The Intergated Scientific Journal, 2006,#13,pp.56-68.

MUSSEL LARVAE-INFECTED ATLANTIC SALMON WITH CANCELED "DEATH PROGRAM" AND LONG LIVED ARCTIC STICKLEBACK ARE POTENTIAL SOURCES OF STRESS PROTECTORS AND ANTICANCER MEDICATION

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ABSTRACT

The present paper analyses a new approach to the development of natural anticancer medications made of hydrobionts selected for longevity and stress resistance. (1) The accelerated senescence program (progeria) was artificially cancelled in arctic threespine stickleback *Gasterosteus aculeatus* by crossing the marine and freshwater forms in ponds and selection of hybrids. The prolongation of life span of fish was accompanied by an increase of production of the antistress exocrine secretion (glue) by the renal epithelium of male sticklebacks (glycoproteins, peptides, and mucoids), required for successful egg development. (2) A unique occurrence was observed when the progeria of Atlantic salmon was cancelled by larval parasite of the gills — freshwater pearl mussel *Margaritifera margaritifera.* As a result, the maximum age of *Salmo salar* fishes hosting the mussel can be as high as 13 years. The mollusk-fish system made it possible to demonstrate that the parasite can inhibit senescence of the host and stimulate nonspecific resistance to stress, i. e. can control longevity. The mussel proved to increase the resistance of wild salmon parrs to epithelioma and cutaneous mycoses. The parasite is perceived to neutralize the senile changes in the salmon regulatory cascade hypothalamus-pituitary-peripheral endocrine glands-hypothalamus. Trials of the new medication prepared from secretion of fish gills (mucus) and kidneys (glue) were made on salmon parr with

epithelioma, guinea pigs with affected skin, mice with transplantable tumors, and human volunteers with depressions and miscellaneous tumors, and demonstrated a good therapeutic effect of natural remedy.

KEYWORDS: anticancer medication, senescence, host-parasite interactions, pearl mussel, salmon, stickleback

INTRODUCTION

The world never stops to search for new antitumor medications from natural organisms that have no side effects on the immune system. Extraction of drugs from aquatic organisms, hydrobionts, always attracts particular attention. Most hopes are pinned to hydrobiont that produces substances efficiently neutralizing microbes and parasites or poisoning/repelling predators. Bioactive substances with anticancer, anti-inflammatory, antimicrobial, and antiviral activities (lipids, mucopolysaccharides, peptides, and glycoproteins) have been recently isolated from oysters, mussels, *Mercenaria*, as well as other mollusks, crabs, shrimps, trepangs, polychaetes, leeches, sea squirts, frogs, and many fishes including halibut, sharks, mullets, loach, and seahorses (Pilat and Ivanov, 2002). Medical significance of seahorses of the genus *Hippocampus* (Gasterosteiformes) is widely known; they are used in traditional medicine of Southeast Asia to treat cardiovascular, metabolic, and sexual disorders as well as certain cancers (www. sea horse project). At the same time, few know the medicinal potential of close relatives of sea horses, sticklebacks (Gasterosteidae), and their wonderful capacity for wound healing. Stickleback fat was successfully used in the blockaded Leningrad during the Second world war to treat wounds and burns, since it contains carotene and vitamin A, which accelerate regeneration and wound healing (Ziuganov, 1991).

Sticklebacks have an amazing ability to release proteins to the environment similar to fibroin filaments of spiders or byssus of oysters. Secondary epithelial cells in the kidney of spawning males release a 200 kDa sticky glycoprotein, which is, together with some other light proteins and mucoid substances, a component of glue used to build a spawning nest from algae (Jakobsson *et al.*, 1999; Jones *et al.*, 2001).

It is well known that Pacific Salmon dies immediately after spawning as a result of accelerated

aging (progeria), which develops when the fish leaves the ocean and swims upstream the river. A signal for progeria proved to be changed from the sea to fresh water. In this case the biological function of suicide seems to be that the remains of the old fish become food for river invertebrates which, in turn, are food for the young fish (Ziuganov et al., 1998).

After spawning in a river Atlantic salmon, in contrast to its Pacific relatives, returns from river to ocean and dies there. If it is the summer race of the fish, it often dies in the fall. However, in general the proportion of fish spawning for the second and third tine is 10- 40%, although fifth and sixth spawnings were reported (Ziuganov, 2005b). Several years we studied larvae of mussel *Margaritifera margaritifera* that develops in the gills of the Atlantic salmon. We found that larvae can somehow "switch off" the "death program" of fish so the larva infected fish live at least one season more than the majority of non infected salmon (some of infected salmon live up to 13 years). An increase of the life span of the host is necessary for the larva to complete their own development. It was shown that the infected fish had no tumors and were more resistant to wounds and burns (Ziuganov, 2005a).

In the present work, a possible medicinal significance of sticklebacks and salmons with cancelled "death program" was analyzed. The remedy composed of the renal secretion of sticklebacks and gill cell extract of salmons was tested on parrs of salmons with epithelioma, guinea pigs with affected skin, mice bearing transplantable Ehrlich carcinoma, and human volunteers with depressions and miscellaneous tumors.

MATERIALS AND METHODS

Glue of Stickleback Gasterosteus aculeatus:

Selected fishes with cancelled accelerated senescence program were used in 2000-2003. Field experiments on producing fish with such biological properties were published earlier (Ziuganov, 1983, 1988, 1991, 1995; Ziuganov *et al.*, 1987; Ziuganov and Tashenov, 2004; Ziuganov and Popkovich, 2005). Glue-producing stickleback spawners were caught with a dip net in ponds and maintained in 100 l aquaria in the littoral zone of a pond. Duckweed was given as a material for nest building. Males were induced for glue production by the spawn extract and ovarian liquid. Glue was collected with a Pasteur pipette from the urogenital foramen of living males (a noninvasive method). In some cases, males were anesthetized with 0.1% phenoxyethanol (Fluka), sacrificed, and the urinary bladder (containing the glue) was dissected using forceps and ophthalmic scalpel. Another

noninvasive method of glue collection included its squeezing from the nest composed of sand and duckweed leaves through a 500 μm mesh. The glue collected by two different methods under the field conditions was stored in Eppendorf tubes for several hours in a portable cooler and then frozen at –16°C. In the laboratory, thawed glue was subsequently filtered (0.45- um Millipore filter, 10 min at 3700 g) to eliminate cells and particular matter, and the samples were maintained in separate glass beakers on ice.

Infestation of Salmon Salmo salar byParasitic Glochidia of Margaritifera margaritifera:

Specifics of the experiment were described in detail earlier (Ziuganov et al., 1994, 1998, 2000, 2001, Ziuganov, 2005 a,b). Atlantic salmon spawners were caught in 1987-2005 in the basins of the Varzuga, Umba, and adjacent rivers (Kola peninsula, Russia) from June to November. Fish were caught to study the behaviour and survival of salmon as a host of pearl mussel *M. margaritifera* larvae (glochidia). Originally experimental goals included establishing the effect of catching and holding in a tank on salmon survival in order to determine the conditions for semiartificial reproduction of the rare and endangered pearl mussel using its natural host, salmon, without adverse effect to the fish. All the fish were held in cylindrical tanks for 5 to 60 days in running river water, in 2 x 5 m river plots bordered with webbing of 10 mm mesh size, or in the standard wooden tanks of the Umba Fish Hatchery (Murmansk province). Initially, females of pearl mussel were induced to release glochidia using the method of Ziuganov *et al* (1994). Then, a glochidial suspension of about 300,000 glochidia per litre was made, and aliquots of this suspension were introduced by medicine dropper into the mouths of salmon being caged. In total, the infestation technique resulted in 10,000 – 12,000 glochidia becoming attached to the gills of each fish. Preliminary experiments determined that the lethal density of infested glochidia is roughly 500,000 per fish. The sublethal dose is about 50,000 glochidia per fish, in order to cause 50% latent mortality of infested fish.

For 19- yr period, more than 400 adult salmons were caught, marked, artificially infested, and released into the river to enhance pearl mussel reproduction. These fish were used in our experiments. Most infested fish were released after a 10 day period of confinement in cages. Assuming a 95% subsequent mortality rate of young mussels, we introduced about 200,000 juvenile mussels into the

Varzuga and Umba river basins. Totally 45 marked adult salmons with cancelled "death program" were caught repeatedly after winter and were held in cylindrical cages for 5 to 15 days in river water.

After detaching glochidial from the host fish the gills were dissected from the fish. Some of gills rinsed in fresh water and fixed in 2.5% glutaraldehyde with 0.05 M cacodylic buffer (pH 7.4) for 2 h at 10 C^0. The specimens were then dehydrated and prepared for scanning electron microscopy as described in our monograph (Ziuganov *et al.,* 1994).

Collection of Fresh Salmon Gill Mucus

Prior to being sacrificed, the fish were anesthetized rapidly in 0.4% 2-phenoxyethanol. After that the parr and adult fish were examined for mussel larvae. The opercula were carefully removed using a scalpel and scissors, and the excised gills were placed between two glass slides. In summer (June-July) juvenils of *Margaritifera* begin to leave the host gills, and at this time one may observe empty cysts which then begin to decrease and a few days later look like small protuberances on the gill filament. Thereafter, no marked histopathological changes in gill tissue were detected. Individual fresh mucus samples were carefully drawn from abandoned by mussels and regenerated gills using a Pasteur pipette (Ziuganov, in press). The mucus was subsequently filtered (0.45- um Millipore filter, 10 min at 3700 g) to eliminate cells and particular matter, and the samples were maintained in separate glass beakers on ice. Finally, glue of stickleback and salmon gill mucus were pooled. Thus medically active compound was obtained.

Characterization of Glue + Mucus

The content of the glue+mucus organic matter was determined in the Russian Federal Research Institute of Fisheries and Oceanography (Moscow). The concentration of solids was 9.1 mg/ml; carbohydrates, 3.0 mg/ml; lipids, 0.64 mg/ml; total nitrogen, mg/ml; nonprotein nitrogen, 1.26 mg/ml; protein nitrogen, 0.34 mg/ml; and mineral matter, 2.1 mg/ml. A detailed biochemical analysis of proteins, peptides, and amino acids was then carried out in the Institute of Genetics and Selection of Industrial Microorganisms (Moscow). Protein concentration was 0.37 and 0.27 mg/ml according to the method of Bradford (1976) and amino acid analysis, respectively. The protein included all 20 proteinogenic amino acids (except tryptophan): Asp.a/Asp, 0.0419 mg/ml; Thr, 0.0181 mg/ml; Ser, 0.0136 mg/ml; Glu.a/Glu, 0.0310 mg/ml; Pro, 0.45– 0.0135 mg/ml; Gly, 0.0103 mg/ml; Ala, 0.0104

mg/ml; Val, 0.0160 mg/ml; Ile, 0.0128 mg/ml; Leu, 0.0181 mg/ml; Tyr, 0.0111 mg/ml; Phe, 0.0119 mg/ml; His, 0.0162 mg/ml; Lys, 0.0161 mg/ml; Arg, 0.0152 mg/ml; Cys, 0.0122 mg/ml; Met, 0.0016; and total amino acids, 0.270 mg/ml. The glue+mucus underwent ultrafiltration through a UM5 membrane. The resulting fraction contained no proteins, included the same proteinogenic amino acids (0.0288 mg/ml in total), and represented the peptides and free amino acids of the mucus. The glue+mucus also demonstrated proteolytic activities of a metalloproteinase, acid proteinase, and aminopeptidase.

The glue of large selected long-lived sticklebacks plus extract from salmon gills preliminary infected by larva mussel were used in experiments as a wound healing, antistress, and antitumor medication. This biodrug was registered under the name "Arktika+" by the Ministry of Health of the Russian Federation. After a trial for toxicological, microbiological, physicochemical, and clinical safety, the drug was given the sanitary–epidemiological certificate no. 77.99.11.915.D.001282.03.02.

Volunteers

First, the author has been testing the preparation in the form of a spirituous tincture (mixed with cognac of premium quality matured for five years) on his own body since 1999. No side effects have been detected. In the course of preclinical tests 200 volunteers were examined complaining of depression disorders of minor and average degree.

RESULTS

Tests on Atlantic Salmon Parrs with Tumors.

Epidermal papillary tumor (epithelioma) is found in parr of Atlantic salmon (*Salmo salar*) in northwest Russia since 1970s. High disease incidence (50%) and death rate (30–90%) of affected fish at the age of 1–4 years is observed in salmon fisheries. Water contamination with carcinogens, cocarcinogens (nitrosamine, benzopyrene, etc.), and viral agents (such as herpesvirus) are considered as the factors of tumor formation. The incidence of epithelioma is 1.5% in adult fish (Ziuganov and Kalyuzhin, 2004).

At the 1st stage, light nodes with rough surface appear on fish skin. At the 2nd stage, pocky lumps with paraffin consis-tency and fine granular surface are formed (Fig. 1). The number and size of the

lumps can vary from single or multiple plaques to their conglomerates. They can be found on body back and sides, head, and pins. At the late 3rd stage, ulcers of different size are formed after tumor rejection, which, after a secondary infection, can be transformed to necrosuppurative inflammations of skin and muscle and eventually can lead to degradation of deep tissues and caudal fin necrosis.

Trials of "Arktika+" were carried out in 2000–2002 on 2-year-old salmon parr with the 2nd stage epithelioma at the Umba Fisheries in the Murmansk Region. Table 1 demonstrates a high curative effect: the survival rate of the parr with stage II disease increased from 7–11% (control) to 89–96% (tenfold on average). The rate of remission (subsiding of the symptoms) increased from 0% (control) to 85–89%. The results were reproduced for three years.

Tests for Skin Wound Healing in Guinea Pigs Cavia porcellus and for Toxicity for Rats and Mice

These tests were carried out in the Laboratory Testing Center of the Institute of Beauty (Moscow) (protocol no. 101, March 3 2000). A 1 × 1 cm derma squares were cut out of 30 guinea pigs (the method of V.S. Peschanskii). The treatment of the Arktika+ accelerated skin wound healing by 62 and 27% by days 8 and 12 after surgery, respectively, as compared to the control group. The medication decreased the healing time by 5 days, which amounted to 19.3% relative to the control group.

Based on these data as well as tests on albino mice (n = 36) and albino rats (n = 30) allowed the Institute to certify the absence of irritating or allergenic effect on the skin and mucous membranes as well as the absence of acute and subacute toxicity and cytotoxic effect on embryonic diploid human cells (protocol no. 104, February 2001). Overall, the Institute of Beauty certified that the medication has a pronounced wound healing effect and recommended to introduce it in curative cosmetics to accelerate skin regeneration.

Tests for Anticancer Activity of the Biodrug in Mice with Implanted Tumors

These tests were carried out at the Petrov Research Institute of Oncology (St. Petersburg). Twenty NMRI mice were intraperitoneally implanted with Ehrlich ascites carcinoma (10^6 cells). Starting from the next day, half of them received the biodrug in their drinking water (1:200 by volume) while the other half were receiving pure water served as the control. The volume of consumed water was measured daily; it amounted to 3.5–4.5 ml per mouse per day in both groups. The day of animal death was recorded. Table 2 shows the extension of the mean mouse lifespan induced by the bio-medication.

According to the report of the head of the Oncology Department Prof. V.N. Anisimov, "All mice of the control group died 9 to 13 days after carcinoma inoculation. In the group of mice receiving the drug, 50% of mice survived day 13. The medication significantly increased the mean lifespan of mice by 16.7% (Table 2). Considering the peptide nature and low toxicity of the mucus, this preliminary result deserves serious consideration and suggests testing the mucus drug for anticancer activity on tumor lines of different histogenesis." Thus, the medication diluted 200-fold (!) demonstrated a significant curative effect on tumor-bearing mice.

Volunteers with Depressions

In the course of preclinical tests 200 adult volunteers of both genders were examined (Table 3) complaining of depression disorders of minor and average degree, cyclothymia, neurocirculatory dystonia of hypotonic type after having an acute respiratory viral infection. The volunteers of the experimental group took the elixir 3 times a day with the dosage of 10-15 ml internally half an hour before meals. The results were compared with those of the control group with the same diagnoses, who took tranquilizers.

The majority of volunteers showed mood improvement, lowering of anxiety level, and vanishing of the ideas of guilt. The volunteers' sleep got normalized, and in this respect there were no side effects, such as somnolence and revocation effect. By the end of the testing period somatic disorders were eliminated: headache, cardiac and vascular activities disorders and gastric and intestinal disturbances. The bio-preparation increases mental workability and reduces fatigue; it is especially effective in case of depression disorders, when the patient has to combine treatment with his labour activities.

Tumor Regression for Volunteers

Below you will find two examples of the bio-preparation successfully curing of hopeless forms of various tumor diseases, for which five-year and even one-year survival are extremely low. For example, regarding **pancreas cancer** the ratio of diseased men to deceased men in 2002 all over the world was 116 thou to 112 thou (with the death-rate of 97%), for women this index makes up 101 thou: 101 thou.

Male patient F., 64 years old, was followed up in Moscow clinical hospital № 15 named after O.M. Filatov since 2002 with the diagnosis: head of pancreas cancer. Cancer of papilla duodeni major. Mechanical jaundice. The following procedures were not conducted: chemical and radiation therapy, surgical treatment.

From the excerpt of his medical case: «In October 2000 the patient was taken to hospital with complaints about weakness, heaviness in the right hypochondrium, sclera and coverlets icteritiousness. Has been ill for the recent two months... Ultrasonic test. Duodenoscopy, retrograde cholangiopancreatography, curative papillosphincterotomy. Nasobiliar catchment. Biopsy. The papilla was cut through for the length of 1 cm, in the course of this operation tissue infiltration was noted along the papilla wound. Diagnosis: tumor of the head of pancreas. Tumor of the distal common bile. In the area of the head of the pancreas the zone of lowered echogenicity to 39 mm is determined. The patient was discharged from hospital with the recommendation of being consulted at the Research Institute named after Sklifosovsky regarding radical surgical treatment».

The patient refused to be operated on and expressed his wish to participate in the pre-clinical test with the "Arktika+" bio-preparation as a volunteer. The 1st cycle of peroral taking of 500 ml of the preparation took 1.5 months. Stable improvement of his general state was noted. The man, who earlier was a bed-patient, returned to normal mobile way of life. During the control examination in December 2002, according to the data of Computer Tomography (CT) the entire regress of the tumor was revealed, common bile was not expanded. The observation is continuing **(Fig.2).**

Cavernous angioma – refers to the diseases of vessel development or vascular malformations of the central nervous system and makes up 5-13% of all the anomalies of the cerebrum.

Female patient M., 49 years old, was observed in the regional clinical hospital of the town of Vinnitsa, the Ukraine, since October 2002. She consulted the doctor complaining of weakness, headache. The primary diagnosis was micro-insult. The CT revealed additionally cavernous angioma of pons cerebelli in the left hemisphere: tumor sized 20 x 20 mm. In November the patient exercised acute worsening of the state with the development of right-side hemiparesis (paralysis), aphasia (speech defect). The desire to live was almost lost. However, the patient agreed to be a volunteer for elixir testing. The improvement of general state with the gradual restoration of speech, apparition of movement in the right extremities, cessation of headaches began during the first weeks of her being treated with the elixir. In the meantime, by April 2003 the measurements of the tumor increased to 23 x 23 mm, **(Fig.3).** In May 2003 the patient already stood on her own feet and was leading an ordinary life of a housewife living in rural steppe area. The CT revealed that by November 2003 (end of treatment with the elixir), the tumor diminished by 90%, it «split» into small islets, and half a year later after the treatment the tumor regressed completely **(Fig. 3**). There were no complications resulting from the treatment. After taking the treatment the patient preserves stability of salutary process which is continuing till present.

DISCUSSION

WHETHER PROGERIA OF FISH AND AGING OF MEN CAN BE "SWITCHED OFF"?

Disabling the accelerated senescence program in fish increases the production of the antistress secretion. In a series of stickleback populations, absolutely all spawners die after the first reproduction act from accelerated senescence (progeria) at the age of 1 year, which is also typical for Pacific salmons. The fish of this type (semelparous) die after spawning as a result of triggering a special biochemical program, in which the production of steroid hormones, particularly, stress hormone cortisol, plays the key role. Accelerated senescence is induced by the activation of the cascade hypothalamus–pituitary–adrenal cortex–high cortisol levels–thymus atrophy (accompanied by depressed immunity)–high blood sugar, fatty acids, cholesterol, and insulin–death of cardiac and renal infarction and insults.

Accelerated senescence of Pacific salmons and sticklebacks can be disabled by gonadectomy or adrenalectomy in juveniles. Such operation doubles their lifespan. Dil'man (1986) was among the first to notice nearly identical hormonal changes during senescence of semelparous fish and human. The difference is a much slower rate of changes in humans than in pink salmon. Recently Maldonado *et al.* (2002) demonstrated a striking similarity between the deposition of β-amyloid plaques in the brain neurons of salmon dying after spawning and the course of Alzheimer's disease in humans. Senescence and related systemic diseases are thought to result from overstrained rather than fading activity of the systems controlling energy processes, adaptation, and reproduction. In particular, the increasing activity decreases the responsiveness of the hypothalamus to the negative feedback signals (Todorov and Todorov, 2003).

Selection of long lived sticklebacks

While studying the genetics and evolution of sticklebacks, we began to think about a gerontological significance of this fish and its medical use 20 years ago. In 1985 we made an attempt to experimentally disable the program of post-reproductive death in holarctic sticklebacks by interspecific hybridization rather than castration and to extend the lifespan of fish normally living for 1 year (Ziuganov *et al.*, 1987; Ziuganov, 1988). Long term selection in natural water bodies (ponds in the polar regions of Karelia) with unusual environmental conditions allowed us to raise hybrid threespine sticklebacks with high behavioral stress resistance and lifespan extended to 7–8 years (Ziuganov and Tashenov, 2004). Hence, monocyclic semelparous fish were transformed to polycyclic iteroparous ones, which die of old age after several acts of reproduction. Noteworthily, these large

(up to 10 cm) long-lived fish with outstanding resistance to wounding as well as infectious and parasitic diseases, demonstrated a 150–200% increased production of the above-mentioned secretion of renal glandular cells—glue. This secretion is produced by all stickleback species. In addition to the main gluing function for the nest, to which the female lays the eggs and which is actively guarded by the male, the secretion can protect the eggs from infections, synchronize their development, protect the male guard from parasites, heal its wounds, and attract females as a pheromone (Ziuganov, 1991). Recently we demonstrated that fish can be used as an antidepressant. Eating the mucus allows sticklebacks to relieve confrontation stress on the spawning grounds and worried depression (Ziuganov and Tashenov, 2004). The selected long-lived fish proved to have less susceptible to diseases, more slowly aged, and lived for extra 5–7 years. Apparently, this was the result from a genetic heterosis, which improved the activity of hypothalamus and other subcortical brain structures and optimized the neuroendocrine regulation of stickleback spawning.

The pearl mussel elongates host life by turns out the program of accelerated senescence in salmon

The fresh-water pearl mussel (family Margaritiferidae, class Unionoida) is the most long-lived species among invertebrate animals. It reaches a maximum age of 200 years (Ziuganov et al., 2000). Paleontological evidence suggests that, in Europe, salmon of the genus Salmo (the Atlantic salmon and brown trout) coevolved with the pearl mussel for 8×10^6 years, since Pliocene; the present range of the mussel coincides with the ranges of these fish species. By the 21^{st} century, several dozens of reproducing M. margaritifera populations remained in Russia, Fennoscandia, and Scotland. When the pearl mussel larva (glochidium) growth was studied on the gills of Atlantic salmon parr and adult fish in various water bodies of northwestern Russia, we found that the larva-infested spawners did not die in autumn after spawning and did not migrate downstream to the sea; they display no signs of progeria and live in the river until the next summer (Ziuganov 2005a). Note that, in winter, wild salmon can carry about 2 - 7 thou small pearl mussel glochidia (50 — 70 microns in diameter) per fish. By the summer, these fish remained nimble, although they lost much weight, and showed normal aggressive behaviour when attacking the spoon lure of an angler. In the White Sea basin, the summer ecological form lived in rivers for a year (from June to the next June), whereas the autumn form, almost two years (for example, from August 2002 to June 2004). Thus, the autumn salmon form can carry pearl mussel larvae twice during each visit from the sea to the river. In summer, both forms of spawners migrate, downstream to the sea and only after this migration most fish die in the sea from exhaustion. The strongest fish survive. The fish that reproduce two or three times constitute 10-40% of the population.

The biological implication of inhibition of the senescence in fish infested by pearl mussel larvae is the following. While growing on fish gills, the larvae increase about ten times size. They need about 1500 degree — days to complete the parasitic phase. In cold rivers of northern Europe, the annual sum of degree-days is 1750; therefore, the mollusk larval phase proved to be extremely long (300 — 350 days). Unlike the parasitic helminth tapeworm ligula, the helminth that kills the intermediate host (fish) to enter the definitive host (bird), the parasitic mollusk must extend the host life, which must not be shorter than the mollusk larval phase, i.e., 8—11 months. Pearl mussel "takes care" that the host salmons (both adult spawners and parrs) that carry larvae on their gills since autumn might not undergo rapid senescence and remain healthy as long as possible — at least until the next summer, to ensure the completion of the mollusk metamorphosis on the fish gills. Later, mature mollusks leave the hosts to become free-living organisms inhabiting the river bottom. During millions of years of coevolution, the mollusk symbiont served as a directional selection factor for host adaptability and longevity, and some longevity assurance genes of the mollusk, e.g., the genes that control resistance to starvation or asphyxia, might have been integrated into the host genome (Ziuganov et al., 2001). Upon larval metamorphosis, the mollusk induces mass necroses of host cells, because the cyst walls are disrupted when it leaves the multiplayer capsule. However, within one or two days after the parasite leaved the fish, thousands of empty cysts with ragged edges rapidly dissolve, which is presumable a result of the stimulation of apoptosis and regeneration of the injured gill tissue. We never observed secondary infection of gills after glochidia leaved fish under natural conditions or in experiments an fish farms (Ziuganov, 2005b).

Note that in the pearl mussel itself, even in old individuals, no diseases, parasites, or tumors were observed until recent climate warming. The individuals of a venerable age die most likely from permanent allometric growth that leads to an excessive increase in shell weight, rather than from aging-related diseases (Ziuganov et al., 2000).

SALMON AND STICKLEBACK EXTRACTS ARE ONCOGEROPROTECTORS

After many successful experiments it has become clear that the bio-preparation developed on the basis of fish secretion, is useful for the treatment of animals and has no side effects, and the idea appeared about its application as a stress protective preparation and geroprotector for the human being. First of all, the author has been testing the preparation on his own body since 1999. No side effects have been detected. In the course of investigations the idea occurred that in case of peroral application of the preparation in its pure form part of curative substances may fail to get into the

blood and the effect of the preparation would be weakened. It would be logical to take the preparation in the form of a spirituous tincture, such an idea was substantiated by the practice of folk medicine that is many centuries old and that uses curative tinctures, balsams and elixirs. In order to strengthen the effect of the bio-preparation, it was decided to mix the preparation with cognac of premium quality matured for five years.

Why are bio-molecules introduced into cognac? Curative substances of hydrobionts are in the form of colloidal solutions of complex compounds (glycoproteins, glycopeptides, mucopolysaccharides), which are difficult to be assimilated by the human body. Digestive juices are not able to break them up, and without being broken up they do not penetrate through the walls of intestines into the lymph and blood. On the contrary, in the course of spirituous extraction many of such compounds decompose into simpler components, which can be preserved for a long time without losses in the dissolved form and are nicely assimilated by our organism. Besides, cognac is known for its curative effect on the vascular system.

In the elixir composition the preservative is a five-year tincture of natural grape spirit from oak, forming compound ethers with curative components (the elixir 2-3 weeks later acquires specific flowery odor), which contributes to quick absorption in the blood already in the mouth cavity and esophagus. Apart from proteins, peptides, aminoacids, natural polysaccharides and lipids, produced by hydrobionts, the elixir also includes vegetable components. By means of cognac spirit from oak the following substances are extracted in the concentration of up to 5 g/l: hemicellulose, tannins (tannic substances), aromatic aldehydes, ethers, organic acids, pigments and polyphenols (flavonoids, anthocyanins, flavonols, resveratrols, carbolic acids, coumarins, quinones), as well as lignine and soluble fibers: pectins and gums. These substances also influence favorably the cardiac and vascular system and metabolism. They inhibit blood condensing: decrease the sticking of blood platelets, expand the diameter of vessels, prevent cholesterol and other “bad” fats from depositing on the walls of vessels and intestines, are of antioxidant action, and reduce the level of hyperinsulinization.

After the author’s regular taking the preparation in the elixir for several years (2-3 times a day in the amount of 10-15 ml thirty minutes before meals) in accordance with the prescription characteristic of many curative balsams (2 weeks of the taking – 2 weeks of leaving it out), it turned out that the elixir compensates to a great extent the effect of overstrain, insomnia, depression, normalizes blood pressure, improves blood formula (enhances hemoglobin up to 150 g/l, normalizes leucocyte formula). The taking of the elixir results in burst of energy, stimulates physical activity, saves from autumnal and spring colds.

Not Everything Is Clear As Far As the Mechanism of Action Concerned

The mechanism of action of the bio-preparation still remains unclear to some certain extent. Stickleback: Most probably, the secretion stimulates programmed death of all atypical cells for wrongly developing fish embryos. The fact is that the survival rate of eggs exposed to the secretion approaches 100%, which has been confirmed many times. At the same time, the survival rate of stickleback embryos in an aquarium with up to date equipment (filtration, aeration, water medication, etc.) is 30-40% at best and embryonic abnormalities are often observed (Ziuganov, 1991). Salmon: It is important to remember that within 1-2 days after larvae of the pearl mussel leaved salmon gills, thousands of empty cysts with lacerated edges are resolved simultaneously, which can also be explained by the stimulation of apoptosis in unwounded tissues of the gills. We never observed secondary infection of gills after glochidia leaved fish under natural conditions or in experiments at fish factories. Therefore, the bio-medication on the basis of this secretion stimulates the death of tumor cells with regard to other fish species and vertebrate animals (rodents and the human being) in general.

After the discovery of the phenomenon of **apoptosis** in the 20th century and the receipt of the evidence of its universality for all the cells of Eucarya, it became evident that apoptosis can play an important role in tumor regression as well. Of course this aspect requires further study. The results given in the present paper confirm the evolution hypothesis of A.V. Makrushin that carcinogenesis is an atavism, perverted preparation of the body for diapause and asexual reproduction by means of budding, in case there are signs for the worsening of environmental conditions (the bio-preparation turns this preparation backwards). According to the hypothesis of A.V. Makrushin (2004), the evolution forerunner of oncogenesis was asexual reproduction and accelerated aging of our distant colonial ancestors 500 mln years ago in Kembria.

ACKNOWLEDGMENTS

The author would like to thank Doctor V.I. Kan for the comment to tomograms, to professor Doctor of Medicine V.N. Anisimov for the conduct of tests of anti-tumor activity of the preparation on mice, and to unknown referee for critical comments. This work was supported by RFBR (grant №05-04-48587).

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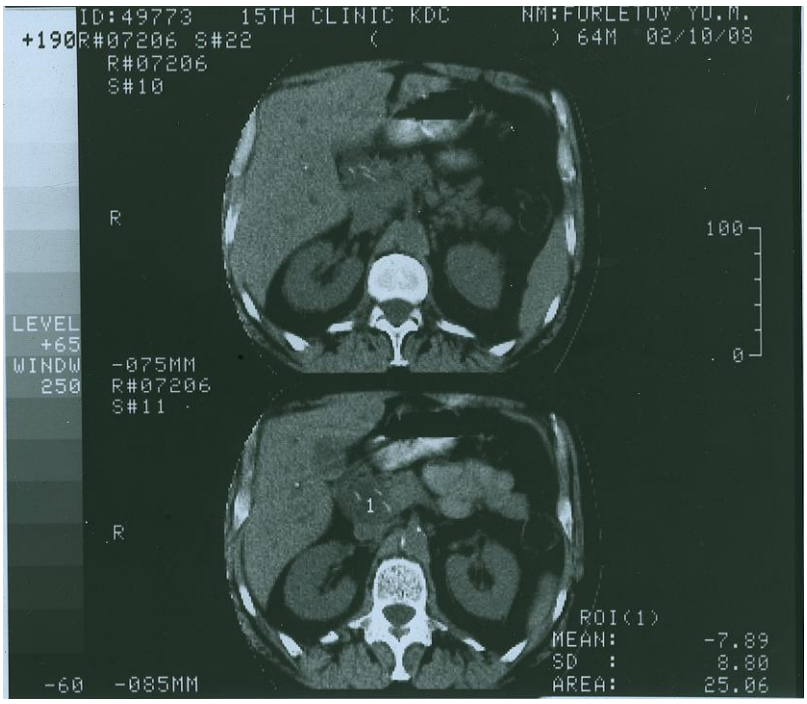
Fig.1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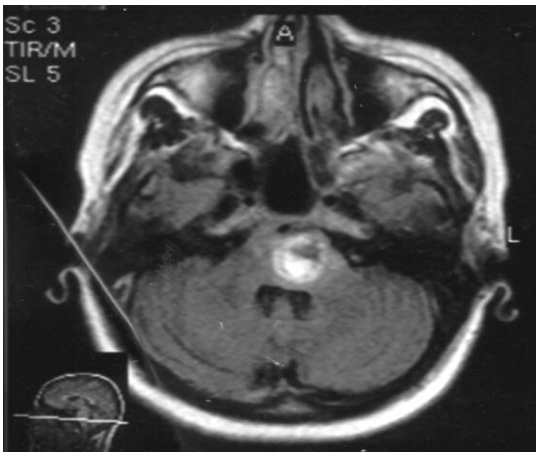
b)

Fig. 2

a)



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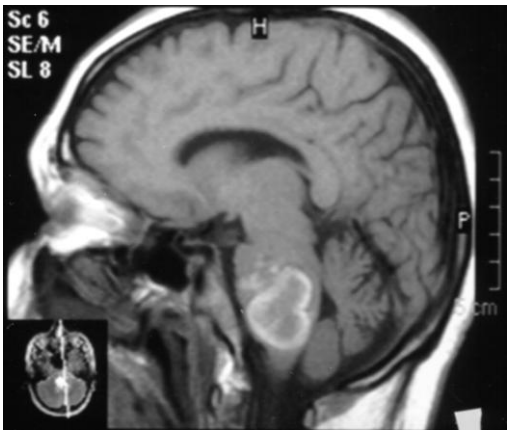

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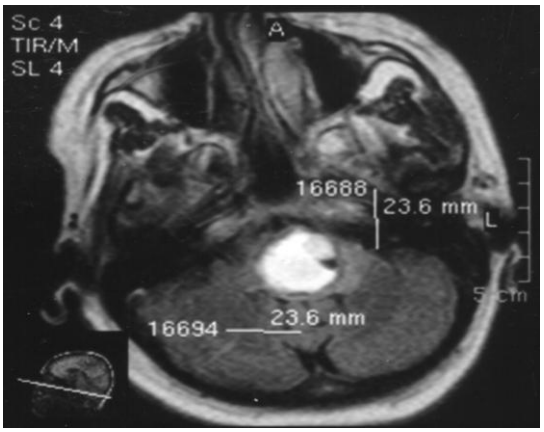

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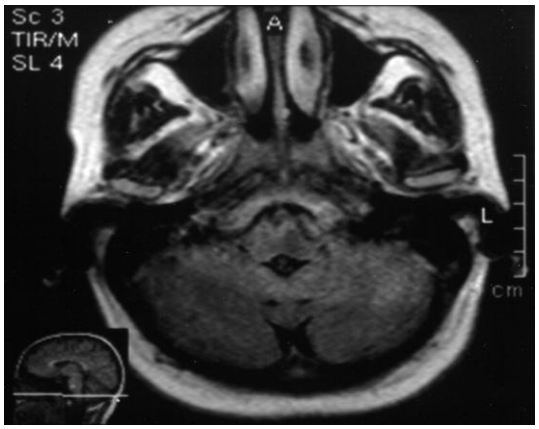
e)



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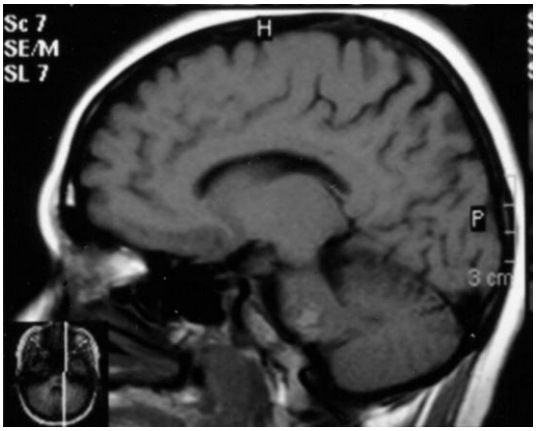

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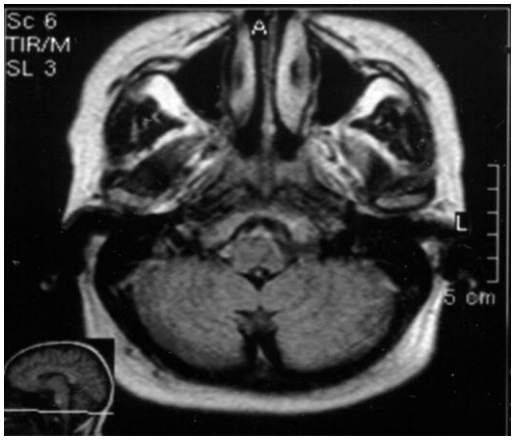

h) **Fig3.**

Captions to the figures

Fig. 1 . Two-year-old Atlantic salmons affected by plaque neoplasms (epitheliomata) complicated by *Saprolegnia* infection (a) and recovered parr (b).

Fig. 2. Computer tomography of the pancreas of patient F., 64 years old (cancer of head of pancreas, cancer of papilla duodeni major. a) – 8 October 2002, before treatment, vermicular volumetric formations (in the photo: of white color) can be seen in the left upper corner on 2 cuts; in one of the photos the tumor is marked with the figure «1» b) – 9 December 2002 after the treatment with the elixir 2 months later the tumor disappeared completely.

Fig. 3. Computer tomography of truncus cerebri (pons cerebelli) of patient M., 49 years old, cavernous angioma of the pons cerebelli in the left hemisphere (a,c,e,g – sagittal plane, b, d, f, h – horizontal plane). a) and b) 23 October 2002 г., before treatment, tumor sized 20 x 20 mm, c) and d) 24 April 2003, commencement of the treatment, measurements of the tumor increased to 23 x 23 mm, e) and f) 5 November 2003, end of the treatment, the tumor decreased by 90%, it «split» into small islets, g) and h) 25 May 2004, six months after the end of the treatment – the tumor regressed completely.

Table 1. Trial of Arktika+ drug on two-year-old salmon *Salmo salar* parr with stage II epithelioma kept for 60 days in nurse ponds in the Umba Fisheries (Murmansk Region) in 2000–2002. Notes: * Differences between the compared groups (control–experiment) were significant at $p < 0.05$. Experimental fish were intramuscularly administered 0.1 ml drug (1 mg solid/10 g body weight or 0.1 g/1 kg) into the caudal peduncle using an insulin syringe three times a week for 2 months. Control fish were administered saline using the same schedule.

Fish groups	Total number of fish survived by the end of the experiment (with the number of fish with tumors in parenthesis)		
	2000	2001	2002
Untreated (control)	10 out of 100 (10)*	14 out of 200 (14)*	22 out of 200 (22)*
Treated with the drug (experiment)	96 out of 100 (14)*	187 out of 200 (24)*	178 out of 200(19)*

Table 2. Trial of Arktika+ drug on NMRI mice with implanted Ehrlich ascites carcinoma carried out at the Petrov Research Institute of Oncology (2004). *Differences between the compared groups (control–experiment) were significant at $p = 0.03$.

Group	Time of mice death, days after implantation									
Control (water)	9	10	10	10	12	12	13	13	13	12 ± 0.5*
Drug (1 : 200)	10	11	12	13	13	15	15	15	16	14 ± 0.6*

Table 3. Effectiveness of the usage of «Arktika+» bio-preparation by volunteers (n=200) with depression symptoms comparing to patients taking tranquilizers.

Clinical symptoms (% of patients)	**Volunteers taking "Arktika+"**			**Patients taking tranquilizers (according to: Pilat, Ivanov, 2002)**		
	1 day	10 day	20 day	1 day	10 day	20 day
Lowering of mood	100	34	8	100	100	80
Sleep disturbances:						
presomnic	37	16	0	40	20	10
intrasomnic	15	4	0	10	5	0
combined	49	12	0	50	20	5
Ideas of guilt	76	38	0	70	60	50
Somatic disorders						
headache	88	44	6	80	80	80
cardiology	35	14	0	40	40	30
gastrointestinal tract	36	20	0	40	40	20