Arctic Environmental Research

Arctic Environmental Research 18(3): 123–131
UDC 599.745.3: 591.111.1
DOI 10.3897/issn2541-8416.2018.18.3.123

Research Article

Features of the blood biochemical composition and adsorption-transport function of erythrocytes in the grey seal (*Halichoerus grypus* Fabricius, 1791) in the early postnatal period of development

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Academic editor: *Yuliya V. Bespalaya* ♦ **Received** 7 August 2018 ♦ **Accepted** 11 October 2018 ♦ **Published** 19 November 2018

Citation: Erokhina IA, Kavtsevich NN (2018) Features of the blood biochemical composition and adsorption-transport function of erythrocytes in the grey seal (*Halichoerus grypus* Fabricius, 1791) in the early postnatal period of development. Arctic Environmental Research 18(3): 123–131. https://doi.org/10.3897/issn2541-8416.2018.18.3.123

Abstract

The results of the study of certain biochemical parameters of blood in grey seals (*Halichoerus grypus* Fabricius, 1791) from birth to weaning are presented. In the blood plasma, 20 indices describing the state of the metabolism of proteins, carbohydrates, lipids, minerals (total protein, urea, creatinine, glucose, lactic acid, total lipids, triglycerides, cholesterol, calcium, phosphorus, sodium, potassium, magnesium, iron, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, creatine kinase, alkaline phosphatase, lactate dehydrogenase) are studied. It has been established that metabolic changes in the early period of postnatal development of grey seals occur in mammals in general, but the expression level of individual reactions may be considered a feature of pinnipeds. By the ratio of transaminase activity, the predominance of catabolism over anabolism was established in animals in the studied period of development. The most significant changes in the metabolism of grey seals were noted during weaning and the transition to self-feeding. The adsorption-transport function of erythrocytes was evaluated by washout on the erythrocyte membranes using 3% sodium chloride solution. It was established that all detected components of blood plasma are found in erythrocyte washings. The level of adsorption, expressed as a percentage of the content of a certain metabolite in the blood plasma, is not the same for different compounds. Enzymes, urea, creatinine, lipids are adsorbed most on the erythrocyte membranes (in some cases more than 100%), and glucose, lactic acid, cholesterol, iron, calcium are adsorbed in a smaller quantity (<50%). The data obtained indicate that the transfer of organic compounds on erythrocytes is more variable and demonstrative than shifts in the corresponding blood plasma parameters, and erythrocytes can participate in maintaining a range of concentrations of a number of substances in the blood plasma.

Keywords

metabolic adaptation of the grey seal, biochemical composition of the grey seal blood, adsorption-transport function of erythrocytes

Introduction

On the Murman shoreland, two large groups of breeding colonies of Atlantic grey seal (*Halichoerus grypus* Fabricius, 1791) are found: the western on the Aynov Islands and the eastern – on the Seven Islands Archipelago. In its international conservation status, the grey seal has a low degree of threat to existence but, in Russia and in several US states, it is a protected species and, in the Murmansk Region of Russia it is subject to blanket protection. This species is interesting owing to its position near the north-eastern limit of the contemporary range, where the adaptive capacities of the species are most pronounced. The first studies of the biochemical status of the grey seal colonies in Murmansk region were held by the Murmansk Marine Biological Institute of the Kola Scientific Centre of the Russian Academy of Sciences in 1991 and were subsequently fragmented. At the same time, it is difficult to exaggerate the importance of such studies in revealing general and particular mechanisms of adaptation of marine animals that determine their distribution and possible habitat in ecologically different environments and in predicting the fate of specific populations under changing environmental conditions. Particularly noteworthy are the early stages of animal development, as they account for the most intensive formation of the structural and functional systems of the body.

Red blood cells are able to adsorb on their surface various compounds from the blood plasma (Zbarskiy and Demin 1949; Dmitrieva and Kirda 1995; Troshkina et al. 2007; Cohen and Solomon 1976; Fulbright and Axelrod 1993; Kumar and Rizvi 2013), although the functional properties of these cells are still associated exclusively with their gas-transport function. Data on adsorption of proteins on the surface of erythrocytes appeared at the beginning of last century (Zbarskiy and Demin 1949), development of this topic having led to the conclusion that the adsorbed proteins affect erythrocyte deformability and blood rheology and, in addition, may be used in an emergency to replenish protein in plasma (Kikuchi and Koyama 1984). Further studies showed that transfer of organic compounds on erythrocytes is a more variable and demonstrative process than changes in relevant indicators of plasma; its adjustability and its dependence on the physico-chemical properties of endoglobular haemoglobin was established. All this is proof of the existence of a second function of erythrocytes, which was called the adsorption-transport function by analogy with the gas transport function (Gareyev 1999). Currently, the role of the adsorption-transport function of erythrocytes (ATFE) is established in the following physiological processes: 1) provision of fast and selective receipt of substances from the blood into tissues; 2) removal of metabolites, denatured proteins, atherogenic lipids and a number of substances with high chemical activity from the blood; 3) maintenance of the normal contents of certain substances in plasma; 4) possibility of biochemical processes on the surface of erythrocytes (Gareyev 2011). The few literature sources on the designated topic are dominated by data about ATFE study in laboratory animals and humans. It has been established that nucleus-free erythrocytes of mammals, as compared to lower organised species of animals, have a higher adsorption capacity. Of particular interest is the study of ATFE in marine mammals, which, owing to their lifestyle, have the features of structural and functional properties of blood, including changes in chemical composition during a prolonged stay underwater.

In connection with the foregoing, the aim of the present work is to study the content of metabolites in blood plasma and adsorbed on the membranes of erythrocytes in grey seals in the early postnatal development period.

Materials and methods

Material for the study was collected during an expedition to the Aynov Islands (Barents Sea) at the end of 2013. The animals studied were divided into three groups, depending on the stage of development, which in the early postnatal period of life are determined by the nature of feeding: infants, aged 0–1 weeks, n=6; actively feeding with mother's milk, aged 2–3 weeks, n=10; animal after milk feeding, aged 4–6 weeks, n=6.

The animal blood was taken from the extradural vein (Geraci and Smith 1975); heparin was used as the anticoagulant. Plasma was separated by centrifuging for ten minutes at a rate of 1500 rpm. Erythrocytes were washed three times with a chilled isotonic solution of sodium chloride. ATFE was evaluated according to the qualitative and quantitative composition of the washouts from the surface of the erythrocyte membrane. The procedure for washout preparation provides for integrity of the membrane structure and includes processing of packed red cells with a 3% sodium chloride solution, with subsequent incubation and centrifuging (Gareyev et al. 1998). A solution of sodium chloride was added to the packed red cells once, in a ratio of 3:1. The erythrocyte suspension obtained was carefully mixed and incubated at 37°C for five minutes, then centrifuged for five minutes at a rate of 1500 rpm; the supernatant (washout) was separated from the erythrocyte mass. As a result of this procedure, the substances adsorbed on the membrane of erythrocytes were transferred to the erythrocyte washout. The content of the main indicators of metabolism of proteins, carbohydrates, lipids, and mineral substances in blood plasma and in washouts from the surface of the erythrocytes was determined using conventional laboratory methods (Guide 2003). Concentration of substances in the washouts was counted per unit volume of packed RBC. The data obtained were processed by statistical methods (Zaitsev 1991). To assess the differences between samples, Student's t-test with a significance level of $p \leq 0.05$ was used. The study results are presented in the form of average values and standard errors ($M \pm m$).

Results

The level of the main indicators of metabolism of proteins, lipids, carbohydrates and mineral substances was determined in blood plasma of the studied groups of seals. The study results are presented in Table 1.

The biochemical composition of blood in newborn animals reflects adaptation associated with birth, when the supply of nutrients from the mother's blood ceases. At this time, the concentration of glucose, which is a key energy substrate, is extremely low. By the end of the period of milk feeding, this figure is reduced by more than half and the main source of energy is the fat from the breast milk.

During active feeding, the biochemical composition of blood changes as follows: reduced glucose and sodium content, reduced activity of aspartate aminotransferase, more than doubling of creatine kinase activity. By weaning, the composition of blood changes significantly in comparison with newborn and actively milk-fed animals. Thus, further reduction of glucose concentration to extremely low values of 0.85±0.12 mmol/l occurs. In addition, changes in the content of lactic acid, total lipids, iron, activity of γ-glutamyltransferase and alkaline phosphatase have been noted. The sodium content remains lower than in newborn animals. The activity of aspartate aminotransferase and creatine kinase is in the same position in relation to the indicators for newborn seals. The following indicators in blood plasma remain stable in animals from birth to weaning: urea, creatinine, triglycerides, phosphorus, magnesium, lactate dehydrogenase.

Along with the study of the biochemical composition of blood plasma in grey seal pups of different ages, the content of the main metabolites in washouts from the surface of erythrocytes was studied (Table 2).

It has been established that the traditionally defined components of blood plasma are also found in erythrocyte washings – total protein, urea, creatinine, glucose, lactic acid, total lipids, triglycerides, cholesterol, calcium, phosphorus, magnesium, iron, sodium, potassium, a number of enzymes (lactate dehydrogenase, aspartate aminotransferase, alanine

Table 1. Biochemical indicators of blood plasma in grey seals in the early postnatal period of development

Indicators	Animal age		
	0–1 week (n=6)	2–3 weeks (n=10)	4–6 weeks (n=6)
Total protein, g/l	69.84±2.10	72.06±1.76	72.40±1.24
Urea, mmol/l	14.09±2.02	14.96±2.52	19.84±3.00
Creatinine, μmol/l	58.22±9.17	59.20±4.85	72.20±5.10
Glucose, mmol/l	1.86±0.24	1.28±0.10*	0.85±0.12* (<0.02)
Lactic acid, mmol/l	7.52±0.68	7.10±0.84	12.25±1.06* (<0.01)
Total lipids, g/l	12.46±0.84	10.92±0.56	10.35±0.48*
Triglycerides, mmol/l	2.32±0.33	2.95±0.89	2.92±0.36
Cholesterol, mmol/l	8.63±1.30	9.20±1.82	11.92±1.47
Calcium, mmol/l	2.42±0.24	2.71±0.35	2.32±0.33
Phosphorus, mmol/l	4.44±0.57	4.16±0.70	3.18±0.89
Magnesium, mmol/l	1.29±0.11	1.05±0.10	1.15±0.14
Iron, μmol/l	59.65±2.23	62.21±3.10	41.26±2.14* (<0.001)
Sodium, mmol/l	169.40±2.25	155.00±2.90*	151.95±1.25*
Potassium, mmol/l	5.44±0.42	5.46±0.83	7.03±1.10
Lactate dehydrogenase, IU/l	1174.50±210.25	1284.23±110.46	1120.74±135.60
Aspartate aminotransferase, IU/l	45.2±3.26	31.72±2.15*	26.19±2.10*
Alanine aminotransferase, IU/l	25.75±1.30	24.12±1.44	20.50±1.12
γ-glutamyl transferase, IU/l	14.59±2.38	9.10±2.08	5.21±0.58*
Creatine kinase, IU/l	6.88±1.40	16.51±2.75*	16.58±3.37*
Alkaline phosphatase, IU/l	60.15±12.80	89.24±10.45	112.10±11.23*

Note. Hereinafter: *n* – number of animals; * are statistically significant differences compared to indicators of newborn animals; brackets denote the level of significance of differences compared to the previous period of development.

Table 2. Biochemical indicators of washouts from erythrocytes membranes in grey seals in the early postnatal period of development

Indicators	Animal age		
	0–1 week (n=6)	2–3 weeks (n=10)	4–6 weeks (n=6)
Total protein, g/l	67.85±2.12	61.47±2.04	60.81±1.20*
Urea, mmol/l	16.39±1.30	16.32±3.51	17.24±3.05
Creatinine, μmol/l	93.62±10.46	87.84±8.23	82.45±9.27
Glucose, mmol/l	0.26±0.04	0.22±0.01	0.16±0.02*
Lactic acid, mmol/l	2.15±0.16	2.84±0.20*	3.10±0.25*
Total lipids, g/l	11.56±1.50	10.24±1.10	12.68±1.45
Triglycerides, mmol/l	10.70±2.21	11.51±1.08	9.38±1.27
Cholesterol, mmol/l	0.86±0.15	1.30±0.24	1.94±0.33*
Calcium, mmol/l	0.12±0.01	0.35±0.04*	0.23±0.05*
Phosphorus, mmol/l	1.70±0.36	2.52±0.42	2.15±0.45
Magnesium, mmol/l	0.34±0.09	0.89±0.24	0.70±0.17
Iron, μmol/l	24.20±1.10	22.36±1.54	16.40±0.85* (<0.01)
Sodium, mmol/l	104.20±1.42	116.56±2.10*	118.25±1.20*
Potassium, mmol/l	2.10±0.16	3.56±0.24*	2.88±0.31*
Lactate dehydrogenase, IU/l	850.26±45.20	924.50±32.10	910.58±51.56
Aspartate aminotransferase, IU/l	35.36±4.10	62.86±6.08*	39.81±4.50 (<0.01)
Alanine aminotransferase, IU/l	19.90±2.10	30.56±4.21*	28.28±3.05*
γ-glutamyl transferase, IU/l	22.58±4.26	25.19±4.51	26.93±4.08
Creatine kinase, IU/l	3.26±0.54	10.23±2.06*	12.24±2.82*
Alkaline phosphatase, IU/l	24.55±2.10	64.60±4.14*	104.26±5.30* (<0.001)

aminotransferase, γ-glutamyl transferase, creatine kinase, alkaline phosphatase).

The biochemical composition of washings of the erythrocyte membranes of seals in the period from birth until weaned is undergoing more pronounced changes in comparison with the composition of blood plasma (Table 1). This is reflected primarily in the number of indicators showing variations in content during the observation period.

Erythrocyte washings of actively feeding seals contains eight such indicators, in seal after completion of milk feeding – 12. In plasma, the numbers are, respectively, 4 and 9. The range of fluctuations in the level of individual biochemical parameters is marked. For example, the activity of alkaline phosphatase adsorbed on erythrocyte membranes, increases more than four-fold by the end of the observation period. At the same time, the activity of this enzyme in plasma increases 1.86 times. A similar pattern is observed for creatine kinase.

A number of indicators (urea, creatinine, triglycerides, phosphorus, magnesium, lactate dehydrogenase) during the observation period did not undergo statistically significant changes, as well as in the blood plasma. It should be noted that the direction of changes in biochemical parameters, with the exception of sodium, are the same in both test fluids.

The level of adsorption, expressed as a percentage of the content of a certain metabolite in the blood plasma, is not the same for different compounds (Fig. 1). For this indicator, the studied compounds can be divided into three groups: 1) low (less than

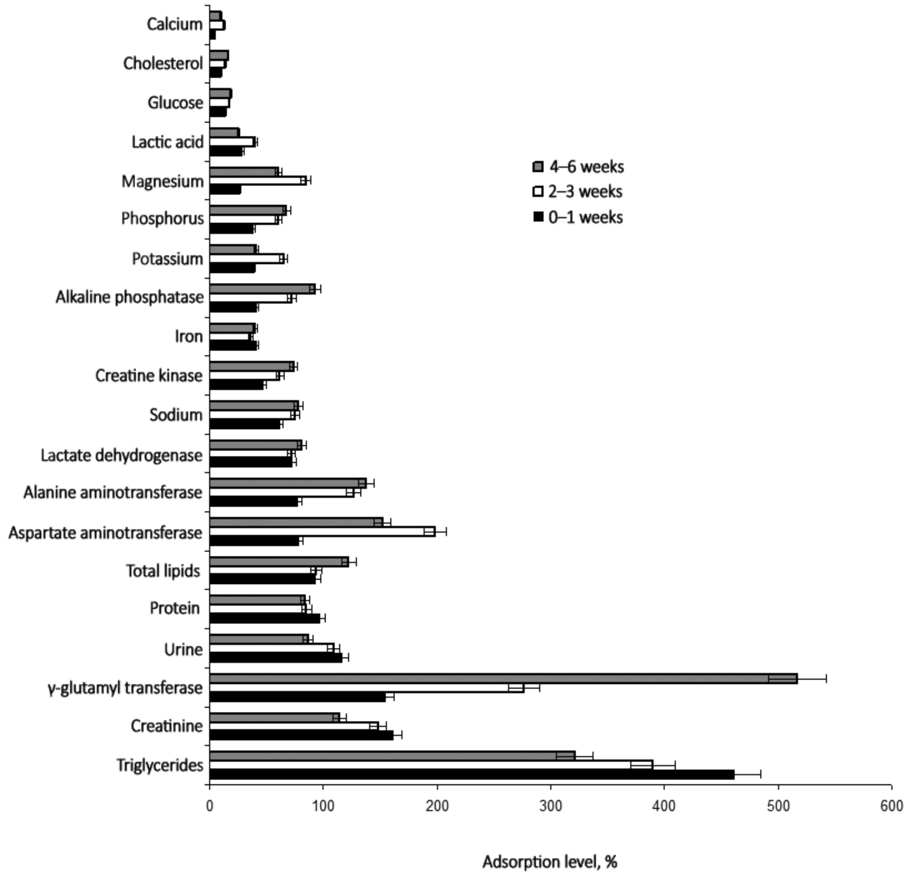

Fig. 1. The level of adsorption of metabolites on the surface of membranes of erythrocytes of grey seals of different ages (percentages in relation to the contents of a particular metabolite in blood plasma)

50%) – calcium, iron, cholesterol, glucose, lactic acid; 2) average (up to 100%) – magnesium, phosphorus, potassium, sodium, total protein, lactate dehydrogenase, creatine kinase, alkaline phosphatase; 3) high (over 100%) – creatinine, urea, total lipids, γ-glutamyl transferase, aspartate and alaninaminotransferase.

Dynamics of adsorption on erythrocytes in seals from birth until they are weaned is different for the studied metabolites. By the end of the observation period, this figure thus decreases for total protein, urea, creatinine, triglycerides, is almost constant for lactic acid, potassium and iron. All other parameters studied demonstrated an increase in the level of adsorption compared with that in newborn animals. The most significant fluctuations in the level of adsorption were observed for triglycerides and γ-glutamyltransferase. The adsorption of triglycerides in newborn animals is 461%, decreasing to 321% by the time they are weaned, thus exceeding the level of adsorption of other metabolites more than two-fold. γ-glutamyl transferase in newborn seals is adsorbed by erythrocytes at 155%; by the end of the observation period this figure rises to 517%. Is should be noted that, at the same time, the concentration of triglycerides in blood plasma is stable, the activity of γ-glutamyltransferase is reduced to almost a third.

Discussion

Early stages of animal development deserve special attention by researchers because they see the most intensive formation of structural and functional systems of the body reflected in the composition of the blood. The peculiarities of the chemical composition of blood plasma in newborn seals includes a low glucose concentration, since the influx of this basic energy substrate from the mother's body has ceased. The level of glucose continues to decline and, by the end of milk feeding, is only 0.85±0.12 mmol/l. The increase in the glucose level in the blood of seals starts at the age of 1.5–2 months (Erokhina 2007), and a significant increase (more than eight times) is observed only after the beginning of self-feeding, obviously as a result of formation of gluconeogenesis mechanisms.

The blood plasma of newborn seals is characterised by high activity of γ-glutamyltransferase (GGTF) and the low activity of alkaline phosphatase (ALP). GGTF is an enzyme associated with cell membranes of many organs (liver, heart, muscle, kidney, pancreas). There is information that GGTF can be used as a marker of passive transfer of immunoglobulins in newborn marine mammals, since colostrum and milk of lactating females are characterised by high activity of GGTF (Bossart et al. 2001). Similar data were obtained for terrestrial domestic mammals (Meyer and Harvey 1992). Yet the values of activity of GGTF for these animals are more than ten times higher than those for the newborn grey seals in our study, as well as the results obtained in the study of harp seal and hooded seal pups (Boily et al. 2006). The formation of passive immunity through immunoglobulin of the mother in pinnipeds thus occurs with less intensity than in land mammals.

Alkaline phosphatase (ALP) found in the blood plasma of adult animals is of hepatic origin and, in the early stages of ontogenesis, there is a significant amount of bone fraction of the enzyme. In marine mammals compared to terrestrial mammals, alkaline phosphatase activity is higher in all age periods (Bossart et al. 2001; Boily et al. 2006). There is evidence that the level of alkaline phosphatase in blood plasma of marine mammals is positively correlated with the intensity of anabolic processes in the body, whereby the concentration of the enzyme can be used as an indicator of animal fatness, as well as differentiating catabolic and anabolic states (Dover et al. 1993). In newborn grey seals, a low activity of alkaline phosphatase is found, which is statistically significantly increased only after they are weaned, reflecting, obviously, intensive growth of bone tissue. The same pattern is observed for creatine kinase (CK). It should be noted that the activity of CK in pinnipeds varies widely – from 21 to 4,572 IU/l (Bossart et al. 2001) and depends on the age (in adults it is several times higher than in young individuals), and this fact is attributed to differences in the physical activity of animals, unless caused by pathological conditions.

According to our data, in the period of active milk feeding, the composition of blood plasma of grey seals does not undergo significant changes, with the exception of four indicators: reduced concentration of glucose, sodium, aspartate aminotransferase and increased activity of creatine kinase. Attention should be paid to the activity of transaminases – aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These enzymes are the link between protein and carbohydrate metabolism, carrying out biochemical regulation of the pool of free amino acids. ALT reflects more the level of anabolism; AST, on the contrary, the intensity of catabolism, and the set of paired activity of ALT ⇔ AST is a simplified general marker of the entire metabolism (Rosliy and Vodolazhskaya 2010). As can be seen from Table 1, a common feature of the state of transaminases in seals in all studied age groups is the prevalence of the activity of AST over ALT, indicating predominance of the processes of catabolism over anabolism. During the observation period, the activity of AST decreases, and the ALT activity is almost constant. The catabolism and anabolism ratio is judged by the de Ritis coefficient – AST/ALT. In newborn seals, this ratio is 1.76, decreasing in the period of active feeding to 1.32 and staying at the same level (1.28) until after weaning. Thus, in conditions of deficiency of glucose (the main energy substrate), transaminases provide for amino acid transamination, involving them in the process of gluconeogenesis.

A noticeable change in the composition of the blood plasma is observed at the end of the milk feeding period. The activity of GGTF continues to decrease. In contrast, the activity of alkaline phosphatase increases, which confirms the above point of view (Dover et al. 1993) about the importance of the enzyme in the body condition score of animals. Transaminases (AST and ALT) and creatine kinase are characterised by the same state as in newborn seals and as in the period of active feeding. One should note the reduction in the concentration of total lipids in blood plasma by the end of milk feeding. We believe this is quite natural, because then the pups of the seals enter the period of natural fasting inherent for all pinnipeds, which lasts 4–5 weeks before the beginning of self-feeding. During this period, fat reserves accumulated in the period of milk-feeding are used up for life support, serving as a source of energy substrates, and are also an important link in the thermoregulation mechanism. From the beginning of self-feeding, the concentration of total lipids increases in the blood plasma of grey seals, like in other pinnipeds (Erokhina 2009).

At the end of milk feeding, the blood plasma of grey seals shows an increase in lactic acid by more than 50 per cent over the previous periods of development, which may be a sign of oxygen starvation of tissues. Yet this phenomenon has no significant effect on the acid-base status of blood: our studies showed that, in the first six months of life, there are no significant changes in the pH of the blood of grey seals and the value of this parameter is in the range of 7.09 to 7.16.

There is a noteworthy decrease in the iron concentration in blood plasma of seals at the end of the observation period to 41.26±2.14 μmol/l, which is, on average, one and a half times less than in newborns and animals in the period of active milk feeding. In healthy animals, the reduction in the level of iron in blood may be caused by its enhanced utilisation by organs and tissues, such as fast growth or increased physical activity. Without excluding this factor, we believe that iron is consumed mainly in the synthesis of haemoglobin. This is indicated by our data on the content of haemoglobin in the blood of the studied groups of seals. Newborn pups of grey seals thus have a rather high concentration of iron in plasma and a relatively low content of haemoglobin (141.32±8.10 g/l). It should be noted that, in representatives of terrestrial mammals the haemoglobin level is one and a half times lower (Yermolina and Sozinov 2005). Thus, at birth, the seals are characterised by a high oxygen capacity of the blood. During the period of active milk feeding, statistically significant changes in haemoglobin concentration are not observed (152.25±6.25 g/l). By the time seals are weaned, their plasma shows a reduction in the level of iron as the concentration of haemoglobin in blood increases to 165.47±7.24 g/l ($p<0.01$), which is comparable to the level in adult animals. It is likely that the further increase in haemoglobin levels at an older age is limited by reserves of iron in the blood plasma.

Against the background of the changes described above, in the biochemical composition of the blood of grey seals from birth to the end of weaning, the composition of metabolites adsorbed on the membranes of erythrocytes undergoes more pronounced changes. This is manifested in the number of compounds changing the concentration and the amplitude of the values of the individual indicators. Currently, it is not possible to discuss our findings comprehensively, given the absence of similar data on marine mammals in the literature. Works performed with erythrocytes of laboratory animals and humans are few in number, the set of studied metabolites is limited. Yet our results are comparable with the literature data and confirm the opinion that the transfer of metabolites on erythrocytes is a more variable and demonstrative process than the changes in corresponding serum parameters. ATFE studies in the works of medical orientation led to the conclusion that erythrocytes adsorbed on the surface of a significant number of metabolites are at risk of reducing the stability of the membranes and even destroying them (Oshakbayev et al. 2007). The content of adsorbed substances, which is much higher than their concentration in plasma, can thus be an indicator of a pathological process. In our study, adsorption of more than 100% for a number of indicators was observed: triglycerides, total lipids, creatinine, urea, γ-glutamyl transferase, aspartate and alanine aminotransferase. Even so, we are not inclined to connect this fact with diseases in the examined animals. External examination and general blood assay showed no signs of disease in these animals. At the same time, morphological and morphometric studies of erythrocytes of grey seals in the first month of life showed the presence in the blood, along with the prevailing normal cells, of reversible (echinocytes, stomatocytes) and irreversible (target cells, schistocyte, spherocytes) forms, immature and young erythrocytes (Minzyuk 2017). This data is indicative of active formation of the hematopoietic system of seals in this period.

Conclusion

It has been established that metabolic changes in the early period of postnatal development of grey seals occur in mammals in general but the expression level of individual reactions may be considered as a feature of pinnipeds. The most significant changes in the metabolism of grey seals were noted during termination of milk feeding and the transition to self-feeding. The ratio of the activities of enzymes of transamination indicates predominance of catabolic pathways of metabolism in animals in the studied period of development.

Erythrocytes of marine mammals are able to adsorb various compounds, as well as the erythrocytes of animals of other taxonomic groups. Possible features of this function are evident in its quantitative characteristics.

The work is performed with the financial support of FASO under state assignment 'Ecology and physiology of marine mammals of the Arctic seas' (SO No. 0228-2018-0017). The authors express their gratitude to the administration of the Kandalaksha state nature reserve for the opportunity to carry out the field work.

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