

Effects of exposure to nanodoses of chemical and physical factors on aquatic animals

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SUMMARY

The biological effect of exposure to nano doses of chemical (inorganic and organic) compounds and physical factors in aquatic organisms is shown.

The paper presents the experimental results obtained when the author studied the effect of more than 25 biologically active and toxic substances in nanodoses on functionally significant reactions in ciliates. In fish of different taxonomic position and at different levels of biological organization, the biochemical effect of nanodose of anxiety pheromone has been identified and compared. The effect of weak physical fields - natural radioactive background and some types of electromagnetic fields and ionizing radiation - on the physiological status of aquatic organisms and the physicochemical parameters of water has been studied. A significant correlation was found between changes in the rhythms of solar activity and the intensity of radio emissions with shifts in biochemical parameters in fish. The universality of the value of small doses for biota is discussed.

Key words: nanodoses, homeopathic doses, biologically active compounds, toxicants, peptides, anxiety pheromone, weak physical fields, heliophysical factors, physiological status of aquatic organisms, ciliates-spirostoma, fish.

RESUME

The biological effect of nanodoses of chemical (Inorganic and organic) compounds and physical factors in aquatic organisms was shown. The article presents experimental results of the author in study of functionally significant influence on the reaction of infusoria of more than 25 biologically active and toxic substances in nanodoses. Biological effect of alarm pheromone nanodose was revealed and identified in fishes of different systematic position and different levels of biological organization. The effect of weak physical fields of natural background radiation, certain electromagnetic fields and ionizing radiation on physiological status of aquatic organisms and physical and chemical parameters of water were studied. Significant correlation of changes on solar activity and intensity of radio emission with shifts in biochemical parameters of fishes were determined. Universal effect of low doses on biota is discussed.

Keywords: nanodose, homeopathic doses, biologically active compounds, toxicants, peptides, alarm pheromone, weak physical fields, heliophysical factors, physiological status of aquatic organisms, infuzoria - spirostomum, fishes.

Introduction

The famous Russian radiobiologist A.M. Kuzin wrote that "the evolution of life on our planet has revealed the ability of living organisms to use ultra-small quantities of many physical and chemical factors, harmful and poisonous in large quantities, (radiation, atmospheric pressure, etc.) for their prosperity" [1]. This, now widely studied phenomenon, called hormesis in biology, at the same time has been the main paradigm of homeopathy for more than 200 years. For an objective assessment of the general biological regularities of the effect of nanodoses, the latter should be studied at various levels of life organization - in biocenoses, at species levels, as well as at suborganic levels - organ, tissue, cellular and molecular. With this approach, we can talk about an impartial assessment of their effect on vital processes, manifestations of which seem to us at times odious. the effect

ultra-small effects, both physical and chemical (in particular homeopathic), this is not an accidental phenomenon in living nature. One of the paradoxical effects of ND is that a biological system can "feel" the influence of a factor introduced into it from the outside in a concentration several orders of magnitude lower than those that exist in the system itself (organ, organism, etc.) [2]. It is known that the tissues and organs of organisms contain on average from 10^{-8} to 10^{-11} M hormones, neurotransmitters, neuropeptides and other biologically active substances. At the same time, the ability of cells and tissues to respond to influences at a concentration of less than 10^{-17} M has been shown [3].

One of the biogenic substances that exhibit their effect in nature in small doses are pheromones, or, as they are figuratively called, environmental hormones. These compounds are released by plants and animals into the environment and affect the behavior or physiological state of other individuals of their own species (this property of pheromones is called species-specificity). The role of pheromones in the life of insects is especially fully studied and widely known. Recently, pheromones of farm animals and even humans have been intensively studied and are being applied in practice. Pheromones in aquatic animals have been studied fragmentarily. In our studies, we tried to discover and compare the effect of small and ultra-small doses of various natural biologically active and synthetic substances,

Purpose of the study: to prove the possibility of perception by aquatic animals of nanodoses chemical and physical factors.

Objects and research methods

The object of our study was an organism - a cell of the water ciliate *Spirostomum ambiguum*. The spirostoma has a characteristic worm-like shape (it is a relative of the well-known ciliate shoe) with dimensions: length - 1–3 mm, diameter - 0.3–0.5 mm. Under optimal conditions, the spirostome carries out a uniform forward movement in the water column at a speed of 2.5 cm / min. The nature of the movement of the spirostom is provided by the mechanism of joint beating of the cilia. The conducted electron microscopic examination indicates the multifaceted morphological organization of the protozoal cell, reflecting the polyfunctionality of the spirostoma. The complex functional organization of ciliates is manifested in a reaction to the action of various stimuli. Among the various "response" reactions of animals, behavior is one of the most sensitive phenomena. An example is the spontaneous locomotor activity inherent in animals of all levels, including protozoa. The criteria for the functional state of spirostom were developed and tested by the staff of the Moscow State University under the guidance of prof. ON THE. Tushmalova [4]. The parameters of the functional state of the ciliates are as follows (as it deteriorates):

1. Changes in the indicator only against the background of exposure to another stimulus in the subthreshold dose.
2. Change in motor activity.
3. Behavior change.
4. Changing the shape of the body.
5. Change in excitability.
6. Changing the timing of survival.

All these behavioral reactions and morphological features of spirostome allowed the creation of a semi-quantitative scale for assessing the degree of stimulus exposure [4, 5]. Changes in the speed of movement, the nature of behavior can serve as an indicator of the functional state of the ciliates. In response to adverse effects, spirostomes change their trajectory and speed. There are "twirls", backward movement and such "harbingers of death" as morphological anomalies of the body. A distinctive feature of these changes is the "all or nothing" response to impact, when all or the overwhelming majority of individuals in the population react to external impact. Experiments were always carried out in

the daytime part of the day (from 9 to 21 hours). Spirostom behavior was investigated by the "open field" method. The quantitative indicator was the change in behavior in unicellular animals, assessed by the criterion of spontaneous locomotor activity (SDA), expressed in relative units. For each point, 20 individuals were used. The measure of behavior was the index of motor activity - the number of times the spirostome crossed the eyepiece of the binocular eyepiece. Observation time is 5 minutes. The exposure time is 60 minutes. The representativeness of the applied criterion is evidenced by its fluctuations in intact spirostomes from 14 to 16 SDA during 18 years of research.

Lower vertebrates - fish were also the objects of our study. The studies were carried out on different types of fish (carp, silver carp, scalar, tilapia and Lena sturgeon). For each point, a group of 3-5 fish was used; in total, more than 80 fish were used in the experiments. The experience of many years of studying the characteristics of stress syndrome in fish (since 1975) has helped us in the adequate formulation and conduct of experiments on the study of stress and anti-stress measures in this series of studies. The physiological status of fish was judged by two criteria: biochemical parameters of mucus in vivo (in vivo diagnostics) and the activity of one of the indicator stress enzymes, acetyl cholinesterase (AChE). In a chronic experiment in vivo, metabolic shifts occurring in the body of fish can be assessed by changes in the composition of biological fluids, in particular, mucus. The biochemical parameters of mucus were determined by the analytical express method used for the first time for fish mucus using reagents immobilized on a solid carrier in a multilayer colored film from Ames [6]. The content of the following components of external mucus was determined: protein, ketones and hemoglobin, as well as changes in pH and mucus density. The content of parameters was estimated in the following units: protein in g / L, ketones in mmol / L, hemoglobin in $\mu\text{g} / \text{L}$. Each of the presented results is the average of 10 determinations [7]. The determination of the activity of the AChE enzyme was carried out according to the method developed by Ellman and colleagues and modified in the laboratory of Professor E.B. Burlakova [8], immobilized on a solid carrier in a multilayer color film of the company "Ames" [6]. The content of the following components of external mucus was determined: protein, ketones and hemoglobin, as well as changes in pH and mucus density. The content of parameters was estimated in the following units: protein in g / L, ketones in mmol / L, hemoglobin in $\mu\text{g} / \text{L}$. Each of the presented results is the average of 10 determinations [7]. The determination of the activity of the AChE enzyme was carried out according to the method developed by Ellman and colleagues and modified in the laboratory of Professor E.B. Burlakova [8], immobilized on a solid carrier in a multilayer color film of the company "Ames" [6]. The content of the following components of external mucus was determined: protein, ketones and hemoglobin, as well as changes in pH and mucus density. The content of parameters was estimated in the following units: protein in g / L, ketones in mmol / L, hemoglobin in $\mu\text{g} / \text{L}$. Each of the presented results is the average of 10 determinations [7]. The determination of the activity of the AChE enzyme was carried out according to the method developed by Ellman and colleagues and modified in the laboratory of Professor E.B. Burlakova [8]. Each of the presented results is the average of 10 determinations [7]. The determination of the activity of the AChE enzyme was carried out according to the method developed by Ellman and colleagues and modified in the laboratory of Professor E.B. Burlakova [8]. Each of the presented results is the average of 10 determinations [7]. The determination of the activity of the AChE enzyme was carried out according to the method developed by Ellman and colleagues and modified in the laboratory of Professor E.B. Burlakova [8].

For convenience in comparing the effect of nanodoses, many of the results obtained are presented in percentages. Statistical processing of all experimental material was carried out by nonparametric methods using the U test (Mann-Whitney), and the Spearman correlation coefficient was also calculated.

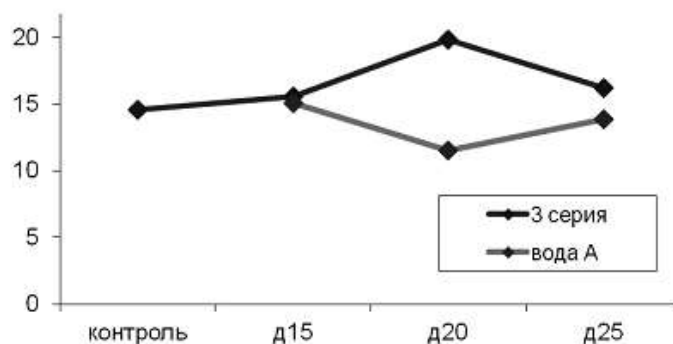
The effect of the patented (patent [9]) preparation cyprin was studied in fish at the level of the whole organism and at the level of the enzyme systems of fish. Its source is water-soluble compounds from fish skin in D15 breeding. Tsiprin is prepared according to the author's technology.

Results and Discussions

The aim of this work is to present and discuss our own data (obtained together with colleagues), proving the perception of nanodoses of physical and chemical factors by aquatic animals. It should be noted that in this work we will focus on the effects of nanodoses comparable to homeopathic doses (potency D3 corresponds to a concentration of 10⁻³). We began to test the regularities of the effect of nanodoses in ciliates by studying their reaction to phenosan, the effect of which was studied in detail by the staff of the laboratory of Professor E.B. Burlakova [10]. The latter prompted us to test the properties of this antioxidant (protein kinase C inhibitor) in ultra-low doses on a spirostome. Investigated a solution of phenosan, prepared by homeopathic technology D15, D20 and D25. For comparison, studies of potentiated water (water A) were carried out. [eleven]. The ability of a differentiated behavioral response by spirostom to the spectrum of studied homeopathic concentrations was revealed. The polymodal nature of the dose dependence inherent in the effect of nanodoses on organisms was revealed.

At the same time, the same doses of phenosan were studied blindly in different laboratories by other scientists. They investigated the effect of an antioxidant on the physical properties of water (electrical conductivity) on the biochemical model of an enzyme-substrate complex (acetylcholinesterase-acetylcholine) on the properties of membranes (viscosity) [12]. Change has been shown

all these criteria under the influence of the tested doses. The correlation and unidirectionality of the action of phenosan on the change in all the indicated parameters were noted. The dependence of the magnitudes of changes on the dilutions used and the potentiation factor was revealed.



Rice. 1. The figure shows one of the replications of the experiment. Results presented are typical for all replicates of the experiment. ** The abscissa is the fetosan concentration, the ordinate is the IDA spirostom.

A large group of substances tested in the next series of experiments is presented in a tabular form for ease of comparison of effects (Table 1).

Table 1

Influence of nanodoses of biologically active substances on ciliates, fish and laboratory animals

No.	Class connections	Ciliates, spirostomas, fish			Fishes, laboratory animals	A source
		Substance	Concentration	SDA effect		
one	Heavy metals	CuSO ₄	3.1 × 10 ⁻⁶ M	Fatal outcome	The change physiological fish status and biochemical composition of mucus and fish blood	and [13, 14]
			3.5 × 10 ⁻⁶ M	Minor the change motor activity		
		6.2 × 10 ⁻⁷ M	1.6 × 10 ⁻⁷ M			
	ZnSO ₄	1.0 × 10 ⁻⁶ M	Minor the change motor activity	The change physiological fish status and biochemical composition of mucus and fish blood	[13, 14]	
	Pb (NO ₃) ₂	1.5 × 10 ⁻⁶ M	7.5 × 10 ⁻⁷ M	Change of SDA by 40%	The change physiological fish status and biochemical composition of mucus and fish blood	[13, 14]
	Amino acids	Alanin	1.0 × 10 ⁻³ M	110%	Attractive action	Unpublished data
Histidine		1.0 × 10 ⁻³ M	180%	Attractive action	Unpublished data	
Alanine + histidine		1.0 × 10 ⁻³ M	133%	Attractive action	Unpublished data	
	Dipeptides	Carnosine	1.0 × 10 ⁻³ M	172%	Protective-migratory behavior in fish	Unpublished data

2	Tripeptides	Thyreoribelin	$1.0 \times 10^{-3}M$	170%	Oppression motor activity in fish	Unpublished data
	Tetrapeptides	ACTH	$1.0 \times 10^{-3}M$	The change SDA at 76 %, the change body shape	Excitation	Unpublished data
	Nanopeptide	Delta peptide sleep	$1.0 \times 10^{-3}M$	The change SDA at 63 %, the change body shape	Oppression	Unpublished data
3	Water-soluble wash low molecular weight lary connections mucus and skin fish	Water-soluble peptides fish slime	$1.0 \times 10^{-3}M$	No change	Minor excitation	[10]
		Pheromone inactivated ny	$1.0 \times 10^{-3}M$	No change	No change	[15]
		Pheromone alarm (leather carp fish)	$1.0 \times 10^{-3}M$	170%	Increase engine noise activity. Care from danger.	[15]
		Tsiprin	$1.0 \times 10^{-3}M$	The change movement spirostom had semi-modal character	In fish, it has Influence at survival, development speed caviar, this midget concentration causes sedation	[15]
4	Narcotic analgesics	Fentanyl	$1.0 \times 10^{-13} M$	Narcotic action, disappearing In 2 hours	Increase threshold pain at about 1.5 times. Dose $1.7 \times 10^{-14} mg / kg$	Own unpublished data
5	Pesticides	Deltamethrin	$1.0 \times 10^{-7} - 1.0 \times 10^{-18} M$	Polymodal character actions	Strengthening action curariform funds for H-XP $1 \times 10^{-18} M$. In lymphoid a number of chicken blood observed at 28 % cases necrobiosis. Dose $3.3 \times 10^{-12} mg / kg$. n = 800 cells	Own unpublished data
		Karbofos	$1.0 \times 10^{-3} - 1.0 \times 10^{-17} M$	Polymodal character actions		Own unpublished data
			$1.0 \times 10^{-13} M$	Oppression of the SDA by 50%, the change behavior		

As you can see from the table. 1, a change in all these criteria was revealed under the influence of nanodose of stimuli of different classes. The study of toxicants of inorganic (group No. 1) and organic (group No. 5) origin revealed that they are perceived by spirostomy. And the degree of the effect depends on the studied concentration. Using the same method, the authors previously estimated the degree of toxicity of water bodies adjacent to the Moscow ring

road, and identified ecologically unfavorable areas [16]. It was possible to detect both the effectiveness of and narcotic substances in nanodoses (group No. 4). Amino-peptide group No. 2 (Table 1) showed different effects of spirostome on the effects of amino acids of different molecular weights. At the same time, peptides, regardless of different molecular weights and biological activity, approximately equally (up to 70%) reduce SDA in spirostom.

I will dwell in somewhat more detail on group No. 3. Many aquatic organisms have well-developed chemoreception, which helps them to adequately perceive the environment with the help of biogenic compounds [17]. These compounds are found in the water body and are effective in ultra-low concentrations. We were able to show that the extremely species-specific biogenic "fright substance" of cyprinids, their anxiety pheromone, is also perceived by unicellular organisms [15]. According to our preliminary data, the anxiety pheromone of cyprinids belongs to peptides (molecular weight less than 1500 Da). Pheromone at a concentration of 10^{-3} to 10^{-8} has a high species-specificity for cyprinids and causes an alarm or danger reaction, accompanied by characteristic behavior and shifts in biochemical parameters inherent in stress syndrome. Its effect was also revealed in spirostom. Based on the paradigm of hormesis [18], it was logical to assume the anti-stress effect of nanodoses of this drug. Investigation of the effect of his scale of dilutions made it possible to reveal such. Concentration 1×10^{-15} has the maximum anti-stress activity. On the basis of the proprietary technology, an antistress drug for fish was created and a patent was obtained for it [9].

We have made an attempt to reveal the effect of some homeopathic medicines on SDA. The following drugs were investigated in preliminary experiments.

It has been shown that the tested homeopathic medicines have a differentiated effect on the SDA of ciliates [18]. However, it should be noted that the effect of the milk sugar (to which the drug is applied) is stronger than the effect of the drug itself. It seems to us that for further testing the efficacy of homeopathic medicines on spirostomes, it is necessary to solve some methodological difficulties. Nevertheless, we found that homeopathic medicines cause changes in SDA that are different from control, which, in turn, indicates the effectiveness of the method.

table 2

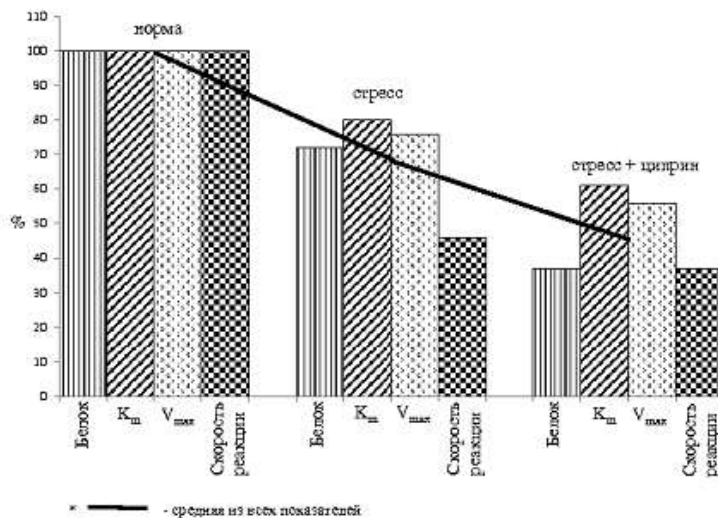
Medicine, concentration	spontaneous motor activity in%
control	one hundred
milk sugar 1 cr in 10 ml	12
milk sugar 1 cr in 100 ml	160
milk sugar 1 cr in 1 l	160
hina D 30 1:10	53
sulfur 6 1 cr in 1 l	130
arsenik 6 1 cr in 1 l	142
arsenic water 1: 10	170

Thus, experiments on unicellular organisms revealed and confirmed the regularities of ND functioning established earlier by other researchers and other methods - the effect and polymodality of their action, hormesis and the absence of species-specific effect.

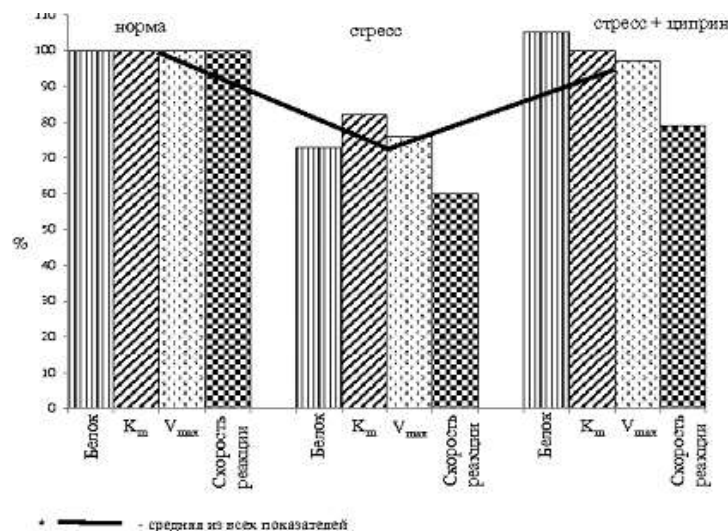
For a comparative biological assessment of the effect of cyprin obtained in invertebrates, the next series of experiments was carried out on highly organized lower vertebrates. The studies were carried out on several types of fish. The choice of the studied species (carp, silver carp, sturgeon, tilapia, and trout) is due to their belonging to systematically distant families. The latter will help to establish such a property of the investigated drug cyprin as its

species specificity. Disorders in the metabolism caused by the state of anxiety (stress), which are accompanied by shifts in biochemical parameters, were previously shown in fish. Changes in the physiological status were judged by shifts in the biochemical composition of a specific fluid - the external mucus of fish [6]. The most informative and specific criterion for stress in fish is the appearance of hemoglobin in their external mucus. The results obtained indicate significant biochemical changes during acute stress in all species of the studied fish: an increase in the content of hemoglobin and protein, as well as a change in the pH and density of mucus. Shifts were also noted in the blood formula. During the experiment, it was visually revealed that the effect of cyprin has an anti-stress effect on fish. The addition of the drug to the water to stressed fish practically normalized the shifts from stress and in terms of the studied biochemical parameters of mucus and blood. Under these conditions, the content of total protein in the brain tissue, which is reduced under stress, also normalizes in stressed fish (Fig. 2a). It is important to pay attention to the fact that the return to the norm of the studied parameters was revealed in all species of the studied fish, even those belonging to the systematically distant groups. It should be especially noted that the found effects differed only in magnitude, and not in its direction. that the return to the norm of the studied parameters was revealed in all species of the studied fish, even those belonging to the systematically distant groups. It should be especially noted that the found effects differed only in magnitude, and not in its direction.

It was of undoubted interest to test the effect of cyprin on tissue and cellular levels in fish. Previously, it was shown that NDs of some physical and chemical influences alter the activity of one of the key stress enzymes, acetylcholine esterase (AChE). The studies were carried out on the tissues of a stressed organism and treated with cyprin in vivo and



Rice. 2a. Influence of ND pheromone on the reaction rate and kinetic characteristics of AChE (in experiments in vivo).



Rice. 2b. Influence of ND pheromone on the reaction rate and kinetic characteristics of AChE (in experiments in vitro).

in vitro in a test tube, when cyprine was added to the membrane fraction and the purified enzyme. In order to reveal the correlation of changes during cyprin treatment of the whole organism with changes in its tissues and cells, the activity and some characteristics of AChE in the brain of fish were assessed. In subsequent experiments, it was possible to show that with the development of stress in fish (in vivo experiments), the activity decreases and the kinetic characteristics of AChE change (Fig. 2a). The addition of ND pheromone to water in stressed fish normalizes the kinetic characteristics of AChE. During the in vitro experiment "in vitro" (addition of ND pheromone to the brain homogenate of stressed carp), changes in the studied parameters were also found. However, it should be emphasized that the direction of the changes was opposite (Fig. 2b). A similar experiment with a fraction of the brain membranes of stressed tilapia reveals a similar effect (Fig. 2b). Studies carried out on aquatic vertebrates made it possible to identify and compare the effect of cyprin in fish of different taxonomic positions and at different levels of biological organization. Under stress, changes in the biochemical parameters in mucus and blood, as well as in the properties and kinetic characteristics of brain AChE, one of the key stress enzymes, were found in fish. The normalizing antistress effect of cyprin on these parameters was revealed. In the study of the effect of cyprine in vitro, it was shown that its addition to the tissues and organelles of the brain also causes significant shifts in the activity of AChE. made it possible to identify and compare the effect of cyprin in fish of different taxonomic positions and at different levels of biological organization. Under stress, changes in the biochemical parameters in mucus and blood, as well as in the properties and kinetic characteristics of brain AChE, one of the key stress enzymes, were found in fish. The normalizing antistress effect of cyprin on these parameters was revealed. In the study of the effect of cyprine in vitro, it was shown that its addition to the tissues and organelles of the brain also causes significant changes in the activity of AChE. made it possible to identify and compare the effect of cyprin in fish of different taxonomic positions and at different levels of biological organization. Under stress, changes in the biochemical parameters in mucus and blood, as well as in the properties and kinetic characteristics of brain AChE, one of the key stress enzymes, were found in fish. The normalizing antistress effect of cyprin on these parameters was revealed. In the study of the effect of cyprine in vitro, it was shown that its addition to the tissues and organelles of the brain also causes significant changes in the activity of AChE. The normalizing antistress effect of cyprin on these parameters was revealed. In the study of the effect of cyprine in vitro, it was shown that its addition to the tissues and organelles of the brain also causes significant shifts in the activity of AChE.

However, these changes of the opposite sign lead to an even greater decrease, rather than normalization of the studied parameters. The addition of cyprine to the water containing stressed fish practically normalized all changes from stress in mucus and blood. The addition of cyprine in stressed fish normalizes the total protein content in the brain tissue, which is reduced under stress. It is important to pay attention to the fact that the return to normal was found in the "distant relatives" of the carp. That is, cyprine retains its biological activity, but loses its species-specificity. During an in vitro experiment "in vitro" (adding cyprine to the brain cells of stressed carp and tilapia), normalization of the physicochemical characteristics of one of the key stress enzymes, acetylcholinesterase, was found. One of the hypotheses which can explain the uniformity of the effect of ultralow doses on the organism of animals, including humans, may be the commonality of molecular mechanisms that determine the responses to these doses. The latter can be considered as an example of the study of phenomena reflecting the effect of small and ultra-small effects of chemical factors on the biota.

Effects of physical factors on animal organisms

I would like to emphasize that the effectiveness of nanodoses of chemical factors for biota is not an unusual and not uncommon phenomenon in nature. Physical factors influenced by

which formed the entire process of evolution, also affect the biota with radiation of various powers. It is important to note the regularity that manifested itself in this case, which is characteristic of ND and called "radiation hormesis" [1]. The effects of small doses of radiation are manifested from a stimulating effect on the biota to the destructive force of the anthropogenic impact of the atomic bomb detonated over Hiroshima. It is known that the reversal of the effect (from negative to stimulating) occurs at doses close to the natural natural radiation background (NRP). This background, often called cosmic or heliophysical factors, consists mainly of gamma, beta and alpha rays with a small admixture of neutrons, protons and other elementary particles. It was found that PRF is necessary for the existence of the biota. Moreover, it was found that

Effects of nano-doses of physical influences on unicellular

In one of our last works with a large team of various specialists under the leadership of Academician A.I. Konovalov, the effect of weak physical fields of the natural radioactive background and some types of electromagnetic fields and ionizing radiation on the physiological status of spirostom and the physicochemical parameters of water has been studied very fully [20]. This work expanded and deepened our understanding of the properties of the environment. Most likely, the state of the physical environment affects the aquatic environment and induces changes in the vital parameters of biological objects in it [21]. The presented results expand and supplement the previously obtained data on the effect of other radiation on behavioral reactions by spirostom [5]. It was revealed that spirostomes differentiate the effects of alpha and beta and their total irradiation on water. Water effect, irradiated with a beta source, on SDA with spirostom, is greater than the effect of water irradiated with an alpha source, and the total effect of these sources causes a significant effect, but with the opposite sign. The found changes in SDA with spirostom from remotely irradiated water ("by stend" effect) are approximately equal to those from directly irradiated water or, in the case of gamma-neutron irradiation, even slightly greater than the "by stend" effect. The "by stend" effect disappears when the aqueous medium is shielded with foil. According to the behavioral criterion, the effect of thermal neutrons and concomitant gamma radiation was studied [22], as well as γ radiation in a wide dose range from 0.01 to 1500 Gy (LD but with the opposite sign). The found changes in SDA with spirostom from remotely irradiated water ("by stend" effect) are approximately equal to those from directly irradiated water or, in the case of gamma-neutron irradiation, even slightly greater than the "by stend" effect. The "by stend" effect disappears when the aqueous medium is shielded with foil. According to the behavioral criterion, the effect of thermal neutrons and concomitant gamma radiation was studied [22], as well as γ radiation in a wide dose range from 0.01 to 1500 Gy (LD but with the opposite sign). The found changes in SDA with spirostom from remotely irradiated water ("by stend" effect) are approximately equal to those from directly irradiated water or, in the case of gamma-neutron irradiation, even slightly greater than the "by stend" effect. The "by stend" effect disappears when the aqueous medium is shielded with foil. According to the behavioral criterion, the effect of thermal neutrons and concomitant gamma radiation was studied [22], as well as γ radiation in a wide dose range from 0.01 to 1500 Gy (LD₅₀ for protozoa more than 1000 Gy) at the cellular (and organismal) levels [20]. The revealed decrease in SDA persisted for many generations (10–15 generations were studied with Spirostom) [5]. Changes in behavior were manifested in a significant number of individuals, i.e. occurred according to the principle "all or nothing" and differed from those usually observed with an increase in the dose of radiation cellular effects by independence of the dose in the range of small and medium doses for spirostom from 0.01 to 800 Gy [5]. A negative effect has been revealed that occurs in a spirostom when one-hour stay in a low-intensity radio-frequency field of an EMF with a radiation frequency of 1 and 10 GHz and a PES of 50 $\mu\text{W} / \text{cm}^2$...

To identify the effect of non-ionized electromagnetic radiation (EMR) on the behavior of the spirostome, they were exposed to stronger than natural, technogenic EMR from a computer and a mobile phone with sparing parameters: water was located for 15 minutes at a distance of 20 cm from the source. Under these conditions, exposure to EMR changed the properties of the experimental water, and the ciliates, being placed in it, increased their IDA by 34%, and also modified their behavior. Longer exposure (up to 2 hours) led to a further increase in SDA up to 160%, which further after another 2 hours led to a sharp decrease in SDA to 80%.

In the study of short-term (from 10 minutes to 10 hours) action of low-intensity EMR) at the frequency of mobile communication (1 GHz) and radars of the military-industrial complex (10 GHz) with an energy flux density (PES) of 5, 10 and 50 $\mu\text{W} / \text{cm}^2$ found:

- a) a decrease in the motor activity of protozoa at all values of the PES (the maximum permissible level for the population of Russia is 10 $\mu\text{W} / \text{cm}^2$ [5];
- b) the presence of a "threshold" exposure, which is shifted towards reducing the permissible

(safe) time spent in EMF with an increase in PES;

c) reaching the "dose plateau", when the magnitude of the effect does not change with increasing exposure. It is shown that the "dose plateau" can be infinitely large;

d) preservation of the biological effect in changing behavior in generations of protozoa (traced 10-15 generations) [5].

All the revealed characteristic features of behavior, in comparison with that of the control individuals, indicate a significant adverse effect of EMR on water.

In another series of experiments, EMP was applied to water in which spirostomes were placed in advance. The latter also showed a change in the studied parameters, the magnitude and nature of which differ from those described above. We believe that our experiments have established a separate effect of EMR on the properties of water and on a living organism. The conditions of the last experiment are close to natural, where the synergism of these effects is observed, as in our second experiment. The results of the studies carried out are consistent with our earlier works on changes in the environment caused by natural cycles, solar activity, etc., which were identified and evaluated using unicellular aquatic organisms. It was revealed that changes in water under the influence of ultra-weak natural cycles,

Effects of nano-doses of physical effects on fish

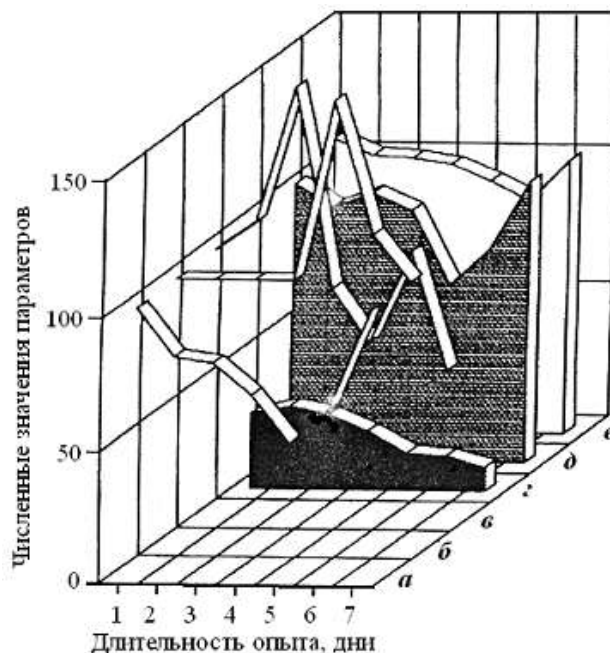
To emphasize the universality of the effect of ultra-low doses on biota, let us give examples of the effect of heliophysical factors in the same doses on the organism of aquatic vertebrates - fish. "At present, a huge empirical material has been accumulated from the results of the study of diurnal and circadian, tidal, annual and sidereal - seasonal rhythms associated with the atmospheric electromagnetic field, which also rhythmically changes under the influence of natural geophysical rhythms of other frequencies, movement of air masses, changes in the activity of the sun and associated with them geomagnetic phenomena, and others still poorly studied, having a long-term duration "[23]. The cosmic effect is clearly manifested in the rhythmology of vital processes in living organisms. Due to the short life cycle of the spirost, the diurnal rhythm was not studied on them. However, in fish, we were able to identify the rhythmic nature of daily changes in physiological status. In a chronic experiment, the rhythmic nature of shifts in biochemical parameters (ketones, hemoglobin and protein) in the external mucus of carp was revealed in normal conditions and under the influence of toxic substances and environmental irritants.

A significant correlation was found between the rhythms of biochemical parameters with changes in solar activity and radio emission intensity. The problem of the combined effect of small doses of various environmental factors on organisms is discussed).

The nature of the rhythms - the revealed synchronization - can be a reflection of the homeostatic process. A change in the nature of the curve under the influence of various stimuli can manifest itself in a change in the amplitude of rhythms and their desynchronization.

Conclusion

The biological effect of exposure to nano doses of chemical and physical factors in aquatic organisms is shown. The reaction to nanodoses of organisms of early stages of evolution (pre-nerve organisms) was revealed. The discovered ability to respond to such doses was retained in highly organized vertebrates. The versatility of the functional significance of nanodoses for all living things is an example of an energetically economical way of development. The meaning of the effect of nanodoses is the minimization of energy costs during metabolism. The essence of the phenomenon of minimum energy consumption lies in its maximum effect. The latter is associated with the need to implement iconic biological phenomena, which is evolutionarily progressive. All of the above and our earlier results allow us to put forward and formulate an assumption:



Rice. 3. Dynamics of heliophysical factors and biochemical parameters of intact fish in chronic experiment. Biochemical parameters: a - ketones, b - hemoglobin level, c - protein content. Physical parameters: d - solar activity Ak, d - Wolf numbers W, e - RF emission level F.

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