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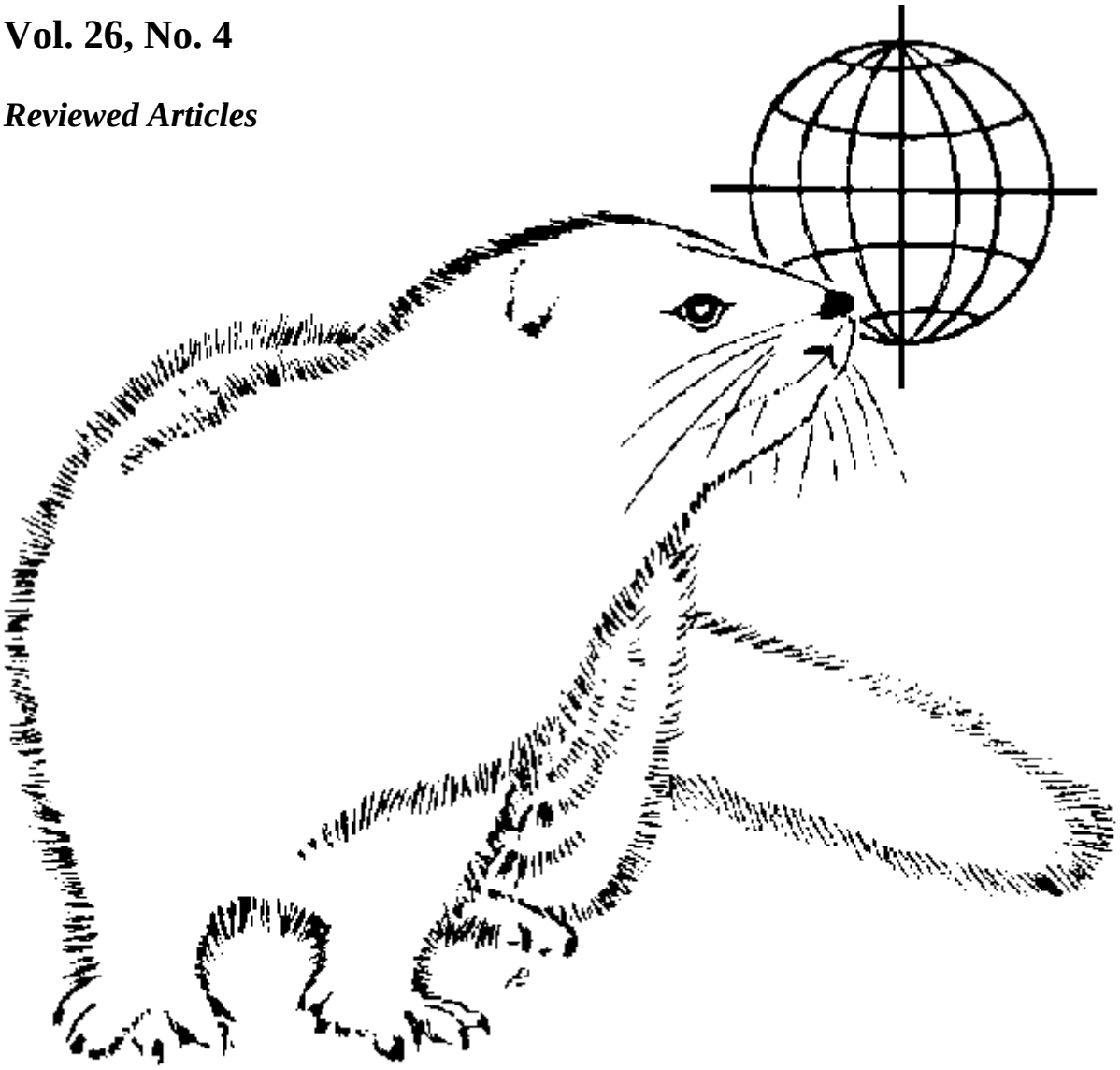
Reviewed Articles



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Notes from the Group of Editors

This electronic version of Scientifur is the fourth issue of volume 26. This issue contains a limited number of reviewed articles. We hope to receive a series of articles with a view to publish these as reviewed articles in volume 27.

It is our plan to continue the publication of one issue per year with reviewed articles only, and we hope that our readers will approve of this change. articles. We hope that our readers will approve of this change.

This issue is the last issue of volume 26, and in the near future the third and fourth issue will be published in a paper version as well.

All the people involved in the publishing of the journal will make every effort to ensure that all the issues of volume 27 will be published in 2003.

On behalf of the
Group of Editors

Birthe Damgaard

Superoxide dismutase and catalase in organs of three Canidae species.

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Abstract

The activities of the protective enzymes, total superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) were measured in organ homogenates of liver, kidney, heart, spleen and lung from three mammalian species of *Canidae* family (raccoon dog, silver and polar fox). Significant distinctions between these enzymes in different species were found in most of the organs. Raccoon dog had higher specific enzyme activity in all organs while in the silver foxes it was the lowest. A difference in the distribution or ratio of these enzymes in various tissues may result in ecological specificity of species.

Key words: catalase, polar fox, raccoon dog, silver fox, superoxide dismutase.

Introduction

The superoxide dismutase and catalase are considered to be specifically involved in the defence of the cell against the partially reduced active forms of oxygen which is necessary for lipoperoxidation and other cellular biochemical processes i.e. synthesis of biologically active substances. Resistance of the organism to environmental impacts and its adaptation capacity largely depend on the level of antioxidant enzymes and first of all superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) activity (Aebi & Wiss, 1978; Fridovich, 1975). SOD and catalase are important enzymes involved in protection of the cell from harmful effects of oxidative degradation. It is supposed that the free radical mechanism is involved in the development, aging and connected with the maximal life span (Lopez-Torres et al., 1993; Perez-Campo et al., 1993, 1994; Sohal et al., 1994). Extensive data about the level of these enzymes in humans and laboratory animals is available from publications (Marklund, 1984; Lopez-Torres et al., 1993; Perez-Campo et al., 1993, 1994). However, interpretation of the data obtained is problematic because investigated animals belong not only to different families, but also different classes and differ considerably in the body mass and metabolic rate. The purpose of the present study was to determine catalase and SOD activity in the liver, kidney, heart, spleen and lung in taxonomically closely related but differing in ecology three *Canidae* species - raccoon dog, silver and polar foxes.

Materials and Methods

Animals

Studies were carried out on 6-7-month-old clinically healthy polar fox (*Alopex lagopus L.*), silver fox (*Vulpes vulpes L.*) and raccoon dog (*Nyctereutes procyonoides Grey*). Blood samples were collected from all investigated animals for clinical-chemical analyses, and no abnormal changes were found in the blood indexes. Furthermore, no infectional diseases were observed on the farm. Five males and 5 females of each species were examined. Animals were kept on a farm under standard conditions and on paste-like diet with two meals a day as recommended for the species and water *ad libidum*.

Sampling and Treatment

Samples of tissues (liver, kidney, heart, spleen and lung) were collected during the slaughter season on

a fur farm. At pelting period (killing of animals was performed according to European Convention [TA-P (96) 19] recommendations) samples of tissues were frozen and stored at -25°C. The homogenates of these tissues were prepared in 0.05 M phosphate buffer, pH 7.0. They were centrifuged at 6000 g for 15 min, and the supematants were assayed for enzymes and proteins.

Assay methods

Total SOD activity was determined using the adrenochromic method (Misra & Fridovich, 1972), and catalase activity was assayed spectrophotometrically according to the amount of decomposed hydrogen peroxide (Beers & Sizes, 1952). The SOD assay is based on the spontaneous autoxidation of epinephrine in alkaline aqueous solution (pH 10.2) at 25°C, with the formation of endproducts which have an absorbance peak at 480 nm. This reaction dependent on the presence of superoxide anions, and it is specifically inhibited by SOD. The amount of enzyme that caused 50% inhibition of epinephrine autoxidation is defined as 1 unit. The method of CAT measurement is based on the reduction of hydrogen peroxide by the enzyme contained in the tissue sample. The rate of decrease of absorbance at 240 nm upon addition of a known amount of homogenate was followed in a spectrophotometer at 25°C. Extinction coefficient for H2O2 was e=43.6/M/cm.

The activity of the enzymes was calculated per 1 g raw tissue and 1 mg protein (specific activity). Protein concentrations were determined as described by Lowry (Lowry et al., 1951) using the bovine serum albumin as the standard.

Statistical analysis

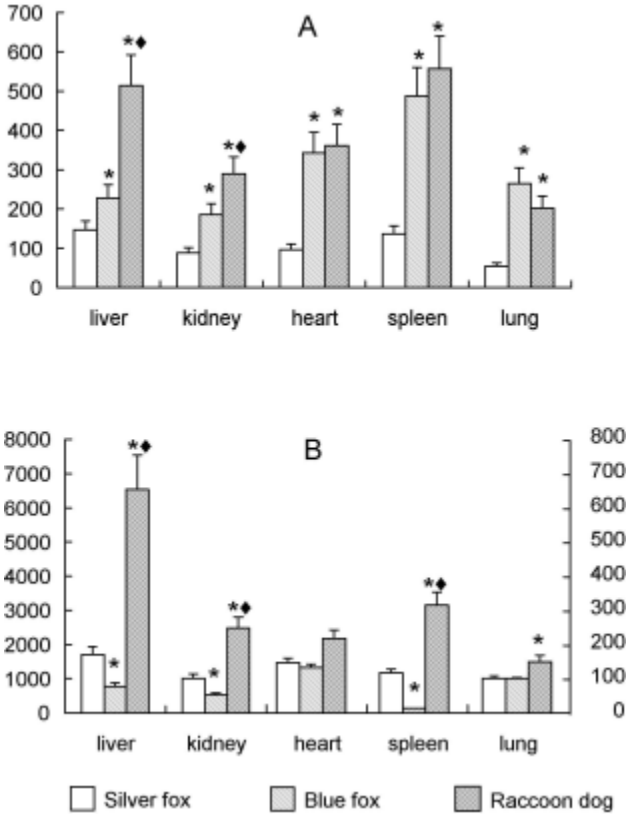
Data are presented as mean ± standard error of the mean (SEM). For statistical analysis of results, Mann–Whitney U test was used with a level of $P < 0.05$ chosen to indicate significant differences. The same organs samples from Silver fox were compared with Polar fox and raccoon dog samples.

Results and discussion.

There were significant differences between individuals as well as species in the SOD and CAT activities in the same organs. Silver fox had the lowest SOD activity in all organs, while raccoon dogs – the highest (except lung). (Fig. 1).

Figure 1

SOD (A) and catalase (B) activities in different organs in Silver fox, Polar fox and Raccoon dog



X-axis: examined organs

Y-axis: activities of SOD (arbitrary unit/g wet weight) and catalase (μmol H_2O_2/g/min).

Left scale: the activity of catalase in liver and kidney.

Right scale: for the rest of the organs.

*: the differences are statistically significant as compared to Silver foxes.

♦: the differences are statistically significant as compared to Polar foxes (P<0,05, Mann-Whitney test).

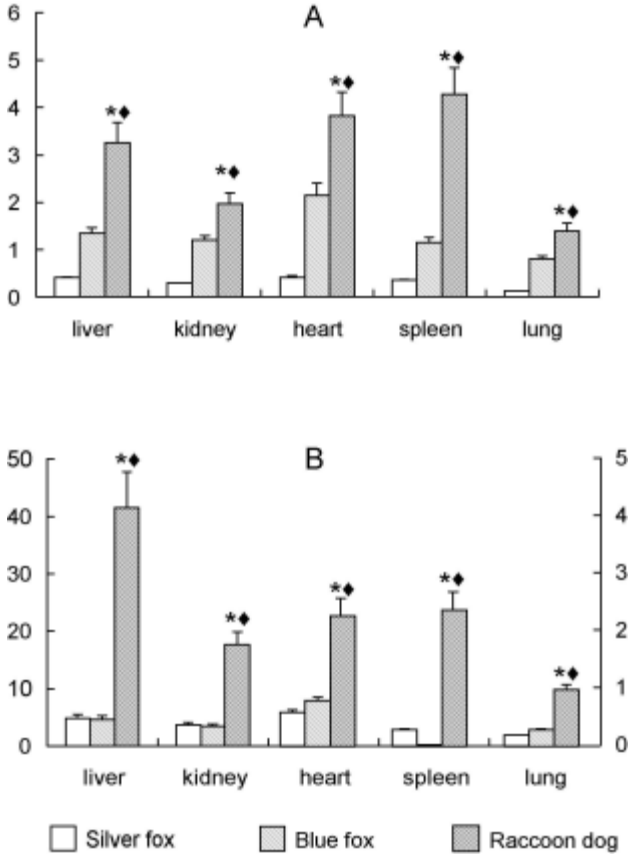
The SOD activity in liver of silver fox was 147.11 ± 22.06 vs. 227.80 ± 34.17 in polar fox and vs.514.65 ± 77.19 U/g wet weight in raccoon dog. In kidney it was 88.44 ± 13.26 vs. 186.15 ± 27.90 and vs. 289.58 ± 43.43 U/g wet weight, respectively. CAT activity was the highest in organs of raccoon dogs, except heart (6544.9 ± 981.73 μM H_2O_2/g/min in liver, 2472.80 ± 321.46 in kidney, 306.01 ± 36.72 in spleen, 145.10 ± 17.41 in lung, respectively). The lowest CAT activity was found in the liver 782.21 ± 101.66 μM H_2O_2/g/min, in the kidney 527.00 ± 63.24, and in the spleen 12.60 ± 0.13 in polar foxes. The heart and lung are the tissues which have a low

concentration of CAT protecting against hydroperoxides. Examination of the data reveals that the degree of changes in both enzymes and their coupling were different in various organs. A difference in the distribution or ratio of these enzymes in various tissues may result in different reactivity of oxygen radicals. It should be kept in mind when considering the above features that SOD synthesis is activated by oxygen (Autor & Stevens, 1978), and CAT synthesis is activated by hydrogen peroxide (Aebi & Wiss, 1978). Hydrogen peroxide is formed not only as a result of superoxide dismutation with SOD participation, but also in enzymatic reactions catalyzed by many oxidases.

The investigated animals considerably differ in parentage and ecological conditions of their ancestors' life. Fox is a native species in Karelia while polar fox is a typical Arctic animal and raccoon dog lives in nature in the Far East (Polar fox., 1985). Changes in SOD and CAT activity in the organs of these vertebrates are influenced by the ecological specificity of their ancestors and first of all their specific diets and metabolic rates. In the liver, kidney, heart and spleen the lowest SOD activity was found in silver fox, the highest - in raccoon dog. Such distribution of activity indicates that the activity of the enzyme in these organs depends not only on basal aerobic capacities of the species. It is shown that the metabolic rate calculated from the level of oxygen consumption in polar fox throughout the year is almost twice lower, than in silver fox (Casey et al., 1979). It should also be taken into account that the sampling of tissues for analysis was made in autumn, when metabolic processes intensify and fat reserves accumulate in the predators (Irwing, 1955; Korhonen et al., 1985). Furthermore, the degree of accumulation of fat reserves correlates with SOD activity - the maximal level (up to 30 % of body mass) was observed in raccoon dog, and the minimum - in silver fox (Brandt, 1989; Korhonen et al., 1985; Rouvinen, 1991). However, increased SOD activity in any organs was not always accompanied by higher CAT activity. Thus, in comparison with silver fox, polar fox had higher SOD activity in liver, kidney and spleen but significantly lower CAT activity in these organs.

Both superoxide dismutase and catalase showed significantly highest activity when calculated per protein in raccoon dog organs in comparison with both fox species (Fig. 2).

Figure 2
Specific SOD (A) and catalase (B) in different organs in Silver fox, Polar fox and Raccoon dog.



X-axis: examined organs
Y-axis: specific SOD (arbitrary unit/mg protein) and catalase (μmol H_2O_2/mg/protein/min) activities.
Left scale: the activity of catalase in liver and kidney.
Right scale: for the rest of the organs.
*: the differences are statistically significant as compared to Silver foxes.
♦: the differences are statistically significant as compared to Polar foxes (P<0,05, Mann-Whitney test).

No significant differences of specific SOD activity in liver of the polar and silver foxes and specific CAT activity in all other organs were observed. Similar pattern of antioxidant enzymes was found in the ground squirell (*Citellus citellus*) (Buzadzic et al., 1998). According to this authors maintenance at 30°C prevents seasonal changes of body temperature and the animals remain euthermic and active. During the winter, a decrease in enzymatic activity and an increase in the level of low molecular antioxidants in all tissues was found. This fact indicates high importance of the antioxidant

system for hibernating mammals and its appreciable conservatism. Specific distribution of the investigated enzymes in organs of raccoon dog is obviously caused by hibernatation they experience even in captivity. SOD seems to be involved more than catalase in the processes of protection against cell damage caused by free oxygen radicals (Fridovich, 1998).

All investigated species had a similar type of SOD and catalase activity distribution in organs characteristic for other mammals with the highest indices in liver. At the same time, some specific features are marked. Thus, SOD activity in heart and spleen of polar fox, and in spleen of raccoon dog was higher than in liver.

The activity of antioxidant enzymes in the same organ in taxonomically-related species with similar body mass and life span differed considerably. Apparently, it was determined, first of all, by specific ecological characteristics of the species.

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THIAMINE STATUS IN MINK BLOOD UNDER ALIMENTARY HYPOVITAMINOSIS B_1 AFTER FEEDING THIAMINASE-CONTAINING FISH

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Summary
The specific functionally interconnected parameters of vitamin B_1 metabolism and some other biochemical parameters were studied in the mink blood under conditions of various provision with vitamin B_1: in thiamine deficiency condition caused by raw thiaminase-containing fish in diet, and with additional vitamin supplement. Our researches have shown that the vitamin deficiency appears in the minks with alimentary thiamine deficiency, and the level of physiologically active thiamine forms and thiamine biotransformation enzymes activity decreases. The earliest thiamine deficiency indicator is the thiamine triphosphate level. Thiamine deficiency was not found for the animals supplied with additional vitamin. Haemopoietic functions, protein and carbohydrate metabolism, as well as productivity were affected insignificant.

Introduction
The role of thiamine in vital functions of carnivorous fur-bearing animals is well known. Prophylactic methods have been designed against hypo- and avitaminosis caused by feeding animals with raw fish containing the enzyme thiaminase which inactivate vitamin B_1 both in food and the organism (Green et al, 1941; Jorgensen, 1977; Juokslahti, 1989; etc). In addition, it is known that thiamine is not synthesized in the organism of carnivores (Helgebostad, 1981). However, despite scientific developments in this field improper feeding system for farmed fur-bearing animals may cause metabolic disturbances and undesirable sequels resulting in decreased productivity and economic losses (Zimmermann, 1981). Therefore, it is obvious that there is a need for regular monitoring of the animal's physiological state using biochemical tests characterizing basic metabolic indices as well as specific organism functions, which depend particularly on thiamine provision. In previous experiments with carnivorous fur-bearing animals only individual metabolic products have been determined: total thiamine in liver, blood and urine, pyruvate level and value of thiamine diphosphate effect (Jorgensen, 1975). For the first time, vitamin B_1 profile complex evaluation with the determination of its physiologically active forms and their biotransformation enzymes in minks and polar foxes in the experiments with alimentary and oxythiamine deficiency was carried out in our laboratory in collaboration with the Institute of Biochemistry, Belarus Academy of Sciences. A number of metabolic changes depending on both the degree of deficiency and the methods of modelling have been found (Izotova et al., 1992; Ilyina et al., 1998; Petrova et al., 1999).
The aim of the present research is to study the complex of functionally interconnected thiamine metabolism parameters in mink blood and a number of other haematological parameters characterising physiological state under alimentary B_1 hypovitaminosis.

Materials and methods
Three groups of healthy male and female dark-brown mink (*Mustela vison*) were used in the experiment. Each group consisted of 60 animals (30 females and 30 males). Dietary thiamine

deficiency was developed in Group 1 as a result of 50-day feeding the minks with raw thiaminase-containing fish (herring Ivasi, *Sardinops Ivasi*) making up 50-90% of meat-fish feed proteins without thiamine additional. The minks of Group 2 were given the standard farm diet, and the food per animal was supplemented with 0,5 mg benphothiamine, thiolyc thiamine form with prolonged effect and resistant to thiaminase (Taranov & Kvartnikova, 1985). The control minks (Group 3) were offered the standard farm diet which included (in 100 kkal of metabolizable energy): beef meat and bones subproducts – 12 g, boiled pork subproducts - 14 g, fish species mixture - 23 g, fish flour - 1,3 g, cereals - 7,5 g, animal fat - 1,5 g, protein-vitamin complex -1,6 g, cabbage - 4 g**.** The blood was collected from the caudal vein in randomly chosen mink (n=18). Blood samples were stabilized with heparin for thiamine parameters determination. All animals were regularly weighed and fur quality was estimated after pelting.

The following parameters characterising the thiamine status in the mink blood were determined by enzymatic and fluorimetric techniques approved at the Institute of Biochemistry, Belarus Academy of Sciences (Chernikevich et al., 1995): a total thiamine content and its biologically active forms - thiamine diphosphate (ThDP) and thiamine triphosphate (ThTP); inorganic phosphate (Pi); activity of thiamine biotransformation enzymes – thiamine kinase (Th kinase), thiamine diphosphate kinase (ThDP kinase), thiamine diphosphatase (ThDPase). The value of ThDP effect was estimated by raised transketolase (TK) activity after preincubation of blood samples with thiamine diphosphate (Bruns et al, 1958).

The following haemopoiesis parameters were determined according to Berestov (1971), Berestov and Kozhevnikova (1981): erythrocyte and haemoglobin content, total protein content and its fractional composition, activity of alanine aminotransferase (ALAT), lactate dehydrogenase (LDG), and alkaline phosphatase (AP).

Results and Discussion

Experimental results revealed the lowest values of thiamine metabolism parameters in Group 1 minks fed thianimase-containing fish - total thiamine content, its both phosphoric ethers (total ThDP and ThTP), Pi, and increased ThDP effect up to 28,9% (Table 1). As a result, the activity of Th-kinase and ThDP-kinase, ThDP and ThTP synthesizing systems, in Group 1 animals was significantly inhibited compared with Group 2 animals, in which adequate provision was observed. The thiamine hydrolysis enzyme activity, ThDPase, was also reduced in Group 1. This is due probably to the necessity of the maintenance of ThDP level in the blood that could explain similar amount of its free form in all groups of animals. The thiamine deficiency estimated as hypovitaminosis was exhibited at biochemical level. The response of thiamine metabolism parameters was obvious. Visually it was exhibited in some decrease of animals' activity.

In Group 2 there was the highest thiamine phosphoric ethers content that ensured sustainable functioning of anabolic thiamine biotransformation enzymes. The value of ThDP effect (18,1%) was found to be practically normal at the expense of additional benphothiamine supply. Some researches have recently come to the conclusion that it is possible to increase a lower rate level of ThDP effect from 15 up to 20 % (Petrova et al, 2000) and even up to 25 % (Kodentsova et al, 1994).

During the experiment, Group 3 developed spontaneous thiamine deficiency as shown by biochemical parameters. It may be connected with the farm feeding deterioration during fore-pelting period, particularly with the use of different fish species mixture in the diet, which could include thiaminase-containing fish as well. Thiamine deficiency in this group was also revealed at the biochemical level: the decreased physiologically active forms content – coenzyme ThDP and the most labile and energy-intensive thiamine form, ThTP; inhibition of the Th kinase activity, phosphorylating thiamine to ThDP; significant increase of ThDP effect (34%).

Other haematological parameters measured are shown in Table 2. The haemopoiesis in Group 1 animals was the most intensive – the number of erythrocytes was higher than in Group 2 ($p<0.05$), and haemoglobin content was higher than in intact animals (Group 3) ($p<0.05$). The total protein level and its fractional composition were within the normal range and similar in animals of all

groups. No significant difference between groups was observed in the serum ALAT activity participating in oxidative catabolism of amino acids and in the LDG activity, key enzyme of carbohydrate metabolism. A specific role in the deposition and transport of thiamine is played by alkaline phosphatase activated on early stages of thiamine deficiency development (Tumanov, 1986). The tendency to its increase in Group 1 and Group 3 was observed in our experiment that confirms the slight changes in thiamine metabolism.

As a result, experimentally developed thiamine deficiency (Group 1) has not affected the pelts quality while spontaneous deficiency (Group 3) has resulted in the insignificant deterioration of pelt quality and somewhat lower body weight of minks. The minks body weight in Group 1 was obviously sufficiently high because of high fat herring content.

Thus, B_1 hypovitaminosis was experimentally modelled in minks and revealed biochemical alterations in the complex of interconnected thiamine metabolism parameters in the blood. First of all, a decreased content of the most responsive physiologically active forms, ThDP and ThTP, and the inhibition of their biotransformation enzymes was observed. They could be used as biochemical markers when estimating vitamin B_1 metabolism in animal organism. The earliest thiamine deficiency indicator, as in experiments with laboratory animals (Chernikevich et al., 1995), is the non-coenzyme form level, thiamine triphosphate, the role of which is to generate and transfer the nerve impulse. The comparative analysis of the thiamine status has shown the thiamine metabolism intensity in the experimental group of animals fed with raw herring Ivasi without additional vitamin B_1, that may be defined as hidden hypovitaminosis. In the control group, the spontaneous character of deficiency was exhibited. At the same time, benphothiamine supplements resistant to thiaminase contained in the farm diet normalized the thiamine metabolism. It became obvious that minks could stay for some period of time without additional supply of thiamine even in metabolic stress due to the biochemical mechanism of maintaining the thiamine status and adaptive responses of the organism.

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Table 1

Phosphate ethers content, Pi (mkmol/l), activity of thiamine metabolism enzymes (nkat) and TK (mkmol/s·*l) in mink blood under B_1 hypovitaminosis (M ± m) (n=6)

Parameter	Group 1 (thiaminase-containing fish)	Group 2 (farm diet + benphothiamine)	Group 3 (farm diet)
Total thiamine	0.16 ± 0.024	0.24 ± 0.050	0.27 ± 0.008
ThDP: total	0.083 ± 0.007*	0.120 ± 0.004†	0.10 ± 0.008
free	0.038 ± 0.006	0.042 ± 0.006	0.033 ± 0.006
bound	0.049 ± 0.012*	0.071 ± 0.012	0.066 ± 0.012
ThTP	0.024 ± 0.002*	0.040 ± 0.001†	0.031 ± 0.002‡
Pi	0.50 ± 0.01*	0.60 ± 0.02†	0.79 ± 0.03‡
Th kinase	0.630 ± 0.08*	1.21 ± 0.10†	0.86 ± 0.09
ThDP kinase	0.031 ± 0.004*	0.052 ± 0.001	0.055 ± 0.003‡
ThDPase	68.2 ± 1.7*	105.8 ± 2.1	107.7 ± 2.53‡
TK	10.02 ± 0.32	10.91 ± 0.38	10.64 ± 0.33
ThDP effect, %	28.9 ± 3.3 *	18.1 ± 2.2†	34.1 ± 3.7‡

* significant difference between Group 1 and Group 2
† significant difference between Group 2 and Group 3
‡ significant difference between Group 1 and Group 3

Table 2.

Haematological and physiological parameters in minks under alimentary B_1 hypovitaminosis (M±m) (n=18)

Parameter	Group 1 (thiaminase-containing fish)	Group 2 (farm diet + benphothiamine)	Group 3 (farm diet)
Erythrocytes, 10^{12}/l	9.7 ± 0.2*	9.1±0.2	9.2±0.2
Haemoglobin, g/100ml	21.6±0.4	21.0±0.4	19.3±0.6†
Total protein g/100 ml	8.3±0.1	8.4±0.2	8.4±0.2
Albumins, %	55.0 ±2.7	57.0±2.0	57.2±1.8
Globulins, %:			
alpha	10.6 ±0.8	11.1±1.1	12.7±1.0
beta	15.7± 0.8	18.0±1.3	16.7±1.5
gamma	20.3 ±4.0	14.4±1.5	13.3±1.5
ALAT, st. unit	40.9 ±3.0	41.5±2.8	44.3±2.8
LDG, st. unit	7.5 ±0.4	7.9±0.3	6.7±0.7
AP, st. unit	11.2±1.7	8.4±1.7	11.0±1.2
Body weight, g (n=30)			
males	2162±41	2212±46	2047±54†
females	1146±31	1217±31	1061±28†

* significant difference between Group 1 and Group 2

† significant difference between Group 2 and Group 3

VITAMIN A, E AND B_1 DURING THE POSTNATAL ONTOGENESIS OF POLAR FOX

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Summary

The aim of our investigation was to determine the vitamins A, E and B_1 level in blood and organs of polar foxes during the postnatal ontogenesis. The study has shown that changes in vitamins level were maximal during the early growing period when metabolic processes most active. The basic tendency of tocopherol level changes in its decrease in tissues during early postnatal period of polar fox. The vitamin A concentration increases sharply after birth. During early growing animals were adequately provided with vitamin B_1. The vitamin levels of adult animals is rather stable and depends considerably on diet, physiological condition and other factors.

Introduction

An important problem in vitamin role study is the research in regularities of the vitamin status formation in association with phases of the individual organism development.

Fat-soluble vitamins A and E play an important role in the regulation of biochemical processes in the organism of animals. Vitamin E (tocopherol) functions in tissues as an antioxidant, protecting unsaturated tissue lipids from peroxidation. It is known that tocopherol in animals acts not only as an structural antioxidant, but also as a regulator of energy metabolism. At the same time, the influence of tocopherol on reproductive functions of animals has been investigated in many works (Nadirov, 1991). The vitamin E importance in the aging changes of fur animals is still not clear. In the organism vitamin A (retinol) is responsible for the functions supporting the stability of cell membranes and ensuring their permeability. Vitamin A participates in photoreception processes, proliferation regulation and cells differentiation, provides normal function of epithelial, nervous, osteal tissues, growth and spermatogenesis of animals. Water-soluble vitamin B_1 (thiamine) affects many functions in the organism, its diphosphorus ester is involved in carbohydrate, lipid, protein and nucleic acids metabolism. It is known that there is no thiamine synthesis in the organism of carnivores (Helgebostad, 1981). The thiamine deficit in the organism of fur animals causes functions disturbance in central and peripheral nervous and reproductive systems, delay of kits growth, fur deviations etc. (Petrova et al., 1987).

The aim of our investigation was to determine to what degree aging changes influence the vitamins A, E and B_1 level during postnatal ontogenesis of polar foxes.

Material and methods

The vitamins A and E (α-tocopherol) concentration in the blood and organs was studied in polar foxes (Alopex lagopus L.). Groups had females and males at the age 5, 35, 50, 90, 180 and 270 days of life. At the age of 5, 35, 50 days the animals were killed and organs (liver, kidney) were taken and frozen (- 25ºC) for chemical determinations. The metabolic thiamine parameters were studied at the age 10, 20, 35, 50, 90, 180 and 270 days of life. All animals were healthy and had standard diet including vitamins. The blood was sampled from the plantar vein.

The concentration of retinol and tocopherol in the blood serum and organs was determined by high performance liquid chromatography method. Proteins in the samples were precipitated by ethanol. Retinol and α-tocopherol were extracted by n-hexane. The ethanol and hexane used for extraction contained butylated hydroxytoluene to prevent the vitamin oxidation during the analytical procedure. Chromatographic separation was carried out by a microcolumn chromatograph with ultraviolet detector. The sample volume introduced into the column was 10 µl (Skurihin & Dvinskaya, 1989).

Some parameters, such as the transketolase enzymatic activity (TK) and the size of thiamine diphosphate effect (ThDP effect) were used to characterize thiamine metabolism. The TK activity was defined by Bruns et al. method (1958). The size of ThDP effect was estimated by a rise in TK activity after blood samples preincubation with thiamine diphosphate (Dreyfus & Lundquist, 1962).

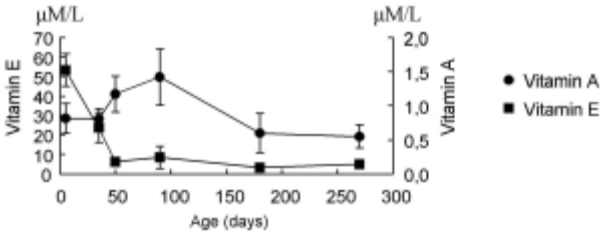
The data expressed as mean ± SD. Mann-Whitney U test was used for comparison of different ages.

Results and Discussion

The study results have shown that at the age of 5 days all the kits had a high concentration of α-tocopherol in the blood serum. Then to 35-day's age it is reduced twice and by the 50-th life day even more. It testifies the lability of vitamin A content in blood and is obviously connected with physiological modifications in organism (Dvořác, 1983). Vitamin A level in the blood at the age of 5 and 35 days remained practically unchanged, but later, at the 50-th day its concentration became higher (Fig.1). The significant difference between females and males of the vitamins level in blood was not detected on early stages of postnatal ontogenesis. It is connected, probably, with the fact that they get the vitamins contained in the mother's milk.

Figure 1
Vitamins A and E concentrations in Polar fox blood serum during postnatal ontogenesis (mean ± SD)

n = 6 for 5, 35, 50, and 180 days
n = 12 for 90 and 270 days

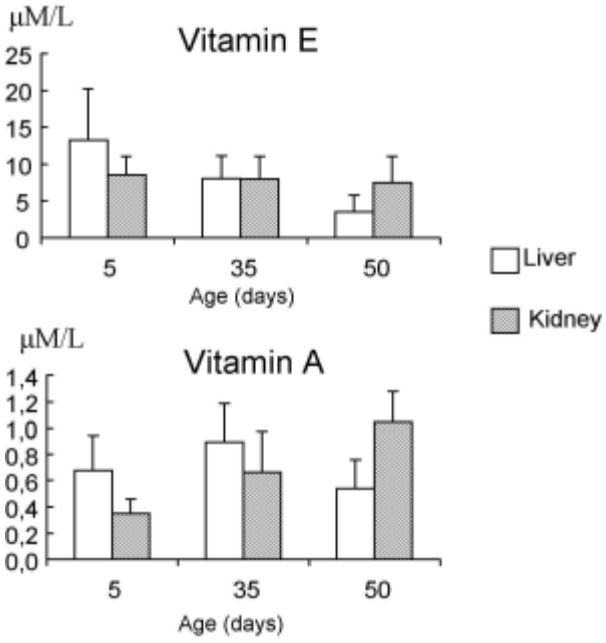


By 3 months some increase of α-tocopherol concentration with the previous research can be seen. In this period the level of vitamin A in males blood serum (10,24 µM/L) was higher than in females (6,47 µM/L). Previously, it has been noted in the literature that young growing males of foxes are more sensitive to vitamin E undersupply (Albert & Wenzel, 1988). The retinol concentration has been found to be higher in females (0,46 µM/L) than in males (0,35 µM/L). Tocopherol in animal organism acts as a regulator of energy metabolism. At the age 3 month the intensity of metabolism grows, which is expressed by a significant consumption of oxygen which results in increased activity of the antioxidant system (Il'ina & Ruokolaynen, 1996). These reasons must have caused increase of the vitamin concentration in the blood. The process of growth is completed by the age of 6 month, the energy dissipated is reduced. At the age of 6 months it achieved a level characteristic for 9-months animals.

In organs the changes of vitamins A and E metabolism intensity has an organo-specific character. The α-tocopherol concentration in the liver considerably decreased to the 35-th and the 50-th days of life that, obviously, is connected with period the kits passed to an independent type of feed after the kits weaning. This fact indicated that tocopherol intensively utilized in organism. A similar pattern of vitamin E depletion in plasma and liver has been reported previously in mink (Treuthardt, 1992). In the kidney no significant changes were noted during the early stages postnatal ontogenesis (Fig. 2, A). For kidneys, which during the significant period of early

growing are differentiated and become more complicated, the age metabolism falling is expressed poorly. In particular, the growing of silver foxes kidneys was marked from newborn to 5-month's age (Garmaeva, 1974). The vitamin A concentration in liver increased at the age of 35-th day but later, after the kits weaning, at 50-th day became lower. The retinol content in kidney increased during early postnatal ontogenesis (Fig. 2, B).

Figure 2
Vitamins A and E concentrations in Polar fox organs during postnatal ontogenesis (mean ± SD; n = 6).



The study has shown the highest values of thiamine metabolism during the early growing period. TK activity to be high, the size of the ThDP effect showed that animals were adequately provided with thiamine (Table 1).

Table 1
TK activity and size of ThDP effect in polar fox blood during postnatal ontogenesis (M ± m)

Age, days	n	TK activity, µM/s·L	ThDP effect, %
10	2	11.90 ± 2.69	6.45 ± 2.47
20	6	12.62 ±0.33	9.9 ± 1.79
35	6	9.85 ± 0.55	9.0 ± 0.6
50	6	10.32 ± 0.17	13.14 ± 4.76
90	12	9.1 ± 0.43	17.79 ± 2.83
180	19	8.78 ± 0.26	26.98 ± 1.18
270	20	10.55 ± 0.43	25.8 ± 2.25

At the age of 3 months the ThDP effect increased, indicating a slight thiamine deficiency. By 6 months no significant changes were noted in comparison with previous research. The TK activity and ThDP effect remained practically unchanged in adult animals. Slight and even moderate thiamine deficiency, not apparent clinically, causes no visible deviations from normal physiological indices. However, the loss of the thiamine in growth and development periods during which a lot of energy is used can have a negative effect on the reproductive functions of animals in the future.

Thus, the present research has demonstrated that the early ontogenesis period the blood serum and liver α-tocopherol contents decreased. The providing of organism with oxygen sharply increased after the birth, the lipid peroxidation speed increased too (Dubinina et al, 1984). This explains, obviously, the high tocopherol concentration in the tissues after birth. During growth and development the level of an oxidizing metabolism continuously reduces. Primary factors here are, on the one hand, the quantitative and qualitative aging changes of tissues and changes in their metabolism, on the other hand, changes in a character of central (nervous and hormonal) regulations of peripheral tissues metabolism. This rapid decrease in the vitamin E status of animals during the early growing period may result from their rapid growth and high requirement for nutrients while growing (Treuthardt, 1992). The tocopherol level stability in late ontogenesis of polar fox and the individuality of this level serve, obviously, as characteristics of homeostasis, which is provided by removing extra tissue

bioantioxidants from the organism (Nadirov, 1991).

The vitamin A concentration in blood increases sharply after birth. The liver plays an active role in vitamins distribution and redistribution because it accumulates and is spent quickly. The vitamin A content in kidneys increases gradually with age and becomes much higher than in liver (Petrova et al.,1987; Schweigert & Thomann, 1995). High TK activity and pentose cycle are characteristic for newborn (Hmelevskiy, 1970). Then the thiamine level in blood and organs of animals gradually decreases, that is confirmed by our own researches and other authors' data.

The results of this investigations showed that changes in vitamin concentrations in polar fox was most active in early ontogenesis, which was linked with the intensive growth and development of animals in this period. Thus, there is reason to suppose that, even at moderately low vitamins deficit, metabolic disturbances can take place and any deficiency should be corrected.

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