

Seasonal changes in the level of endogenous ethanol and acetaldehyde in the blood of large herbivorous mammals of the Arctic and Subarctic

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Abstract. *We investigate seasonal changes in the state of endogenous system of ethanol/acetaldehyde metabolites in the organisms of the large herbivorous mammals of the Arctic and Subarctic – reindeer (R) and Yakut breed horse (YH). We focus on the content of endogenous ethanol (EE) and endogenous acetaldehyde (EA) in the blood of the animals, along with the activity and concentration of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in their livers. We analyzed the involvement of the endogenous system in the adaptation mechanisms of the aboriginal herbivorous mammals to the extreme climate conditions at the high latitudes. The biomaterial (blood, liver) for the study was collected immediately after the slaughter in winter (December–January) and summer (June–July) in 2017–2019. The animals led an active lifestyle throughout the year in native environment. The number of R in each season made 30, the number of YH – 40. The seasonal temperature fluctuations ranged from –50 °C (in winter) to +38 °C (in summer). We used the method of gas chromatography with mass spectrometry (GC-MS) to determine the EE and EA concentration in the animals' whole blood. It has been shown that the levels of EE and EA in the blood of the large herbivorous mammals under study were significantly higher than those of the laboratory animals and humans. We detected seasonal dynamics in the content of metabolites, namely, a synchronous increase in their concentrations in the blood during the period of low ambient temperatures. The mechanism of the latter included seasonal changes either in isozyme forms of ADH and ALDH, which differed in their catalytic and physicochemical parameters in R, or in the concentration of enzymes in YH. These changes represent physiological and biochemical adaptive adjustments that increase resistance of the animals to the cold. An increase in the content of endogenous ethanol in the blood of YH and R prove that their reserve catabolic materials capable of generating biochemically useful energy under stressful conditions are included in the energy metabolism. Furthermore, an increase in the content of endogenous acetaldehyde represents a mechanism for reducing the overall level of bioenergetic processes with a redistribution of their intensity towards the increased generation of thermal energy. The physiological function of the system of endogenous ethanol, acetaldehyde and their metabolizing enzymes is to regulate (increase) the body's resilience to the stressful impact of the cold.*

Keywords: biorhythms, seasons, metabolites, ethanol, acetaldehyde, dehydrogenases, cold, adaptation, reindeer, Yakut horse

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Сезонные изменения уровня эндогенных этанола и ацетальдегида в крови крупных растительноядных млекопитающих Арктики и Субарктики

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Аннотация. Проведено исследование сезонных (зима, лето) изменений состояния эндогенной системы метаболитов этанол/ацетальдегид (содержание эндогенного этанола (ЭЭ) и эндогенного ацетальдегида (ЭА) в крови, активность и концентрация алкогольдегидрогеназы (АДГ) и альдегиддегидрогеназы (АльДГ) печени) в организмах северного оленя (СО) и лошади якутской породы (ЯЛ), ее участия в механизмах адаптации аборигенных крупных северных растительноядных млекопитающих к экстремальным климатическим условиям высоких широт. Объекты исследования (СО и ЯЛ) относятся к аборигенным северным холодаадаптированным животным, ведущим круглогодично активный образ жизни в нативных условиях среды. Сбор биоматериала (кровь, печень) проводили сразу после забоя в нативных условиях в 2017–2019 гг. в декабре–январе и в июне–июле; nСО в каждом сезоне составило 30, nЯЛ – 40 особей. Сезонные колебания температуры в период проведения исследований составляли от -50°C (зимой) до $+38^{\circ}\text{C}$ (летом). Для определения концентрации ЭЭ и ЭА в цельной крови животных использовали метод газовой хроматографии с масс-спектрометрией (ГХ-МС). Показано, что у крупных растительноядных млекопитающих Арктики и Субарктики (ЯЛ, СО) уровни ЭЭ и ЭА в крови значительно выше, чем у лабораторных животных и человека во все сезоны года. Выявлена выраженная сезонная динамика содержания изученных метаболитов, заключающаяся в синхронном повышении их концентраций в крови в период низких температур окружающей среды. Механизм последних – сезонные изменения либо изоферментных форм АДГ и АльДГ, различающихся своими каталитическими и физико-химическими параметрами (СО), либо концентраций ферментов (ЯЛ). Эти изменения и являются теми физиолого-биохимическими адаптивными перестройками, которые увеличивают устойчивость организма к холоду. Повышение содержания в крови ЯЛ и СО эндогенного этанола является свидетельством включения в энергетический обмен резервного катаболического сырья, способного генерировать биохимически полезную энергию в стрессогенных условиях, а повышение содержания эндогенного ацетальдегида является механизмом снижения общего уровня биоэнергетических процессов с перераспределением их интенсивности в сторону повышения генерации тепловой энергии. Физиологической функцией системы эндогенных этанола, ацетальдегида и ферментов их метаболизирующих является регуляция (повышение) устойчивости организма к стрессирующему воздействию холода.

Ключевые слова: биоритмы, сезоны, метаболиты, этанол, ацетальдегид, дегидрогеназы, холод, адаптация, северный олень, якутская лошадь

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Introduction

The organism of northern animals, adapted to cold, has specific morphological, physiological and biochemical characteristics, well-developed physi-

cal and chemical thermoregulation, established during the evolution and allowing them to exist in a sharply continental climate with an annual range of temperatures of about 100°C [1–4]. During the

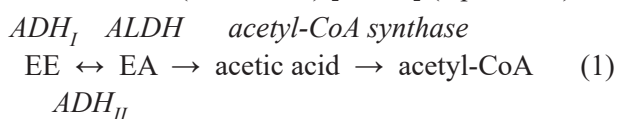
evolution under extreme climatic conditions (exposure to extreme cold, long light (in summer) or dark (winter) periods, sharp diurnal temperature differences (over 20 °C) geomagnetic disturbances, etc.), as well as with limited food availability in winter, the mechanisms for maintaining temperature homeostasis are formed and genetically inherited in the body of northern animals, which allow them to optimally perform their vital functions. Bioenergetic adjustments are an integral part of implementing the genetically inherent adaptation mechanisms [5–8].

Reindeer (*Rangifer tarandus*) and Yakut breed horse (YH) can be classified as large cold-adapted herbivorous mammals inhabiting in the Arctic and Subarctic. Reindeer (R) is naturally distributed in the Arctic and subarctic regions and is the only domesticated species among *Cervidae*. Yakut breed horse is the only horse breed with the unique properties that allow it to adapt to a year-round free grazing (in summer at the temperatures above +30 °C, in winter – below –50 °C). As a breed, YH has formed under the influence of extreme natural conditions in the high latitudes. Cold hardiness of R and YH is ensured by a well-developed physical and chemical thermoregulation and ethological features, allowing them to maintain a stable body temperature even under a very long exposure to very low ambient temperatures [1, 8, 10, 11] during the annual cycle. The ability of R and YH to endure winter cold is also associated with the high heat-insulating properties of their hair cover, which determines the minimum range of changes in their body temperature during the day [1, 4]. In preparation for winter, a decrease in the R body temperature has been identified, contributing to a decrease in the “environment-animal” temperature gradient and a more successful wintering [1, 3]. In winter, the mobility of the animals decreases; a statistically significant decrease in the level of metabolism and oxygen consumption is observed [1, 2]. In purebred YH, the minimum annual temperature does not fall below 31 °C. The level of metabolism of YH changes with a decrease in the ambient temperature, and it was found that at the temperature of minus (30–35) °C, the “winter” level of metabolism starts in the body, which is characterized by a decrease in the rate of metabolic reactions of the basal metabolism [1, 10]. On average, in winter, the horses’ level of metabolism is 40 % lower than in summer, and a decreasing respiratory quotient reaches the values that are not typical for herbivores. In summer months, R has the

highest average daily body temperature of R – up to +39.9 °C, compared to the minimum winter temperature of +33.0 °C [1]. In summer, YH body temperature can be higher for a longer time than in winter and depends on the ambient temperature to a lesser extent. Previous physiological and biochemical studies have shown that animals living in the North and leading an active lifestyle have less intensive respiration in winter, and oxygen aeration of organs and tissues drops by 38–40 % [1].

Under these conditions, the viability of organisms is to a certain extent ensured by switching of catabolic reactions from the oxidase to dehydrogenase metabolic pathways. At that, there emerges an additional opportunity of releasing the heat energy and maintaining a constant body temperature due to the residual activity of oxidase systems during the uncoupling of oxidative phosphorylation. When the level of aerobic reactions decreases, one of the possible adaptive mechanisms may be reservation and increase in the reduced entities’ content in the cells, that can serve as energy substrates, with endogenous ethanol (EE) being the most energetically intensive and, at the same time, rapidly involved in the metabolic processes. In terms of its bioenergetic efficiency (the amount of ATP per 1 g of bioenergetic substrate), ethanol is 1.75 times higher than glucose and only 1.38 times inferior to tripalmitin fat; its value as a bioenergetic substrate sharply increases when a nonspecific adaptive reaction “stress” is formed in the body [25].

In this regard, an important bioenergetic and regulatory role in the adaptation of animals to cold is played by the substrate-enzymatic system, which includes EE, endogenous acetaldehyde (EA), and their metabolizing enzymes: alcohol dehydrogenase – ADH (AP 1.1.1.1), and aldehyde dehydrogenase – ALDH (AP 1.2.1.3) [12, 13] (equation 1).



It is known that EE and EA are important metabolites of a number of strains of microorganisms, plant tissues, homeothermic and heterothermic animals [12–18]. The formation of EE can occur during the EA reduction, formed during pyruvate dehydrogenase during the abstraction of an intermediate product in the pyruvate decarboxylase complex [16], as well as during the decarboxylation of lactate accumulated in the red skeletal muscles during glycolysis under oxygen deficiency [18]. The metabolism

of EE and EA is associated with the basal metabolism through the formation of acetyl-CoA. EA is involved in the metabolism of biogenic amines, which, among other things, provide synaptic transmission of nerve impulses [17], as well as involve in the regulation of mitochondrial terminal oxidation [16].

The content of EE and EA and the ratio of their levels are mainly maintained by the dehydrogenase enzymatic system, consisting of two NAD-dependent enzymes: ADH and ALDH. ADH-dependent reaction is reversible, whereas ALDH-dependent reaction is irreversible. ADH-dependent conversion of EE occurs through its oxidation first to EA, then to acetic acid, followed by the formation of its conjugate with coenzyme A – acetyl-CoA (see equation 1), which further enters into a wide variety of catabolic and anabolic transformations. It has been shown that up to 95 % of ADH is contained in the liver, about 3 % in the surface mucous cells of stomach, and about 0.025 % in the brain [15–18]. Similar to ADH, two groups of ALDH isoforms are distinguished: with high affinity to aldehyde (Michaelis constant $K_{\text{Mald.}} = 0.1\text{--}1.0\text{ mcM}$) – ALDH_1 , and with low affinity to it ($K_{\text{Mald.}} = 0.1\text{--}1.0\text{ mM}$) – ALDH_2 [13, 16]. Due to the heterogeneity of the isozyme composition of both enzymes, the levels of EE, EA and their ratios can vary over a fairly wide range.

EE and EA are seen as metabolites that control a large part of the homeostasis mechanisms, ensuring the optimal vital functions of living organisms in various living conditions [14, 15, 17, 18]. In the course of evolution, the predominant catabolic function of the EE-EA system was replaced by the regulatory one [12, 14, 15]. The normal level of homeostasis in laboratory animals and humans is maintained with the EE concentrations of about 0.05–0.2 mM, and EA concentrations of about 0.3–0.8 mcM in the blood. The biological role of EE is diverse. (1) It is a high-energy compound, and under normal conditions it can provide up to 10 % of the body's energy needs; (2) it involves in maintaining the liquid-crystalline, fluid state of the lipid layer of membranes, fluidifying them [18–20, 21]; (3) it is a regulator of lipid peroxidation (LPO) in the cell membranes, showing the properties of a free radical scavenger and activating cholesterol synthesis [20, 21]. Dissolving well both in the glyco-protein and the lipid layer of the membranes, and reducing their viscosity, EE changes the conformation of membrane receptors, modulating their affinity to intracellular and intercellular regulators. (4) Ethanolamine molecules – a component of one of the most common types of

phospholipids in the cell membranes – phosphatidylethanolamines, are synthesized from EE [20, 21]. (5) EE is a cellular depot and a form of transportation for an important metabolic regulator, EA.

EA is chemically very active. It does not penetrate cell membranes, but it can change their permeability to other substances. The biological functions of EA are: (1) regulation (inhibition) of bioenergetic reactions in the chain of terminal oxidation of electron transfer from NADH to FAD by flavin enzymes; (2) regulatory modification (through the formation of Schiff's bases) of opioid peptides. (3) EA is involved in the synthesis of endogenous morphine and morphine-like compounds; (4) it regulates the metabolism of the most important neurochemical mediators of amine nature: dopamine, noradrenaline, serotonin; adrenaline hormone [16, 17]. (5) There is information about the effect of EA or its condensation products with catecholamines (salsolinol) on the functional state of opiate receptors [17]. All the above allows us to consider the EE-EA system an important element of nonspecific regulatory systems of the body, providing the body's adaptive capabilities to stress, including, apparently, the cold stress.

The aim of this work is to study seasonal (winter, summer) changes in the state of the endogenous ethanol/acetaldehyde system (the content of EE and EA in the blood, the activity of ADH and ALDH of liver) in the body of reindeer and Yakut breed horse, its involvement in the mechanisms of adaptation of aboriginal large northern herbivorous mammals to the extreme climatic conditions of the high latitudes.

The working hypothesis of the study is the assumption that an increase in the content of EE in the YH and R blood proves the inclusion in the energy metabolism of a reserve catabolic raw material, capable of generating biochemically useful energy under stressful conditions, and an increase in the EA content is a mechanism for a decrease in the overall level of bioenergetic processes with a redistribution of their intensity towards the increase in the generation of heat energy.

Material and method

The objects of study: reindeer (*Rangifer tarandus L.*) and Yakut breed horse, being the aboriginal northern cold-adapted animals, with an all-year-round active lifestyle in their native environmental conditions. The biomaterial (blood, liver) was collected immediately after a slaughter in the native conditions in 2017–2019 in December–January and

June–July; the number of R in each season was 30, whereas the number of YH made 40 individuals. The seasonal temperature fluctuations during the research period ranged from -50°C (in winter) to $+38^{\circ}\text{C}$ (in summer).

All research was conducted in compliance with the International Guiding Principles for Biomedical Research Involving Animals, based on the protocol of the Bioethics Committee of Yakutsk Scientific Center for Complex Medical Problems of the Siberian Branch of the Russian Academy of Sciences. Biochemical studies were carried out on the basis of the Department of Ecological and Medical Biochemistry and Biotechnology of the Institute for Biological Problems of Cryolithozone of the Federal Research Center, Yakutsk Scientific Center, SB RAS (Yakutsk, Russia).

To determine the EE and EA concentration in the animals' whole blood, the method of gas chromatography with mass spectrometry (GC-MS) was used [22]. To prepare the samples, 100 μl of whole blood was added by 100 μl of the internal standard (1-propanol), and dissolved in 500 μl of a 1.2 % solution of Triton X-100 in acetonitrile. It was centrifuged at 10,000g for 5 minutes, the supernatant was collected and put into the chromatograph. Agilent 7820A gas chromatograph with Agilent MSD 5975 mass spectrometric detector was used. Agilent HP-INNOWAX column (30 m $\cdot$ 0.25 mm, DF = 0.25 μm). The injector temperature was 220°C , forline temperature 280°C . The carrier gas was helium, the flow rate was 1.5 ml/min. The temperature gradient: $40^{\circ}\text{C} - 2 \text{ min}$, $40-200^{\circ}\text{C}$, 5°C/min , $200^{\circ}\text{C} - 5 \text{ min}$. The sample input volume made 5 μl , the input mode – with a split flow in a ratio of 1:100 and a speed of 150 ml/min. Detection conditions: source temperature (MS Source) 230°C , quadrupole temperature (MS Quad) 150°C . The scanning parameters from 10 to 500 masses, the gain factor – 16 (2518 V). The scanning speed is normal. When constructing a calibration curve, standard solutions of ethanol, acetaldehyde, and an internal standard were used in the concentration range from 0.02 mg/l to 1 mg/l.

For kinetic studies, the following procedure was used to purify the enzymes from the animals' liver. The frozen liver was washed from blood by repeatedly perfusing it in a cooled physiological saline solution ($T = 0^{\circ}\text{C}$). After perfusion, the liver should be pale yellow. In the cold, the liver was chapped and then weighed. Liver was homogenized

in a mechanical homogenizer; the end-point dilution of the homogenate was usually 2:1 (2 ml of 0.05 M glycine solution and 1 g of liver). To remove the completely destroyed cells and nuclei, the tissue homogenate was centrifuged for 30 minutes at 7000 G ($T = +2^{\circ}\text{C}$). The supernatant was carefully tapped out. The loose sediment containing nuclei and intact cells was discarded. Cooled ethanol (0°C) of a final concentration of 20 % was added to the supernatant. The mixture was intensively shaken and then re-centrifuged. The supernatant was passed through a column with Sephadex G-100 (1.5 $\times$ 90 cm), equalized with 0.5 M glycine solution ($T = +2^{\circ}\text{C}$). In the selected fractions, the activity of alcohol and aldehyde dehydrogenases was determined. The fractions with the maximum content of enzymes were selected for studying, by measuring the absorption of proteins at 280 nm.

When determining the concentration of liver ADH, the ADH active centers titration method with hydroxymyrcuribenzoate (HMB) was used, based on the high affinity of mercury-containing inhibitors to the SH-groups of the enzyme's active center [20]. Conditions: 0.1M Na-phosphate buffer, pH 6.5; concentrations: HMB – $0.02 \div 3.0 \text{ mM}$; NADH – 0.45 mM; acetaldehyde – 4 mM. The ADH concentration was calculated taking into account its dimeric form (2 inhibitor molecules are used for the titration of one ADH molecule).

The heterogeneity of liver ADH was studied by starch electrophoresis, using a modified technique described in the work of (Tsukamoto et al, 1980).

The ADH and ALDH activity in micromole/min per 1 g of liver [13], the ADH and ALDH Michaelis constant (K_M) for ethanol and acetaldehyde [19] was determined by a two-beam recording spectrophotometer Shimadzu UV – 2600 (Japan). The ALDH isoforms were differentiated into two groups by the kinetic method [22].

The conducted research is based on a simple random sampling. The normal distribution laws were checked using the Shapiro-Wilk test. The following characteristics of the sample were calculated: sample mean (M), standard error of the mean (m). The result was considered statistically significant at $p < 0.05$. To test the hypothesis about the presence of mean differences in the groups, a two-tailed Student's test (t) was used. To process the results, we used a statistical processing experimental data package in MS Excel and the statistical program Stat Plus 2007, Professional.

Results and discussion

The obtained results prove seasonal (winter-summer) changes in the EE and EA content in the blood and in the ADH and ALDH activities of liver, of both YH and R (table). Moreover, in all seasons under study, the EE concentration in these animals'

blood was significantly higher than in laboratory animals under the conditions of the North [14]: for YH in summer by 3.53 times, in winter – by 5.75 times; for R, by 11.8 times in summer and 8.8 times in winter.

The blood of the studied animals has shown a statistically significant increase in the EE concentration

Seasonal characteristics of the endogenous ethanol/acetaldehyde system in the body of large herbivorous mammals of the high latitudes

Сезонная характеристика эндогенной системы этанол/ацетальдегид в организме крупных растительноядных млекопитающих высоких широт

Parameters	Yakut horse		Reindeer	
	Winter ($n = 40$)	Summer ($n = 40$)	Winter ($n = 30$)	Summer ($n = 30$)
Activity of $ADH_I^{(1)}$ (mcM/ min · g liver)	2.12 ± 0.21	2.41 ± 0.20	0.55 ± 0.04	1.10 ± 0.09 * ($p = 0.000001$)
Activity of $ADH_{II}^{(2)}$ (mcM/ min · g of liver)	6.04 ± 0.40	6.92 ± 0.43	1.45 ± 0.12	5.81 ± 0.30 * ($f = 58$; $p = 0.000000$)
Concentration of ADH (nmol/g of liver)	103.08 ± 12.13	119.12 ± 12.02	39.32 ± 3.13 ** ($f = 68$; $p = 0.000000$)	38.94 ± 3.02 ** ($f = 68$; $p = 0.000000$)
$k_{cat} ADH_I^{(1)}$ (min^{-1}) (nmol/g of liver)	20.57 ± 2.42	20.23 ± 2.04	14.00 ± 1.11 ** ($f = 68$; $p = 0.000000$)	28.24 ± 2.19 ** ($f = 68$; $p = 0.000000$)
$k_{cat} ADH_{II}^{(2)}$ (min^{-1}) (nmol/g of liver)	58.60 ± 6.85	58.09 ± 5.85	36.88 ± 2.88 ** ($f = 68$; $p = 0.000000$)	149.20 ± 11.27 ** ($f = 68$; $p = 0.000000$)
$Km_{ADH-ethanol}^{(3)}$ (mmol/l)	0.50 ± 0.06	0.41 ± 0.05	0.80 ± 0.07	0.60 ± 0.04 * ($p = 0.016088$)
$Km_{ADH-aldehyde}^{(4)}$ (mmol/l)	0.42 ± 0.03	0.30 ± 0.03 * ($p = 0.0059$)	0.60 ± 0.05	0.40 ± 0.03 * ($p = 0.001129$)
Activity of ALDH mcM/min · g of liver) including the share (%):				
ALDH _I ⁽⁵⁾	35.12 ± 3.25	35.23 ± 3.92	35.12 ± 3.25	45.23 ± 3.92
ALDH ₂ ⁽⁶⁾	64.88 ± 5.63	64.77 ± 4.76	64.88 ± 5.63	54.77 ± 4.76
Km_{Aldh1} (mcM/l) ⁽⁷⁾	0.83 ± 0.32	1.02 ± 0.21	1.21 ± 0.14	1.02 ± 0.11
Km_{Aldh2} (mcM/l) ⁽⁸⁾	70.02 ± 10.02	73.17 ± 11.12	100.13 ± 12.02	90.14 ± 12.11
[ethanol] _{blood} mmol/l	0.92 ± 0.08	0.60 ± 0.05 * ($p = 0.001098$)	1.89 ± 0.11 ** ($f = 68$; $p = 0.000000$)	1.41 ± 0.10 * ($p = 0.002064$) ** ($f = 68$; $p = 0.000000$)
[acetaldehyde] _{blood} mcM/l	9.51 ± 0.75	2.60 ± 0.24 * ($p = 0.000000$)	12.62 ± 0.76 ** ($f = 68$; $p = 0.004865$)	3.06 ± 0.28 * ($p = 0.000000$)
[EE]/[EA]	96.84	230.77	149.8	460.8

Note. The data is presented as $M \pm m$. * Reliability of difference as compared to a winter season. ** Reliability of difference between species in the same season.

⁽¹⁾ – activity of ADH in the ethanol oxidation reaction; ⁽²⁾ – activity of ADH in the acetaldehyde reduction reaction; ⁽³⁾ – ADH Michaelis constant for ethanol; ⁽⁴⁾ – ADH Michaelis constant for acetaldehyde; ⁽⁵⁾ – ALDH fraction is the most sensitive to acetaldehyde; ⁽⁶⁾ – ALDH fraction is less sensitive to acetaldehyde; ⁽⁷⁾ – ALDH_I Michaelis constant for acetaldehyde; ⁽⁸⁾ – ALDH₂ Michaelis constant for acetaldehyde.

in winter, compared to the summer period: YH by 1.53 times ($p = 0.001098$), R – by 1.34 times ($p < 0.01$). Moreover, in the blood of R, the level of EE both in winter and in summer was 2.0–2.4 times higher than of YH ($f = 68$; $p = 0.000000$; see the Table).

It was found that the EA level in the blood of the studied animals is higher than in the blood of laboratory animals (0.9–1.0 mM/l) in summer – by 2.9–3.4 times, in winter – by 9.5–12.6 times. The winter increase in the EA concentration in blood, as compared to the summer one, is 3.66 times for YH ($p = 0.000000$), for R – 4.12 times ($p = 0.000000$). At that, if in summer the content of EA in the blood of R is only 1.18 times higher than in the blood of YH, which is not significant, in winter these differences (1.33 times) become significant ($f = 68$; $p = 0.004865$).

We associated the obtained interspecies and seasonal changes in the EE and EA concentration with the corresponding changes in the activity and isozyme composition of ADH and ALDH.

In winter, in R liver of, the activity of ADH is reliably twice lower in the reaction of EE oxidation (ADH_I) and 4 times lower in the reaction of EA reduction (ADH_{II}), primarily due to a 2-fold decrease in the k_{cat} of ADH_I and a 4-fold decrease in the k_{cat} of ADH_{II} , i.e. due to a change not in the concentration of the enzyme, but in its isozyme spectrum in favor of less active forms of ADH, especially in the reaction of EA reduction. The calculation shows that this is what leads to a winter increase in the EE concentration in R blood by 1.34 times, and a simultaneous decrease in the activity of $ALDH_I$ by 1.29 times – to an increase in the EA concentration by 4.12 times. Therefore, the ratio of EE to EA concentrations in R blood in winter decreases, as compared to the summer period, by 3.08 times.

In YH liver, the activity of both ADH_I and ADH_{II} decreases in winter by 14–15 % due to a 16 % decrease in the ADH concentration. At that, the ADH concentration in Yakut horse liver is more than 10 times higher than in a human liver, 38 times higher than in laboratory rats, and in all seasons higher than in reindeer (2.62 times in winter and 3.06 times in summer; see the Table). In contrast to a relatively small winter decrease in the ADH concentration in YH liver, the EE concentration begins to decrease by 22 % due to the affinity of ADH for ethanol ($Km_{ADH-ethanol}$) and by 40 % for acetaldehyde ($Km_{ADH-ald}$), i.e., changes in the isozyme composition of ADH. The calculation shows that this is what leads to a winter increase in the EE con-

centration in R blood by 1.53 times, and a simultaneous decrease in ALDH activity by 1.64 times – to an increase in the EA concentration by 3.66 times. Therefore, the ratio of the concentrations of EE to EA in YH blood in winter decreases, in comparison with the summer period, by 2.38 times.

The obtained results confirm the validity of the working hypothesis. Apparently, the main goal of the winter increase in the EA level is the need for significant inhibition of aerobic bioenergetic processes aimed to reduce the level of basal metabolism and oxygen consumption in the body of YH and R, and, as a result, of the heat transmission coefficient, as well as switching of most of the processes of terminal oxidation of NADH and FADH to the heat energy production. The summer decrease in the EA concentration in the animals' blood aims to reduce the degree of inhibition of the terminal mitochondrial oxidation, which leads to the activation of aerobic bioenergetic processes and an increase in oxygen absorption, and thus, to the activation of heat production processes and an increase in summer body temperature [1, 2]. The winter increase in the EE concentration in the blood of R and YH contributes to maintaining the intensity of bioenergetic processes at a level sufficient to ensure the vital activity of the animals in the conditions of cold and anaerobic stress, due to the inclusion of reserve sources of biochemically useful energy in the bioenergetic exchange, whose dehydrogenase oxidation can be initiated under oxygen deficiency.

Both species of the studied animals are in open spaces all year round; they feed on poor food for a significant part of the annual cycle [1–4]. Moreover, since the evolution of a “Reindeer” species in the extreme conditions of the North by natural selection took much longer than that of a Yakut breed horse, the absolute concentrations of EE and EA in the blood and their seasonal changes in R are higher than in YH.

Conclusion

It has been shown that large herbivorous mammals of the Arctic and Subarctic (Yakut breed horse, reindeer) have significantly higher levels of EE and EA in their blood than laboratory animals and humans in all seasons of the year. Significant seasonal dynamics of the content of the studied metabolites was revealed, consisting in a synchronous increase in their concentrations in blood during the period of low ambient temperatures. The mechanism of the latter ones is seasonal changes either in isozyme

forms of ADH and ALDH, which differ in their catalytic and physicochemical parameters (reindeer), or in the concentration of enzymes (Yakut breed horse). These changes are those physiological and biochemical adaptive adjustments that increase the body's resistance to cold. The increased EE content in the blood of YH and R, apparently, makes it possible to maintain a body temperature during sharp (daily and seasonal) drops in the ambient temperature, to maintain a sufficiently wide thermoneutral zone and stable homeostasis within these temperatures.

The results of the studies indicate that during the evolution, the conjugated metabolites of EE and EA in the bodies of large homeothermic cold-adapted mammals with a year-round active lifestyle in the North form one of the systems for regulating the metabolic rate, that ultimately reduce the intensification of the bioenergetic exchange. Moreover, since the period of reindeer adaptation to northern conditions significantly exceeds that of a Yakut breed horse, the molecular mechanisms of the adaptation process at the level of epigenetic rearrangements have been formed in a R organism – the activation of alleles (loci) of the genome encoding ADH and ALDH isoforms with increased molecular activity. In the body of YH, the adaptation is simpler – a slight increase in the expression of genes encoding ADH and, apparently, ALDH, resulting in their increased concentrations.

This greatly expands the possibilities for the existence of northern species in extreme conditions. The physiological function of the system of endogenous ethanol, acetaldehyde and their metabolizing enzymes is to regulate (increase) the body's resistance to the stressful effects of cold.

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