex by 30% during artificial hypobiosis [37] may be a consequence of export of cholesterol from the tissue to the bloodstream. Nuclei of neurons and glia express mRNA of receptors participating in functioning of the hematoencephalic barrier and responsible for cholesterol homeostasis [60]. Changes in cholesterol concentration in cell nuclei of neocortex may be related with this process. Sphingomyelin of nuclei plays an important role in cell functions and vitality [42]. Increased sphingomyelin concentration seems to be caused by inhibition of sphingomyelinase, possibly for suppression of production of proapoptotic ceramides and growth of vitality of glia cells.

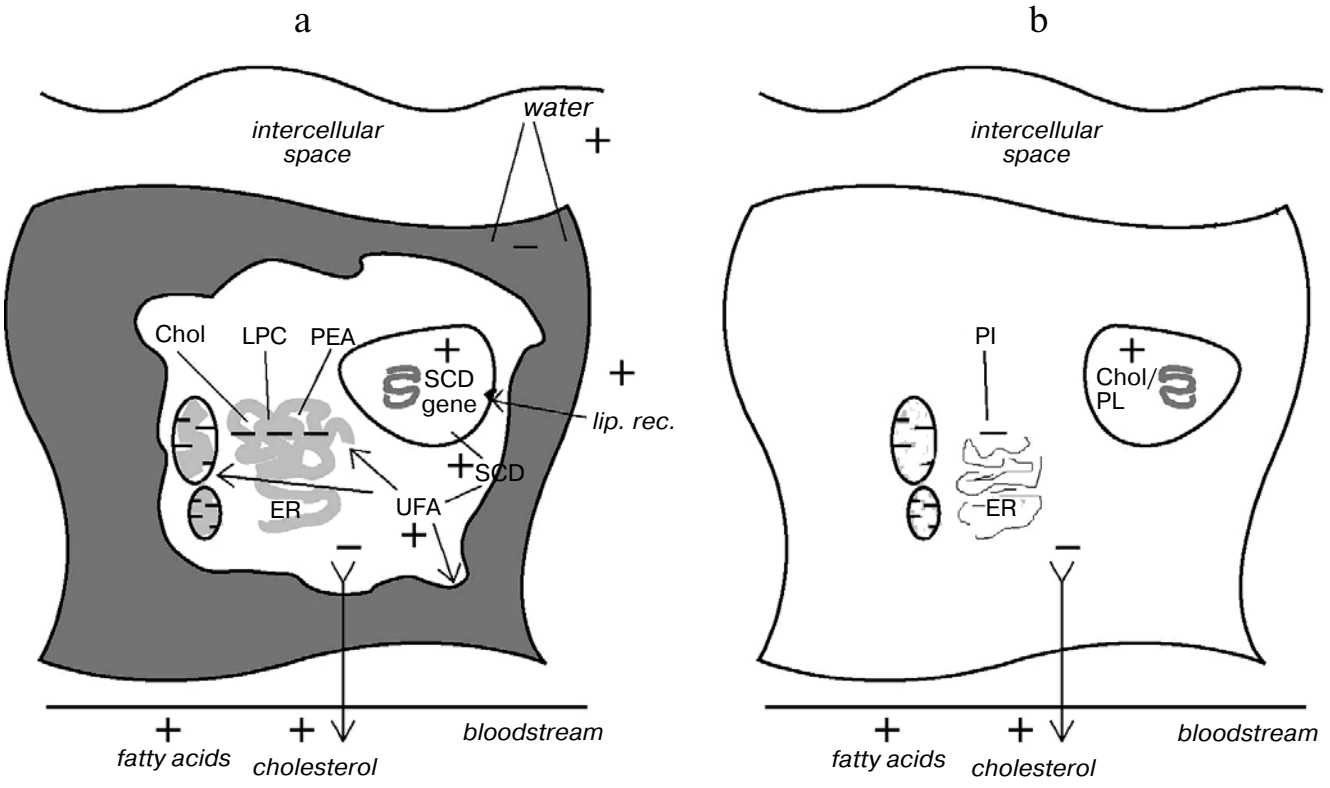
For rats, the concentration of phosphatidylinositol in microsomes of neocortex decreased by 30% in the state of anabiosis, the concentration of phosphatidylinositol being unchanged in neocortex tissue, the nuclei of neurons and glia [61]. It was shown that entering into artificial hypobiosis is accompanied by enhanced release of catecholamines into blood [16]; catecholamines can cause a decrease in phosphatidylinositol concentration in membranes of endoplasmic reticulum [62]. It is known that hydrolysis of phosphatidylinositol by phosphatidylinositol-dependent phospholipase C results in production of inositol-3-phosphate and diglycerides, which activate cell metabolism. Thus, changes in lipid composition of ER membranes (microsomal fraction) of rat neocortex in the state of artificial hypobiosis are totally different from changes in lipid composition of the microsomal fraction of neocortex of Yakutian ground squirrel in torpor during hibernation [46, 59].

Lipids and morphology of neurons during hibernation. As mentioned earlier, when animals enter into hibernation, it was found that membranes of endoplasmic reticulum and dictyosomes in neurons disassembled [27], morphology of synapses changed, spicules in hippocampus retracted [63, 64], and neuron body areas of various brain sites decreased by 35-40% as compared with those for the active state of the ground squirrel *Spermophilus lateralis* [65, 66]. Morphologic changes (construction of mitochondrial structures) in torpor were also observed in liver [67]. Suppression of mitochondrial functions was ascribed to the condensed state of structures in animals in

torpor. Using electronic microscopy, it was found that liver mitochondria of hibernating ground squirrels maintain the condensed state for a long time [67]. The role of condensation of cell structures in the resistance of the organism to low temperatures was indicated by N. N. Timofeev [16].

Changes in cell morphology in hypobiosis under conditions of hibernation can be based on changes in structure of macromolecules—proteins and nucleic acids—perhaps by the action of regulatory signals organizing hibernation. A study of the temperature dependence of enzyme activity for active and hibernating ground squirrels [68] showed that for hibernants changes in thermotropic properties of enzymes are governed by preparation for torpor rather than the effect of temperature. An inflection temperature on the Arrhenius curve for the temperature dependence of enzyme activity appeared to be different for several enzymes of various endotherms during hibernation [68]. The inflection point on the Arrhenius curve for the enzyme of internal mitochondrial membrane is not related with the phase transition of lipids of this membrane [69]. These data suggest that the structural state of proteins plays the main role in change in thermotropic properties of enzymes in torpor.

Considering physiochemical factors defining the direction of biological evolution, S. E. Schnoll indicated the possible role of temperature dependence of structural transitions of all the three basic components of cell—proteins, water, and lipids—in the origin of homoiothermy [2]. Specific properties of water as the main mass component of cell structures are suggested to be a basis for the origin of homoiothermy [70, 71]. Using microdialysis, it was shown that water homeostasis is involved in hibernation of hamster brain: water was observed to exit from cells to the intercellular space in torpor [72]. This observation agrees well with data on a decrease in neuron body areas in torpor [65, 66]. On entering into torpor, water loss from the cell is a stage when structures are condensed, and thus their conservation under the conditions of suppressed metabolism is provided [16]. Membrane properties are significant for the dynamics of water. Changes in lipid composition influence the lifetime of



Participation of lipids in metabolism and morphology of neocortex cells in torpor during hibernation of hibernating animals at body temperature from –2 to +4°C (a) and during artificial hypobiosis of rats by the "closed chamber" method [15] at body temperature 15-22°C (b). The scheme was drawn using the references given in square brackets. Parameter increase and decrease in torpor are denoted by "+" and "–", respectively. a) *Water* [72], shaded region – cell area decrease in torpor [65, 66]; Chol, LPC, and PEA designate cholesterol, lysophosphatidylcholine, and phosphatidylethanolamine of ER and dictyosomes [27, 46]; *lip. rec.*, lipophilic nuclear receptors [39]; SCD gene, gene of stearoyl coenzyme-A desaturase; SCD, stearoyl coenzyme-A desaturase [44]; UFA, unsaturated fatty acids [67-69]; ER, endoplasmic reticulum; *cholesterol* [26, 27], *fatty acids* [30]. b) PI, phosphatidylinositol [61]; Chol/PL, cholesterol/phospholipids ratio [59]; *fatty acids* [16], *cholesterol* [37, 57]

pores through which water passes through the lipid membrane. Peptides and proteins also influence permeability of water pores in lipid membrane [73]. Preparation for structural changes is regulated by the seasonal cycle and includes specific changes in metabolism of lipids. The data indicate that lipids participate in changes in cell morphology in torpor during hibernation, providing cell resistance and reversibility of structural changes. The participation of lipids in metabolism of neocortex cells in torpor during hibernation of hibernating mammals and in artificial hypobiosis is presented in the figure.

Lipids participate in phylogenetically developed adaptation (hibernation) of mammals to low ambient temperatures at all the levels of organization, beginning from the annual rhythm, regulation of hibernation parameters, activity of genes, energy metabolism, and the state of intracellular structures. Lipids play a role in regulation of hypometabolic states of cells during natural mammalian hypobiosis via signal mechanisms, influencing gene expression, thus changing membrane viscosity and receptor functions. Special features of phylogenetic adaptation of mammals to low ambient temperatures are revealed in comparison with the effects of artificial hypobiosis. The role of lipids in system response of mammals to low temperatures is shown in comparative study of lipids in organs and tissues under conditions of natural hibernation and artificial hypobiosis. Changes in fatty acids and cholesterol concentrations in blood, liver, and brain are similarly directed in torpor. Different character of changes in lipid metabolism in torpor during hibernation and artificial hypobiosis are distinctly shown for membrane lipids. Comparison of changes in morphology and lipid composition of membranes suggests that lipids participate in formation of the structural state of low molecular weight cell components on phylogenetically developed adaptation of mammals to low ambient temperatures. Comparative study of lipid metabolism during natural and artificial hypobiosis is promising for elucidation of the participation of lipids in regulation of hypobiosis and their role in mechanisms of enhancing mammalian resistance to unfavorable environment conditions.

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