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METOVITAN INCREASES THE RESISTANCE OF THE BODY TO HYPOXIA

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Aim. To study the effectiveness of the vitamin preparation Metovitan and the multivitamin preparation Decamevit in the prevention of the development of oxidative stress in the tissues of rats and the survival rate of rats under hypoxic hypoxia conditions.

Methods. Experiments were performed on Wistar rats, 160–220 g. A model of hypoxic hypoxia was induced by lifting the rats in a barocamp to a conditional altitude of 11 thousand meters above sea level (pressure 190 mm Hg). Tissue metabolite concentrations and enzyme activities were measured using conventional biochemical methods.

Results. The results of the study showed that the positive effect of Metovitan, which contained methionine and vitamins E, B_1, and B_3, on many indicators, including the activity of antioxidant defense enzymes, was significantly higher than the effect of Decamevit, which contained the same components plus vitamins A, B_2, B_6, B_9, B_{12}, vitamin C, and the bioflavonoid rutin. The survival time of rats treated with Metovitan before the experiment was one and a half times longer than that of Decamevit.

Conclusion. A preparation containing a limited amount of vitamins acting synergistically on narrow links of cellular metabolism was more effective in preventing oxidative stress than a conventional multivitamin preparation.

Key words: vitamin preparation, hypoxia, oxidative stress, survival under adverse conditions, Metovitan.

Abreviature

AOA — anti-oxidant activity
GPO — glutathione peroxidase (EC 1.11.1.9)
GR — glutathione reductase (EC 1.11.1.6)
GSH — glutathione
GSSG — glutathione oxidize
DNPH — 2,4-dinitrophenylhydrazine
KGDC — α-ketoglutarate dehydrogenase complex
MDA — malonic dialdehyde
PDC — pyruvate dehydrogenase complex
SDH — succinate dehydrogenase(EC 1.3.99.1)
SOD — Superoxide dismutase (EC 1.15.1.1.1)
TBA — thiobarbituric acid
ROS — reactive oxygen species

Exposure of the human body to unfavorable exogenous and endogenous factors often causes a stable imbalance in cellular metabolism, which can lead to serious health consequences. The search for ways to correct the cellular metabolism in order to increase the survivability of the organism under unfavorable conditions is becoming more and more urgent. One of the universal manifestations of the influence of adverse factors on cell metabolism is hypoxia. Hypoxia is a widespread phenomenon that occurs both in conditions of oxygen deficiency in the external environment and as a result of various pathologies, the consequence of which is the development of oxidative stress [1, 2].

Oxidative stress due to increased generation of reactive oxygen species (ROS) and decreased efficiency of the antioxidant

defense system is considered a universal mechanism of destructive changes in tissues and organs in various pathological conditions. Pharmacological agents or non-medical methods can increase resistance to hypoxia by increasing oxygen supply and/or efficiency of oxygen utilization. Vitamin preparations belong to the category of pharmacological agents, and various multivitamin preparations are often used to correct metabolic disorders [3]. However, not all multivitamins are equally effective.

The studies showed that the stable functioning of the body depended not only on receiving sufficient amounts of all vitamins, but also on the ability of tissues to assimilate and convert them into biologically active forms. The latter required the presence of other biologically active compounds that contributed to the activation of vitamin metabolism [3–7]. In case of simultaneous intake of several vitamins into the body as part of widely advertised multivitamin preparations, some of them may compete with each other in the process of transport, metabolism, and even functioning. Practice has shown that it is more rational to develop vitamin preparations containing a limited number of components that act synergistically on individual links of cellular metabolism.

All the above-mentioned factors were taken into account when creating the composition of biologically active substances that form the basis of the complex preparation "Metovitan". The preparation was created based on experimental studies on the relationship between methionine transformations in the tissues of rats and vitamin E intake into the body. These studies were conducted for a long time under the supervision of Stutman [7–10]. The study showed that a certain range of vitamin E concentrations increased the uptake and conversion rate of administered methionine in the rat liver. Activation of glutathione peroxidase [10], an enzyme that neutralizes lipid peroxides in animal tissues with the participation of glutathione, a methionine conversion product, was observed only when these compounds were co-administered. Further studies showed that the joint administration of methionine and vitamin E in a certain ratio significantly enhances not only the metabolism of sulfur-containing compounds, but also other cellular processes [5]. This discovery became a key factor in the development of the drug Metovitan.

This study compares the efficacy of Metovitan and Decamevit, a multivitamin preparation, in preventing the development of oxidative stress in rat tissues and their survival under hypoxic hypoxia.

Materials and Methods

Materials. Most biochemical compounds and reagents were purchased from Sigma-Aldrich (USA) unless otherwise noted. All other reagents were of the highest purity available. Solutions were prepared in double-distilled water.

Composition of vitamin supplements, percent of active ingredient according to Instruction (weight percentages): Metovitan: Vitamin E (α-tocopherol acetate) — 2.5; Vitamin B_1 (thiamin hydrochloride) — 0.3; Vitamin B_3 (PP, nicotinamide) — 0.23; DL-methionine — 96.9; zinc sulfate heptahydrate — 0.05%.

Decamevit: Vitamin A — ~0.37; Vitamin E (α-tocopherol acetate) — ~1.87; Vitamin B_1 (thiamin hydrochloride) — ~3,74, Vitamin B_2 (riboflavin) — ~.87; Vitamin B_3 (PP, nicotinamide) — ~9.36; Vitamin B_6 (pyridoxin) — ~ 3.74; Vitamin B_9 (folic acid) — ~0.37; Vitamin B_{12} (cyancobalamin) — ~0.019; Vitamin C — ~ 37,44; Vitamin P (rutin) — ~ 3.74; Methionine — ~ 37.44.

Ethical Statement. All animal experiments were performed in accordance with the Helsinki Declaration on the Guide for the Care and Use of Laboratory Animals, which defines the conduct of ethical research involving animals, and with the "Rules of work with experimental animals" approved by the Commission on the Care, Maintenance and Use of Experimental Animals of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine.

Animals and procedures. Male Wistar rats weighing 160–220 g were used in the experiment. The animals were kept in the animal house (or vivarium) of the Palladin Institute of Biochemistry. They were housed in quiet, temperature-controlled rooms and provided with water and food pellets ad libitum. The animals were divided into three groups one week before the experiment. Two experimental groups received daily oral administration of either Metovitan or Decamevit through a probe. The drugs were administered in saline suspension at 50 mg active substance per 1 kg body weight. The control group received saline daily in the same manner.

A model of hypoxic hypoxia was induced by raising rats in a barocamera to a conditional altitude. For biochemical studies, animals

were raised in a barocamber to a conditioned altitude of 9,000 m (pressure — 230 mm Hg) for 20 min, after which the pressure was reduced and the rats were sacrificed by cervical dislocation. To determine survival in hypoxia, animals were raised to a conditional altitude of 11,000 m above sea level (pressure 190 mm Hg) Climb time — 4 min. Ascent rate —37.5 m/s.

Biochemical parameters

Enzyme Activity Assays. Antioxidant enzyme activity was measured in blood, brain and heart tissue as previously described [11].

Superoxide dismutase (SOD, EC 1.15.1.1.1). The activity was measured by the method of Misra and Fridovich [12], modified by Protas and Chayalo [13]. The method was based on the inhibition of adrenaline auto-oxidation (0.01% solution) at pH 10.2 by SOD contained in the tested samples. The unit of SOD activity was defined as the amount of protein that caused 50% inhibition of the rate of conversion of adrenaline to adrenochrome under the specified conditions. The amount of enzyme required to inhibit the initial rate of adrenaline auto-oxidation by 50% was taken as the conditional unit of activity. The results were expressed as the specific activity of the enzyme in units per ml of plasma.

Catalase (EC 1.11.1.6) activity was measured according to the method described in [14]. The method for estimating catalase activity was based on the determination of colored products formed by the reaction of hydrogen peroxide with ammonium molybdate, and the amount of hydrogen peroxide decomposed in the presence of a catalase-containing sample was used to estimate catalase activity.

The *glutathione peroxidase* (GPO, EC 1.11.1.9) activity was determined by measuring the rate of oxidation of reduced glutathione during H_2O_2 decomposition and the color reaction of HS groups of glutathione with Ellman's reagent [15] . The reaction was performed in the presence of sodium azide, which acted as an inhibitor of catalase. Enzyme activity was expressed in nmoles of GSH consumed per minute per 1 mg of protein.

The activity of pyruvate and alpha-ketoglutarate dehydrogenase complex was determined in the whole system by adding NAD and CoA to the incubation medium as described [16]. The reaction was monitored by the formation of NADH at 340 nm.

Succinate dehydrogenase (SDH, EC 1.3.99.1) *activity* was determined using iodonitrotetrazolium chloride as electron acceptor [17].

Determination of biomarkers of oxidative stress and metabolites

The intensity of lipid peroxidation processes was determined by the accumulation of TBA-reactive products [11]. The content of free SH groups of low molecular weight compounds was determined by reaction with Ellman's reagent as previously described [18].

Protein carbonyls, which are the products of oxidative protein modification, have been detected in plasma by reaction with 2,4-dinitrophenylhydrazine (DNPH) [19]. This reaction led to the formation of protein ketone-2,4-dinitrophenylhydrazones in the presence of electron donors and metals of variable valence, primarily iron or copper ions. An automated μQuant microspectrophotometer (Biotek, USA) was used to record changes in optical density.

Total NO concentration (NO_3^- + NO_2^-) was determined by the interaction of the stable metabolite of nitrite anion (NO_2^-) with the Griess reagent (a mixture of 1% sulfonic acid with 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride) as described in [20]. The principle of this assay is the reduction of nitrate by vanadium(III) combined with detection by the acidic Griess reaction.

The concentration of lactate and pyruvate in the enzymatic reaction with lactate dehydrogenase was measured according to the accepted methods [21]. The ratio of free NAD+/NADH in heart tissue was also calculated using these methods.

Statistical analysis

Experimental data were processed using generally accepted statistical methods. Unless otherwise noted, data are expressed as mean ± standard error of the mean (SEM). Data from 3 independent experimental series were pooled. A value of $P < 0.05$ was considered statistically significant. Student's t-test was used for statistical analysis. Microsoft Excel (Office 365 package) with BioStat add-on from AnalystSoft and GraphPad Prism, version 8.0 (USA) were used for calculations and graphical presentation of the obtained results.

Results and Discussion

Redox reactions are inseparable from energy metabolism in living cells and are essential for the physiology of the organism

and for life itself [22, 23]. The cellular redox signaling network is maintained by a balance of pro-oxidant and antioxidant processes. Pro-oxidant factors include reactive oxygen species (ROS), such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2), reactive nitrogen species (RNS, namely nitric oxide NO^-) and their derivatives (especially peroxynitrite $ONOO^-$), which are produced by NAD(P)H oxidases and nitric oxide synthases or formed during the functioning of the mitochondrial electron transport chain. The action of pro-oxidant factors in cells is counterbalanced by the antioxidant defense system. This system includes, first of all, antioxidant enzymes such as superoxide dismutases SOD1-3, catalase, glutathione peroxidase and others, as well as non-enzymic low-molecular compounds such as glutation (GSH), α-tocopherol, ascorbate, etc. [24].

Living organisms possess finely regulated systems for maintaining the balance between pro- and antioxidant processes. However, under certain circumstances, this balance can be disturbed to the point of developing oxidative stress [24, 25], a physiological state characterized by elevated steady-state levels of ROS and accompanied by certain physiological effects.

The development of oxidative stress can be prevented by introducing exogenous antioxidants into the body or otherwise activating the antioxidant defense system. The ability of Metovitan and Decamevit to prevent the development of oxidative stress was tested in the model of hypoxic hypoxia. The preparations were administered to the experimental animals prior to placement in the barocamera as described in the "Materials and Methods" section. At the end of the exposure in the barocamera, the animals were killed in an ethical manner and blood, brain, and heart muscle were collected for analysis. Table 1 shows the data from the analysis of some key biochemical parameters in the myocardium of animals from all experimental groups: control group, animals exposed to hypoxia without and with prior drug administration.

Cardiac muscle is one of the tissues with high oxygen supply and predominance of aerobic processes in metabolism. It is believed that under hypoxia there is a switch of oxidative processes at the level of citric acid cycle (CTC) from NAD-dependent substrates to succinate, which prevents the deficit of reducing and energy equivalents in cells [26].

Under hypoxic conditions, PDC activity in heart tissue increased (by 65.1%) and KGDC activity increased by 24.3% (Table 3). Generation by these enzymes of reducing equivalents in the form of NADH characterizes the growth of redox potential in the key compartments of cells — cytosol and mitochondria, namely, changes in the ratio of free NAD^+/NADH calculated in this work from the concentration of lactate, pyruvate and the equilibrium constant of lactate dehydrogenase, which supports the stoichiometry of substrates and coenzymes in this reaction [27].

Administration of both drugs prevented the increase of KGDC activity, and the activity of PDC was even significantly decreased compared to the control group. Administration of both drugs also prevented excessive increase of SDH activity under hypoxia, although the final activity of this enzyme remained slightly higher than in the control group by 28.1 and 48%, respectively, in the groups of animals administered Metovitan or Decamevit. The content of free SH groups of low molecular weight compounds decreased by 18.4% in heart tissue under hypoxia (Table 1). Both drugs significantly prevented this decrease. In living cells, the pool of free SH groups is predominantly represented by reduced glutathione (GSH). GSH is the most abundant thiol antioxidant in animal cells. In addition, GSH is involved in cellular redox signaling, regulation of cell proliferation and death, and detoxification of xenobiotics through S-glutathionylation of proteins. The redox couple $NADP^+$/NADPH and glutathione GSH/GSSG form a dual system that ensures the maintenance of cellular redox homeostasis. GSH is a cosubstrate for glutathione peroxidases (GPxs) in the scavenging of hydrogen peroxide (H_2O_2), and NADP(H) functions as an essential cofactor for glutathione reductase (GR), which catalyzes the reduction of GSH from its oxidized form (GSSG), and thioredoxin reductase (TRs) [28].

Warburg effect, i.e. it is accompanied by an increase in glycolytic processes and accumulation of lactic acid in tissues. In our experiments, the lactate content in heart tissue increased by 84.2%. According to the calculations, the ratio of free NAD^+/NADH in the cytosol of heart cells decreased by 51.2% (Table 1). Administration of both drugs prevented the increase of lactate in the myocardium under hypoxic conditions and inhibited the decrease of the ratio of free NAD^+/NADH in the cytosol of heart cells. It should be noted that the effect of Metovitan was more pronounced in this case.

Table 1

Activity of PDC, KGDC, SDH, content of SH-groups, pyruvate/lactate and NAD+/NADH ratio in the heart muscle of experimental animals

Parameters under study	Control	Hypoxia	Hypoxia + Metovitan	Hypoxia + Decamevit
PDC activity in heart muscle, nmol NADH/min×mg protein	20.0±3.3	33.0± 6.7*	11.3±0.6#	7.3±0.5#
KGDC activity in cardiac muscle, nmol NADH/min×mg protein	3.7±0.5	4.6±0.4*	4.0±0.5	4.2±0.6
SDH activity in cardiac muscle, μmol NADH/min×mg protein	19.91±2.34	37.41±3.81*	25.51±2.01#	29.46±1.68#
Free SH-groups of low molecular weight compounds, nmol/mg protein	4.34±0.28	3.56±0.19*	4.98±0.29#	5.01±0.32#
Lactate content in heart muscle tissue, μmol/g tissue	1.01±0.07	1.86±0.09*	1.17±0.06#	1.29±0.07#
Pyruvate content in heart muscle tissue, μmol/g tissue	0.071±0.003	0.064±0.002*	0.070±0.005#	0.065±0.004
Pyruvate/lactate ratio	0.070±0.003	0.034±0.001*	0.060±0.002#	0.050±0.003# ‼
The ratio of free NAD^+/NADH in the cytosol of cardiac myocytes according to the stoichiometry of the lactate dehydrogenase system.	633.0±42	310.0±18*	539.0±20#	454.0±27# ‼

*/ — The difference compared to control is significant ($P < 0.05$)
#/ — The difference from the hypoxia effect is significant. ($P < 0.05$)
‼ / — The difference from the effect of Metovitan is significant. ($P < 0.05$)

The redox state of nicotinamide adenine dinucleotides NAD^+/NADH and $NADP^+$/NADPH is an important factor in maintaining cellular redox homeostasis and regulating cellular metabolism. The $NAD(P)^+$/NAD(P)H redox couple controls energy metabolism, namely substrate phosphorylation in glycolysis and oxidative phosphorylation in the mitochondrial electron transport chain. The intracellular distribution of NAD(H) and NADP(H) is clearly compartmentalized according to the specific localization of the enzymes that use them to catalyze redox reactions. Such compartmentalization provides the possibility of simultaneous occurrence in cells of catabolic processes accompanied by generation of reductive equivalents of NADH and processes of reductive biosynthesis using reductive equivalents of NADPH [29].

The pleiotropic effects of NAD(H) and NADP(H) are due to the fact that they are not only coenzymes of oxidoreductases, but also serve as substrates and cofactors of at least three other classes of enzymes: The poly(ADP)-ribose polymerase family (PARP1-2), cADP-ribose synthases (CD38, CD157) and sirtuin deacetylases (SIRT1-7), which perform extremely important regulatory functions, ensuring the processes of cell proliferation, differentiation and apoptosis. It was found that poly(ADP)-ribose polymerases provide remodeling of chromatin structure, are involved in the processes of repair of single-stranded DNA breaks and regulate the transcriptional activity of genes, cADP-ribose as a product of cADP-ribose synthases controls Ca2+-dependent signaling in cells, and SIRT1-7 by deacetylation of transcription factors provide post-translational modification of proteins, regulate biogenesis and mitochondrial functions [30].

The progression of hypoxic hypoxia in rats raised to a conventional altitude of 9 thousand meters (pressure — 230 mm Hg) in a barocamber for 20 min was accompanied by changes in all tissues. Table 2 shows the changes in TBA reactive products, free SH groups, NO (NO^{3-}+ NO^{2-}) and carbonyl groups of proteins in brain tissue and serum.

As shown in Table 2, the content of TBA-reactive products in rat brain tissue under hypoxia increased significantly (more than

Table 2

Content of free radical oxidation products in rat tissues under hypoxic hypoxia and the effect of Metovitan and Decamevit. $M \pm m$, $n = 9$

Parameters under study	Control	Hypoxia	Hypoxia + Metovitan	Hypoxia + Decamevit
Brain tissue				
TBA-reactive products (MDA), nmol/mg protein	0.61 ± 0.04	2.72 ±0.17*	0.93 ± 0.06#	1.85 ± 0.11*# !!
Free SH-groups of low molecular weight compounds, nmol/mg protein	1.69 ± 0.11	1.25 ± 0.07*	1.47 ± 0.06#	1.55 ± 0.12
NO (NO_3^- + NO_2^-) nmol/mg of protein	22.73 ± 1.32	33.78 ± 2.05*	25.31 ± 1.94#	29.46 ± 2.25
Carbonyl groups of proteins, nmol/mg of protein	0.47 ± 0.02	0.93 ± 0.06*	0.51 ± 0.04#	0.60 ± 0.03#
Blood serum				
TBA-reactive products (MDA), nmol/mg protein	3.41 ± 0,19	4.69 ± 0.22*	3.80± 0.17**	4.42 ± 0.25
NO (NO_3^- + NO_2^-) nmol/mg of protein	15.4 ± 0.72	35.8 ± 2.14*	21.3 ± 1.17#	29.4 ± 1.78# !!
Carbonyl groups of proteins, nmol/mg of protein	1.52 ± 0.04	2.87 ± 0.15*	1.51 ± 0.06#	1.89 ± 0.09# !!
blood pH	7.31 ± 0.26	7.22 ± 0.31*	7.29 ± 0.24	7.25 ± 0.35

*/ — The difference compared to control is significant $P < 0.05$)
#/ — The difference from the hypoxia effect is significant ($P < 0.05$)
!!/ — The difference from the effect of Metovitan is significant ($P < 0.05$)

threefold), the most prominent of which was the content of malonic dialdehyde (MDA), which is the most potent protein modifier. To a lesser extent, the content of protein carbonyl groups increased (by 49.5%) and the total NO content, which is the sum of NO^{3-} and NO^{2-} (by 48.6%). At the same time, the content of free SH groups of low molecular weight compounds, the predominant component of which is reduced glutathione (GSH), decreased by 26.1% (Table 2). A similar pattern in the generation of reactive oxygen species under hypoxia of different severity occurred in the serum. TBA reactive products content increased by 37.5%, protein carbonyl groups content by 88.8%, and total NO content by 132.4% (Table 2).

As we can see from the table 2, under the influence of Metovitan preparation in brain tissue the content of TBA reactive products (MDA) decreased by 65.9%, protein carbonyl groups by 45.2% and NO by 25.1. On the contrary, the content of free SH groups of low molecular weight compounds increased by 17.6%. The positive effect of Metovitan preparation on MDA content was also observed in blood serum, as well as normalization of blood pH.

In the conditions of our experiment under the influence of hypoxia, the total NO content, which is the sum of NO^{3-} and NO^{2-}, increased by 48.6% in brain tissue and by 132.4% in blood serum (Table 2). In addition, it should be emphasized that nitric oxide is a universal regulator of metabolic processes in various cells and tissues of the body, and its level significantly increases in various pathological conditions accompanied by ischemia and inflammatory processes [31].

Under normal physiological conditions of free radical formation, the level of intermediate and final products of lipid peroxidation is regulated by a complex, multicomponent system of antioxidant protection, which includes a number of enzymes. The enzymes of antioxidant defense that neutralize free radical compounds, peroxides and aldehydes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPO) and some others. SOD

Table 3

Activity of antioxidant enzymes in rat tissues under hypoxic hypoxia and the effect of the studied drugs
M±m, n = 9

Parameters under study	Control	Hypoxia	Hypoxia + Metovitan	Hypoxia + Decamevit
Brain tissue				
Superoxide dismutase, U/min×mg protein	179.6±9.14	115.2±6.1*	175.2±8.5#	114.1±5.2 !!
Catalase, nmol of decomposed H_2O_2/min×mg of protein	85.5±4.29	58.6±3.06*	83.2±2.91#	69.7±3.14# !!
Glutathione peroxidase, nmol GSH/min×mg protein	15.9±0.74	4.75±0.32*	9.8±0.60* #	7.1±0.46* # !!
Blood serum				
Superoxide dismutase, U/min×mg protein	146.3 ± 6.56	42.7±2.35*	122.8±5.12#	101.9±4.87*#
Catalase, nmol of decomposed H_2O_2/min×mg of protein	23.8±1.21	14.7±0.6*	21.5±1.21#	18.3±1.13* #
Glutathione peroxidase, nmol GSH/min×mg protein	13. 4±0.1	6.86±0.1*	11.8±0.46#	9.47±0.52* # !!
AOA (% inhibition)	28.0±2.1	13.4±3.9*	51.2±4.6* #	42.8±3.4* #

*/ — The difference compared to control is significant ($P < 0.05$),
#/ — The difference from the hypoxia effect is significant ($P < 0.05$)
!! / — The difference from the effect of Metovitan is significant ($P < 0.05$)

continuously dismutes and neutralizes O^{2-} to form hydrogen peroxide. Hydrogen peroxide (H_2O_2) is a common metabolite of cells, the concentration of which can vary depending on the intensity of metabolic processes in the cell. The main enzyme involved in the breakdown of H_2O_2 is catalase. In addition, H_2O_2 can be utilized by the enzyme glutathione redox system, which includes glutathione peroxidase and glutathione reductase. Under conditions of hypoxia, hydrogen peroxide H_2O_2 can serve as a source of endogenous O_2 formation [32]. Under the influence of unfavorable factors on the body, it is the system of antioxidant defense that takes the first blow.

As can be seen from the data presented in Table 3, acute hypoxic hypoxia causes a decrease in the activity of antioxidant defense enzymes: in brain tissue, SOD activity decreased by 35.8%, catalase activity by 31.5%, and especially glutathione peroxidase activity by 70.1%. The peculiarity of the changes in serum was a much more pronounced decrease (by 70.8%) in SOD activity and a decrease (by 52.1%) in total antioxidant activity.

Nitric oxide plays a significant role in the functioning of antioxidant protection enzymes under hypoxic hypoxia, the increase in its content is observed due to the endogenous formation of NO during the reduction of nitrite ions by heme-containing proteins, which are mainly in the deoxyform under hypoxia. Relatively stable NO radical interacting with superoxide radical forms an extremely toxic product —

peroxynitrite $O^{2-} + NO \rightarrow ONOO^-$ [33].

In addition, nitric oxide protonation products are formed, which are particularly dangerous for enzymes because they can change the composition of amino acid residues. For example, the interaction of NO with the iron-porphyrin complex in catalase leads to the formation of nitro derivatives that block the binding of H_2O_2 and thus reduce the efficiency of the enzyme reaction. Decrease in activity of antioxidant defense enzymes can be mediated by direct interaction of NO with Cu^{2+} ions in the active center of SOD, catalase and cytochrome oxidase [34].

Table 4

Survival time (min) in hypoxic conditions of animals treated with "Metovitan" or Decamevit for 7 days

Dose, mg per 1kg of weight			
	Without injecting the drug	Metovitan administration	Decamevit administration
50	23.0±1.5	36.0±2.1*# $P < 0.05$	29.2±1.9*!! $P < 0.05$

Note: here the index "*/" denotes the index that significantly (at $P < 0.05$) differs from the same in the group without drug administration; the index "#/" — from the group of animals administered Prototype (at $P < 0.05$).

Thus, it is shown that under our conditions experimental acute hypobaric hypoxia was accompanied by the development of oxidative stress, activation of lipid peroxidation (with an increase in the content of TBA-reactive products, carbonyl groups of proteins, and a decrease in the content of free SH groups of low molecular weight compounds) and inhibition of the activity of antioxidant defense enzymes (SOD, catalase, and GPO) in the brain and blood of rats. Under hypoxia, an increase in the content of reducing equivalents of NADH was observed in heart tissue (NAD^+/NADH ratio decreased) as a result of increased activity of pyruvate dehydrogenase and ketoglutarate dehydrogenase complexes. At the same time, the increase in succinate dehydrogenase activity ensured efficient utilization of reductive NADH equivalents in the terminal parts of the electron transport chain.

The preparation Metovitan, which contains only methionine, vitamin E and vitamins B_1 and B_3, enhances the processes of transsulfuration, methylation and has a strong antiradical effect, reduces the content of peroxidation products and thus provides activation of antioxidant defense enzymes. Giving Metovitan to the animals before immersing them into hypoxic conditions practically completely inhibited the decrease in SOD and catalase activity in brain tissue, which is not observed when Decamevit is administered.

The increase in the activity of SOD, catalase and glutathione peroxidase in the blood under the effect of the drug provided a significant (almost fourfold) increase in the antioxidant activity of the blood (Table 2). The preparation Decamevit, which contains almost all essential vitamins, also showed a positive, although less pronounced, effect on the normalization of the content of intermediate and final products of POL and the activity of antioxidant defense enzymes in the group of animals exposed to hypoxia.

The advantage of Metovitan preparation was more convincingly shown by the results of the test of survival of experimental animals under hypoxia (Table 4). In order to assess how the above-mentioned effect of the studied preparations on metabolic processes affects the viability of animals, an experiment was conducted to evaluate the survival time of rats in hypoxia without and after drug administration. As can be seen from Table 4, the preliminary administration of Decamevit at a dose of 50 mg per 1 kg prolonged the life of animals under hypoxic conditions by more than one and a half times.

Thus, the results of tests on animals subjected to hypoxia indicate that administration of Metovitan to animals during 7 days before lifting them to a conditional height prevented a significant part of disorders caused by hypoxia, increased the rate of adaptation to new conditions, which in general contributed to an increase in survival of rats in hypoxia by 1.5 times. Administration of Decamevit in these conditions also had a positive effect, but it was two times weaker than the effect of Metovitan.

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МЕТОВІТАН ПІДВИЩУЄ СТІЙКІСТЬ ОРГАНІЗМУ ДО ГІПОКСІЇ

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Мета. Дослідити ефективність вітаміновмісного препарату Метовітан і полівітамінного препарату Декамевіт у запобіганні розвитку окисного стресу в тканинах щурів та їхньому виживанні за умов гіпоксичної гіпоксії.

Методи. Експерименти проведено на щурах лінії Вістар 160–220 г. Модель гіпоксичної гіпоксії індукували підняттям щурів у барокамері на умовну висоту 11 тис. м над рівнем моря (тиск 190 мм рт. ст.). Концентрацію метаболітів та активність ензимів у тканинах вимірювали з використанням традиційних біохімічних методів.

Результати. Показано, що позитивний ефект препарату Метовітан, який містить метіонін, вітаміни Е, B_1 і B_3, на багато показників, зокрема на активність ензимів антиоксидантного захисту, виявляється достовірно вищим, ніж ефект препарату Декамевіт, який містить ті ж самі компоненти, а також додатково вітаміни А, B_2, B_6, B_9, B_{12}, вітамін С і біофлавоноїд рутин. Час виживання щурів, які отримували препарат Метовітан до початку експерименту, був у півтора рази більшим, ніж у випадку Декамевіту.

Висновки. Препарат, що включає обмежену кількість вітамінів, які синергічно діють на вузькі ланки клітинного метаболізму, ефективніший у запобіганні окислювальному стресу, ніж звичайний полівітамінний препарат.

Ключові слова: вітамінний препарат, гіпоксія, оксидативний стрес, виживання за несприятливих умов, Метовітан.

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ПІДВИЩЕННЯ ВРОЖАЙНОСТІ ВІГНИ СПАРЖЕВОЇ (*Vigna unguiculata* L. Walp.) ЗА ДОПОМОГОЮ ЗЕЛЕНОГО СИНТЕЗУ НАНОЧАСТИНОК МАГНІЮ (MgO) З ВИКОРИСТАННЯМ ЕКСТРАКТУ ЛИСТЯ *Jatropha tajonensis*

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Метою роботи було дослідити вплив синтезованих наночастинок оксиду магнію з екстракту листя *Jatropha tajonensis* на ріст і врожайність вігни спаржевої (*Vigna unguiculata* (L.) Walp.).

Матеріали та методи. Підготовка і посадка насіння коров'ячого гороху; екстракція екстракту листя *Jatropha tajonensis* у водному розчині. Синтез наночастинок оксиду магнію (MgO) з екстракту, а також їхня характеристика для підтвердження утворення — методи УФ-ВІС, Фур'є-ІЧ-спектроскопія (FTIR), сканувальна електронна мікроскопія з енергетично-дисперсійним рентгенівським аналізом (SEM-EDX) та рентгенівська дифрактометрія (PXRD). Вивчено вплив MgONPs на рослини *Vigna unguiculata* (L.) Walp. в польових умовах для оцінювання їх використання з метою покращення їхнього росту і врожайності.

Результати показали, що різні дози наночастинок MgO (MgONPs), застосовані до рослин *Vigna unguiculata* (L.) Walp., суттєво впливали на всі вимірювані параметри розсади *Vigna unguiculata* (L.) Walp. в польових умовах позитивним чином. Найкращі результати щодо росту, врожайності та фенологічних параметрів було отримано у рослин, оброблених високими дозами MgONPs (100 мг/л). Зазначено, що різні дози MgONPs мають значний вплив на вегетативний ріст і врожайність *Vigna unguiculata* (L.) Walp. Значне збільшення кількості вегетативних параметрів спостерігалося у горщиках із різними дозами MgONPs (20, 40, 60, 80 та 100 мг/л) у порівнянні з контролем. Різні обробки MgO (з наночастинками або без них) призвели до суттєвих відмінностей у формуванні пагонів ($P < 0,01$). Відповідно до впливу різних доз наночастинок магнію на вігну спаржеву, висота рослин варіювалася від 18,88 ± 27,51 до 21,35 ± 3,25 см. Найвищу висоту, 21,35 ± 3,25 см, було отримано при застосуванні MgONPs у дозі 100 мг/л, а найнижча висота, 17,48 ± 3,83 см, спостерігалася при застосуванні MgONPs у дозі 17,48 мг/л.

Висновки. Встановлено, що наночастинки оксиду магнію (MgONPs) значно вплинули на параметри росту рослин та інші вимірювані характеристики. Крім того, було виявлено, що ефективність росту та врожайність *Vigna unguiculata* (L.) Walp. можна покращити шляхом збільшення застосування MgO у формі наночастинок. Також було підкреслено можливість використання MgONPs для підвищення врожайності інших культур, що може задовольнити потреби зростаючої чисельності населення світу.

Ключові слова: наночастинки оксиду магнію, *Jatropha tajonensis*, нанодобриво, *Vigna unguiculata* L. Walp., фенологія.