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of the Institute of Plant Biology and Biotechnology

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DEVELOPMENT OF A BIOSENSOR OF UREA WITH THE APPLICATION OF POLYMER TECHNOLOGIES FOR BLOOD AND URINE ANALYSIS

Abstract. Based on polymeric nanotechnologies, enzyme sensors and microreactors have been developedin the way, that they can determine urea in liquids. The technology of manufacturing an enzymatic biosensor does not differ significantly from the known technology of manufacturing microcapsules with an enzyme by the laer-by-laer method. This allows us, when constructing a biosensor, to use the information obtained on encapsulated enzymes by other authors. It is shown that urea biosensor is able to work for a long time (up to 2 months) without significant loss of enzyme activity. Polymer technology for manufacturing sensors is less laborious and expensive compared to other similar technologies. We propose to develop biosensor devices – urea analyzers with polymer enzyme chips for express diagnostics of biological fluids (blood, urine). One of the significant results of this work from our point of view is two factors. The first factor is the optimization of the conditions for the production of a functionally active enzyme immobilized in a polyelectrolyte coating, when the enzyme after the immobilization procedure shows an activity comparable to that of a freshly prepared free enzyme. Such a result will allow reducing the cost of enzymes when creating a sensitive layer of the developed urea analyzer. And the second factor is that the polymer coating with the enzyme is able to work not only as an enzyme electrode, but also as an enzyme microreactor, without decreasing the rate of signal registration after passing the catalytic urease-urea reaction.

Keywords: enzyme biosensors, polymeric nanomaterial, portable analyzer, microreactor, microcapsules, urea.

Introduction. The volume of laboratory research worldwide is steadily increasing and reaches 45 billion analyzes per year, and in industrialized countries the number of analyzes per person reaches 40-60 per year. Universal biochemical analyzers analyze any biological fluids (substrates, enzymes, lipids, drugs, hormones, proteins, electrolytes, drugs). They are produced by about 60 companies, the main producers are Abbott (USA), ABC1 (Austria), Koné (Finland), Nova (USA), Corning (England), Beckmann (USA), "Radiometer" (Denmark). Ready-made sets of reagents are in great demand. Their market is about 27 billion dollars in the world market of laboratory instruments in 6 billion dollars.

Spectroscopic analyzers are used for biochemical studies (determination of organic and inorganic chemicals, such as potassium, sodium, calcium, magnesium, lithium, chlorine, substrates, metabolites, enzymes of biochemical processes in blood and other human biological fluids). Universal biochemical analyzers with the help of which an analysis of any biological fluids for the content of various components are recognized as promising. However, at the present time there are no portable devices of this class. The development of portable devices for the analysis of biological fluids is an urgent task of modern medical diagnostics. Of particular interest among portable analyzers of various substances undoubtedly represent analyzers based on biosensors. Any biosensor consists of two functional elements: a biosensor containing a bioselective material, and a physical converter that transforms any generated signal (ion concentration, mass, color, etc.) into an electrical signal. In the role of biosecting material are all types of biological structures - enzymes, antibodies, receptors, nucleic acids and even living cells. In biosensors are used a variety of physical converters: amperometric, conductometric, optical, luminescent, fluorescent, acoustic, gravitational, etc.

The development of biosensors is an extremely time-consuming process. The most important stage in the development of enzyme sensors is proper immobilization of enzymes on solid supports (substrates). We have developed a method for immobilizing enzymes using polymer technologies, in which the immobilized enzyme is in a functionally active state [1-3]. Immobilization of enzymes was carried out in a biosensor sensitive coating, which is a combination of nanometer polyelectrolyte layers and micro-encapsulated enzymes placed between these layers (figure 1).

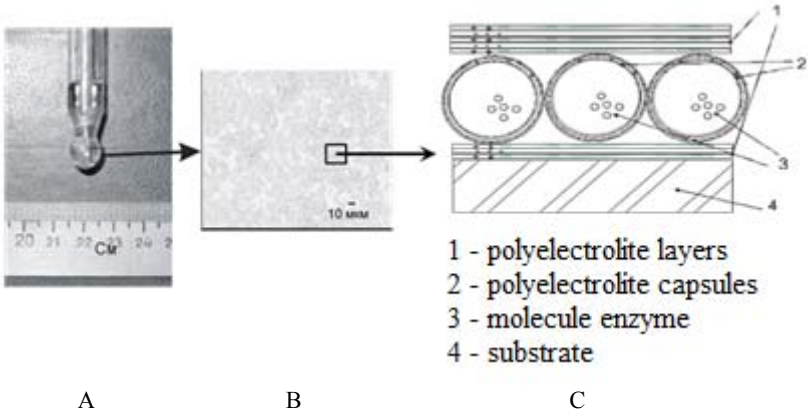

Figure 1 – Enzyme electrode with sensitive biosensor coating:
A – is a glass pH electrode with a sensitive coating containing the enzyme urease;
B – image of a polyelectrolyte coating with microcells in a light microscope;
C – is a schematic representation of a sensitive coating with an enzyme

As it was shown in these works, enzymes in microcells of a polymeric material are reliably protected from aggressive influences of environment (microbes, proteases, etc.); able to detect substrates for a long time (up to 3 weeks in storage at room temperature). This work continues to improve the characteristics of the developed urea biosensor.

Materials and methods. For the production of enzymatic biosensors and enzyme micro-reactors, lyophilized urease (EC 3.5.1.5) was used from the Canavalia ensiformis beans of Sigma and Fluka, an urease solution from the Urea KT(200) kit, (Deacon-DS) with an activity of 253000 U/l., Urea extra clean (Reachim), MES buffers (Sigma), Tris-HCl (Sigma). Salts of $CaCl_2$, Na_2CO_3, NaCl and KCl had a gradation of chemically pure or pure for analysis. Ethylene glycoltetraacetic (EGTA) and ethylenediaminetetraacetic (EDTA) acid (both Sigma-Aldrich, USA). To form films and shells of microcapsules, domain enzymes, polyelectrolytes were used: polyethyleneimine (PEI) weight 600000-1000000, polystyrene sulfonate (PSS), polyallylamine hydrochloride (PAAH), (all - Aldrich) with a mass of 60000-70000. The test substances were used as solutions in 0.33 M NaCl. All salt solutions were prepared on deionized water obtained by purifying distilled water with Arium 611-UF (Sartorius). The conductivity of the water was 1 μS/cm.

The following instruments were used in the work: spectrophotometer Bekman UV/Vis DU 520 (USA), Nikon eclipse E200 microscope, 4-channel potentio-microamperometric analog-digital amplifier "Record-4usb" with computer connection (development of IBK RAS), pH- meter Bekkman F 690 pH / Temp/mV/ISE Meter (USA), Axiovert 200 microscope, photometer (model 680 BIO-RAD, USA), Vortex (shaking and mixing device), ultrasonic bath, magnetic stirrer, table centrifuge, semi-automatic micro-pipette for 2-20 μl, 20-200 μl, 200-1000 μl, 5000 μl, chamber Goryaev.

Preparation of enzyme-containing calcium carbonate crustal particles. Composive microspherolites $CaCO_3$– protein were used as core microparticles for the preparation of polyelectrolyte capsules.

$CaCO_3$ microspherolites were obtained by the ion exchange reaction when mixing solutions of calcium chloride and carbonate in the presence of protein (enzyme) – by biomineralization [4-7].

Preparation of enzyme-containing polyelectrolyte microcapsules. Polyelectrolyte microcapsules with urease were produced by the method of alternate layer-by-layer adsorption with the application of polystyrene sulfonate (PSS) and polyallylamine hydrochloride (PAAH) molecules to composite calcium-carbonate spherulites containing urease as described in [4-6, 8].

Alternate layering of oppositely charged macromolecules of polyelectrolytes on colloidal particles was carried out three to five times, obtaining three/five shells with the architecture of $PAAH/(PSS/PAAH)_n$ and $PSS/(PAGE/PSS)_n$ where n=1.2. The procedure for the formation of microcapsules was carried out at room temperature (15-25°C). Microcapsule size and sphericity of calcium carbonate particles were monitored with a Nikon eclipse E200 light microscope. The removal of calcium carbonate particles from the microcapsules was carried out while maintaining the solution with microcapsules in dialysis bags for 3 hours to 12-15 hours in 25 mM EGTA or EDTA at a temperature of 4°C or 20°C with basic alkalinization (pH 7.2-7.5). The number of capsules in the solution was counted using a cameraGoryaev.

Potentiometric method for determination of urea concentration with a standard pH electrode. Using the technique described in [1, 2], a potentiometric polymer biosensor of urea was prepared on the basis of a modified glass pH electrode (figure 1A). Measurements of the hydrogen ion concentration in the test solution were carried out using a four-channel ADC – "Record 4usb". The solution was stirred with a magnetic stirrer and maintained at 25 ± 1 °C with a U-1 thermostat (Germany). Then, the enzyme preparation was added thereto in the required quantities or a modified pH electrode was introduced. The alkaline pH shift recorded (in mV) was saturated for 20-30 seconds.

Results and discussion. For the first time, the possibility of measuring the urea concentration by a modified glass pH electrode on which an ultrathin sensitive polymer coating with urease was deposited was demonstrated by us in [1, 2]. The following properties of the polymer coating provided this possibility: good permeability of polyelectrolyte multilayers for the substrate (urea) and its decomposition products by urease; impermeability of these layers for the enzyme; preservation of the enzyme in the cells of the coating, high activity for a sufficiently long time; as well as significant alkalization of the medium during the decomposition of urea to carbon dioxide and ammonia. Improving the characteristics and properties of the polymer sensitive coating of the urea sensor is associated with an increase in the initial activity of the immobilized enzyme, an increase in the duration of the sensor operation, and the ability to measure urea in biological fluids. As was shown in [9], we managed to achieve a sufficiently high activity of the immobilized enzyme, which amounted to 40-50% of the activity of the free freshly prepared enzyme.

In this paper, data are presented on the continuation of studies related to an increase in the initial activity of the urease sensor. Figure 2 shows the data on the dependence of the response of the glass pH electrode on urea concentration in the measuring cell for the free (line 1) and encapsulated (line 2) enzyme.

It can be seen from the figure that the activity of the encapsulated enzyme is comparable to the activity of a free freshly prepared enzyme and was about 75% of its activity. In the encapsulation process, the enzymes are partially damaged, and in the first studies on capsules with the enzyme, a high initial

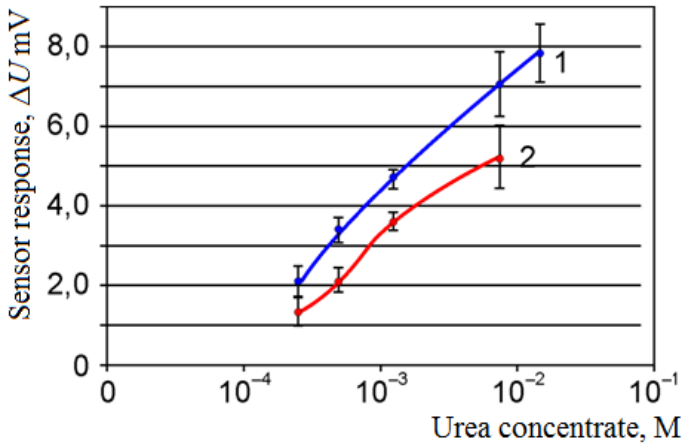

Figure 2 – Dependence of the response of the glass pH electrode on the urea concentration (0.5 μg enzyme concentration was determined by the Bradford method): 1 - free enzyme (urease); 2 - encapsulated enzyme contained in microcapsules with the architecture of the PSS-PAAG-PSS envelope.

Study medium: 1 mM Tris-HCl, 1 mM MES, 100 mM NaCl, initial pH 5.3.

activity of the encapsulated enzyme was not achieved. Usually, the activity of encapsulated enzymes decreased by a factor of 6-7 [1, 6, 10-15]. Close results to our experimental data presented in this study on encapsulated urease were obtained in [16-19]. Authors, using the enzyme dextranase, obtained encapsulated enzymes with a catalytic activity equal to 80% of the activity of the free enzyme (the capsules were formed from calcium alginate with the inclusion of silica).

Since unmodified glass pH electrodes were used for measurements in cells with a free and encapsulated enzyme, we tried to compare pH measurements during the passage of the urease-urea catalytic reaction using a modified by our method an electrode and an unmodified electrode that were simultaneously placed in a measuring cell. In this case, the decomposition reaction of urea passed in the biosensitive layer of the modified electrode (figure 3).

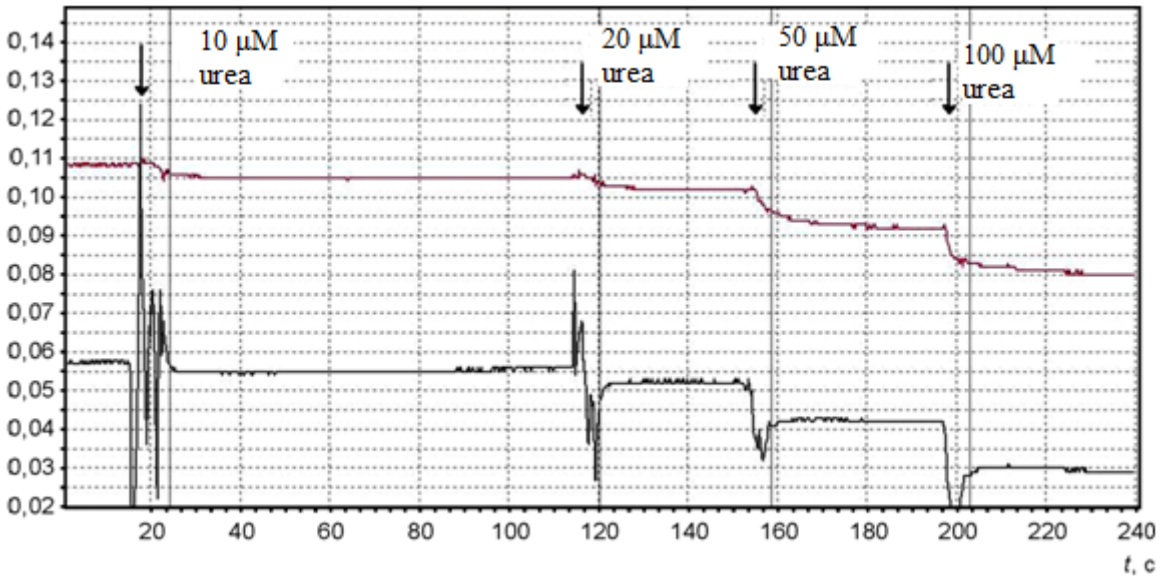

Figure 3 – Diagram of the experiment for measuring urea concentration with unmodified (upper curve) and modified pH electrodes.

A sensitive coating with urease is deposited on the ball of the lower electrode. Microcells of sensitive coating with the architecture of the shell of PAAG-PSS-PAAG.

Study medium: 1 mM MES, 100 mM NaCl, initial pH 6.0

Since unmodified glass pH electrodes were used for measurements in cells with a free and encapsulated enzyme, we tried to compare pH measurements during the passage of the urease-urea catalytic reaction using a modified by our method an electrode and an unmodified electrode that were simultaneously placed in a measuring cell. In this case, the decomposition reaction of urea passed in the biosensitive layer of the modified electrode (figure 3).

It can be seen from the experimental diagram that the response time after the catalytic reaction of the enzyme-substrate with the help of the modified and unmodified electrodes is practically the same. This is due to the fact that the substrate – urea and the decay products of the urease-urea catalytic reaction – carbon dioxide and ammonia easily penetrate through the nanometer polyelectrolyte shell that separates urease from the external solution. Such experimental results allowed us to create not only enzyme electrodes, but also enzyme microreactors (when the recording electrode is separated from the sensitive layer).

As a microreactor, plastic and glass cuvettes with a polyelectrolyte coating were applied, the same as for a ball of a modified pH electrode. This coating, which is a multilayer film, between layers of which was a layer of polyelectrolyte capsules with a diameter of about 2-5 microns filled with urease molecules, was applied to one of the walls of the cuvette. The presence of several, not less than five polyelectrolyte layers separating enzymes from the external environment, prevented the latter from inactivation, for example, by foreign enzymes or microbes. One of the features of the coating was that the total thickness of the polymer layers was less than 2% of the inner cell diameter in it.

Figure 4 presents data on the catalytic activity of the urease microreactor.

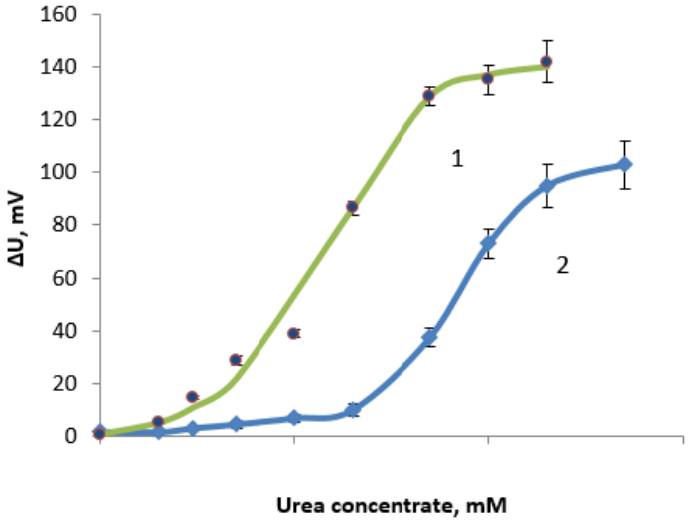

Figure 4 – Dependence of the response of the glass pH electrode on urea concentration (enzyme concentration 3 μg was determined by the Bradford method):
1 - free enzyme (urease); 2 - enzyme immobilized on the lateral surface of the spectrophotometric cell and contained in the microcells of the sensitive coating with the architecture of the PAAG- (PSS-PAGE) 2 shell.

Study medium: 1 mM MES, 100 mM NaCl, initial pH 6.0

Thus, it has been shown that by potentiometric method using a new type of polymeric urease sensor on a glass pH electrode it is possible to measure urea concentrations ranging from 10-20 μM. The upper limit of the measurement depends on the concentration of urease placed in the polyelectrolyte coating and on the properties of the electrode. We were able to measure more than 100 mM urea. In fact, when developing a urea sensor for medical diagnosis, it is not necessary to measure such high urea concentrations, since the normal urea content in the human blood is between 1.8 and 7.5 mM, depending on the age.

Studies of the stability of a new type of urea sensor showed that when stored in distilled water at a temperature of 4 °C, the sensor is capable of operating for up to 2 months. At the same time, the decrease in activity of immobilized urease is initially 40-50%. The stability of the sensor over time can, among other things, be due to the stability of the polyelectrolyte shells that protect the enzymes from the external environment in the microcells of the coating. Perhaps in this case, the size of the microcapsules, as well as the number of polyelectrolyte layers forming the microcapsule shell, will be important for increasing the stability of the polyelectrolyte coating.We carried out preliminary studies of the strength of 10 μm microcapsules containing a calcium-carbonate core with the help of a NanoScan-4D nanodidomer [20]. It was shown that the destruction of a single microcapsule occurred when it was compressed by 1.1 μm and a load value of 25 mN. Investigation of the strength of microcapsules with a remote calcium carbonate nucleus depending on the size of microcapsules and the number of layers of capsule shell polyelectrolytes is of interest for improving the stability of microcapsules and a new polyelectrolyte coating.

The necessary component of research in the development of biosensors is testing on biological fluids. Investigations of the urease polymer sensor for the determination of urea in biological fluids were conducted using urine and blood as an example. These experiments are presented in [9], from which it follows that if the accuracy of measuring by our method the concentration of urea in daily urine diluted 100 times approaches the error obtained during dilution of urine, then a different picture is observed when measuring urea in serum. Blood in different people has its own pH and buffer capacity, so when measuring urea in blood serum, we tested the "double additives" method, which increased the accuracy of measurements to 5%.

The sensitivity of the urease sensor can be significantly increased by using pH-sensitive field effect transistors (figure 5).

As can be seen from the figure, the field effect transistor has increased the sensitivity of the sensor by more than an order of magnitude. The enzyme consumption during the creation of a sensitive field-transistor coating was 20 μl from the 3 ml set (see Materials and Methods). This amount of enzyme is used for one or two measurements in enzyme analysis by the usual spectral method in polyclinics.

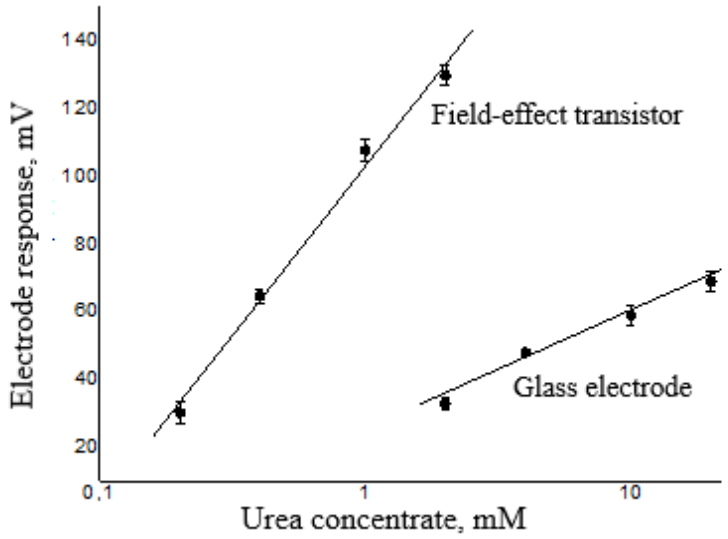

Figure 5 – Comparison of the response of the pH-sensitive field-effect transistor and the glass pH electrode to the urea concentration.
The sensitive coating is applied to the glass electrode ball and to the surface of the recording element of the transistor.
Study medium: 2 mM Tris-HCl, 200 mM NaCl, initial pH 7.8

Conclusion. The urea biosensor manufactured with the help of polymer technologies and representing a combination of polyelectrolyte layers and microcapsules with an enzyme inside and a shell of the same polyelectrolytes, as shown by the experimental data, is perfectly suitable for determining the urea concentration in blood and urine. The technology of manufacturing an enzymatic biosensor does not differ significantly from the known technology of manufacturing microcapsules with an enzyme by the laer-by-laer method [4-6]. This allows us, when constructing a biosensor, to use the information obtained on encapsulated enzymes by other authors. In this case, the urea biosensor is able to work for a long time (up to 2 months) without significant loss of enzyme activity. One of the significant results of this work from our point of view is two factors. The first factor is the optimization of the conditions for the production of a functionally active enzyme immobilized in a polyelectrolyte coating, when the enzyme after the immobilization procedure shows an activity comparable to that of a freshly prepared free enzyme. Such a result will allow reducing the cost of enzymes when creating a sensitive layer of the developed urea analyzer. And the second factor is that the polymer coating with the enzyme is able to work not only as an enzyme electrode, but also as an enzyme microreactor, without decreasing the rate of signal registration after passing the catalytic urease-urea reaction. This is due to the fact that the layers of polyelectrolytes separating the enzyme from the external analyte solution have a nanometer thickness and are easily permeable to urea and decomposition products of the urease-urea catalytic reaction. Separation of the sensitive sensor from the recording electrode provides many opportunities for designers of urea analyzers based on a polymer ultrathin coating.

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ҚАН ЖӘНЕ НЕСЕПТІ ТАЛДАУ ҮШІН ПОЛИМЕРЛІ ТЕХНОЛОГИЯЛАРДЫ ПАЙДАЛАНУ АРҚЫЛЫ МОЧЕВИНА БИОДАТЧИГІН ЖАСАУ

Аннотация. Полимерлі нанотехнологиялар негізінде сұйықтықтарда мочевинаны анықтай алатын ферментті тіркеуіштер мен микрореакторлар жасалды. Ферментті тіркеуішті жасау технологиясы laer-by-laer әдісімен ферментті микрокапсулалар жасаудың белгілі технологиясынан айтарлықтай ерекшеленбейді. Бұл бізге басқа авторлармен инкапсуляцияланған ферменттерден алынған мәліметтерін биотіркеуіш жасауда ақпарат ретінде мүмкіндік береді. Мочевина биосенсоры ұзақ уақыт бойы ферменттің белсенділігін айтарлықтай жоғалтпай ұзақ уақыт бойы (2 айға дейін) жұмыс жасай алатындығы табылды. Полимерлі технология басқа да ұқсас әдістерге қарағанда жеңіл және арзан болып табылады. Биологиялық сұйықтарды (қан, несеп) экспресс анықтау үшін полимерлі ферментті чипі бар мочевина анализаторы ұсынылады. Бұл жұмыста біздің ойымызшаайтарлықтай екі артықшылық факторы бар. Бірінші фактор – полиэлектролитті жабынға иммобилизацияланған функционалды-белсенді фермент алу жағдайын оңтайландыру, мұнда иммобилизация әрекетінен кейін фермент жаңа даярланған бос ферменттің белсенділігіне ұқсас белсенділік көрсетеді. Мұн-

дай нәтиже мочевина анализаторы қондырғысын жасауда сезімтал қабатты дайындауда ферменттерге кететін шығындарды арзандатады. Екінші фактор, ферменті бар полимерлі жабын ферментті электрод ретінде ғана жұмыс жасап қоймай, уреаза-мочевина каталитикалық реакциясы өткеннен кейін тіркеу жылдамдығын төмендетпей ферментті микрореактор ретінде де іс атқарады.

Түйін сөздер: ферментті биосенсорлар, полимерлі наноматериал, портативті анализатор, микрореактор, микрокапсулалар, мочевина.

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РАЗРАБОТКА БИОДАТЧИКА МОЧЕВИНЫ С ПРИМЕНЕНИЕМ ПОЛИМЕРНЫХ ТЕХНОЛОГИЙ ДЛЯ АНАЛИЗОВ КРОВИ И МОЧИ

Аннотация. На основе полимерных нанотехнологий созданы ферментные датчики и микрореакторы, способные определять мочевину в жидкостях. Технология изготовления ферментного биодатчика существенно не отличается от известной технологии изготовления микрокапсул с ферментом методом laer-by-laer. Это позволяет нам при конструировании биодатчика пользоваться информацией, полученной на инкапсулированных ферментах другими авторами. Показано, что биосенсор мочевины способен работать в течение длительного времени (до 2 месяцев) без значительной потери активности фермента. Полимерная технология изготовления датчиков менее трудоемкая и дорогостоящая по сравнению с другими аналогичными технологиями. Предлагаются к разработке биосенсорные приборы – анализаторы мочевины с полимерными ферментными чипами для экспресс-диагностики биологических жидкостей (кровь, моча). Одним из существенных результатов настоящей работы с нашей точки зрения являются два фактора. Первый фактор – это оптимизация условий получения функционально-активного фермента, иммобилизованного в полиэлектролитное покрытие, когда фермент после процедуры иммобилизации показывает активность сравнимую с активностью свежеприготовленного свободного фермента. Такой результат позволит удешевить расходы на ферменты при создании чувствительного слоя разрабатываемого прибора-анализатора мочевины. И второй фактор, это то, что полимерное покрытие с ферментом способно работать не только как ферментный электрод, но и как ферментный микрореактор, при этом не уменьшая скорость регистрации сигнала после прохождения каталитической реакции уреаза-мочевина.

Ключевые слова: ферментные биосенсоры, полимерный наноматериал, портативный анализатор, микрореактор, микрокапсулы, мочевина.

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GENERAL CHARACTERISTICS OF INFLUENZA VIRUS A MOLECULAR STRUCTURE

Abstract. The influenza virus is one of the most abundant viruses in the world. It causes both mild seasonal infections and severe pandemics killing thousands of people and mammals. Two main extracellular receptors – neuraminidase (NA) and hemagglutinin (HA) are responsible for infection symptoms development and spread. Error-prone RNA-polymerase incorporates mutations into both neuraminidase and hemagglutinin per replication cycle, which complicates the development of highly effective drugs against animal influenza. Incorporated mutations are also involved in the transition of influenza from animal to human species and vice versa. Transited influenza subtypes are the most dangerous, because it is unpredictable now, where the mutation might arise. However, it starts to become clear, which molecular regions are the most common for the mutation to occur.

This article revises the molecular structure of influenza extracellular receptors, including critical regions of receptors binding sites and susceptible mutation sites. The clear understanding of molecular structures and critical regions of HA and NA might facilitate the development of an effective vaccine and/or drug development.

Key words: influenza, neuraminidase, hemagglutinin, mutation, sialidase, virus.

INTRODUCTION. General description of influenza viruses. Influenza A viruses belong to a viral family of *Orthomyxoviridae*, what can be interpreted from Greek language as viruses which bind to mucoproteins. Influenza A viruses cause flu in many representatives of animal species, the most common hosts are: salmon, pigeon, poultry, fowl, especially chicken, swine, camel, bats and human. It should be considered that an avian virus does not cause such a wide range of symptoms in birds but as it is transmitted to humans, disease conditions might be very severe [1].

There are several types of influenza viruses: A, B and C types. Influenza A is the most studied and common type: has a wide range of hosts including mammals, fish and birds and is responsible for most flu epi- and pandemics. Influenza B virus infects only humans, and influenza C infects both human and swine [1, 2].

Influenza A virus causes a wide range of non-specific symptoms: high fever, sore throat, mild headache, chills, malaise, cough and muscular pain. These symptoms do not necessarily indicate that a person has flu. Otherwise, additional analyses should be certainly made to ensure a final diagnosis. Most harm done by influenza is towards infants and elders due to their suppressed or non-developed immune system. Sometimes it could be even fatal due to chronic illness or lack of special medical care and medicine. However, as vaccines and drugs are being improved, those fatal cases are getting much lower than in previous century [1].

There were several pandemics caused by initially swine or avian flu through the development of their transmittance to humans. It is uncommon that once in the future another pandemic virus would arise leading to enormous problems including lack of effective vaccine, inefficiency of available drugs, and may be financial losses. It is very hard to predict next pandemics and subtype of virus involved in particular, so detailed studies of viral subtypes might be a solution to overcome next potential pandemics.

Molecules mostly involved in the development of influenza infections are envelope proteins: hemagglutinin (HA) and neuraminidase (NA). Most of the drugs against flu use HA or NA as their main targets through reducing their affinity to sialic acid, destroying covalent inter- and intramolecular bonding or by using antagonistic features. For example, zanamivir and oseltamivir are NA-inhibiting drugs [1].

So, in this review we would consider molecular structure, functions and subtypes of envelope glycoproteins HA and NA, discuss interactions between viral and host molecules and briefly mention how the transition from avian to human flu is established, which is the main cause of unexpected pandemics [1].

Hosts of influenza A virus. Influenza A virus has a variety of hosts. Well known examples are chicken, domesticated birds, swine, camels, horses, bats and humans.

There is a great variety of bird species susceptible to influenza A virus: ducks, wigeons, teals, mergansers, geese, swans, redshanks, gulls, grebes and etc. Most of the avian hosts are waterfowl and only several are terrestrial such as pigeons and chicken. The most known birds infecting influenza A subtypes are highly pathogenic H5N1 initially identified in Hong Kong, H7N3 outbreak in England and Australia in 1963 and 1992-1994 respectively, H7N7 several outbreaks in Germany, England, Australia and Netherlands and H5N2 with outbreaks in USA, Mexico, Italy and China.

Swine influenza is another major source of virus reservoir in wild environment. Outbreaks of swine influenza were firstly identified in Spanish influenza pandemic in 1918 year and had occurred several times after that. Major swine influenza subtypes: H1N1, H1N2 and H3N2 [3].

Equine influenza was described as a disease having similar symptoms as human influenza since Romanian times. It has potentially evolved together with human influenza virus due to their living in close proximity with horses, mules and donkeys. Influenza virus is still considered as the most important respiratory pathogen in horse and related species. The first isolate of equine influenza was obtained in Eastern Europe in 1956 year and identified as H7N7 subtype. Another example of horse influenza is H3N8, which was isolated in 1963 and since those times is considered as enzootic in Europe and America [3].

Recently two novel influenza viral subtypes from bat species were identified and studied– H17N10 and H18N11. However, they are considered as influenza-like viruses, because their characteristics are rather distinct: a different binding site to sialic acid receptors and neuraminidase not being a sialidase [4].

However, human infecting influenza subtypes have emerged from animal infecting subtypes through the adaptation to the surroundings and incorporation of mutations per replication cycle. So, wild subtypes of influenza virus should be thoroughly studied to prepare and possibly prevent next pandemics.

General description of influenza A virus. Influenza virions have roughly spherical or filamentous shape. Newly synthesized ones have more filamentousvirions, and as they become mature, shape becomes roughly spherical. Gene segments are wrapped into helical nucleocapsid, which is then packaged into lipid envelope mostly derived from a host's plasma membrane.

Influenza A viruses are encoded by six-eight strains of negative-sense RNA. RNAs are error-prone due to RNA-dependent RNA-polymerase, which drives rapid adaption and evolution of influenza [5]. Each of the genome segments encodes one or two proteins, functions of proteins encoded together on one gene segmentare rather similar. Envelope glycoproteins, which are located on the outermost layer, HA and NA are encoded by genome segments 4 and 6, respectively [1]**.** Three RNA polymerase proteins (PA, PB1 and PB2) are encoded by distinct genome segments from 1st to 3rd. 7thgene segment codes for an integral membrane protein (M1) with ion channel activity and an envelope protein by subsequent splicing of mRNA. Influenza A virus expresses 11 proteins in total, and 9 of them are packaged into new virions. Two proteins, which are not packaged, facilitate assembly of viral particles.

Only in case all RNA gene segments are packaged into a viral particle, the virus is capable of survival and infection [5].

Influenza A virus replicates in the host nucleus, unlike most other RNA viruses such as bynuaviruses, paramyxoviruses and rhabdoviruses replicating in the cytoplasm. Viral mRNA of influenza stealscapped 5'ends of cellular mRNA in the nucleus, which facilitates rapid synthesis of new virion particles. Rapid evolution of these viruses is due to the presence of both error-prone RNA-polymerase and frequentreassortment (antigenic shift) of whole genome segments or some parts between related strains. This reassortment is the one most responsible of pandemics due to mutated surface antigens NA and HA [1].

Influenza A viruses are classified according to NA and HA subtypes, which together show different antigenic reactivity to poly- and monoclonal antibodies and show different nucleotide sequences [6].

The main difference between avian and human adapted viruses is their preferential binding to sialylated glycan receptors, hemagglutinin in particular. Human viral hemagglutinin exemplified by H1N1, H2N2, H3N2 subtypes preferentially binds to long α2-6 sialylated glycan receptors, which are mostly expressed in human upper respiratory epithelium. HA of avian influenza exemplified by H1N8, H2N9, H3N2, H3N8, H5N8 binds to short α2-3 sialylatedglycans. Due to this feature, there are only several avian influenza viruses capable tocause diseases in humansuch as H5N1, H7N7, H7N3, H9N2 serotypes. Differences in receptor specificity of human and avian influenza A viruses are also considered as features responsible for tissue tropism, host species barrier and interspecies transmission blocking. So, there is a need to constantly monitor these avian viruses to be able to detect changes in external glycan structure as they play the most important role in influenza evolution [6].

Swine influenza viruses are able to bind sialic acid in both α2-3 and α2-6 linkages, so they combine features of both human and avian viruses.

HA and NA both recognize sialylated receptors on the outside of a host cell membrane. Influenza infection is promoted by multiple HA binding to sialic acids found on the carbohydrate side chains outside regions: on surface glycoproteins and glycolipids. NA's primary function is to release accidentally bound newly synthesized HA from sialylated glycoproteins and glycolipids, however it performs other functions as well.

Neuraminidase. NA is a viral cell surface receptor of a tetramericglycoprotein nature. It is encoded by 6^{th}gene segment of influenza A genome. Its length is approximately 1413 base pairs in length with slight variations, mature protein size is 454 Daltons.

It consists of four identical polypeptides of approximately 470 amino acids with slight variations in sequence. Four domains of this protein are a membrane-anchoring hydrophobic domain, a thin variable stalk, a globular head domain which is a carrier of enzyme active site and a calcium-binding site [7].

The stalk domain's length varies significantly, and its shortening is associated with adaptation of waterfowl to poultry [8]. Subunits consist of six bladed propeller-like structures, and blades are made up of four antiparallel β-strands [9]. An enzyme active site with conserved charged amino acid residues can be found in the central region of each subunit.

The function of NA is to cleave sialic acid residues from cellular and viral glycoproteins expressed outside the host cell membrane. It is crucial to prevent HA mediated aggregation of newly synthesized viral particles at the surface right after their leakage from damaged host cell, because this would prevent further dissipation. NA fulfills that function by removing newly synthesized HA, which were accidentally bound to sialylatedreceptors of dead cell [10].

Besides the release of budding virion particles through HA release, NA plays a role in promoting cellular infection by promoting glycosylation of the HA and cleaving potential inhibitory Sia-s from mucins. It was shown, that NA recognizes sialic acid residues on host glycoproteins and glycolipids in a different manner in comparison with HA [11].

Different structural features of NA and HA form influenza subtypes.

Neuraminidase subtypes. 10 structurally different NA circulate in birds, which are classified into two main groups [12]. The group 1 includes N1, N4, N5 and N8, and group 2 includes N2, N3, N6, N7 and N9. The classification is based on similar features within the following regions: in the 150-loop (residues 147-152), the 270-loop (residues 267-276), and the 430-loop (residues 429-433), which are regions adjacent to the enzyme's active site.

The only conserved site for all influenza A and B types NA is Asn146 glycosylation region, which is located on the membrane-distal surface close to the active site [13]. Besides that highly conserved region, neuraminidase shows a great diversity in nucleotide sequence, which results in various structural conformations. Mutations incorporated per replication cycle add changes into already present pool of neuraminidase structural diversity.

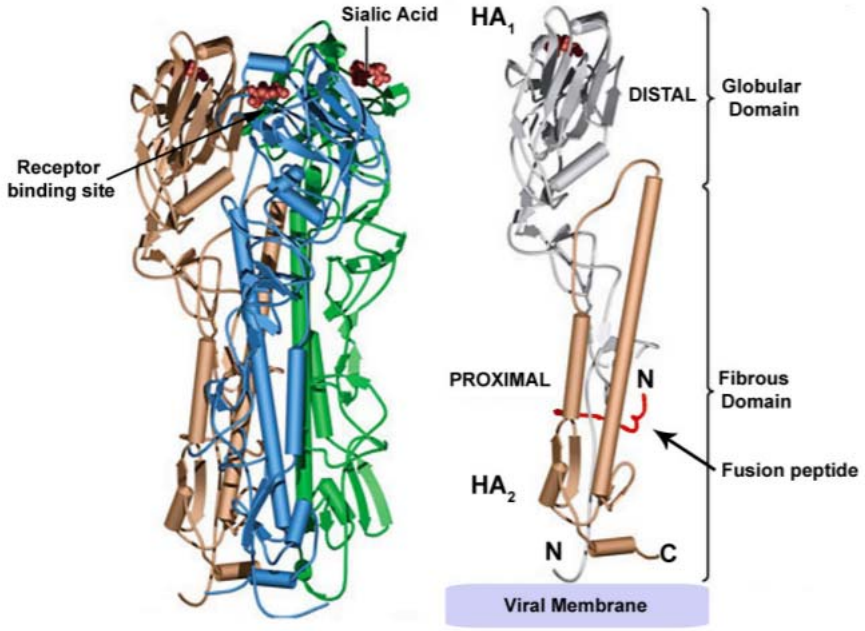
Hemagglutinin. Name of this protein comes from its ability to form aggregates of red blood cells, the carriers of hem – hem agglutinating protein. Widely spread, rapid and moderately sensitive technique of most viruses' identification – is the hemagglutination assay, in which hemagglutinin glycoproteins

clump cells by binding to their surface receptors. There are 18 subtypes of hemagglutinin, and two recent ones H17 and H18 were found in bats [14].

HA is expressed as a trimeric surface receptor on the outside of the viral membrane. It facilitates entry through the receptor binding to the target cell and recognizes sialylated cell receptors for consecutive chemical binding. It is encoded by 4th gene segment of influenza A viral genome with approximately 566 nucleotides in length, although some nucleotide variations are possible.

Sialylated receptor-bound viruses fuse with the cell membrane, and then are engulfed into endosomes once entering cytoplasm. HA becomes acidified by endosomal enzymes, which is followed by conformational changes in its molecular structure and subsequent activation. Different kinds of conformational changes constitute HA subtypes classification system.

The linkage between glycan structures and galactose is crucial in host determination. Avian viruses are characterized by binding to α2-3Sia and are so-called avian-type receptors, while mammalian viruses bind to α2-6Sia and are so-called human-type receptors. However, it should be noted, that cells in human upper epithelium mostly possess α2-6Sia receptors, whereas cells in lower epithelium possess α2-3Sia cell surface receptors. This means that influenza bearing only avian-type receptors is able to cause a moderately mild infection without any serious consequences.



The schematic representation of hemagglutinin structure [15]

However, influenza viruses typically reproduce in cells of human upper respiratory tract through the recognition of sialic acid, or N-acetylneuraminic acid, by HA. N-acetylneuraminicacid is terminated by glycan structures, which are linked to galactose in a β1-4 linkage to glucosamine, and thislinkage in particular is associated with HA recognition and binding to the target cell [10, 16].

The HA is a homotrimer consisting of a globular head with sialic acid binding domain and a fibrous stalk region. Three identical subunits have resulted from proteolytic cleavage of a single precursor, and the process occurs by cleaving single arginine residue extracellularly by serine proteases before entering the host cell. However, some members of H5 and H7 subtypes have acquired several cleaving residues (arginine and lysine), which is partly responsible for H5 and H7 high infectivity and pathogenicity [9].

The coiled-coil structure of the stalk domain stabilizes HA trimers and anchors the protein in the membrane through its transmembrane subdomain [12]. The only conserved amino acid throughout all subtypes of the stalk domain is Lys51.

As it was mentioned before, HA recognizes sialylated cell surface receptors of a target cell, so let's consider these interactions more closely. The interaction occurs through hydrophobic and hydrogen bonding between HA residues from the 130-, 220-loops, 190-helix and sialylated receptors [17].

Sialic acid binding site contains four main structural regions made up from antiparallel β-sheets [12]: a base with highly conserved Tyr98, Trp153 and His183, a 190-α helix (residues 184-190), a 130-loop (residues 126-135) and a 220-loop (residues 215-224) [19].

Amino acids Tyr98, Trp153, His183, Glu190 and Tyr195 directly interact through hydrogen bonding with the side chains of sialic acid, which was shown on H3 subtype in particular.

The 130-loop with crucial residues at 135-137 forms chain interactions with receptor's sialic acid moiety. Mutations within 220-loop constitute differences in host specificity due to slight changes in loop conformation associated with glycosidic linkage type [20]. The 190-helix plays a role in species specificity determination. Double mutations in the HA receptor binding domains of H1N1 at Glu190Asp and Gly225Asp and H2N2/H3N2 at Gln226Leu and Gly228Ser influenza A subtypes are associated with the adaptation of avian viruses into human pandemic viruses.

In addition, four amino acid substitutions of HA in H5N1 at Ser123Pro, Ser133Ala, Thr156Ala, and Gln192Lys are associated with increased binding of the virus to mammalian receptors [20]. Mutation of Asn158 and Thr160 were shown to increase virus affinity to human-type receptors due to the loss of the same glycosylation site on the top of the HA globular head.

In HA2, HA3 and HA9 subtypes Leu226 enables influenza A virus replication in the human airway epithelium.

HA binds to sialic acid through hydrophobic interactions and hydrogen bonding to the conserved amino acids within 130- and 220-loops, although responsible amino acid residues differ from one subtype to another [9]. For example, in HA1, glutamic acid and glycine residues at positions 190 and 225, respectively, are responsible for binding to avian SIA-receptors, whereas HA1 proteins that carry aspartic acid residues at these two positions interact with human SIA receptors. For HA2 and HA3, mutations of Gln226Leu and Gly228Ser correlate with a shift from avian to human receptor specificity [21]. So, slight mutations in amino acid sequenceconstitute the basis of HA subtypes classification.

Hemagglutinin subtypes. Two main groups of 18 HA circulatingin many different hosts are classified according to sequence comparisons and structural characteristics. Group 1: H1, H2, H5, H6, H8, H9, H11, H12, H13 and H16. Group 2: H3, H4, H7, H14, H15 and H10 [22]. The classification also considers conformational changes within HA molecular structure triggered by acidification due to endosomal enzymes functioning.

Ha subtypes could be also relatively classified according to their main host species. For instance, HA1, HA2 and HA3 subtypes circulate mostly in human populations.

A computer analysis by using free access applications has shown close evolutionary relationships between HA subtypes[14]. Very close relationship was shown between HA7, HA15 and HA10 constituting one clade and HA4, HA14 and HA3 constituting another. The common origin was shown for HA8, HA12 and HA9 as well as for HA13, HA16 and HA11[14]. Recently described HA17 and HA18 subtypes show closest evolutionary relations towards another clade including HA1, HA2, HA5 and HA6. This evolutionary relationship formed during last century indicates rapid evolution of influenza viruses due to reassortment between different viral subtypes.

Transition between avian and human influenza types. The transition between avian and human influenza types had occurred several times and had caused several pandemics during human history.

The transition between avian and human influenza types is not so uncommon because of several reasons underlying this process. They include high degree of genetic recombination between different subtypes, error-prone RNA polymerase, which enables a multitude of mutations to occur per replication cycle, rapid generation time of virions and fast replication of virion particles inside of a host nucleus.

The major receptor binding site substitution between avian and human HA10 is Lys137Arg, although some others might also emerge due to incorporation of mutations per replication cycle. Such transitions between avian/swine/human influenza should be studied in details for the trafficking influenza evolution [22].

Conclusion. So far we have discussed some important structural features of HA and NA – envelope proteins responsible for influenza A virus infection occurrence and spread. HA plays a significant role in binding to receptors in host's upper epithelium cells. This is the most common way for a virus to enter a cell. NA's primary function is to release newly synthesized virions from sialylated receptors of host cell. However, NA is also responsible for facilitating HA's glycosylation for viral infection spread. These two

proteins obtain a variety of mutations per replication cycle due to error-prone RNA polymerase. This constitutes a feature of influenza A virus to quickly evolve into new subtypes, which is a great obstacle for modern vaccines. Vaccines are designed mostly against widely spread influenza A pathogens, and if a novel pandemics occurs, the use of vaccines would not be effective.

So, it is very important to understand structural and functional features of influenza A virus proteins to be able to synthesize modern vaccines against it.

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ТҰМАУ А ВИРУСЫНЫҢ МОЛЕКУЛАЛЫҚ ҚҰРЫЛЫМЫНЫҢ БАЗАЛЫҚ ҚАСИЕТТЕРІНІҢ СИПАТТАМАСЫ

Аннотация. Тұмау вирусы – кен таралған вирустардың бірі. Ол жеңіл маусымдық және ауыр пандемияны туғызып, миллионнан астам адамдардың қайтыс болғанына себебші болған. Екі негізгі жасушадан тыс рецепторы – нейраминидаза (NA) мен гемагглютинин (HA) тұмаудың белгілерін дамытуына және аурудың таралуына жауапты.

Қателікке бейімді РНК-полимераза әртүрлі мутацияларды нейраминидаза мен гемагглютининге әр бөлінген сайын енгізе алады. Осы қасиеті тұмауға қарсы көп эффективті дәріні құрастыруды қиындатады. Пайда болған мутациялар вирустыжануарлардан адамдарға жұқтырады. Осы вирустардың типтері өте қауіпті, өйткені мутациялайтын жерлерді алдын ала болжау қиын. Қазіргі таңда вирустарда қандай молекулалық аймағында мутациялар жиі кездесетіндігі анықталды.

Осы мақалада жасушадан тыс рецепторлардың молекулалық құрылымы, қиын аймақтарға рецепторлардың жалғасу механизмдері мен таралған мутациялар аймақтары қарастырылған. Гемагглютинин мен нейраминидаза молекулалық структурасын және қиын аймақтарын зерттеу эффективті вакцина мен дәрілерін дамытуға ықпал ете алады.

Түйін сөздер: тұмау вирусы, нейраминидаза, гемагглютинин, мутация, сиалидаза, вирус.

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БАЗОВЫЕ ХАРАКТЕРИСТИКИ МОЛЕКУЛЯРНОГО СТРОЕНИЯ ВИРУСА ГРИППА А

Аннотация. Вирус гриппа является одним из самых распространенных вирусов в мире. Вирус способен вызывать как умеренные сезонные инфекции, так и пандемии, которые приводят к гибели сотен тысяч людей и животных. Нейраминидаза и гемагглютинин являются основными внешними рецепторами вирусной частицы и участвуют в таких процессах, как проникновение в клетки хозяина и распространение между клетками. РНК – зависимая РНК – полимеразаспособна в процессе репликации допускать ошибки, что приводит к быстрой эволюции вируса и соответственно снижению эффективности разработанных лекарств. Кроме того, высокая мутабельность вируса гриппа приводит к возможности определенного субтипа вируса расширить круг хозяев. Транзитные вирусы являются наиболее опасными и, как правило, способны вызывать пандемии. Однако возможно предсказать какие области генома вируса обладают высокой мутабельностью.

Данная статья описывает молекулярную структуру внешних рецепторов вируса гриппа А, включая рецептор – связывающие сайты и вариабельные участки. Углубленное изучение молекулярной структуры гемагглютинина и нейраминидазы по способствует разработки более эффективных лекарств и вакцин против гриппа.

Ключевые слова: грипп, нейраминидаза, гемагглютинин, мутация, сиалидаза, вирус.

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USING OF COMPUTER PROGRAMM «BD-PLANT-KZ» FOR CADASTRAL REGISTRATION OF PLANTS OF THE NATURAL FLORA OF KAZAKHSTAN

Abstract. The description of the computer “BD-PLANT-KZ” program, intended for input and storage in memory of the computer of various botanical information on plants of natural flora of Kazakhstan is provided. 11 points of the program are a part of the Main menu: "File", "Editing", "Input", "Search", "Viewing", "Lists", "Herbarium", "Communities", "Databases", "Service" and "Reference". The program allows carrying out quick search of data, printing, exporting to various formats, drawing up reports and lists in the set taxonomical, bioecological, decorative and other parameters. “BD-PLANT-KZ” has undergone successful approbation in two botanical gardens of Kazakhstan (Altai and Mangyshlak). Now floristic the database of the program includes information on natural flora for 882 taxons from 4 departments, 6 classes, 12 subclasses, 26 suborders, 59 orders, 10 suborders, 80 families and 300 gena. Approbation of the program has allowed making the summary characteristic of natural flora of the Western Kazakhstan on the example of the Mangystau, Atyrau, Aktyubinsk and West-Kazakhstany regions. Lists of taxons are determined by geographical points and floristic areas, geographical novelties are revealed.

Key words: computer program, «BD-PLANT-KZ», cadastral, registration of plants, Data Base.

Introduction. During creation of the information databases (DB) which containing a large number of accounts and variables, such as the inventory of plants, development of the instrument of formation of DB - the special computer program adapted with modern operating systems, graphic and text editors has essential value. Storage and processing of botanical information is widely applied in the countries of CIS and beyond. However, in Kazakhstan researches in this direction weren't carried out earlier.

In 2011-2012 on the basis of Republic State Entertainment "Mangyshlak Experimental Botanical Garden" of Science Committee of the Ministry of Education and Science within implementation of the project "Development of Scientific-Methodical and Information Base for Creation of the Inventory of Plants of the Republic of Kazakhstan" the special computer program “BD-PLANT-KZ” has been developed. There are electron shells inside allowing entering into databases several information on taxonomical structure of vascular plants with the description of their morphology, ecology, economic and biological properties, geographical GPS coordinates, herbarium samples, vegetative communities, raw resources, geographical and floristic areas, illustration photos and maps of areas.

The purpose of this scientific work is assessment lies in possibility of application of computer program for accounting of plants of natural flora of Kazakhstan.

Materials and methodlogy. During construction of “BD-PLANT-KZ" four programming languages have been used: Microsoft Visual FoxPro 9 SP2, Visual Basic For Applications 7.0, HTML 4.0 and JavaScript API 2.1.

For simplification of input of taxonomical units in the DB program is used the list of plant gena according to R.K. Brummitt [1]. In the DB for systematization of information is used the phylogenetic system of A.L. Takhtadzhyan [2, 3].

At the description of vegetable communities in "BD-PLANT-KZ" the scheme of geobotanical inspection of the deserts of Mangyshlak is accepted according to I.N. Safronova [4]: vegetation type, group of formations, formation, association. The volume of information on each record of DB is 25-30 (with drawings and the map - to 150-200) kB.

The Install Shield 2012 Premier Edition SP1 program was applied to formation of an adjusting compact disk and the uniform distributive Setup.exe file.

Effective work of "BD-PLANT-KZ" is possible at implementation of the following system requirements to computers: the Microsoft Windows XP SP 2-3, Vista SP 1-2 operating system or 7, 8 and 10 (32-bit or 64-digit), existence of Microsoft Office 2007, 2010 or 2016, is also more modern than Adobe Reader 7 or more of the late version, Internet Explorer 9; processor: Intel Pentium 4 or above; The RAM of 512 MB and more, is recommended – 2048; free disk memory - 700 MB; minimum resolution of the monitor not less than 1024 x 768. For the maximum use of opportunities for hardware acceleration graphic video cards, compatible DirectX, with the built-in video memory not less than 128 MB are recommended.

Results and their discussion. The structure of the program is reflected by its Main Menu (MM) which included 11 points: "File", "Editing", "Input", "Search", "Viewing", "Lists", "Herbarium", "Communities", "Databases", "Service" and "Reference" (figure 1).

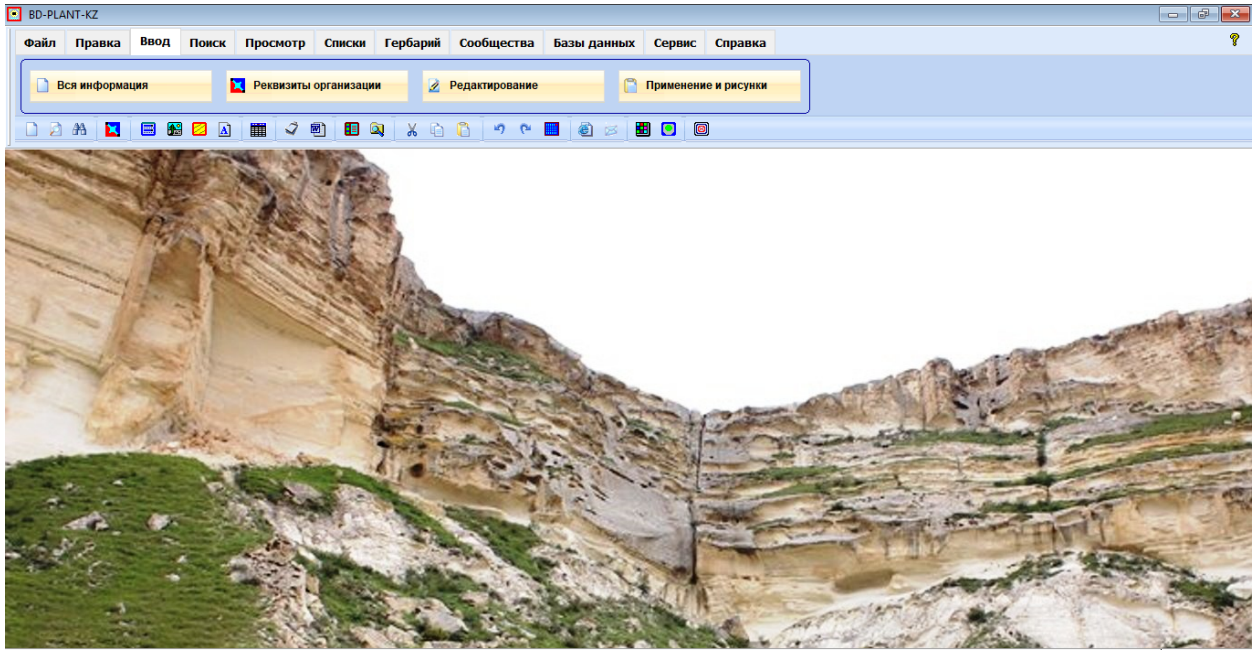

Figure 1 – The main menu of the Program

Point of MM “File” includes a standard set of sub points: "To open...", "My computer", "Press", “Filer”, "Search of files", "Server", “Internet”, "Mail" and "Exit" and intends for creation new and works with the available files, printings of information, contact with the server and Internet resources, for sending electronic messages and to make exit from the program. Point “Editing” is necessary for editing active text fields of forms of input and viewing of information, and also for searching and replacement of words and expressions, control of their font, color of letters and a background. From point “Input” is formed filling of new and changing earlier entered information. This point includes three sub points – "All information", "Requisites of the organization", "Editing" and "Application and figures".

Point “Search” allows to find plants in the DB using the following options: according to the identification number; according to the Latin name of taxon, according to the Russian name, according to the national name, family name, on floristic and geographical areas and by any word or a fragment of word from Latin, Russian and Kazakh names. In point "Advanced search" practically all above-mentioned ways are integrated. Point "Viewing" is used for work with already entered information with opportunities of its press and export in external editors and programs in various formats - doc, docx, rtf, txt, pdf, xml, etc.

Applying the point “Lists” it is possible to form the most various reports about plants according to taxonomical, morphological and other characteristics.

Three points "Input and viewing", "Reports" and "Export" of MM “Herbarium” realize a possibility of full work with information of Herbarium fund of botanical establishments.

Point "Communities" include only one sub points "Input and viewing" which is necessary for work with vegetation populations.

Point of MM of “Data base” is intended for implementation of the following commands: "Copying", "Restoration", "Export", "Import", “Re-indexation”, "Repair of indexes" and "DB Information".

Under MM there is a system Push-button menu for a fast loading of the most often used forms of input, viewing, printing of information, etc.

After “BD-PLANT-KZ “ installation at the first start of the program requisites of botanical establishment are without fail entered, using for this purpose the sub point "The main menu \Input \Requisites of the organization". It is required for a binding of all taxa at input of information to a certain organization.

All data on a plant are divided on forms of input and viewing into 11 groups (pages): Taxonomy, Names, Areas, Map, Morphology, Ecology, Application, Addition information, Herbarium, Figures and Text. On all pages are provided menus and buttons of the fast choice of the standard or already available in DB information for the purpose of its operating input (figure 2).

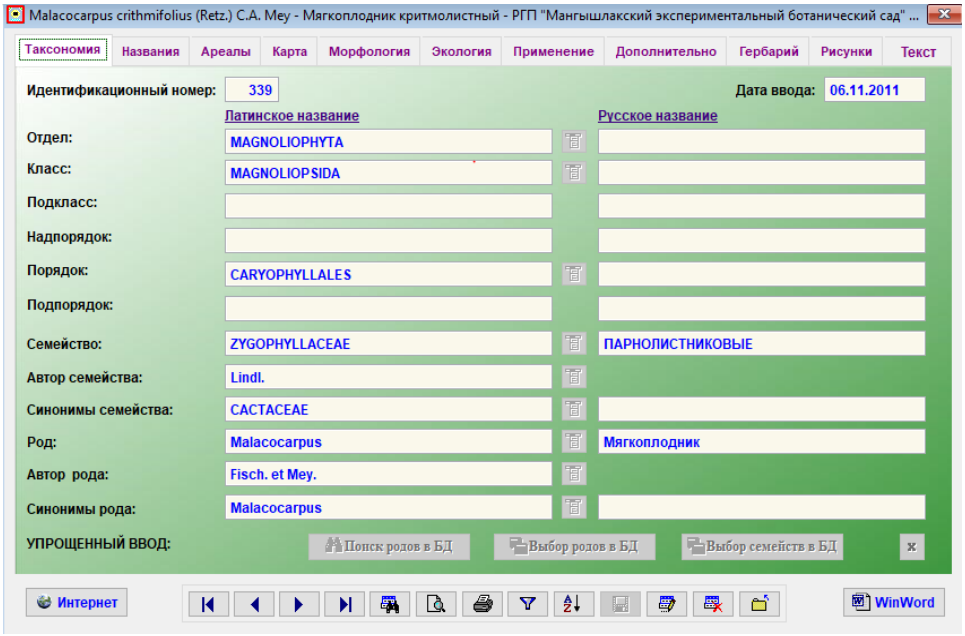

Figure 2 – Page «Taxonomy» – forms of entering and viewing

Forms "Input" and "Viewing" of plants’ data differ only functionally and according to the lower push-button menu of commands. The push-button menu on a form "Input" includes 5 points: "To keep" - it is used for addition into DB new record after entering all information about plant; "Copy" - serves for copying from DB already entered data on taxa for further editing and preservation that facilitates input of information; "Check" is necessary for search of a plant in DB that exclude duplication; "Dumping" - removal of all information from a form of "Input" and "Exit" - for its closing. On form "Viewing" is located 11 command buttons which perform various functions of work with earlier entered data about plants, 4 of them are placed at the left and are included for navigation on a DB. On the page “Taxonomy” is entered or checked all systematic characteristic of a plant.

The program enters full "Names" of plants automatically, adding the name of species, a form, etc. through a gap to a sort. For authors certain fields are provided. The structure of fields of the section "Areas" has included old and new names of floristic areas, administrative and geographical regions in the explored territory, the general distribution, etc. Places of occurrence of plants can be displayed on the page “Card”.

The description of morphological features of taxa is conducted on the page “Morphology” (figure 3) and used the following indicators: growth form, vital form according to A. Raunkier, classification by frequency of fructification, pollination type, data of blossoming and pollination, coloring of flowers, fruits and leaves, the characteristic of a morphological structure.

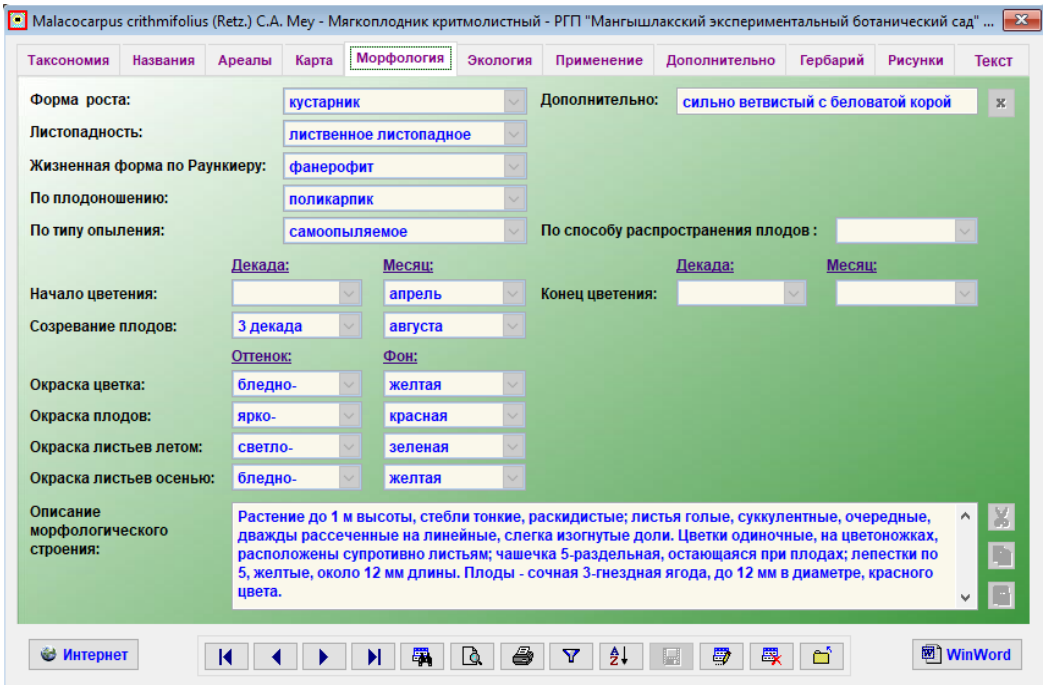

Figure 3 – Page «Morphology» – forms of entering and viewing

Ecological features are displayed on the page “Ecology” (a natural area, habitats, the phyto security status, an endemic, relicts and aboriginal status, classification in relation to light, water, fertility and salinity of the soil, etc.).

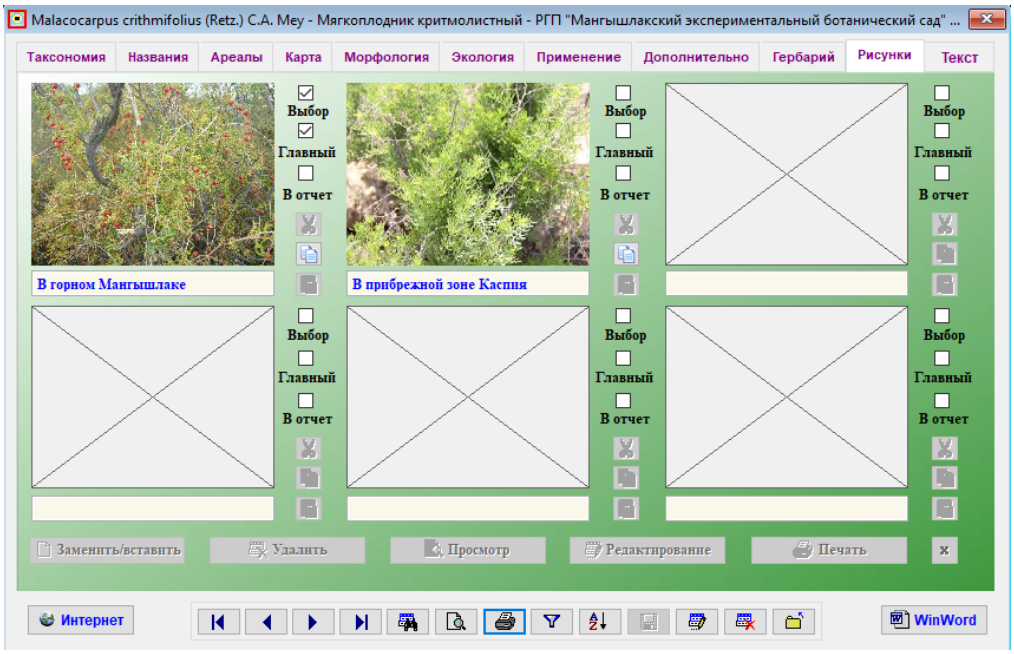

Figure 4 –Page «Figures» - forms of input and viewing

Economic and biological value and reproductive ability of plants is collected in fields of DB – "Application". On the page “Addition information” are placed references and data about user - organization. The section "Herbarium" is made for input and viewing of places and geographical coordinates of herbarium samples (until 3 samples). On the page “Figures” is possible to insert into DB until 6 files of plant images with their names (figure 4). The page “Text” is made for the purpose of input and storage of big text information on taxa (including of files).

The great value in "BD-PLANT-KZ" is given to quick search of taxa. The special form is made which allow to filter taxa by institutions, families and gena or to choose a concrete plant (figure 5).

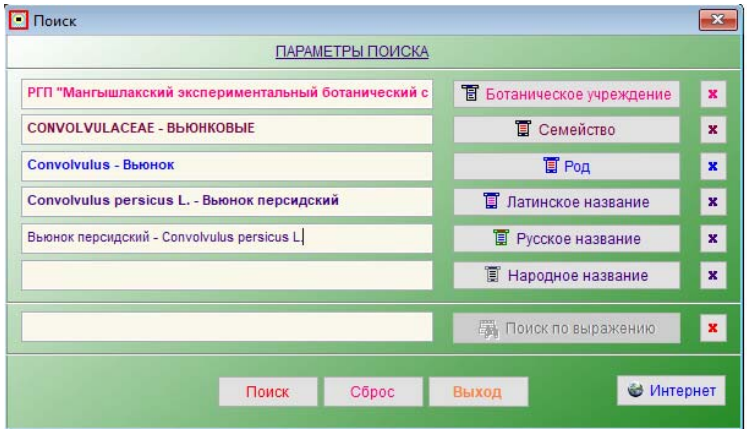

Figure 5 – Form «Advances search»

Using of "BD-PLANT-KZ" it is possible to export plant information to 9 formats (txt, doc, docx, xls, xlsx, rtf, pdf, tif and xml) for the subsequent editing in external text and graphic editors. The call of a form of export (figure 6) is carried out through MM - "The main menu \Viewing \In WinWord". On completion of translation of data in the chosen format, the created file opens in the corresponding editor. The example of export to Microsoft Word is shown in the figure 7.

Access to the form of the list of taxonomical units is carried out by the button “Systematization” in point “Viewing” of MM (figure 8). At the choice of any unit of systematization in the right text field there is a list of the taxa of DB. Here it is possible to obtain also information, as about all taxonomy of organization, so inside DB in general.

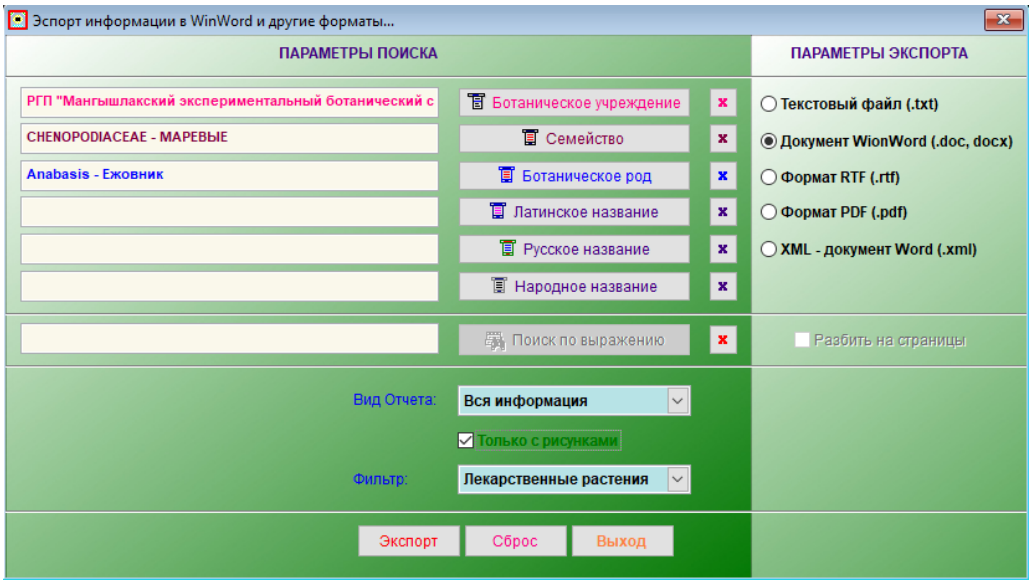

Figure 6 – Form «Export information in WinWord and other formats»

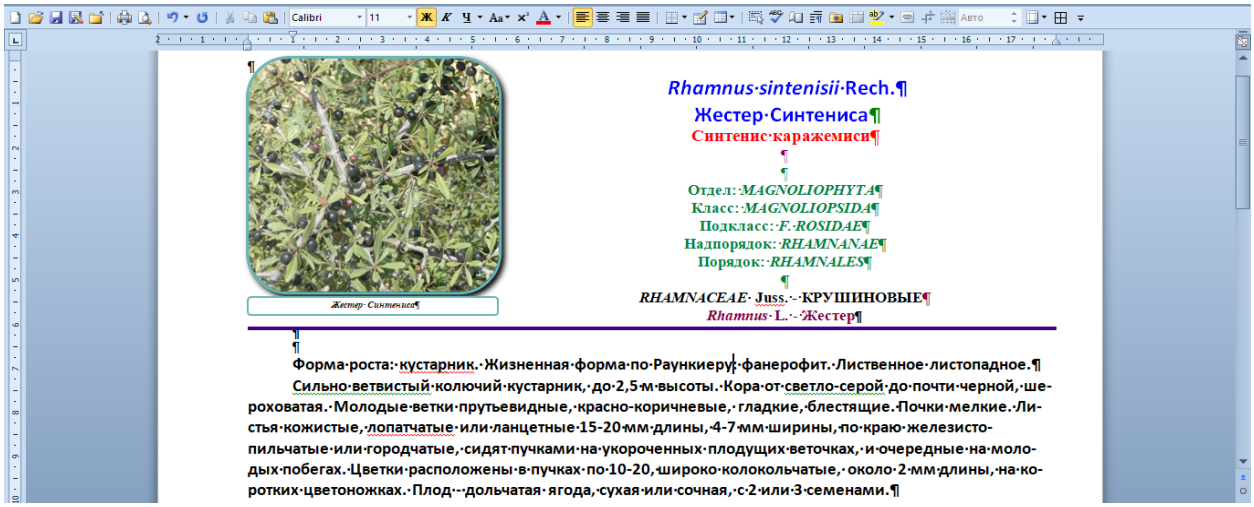

Figure 7 – Example of export of information in pdf – format

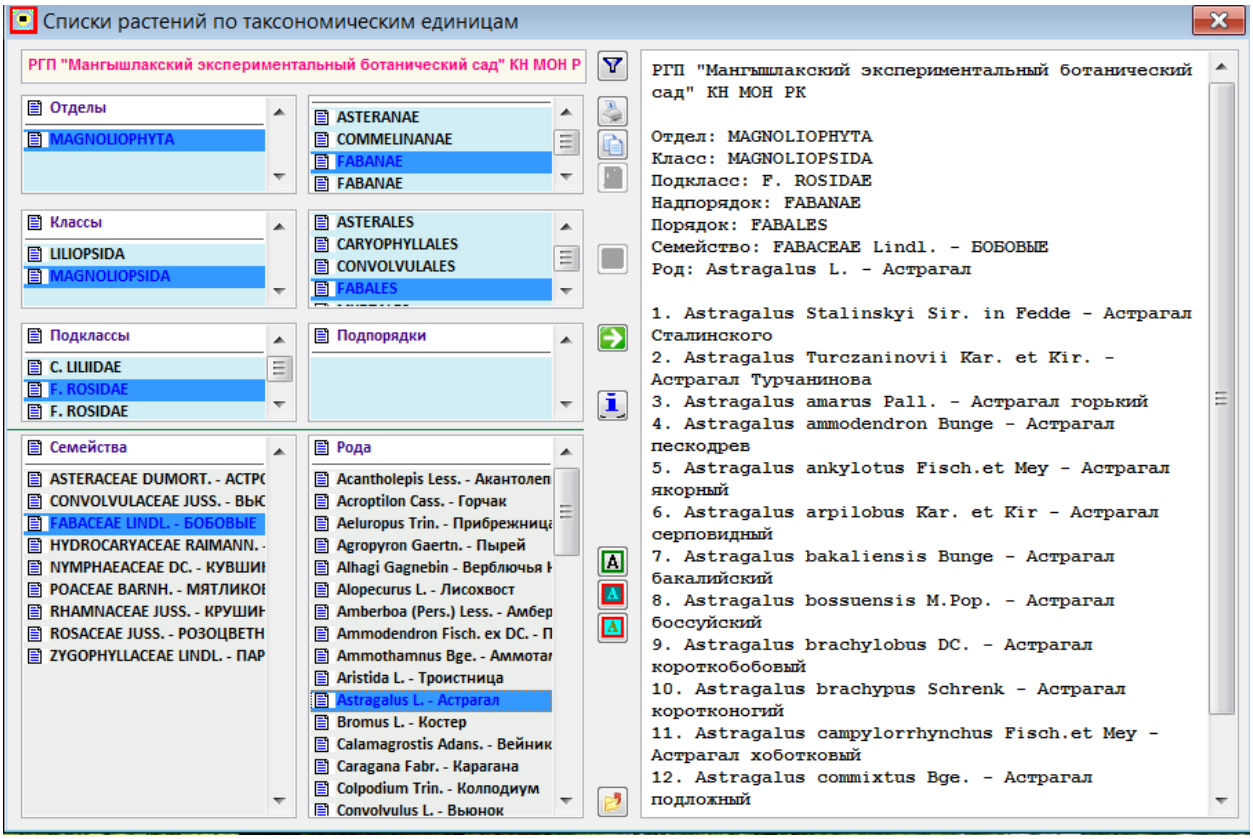

Figure 8 – Form of the list of taxonomical units

The program has provided formation of the most various lists of plants according to taxonomical, morphological and other characteristics.

More detailed task of parameters of creation lists is possible when using a form with the similar name which is started by command “Choice …” of point of MM. Creation lists is carried out to Excel with use of the form which represented in the figure 9.

The command “Main Menu \Herbarium” is applied to work with Herbarium fund. At the same time on the screen there will be a special form on which all list of plants of botanical organization by default will be displayed. By using of the lower Push-button menu it is possible to execute search of the necessary

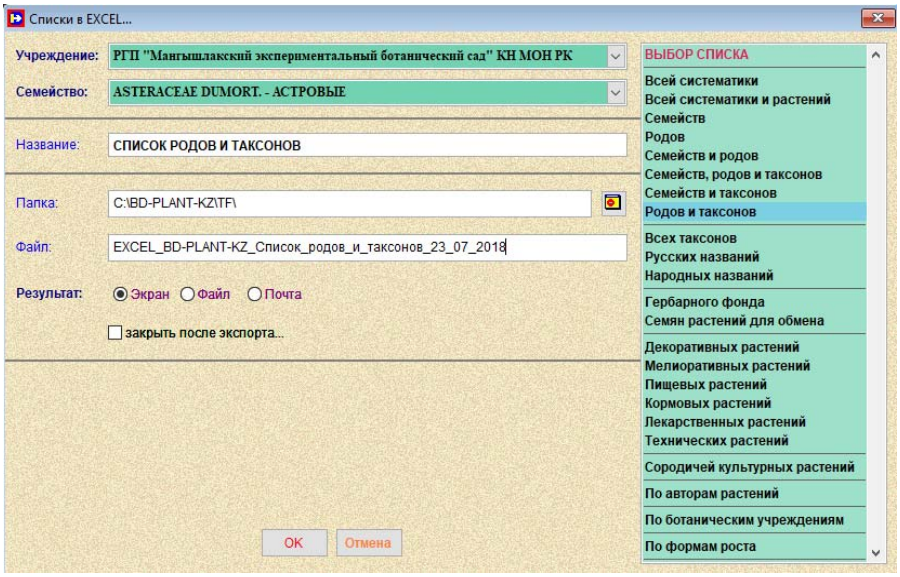

Figure 9 – Form «List in Excel…»

taxon, to view and print out all list, and also to edit it regarding inclusion or an exception of a plant of herbarium fund. If herbarium samples are available, the button “Samples” is activated by means of which the form intended directly for editing information is started. The Command “Editing” makes available for editing of the field DB. The current record of the Herbarium can be copied and removed. The modes “Reports” and “Export” gave the chance of a conclusion of herbarium data in two options: "All information" and "Labels".

For plant communities in "BD-PLANT-KZ" is provided the forms of input and viewing information included 5 pages (groups) of information: "Location", "Communities", "Tier", "In addition" and "Figures" (figure 10).

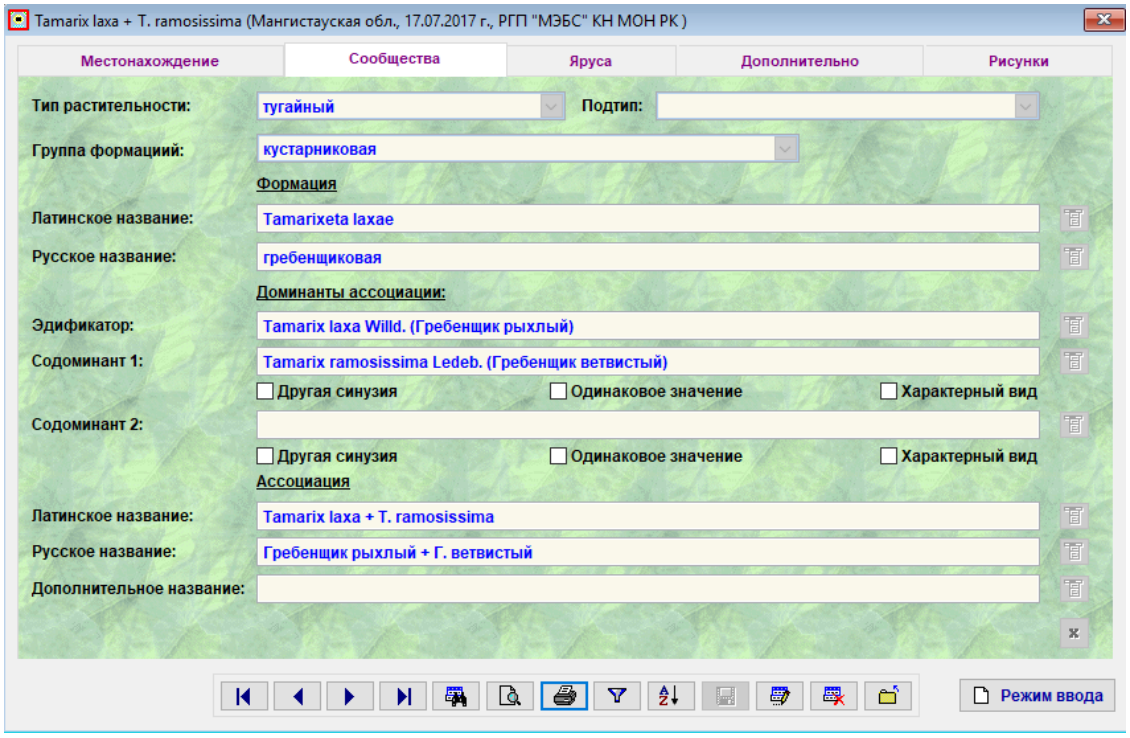

Figure 10 – Page «Community» - forms of input and viewing information

The page “Location” is concentrated fields of DB characterized administrative and geographical location of population, GPS coordinates, name of natural zone and conditions of growing. The group of variables "Community" is devoted to directly geo botanical units.

Many of them can be chosen or created from the lists revealing the corresponding buttons. The correct connection of dominant in the name of association can be executed automatically by means of a combination of installation or removal of a tick to the left of the words "Other sinuzia", "Identical Meaning" and "Characteristic Species".

By using of Latin names of communities, the name of ediphicator is put on the first place, Russian – on the contrary [4]. Taxa of various sinuzia connect in population the sign of a hyphen "-", one – plus "+". In case plants have identical value and belong to one sinuzia, then they are listed through a comma. Species which are characteristic of community, consist in their names in square brackets "[…]".

The page "Tier" includes as the general list of the plants entering into structure of population, and their level accessory with the indication of a projective covering, abundance according Drudae, occurrences and heights. To group “Addition” are carried out geo botanical districts, areas and sub-districts, existence of raw resources, the note of a text format unlimited on length, botanical organization, a position, degree and Full name of the performer. "Figures" (page No. 5) are necessary for work with graphic material on communities which can be observed in three modes: "Clip", "Isometry" and "Stretched".

Thus, structurally three main databases are a part of the program: 1) floristic, 2) herbarium and 3) geo botanical, consisting, respectively, from 211, 60 and 131 fields of numerical, symbolical and logical types in the total length - 10602, 2703 and 8161 symbol.

Commands of point MM “Data Base” have the following functional purpose:

1) "Copying" - creation of the insurance copy of all DB, setting up the program and files of images on a case of loss of information;

2) "Restoration" - a complete recovery of a DB and settings;

3) "Export" - creation of the copy of a floristic, herbarium and geo botanical DB for transfer on other personal computer (PC) or in other botanical organizations;

4) "Import" - addition of records on plants, herbarium fund and communities from other personal computer;

5) “Re-indexation” - updating of the DB indexes and their packing;

6) "Repair of indexes" - creation of new indexes instead of spoiled in the process of work, if that happens;

7) "Information" - obtaining data on the maintenance of DB.

After using the first two sub points is also possible copying of DB on other personal computer. The folder created in the mode “Export” can be archived and sent at once to other botanical organization by e-mail or via the server for formation of a uniform DB according to the inventory of plants of natural flora of Kazakhstan.

Present days floristic DB includes the fullest taxonomical, geographical, ecological - biological and graphic information for 882 taxa from 4 departments, 6 classes, 12 subclasses, 26 above orders, 59 orders, 10 sub orders, 80 families and 300 gena is entered. More than a half (66,0%) of taxa (582) of DB is represented by representatives of 7 families (table 1); the most numerous are 4 families: Asteraceae Dumort. (124), Chenopodiaceae Vent. (152), Fabaceae Lindl. (111) and Poaceae Barnh. (138). Among taxa complexes in the database are considerably prevailed the following gena - Artemisia L. (53 - 6,0%), Astragalus L. (80 - 9,1%), Elymus L. (34 - 3,9%) and Salsola L. (20 - 2,3%). In the natural conditions plants grow in 37 floristic regions of Kazakhstan. 96 species meet on all territory of the republic. The greatest number of taxa is dated for the following floristic areas: "3. Tobolsk and Ishim (101), "4. Ural" (33), "5. Aktyubinsk" (45), "6. Turgai" 63), "16. Mangystau" (40) and "30. Altai" (119). At present in the DB there are 976 graphic files (figures, images and maps).

Now in herbarium database of program “BD-PLANT-KZ” is contained data recording for 765 samples of 281 species and a form of plants of natural flora from 53 families and 162 gena collected in 74 locations of 320 geographical regions (areas) of 14 administrative regions of 4 areas of Kazakhstan.

The greatest number of samples has been collected in Beyneu (99), Karakiyansky (150), Mangystau (170) and Tupkaragan areas (65) of the Mangystau Region; and also in Zhylyoy (53) and Kzylkoginsky areas (126) of the Atyrau regions (table 2). Amongfloristic areas on number of herbarium information

Table 1 – The most representative families and gena of plants of the floristic database

Family	Taxa	%	Gene	Taxa	%
Asteraceae Dumort.	124	14,1	*Artemisia* L.	53	6,0
Brassicaceae Burnett.	26	2,9	*Astragalus* L.	80	9,1
Chenopodiaceae Vent.	152	17,2	*Atriplex* L.	15	1,7
Fabaceae Lindl.	111	12,6	*Chenopodium* L.	16	1,8
Lamiaceae Lindl.	14	1,6	*Elymus* L.	34	3,9
Poaceae Barnh.	138	15,6	*Salsola* L.	20	2,3
Scrophulariaceae Juss.	17	1,9	*Suaeda* Forks.	14	1,6
Total:	582	66,0	Total:	232	26,3

Table 2 – Distribution of herbarium samples of the database by the administrative and floristic regions of Kazakhstan

Administrative region (oblast)	Taxa	%	Floristic region	Taxa	%
Beineu (Mangystau)	99	12,9	5. Aktobe	23	3,0
Bokeiorda (Western Kazakstan)	1	0,1	15. Bosaschy	6	0,8
Zhylyoy (Atyrau)	53	6,9	13a. Bekeev	1	0,1
Inder (Atyrau)	39	5,1	16. Mangystau	381	49,8
Isatai (Atyrau)	20	2,6	13. Caspian	1	0,1
Karakiyansky (Mangystau)	150	19,6	17. Northern Ustyurt	96	12,5
Kzylkoginsky (Atyrau)	126	16,5	6. Turgay	1	0,1
Makat (Atyrau)	14	1,8	4. Ural	256	33,5
Mangystau (Mangystau)	170	22,2			
Makhambet (Atyrau)	22	2,9			
Mugadzhary (Aktobe)	1	0,1			
Munailinskyi (Mangystau)	4	0,5			
Tubkaragan (Mangystau)	65	8,5			
Khobda (Atyrau)	1	0,1			
Total:	765	100,0	Total:	765	100,0

entered into a DB considerably dominate "16. Mangystau" (381 - 49,8%), "17. Northern Ustyurt" (96 - 12,5%) and "4. Ural" (256 - 33,5%). Herbarium samples of the database are illustrated by 465 photos.

Conclusion. The computer program has successfully approbated in two botanical gardens of Kazakhstan (Altai and Mangyshlak), and has shown high reliability and efficiency of work with floristic and herbarium information on plants of natural flora of Kazakhstan. Lists of taxa are revealed according to systematic accessory, ecological and biological properties, geographical points, floristic areas, etc. It is determined geographical novelties of plants.

"BD-PLANT-KZ" is registered in Committee on Intellectual Property Rights of the Ministry of Justice of the Republic of Kazakhstan (the certificate on the state registration No. 1408 of December 25, 2012, IS 0009258).

Introduction of the program into practice of the cadastral registration has considerably simplified creation information databases, has allowed carrying out quickly search of taxa and, in general, has expanded possibilities of work with information about plants and their communities

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ҚАЗАҚСТАННЫҢ ТАБИҒИ ФЛОРАСЫНЫҢ ӨСІМДІКТЕРІН КАДАСТРЛІК ЕСЕПКЕ АЛУ ҮШІН «BD-PLANT-KZ» КОМПЬЮТЕРЛІК БАҒДАРЛАМАСЫН ҚОЛДАНУ

Аннотация. Қазақстанның табиғи флорасының өсімдіктерінің ботаникалық алуан түрлігігі жөнінде ақпаратты компьютер жадына енгізу және сақтау үшін арналған «BD-PLANT-KZ» компьютерлік бағдарламасының сипаттамасы берілген. Бағдарламаның негізгі құрылымының құрамы 11 мәтіннен тұрады: «Файл», «Өңдеу», «Енгізу», «Іздеу», «Қарау», «Тізім», «Кеппе шөп», «Қауымдастық», «Деректер базасы», «Сервис» және «Анықтама». Бағдарлама деректерді жылдам іздеуге, басып шығаруға, әртүрлі форматта экспорттауға, берілген таксономикалық, биоэкологиялық, сәндік және басқа параметрлер бойынша тізімдер мен есептерді жасауға мүмкіндік береді. «BD-PLANT-KZ» Қазақстанның екі ботаникалық бақтарында (Алтай және Маңғыстау) сынақтан сәтті өтті. Қазіргі уақытта бағдарламаның флористикалық деректер базасында табиғи флораның 4 бөлімдер, 6 класс, 12 класс асты, 26 қатарүсті, 59 қатар, 10 қатар асты, 80 тұқымдастан және 300 туыстан тұратын 882 таксон үшін ақпарат енгізілген. Бағдарламаның сынағы Батыс Қазақстанның мысалға Маңғыстау, Атырау Актөбе және Батыс Қазақстан облыстарының табиғи флорасының жиынтық сипаттамасын құрастыруға мүмкіндік берді. Географиялық нүктелер мен флористикалық аудандар бойынша таксондар мен географиялық жаңа тізімдер анықталды.

Түйін сөздер: компьютерлік бағдарлама, BD-PLANT-KZ, кадастр, өсімдік есебі, деректер базасы.

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ИСПОЛЬЗОВАНИЕ КОМПЬЮТЕРНОЙ ПРОГРАММЫ «BD-PLANT-KZ» ДЛЯ КАДАСТРОВОГО УЧЕТА РАСТЕНИЙ ПРИРОДНОЙ ФЛОРЫ КАЗАХСТАНА

Аннотация. Приводится описание компьютерной программы «BD-PLANT-KZ», предназначенная для ввода и хранение в памяти компьютера разнообразной ботанической информации о растениях природной флоры Казахстана. В состав Главного меню входят 11 пунктов программы: «Файл», «Правка», «Ввод», «Поиск», «Просмотр», «Списки», «Гербарий», «Сообщества», «Базы данных», «Сервис» и «Справка». Программа позволяет осуществлять оперативный поиск данных, вывод на печать, экспорт в различные форматы, составление отчетов и списков по заданным таксономическим, биоэкологическим, декоративным и иным параметрам. «BD-PLANT-KZ» прошла успешную апробацию в двух ботанических садах Казахстана (Алтайский и Мангышлакский). В настоящее время флористическая база данных программы включает информацию по природной флоре для 882 таксонов из 4 отделов, 6 классов, 12 подклассов, 26 надпорядков, 59 порядков, 10 подпорядков, 80 семейств и 300 родов. Апробация программы позволила составить сводную характеристику природной флоры Западного Казахстана на примере Мангистауской, Атырауской, Актюбинской и Западно-Казахстанской областей. Определены списки таксонов по географическим точкам и флористическим районам, выявлены географические новинки.

Ключевые слова: компьютерная программа, «BD-PLANT-KZ», кадастр, учет растений, базы данных.

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CHANGES OF THE THYROID HORMONES CONCENTRATION AND THE BIOCHEMICAL PARAMETERSFEAUTURES OF LYMPH AND BLOOD OF RAT WITH HYPOTHYROIDISM

Abstract. The article considers the influence of the mercazolilum management on the hormonal state in the lymph and blood of rates. The study shows that after mercazolilum management and the hypothyroidism signs appearance in rats, there was a decrement of lymph flow from the thoracic lymphatic duct. Changes in the hormonal and biochemical composition of the lymph were observed. There were disturbances not only of the metabolism in the lymph, but also of the functional state of the body. There were disturbances of rheologic and physicochemical parameters of lymph and blood at the experimentative hypothyroidism of rats: viscosity has been increased, coagulability and number of erythrocytes have been decreased, thrombocytes and leucocytes in blood have been increased. It has been led to a decrease of rats of the 2nd experienced group in T_3, T_4 content and increase of thyrotropic hormone in lymph and blood which reflected the occurrence of their hypothyroidism. Experiments showed that T_3content in rats with hypothyroidism has decreased by 45.7% and $T_4$by 35.6% in lymph, and in blood $T_3$by 43,5% and $T_4$by 41,6% have decreased compared with the control group of animals.

Key words: hormones, blood, rats, lymph, lymph flow, thyroxin, triiodothyronine.

The most relevant topic among the current medical and social issues is thyroid bodypathology. This is due to the fact that thyroid body diseases depend on many factors and conditions: geochemical, demographic, social and ecological and climatic, etc. [1, 2]. Hypothyroidism is one of the most prevalent pathologies of endocrine system due to persistent and long lasting deficit of thyroid hormones in the body or a decrease of their action on target organs [3-5].

Hypothyroidism in popularity is among the highest in all endocrine diseases, and its prevalence increases with age. Primary hypothyroidism is associated with thyroid body pathology leading to a decrease in glandular tissue mass of thyroid body and inhibition of the synthesis of thyroid hormones. This may be due to aplasia or agenesis of the thyroid body, autoimmune processes, deficit iodine, Selena deficit [6]. Secondary hypothyroidism ("central") is associated with loss of the hypophysary tropic function (decrease in thyreotrophin production). Insufficient intake of thyroid body hormones leads to disturbance of protein and carbohydratemetabolism, flattening of the sugar curve after glucose loading, to disturbance of lipid and water-salt metabolism [7]. The variety of clinical symptomatology inherent in underactive thyroid body is largely due to metabolism processes disturbance associated with thyroid hormone deficit.Thyroid body pathology reflects on the provision of endocrine, immunological, energy homeostasis of the body [8, 9]. Hypothyroidism occurs at approximately 19 per 1,000 in women and 1 per 1,000 in men. Despite its prevalence, hypothyroidism is very often detected late [10].

Taking into account the important role of lymphatic system in homeostasis maintaining in the body [11, 12], the study of its role at thyroid insufficiency is of current importance. The literature has no data of the functional state of the lymphatic system at hypothyroidism. The purpose of this research is the study

of experimental hypothyroidism on lymphatic flow, hormonal and biochemical parameters in the lymph and blood in rats.

Materials and methods. The work has been done on 55 white sexually mature non-pedigree rat-males weighing 230-250 g. Rats feeding has been carried out according to standard diet of the vivarium. Rats of the 1st group (15 rats) were control. They were in the same conditions of feeding and keeping with animals of experienced rats. The 2nd group (20 rats) and the 3rd group (20 rats) were experienced. Experimental hypothyroidism of trial rats has been modeled according to Orlov method, 2002 [13, 14]. The rats of the experienced groups have been administered mercazolilum in a dose of 20 mg per 100 g of body mass with drinking water daily within 15 and 30 days for the state development of experimental hypothyroidism. All groups of animals were in the same conditions of feeding and keeping.

The speed of lymph flow and its rheological properties of control and experimental groups of animals have been studied. In lymph of all groups of animals physical and chemical parameters, coagulation time, lymph according to Sukharev, viscosity using a viscometer CT-4, biochemical parameters in lymph, blood plasma of control group of animals have been determined. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level, total protein, cholesterin, triglycerides, bilirubin, urea, creatinine have been determined in samples of lymph and blood using automatic biochemical analyzer COBOS INTEGRA 400 [15].

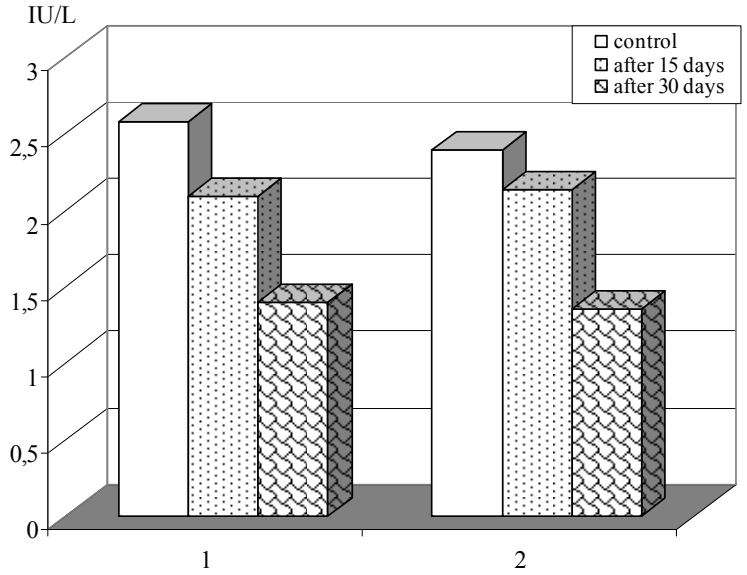
The development of hypothyroidism has been controlled by the level of thyroid-stimulating hormone, triiodothyronine, thyroxin in blood and lymph. The concentration of thyroid-stimulating hormone (TSH), triiodothyronine and thyroxin in lymph and blood of intact animals on 15th and also on 30th days of experimental hypothyroidism have been determined by electro-chemiluminescent method using standard test-system in accordance with enclosed production instructions IMMUNOTECH (Czechia), with further processing of the results obtained on the analyzer COBOS INTEGRA 400 (USA). Experience results have been processed by variation statistics method on ECM using Student's t-test. The results have been considered accurate at $p<0,01$, $p<0,05$.

Results and its discussion. The results show that hypothyroidism in rats has been formed after mercazole management, it was characterized by a decrease in body mass and change in their behavior – hyperactivity has been watched. The research showed that a decrease of thyroid hormone in lymph and blood has been occurred at hypothyroidism. There was a decrease of concentration in lymph T_3 1,2 times, T_4 1,14 times in the second week, that is, after 15 days of the research, and hormonal state of the parameters under the research have been decreased 1,8 and 1,7 times accordingly on the 30th day of the research.

Similarly, the picture of the hormonal state showed that T_3иT_4 level in blood had reduced 1,12 times after 15 days, and accordingly 1,8 and 1,6 times after 30 days (table 1, figure 1, 2).

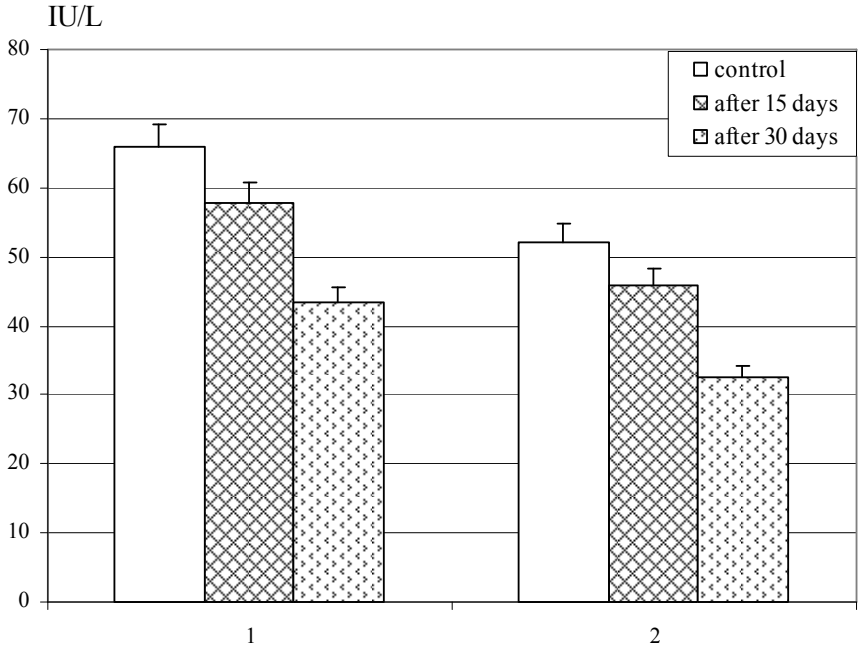
Table 1 – Change of thyroid hormones in lymph and blood of rats of control group and at experimental hypothyroidism

Parameters	Control	Experimental	
		15 days	30th day
Lymph			
TSH – thyroid-stimulating hormone, IU/L	0,14±0,002	0,26±0,001**	0,27±0,003**
T_3 – triiodothyronine, IU/L	2,58±0,01	2,09±0,01*	1,40±0,01**
T_4 – thyroxin, IU/L	65,8±3,3	57,8±2,5*	42,4±1,7**
Blood			
TSH – thyroid-stimulating hormone, IU/L	0,03±0,001	0,05±0,001**	0,07±0,002**
T_3 – triiodothyronine, IU/L	2,39±0,03	2,13±0,01*	1,35±0,07**
T_4 – thyroxin, IU/L	52,2±2,4	45,8±2,1*	32,5±1,3**
Note - * accurate compared to control $p<0,05$,* -$p<0,01$**.			



Notations: By Y-axis: triiodothyroninelevel (T_3) in IU/L. By X-axis: 1-lymph, 2 – blood plasma.

Figure 1 – Triiodothyronine content (T_3) in lymph and blood in control and at experimental hypothyroidism

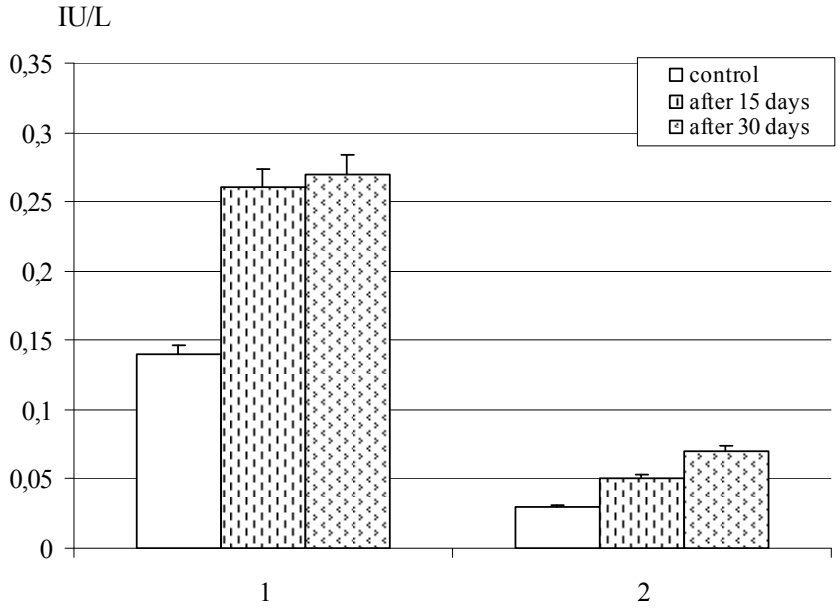


Notations: By Y-axis: thyroxin level (T_4) in IU/L. By X-axis: 1-lymph, 2 – blood plasma,

Figure 2 – Thyroxin content (T_4) in lymph and blood in control and at experimental hypothyroidism

There was a decrease in T_3, T_4content and increase of thyrotropic hormone in lymph and blood in rats of the 2nd experienced group with hypothyroidism, which reflect the occurrence of their hypothyroidism. Experiments have shown that in rats with hypothyroidism T_3 content decreased by 45.7% and T_4 by 35.6% in lymph, and inblood T_3 decreased by 43.5% and T_4 by 41.6% compared with the control group of animals.

The level of thyroid-stimulating hormone (TSH) in the lymph and blood showed an increase of 92.9 and 133.3%, accordingly, compared to intact animals (table 1, figure 3).



Notations: By Y-axis: Thyroid-stimulating (TSH) level, in IU/L. By X-axis: 1-lymph, 2 – blood plasma.

Figure 3 – Thyroid-stimulating hormone level (TSH) in lymph and blood in control and at experimental hypothyroidism

Lymph flowspeed from thoracic duct of rats showed that lymph flow decrease at experimental hypothyroidism was from 0,34±0,02 to 0,25±0,02 ml/hour, it is 26% higher compared to parameters of control group.

The results of the research showed that hemoglobin contents in blood of rats of the 1st group did not have significant changes during experiment, it was within 152.3 to 156.6 g / l. Hemoglobin content has decreased compared to control group by 1,24-1,25 times (by 122.6-124.8 g / l) after mercazolilum management in 15 days at experimental hypothyroidism. Its value has been decreased compared to the control group by 1.41 times (70%, by 112.2 g / l) / by the 30th day from the beginning of the experiment.

The erythrocyte parameters in the blood of the control groups of animals ranged from 7.59 to 7.84 ± 0.9 × 1012 / l. Its value in the blood in the experimental groups showed a decrease of 31% from the initial level. Experiment has observed a decrease of hematocrit by 10.7% compared to control group after mercazolilum administering. The background value of thrombocyte in the blood of rats was detected in the range from 225 to 243.3 ± 11.3 h109 / l.

The thrombocytes content of animals of the 2nd group, which received mercazolilum within a month, increased by 1.6 times (experimental groups 395.7 ± 10x109 / l) compared to the control group. Leukocyte parameters in experimental hypothyroidism have increased by 87.9% compared to rats of the control group. The leukocytes level of intact animals showed from 12.87 to 14.38 ± 1.7x109 / l. According to the biochemical research data there was a decrease in the concentration of total protein and alkaline phosphatase in the lymph by 26-29 and 50.6%, in the blood plasma by 20-25 and 50.2%, accordingly, as well as an increase in the enzymatic activity of alanine aminotransferase and aspartate aminotransferase compared with intact rats in lymph by 63-61%, and blood by 187-131% (table 2) at experimental hypothyroidism.

Data obtained shows that leukocytosis and thrombocytosis, a slight decrease in the number of erythrocytes compared with the control are in evidence at hypothyroidism. Analysis of the research results showed a decrease in the volumetric flow rate of the lymph flow and changes in the biochemical and rheological properties of lymph. We found that a decrease in the viscosity of the lymph had contributed to a decrease in the speed of lymph movement. Changes in the physicochemical parameters and rheological properties of lymph contribute to changes in lymph viscosity in our experiments.

The research results showed that the thyrostatic administering of thyrostatics, i.e. mercazolilum causes experimental hypothyroidism state in animals. According to scientific literature data mercazo-

Table 2 – Biochemical parameters of blood of control rats and at hypothyroidism

Parameters	Control	Hypothyroidism
Lymph		
Total protein, g /l	42,8±1,4	30,3±1,7
Alanine aminotransferase, mmol/l	84,4±2,9	138,2±10,1
Aspartate aminotransferase, mmol/l	159,7±10,8	257,5±10,9
Alkaline phosphatase, mmol/l	584,3±10,6	295,7±10,5
Blood		
Total protein, g/l	65,2±2,3	52,8±1,5
Alanine aminotransferase, mmol/l	78,4±2,3	220,7±10,6
Aspartate aminotransferase, mmol/l	139,6±10,1	323,6±10,5
Alkaline phosphatase, mmol/l	673,2±10,7	338,3±10,4
Note - * accurate compared to control p<0,05,* -p<0,01**		

lilumis a specific synthetic thyreostatic that inhibits the activity of thyroid body hormones involved in the synthesis and also inhibits thyroxinsynthesis, lowers the synthesis of basic metabolism [16, 17]. Research has shown that at Thyroid-stimulating (TSH) level in blood has risen, T_3 and T_4 level has decreased. We have showed a prevailing amount of thyroid hormones in the lymph than in the blood of experimental animals.

The amount of thyroid hormones in the body of animals has decreased in parallelat mercazolilum management, both in the blood and in the lymph, but the amount of hormones in the lymph was inhibited. Thus, it can be concluded that hypothyroidism in rats has been developed at mercazolilum managementin an average daily dose of 20 mg / 100 g within 30 days with water. It is characterized by features of the general state of rats, wool in the tail area of some individuals has lost, body weight has decreased, aggressive and hormonal, biochemical composition of lymph and blood, changes in physico-chemical and rheological properties of blood and lymph have been observed in the behavior of rats.

Conclusion. The results obtained in the presented research data, especially the results in the lymph can have important theoretical implications to understand the biochemical mechanisms at experimental hypothyroidism. Thus, we obtained experimental hypothyroidism in rats that showed a decrease in the level of thyroid hormones in the blood and lymph.

The level of thyroid hormones in the lymph slightly exceeded their level in the blood, both in normal conditions and also at hypothyroidism. Lymph flow has decreased and hormonal, biochemical, rheological lymph parameters have changed dramatically at hypothyroidism. Our findings suggest that experimental hypothyroidism in rats has been obtained. Thyroid body disturbance in animals occurs at mercazolilum management, it shows by a decrease of hormonal state both in the lymph and in the blood.

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ЕГЕУҚҰЙРЫҚТАРДА ГИПОТИРЕОЗ КЕЗІНДЕГІ ЛИМФА МЕН ҚАНДАҒЫ ГОРМОНДАР КОНЦЕНТРАЦИЯСЫНЫҢ ӨЗГЕРІСТЕРІ ЖӘНЕ БИОХИМИЯЛЫҚ КӨРСЕТКІШТЕРІНІҢ ЕРЕКШЕЛІКТЕРІ

Аннотация. Мақалада егеуқұйрықтардың лимфасы мен қанының гормоналды көрсеткіштеріне мерказолил препартының әсері қарастырылған. Зерттеуде көрсетілгендей, мерказолилді енгізгенен кейін егеуқұйрықтарда гипотиреоз белгілері байқалғанда кеуде арнасында лимфа ағысының төмендегені байқалады. Лимфа құрамында гормоналды және биохимиялық өзгерістер байқалды. Зат алмасу өзгерісі тек лимфада емес, сонымен бірге организмнің функционалды жағдайында байқалды. Эксперименталды гипотиреоз кезінде егеуқұйрықтардың лимфасы мен қанында реологиялық және физика-химиялық көрсеткіштердің өзгеретіндігі байқалды: тұтқырлық артты, қанның ұюы мен эритроциттер санының төмендеуі, қанда тромбоциттер мен лейкоциттер мөлшері артты. 2-ші тәжірибелік топтағы гипотиреозды егеуқұйрықтардың лимфасы мен қанында T_3, T_4 мөлшерінің төмендеуі және ТТГ артуы, яғни оларда гипотиреоз белгісінің пайда болғандығын көрсетті. Эксперимент нәтижесінде, гипотиреозды егеуқұйрықтар лимфасында T_3 мөлшері 45,7% және T_4 35,6%-ға төмендегені, ал қанда бұл көрсеткіштер бақылау тобы жануарларымен салыстырғанда T_3 43,5% және T_4 41,6%-ға төмендегенін көрсетті.

Түйін сөздер: гормондар, қан, егеуқұйрық, лимфа, лимфа ағысы, тироксин, трийодтиронин.

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ИЗМЕНЕНИЕ КОНЦЕНТРАЦИИ ТИРЕОИДНЫХ ГОРМОНОВ И ОСОБЕННОСТИ БИОХИМИЧЕСКИХ ПОКАЗЕТЕЛЕЙ ЛИМФЫ И КРОВИ У КРЫС ПРИ ГИПОТИРЕОЗЕ

Аннотация. В статье рассмотрено влияние препарата мерказолила на гормональный статус в лимфе и крови у крыс. В исследовании показано, что после ведения мерказолила и появление признаков гипотиреоза

у крыс наблюдалось снижение лимфотока из грудного лимфатического протока. Наблюдались изменения в гормональном и биохимическом составе лимфы. Наблюдались нарушения не только обмен веществ в лимфе, но и функциональной состоянии организма. При экспериментальном гипотиреозе у крыс наблюдались нарушения реологических и физико-химических показателей лимфы и крови: повышалось вязкость, снижалась свертываемость и число эритроцитов, увеличилась тромбоцитов и лейкоцитов в крови. У крыс 2-ой опытной группы с гипотиреозом приводило к возникновению снижению в содержании T_3, T_4 и повышение ТТГ в лимфе и крови, которые отражали возникновение у них состояния гипотиреоза. Эксперименты показали, что у крыс с гипотиреозом содержание T_3 снижалось на 45,7% и T_4 на 35,6% в лимфе, а крови T_3 на 43,5% и T_4 на 41,6% понижалось по сравнению с контрольной группой животных.

Ключевые слова: гормоны, кровь, крысы, лимфа, лимфоток, тироксин, трийодтиронин.

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MOLECULAR-GENETICAL INVESTIGATION OF OIL POLLUTION EFFECT ON AGUABIOTA OF KAZAKHSTANY ZONE OF CASPEAN SEA

Abstract. The Study of the impact of pollutants on the aquatic ecosystem for the purpose of biological indication of areas subject to anthropogenic pollution is extremely important for the Caspian sea. The article presents the restriction DNA analysis of fishes (*Neogobiusgorlap*) and polychaetes (*Nereisdiversicolor*) from three habitats of the coastal zone of the Caspian sea. Currently, the method of DNA analysis, polymerase chain reaction (PCR), developed in 1985, is widely used to assess genome stability [1]. The sensitivity of PCR makes it possible to successfully analyze even degraded DNA from Museum and archaeological samples. In this paper, we used the RAPD-PCR analysis method. In the total selected sample of 3 biotopes, 4 to 11 fragments were evaluated. Visual analysis of DNA fragments obtained for samples had a very high degree of differences (difference). All selected primers showed a different picture of inter-species differentiation. It is absolutely reliable markers (interbreeding specific DNA fragments) to identify phenotypic interbreeding, studied fishes. The most important primers for the samples were OPA-09 and OPA-10. During the analysis of polymerase chain reaction spectra with polymorphic and monomorphic DNA of fish caught in sites with different levels of pollution were found. The specific properties of the DNA spectra of the studied fish and polychaetes were determined. A unique DNA fragment was found in polychaetes from the polluted environment, which can be used as a DNA marker.

Key words: RAPD-PCR, DNA, electrophoresis, enzyme, restrictase, primers, polymerase chain reaction, polymorphism.

Introduction. Restriction DNA analysis of any organism is widely used in molecular genetic research and is one of the most important tools in the study of violations in the DNA molecule. DNA molecule cleavage products are analyzed using gel electrophoresis in agarose or polyacrylamide and the resulting pattern of DNA molecule fragment separation in the form of a specific, different for different enzymes, a set of bands and is the result of restriction analysis of the studied DNA. Many restrictase allow cleavage of DNA at more than 150 sites of recognition. Restriction analysis is carried out for a variety of DNA, ranging from small fragments of several tens of nucleotide pairs, and up to the entire eukaryotic genomes of more than 1 billion base pairs. It was found that in most cases the cleavage of chromosomal DNA by restriction endonucleases resulted in the formation of clearly visible fragments of a certain length, which allowed to judge about genome disorders of the studied animals, in particular polymorphism in individuals, which living at strong polluted area [1-3].

Actuality of the work. The study of the impact of pollutants on the ecosystem for the purpose of bioindication of technogenic contaminated areas is of extreme relevance for the Caspian sea area. Molecular genetic analysis of species of fish and the polychaetes from the coastal zone of the Caspian sea for assessing the impact of pollutants on the stability of the genome for the first time. Determination of the accumulation of polycyclic aromatic hydrocarbons (PAHs) and their metabolites as specific xenobiotics in

the area of oil production, processing and transportation is an extremely urgent problem of the Kazakhstan shelf of the Caspian sea.

Purpose of research. The purpose of this work is to conduct a reconnaissance study of the effect of oil pollution on the genome stability of test objects by molecular genetic analysis methods

Tasks:

- analysis by polymerase chain reaction of DNA spectra of fish and polychaetes selected from the coastal zone of the Caspian sea with different levels of pollution;
- quantitative and qualitative assessment of the isolated DNA from the body of test objects

Research materials and methods. The objects of research were selected goby fish (Neogobius-gorlap) and polychaetes (Nereisdiversicolor) caught in the coastal zone of the Caspian sea. Three points were selected for the analysis by polymerase chain reaction of fish and polychaete DNA spectra: Atyrau, of the river Ural delta and the coastal zone of the Caspian sea with different levels of pollution. These types of aquatic organisms fully meet the requirements for biomonitoring objects: widespread distribution in the reservoir, well-studied biology, do not make long migrations. The material for the study served as the fins of fish and the tissue of the polychaetes.

Quantitative and qualitative assessment of the isolated DNA was performed using spectrophotometric and electrophoretic analysis. For spectrophotometric analysis, adsorption of aqueous DNA solutions was measured at three wavelengths: 260 nm, 280 nm and 320 nm. The size of DNA molecules and the presence of RNA impurities were determined by electrophoresis in 0.7% agarose gel after staining with bromide ethidium. Visualization of DNA, RNA was carried out using a transilluminator in ultraviolet light.

Treatment of DNA by enzymes. Ferments produced of company “Fermentas”, Vilnius, Lithuania were used in the work." The cleavage of DNA was carried out in the manufacturer's recommended buffers for restriction of DNA at the optimum temperature for 16 hours. Restriction was carried out in 20 µl of the reaction mixture to which 5 µl of DNA and 0.5 µl of the enzyme were added. Activity of the enzymes used: AluI - 10 u / µl, EcoRI - 10 u/µl, BsuRI - 10 u / µl.

Electrophoresis. To identify fragments of length from 40 to 2000 p.b. used the electrophoresis in 8% polyacrylamide gel (on the track we applied 5 µl of the treated DNA) ("Sigma", USA). After the electrophoresis, DNA visualized with ethidium bromide and photographed under UV light.

DNA sample. For genomic DNA extraction a set of reagents QIA amp DNA Mini Kit (Qiagen, USA) was used. Quantitative and qualitative assessment of the isolated DNA was performed using DNA photometer (Biofotometer Plus, Eppendorf, Germany) and electrophoretic analysis. For photometric analysis, adsorption of aqueous DNA solutions was measured at three wavelengths: 260 nm, 280 nm and 320 nm. The size of DNA molecules, as well as the presence of RNA impurities, was determined by electrophoresis in 0.7% agarose gel after staining with bromide ethidium. Visualization of DNA, RNA was carried out using a transilluminator in ultraviolet light. PCR mixture with Taq polymerase, PCR Master Mix (Thermo Scientific, Lithuania) was used for DNA amplification of the studied and control samples. Amplification was performed automatically on the programmable master cycler nexus Gradient amplifier (Eppendorf, Germany) using the "hot start"method. The tubes with reagents were placed in an amplifier heated to a temperature of 93-94°C. This technique allows to avoid non-specific annealing of primers.

RAPD-PCR analysis. Polymerase chain reaction was carried out with ten members oligonucleotide primers synthesized in RSE "Institute of General Genetics and Cytology" (Kazakhstan) on a synthesizer ASM-800 of Bioset company, (Russia) (table). To minimize the error, the reaction was optimized by selecting the necessary concentrations of each component and preparing the total mixture for the entire sample.

The PCR reaction was carried out in the following temperature mode: initial denaturation at 94°C for 2 min, 40 cycles consisting of four stages, including 45 C at 92°C, 30 C at 37°C, 15 C at 45°C and 2 min at 72°C. the Reaction completed a 10-minute elongation stage at 72°C. Negative reaction control (contamination test) contained a reaction mixture without the addition of DNA.

Electrophoresis. Electrophoresis of amplified DNA fragments was carried out in 2% agarose gel in Tae_buffer (0.89 M Tris, 0.1 M sodium acetate, 0.05 M EDTA), pH 7.8 with bromide etide (5 µg/ml) and was photographed in transmitted UV light. The size of each fragment was determined by comparison with marker DNA fragments Gene Ruller 100 kbDNALadder (ThermoScientific, Lithuania).

Primers used for RAPD-PCR DNA analysis of fish and polychaetes

Primer	5′ → 3′
OPA-02	TGCCGAGCTG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT

Results. At the first stage of the research, the most informative primers for PCR analysis based on literature data were selected. Primers with the nucleotide sequence OPA-02 (TGCCGAGCTG); OPA-09 (GGGTAACGCC); OPA-10 (GTGATCGCAG) and OPA-11 (CAATCGCCGT). Then the variability of randomly amplified DNA was analyzed by RAPD-PCR method with selected standard 10-nucleotide primers.

During the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught in the vicinity with different levels of pollution, both polymorphic and monomorphic DNA were found. According to the literature, such monomorphic DNA should be distinguished from polymorphic and considered as a manifestation of genetic monomorphism at the DNA level [4, 5]. The features of the DNA spectra of the studied fish and polychaetes in two areas with different levels of pollution of the environment (biotopes) were revealed. A unique DNA fragment was found in individuals living in a more polluted environment. The results of studies of the influence of anthropogenic factors on the features of the DNA spectra of the fish studied, obtained by PCR analysis are shown in figure 1.

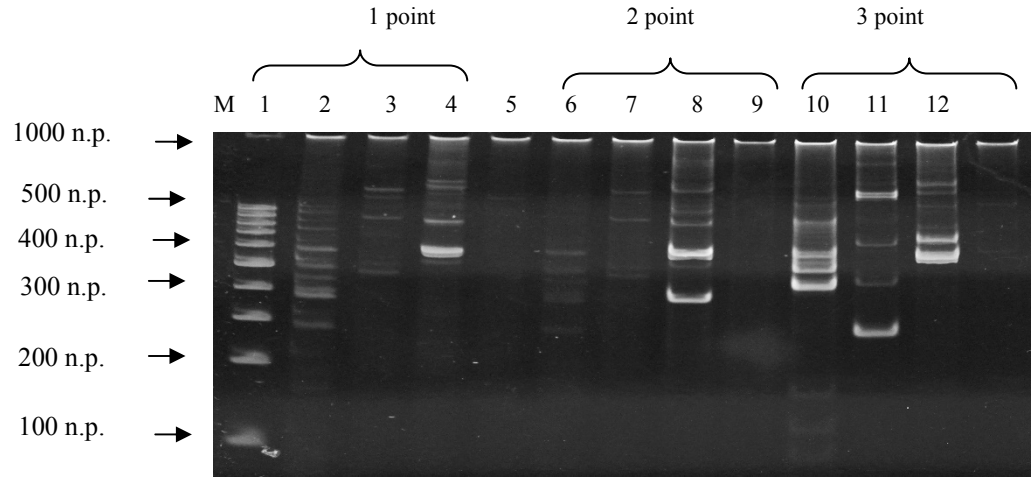

Figure 1 – RAPD - polymorphism of fish detected with primers OPA-02, OPA-09, OPA-10 and OPA-11.
1 point – the coastal area of the Caspian sea; 2 – point Delta of the Ural river; 3 point – coastal area of the city of Atyrau;
M – DeMarker (GeneRuller 100 kb DNA Ladder). 1 – the primer OPA-02; 2 – primer OPA-09; 3 – primer OPA-10;
4 – primer OPA-11; 5 – primer OPA-02; 6 – primer OPA-09; 7 – primer OPA-10; 8 – primer OPA-11;
9 – primer OPA-02; 10 – primer OPA-09; 11– primer OPA-10; 12 – primer OPA-11

The total sample of 3 selected points revealed from 4 to 11 fragments. In visual analysis of DNA fragments obtained for species samples had a very high degree of dissimilarity (differences). All selected primers showed different patterns of inter-species differentiation. But it is absolutely reliable markers (i.e. hybridizing DNA fragments) to identify phenotypic hybrids studied fish have been identified. For the studied samples, the most indicative primers were OPA-09 and OPA-10. Figure 1 shows distinct strips (DNA fragments) of different sizes.

Thus, during the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught from different degrees of contamination of biotopes, polymorphic DNA was found. In the studied fish samples monomorphic DNA was also revealed. Figure 1 shows that polymorphic DNA markers may be responsible for different characteristics of individuals.

The next stage of the study was the analysis of polychaetes caught in the same biotopes as fish. The same primers OPA-02, OPA-09, OPA-10 and OPA-11 were used. The studies have shown that the DNA spectrum of polychaetes contains from 6 to 9 randomly amplified DNA fragments length from 100 to 1200 nucleotide pairs (figure 2). It should be noted that the fragment on the 12 sample, which contains more than 1000 BP, is the most indicative and is of interest. DNA fragments found in other individuals can serve as markers of different processes and characteristics that distinguish these individuals.

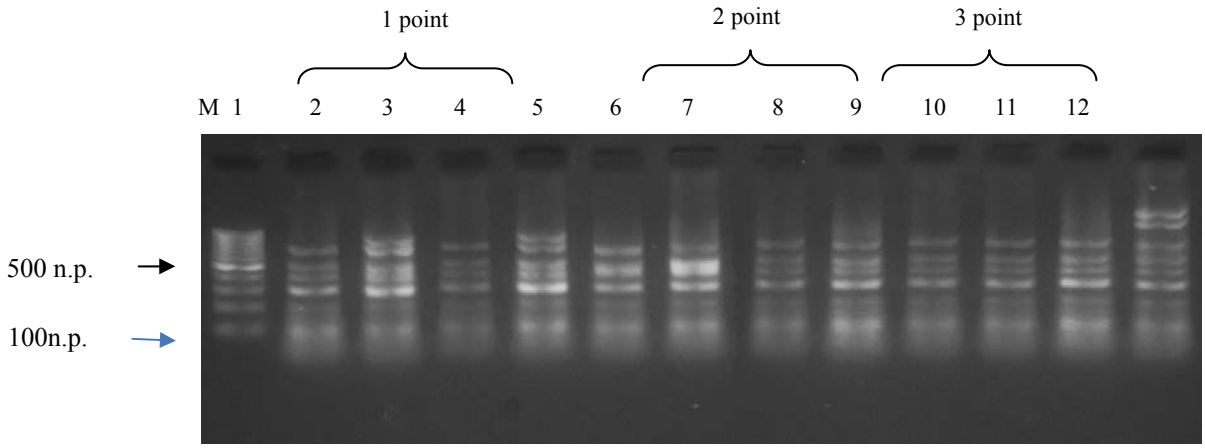

Figure 2 – RAPD-polymorphism of policeto identified by using primers OPA-02, OPA-09, OPA-10 and OPA-11.
1 point – the Delta of the Ural river; 2 point – intensely polluted coastal area of the Caspian sea;
3 point – low polluted coastal area of the Caspian sea; M – DeMarker (GeneRuller 100 kb DNA Ladder).
1 – the primer OPA-02; 2 – primer OPA-09; 3 – primer OPA-10; 4 – primer OPA-11; 5 – primer OPA-02; 6 – primer OPA-09;
7 – primer OPA-10; 8 – primer OPA-11; 9 – primer OPA-02; 10 – primer OPA-09; 11– primer OPA-10; 12 – primer OPA-11

During the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught from different degrees of contamination of biotopes, polymorphic DNA was found. Monomorphic DNA was also revealed in the studied fish samples. DNA spectrum of polychaetes contains from 6 to 9 randomly amplified DNA fragments with length from 100 to 1200 nucleotide pairs. Fragment 12 the sample contains more than 1000 BP is the most striking manifestation of polymorphism. In turn, DNA fragments detected in other individuals can serve as markers of different processes and characteristics that distinguish these individuals. A DNA fragment 300 BP long was detected, which was found in all polychaetes (100% frequency of occurrence). This fragment can be considered as a manifestation of genetic monomorphism at the DNA level. Later it can also be used as a molecular marker for specific species under study.

Discussion. From the literature data it follows that RAPD-markers are localized mainly in the non-coding region of DNA, because it it is the vast majority of the eukaryotic genome. The rate of mutation in non-coding DNA is about twice higher than in coding. In addition, RAPD markers are sometimes amplified from regions of repetitive DNA and thus may reflect high rates of their mutation, which can be induced by radiation or chemical factors of environmental pollution. On this basis, a method of Express identification of species belonging of living sturgeon tissue samples, products of their processing, including caviar, using a multiplex polymerase chain reaction (IGCC), performed in one tube, which allows simultaneously amplifying the unique sites of the mitochondrial genome of different species of sturgeon to obtain species-specific multi-dimensional amplicons, divided in electrophoresis into polyacrylamide or agarose gel [6,7].Thus, in the works of De Salle, 1996, Birstein, 1998, unlike the previously proposed test systems, the developed technique allows to discriminate unambiguously against Siberian and Russian sturgeons, including carriers of the mitochondrial "baerii-like" haplotype, which was previously impossible, and repeatedly led to an erroneous interpretation of the origin of caviar products exported from the Caspian region to the United States and other countries [8, 9]. Various methods of DNA polymorphism assessment depending on the goals are successfully used at different levels: from the individual and the population to the orders and the superorder categories. In many cases, variable DNA markers can detect genetic differences that are not recognized by protein electrophoresis.

RAPD is a DNA polymorphism. The RAPD (Random Amplified Polymorphic DNA) method is based on PCR-amplification of anonymous DNA sites using one, usually short (up to 10 nucleotide pairs, n) primer of arbitrary sequence, which induces in each reaction the synthesis of up to several tens of DNA fragments of random localization. The ability to investigate the polymorphism of the entire genome without prior knowledge of specific DNA sequences is a major advantage of RAPD analysis. The high level of polymorphism determined by the RAPD-method is quite informative in population studies, to identify hidden genetic polymorphism in lines and closely related species, as well as individual identification [9]. It should be noted that RAPD-markers are inherited as dominant features, which does not allow to distinguish the dominant homozygote from heterozygote and recessive homozygote from unamplified sequence. To overcome this disadvantage of the method, an original mathematical apparatus was developed, with which the frequency of the recessive conditional "zero-allele"is taken into account. The low reproducibility of RAPD-analysis due to non-specificity deserves attention from the negative sides primers inducing amplification of bands of different intensity, formation of heteroduplexes. The application of this technique is limited by the high quality requirements of the matrix and the potential non-homologation of RAPD bands (one fragment may contain different nucleotide sequences of the same size on an electrophoregram). Note that the development of the method emphasized the potential possibility of constructing with the help of RAPD-markers maps of adhesion to specific loci. However, the progress in this area is more than modest, besides such work requires quite expensive large-scale screening. For example, 310 random primers of different structures were used to identify DNA markers that diagnose the gender of Beluga, but none of the 4146 obtained bands was linked to the sex [10, 11]. RAPD-PCR is a PCR with random amplification polymorphic DNA is used to study the variability of similar genetic sequence of organisms, for example, different varieties of crops, breeds of dogs or closely-related microorganisms. This method usually uses one primer of small size (about 10 BP). This primer may be partially complementary to random DNA regions of the organisms under study. The literature data suggest that the increased content of PAH, heavy metals (copper, iron and manganese, etc.) may cause an increase in the level of DNA damage in fish erythrocytes [11-13]. Among environmental pollutants, PAHs and metals are of particular concern due to their potential toxic effects and bioaccumulative potential in aquatic ecosystems. Thus, the genotoxicity of copper and zinc is shown in a separate and combined action using a micro-nuclear test, copper causes an increase in the level of micronuclei in fish Oncorhynchusmykiss. The increase in the level of DNA damage in erythrocytes of fish Prochiloduslineatus by the action of aluminum is shown by the method of DNA-comets. A possible mechanism of iron toxicity may be the induction of DNA damage due to the generation of free oxygen radicals, which can cause site-specific oxidative damage [14, 15].

Conclusion. Thus, hydrobionts (fish and polychaetes) can be the most adequate object for the assessment of water pollutants, as they metabolize and accumulate in the body the chemical compounds contained in water. In our case, both fish and polychaetes react to toxic compounds similar to higher vertebrates. They can be used for screening chemical compounds, potentially mutagenic and carcinogenic to humans.

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КАСПИЙДІҢ ҚАЗАҚСТАНДЫҚ АУМАҒЫНЫҢ АКВАБИОТАСЫНА МҰНАЙМЕН ЛАСТАНУ ӘСЕРІН МОЛЕКУЛАЛЫҚ-ГЕНЕТИКАЛЫҚ ЗЕРТТЕУ

Аннотация. Антропогендік ластануға ұшыраған аймақты биологиялық индикациялау мақсатында ластаушы заттардың су экожүйесіне әсерін зерттеу Каспий теңізі үшін өте маңызды. Мақалада Каспий теңізінің жағалау аймағының үш ареалынан алынған балық (*Neogobiusgorlap*) пен полихеттің (*Nereis diversicolor*) ДНҚ-ның рестрикциялық талдауы келтірілген. Қазіргі таңда геном тұрақтылығын бағалау үшін 1985 жылы жасалған ДНҚ талдауының полимеразды тізбекті реакция (ПТР) әдісі қолданылады [1]. ПТР сезімталдығы мұражайлар мен археологиялық ескірген ДНҚ-ды да талдауға мүмкіндік береді. Бұл жұмыста RAPD-PCR талдау әдісін қолдандық. Үш биотоптан алынып іріктелген 4-тен 11-ге дейін фрагменттер бағаланды. Үлгілерден алынған ДНҚ фрагменттерін визуалды талдау кезінде үлкен айырмашылықтар байқалды. Алынған барлық праймерлер тұраралық дифференциацияның әртүрлі көріністерін көрсетті. Бұл зерттелген балықтардың фенотиптік гибридтерін біртектендіру үшін сенімді маркерлер. Зерттелген үлгілер үшін OPA-09 және OPA-10 біршама маңызды праймерлер болып табылды. Полимеразды тізбекті реакция талдауы кезінде ластану деңгейі әртүрлі жерлерден алынған балық ДНҚ-да полиморфты және мономорфты спектрлер байқалды. Зерттелген балық пен полихеттердің ДНҚ-спектрлерінде арнайы қасиеттері анықталды. Ластанған аймақтан алынған полихеттерде ерекше ДНҚ-фрагмент табылды, ол фрагментті ДНК маркер ретінде қолдануға болады.

Түйін сөздер: RAPD-PCR, ДНҚ, электрофорез, фермент, рестриктазалар, праймерлер, полимеразалы тізбекті реакция, полиморфизм.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЛИЯНИЯ НЕФТЯНОГО ЗАГРЯЗНЕНИЯ НА АКВАБИОТУ КАЗАХСТАНСКОЙ ЗОНЫ КАСПИЯ

Аннотация. Изучение влияния загрязняющих веществ на водную экосистему с целью биологической индикации территорий, подверженных антропогенному загрязнению крайне актуально для Каспийского моря. В статье представлен рестрикционный анализ ДНК рыб (*Neogobiusgorlap*) и полихет (*Nereisdiversicolor*) из трех ареалов прибрежной зоны Каспийского моря. В настоящее время для оценки стабильности генома широко используется метод анализа ДНК, полимеразной цепной реакции (ПЦР), разработанный в 1985 году [1]. Чувствительность ПЦР позволяет успешно анализировать даже деградированную ДНК из музейных и археологических образцов. В данной работе мы использовали метод анализа RAPD-PCR. В общей отобранной выборке из 3-х биотопов оценивали от 4 до 11 фрагментов. Визуальный анализ фрагментов ДНК, полученных для образцов, имел очень высокую степень различий (различие). Все выбранные праймеры показали различную картину межвидовой дифференциации. Это абсолютно достоверные маркеры (gibridspecies DNA fragments) для идентификации фенотипических гибридов, изученных рыб. Для исследуемых образцов были наиболее важными праймеры OPA-09 и OPA-10. Во время анализа полимеразной цепной реакции были обнаружены спектры с полиморфной и мономорфной ДНК рыб, пойманных в местах с различным уровнем загрязнения. Определены специфические свойства ДНК-спектров исследуемых рыб и полихет. Обнаружен уникальный ДНК-фрагмента у полихет из загрязненной среды обитания, который может быть использован в качестве ДНК маркера.

Ключевые слова: RAPD-PCR, ДНК, электрофорез, фермент, рестриктазы, праймеры, полимеразная цепная реакция, полиморфизм.

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ACTIVITY OF ANTIBIOTIC ROSEOFUNGIN AGAINST CLINICAL PATHOGENS OF VAGINAL CANDIDIASIS

Abstract. The polyene antibiotic roseofungin (registration number RK-LS-5No23224) is a new drug substance developed by the Kazakh scientists, on the basis of which the antifungal dosage form "Roseofungin-AS, ointment 2%" (registration number RK-LS-5№023225) was prepared for external application. In order to prepare a new dosage form for the treatment of vaginal candidiasis, activity of the antibiotic roseofungin was examined by the agar diffusion technique against 15 clinical fungal strains of the genus Candida: Candida albicans (9 strains), Candida krusei (2 strains), Candida tropicalis (2 strains), Candida glabrata (1 strain), Candida parapsilosis (1 strain). The antibiotic roseofungin exhibited high antifungal activity against clinical pathogens of vaginal candidiasis, the minimum inhibitory concentration varied in the range of 1.66-2.5 μg/mL. The highest activity of the antibiotic roseofungin was observed against clinical Candida albicans strains, MIC values were within the 1.66-2.0 μg/L range. Activity against Candida non-albicans strains was lower: MIC for Candida tropicalis was of 2.0 μg/mL, for Candida krusei, Candida glabrat, and Candida parapsilosis of 2.5 μg/mL. The presence of high activity in the antibiotic roseofungin against clinical pathogens of candidiasis indicates the possibility of developing new drugs on its basis that can improve the condition of patients in Kazakhstan and beyond.

Key words: antibiotic roseofungin, antifungal activity, minimum inhibitory concentration, vaginal candidiasis.

Introduction. Candidal vulvovaginitis relates to infectious diseases that cause inflammation of the vulvar and vaginal mucosa with yeast-like fungi belonging to the genus Candida [1]. Treatment and prevention of candidal vulvovaginitis is currently an urgent problem in gynecology. This disease occupies a leading position among vaginal infections, since almost every woman had at least one episode of the disease during her life, and more than 70% of women experienced relapses [2-5]. The major cause of candidal vulvovaginitis is an infection with yeast-like fungi of the genus Candida against the background of decreased immune status [6-7].

Currently, there are more than 170 species of Candida, of which no more than twenty species are registered as infection causative agents in humans [8]. Although Candida albicans is the most common cause of vulvovaginal candidiasis, the frequency of this disease caused by other Candida species, such as C. tropicalis, C. glabrata, and C. krusei, is increasing, especially in HIV-infected women [9]. The diversity of Candida spp. that are encountered in infections is expanding, and other species are emerging that have rarely been seen before [10, 11].

Polyenes, azoles, echinocandins, nucleoside analogs and allylamines are used with different effectiveness to treat infections caused by fungi of the genus Candida, depending on the type and location of infection and susceptibility of Candida species [12-15]. Candidal vulvovaginitis is usually treated with

local antimycotic drugs, which improve the microscopy parameters of the vaginal and cervical canal discharge (reduced leukocytosis, decrease in the number of coccal and bacterial flora, and disappearance of fungi) in 93.3% of patients [16]. The treatment of candidal vulvovaginitis varies significantly, and the most common drugs include azole agents [17], of which fluconazole is the most frequently prescribed antifungal agent. The widespread use of these drugs as preventive and therapeutic agents contributes to the emergence of resistant Candida strains, thereby causing serious problems in the successful treatment of vulvovaginitis [18]. There is a higher level of resistance, especially to azoles, in most species of Candida non-albicans, many of which have a natural resistance to antifungal agents [19, 20]. C. glabrata has the highest resistance to azoles among clinical isolates of Candida and exhibits a natural reduced susceptibility to this group of chemical compounds [21, 22].

In connection with the foregoing, the search for new antifungal drug substances and the development of effective drugs on their basis for treatment of candidal vulvovaginitis accessible to the general population is an urgent necessity.

The purpose of this study was to examine the activity of the antifungal polyene antibiotic roseofungin against the clinical pathogens of vaginal candidiasis and assess the possibility of its use for the development of a new dosage form.

Materials and Methods. The object of the study was a polyene antibiotic roseofungin, registered in the Republic of Kazakhstan under the number RK-LS-5N023224 [23].

Antifungal activity of the antibiotic roseofungin was examined against 15 clinical fungal Candida strains: 9 strains of Candida albicans (Berkhout, 1923) (strains R-11, R-25, R-28, R-29, R-33, R-41, R 44, R-46, R-50), 2 strains of Candida krusei (Berkhout, 1923) (R-19, R-47), 2 strains of Candida tropicalis (Berkhout, 1923) (R-5 .R-39), 1 strain of Candida glabrata (SA Mey. & Yarrow, 1978) (R-17), 1 strain of Candida parapsilosis (Langeron & Talice 1932) (R-14). Fungal isolates belonging to the genus *Candida* were obtained from patients living in the Almaty region in the microbiological laboratory at the Regional Dermatovenerologic Dispensary under the Ministry of Health of the Republic of Kazakhstan and bacteriological laboratory at the Central Clinical Hospital. Identification of clinical fungal strains of the genus Candida was carried out using the BIO MERIEUX automated MINI API bacteriological analyzer. The bacteriological analyzer has an expert system for interpreting the results obtained on antibiotic resistance and species identification of microorganisms based on international standards (NCCLS).

Antifungal activity of the antibiotic roseofungin was studied by the agar diffusion method [24]. The nutrient agar medium F was used to determine the biological activity.

The composition of medium F (g /L): peptone - 9.4; yeast extract - 4.7; beef extract - 2.4; sodium chloride - 30.0; glucose monohydrate - 10.0; agar - 23.5; distilled water - up to 1000 ml, pH after sterilization - 7.0 ± 0.1.

Clinical fungal strains of the genus Candida were grown in Petri dishes on the surface of medium F for 24 hours at a temperature of 30 to 37 ^{0}C; typical colonies were selected, reinoculated onto agar slants of the same composition and grown under the conditions described above. The grown culture was washed from the slanted nutrient medium F with 10 ml of a sterile solution containing 9 g/L of sodium chloride. A working suspension was prepared from the resulting microbial suspension of such a density, which when diluted with a sterile solution containing 9 g/L of sodium chloride corresponded to the L.A. Tarasevich SISC turbidity standard (10 units).

The inoculation dose of the test microorganism was 1.0 ml of a working suspension per 100.0 ml of medium F. The inoculation was carried out at a temperature of 48-500 °C. 15.0 ml of the inoculated medium F was poured into each Petri dish, so that a uniform layer with a thickness of 2 mm to 5 mm was formed therein. Filling of the inoculated medium F into Petri dishes was carried out on a horizontally flat surface.

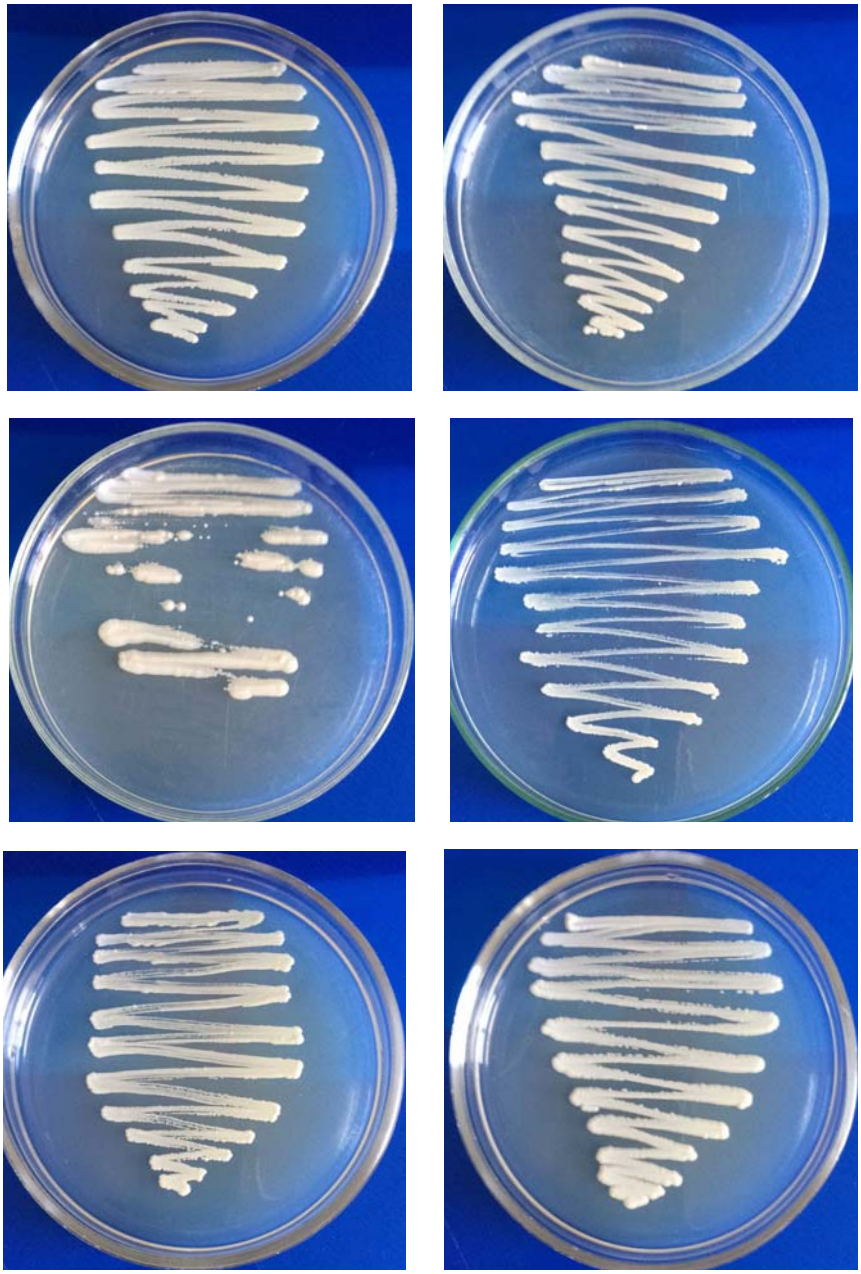
10.0 mg of roseofungin powder was placed in a 10.0 ml volumetric flask and dissolved in 5.0 ml of dimethylsulfoxide. The volume of the solution was adjusted to the mark with the same solvent and stirred. Further dilution of the stock solution was made with a phosphate buffer solution (pH 6.0) to the desired concentration.

To prepare a phosphate buffer, 50.0 ml of a 0.2 M potassium dihydrogen phosphate solution was transferred to a 200 ml volumetric flask, 5.7 ml of 0.2 M sodium hydroxide was further added and mixed. The resulting solution was then made up to the mark with distilled water.

Solutions with the calculated concentration were added to the wells of 7 mm in diameter prepared in the inoculated nutrient medium F. All wells were filled with equal volumes of solutions. The dishes were incubated at 37^0C for 18-24 hours. To reduce the influence of the time difference between addition of solutions and to refine a regression line, preliminary diffusion was used at a temperature of about 4 °C with 4-hour duration.

All studies were carried out in three to five replicates. The standard methods for finding mean values and their mean errors were used for mathematical processing of the results [25].

Results and discussion. As a result of the studies, activity of the antibiotic roseofungin was determined against 15 clinical fungal strains of the genus Candida: Candida albicans (9 strains), Candida krusei (2 strains), Candida tropicalis (2 strains), Candida glabrata (1 strain), and Candida parapsilosis (1 strain). Figure shows the growth of clinical pathogens of vaginal candidiasis on meat peptone agar.



The growth of clinical pathogens of vaginal candidiasis on meat peptone agar:
1 - *C. albicans* R-25; 2 - *C. albicans* R-33; 3 - *C. krusei* R-19; 4 - *C. glabrata* R-17;
5 - *C. tropicalis* R-39; 6 - *C.* parapsilosis R-14

Activity of antibiotic roseofungin against clinical pathogens of vaginal candidiasis

SN	Strain No.	Species of test microorganism	Minimum inhibitory concentration, μg/mL
1	R-11	Candida albicans	2,0
2	R-25	Candida albicans	2,0
3	R-28	Candida albicans	1,66
4	R-29	Candida albicans	1,66
5	R-33	Candida albicans	2,0
6	R-41	Candida albicans	1,66
7	R-44	Candida albicans	2,0
8	R-46	Candida albicans	1,66
9	R-50	Candida albicans	1,66
10	R-19	Candida krusei	2,5
11	R-47	Candida krusei	2,5
12	R-5	Candida tropicalis	2,0
13	R-39	Candida tropicalis	2,0
14	R-17	Candida glabrata	2,5
15	R-14	Candida parapsilosis	2,5

Results of determination of roseofungin activity are given in table.

The minimum inhibitory concentration (MIC, μg/mL) for the examined strains of the causative agents of vaginal candidiasis varied between 1.66-2.5 μg/mL. The highest activity of the antibiotic roseofungin has been observed against clinical Candida albicans strains, MIC values were within 1.66-2.0 μg/mL. Activity against Candida non-albicans strains was lower: MIC for Candida tropicalis was of 2.0 μg/mL, for Candida krusei, Candida glabrata, and Candida parapsilosis of 2.5 μg/mL. Our results correspond to the literature data according to which the causative agents of vulvovaginal candidiasis Candida non-albicans have less natural susceptibility to antifungal drug compounds [19, 20].

High antifungal activity of the antibiotic roseofungin has been thereby established against clinical pathogens of candidiasis (strains of Candida albicans, Candida krusei, Candida tropicalis, Candida glabrata, and Candida parapsilosis); the minimum inhibitory concentration was in the range of 1.66-2.5 μg/mL. The presence of high activity in the antibiotic roseofungin against clinical pathogens of candidiasis demonstrates the necessity for developing new drugs on its basis, including those for the treatment of candidal vulvovaginitis, which can improve the condition of patients in Kazakhstan and beyond.

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РОЗЕОФУНГИН АНТИБИОТИГІНІҢ ВАГИНАЛЬДІ КАНДИДОЗДЫҢ КЛИНИКАЛЫҚ ҚОЗДЫРҒЫШТАРЫНА ҚАТЫСТЫ БЕЛСЕНДІЛІГІ

Аннотация. Розеофунгин полиенді антибиотигі (тіркеу нөмірі ҚР-ДЗ-5№023224) Қазақстан ғалымдары әзірлеген жаңа дәрілік субстанция болып табылады, оның негізінде сыртқа қолдануға арналған «Розеофунгин-АС, 2% жақпамайы» (тіркеу нөмірі ҚР-ДЗ-5№023225) зеңге қарсы препаратының дәрілік үлгісі дайындалды. Вагинальді кандидозды емдеу ішін жаңа дәрілік үлгіні дайындау мақсатында Candida туысына жататын: Candida albicans (9 штамм), Candida krusei (2 штамм), Candida tropicalis (2 штамм), Candida glabrata (1 штамм), Candida parapsilosis (1 штамм) саңырауқұлақтарының 15 клиникалық штамдарына қатысты агарға диффузиялау әдісі арқылы розеофунгин антибиотигінің белсенділігіне зерттеу жүргізілді. Розеофунгин антибиотигі вагинальді кандидоздың клиникалық қоздырғыштарына қатысты жоғары антифунгальді белсенділікті көрсетті, минимальді тежейтін концентрациясы 1,66-2,5 мкг/мл дейін өзгерді. Розеофунгин антибио-

тигі Candida albicans түрінің клиникалық штамдарына қатысты ең жоғары белсенділікке ие болды, МТК – 1,66-2,0 мкг/мл. Candida non-albicans штамдарына қатысты белсенділігі төмен болды: МТК Candida tropicalis 2,0 мкг/мл, Candida krusei, Candida glabrata және Candida parapsilosis – 2,5 мкг/мл құрады. Розеофунгин антибиотигінің кандидоздың клиникалық қоздырғыштарына қатысты белсенділігінің болуы оның негізінде Қазақстан және одан тысқары жерлердегі науқастардың жағдайын жақсартатын жаңа дәрілік препараттарды әзірлеп шығарудың мүмкіндігін көрсетеді.

Түйін сөздер: розеофунгин антибиотигі, антифунгальді белсенділігі, минимальді тежейтін концентрация, вагинальді кандидоз қоздырғыштары.

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АКТИВНОСТЬ АНТИБИОТИКА РОЗЕОФУНГИНА В ОТНОШЕНИИ КЛИНИЧЕСКИХ ВОЗБУДИТЕЛЕЙ ВАГИНАЛЬНОГО КАНДИДОЗА

Аннотация. Полиеновый антибиотик розеофунгин (регистрационный номер РК-ЛС-5№023224) является новой лекарственной субстанцией, разработанной учеными Казахстана, на основе которой создана лекарственная форма противогрибкового препарата «Розеофунгин-АС, мазь 2%» для наружного применения (регистрационный номер РК-ЛС-5№023225). С целью создания новой лекарственной формы для лечения вагинального кандидоза изучена активность антибиотика розеофунгина методом диффузии в агар в отношении 15 клинических штаммов грибов рода *Candida: Candida albicans* (9 штаммов), *Candida krusei* (2 штамма), *Candida tropicalis* (2 штамма), *Candida glabrata* (1 штамм), *Candida parapsilosis* (1 штамм). Антибиотик розеофунгин проявил высокую антифунгальную активность в отношении клинических возбудителей вагинального кандидоза, минимальная подавляющая концентрация изменялась в пределах 1,66-2,5 мкг/мл. Наиболее высокой активностью антибиотик розеофунгин обладал в отношении клинических штаммов вида *Candida albicans*, МПК – 1,66-2,0 мкг/мл. Активность в отношении штаммов Candida non-albicans была ниже: МПК для *Candida tropicalis* составила 2,0 мкг/мл, для *Candida krusei, Candida glabrata* и *Candida parapsilosis* – 2,5 мкг/мл. Наличие высокой активности у антибиотика розеофунгина в отношении клинических возбудителей кандидоза свидетельствует о возможности разработки на его основе новых лекарственных препаратов, способных улучшить состояние больных в Казахстане и за его пределами.

Ключевые слова: антибиотик розеофунгин, антифунгальная активность, минимальная подавляющая концентрация, возбудители вагинального кандидоза.

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ANALYSIS OF CANDIDATE POLYMORPHISMS AT EPILEPSY PATIENTS WITHOUT MECHANICAL DISTURBANCES

Abstract. The article presents the results of a molecular-genetics research on patients with diagnosed epilepsy without mechanical disturbance. The aim of this research was the analysis of candidate gene polymorphisms in development of different forms of epilepsy excepting mechanical reasons. 78 patients of V.M. Savinov SVS clinic with different forms of epilepsy were selected for the molecular-genetic analysis. Genotyping on candidate polimorphisms of gene coding the methyl-CpG-binding protein 2 (*MECP2,* 3 polymorphisms), the genes of sodium (*SCN1A,* 4 polymorphisms) and potassium (*KCNT1,* 2 polymorphisms) channels was performed by a site-specific PCR-RFLP method. Molecular genetic analysis revealed the presence of normal functioning alleles for 3 investigated candidate polymorphisms (p.Thr158Met, p.Thr197Met, p.Arg306Ter) of 3^{d} exon of *MECP2* gene at all epilepsy patients. However, 1 case (patient suffering from Dravet syndrome) of *de novo* mutation was defined for sodium channel gene (*SCN1A* p.Ala1783Thr) and 3 cases (2 patients suffering from temporal epilepsy and 1 patient with residual encephalopathy) of new mutations in gene responsible for potassium channel (*KCNT1* p.Ala934Thr). To determine the inherited *SCN1A* and *KCNT1* mutations, the molecular-genetics analysis was conducted for close relatives of patients. As a result, we conclude that, candidate polymorphisms of SCN1A p.Ala1783Thr and *KCNT1* p.Ala934Thr, disrupting the ion channels normal functioning, can be involved in development of non-mechanical forms of epilepsy.

Keywords: epilepsy, gene polymorphism, mutation, *MECP2, SCN1A, KCNT1*.

Introduction. Epilepsy is one of the most common and heterogeneous neurological diseases with chronic appearance characterizing by recurrent, unprovoked seizures.

Non-mechanical forms of epilepsy are diagnosed if patient had two unprovoked seizures that were not caused by a known and reversible disease, such as seizures after a brain concussion on the fever background, alcohol withdrawal, or an excessively low level of sugar in the blood.

According to the Code of the Republic of Kazakhstan "About people health and health care system" (Article 7, item 89), dated by September 18, 2009, epilepsy refers to socially significant diseases. This disease is one of the most common serious neurological disorders that affects about 1% of people worldwide (50 millions) [1]. In Kazakhstan, more than 45,000 people suffer from epilepsy, 40% of them are children, adolescents and young people, 38% of patients become disabled, and their life quality reduces by 85% on average [2].

The greatest number of children suffering from epilepsy is registered at the age of 4 to 7 years (31.75%), which is probably due to better diagnosis and clinical manifestations in this age group. The next age range for epilepsy frequency is the age from 1 year to 3 years - 27.48%. The frequency of epilepsy in age from 8 to 14 years is 20.78%. And the lowest frequency of epilepsy patient (19.97%) is registered for children before 1 year [2]. In recent decades, in view of untimely diagnosis and wrong treatment, infant mortality from epilepsy remains at a high level. For an example, mortality rate from sudden unexpected

death in epilepsy (SUDEP) reaches 8.2-10 per 1000 individuals. The main perspectives in reducing such high rates of morbidity and mortality associate with the improvement of diagnostic methods that have scientifically based effectiveness.

Both hereditary and environmentally acquired factors are involved in epilepsy pathogenesis. The molecular mechanisms underlying the various epileptic seizures have been intensively studied for more than two decades. The genetic impact plays a big role in the etiology of epilepsy idiopathic forms. Approximately 20-30% of epilepsy cases by acquired conditions, such as stroke, tumor, or head trauma. However, recent data indicate that remaining 70-80% of cases development due to genetic background [3].

Most of the epilepsy hereditary forms with established gene mutations are caused by the damage of ion channels that ensure the neuronal membrane polarization. Such epilepsy forms are referred to the channelopathy group. First of all, they include the genes of sodium, potassium, calcium and chloride channels (SCN1A, SCN2A, CACNA1A, KCNJ10, KCNQ2) [4-9].

Mutations in the sodium channel genes - SCN1A and SCN2A, were described for 70% of children suffering from Dravet syndrome, most of the mutations had spontaneous nature [4, 6]. SCN1A mutations can cause the development of severe myoclonic epilepsy of infancy (SMEI), which related to symptomatic forms [10]. The dominant mutations in the KCNT1,sodium potassium channel gene intensely expressed in the brain, cause autosomal dominant night frontal lobe epilepsy (ADNFLE) and malignant migrating partial seizures of infancy (MMPSI) [7]. Mutations in this gene increase the membrane permeability that leads to unregulated excitation of neurons in the brain.

The genes responsible for DNA methylation are also involved in the pathogenesis of epilepsy and the development of mental retardation. Using a systematic approach to gene screening, Zogby and coauthors [11] identified mutations in the gene for methyl-CpG-binding protein 2 (MECP2), which were responcible for development of some cases of Rett's syndrome. MeCP2 is a chromosome-binding protein that selectively binds 5-methylcytosine residues in symmetrically located CpG-dinucleotides [12].

The list of candidate genes for epilepsy is not restricted by mentioned variants. The spectrum of epilepsy genes acquires specificity, largely due to the results of large-scale genome-wide studies (GWASs) [13]. Leading manufacturers develop genetic panels and biochips for epilepsy diagnosis based on massive parallel sequencing (NGS) and full exome sequencing. But, despite the obvious progress in epilepsy genetics a lot remains to be understood.

The aim of this research was the analysis of candidate gene polymorphisms of *MECP2* (c.473C>T - p.Thr158Met; c.590C>T - p.Thr197Met; c.916C>T - p.Arg306Ter)*, SCN1A* (c.5492T>C - p.Phe1831Ser; c.5020G>C - p.Gly1674Arg; c.5347G>A - p.Ala1783Thr; c.4969C>G - p.Pro1657Ala)*, KCNT1* (c.2782C>T - p.Arg928Cys and c.2800G>A - p.Ala934Thr) genes in development of different forms of epilepsy excepting mechanical reasons.

The identification the association of key genes mutations or polymorphisms with epilepsy symptoms will help to develop the successful early diagnosis and therapy tools.

Materials and methods.

Study objects. For molecular genetic analysis, we collected the EDTA-treated peripheral blood samples presenting 78 patients of SVS clinic named after V.M. Saminov, who were epilepsy diagnosed in accordance with the criteria of the ILAE Commission for Classification and Terminology (1989). The excepting criterium was mechanical reason of epilepsy development. Before collection of blood samples we asked people the voluntary consent to participate in genetic research. A detailed questioning was done after obtaining the signed voluntary informed consents. The study was approved by the Ethics Committee of the Kazakh-Russian Medical University.

Isolation of genomic DNA. DNA was isolated from frozen (-20°C) peripheral blood samples containing EDTA as an anticoagulant. Isolation was carried out using the GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) in accordance with protocol recommended by the manufacturer. Quantitative and qualitative evaluation of DNA preparations was carried out by spectrophotometric and electrophoretic analysis. After isolation, the DNA samples were stored at -20°C.

Site specific PCR for critical regions of MECP2, SCN1A, and KCNT1 genes. PCR was carried out with specific primers, the design of which was selected using the online program - PrimerQuest Tool, the PCR conditions were optimized for each primer (table 1).

Table 1 – Sequence of primers for site specific PCR

Gene, location	Primers, 5'→ 3'	PCR conditions
SCN1A, 26 exon	F-CCCGACTGTGACCCTAATAAAG	94°C - 4 min. 94°C - 40 sec. 55°C - 30 sec. 72°C - 40 sec. 72 °C - 8 min. 35 cycles
	R-GTTTGGTTGTGGCAGATTGAG	
	F -GTTTCTTGCCGAGCTGATAGA	
	R -CGATCCCAACTTCCCTCTTAAC	
	F-ACCGGATCCACTGTCTTGATA,	
	R-CGTCTGTAAGCACGCTGAATAA.	
KCNT1, 24 exon	F-CACCCTGAGACCTCCTACAA	95°C - 3 min. 95°C - 30 sec. 58°C - 30 sec. 72°C - 30 sec. 72 °C - 10 min. 35 cycles
	R-CCCTTTCTCCCACTCTTTCTG	
MECP2, 3 exon	F-ATGGGAGTTGATTGCGTACTT	95°C - 3 min. 95°C - 30 sec. 58°C - 30 sec. 72°C - 30 sec. 72 °C - 10 min. 35 cycles
	R-CAGTCCTTTCCCGCTCTTC	

The PCR was carried out in 0.2 ml microtube on the Thermocycler Eppendorf ™ Mastercycler ™ Nexus Thermal Cycler with a set of programs that determine the temperature of the PCR. To evaluate the amount and specificity of the resulting PCR products, the amplified DNA fragments length were checked by electrophoresis in 1.5% agarose gel. The correspondence of the molecular weights of the amplicons of each gene was assessed using the DNA Ladder GeneRuler 100 bp marker (ThermoFisher Scientific, USA). A samples which not contained DNAs were used as a negative control of PCR.

RFLP – analysis of candidate mutations/polymorphisms. For each polymorphic site, a restriction enzyme was selected using the online software WatCut. Table 2 indicates the restrictases, the restriction products for each selected candidate polymorphism.

Table 2 – RFLP identification of candidate polymorphisms

Gene, location	Mutation/Polymorphism	Restriction endonuclease	DNA fragments length and corresponding genotype
SCN1A, 26 exon	c.5492T>C (p.Phe1831Ser)	PstI	TT - 321bp; CC -282 и 39 bp; TC - 321, 282 и 39 bp
	c.5020G>C (p.Gly1674Arg)	HaeIII	GG - 90, 83 и 75 bp; CC - 173 и 75 bp; GC - 173, 90, 83 и 75 bp
	c.4969C>T (p.Pro1657Ala)	BamHI.	CC - 140 и 108 bp;TT - 248 bp;CT - 248, 140 и 108 bp.
	c.5347G>A (p.Ala1783Thr)	Acc II	GG - 188 и 133bp; AA - 321 bp; GA - 321, 188 и 133bp
KCNT1, 24 exon	c.2782C>T (p.Arg928Cys)	HpyF10VI	CC - 116 и 117 bp; 233 bp; 233, 117 и 116 bp
	c.2800G>A (p.Ala934Thr)	Acc II	GG - 128 и 105 bp; AA - 233 bp; GA - 233, 128 и 105 bp
MECP2, 3 exon	c.473C>T (p.Thr158Met)	TaaI	CC - 424, 75, 70 и 36 bp; TT - 493, 75 и 37 bp CT - 493, 424, 75, 70, 37 и 36 bp
	c.590 C>T (p.Thr197Met)	Hin1II	CC - 334 и 207 bp;TT - 207, 187 и 141 bp;CT - 334, 207, 187 и 141 bp.
	c.916 C>T (p.Arg306Ter)	HhaI.	CC - 513 и 91 bp;TT - 604 bp;CT - 604, 513 и 91 bp.

The PCR products were subjected to restriction by endonuclease (all used restrictases were taken from ThermoFisher Scientific, USA) digestion at an incubation temperature of 37°C for 5 hours. Then the RFLP-products were analyzed in an 8% polyacrylamide gel (PAGE) with staining by ethidium bromide. Evaluation of the fragments obtained was carried out using a DNA Ladder GeneRuler 100 bp marker (Thermo Fisher Scientific, USA) and a gel-documenting system QuantumSTS (VilberLourmat France).

Results and their discussion. The demographic and clinical data of 78 epilepsy patients, who voluntary agreed to participate in the study, are summarized in Table 3. Clinical examination of studied cohort with different forms of epilepsy revealed that the neurological status of all 78 patients was without meningeal signs and cerebral symptoms. A decrease of psycho-emotional memory, attention and emotional lability were registered at 1 patient.

Table 3 – Clinical characteristics of patients

Total number of patients	78
Age	
Average age	34±11,15
Year of birth	1954-2015 yy
Sex	
Males	44
Females	34
EEG data	
Pathological variant of EEG	72
Flat-EEG	6
Seizures type	
Generalized seizures	45
Partial seizures	33

According to the clinical diagnosis, 11 individuals were suffering from temporal epilepsy, 10 individuals had epilepsy with tonic clonic seizures, 4 individuals - juvenile and child absent epilepsy, 6 individuals - primarily generalized epilepsy, 5 individuals - secondary generalized epilepsy, 6 - residual encephalopathy, 6 - juvenile myoclonic epilepsy, 4 persons had autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), 10 persons - idiopathic epilepsy, 3 - frontal epilepsy, 1 patient was diagnosed by Vest syndrome, and 12 persons had symptomatic epilepsy.

The DNA samples were extracted, characterized and genotyped using PCR-RFLP analysis for screening the fallowing candidate polymorphisms: *MECP2* (c.473C>T - p.Thr158Met; c.590C>T - p.Thr197Met; c.916C>T - p.Arg306Ter), *SCN1A* (c.5492T>C - p.Phe1831Ser; c.5020G>C - p.Gly1674Arg; c.5347G>A - p.Ala1783Thr; c.4969C>G - p.Pro1657Ala), *KCNT1* (c.2782C>T - p.Arg928Cys and c.2800G>A - p.Ala934Thr).

The molecular genetic analysis of 3 exon of *MECP2* gene coding the methyl-CpG-binding protein 2 did not revealed the mutant alleles in critical sites (c.473C>T, c.590C>T, c.916C>T) in all studied DNA samples. The figure 1 demonstrates the normal alleles by 3 investigated sites of 3 exon *MECP2*.

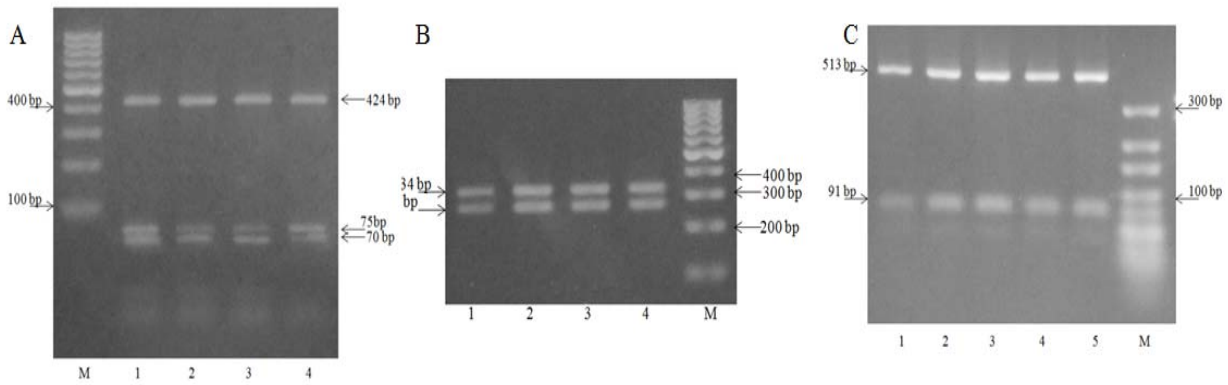

Figure 1 – PCR-RFPL analysis for mutation of *MECP2*gene:
M - 100 bp DNA ladder. A - RFPL analysis for mutation p.Thr158Met,
B - RFPL analysis for mutation p.Thr197Met, C - RFPL analysis for mutation p.Arg306Ter

As known from literature data, the mutations of the X chromosome gene-*MECP2*, which encodes the methyl-CpG-binding protein 2, can result in development of Rett syndrome, which clinically characterized by epilepsy and mental retardation [26]. Mutations in the *MECP2* gene exclusively affects the females because for the males they associate with lethal effect. But recently, there has been published evidence of detection of *MECP2* mutations at males, including the epilepsy patients who suddenly died from unknown reasons [26].

Genotyping of 4 candidate *SCN1A* gene polymorphisms of 26 exon (c.5492T>C - p.Phe1831Ser; c.5020G>C - p.Gly1674Arg; c.5347G>A - p.Ala1783Thr; c.4969C>G - p.Pro1657Ala) revealed only 1 case of mutation (c.5347G> A) in heterozygouse state at patient (2.5 years old) with the Dravet syndrome (figure 2). The analyses of close relatives (father, mother, 3 month-aged sister) of this child did not revealed the mutant alleles. We conclude that the detected variant represent *de novo* mutation of sodium channel gene *SCN1A*.

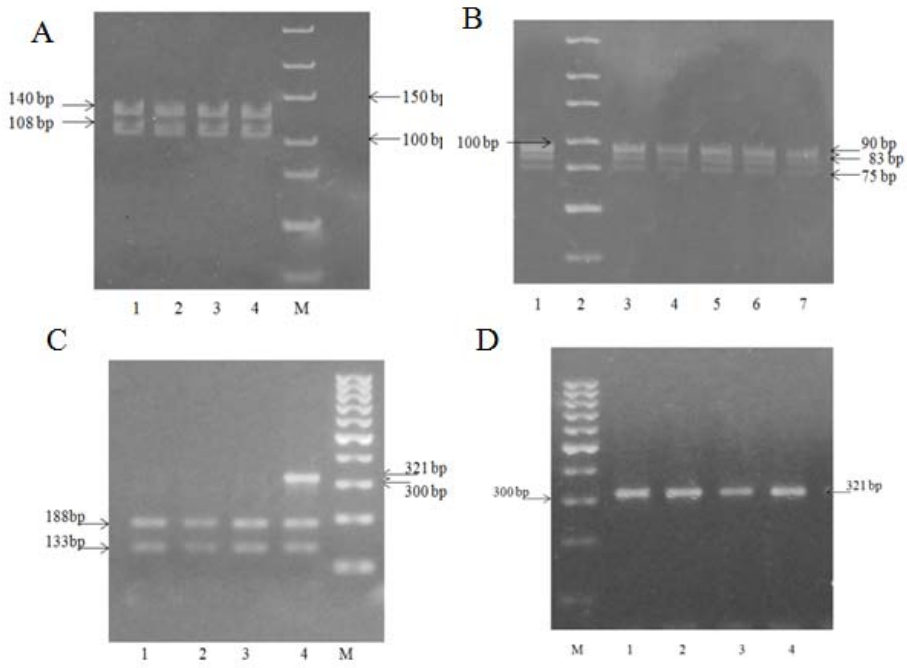

Figure 2 – PCR-RFPL analysis for mutation of *SCN1A* gene:
M - DNA ladder. A - RFPL analysis for mutation p.Pro1657Ala, B - RFPL analysis for mutation p.Gly1674Arg,
C - RFPL analysis for mutation p.Ala1783Thr (Lanes 1–3 normal samples, lane 4 – sample with a heterozygous mutation),
D - RFPL analysis for mutation p.Phe1831Ser

The clinical data indicate that convulsions at this patient first time detected at the age of 3 months and were repeated 2 times per month with different semiotics. The febrile convulsions were not detected. *De novo* mutation of the gene *SCN1A* (p.Ala1783Thr), which led to a disruption of the sodium channel, is evidence of the Dravet syndrome. The Dravet syndrome is a cryptogenic epileptic syndrome that has features of both focal and generalized seizures and in which convulsions usually do not respond to treatment and are associated with mental disability.

The treatment of the patient by Valproate led to only slight improvement. The replacement of therapy by Topiramate led to decreasing the frequency of seizures, but not significantly. The treatment by Oksarbazepine was unsuccessful because of worsening of the patient's condition. Based on this experience, the patient was again appointed to Topiramate in combination with Valproate and Dexamethasone. But despite this, seizures arose daily with myoclonus of the eyes and shoulders [13-15].

We also conducted the molecular genetic analysis of 2 critical for epilepsy candidate sites (c.2782C>T - p.Arg928Cys and c.2800G>A - p.Ala934Thr) of potassium channel *KCNT1* gene, exon 24. The result of PCR-RFLP analysis of *KCNT1* gene is presented on figure 3. The 3 cases of mutant variants (c.2800G>A) were detected regarding the polymorphism of 934 codon. All mutations were in heterozy-

gous state. 2 (1972 and 1988 yy. of birth) of 3 patients, who carrying the mutation, were suffered from temporal epilepsy. They had partial and generalized attacks of psychomotor automatism. The seizures frequency was 1-2 times per month. The another patient (born in 1987), carrying the mutation of 934 codon of *KCNT1* gene in heterozygous state, was diagnosed by residual encephalopathy. He had primary generalized convulsions. The frequency of seizures was 1 time per 1-2 months.

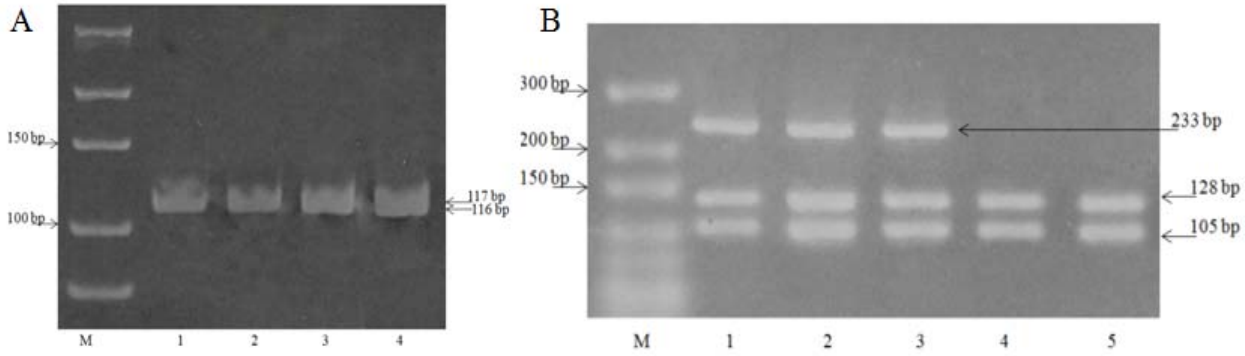

Figure 3 – PCR-RFPL analysis for mutation of *KCNT1* gene:
M - 25 bp DNA ladder. A - RFPL analysis for mutation p.Arg928Cys (Lanes 1-4 – normal samples),
B - RFPL analysis for mutation Ala934Thr (Lanes 1-3 – sample with a heterozygous mutation, lanes 4 and 5 normal samples)

Molecular genetic analysis of close relatives of these 3 patients (mothers, farthers, sisters, brothers) did not revealed the mutant variants of 934 codon of *KCNT1* gene. That confirmed the *de novo* occurrence of *KCNT1* p.Thr934 allele in all 3 families.

Mutations in the potassium channel gene of *KCNT1* were detected at various epileptic syndromes: ADNFLE [16], epilepsy of infancy with migratory focal seizures (EIMFS), previously known as malignant migratory partial seizures of infancy (MMPSI), or recently, as malignant migratory fetal seizures of infancy (MMFSI) [17], early onset epileptic encephalopathy (EOEE) [18], and Okhtahara Syndrome (OS) [19]. Patients with mutation in the *KCNT1* gene were characterized by high level of severe psychic inferiors and mental retardation.

Literature data [20] shows that the indicated mutation of 934 codon of the *KCNT1* potassium channel gene should be specific for malignantly migrating partial infantile seizures (MMPSI). But we have identified the *de novo* mutation p. Thr934 *KCNT1* in patients suffering from temporal epilepsy (TLE). And we did not detected this mutation at 4 studied ADNFLE patients.

Thus, we conclude that mutations in the *KCNT1* potassium channel gene can cause not only an autosomal dominant nocturnal epithelial frontal lobe (ADNFLE), but other forms of epilepsy.

So, the primary analysis of the range of candidate polymorphisms of key epilepsy genes allows to conclude that candidate polymorphisms of *SCN1A* p.Ala1783Thr and *KCNT1* p.Ala934Thr, disrupting the ion channels normal functioning, can be involved in development of non-mechanical forms of epilepsy. Mutations of *MECP2* are rare, and, possibly, to detect them we need to increase the case number and examined the lethal cases.

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МЕХАНИКАЛЫҚ СИПАТТАҒЫ БҰЗЫЛЫССЫЗ ЭПИЛЕПСИЯМЕН АУЫРАТЫН НАУҚАСТАРДА КАНДИДАТ ПОЛИМОРФИЗМДЕРДІ ТАЛДАУ

Аннотация. Мақалада механикалық сипаттағы емес эпилепсия диагнозы қойылған науқастарды молекулярлы-генетикалық зерттеу нәтижелері келтірілген. Жұмыстың мақсаты механикалық бұзылыстарды қоспағанда эпилепсияның әртүрлі формаларының дамуына кандидат полиморфизмдердің әсерін талдау. Молекулярлы-генетикалық талдау жүргізу үшін В.М. Савинов атындағы SVS клиникасында емдеуде болған эпилепсияның әртүрлі формаларымен ауыратын 78 науқас таңдалынып алынды. Генотиптеу метил-CpG-байланыстырушы белок 2 (*MECP2,* 3 полиморфизм) гені, натрий (*SCN1A,* 4 полиморфизм*)* және калий (*KCNT1,* 2 полиморфизм) каналдары гендерінің кандидат полиморфизмдері бойынша сайт-спецификалық ПТР-ПДРФ әдістерінің көмегімен жүргізілді. Молекулярлы-генетикалық талдау нәтижесі барлық науқастардың *MECP2* генінің 3-ші экзонының 3 зерттелген кандидат полиморфизмі (p.Thr158Met, p.Thr197Met, p.Arg306Ter) бойынша қалыпты функциональды аллелді көрсетті. Алайда 1 науқаста (Драве синдромымен ауыратын) натрий каналы гені бойынша *de novo* мутация (*SCN1A* p.Ala1783Thr) және 3 науқаста (2 науқас самайлық

эпилепсиямен және 1 науқас резидуальды энцефалопатиямен ауыратын) калий каналы генінің жаңа мутациялары (*KCNT1* p.Ala934Thr) анықталды. *SCN1A* және *KCNT1* гендерінің тұқым қуалаушы мутацияларын анықтау үшін науқастардың жақын туыстарына молекулярлы-генетикалық талдау жүргізілді. Нәтижесінде *SCN1A* p.Ala1783Thr және *KCNT1* p.Ala934Thr кандидат полиморфизмдері ионды каналдардың қалыпты жұмысын бұзып, механикалық емес сипаттағы эпилепсияның дамуымен байланысты болуы мүмкін деген қорытынды жасалынды.

Түйін сөздер: эпилепсия, гендер полиморфизмі, мутациялар, *MECP2, SCN1A, KCNT1*.

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АНАЛИЗ КАНДИДАТНЫХ ПОЛИМОРФИЗМОВ У БОЛЬНЫХ ЭПИЛЕПСИЕЙ БЕЗ НАРУШЕНИЙ МЕХАНИЧЕСКОГО ХАРАКТЕРА

Аннотация. В статье приведены результаты молекулярно-генетического исследования пациентов с диагностированной эпилепсией не механического характера. Целью данной работы был анализ участия кандидатных полиморфизмов в развитии различных форм эпилепсии, за исключением механических повреждений. Для проведения молекулярно-генетического анализа были выбраны 78 пациентов с разными формами эпилепсии, которые находились на лечении в SVS клинике им. В. М. Савинова. Генотипирование проводили методами сайт-специфической ПЦР-ПДРФ по кандидатным полиморфизмам гена метил-CpG-связывающего белка 2 (*MECP2,* 3 полиморфизма), генам натриевого (*SCN1A,* 4 полиморфизма*)* и калиевого (*KCNT1,* 2 полиморфизма) каналов. Молекулярно-генетический анализ показал наличие нормальных функциональных аллелей по 3-м изученным кандидатным полиморфизмам (p.Thr158Met, p.Thr197Met, p.Arg306Ter) 3 экзона гена *MECP2* у всех пациентов с эпилепсией. Однако, 1 случай (пациент с синдромом Драве) *de novo* мутации был установлен в гене натриевого канала (*SCN1A* p.Ala1783Thr) и 3 случая (2 пациента с височной эпилепсией и 1 пациент с резидуальной энцефалопатией) новых мутаций гена калиевого канала (*KCNT1* p.Ala934Thr). Для установления наследуемых мутаций генов *SCN1A* и *KCNT1* проводили молекулярно-генетический анализ родственников пациентов ближайшей степени родства. В результате установлено, что кандидатные полиморфизмы *SCN1A* p.Ala1783Thr и *KCNT1* p.Ala934Thr, нарушающие нормальную работу ионных каналов, могут быть связаны с развитием эпилепсии немеханического характера.

Ключевые слова: эпилепсия, полиморфизм генов, мутации, *MECP2, SCN1A, KCNT1*.

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БИОРАЗНООБРАЗИЕ ПОЛУЖЕСТКОКРЫЛЫХ (HETEROPTERA) ГОРОДА АЛМАТЫ (ЮГО-ВОСТОЧНЫЙ КАЗАХСТАН)

Аннотация. В результате проведенных исследований г. Алматы выявлены из 5 семейств 47 видов полужесткокрылых насекомых.По видовому многообразию выделяются семейства Miridae (27 видов – 57,4%), Tingidae (8 видов – 17,0%), Nabidae – (6 видов – 12,8%), Anthocoridae – (4 вида – 8,5%), Reduviidae – (2 вида – 4,3%). По приуроченности к местам обитания полужесткокрылые города Алматы подразделяются на несколько групп: дендробионты (3 вида), дендро-тамнобионты (3 вида), дендро-хортобионты (3 вида), тамно-хортобионты (2 вида), герпето-хортобионты (2 вида) и хортобионты (34 вида). Из них мезофилы 45 видов, мезоксерофилы – 1 вид, гигромезофил – 1 вид, среди них фитофаги – 27 видов, зоофаги – 13 видов, мицетофаги – 1 вид, зоофитофаги – 6 видов.

Ключевые слова: полужесткокрылые, город Алматы, Юго-Восточный Казахстан.

Введение. Полужесткокрылые насекомые имеют большое значение в природе. Образ жизни очень разнообразен. Наземные полужесткокрылые живут чаще открыто на растениях, иногда на поверхности почвы и в верхнем слое её, в подстилке, по берегам водоёмов, под корой и т.п. Основу фауны полужесткокрылых составляют наземные растительноядные виды. Они питаются соками растений, главным образом их генеративных органов и семян. Среди растительноядных клопов много вредителей сельского и лесного хозяйства. Некоторые полужесткокрылые, будучи хищниками, истребляют вредителей сельского и лесного хозяйства.

Основой для данной работы послужили сборы и полевые наблюдения авторов, сделанные в 2017-2018 гг. по территории города Алматы.

Методы исследования. Сбор полевых материалов осуществлялись в летний период 2017-2018 гг. Изучение фауны и экологии полужесткокрылых проводилось методами маршрутных обследований и стационарных наблюдений. Для сбора клопов применялись различные методики: кошение энтомологическим сачком, сбор эксгаустером, лов на свет и др. [1-5].

Результаты исследования. В результате проведенных исследований были отмечены следующие виды полужесткокрылых. Список включает в себя краткие описания по биологии и экологии каждого вида.

Семейство Miridae – Слепняки

Bothynotus pilosus (Boheman, 1852). г. Алматы, реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 эк.; 18.07.2017. 2 экз.,20.08.2018. 5 экз.;г. Алматы,Жарбулак (Казачка), 15.06.2017. 3 экз., 16.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2017. 4 экз., 18.07.2018. 3 экз, 21.08.2017. 2 экз. Злаково-осоковый луг, в мезофитных биотопах, в сырых местах, полифитофаг [6]. Летит на свет.

Deraeocoris punctulatus (Fallen, 1807). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2017. 3 экз., 18.07.2018. 2 экз., 21.08.2017. 2 экз.; окр. Есентай (Весновка) 22.06.2017. 3 экз., 20.07.2018. 3 экз., 21.08.2018. 4 экз.; проспект аль-Фараби, 16.06.2018. 3 экз., 21.07.2018.

4 экз., 18.08.2018. 2 экз. На травянистых растениях: *Rumex, Artemisia*, отмечен на низкогорном и субальпийском лугу, на высоте 800-1800 м; питается мелкими насекомыми: тлями, трипсами [7].

Deraeocoris serenus (Douglas & Scott, 1868). г. Алматы, проспект аль-Фараби, 16.06.2018. 2 экз., 18.07.2017. 2 экз.; 21.07.2018. 3 экз.; окр. КазНУ им. аль-Фараби, 17.06.2018. 3 экз., 22.07.2018. 3 экз., 15.08.2018. 3 экз.; г. Алматы, Ремизовка, 23.06.2017. 3 экз, 25.07.2018. 2 экз.,17.08.2018. 2 экз. На различных травянистых растениях; в мезофитных биотопах, низкогорный луг, 800-1100 м; питаетсямелкими насекомыми [8].

Deraeocorisventralis Reuter, 1904. г. Алматы, окр. КазНУ им. аль-Фараби, 15.06.2018. 2 экз., 19.07.2018. 2 экз, 19.08.2018, 2 экз; окр. Есентай (Весновка), 22.06.2017. 2 экз., 20.07.2018. 3 экз., 21.08.2018. 4 экз.; г. Алматы,Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 2 экз. На сложноцветных и злаковых; в горах низкогорные луга до 800 м; зоофитофаг.

Deraeocoris scutellaris (Fabricius, 1794). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз., 18.07.2017. 2 экз., 20.08.2018. 2 экз.; г. Алматы, проспект аль-Фараби, 16.06.2017. 2 экз., 21.07.2017. 2 экз.; 19.08.2018. 3 экз., окр. КазНУ им. аль-Фараби, 18.06.2018. 3 экз., 19.07.2018. 3 экз., 15.08.2018. 3 экз.; На различных травянистых растениях, на мезофитных разнотравных лесных лугах; зоофитофаг.

Deraeocoris ruber (Linnaeus, 1758). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2017. 3 экз., 15.07.2018. 2 экз., 20.08.2018. 3 экз.; г. Алматы, проспект аль-Фараби, 16.06.2018. 2 экз., 21.07.2017. 3 экз. 19.08.2018. 2 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 3 экз., 21.08.2018. 4 экз.; На травянистых растениях, на пойменных лугах; зоофитофаг.

Adelphocoris lineolatus (Goeze, 1778). г. Алматы,окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 15.08.2018, 2 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 20.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад.19.06.2018. 3экз.,14.07.2018. 2 экз, 16.08.2018. 3 экз. На сложноцветных, маревых и бобовых растениях. Самый массовый вредитель бобовых. При значительной заселенности полей этим видом наблюдается опадениегенеративных органов до 75%, что ведет к резкому снижению урожая семян люцерны [9].

Adelphocorisquadripunctatus (Fabricius, 1794). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2017. 3 экз., 15.07.2018. 2 экз., 15.08.2018. 2 экз.; г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 2 экз. 22.08.2018. 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 3 экз., 20.08.2018. 3 экз. На пойменных лугах, на сложноцветных, маревых и бобовых растениях.

Agnocorisrubicundus (Fallen, 1807). г. Алматы,окр. реки Большая Алматинка и Малая Алматинка, 15.06.2017. 3 экз., 15.07.2018. 2 экз., 20.08.2018. 2 экз.; г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; На лиственных, плодовых деревьях и кустарниках, чаще на иве; в поймах, в горах 800-2300 м; полифитофаг. Приводится в числе вредителей плодовых культур [10, 11], что, по-видимому, не соответствует действительности.

Apolyguslimbatus (Fallen, 1807). г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 20.06.2017. 3 экз., 20.07.2018. 2 экз., 20.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз.;. 16.08.2018. 3 экз. Преимущественно на ивах, а также на березе.

Apolygus lucorum (Meyer-Dur, 1843). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2017. 3 экз., 15.07.2018. 2 экз., 20.08.2018. 3 экз.; г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 2 экз. 17.08.2018. 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; На различных травянистых растениях: *Artemisia, Tanacetum, Urtica* и др.; на разнотравных лугах и степных склонах гор, в поймах; полифитофаг [7, 12].

Liocoris tripustulatus (Fabricius, 1781). г. Алматы,окр. КазНУ им. аль-Фараби, 20.07.2018. 2 экз., 21.06.2017. 3 экз.; 22.07.2018. 2 экз, 19.08.2018, 3 экз; окр. Есентай (Весновка), 16.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 21.07.2017. 3 экз, 16.08.2018. 3 экз. На травянистых растениях; на мезофитных разнотравных лугах и степных склонах гор, 900-1300 м; полифитофаг (чаще на *Urtica, Artemisia* и др.) [13].

Lygus gemellatus gemellatus (Herrich-Schaeffer, 1835). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз., 18.07.2018. 2 экз., 20.08.2018. 3 экз.; г. Алматы, проспект

аль-Фараби, 16.06.2018. 3 экз., 21.07.2017. 3 экз. 19.08.2018. 2 экз; окр. Есентай (Весновка), 20.06.2017. 3 экз., 21.07.2018. 2 экз., 18.08.2018. 2 экз.; На *Artemisia* и других различных травянистых растениях. Летит на свет. Повсеместно вредит зерновым, бобовым культурам [9].

Lygus pratensis (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 20.07.2018. 2 экз., 21.06.2017. 3 экз.; 22.07.2018. 4 экз, 21.08.2018, 3 экз; микрорайон «Казахфильм», 16.06.2017, 2 экз; 20.07.2018, 3 экз, 19.08.2018, 2 экз. В пойме рек, в низкогорном и субальпийском лугу, 800-2000 м; полифитофаг (зерновые, бобовые и др.) [14].

Orthops campestris (Linnaeus, 1758). г. Алматы,проспект аль-Фараби, 16.06.2018. 3 экз., 21.07.2017. 3 экз. 19.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. В горах до высоты 800-2300 м; широкий олигофитофаг - на зонтичных; вредитель всех зонтичных, возделываемых на семена [15].

Orthops kalmi (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 2 экз. 17.08.2018. 3 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 3 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. На различных травянистых растениях; низкогорные и субальпийские луга, 950-2300 м; широкий олигофитофаг (на зонтичных). Зарегистрирован также на плодовых деревьях [16].

Polymerus unifasciatus (Fabricius, 1794) г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 3 экз.;г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. Мезофитные разнотравные луга, в горах до 800-1300 м; полифитофаг (на подмаренниках *Galium* и различных травянистых растениях) [7].

Myrmecorisgracilis (R.F.Sahlberg, 1848) г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз, 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 3 экз. На травянистых растениях; под луговыми травами на склонах в высокогорье около 2500 м над ур. м.; зоофаг. [17].

Notostiraerratica (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2018. 2 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. На луговых злаковых растениях; в горах на диких злаковых: *Agropyrum, Phleum, Poa, Elymus* и др.) [18].

Stenodema virens (Linnaeus, 1767). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 3 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз.Низкогорные, субальпийские (850-2400 м), пойменные луга, еловое редколесье, поляны и опушки лесов; широкий олигофитофаг (на диких и культурных злаковых).

Halticus apterus apterus (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. Различные мезофитные луга, в горах 800-1400 м; широкий олигофитофаг (на бобовых травах: *Ononis, Vicia* и др.) [7].

Chlamydatus pulicarius (Fallen, 1807). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз. На различных лесных травянистых растениях; на лугах, степных участках, опушках и лесных полянах, в горах до 1000-1500 м; полифитофаг (на бобовых, сложноцветных и других травянистых растениях) [19].

Chlamydatuspullus (Reuter, 1870). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 3 экз; 21.07.2018, 3 экз, 19.08.2018,

2 экз.В горах и на подгорных равнинах, на лугах; на бобовых, сложноцветных и других травянистых растениях. Известен как вредитель бобовых культур [20].

Criocorisquadrimaculatus (Fallen, 1807). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 5 экз.;г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз.На пойменных среднеувлажненных и сырых лугах, субальпийских лугах, 1300-2300 м; узкий олигофитофаг (на подмареннике) [21].

Plagiognathus arbustorum arbustorum (Fabricius, 1794). г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 3 экз.На различных травянистых растениях; на лугах, предпочитает влажные места, в горах 800-1000 м; полифитофаг (на *Urtica, Carduus, Cirsium, Melandryum*) [22].

Plagiognathus chrysantemi (Wolff, 1804) – слепняк малый люцерновый.г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 5 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. . Разнотравно-злаковые луга, в горах до 800-1300 м; полифитофаг (на сложноцветных, бобовых, злаковых и других травянистых растениях, сосет молодые листья, бутоны, цветки и зеленые бобы [23].

Psallus anticus (Reuter, 1876). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 18.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз. На иве, дубе, спирее и карагане; лиственные леса, луга, в долинах горных рек, в горах до 900-1500 м; зоофитофаг [24].

Семейство Tingidae – Кружевницы

Acalypta gracilis (Fieber, 1844). г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 3 экз.Среди детрита, на травянистых растениях: *Ajuga, Potentilla, Hieracium, Thymus, Sedum*; более и менее сухие места, высокогорные степи, прогреваемые солнцем [25].

Agramma confusum (Puton, 1879). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз.. В высокогорных степях, на субальпийском лугу, 800-2000 м; на ситниковых: *Juncus* и осоковых: *Carex, Blysmus, Eriophorum*). Имаго и личинки чаще всего держатся на соцветиях кормовых растений [26].

Dictylaechii (Schrank, 1782). г. Алматы, проспект аль-Фараби, 16.06.2018.2 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз.Самые разнообразные биотопы, избегает сырых и сильно затененных мест; горно-лесной пояс; на *Symphytum, Echium, Pulmonaria*. Известно, что имаго и личинки могут питаться соками более 20 различных растений [25-27]. Однако основными кормовыми растениями им служат бурачниковые (Borraginaceae).

Dictylalupuli (Herrich-Schaeffer, 1837). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 3 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 20.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. Низкогорные и субальпийские луга, до 2500 м; узкий олигофитофаг (незабудка *Myosotis palustris,* окопник *Symphytum* из бурачниковых [25].

Galeatus sinuatus (Herrich-Schaeffer, 1838). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. Приурочен к опушкам лесов, зарослям кустарников, речным долинам, залежам, низкогорные и субальпийские луга, 1000-2000 м; на бурачниковых, губоцветных. По некоторым данным [28, 29],

основным кормовым растениям имаго и личинкам служит зопник клубненосный (*Phlomistuberosa*), в отдельных случаях клопы могут питаться на яснотке (*Lamium*), шалфее (*Salvia*).

Lasiacanthacapucinacapucina (Germer, 1837). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 5 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 20.06.2018. 2 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. На растениях и под ними, среди растительного детрита; на опушках леса, в горах до 800-2400 м, субальпийские луга; на губоцветных: тимьян *Thymus* и др. [30].

Physatocheilasmreczynskii China, г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 17.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 3 экз. На кустарниках и деревьях из сем. Розоцветных; в лесных биотопах, в горах до 900-1300 м [25].

Tingispilosa (Hummel, 1825). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. .08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз.Самые разнообразные мезофитные биотопы: пойма, низкогорные луга 800-1300 м, разреженные леса, парки, окраины садов и другие участки, на различных растениях, чаще на губоцветных: *Phlomistuberosa, Lamiumalbum, Galeopsisbifida* и др. Более 10 видов растений указывают [25, 26], на которых питаются взрослые и личинки.

Семейство Nabidae

Himacerusmaracandicus (Reuter, 1890). г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 3 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 17.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 3 экз. Держится на высоких травянистых растениях, особенно зонтичных, на почве, иногда на кустах; на высокотравных лугах и в зарослях кустарников в горах на высотах от 400 до 3000 м над ур. м. [31].

Himacerus apterus (Fabricius, 1798). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 3 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. В лиственных, хвойно-широколиственных и сосновых лесах, парках, садах, пойменных древесно-кустарниковых зарослях, личинки 1-го и 2-го возрастов держатся в траве, с 3-го возраста они переходят на кустарники, а затем и на деревья [31]; горный лесной, поднимается в субальпийские луга.

Nabis flavomarginatus Scholtz, 1847. г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 17.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз.;. 16.08.2018. 3 экз. Широко распространен по лесной и лесотундровой зонам, разнотравные луга, опушки леса, лесные поляны, в горы поднимается до 2000 м, в субальпийских лугах; на мезофитных и сырых лугах; питается мелкими насекомыми [31].

Nabis brevis brevis Scholtz, 1847. г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. Живет на лугах в травостое, преимущественно на злаковых; по мезофитным участкам: западины, луговинки близ родников и т.д., поднимается в горы до высоты 3600 м; зоофаг (широко многояден) [31].

Nabisferus (Linnaeus, 1758). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 5 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 21.08.2017. 2 экз. Очень обычный в лесной зоне, приурочен главным образом к берегам морей, рек, озер и родников, в горах до высоты 2500 м; многоядный вид, питающийся мухами, тлями, цикадами, клопами и другими насекомыми; является самым полезным видом из полужесткокрылых в сельском хозяйстве.

Nabisrugosus (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 22.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. Вразличных биотопах на травянистой растительности, в травостое в тенистых местах, в основном под пологом широколиственного леса, на лесных полянах и опушках; в горах до высот около 2000 м [31]; зоофаг (питается тлями, личинками цикадок и клопов-слепняков, другими насекомыми).

Семейство Anthocoridae

Acompocorisalpinus Reuter, 1875. г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 22.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз. На хвойных деревьях: *Abies, Picea. Larix, Pinus*), поднимается в горы до 1200 м над ур. м. и выше; в лесной зоне, большей части в горах; зоофаг (главным образом питается тлями).

Anthocorisnemorum (Linnaeus, 1761). г. Алматы, окр. реки Большая Алматинка и Малая Алматинка, 15.06.2018. 3 экз.; 18.07.2017. 2 экз., 20.08.2018. 5 экз.; г. Алматы, Жарбулак (Казачка), 15.06.2017. 3 экз., 18.07.2018. 2 экз., 20.08.2017. 3 экз.; г. Алматы, микрорайон Карасу, 18.06.2018. 4 экз., 16.07.2018. 3 экз, 19.08.2017. 2 экз. На различных травянистых, кустарниковых и древесных растениях, реже на траве; горные леса, альпийские и субальпийские луга, до 1000-3000 м над ур. м., встречается в садах, где играет большую роль в регулировании численности вредителей яблони (Пучков, 1961); широкий полифаг, питается тлями, клещами, червецами, трипсами, яйцами и гусеницами совок, яйцами Miridae [32].

Orius horvathi (Reuter, 1884). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 2 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 23.07.2018, 3 экз, 19.08.2018, 2 экз.На различных травянистых растениях: *Medicago, Trofolium* и др.; от пустынь до высокогорий, в поймах рек; зоофаг (тли, листоблошки, трипсы, мелкие гусеницы бабочек, клещи и их яйца, яйцами вредной черепашки, хлебного клопа) [33].

Orius vicinus (Ribaut, 1923). г. Алматы, проспект аль-Фараби, 16.06.2018. 3 экз., 18.07.2017. 3 экз. 17.08.2018. 2 экз; окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; микрорайон «Казахфильм», 17.06.2017, 2 экз; 21.07.2018, 3 экз, 19.08.2018, 2 экз.. На цветах и листьях различных травянистых растений, кустарниках, деревьях; от пустынь до высокогорий до 2000 м и более; зоофаг (широкий полифаг, в основном щитовками и другими мелкими насекомыми) [33].

Семейство Reduviidae

Rhynocoris annulatus (Linnaeus, 1758). г. Алматы, проспект аль-Фараби, 16.06.2017. 2 экз., 18.07.2018. 3 экз., 17.08.2018. 2 экз; г. Алматы, Ремизовка, 23.06.2018. 3 экз, 25.07.2018. 2 экз., 17.08.2018. 2 экз.,окр. КазНУ им. аль-Фараби, 15.06.2018. 3 экз., 20.07.2018. 3 экз., 15.08.2018. 3 экз. На деревьях: сосна, ель, можжевельник, береза, лещина, ольха, дуб, осина; на различных кустарниках и травянистой растительности: зонтичных, бобовых, сложноцветных; лесная, лесостепная зоны, приречные леса; многоядный зоофаг (листоеды, осы, пчелы, гусеницы бабочек и др.). [34].

Rhynocoris iracundus (Poda, 1761). г. Алматы, окр. КазНУ им. аль-Фараби, 21.06.2017. 3 экз.; 19.07.2018. 4 экз, 18.08.2018, 3 экз; окр. Есентай (Весновка), 22.06.2017. 3 экз., 20.07.2018. 2 экз., 21.08.2018. 3 экз.; г. Алматы, Алматинский ботанический сад, 19.06.2018. 2 экз., 14.07.2018. 2 экз, 16.08.2018. 3 экз. Различные природные зоны: от остепненных долин и жарких, поросших редколесьем склонов предгорий и низкогорий до высокогорных лесных полян и субальпийских лугов до 2000 м, на деревьях, кустарниках и травянистой растительности; зоофаг (подстерегают добычу на высоких цветущих растениях и охотно ловят различных насекомых: листоедов, ос, пчел, гусеницы бабочек и др.) [35].

Обсуждение результатов. В результате проведенных исследований выявлены из 5 семейств 47 видов полужесткокрылых (таблица).

Из приведенной таблицы видно, что по видовому многообразию выделяются семейства Miridae (27 видов – 57,4%), Tingidae (8 видов – 17,0%), Nabidae – (6 видов – 12,8%), Anthocoridae – (4 вида – 8,5%), Reduviidae – (2 вида – 4,3%).

Видовой состав полужесткокрылых г. Алматы.

№	Семейство	Виды	Число видов	%
1	Miridae	*Bothynotuspilosus* (Boheman, 1852) *Deraeocoris punctulatus* (Fallen, 1807) *Deraeocoris serenus* (Douglas & Scott, 1868) *Deraeocorisventralis* Reuter, 1904 *Deraeocoris scutellaris* (Fabricius, 1794) *Deraeocoris ruber* (Linnaeus, 1758) *Adelphocoris lineolatus* (Goeze, 1778) *Adelphocorisquadripunctatus* (Fabricius, 1794) *Agnocorisrubicundus* (Fallen, 1807) *Apolyguslimbatus* (Fallen, 1807) *Apolygus lucorum* (Meyer-Dur, 1843) *Liocoris tripustulatus* (Fabricius, 1781) *Lygus gemellatus gemellatus* (Herrich-Schaeffer, 1835) *Lygus pratensis* (Linnaeus, 1758) *Orthops campestris* (Linnaeus, 1758) *Orthops kalmi* (Linnaeus, 1758) *Polymerus unifasciatus* (Fabricius, 1794) *Myrmecorisgracilis* (R.F.Sahlberg, 1848) *Notostiraerratica* (Linnaeus, 1758) *Stenodema virens* (Linnaeus, 1767) *Halticus apterus apterus* (Linnaeus, 1758) *Chlamydatuspulicarius* (Fallen, 1807) *Chlamydatuspullus* (Reuter, 1870) *Criocorisquadrimaculatus* (Fallen, 1807) *Plagiognathus arbustorum arbustorum* (Fabr., 1794) *Plagiognathus chrysantemi* (Wolff, 1804) *Psallus anticus* (Reuter, 1876)	27	57,4
2	Tingidae	*Acalyptagracilis* (Fieber, 1844) *Agramma confusum* (Puton, 1879) *Dictylaechii* (Schrank, 1782) *Dictylalupuli* (Herrich-Schaeffer, 1837) *Galeatus sinuatus* (Herrich-Schaeffer, 1838) *Lasiacanthacapucinacapucina* (Germer, 1837) *Physatocheilasmreczynskii* China, 1952 *Tingispilosa* (Hummel, 1825).	8	17,0
3	Nabidae	*Himacerusmaracandicus* (Reuter, 1890) *Himacerus apterus* (Fabricius, 1798) *Nabis flavomarginatus* Scholtz, 1847 *Nabis brevis brevis* Scholtz, 1847 *Nabisferus* (Linnaeus, 1758) *Nabisrugosus* (Linnaeus, 1758)	6	12,8
4	Anthocoridae	*Acompocorisalpinus* Reuter, 1875 *Anthocorisnemorum* (Linnaeus, 1761) *Orius horvathi* (Reuter, 1884) *Orius vicinus* (Ribaut, 1923).	4	8,5
5	Reduviidae	*Rhynocoris annulatus* (Linnaeus, 1758) *Rhynocoris iracundus* (Poda, 1761)	2	4,3
ВСЕГО			47	100

При выделении экологических группировок оценивался диапазон потребностей каждого вида. Учитывались сведения о распространении, трофические связи и другие особенности каждого вида, известные как по собственным наблюдениям, так и взятые из многочисленных литературных источников [5-9]. Разные виды полужесткокрылых имеют различные требования к степени увлажненности местообитания. По этому признаку выделены следующие экологические группы видов:

мезо-ксерофилы, мезофилы, гигро-мезофилы. Мезофилы населяют открытые и затененные местообитания с умеренной степенью увлажненности, в них отмечено 45 видов. Мезоксерофилы- обитатели остепненных лугов, сухих луговых степей, сухих лесных местообитаний. Здесь встречается *Apolygus lucorum*. Гигро-мезофилы – населяют сырые луга и переувлажненные участки, такие биотопы населяет *Dictylalupuli*.

По приуроченности к местам обитания полужесткокрылые города Алматы подразделяются на несколько групп: дендробионты (3 вида), дендро-тамнобионты (3 вида), дендро-хортобионты (3 вида), тамно-хортобионты (2 вида), герпето-хортобионты (2 вида) и хортобионты (34 вида).

Питание полужесткокрылых чрезвычайно разнообразно, среди них выделяются фитофаги (27 видов), зоофаги (13 видов), мицетофаги (1 вид), зоофитофаги (6 видов), потребляющие как растительную, так и животную пищу.

Выводы. Из отмеченных полужесткокрылых доминирующими по числу видов являются сем. Miridae – 27 видов, Tingidae – 8 видов, Nabidae – 6 видов, из остальных семейств известно всего по 2-4 вида. Из них мезофилы 45 видов, мезоксерофилы – 1 вид, гигромезофил – 1 вид, среди них фитофаги – 27 видов, зоофаги – 13 видов, мицетофаги – 1 вид, зоофитофаги – 6 видов.

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АЛМАТЫ ҚАЛАСЫНЫҢ (ОҢТҮСТІК-ШЫҒЫС ҚАЗАҚСТАН) ЖАРТЫЛАЙҚАТТЫҚАНАТТЫЛАРЫНЫҢ (HETEROPTERA) АЛУАН ТҮРЛІЛІГІ

Аннотация. Зерттеу нәтижесінде Алматы қаласының жартылай қаттықанаттылардың 5 тұқымдасына жататын 47 түрі анықталды. Бұлардың арасында түр құрамының көптігімен ерекшеленетін тұқымдастар Miridae (27 түр – 57,4%), Tingidae (8 түр - 17,0%), Nabidae – (6 түр - 12,8%), Anthocoridae – (4 түр – 8,5%), Reduviidae – (2 түр - 4,3%). Олардың ішінде мезофилдер - 45 түр, мезоксерофилдер - 1 түр, гигромезофил - 1 түр, бұлардың арасында фитофагтар - 27 түр, зоофагтар - 13 түр, мицетофаг - 1 түр, зоофитофагтар - 6 түр.

Түйін сөздер: жартылай қаттықанаттылар, Алматы қаласы, Оңтүстік-Шығыс Қазақстан.

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BIODIVERSITY OF HETEROPTERA OF THE ALMATY CITY (SOUTH-EASTERN KAZAKHSTAN)

Abstract. As a result of the research conducted in Almaty city, 47 families of true bugsinsects were identified from 5 families. Miridae families are distinguished according to the species diversity (27 species - 57.4%), Tingidae (8 species - 17.0%), Nabidae - (6 species - 12.8%), Anthocoridae (4 species - 8.5%), Reduviidae - (2 species - 4.3%). True bugs of the Almaty city are divided into several groups according to their confinement to habitats: dendrobionts (3 species), dendrotamnobionts (3 species), dendrohortobionts (3 species), tamnohortobionts (2 species), herpetochortobionts (2 species) and chortobionts (34 species). Of these, 45 species of mesophyles, 1 species of mezoxerophiles, 1 species of hygromesophylus, 27 of them phytophagous, 13 species of zoophagous, 1 species of mycetophagous, 6 species of zoophytophagous.

Keywords: True bugs, Hemiptera, city, Almaty, south-eastern Kazakhstan.

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МАЗМҰНЫ

СОДЕРЖАНИЕ

CONTENTS

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