This scripture deals with new unique biotechnologies aimed at human health promotion and wellbeing of the people on the Earth. Climate change creates the most dangerous threat of our time because of careless use of earth resources, destroying the ecosystem and leads to the loss of biodiversity in the world. The task of careful and most responsible regulatory impact upon both healthy and sick organisms of living beings is no longer the concern just of scientists. For over fifty years we have been studying the problems of regulation of biological processes under health and disease conditions, especially under so-called stress conditions and induced fatal diseases. The technology has been elaborated to struggle against widespread viral diseases with drug izatizon, which has a broad spectrum of antiviral activity, including DNA-and RNA-containing viruses, viral and microbial associations. The role of natural biologically active substances, their analogues and modification products in a human and environmental molecular-genetic sanitation can hardly be overestimated.

Anatoly Potopalsky

Prof. Anatoliy Potopalsky Ph.D. in Medicine created his system of molecular genetic rehabilitation of human and the environment on the base of new modified biologically active compounds and molecular study of their action. He has over 300 publications, 53 author's certificates of inventions and patents, 13 monographs.

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New Molecular genetic methods

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New methods for molecular genetic recovery of humans and environment

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Introduction

This scripture deals with new unique biotechnologies aimed at human health promotion and wellbeing of the people on the Earth.

Climate change creates the most dangerous threat of our time because of careless use of earth resources, destroying the ecosystem and leads to the loss of biodiversity in the world. The task of careful and most responsible regulatory impact upon both healthy and sick organisms of living beings is no longer the concern just of scientists. By thousands years of history humanity has certainly proved to have inexhaustible self-regulating abilities and reserves of wild Nature.

For over fifty years we have been studying the problems of regulation of biological processes under health and disease conditions, especially under so-called stress conditions and induced fatal diseases. The technology has been elaborated to struggle against widespread viral diseases with drug izatizon, which has a broad spectrum of antiviral activity, including DNA-and RNA-containing viruses, viral and microbial associations. The role of natural biologically active substances, their analogues and modification products in a human and environmental molecular-genetic sanitation can hardly be overestimated.

Keywords: modification, natural substances, amitozyn, izatizon, new varieties, biotechnologies.

I. The role of natural biologically active substances, their analogues and modification products in a human and environmental molecular-genetic sanitation.

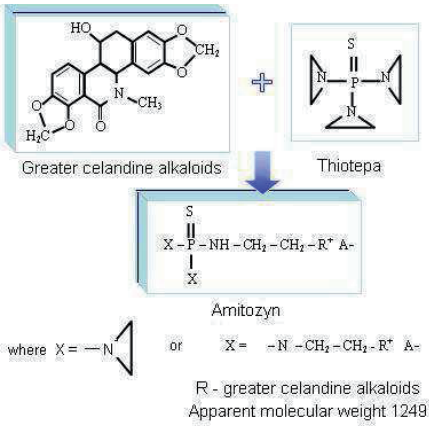
Due to our new method of purposeful modifying natural molecules by alkylation, it is possible to enhance the resistance of living beings to unfavorable environmental factors, to prevent mass viral and bacterial diseases, including tumour growth, immunoagressive and immune deficiency conditions. Of special note is a possible regulatory effect of such substances upon inherited disorders and radioactive poisoning.

The approach for obtaining a large group of antitumor products of alkylation of isoquinoline alkaloids of triethyphoms, elaborated in collaboration with M. Turkevich, M. Oliyovskaya, V. Ya. Novitsky in 1969, was patented in 16 countries.

Altogether there are over 60 preparations registered as biologically active substances of this new type in the public register of the former USSR. They were created by the author in collaboration with a team of scientists in Lviv and Kiev and protected by 40 author's certificates, 5 patents of Ukraine and 14 international patents.

The first substance of this type, that able together with its analogues to stop destructive actions, was antitumour, antiviral and antimicrobial, immune-regulating preparation amitozyn, which was devised in 1959 through alkylation of the sum of alkaloids extracted from a well-known plant – greater celandine (*Chelidonium majus* L.) by antitumour preparation thiophosphoamide (ThioTEPA) (A. I. Potopalsky, 1961). Here is the scheme 1 of amitozyn synthesis.

1. Scheme of amitozyn synthesis



1.1. The obtaining of antitumor preparations

It is well known that electrophilic alkylating agents possess a mutagenic and carcinogenic action, while di- and polyfunctional compounds may also exert an antitumoral effect due to their ability for cross-linking the double strands of DNA [1. P.D. Lawley and P. Brookes: "Alkylation of Nucleic acid their consistent nucleotides", *Biochem. J.,* V. 89, (1963), pp. 127-138]. Among such compounds are ethyleneimine derivatives — thiotepa (N,N',N''-triethylenethiophosphoramide), benzotepa (N-benzoyl-N',N''-diethylenephosphoramide), imiphos (2-methyl-3-thiazolildiethylenephosphoramide) and others, which are being used as an antitumoral preparations for a long time [2, 3].

Thiotepa is being successfully employed in chemotherapy of malignant neoplasm and being under extensive study as yet [4], [5], [6], [7]. Besides its antitumoral effect thiotepa generally exerts a pronounced toxic effect [8], [9], [10], [11]. Minor doses of thiotepa were found to have cytostatic and cytotoxic effects, while higher ones exhibited mutagenic effect [12], [13]. The most effective

antitumoral drugs have proved to be products of the alkylation by thiotepa of some biologically active substances such as alkaloids, particularly the celandine (Chelidonium majus) alkaloids, (the drug amitozyn [14] and nucleic acids [15]. In the experimental models of the animal transplantable tumors (Shvets' erythromyelosis, Ehrlich's carcinoma, cancer of ovaries etc.), thiotepa-modified DNA and RNA inhibited tumor growth by 90-100%, while the efficacy of the thiotepa action *per se* usually does not exceed 50-55% and nucleic acids themselves possess no pronounced antitumoral effect. It was also shown that both embryonic and thiotepa-modified human DNA have weak mutagenic effects with almost equal activities. They increase the frequency of spontaneous mutations only three times, whereas the mutagenic effect of thiotepa is 20 times as high. Alkylation effect appeared to be somewhat lower in plants: both thiotepa-modified DNA and RNA inhibited neoplasm growth in tomato by 41% and 62%, respectively [15].

Pioneering studies on alkylation of nucleic acids and their components, next isolation and identification of modification products have been started 30-40 years ago. The authors of these works were Lawley, Price, Hemminki et al. [1], [16], [17], [18], [19], [20], [21]. Besides the common alkylating agents (diazomethane, dialkyl sulfates, alkyl methane sulfonates and others), they used mustard gas and its nitrogen-containing analogs – N-2-chloroethylmorpholine and N,N-diethyl-2-chloroethylamine (nitrogen mustard, $(C_2H_5)_2NCH_2CH_2Cl$). Similar to ethyleneimine and its derivatives, two last named compounds form an active intermediate, immonium cation, during alkylation [20]. Among others, a special attention should be given to Singer's review on alkylation of DNA and its components by almost all the alkylating agents, being used at that time, except thiotepa [22].

The early works on thiotepa alkylation of nucleic acids and their components appeared later on and dealt primarily with pharmacokinetic studies [23], mutagenic and toxic effects of this alkylating agent itself in comparison with the same effects of intact and thiotepa-modified nucleic acids [15]. However, these authors neither isolated nor identified the products of reactions. The interaction between thiotepa and

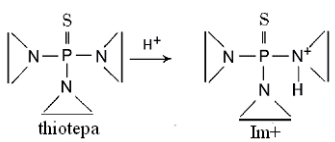
nucleic acids was intrinsically registered by sulfur and ethyleneimino groups in alkylated products. Nucleic acids modification by thiotepa was also experimentally proved by changes in their melting temperature curves [24] or DNA luminescence that indicates DNA alkylation at the position 7 of guanine [25]. Based on mass-spectrometric data on thiotepa interaction with the methylated nitrogenous bases, it was suggested that all canonical bases in DNA are able to be alkylated [26]. In nucleotides, on the contrary, alkylation reaction was reported to occur at the phosphate group, while the nitrogenous bases were not alkylated, with the exception of guanine in GMP and DNA [27].

We first isolated the thiotepa-alkylated products of heterocyclic bases [28], which were subsequently used as markers in identification of alkylated nucleosides, nucleotides and polynucleotides [29], [30], [31].

Having a high pKa (7.8), thiotepa is able to alkylate the nucleophilic centers even in neutral media, however the yield of modified products is rather low [27]. The alkylation rate substantially increases in the presence of a proton donor, which facilitates the formation of active alkylating particle, the immonium cation (Im+), generated by protonation of the ethyleneimine (aziridine) ring [20], [28], [32] (see scheme 2).

But, thiotepa may directly alkylate the acids themselves, which are proton donors. The stability of the immonium cation and therefore the efficiency of alkylation reaction are determined by the nature of proton donor, i. e. the nucleophility of its anion.

Scheme 2



For alkylation reactions performed at pH 4.5-7.0, perchloric acid ($HClO_4$) was chosen as the most preferable proton donor [28]. Its anion possesses the last place in

the row of anion nucleophility, and so the product of its interaction with thiotepa displays the ionic structure, while HCl is covalently linked to thiotepa and the chloride-anion competes with nucleophils in alkylation reaction, including the nucleic acid bases. It was reported that if the reaction is performed in the presence of immonium cation, a temperature increase not only accelerates the hydrolysis of the P—N bonds, but also provokes (through the intermediate formation of thiotepa mercapto derivatives) a sulfur release as a hydrogen sulfide, which reacted with lead acetate yields a black precipitate PbS. This results in a complex mixture of thiotepa degradation products, including various phosphoric acid derivatives.

With taking all above concerns into account, the alkylation reactions in the presence of $HClO_4$ were performed at temperature not higher 37°C. When the reactions were conducted under the neutral conditions, the yield of products was increased either by heating up to 100°C or by raising the alkylating agent concentration (adenine-thiotepa, 1:5). Since in great excess of thiotepa the medium was being weakly alkalized, it was neutralized by adding diluted $HClO_4$.

To get information on the alkylation of bases by thiotepa in comparison with other alkylating agents – ethyleneimine (EI), monoaziridinediethylphosphate (MAEP) and foregoing agents from B. Singer review [22] the following methods and materials were used.

The nucleic acid bases were purchased from Serva (Germany). *The alkylating agents (*thiotepa, ethyleneimine and monoaziridinediethylphosphate) were synthesized as described previously [33], [34]. *UV spectra* were recorded with a Specord UV-VIS spectrophotometer (Karl Zeiss, Germany).

The alkylated mixtures were separated by using a Bio-Rad HPLC system (USA) with a flow-through UV detector of the LTV monitor type, model 1306, of the same firm. The chromatography of the alkylation products of the bases was carried out under the conditions of reversed-phase HPLC on a Bio-Sil ODS-5S column (4 x 150 mm) at a rate of elution of 0.7 ml/min in a 0-20% concentration gradient of acetonitrile in 0.05 M sodium phosphate buffer, pH 7.0.

Alkylation products were identified by comparison of their UV spectra with UV spectra of bases alkylated by other alkylating agents reported in [22].

Filtrak FN-12 paper (Germany) and *Silufol*^{R} *UV-254 plates* (Czechoslovakia) were used for separating the alkylation products by means paper (PC) and thin-layer (TLC) chromatography accordingly.

Alkylation of the nitrogenous bases:

a) *In acidulous medium.* 13 -15 mg (≈ 0.1 mmol) of bases were dissolved upon heating in 3-5 ml of water and then added to freshly prepared solution of Im+ obtained by mixing of 19 mg (0.1 mmol) thiotepa and 17.4 mg (0.1 mmol) $HClO_4$ both dissolved in 1 ml of water. The mixture was stirred at 20°C for 3 h and kept at 37°C for 18 h. Following ether extraction of non-reacting thiotepa and solution concentrating at 35-40°C in the vacuum of the water-jet pump, the 0.2-0.4 mg base aliquot was chromatographed on a column.

b) *In neutral medium*. 0.1 mmol of bases were dissolved in 4-6 ml of water and mixed with 0.5 mmol of alkylating agent**.** To neutralize the weak alkaline medium, the diluted (1:200) 5.74 N $HClO_4$ was added. The mixture was incubated at 37°C for 24 h, and then analyzed as described above.

c) *In the absence of* $HClO_4$ (water solutions of thiotepa have pH≈8, those of EI have pH≈10**).** A mixture containing 100 mg of adenine and 300 mg of thiotepa in 10 ml of water was heated at 100°C under reflux for 8-10 h. After cooling, filtration, and extraction with ether, the mixture was analyzed as described above.

To identify the alkylation sites and structures of alkyl radicals of modified bases, the reactions of adenine alkylation by thiotepa under different conditions (pH and temperature) were studied in details. As seen from Figures 1a and 2a, alkylated adenines are eluted both before and after adenine fraction.

By HPLC method the reaction mixture containing products of adenine alkylation in the presence of $HClO_4$ was separated into four fractions. Three fractions are located far before the adenine fraction (Fig. 1a). The UV spectra of these fractions were completely identical to those of the products of adenine substitution in the

positions N1, N3 and N9 of the heterocycle (Scheme 3, Table 1), which have been reported in Singer's review [22]. Remarkably, the isolated products were eluted from a column in the order correlating with their pKa values (Table 1).

To explain the different chromatographic mobility of thiotepa alkylated adenines and to determine the structures of alkyl radicals, we carried out the alkylation reactions with EI and MAEP. These agents are monofunctional analogs of thiotepa that contribute to the interpretation of the experimental data on thiotepa. Particularly, to compare with thiotepa, MAEP has a single amide bond which can be hydrolyzed. Fig.1 demonstrates that the chromatogram of the mixture of adenines alkylated by thiotepa in the presence of $HC10_4$ is rather similar to that of ethyleneimine alkylated adenines in the absence of $HClO_4$. Under the alkylation by MAEP two types of products.

Conditions for alkylation reaction and mixture separation are given in the text above N1, N3, N9 – sites of adenine alkylation. Ade – peak of adenine were separated (Fig. 3a).

Both types have the same spectral characteristics corresponding to alkylation at the N1 and N3 positions, but having different elution times (before and after adenine). Contrary to alkylation of adenine by ethyleneimine and thiotepa, the N9-substituted adenine was not detected before adenine, but it appeared after the acid hydrolysis of the mixture (Fig. 3b).

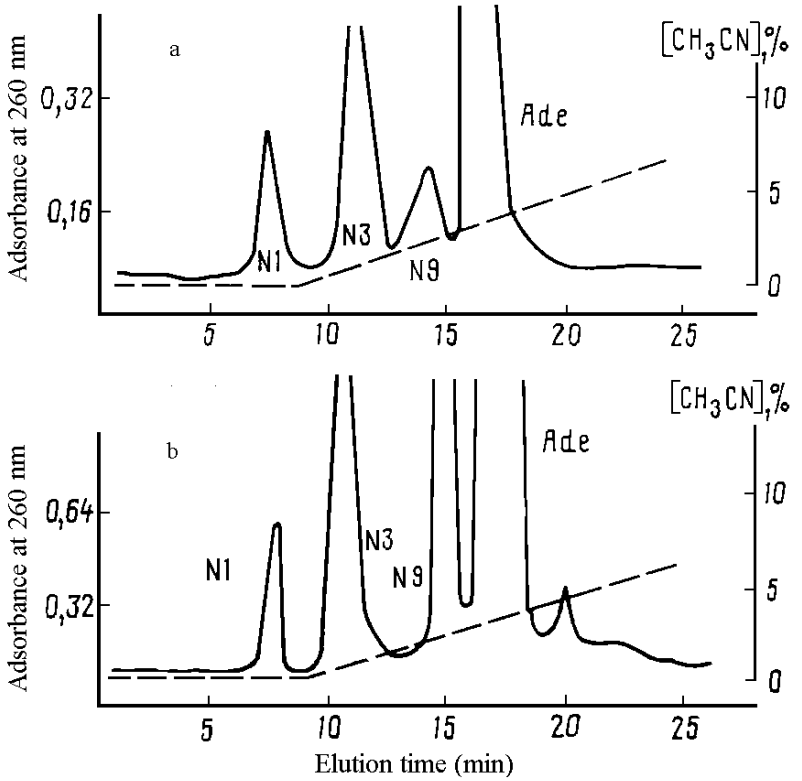
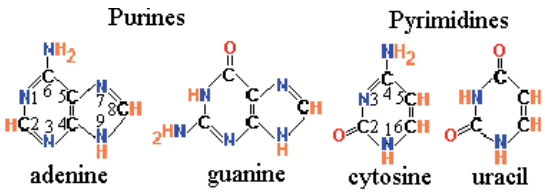


Fig.1. HPLC of adenine alkylation products: a) by thiotepa in the presence of the perchloric acid (starting pH 4.5) and b) by ethyleneimine in the absence of proton donor (pH 8.0).

Scheme 3



Based on the results obtained for adenine alkylation by MAEP at neutral pH and the structure of alkylating agent, one may suppose that alkylation could be

mediated by the two types of the alkyl radicals: phosphoraminodiethyl one – R^*, resulting from opening of the protonized aziridine cycle, and the aminoethyl radical – R, resulting from the hydrolysis of the amide (P-N) bond of radical R*. The following structures are proposed for these MAEP radicals (Scheme 4):

Scheme 4

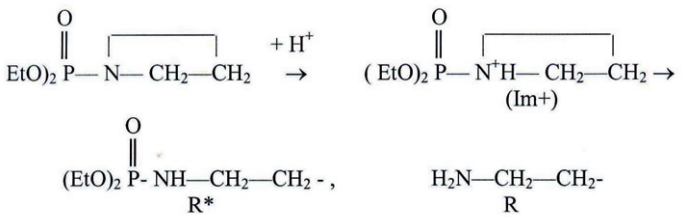


Table 1. UV spectral characteristics and dissociation constants of adenines alkylated by thiotepa and isolated by reversed-phase HPLC.

Sites of alkylation	pH	λ_{max} - λ_{min}, nm		pKa [22]
		A	B	
Adenine	1	262.5 – 228	-	4.15
	12	269.5 – 237	-	
N1	1	263 – 235	262 – 233	7.2
	12	272 – 246	271 – 242	
N3	1	276 – 233	275 – 236	6.0 – 6.5
	12	274 – 245	274 – 245	
N^6	1	272 – 233	272 – 234	4.2
	12	273 – 244	273 – 236	
N9	1	259 - 228	258 - 227	4.0
	12	261 – 233	261 – 229	
(N7)*	1	272 – 235	273 – 237	3,6
	12	270 - 234	270 -230	

A – our data, B – data from B. Singer review [22].
* - minor product obtained by means PC and TLC chromatography (from Fig.4).

Amide bond may be cleaved both during the alkylation reactions and following the storage of alkylated mixtures. R^* radical seems to impart hydrophobic properties to the product, while R - hydrophilic ones (together with an increase in basicity), which explains their emergence after and before adenine, respectively.

This also provides an explanation for different chromatographic mobility of the modified products under various alkylation conditions. Upon alkylation in weak acid media (with Im^+ involved) the aminoethylation products (with R radical) are produced as a result of the preferential amide bond hydrolysis, whereas in neutral media the phosphoraminoethylation products (with R^* radical) are produced and eluted after adenine (Figs 2 and 3).

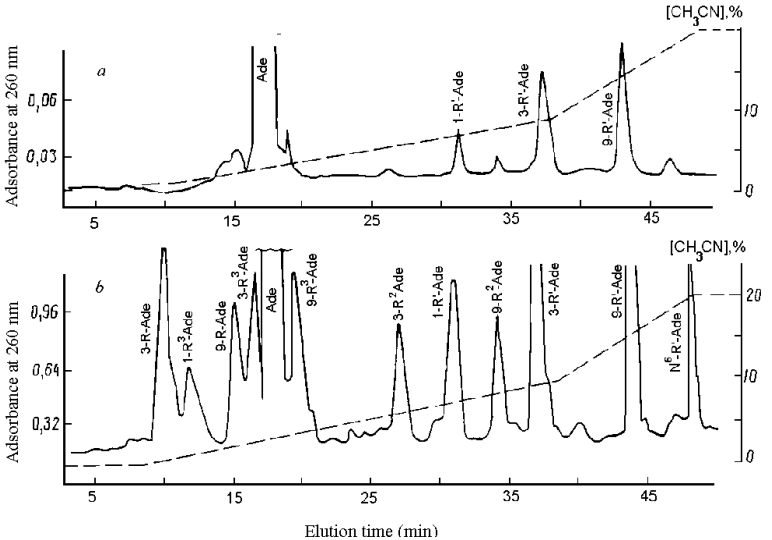


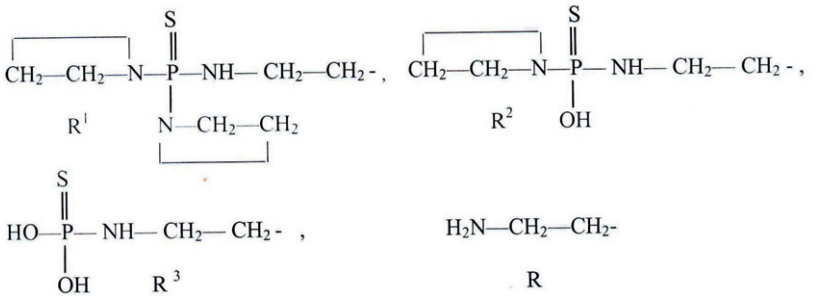
Fig.2. HPLC on the column (4x150 mm) Bio-Sil ODS-5S for the mixture of adenines alkylation by thiotepa during a day in neutral conditions at room temperature and in adenine-thiotepa-$HClO_4$ 3:15:0.1 ratio (a) and after 8 h of heating at 100°C and in adenine-thiotepa 1:2 ratio (b). 1, 3, and 9 – sites of adenine alkylation. The structures of R, R^1, R^2 and R^3 radicals are presented in scheme 4.

By analogy, having three phosphor amide bonds, thiotepa is suggested to form four types of alkyl radicals at the each position and therefore four types of alkylation products produced by the consecutive cleavage of three amide bonds. In common case, four structures of thiotepa radicals are depicted in scheme 5.

Products with these radicals were isolated from the mixture of alkylation products upon boiling of adenine with thiotepa. As seen from Fig. 2b, the mixture, in

particular, contains four products with spectral characteristics of 3-substituted adenines and as many products, alkylated at N9 position of the heterocycle. Identification of the fractions on the chromatogram was accomplished by comparison with the appropriate peaks in Figs 1b and 3a, where the radicals were determined as R (this radical is the only one for EI) and R^*

Scheme 5



(R^1 for thiotepa). Decrease of product hydrophobity with the hydrolysis of its amide bonds (R^2 and R^3) was also considered to identify fractions. The last product eluted from the column was adenine substituted at the exocyclic nitrogen in the N^6 position. Direct alkylation at this position proceeds extremely rarely and with low efficiency.

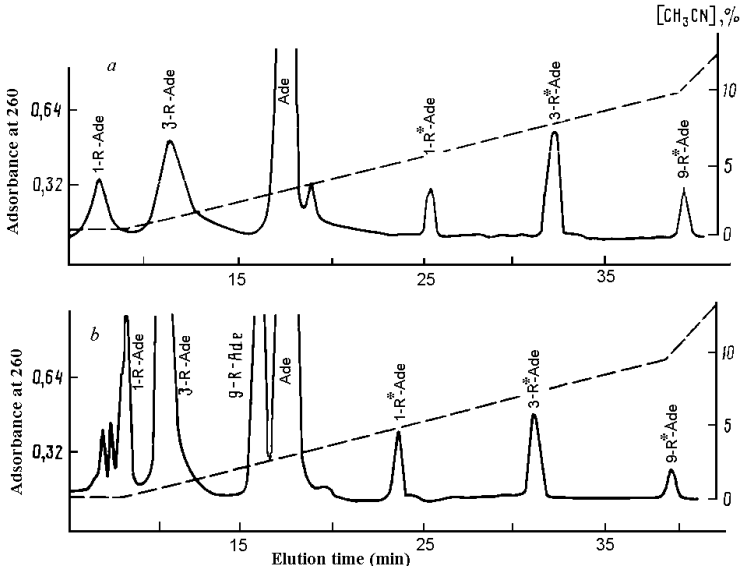


Fig.3. HPLC on the column (4x150 mm) Bio- Sil ODS-5S for mixture of alkylated by monoaziridinediethylphosphate adenines at 37°C during a day and in adenine-MAEP-$HClO_4$ 1:1:0.1 ratio (a), the same mixture after acid (0.5 N HCl, 100°C, 1 h) hydrolysis (b). R – radical with amide bond cleavage, R^* – radical without such cleavage.

Adenine N^6-derivatives are generally formed from 1-substituted products as the result of a Dimroth rearrangement at relatively low alkalinity (pH 8-9) and even in neutral media [20], [30]. Such transformation into N^6-derivative may be a reason for the absence of 1-aminoethyladenine (1-R-Ade) fraction in Fig. 2b**.** N^6-R-Ade is eluted together with adenine (their pKa values are very close, Table 1), both are separated on the column with sephadex G10. In acidulous media with unfavorable conditions for such regrouping, 1-substituted adenine can amount to 20% of the bulk of alkylated products (Fig. 1a).

Spectral characteristics of adenine alkylated at the exocyclic amino group and isolated both by thin layer (TLC) and paper (PC) chromatography (Table 2, Fig. 4) slightly differ from those of N^6-alkyl adenine isolated by HPLC. At the same time, both products have analogs reported in literature [22]. Thus, UV absorption spectra of N^6-(2-hydroxyethyl)adenine were analogous to those of adenine alkylated by thiotepa

without cleaving P-N bond (N^6-R^1-Ade), while UV spectra of N^6-Me- (or Et-) adenines correspond to that of the N^6-aminoethyladenine isolated in Silufol plates. Apparently, the N^6 of adenine is the only position for which UV spectra are determined not only by the alkylation site but also by the structure of the alkylating particle and possibly by conditions of product isolation (neutral medium in case of HPLC and alkalescent in case of PC and TLC).

Table 2. UV spectral characteristics of alkylated adenines isolated by TLC method. Solvent systems: A - methanol-ammonia (25%)-water (5:1:7), B - isopropanol-ammonia (25%)-water (7:1:2).

Sites of alkylation	pH	λ_{max} - λ_{min}, nm	R_f (against Ade), in system		A_{280}/A_{260} (isolated in system B)
			A	B	
N3(R^1)	1 12	275-236 273-2 44	0, 6-0,7	0,8	1,3 1,3
N^6	1 12	267-233 273(280)-244	(0.9)	(0.47)	(0.9) (1,1)
N3(R)	1 12	275-238 273-247	I .45	0,7	0,87 0.85
N9	1 12	258-231 2 61-233	1,16	0,85	0,23 0,16
Adenine	1 12	262.5-228 269.5-237	1.0	1,0	-
N7	1 12	272-235 270-234	-	1,15	-

The values in parentheses are presumably attributed to N1-substituted adenines, which were converted into N^6-derivatives under separation in alkaline medium (on their re-elution R_f=R_f adenine).

Besides the products isolated by HPLC, we isolated the minor product by means of PC and TLC. When bulk of the products of alkylation remained close to the start in a neutral medium (Fig. 4a, R_f 0-0.3), we managed to isolate the minor product with R_f 1.36 in sufficient amount. This product displays UV spectral characteristics of N7-alkyladenine. Since it goes before the adenine in chromatogram, its pKa is supposed to be lower than that of adenine. This is the second supporting evidence that this product is N7-substituted adenine, which has pKa 3.6 [22].

The analogous product was detected on the Silufol UV-254 (R_f 1.15, Fig. 4b) plates. It is noteworthy, that reaction mixture after alkylation in acidulous medium was divided into three spots apart from adenine. Apparently, these are the products of substitution with various alkyl radicals (R^1, R^2 and R^3, in the order of their hydrophobity decrease). The data obtained in different conditions of alkylation and separation showed that the main products of alkylation in neutral medium are formed without the cleavage of the P—N bonds of thiotepa, while the more mobile ones are formed through aminoalkylation and yield decreased with pH raising (Fig. 4b).

Thus, alkylation of adenine by thiotepa proceeds similarly to alkylation by other electrophilic agents at the N3, N9 and N1 positions with decreasing efficiency and with partial regrouping of the last into N^6-derivative in alkalescent media. Less effective alkylation may also proceed at the N7 position of heterocycle. Unlike the reactions with monofunctional alkylating agents (dialkyl sulfates, alkyl halides, alkylene oxides etc.), the reactions with thiotepa are rather dependent on pH, temperature and duration of reaction. Under reaction performed within the pH range 4.5-5.0 and at room temperature, the products with R radical were obtained. In neutral and alkalescent media (without proton donor) alkylation is preferentially realized by the uncleaved molecule of alkylating agent (R^1 radical). In acidulous media, where the alkylation reactions are generally performed, radicals of various types can appear, since the starting pH 4.5 can rise as the reaction proceeds. Such radicals can also be produced upon heating (up to 100°C and more) and long-term storage (about a year) of alkylated mixtures. Upon separation by reversed-phase HPLC, the modified products with R, R^3, R^2 and R^1 radicals were sequentially eluted from the column. Remarkably, the products with R^1 radical exhibit a higher antitumoral effect than those with other radicals [29].

The thiotepa-alkylated products of other heterocyclic bases were also obtained and then identified (Table 3). Guanine is preferably alkylated at the N9 and N7 positions and to a lesser extent at the N1 position. As in the case for adenine, UV absorption spectra for alkyl derivatives of guanine, cytosine and uracil appeared to be

practically identical for all the alkylating agents used (EI, MAEP, thiotepa) and the data obtained are consistent with the data of B. Singer review.

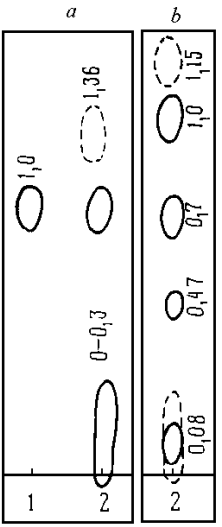


Fig.4. Separation in the system i-propanol- ammonia (25%) – water 7:1:2 ratio of adenine products alkylated by thiotepa (37°C, 24 h) on the paper Filtrak FN-12 (a) and plates Silufol UV-254 (b): 1 – adenine, 2 – reaction mixture. Numerals near the spots – their R_f comparative to adenine. Starting pH - 7 (a) and 4.5 (b). Operating conditions for experiment are presented in section "Materials and methods".

Pyrimidine bases of nucleic acids are alkylated much less effectively than purinic ones and with very small output (to 1-2%), preferably at the N3 position, while cytosine is also alkylated at N^4.

Thus thiotepa alkylated products of two purine and two pyrimidine bases were isolated and identified by reversed-phase HPLC and UV spectroscopy. Through alkylation of adenine by trifunctional alkylating agent thiotepa and its monofunctional analogs, monoaziridinediethylphosphate and ethyleneimine, it was found that adenine alkylation by thiotepa yields four types of alkyl radicals, while its alkylation by monoaziridinediethylphosphate produces two types of radicals**.** Our findings indicate the alkylation sites of heterocyclic bases and provide evidence for the structure of alkyl radicals. Adenine alkylation proceeds most efficiently at the N3, N1, and N9 positions of heterocycle, less efficiently at the N6 position (mainly due to

regrouping of N1 in weak alkaline and neutral media) and much less effectively at the N7 position. Guanine is preferentially alkylated with high activity at the N7 position (with insignificant imidazole ring cleavage) and with a much less efficiency at the N9 and N1 positions. Pyrimidine bases are slightly alkylated and preferentially at the N3 position, while the cytosine is also alkylated at the N4 position.

Table 3. UV spectral characteristics of alkylated guanines, cytosines and uracil isolated by reversed-phase HPLC.

Sites of alkylation	pH	λ_{max} - λ_{min}, nm	
		A	B
		Guanine	
N1	1	253(272) – 233	251(274) – 229
	12	277(261) – 246	278(260) – 243
N7	1	252(272) – 232	249(272) – 233
	12	283 – 257	280 – 258
N7*	1	262 – 230	262 – 221
	12	260 – 248	261 – 243
N9	1	253(277) - 231	251(276) -230
	12	256(269) - 241	(258)268 -238
		Cytosine	
N3	1	277 - 246	275 – 242
	12	297 – 258	294 – 254
N^4	1	280 – 248	277 – 244
	12	289 – 259	284 – 253
		Uracil	
N3	1	262 – 235	259 – 230
	12	281,5 – 248	218, 283 – 245

A – our data, B – data from B. Singer review [22].
* with cleavage of the imidazole ring leading to 5 – R and R'-pyrimidines.

The data provide evidence for alkylation sites and the structures of alkyl radicals.

Thiotepa as a polyfunctional alkylating agent with antitumoral effect was distinguished by cross-linking of DNA double strands. There is no literature data on the chemical nature of cross-linking induced by thiotepa. In our study, we failed to establish cross-linking between two molecules of any base under alkylation by thiotepa. Most likely, thiotepa induces cross-linking only in DNA, one molecule of

thiotepa alkylating two guanines at the N7 position of heterocycle, as shown in case of "nitrous mustard gas" [30].The obtained data provide evidence for alkylation sites and the structures of alkyl radicals.

Based on DNA and RNA preparations, their components and analogues, the biotechnology has been developed for regulation of biological process and accelerated production of new forms, varieties and species of agricultural and medicinal plants resistant to extreme environmental factors: drought, frost, salinity and nitrogen-depleted soils. The objects of our study are natural substances, life products of microorganisms, plants, fungi, insects and animals. These are alkaloids, biogenic amines, aminoacids, proteins, carriers of hereditary information – nucleicacids and their precursors.

The complete understanding of the therapy mechanism action of drugs is impossible without studies of the interaction of these compounds with biological objects on the molecular level. In cooperation with Physics Department.Kyiv National Taras Shevchenko University the luminescent manifestation of the DNA – amitozyne's alkaloid interaction were performed

The absorbtion, fluorescence and phosphorescence of amitozyn were studied in water solution without and in presence of the DNA. The fluorescence maximum amitozyn without DNA depends on excitation wavelength but fluorescence maximum amitozyn in presence DNA doesn't depend. Simultaneously the fluorescence intensities increase approximately 10 times (see Fig.5). This phenomenon is connected, to our opinion with the adsorption one of the one amitozyn's alcaloid on the DNA macromolecules (amitozyn molecule consists from several alkaloids).

According to our investigations the triplet excitations in DNA are localized mainly on amitozyn's alkaloid – berberine (the phosphorescence spectra DNA+berberin are very close to berberine water solution spectra). It was obtained from studies of the phosphorescence dependence of DNA+berberine solution on berberine concentration, that average value of the triplet excitation displacement at least reaches the 20 base sequence length (7 nm.) [38]

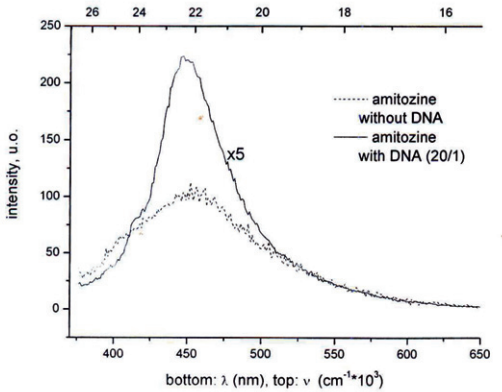


Fig. 5. The fluorescence spectra amitozine (amitozyn) and DNA+amitozine water solutions, T=293 K, λ_{exc}=366 nm.

These data open the way to establish the molecular mechanism of the amitozyn therapeutic action.

Nucleic acids (NA) and their several components (bases, nucleosides and nucleotides), being initially biologically active substances, after their alkylation with substances possessing antitumor action, obtain another antitumor effect, but practically without mutagenicity that is inherent to alkylating agent.

The objects of study are natural substances, synthesized by microorganisms, plants, fungi, insects and animals. These are alkaloids, biogenic amines, amino acids, proteins, nucleic acids as carriers of hereditary traits and their precursors. Due to our new method of a directed change of natural molecules through alkylation, it is possible to enhance the protection of living beings against hazardous environmental factors, to prevent mass virus and microbe diseases, including tumors, immunoagressive and immunodeficiency states. Particularly we should note the possibility of regulatory effect of such substances upon hereditary diseases and radiation damages.

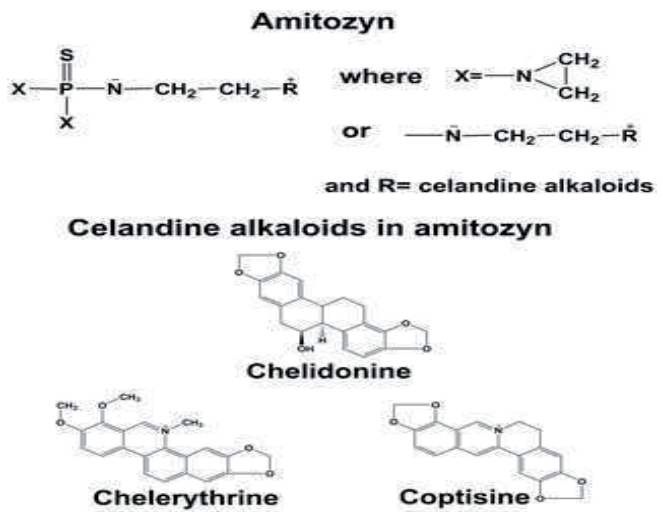
Among promising alkylated derivatives of alkaloids of plant origin and biogenic amines of animal origin there are preparations berberin, sanguinarine,

chelidonine, quinine, vinblastin, salsoline, colchamine, adrenalin, noradrenaline, serotonin and some others.

Especially promising preparations were devised on the basis of alkylated nucleic acids and their precursors and analogues (nucleorhexin, the first preparation of the type, created by A.I. Potopalsky, 1979) and they possess antitumor, antiviral, antibacterial, radioprotective and immunoregulatory actions. The substances of this new class can be used for the correction of severe hereditary diseases and disorders in the creation of novel resistant to hazardous weather conditions plant lines.

Thus, in the number of our works, devoted to NA components alkylated with different agents it was determined that molecules, modified with polyfunctioning alkylating agents, depending on the conditions of reaction's carrying out, may contain different alkyl radicals [28-30]. This fact concerns, first of all, trifunctional alkylating agent thioteph (N',N",N"' – triethylenethiophosphoramide, thiophosphamide) that is able to give four types of alkyl radicals as sequential aziridine cycles breakdown (Scheme 6).

Scheme 6



The molecules, modified by R1 radical, have the highest antitumor activity, and the lowest – R radicals [39]. This can be explained by the fact that preparations with R1 radical are not only alkylated as the preparations with R radical, but also themselves have property to alkylate i.e., likewise thioteph, are the alkylating agents.

From the table given below, it is seen that thioteph, which until now used in clinics as an antitumor preparation, has lower effectiveness than alkylated DNA (DNT) and also than its several components.

In the experiment on Ehrlich's ascites tumor (EAT) cells of the mice *in vitro* it was shown that DNA and especially its guanine nucleotide (GMP) are the stimulants of the tumor growth, whereas DNT inhibits the growth of tumor cells more than even antitumor preparation thioteph (Table 4).

On the adenine example, it is also shown that not so much the fact of alkylation is as of an importance, rather than the place in basis molecule, where the reaction took place. The most active preparations among investigated ones are the adenine basis and nucleoside, modified by nitrogen in N1 thiotheph position with one open aziridine cycle (radical R1). N1-substituted guanosine nucleoside and nucleotide with R1 radical have rather less activity (besides, comparable to thioteph antitumor activity, but without its mutagenic effect).

Antitumor action of the thioteph alkylated DNA preparations of different derivation (cattle spleen, salmon sperm, chicken erythrocytes), yeast RNA and mononucleotides – ATP and GTP proved in the experiment *in vivo* on the mice with transplantable Ehrlich's carcinoma. Therapeutic doses (100 – 300 mg/kg of weight) of the mentioned preparations provide inhibition of tumors growth on 90-100% at toxic LD_{50} up to 2 g/kg.

Tabl 4. Influence of the biologically active preparations on labeled nucleosides inclusion in NA of EAT cells in vitro*

Preparations	Nucleosides inclusion in % comparing to control	
	^{3}H-thymidine	^{3}H-uridine
EAT DNA (50 mkg/ml)	105.1 ± 11.7	102.0 ± 7.3
EAT DNT (50 mkg/ml)	46.0 ± 4.0	70.7 ± 21.1
Thioteph (1.5 mM) mkg/ml)	57.0 ± 16.4	80.1 ± 3.7
1-R-adenine	97.1 ± 3.6	87.4 ± 9.6
1-R1-adenine	39.4 ± 4.2	78.6 ± 11.7
3-R-adenine	96.3 ± 4.5	92.5 ± 5.7
3-R1-adenine	56.4 ± 7.7	83.4 ± 6.5
9-R-adenine	93.6 ± 4.8	98.6 ± 8.4
9-R1-adenine	74.0 ± 4.9	101.8 ± 9.1
1-R1-adenosine	37.3 ± 10.7	55.7 ± 7.8
AMP	96.0 ± 1.3	48.6 ± 5.2
1-R1-AMP	76.5 ± 4.6	101.5 ± 8.5
Guanosine	84.0 ± 6.3	118.0 ± 4.3
7-R1-guanosine	52.2 ± 6.8	86.6 ± 7.3
GMP	203.5 ± 15.7	123.0 ± 16.5
7-R1-GMP	57.9 ± 4.7	74.5 ± 3.2

*1. The time of cells incubation with DNA, DNT, thioteph and other preparations (the upper part of the table) – is 30 min, with NA alkylated monomeric components – 90 min.
2. The time of incubation with labeled nucleosides: thymidine – is 105 min, uridine – 20 min. 3. R – aminoethyl, R1 – phosphaminoethyl radicals (scheme). 4. Concentration of the preparations (except for mentioned in the table) – 1 mM.

The introduction on the later stages (for instance, on the 8th day) is not effective, as it is accompanied by intensive tumor cells death that leads to high intoxication of the animals and to their death.

Antitumor and immunomodulating activities are inherent to amitozyn antitumor preparation. But the mechanisms of its influence on the cell are not completely elucidated. Thus, studying of the mechanisms of amitozyn and its alkaloids action is of current importance, as it gives new possibilities of amitozyn wide use in clinics.

So, to elucidate molecular mechanisms of preparations interaction with DNA and RNA, luminescent and spectrophotometric analyses, that are the most informative in biopolymers and ligands interaction, were used. In this investigation for studying of competitive connection of two ligands and DNA. we used amitozyn, berberin chloride (Bch), thymus DNA, transfer RNA, ethidium bromide (EB) and actimicine D analog – ActH.

Fluorescence and phosphorescence spectra were registered using spectrometer, constructed in laboratory (of Kyiv Taras Shevchenko National University), and "Cary Eclipse" and "Hitachi" (Model 850) spectrofluorimeters. Absorption spectra were recorded using Specord UV VIS spectrophotometer.

The research of lymphocytes blastogenic response revealed that amitozyn, in therapeutic doses, significantly stimulates DNA synthesis [40]. This fact suggests that amitozyn interacts with DNA. Researches of fluorescence and phosphorescence of amitozyn water solutions and one of the preparation alkaloids – berberin, at DNA presence, showed that amitozyn fluorescence spectrum depends on the wave length of exciting radiation (that reveals the preparation as an multi-component one) [41]. Nevertheless, berberin fluorescence spectrum, as it was expected, does not depend on the exciting wave length. Furthermore, it was determined that berberin fluorescence intensity growths at DNA presence approximately 60 times more comparing to berberin water solution without DNA. In our opinion, this fact may be connected to DNA binding with berberin molecule (probably by intercalation). This effect, as is well known, related to decreasing of radiationless relaxation exciting probability and corresponding increasing of quantum yield of fluorescence. Similar effect is observed also for the amitozyn water solutions, however, the obtained results analysis indicates that not only berberin among all amitozyn alkaloids interacts with DNA. Amitozyn fluorescence spectrum at DNA presence differs from bound with DNA berberin fluorescence spectrum, although, fluorescence intensity of the preparation significantly increases. To identify amitozyn components, binding with DNA, further researches is necessary.

Investigation of phosphorescence spectra showed that triplet excitation is transferred through DNA macromolecule and localizes on berberin molecules (berberin phosphorescence spectrum has insignificant difference comparing to phosphorescence spectrum of berberin with DNA). Investigation of phosphorescence intensity depending on berberin concentration ratio and pairs of DNA bases revealed that minimal distance of the excitation effect is 20 pairs of DNA bases or 7 nanometers (3).

Thus, the data described above prove amitozyn and berberin interconnection with DNA. Therefore, the purpose was to clarify the way of amitozyn and berberin binding to DNA. One of the approaches was spectrophotometric analyses of visible and ultraviolet spectral regions. Different DNA concentrations and permanent concentrations of the preparations were used. As the spectrophotometric measurements showed, absorption band displacement took place in the visible and ultraviolet spectral regions, together with its intensity decreasing and appearance of isobathic points. This fact indicates the connection of these substances with DNA matrix and presence of the only one connection type in the field of P/D small values. Such spectrophotometric manifestations are typical for ethidium bromide (EB) binding with thymus DNA, and forming of the complex of intercalation type is typical for the last one. And it is significant, at the high DNA concentrations, Bch spectra of compounds absorption do not pass through isobathic points, that indicates forming of the other type of complexing connection.

The exact constants and values of preparations connection, using different methods of calculating programs, were determined. It is essential to notice that constant of EB connection with thymus DNA was 2 times higher comparing to alkaloids we had studied.

For obtaining fundamental understanding of mechanism of amitozyn alkaloids and DNA connection, we have conducted research of competitive connection of its Bch alkaloid and DNA at EB intercalator presence. The research results showed that

EB influences on Bch and DNA connection and is collateral for the place of connection.

Using spectrophotometric and fluorescence methods we showed that at binding amitozyn preparation with DNA, other type of connection is carried out – furrow binding.

Study of berberin alkaloid and tRNA spectral interaction showed that spectrum shifts from 480 nm up to 535 nm and at the same time fluorescence intensity increased. Calculations of association constant and stoichiometry of binding, conducted on one of the category of Bch binding site on tRNA molecule, showed that at alkaloid and tRNA interaction, specific Bch complex with tRNA ordered space structure has formed. And it is significant to notice that stoichiometry indicates the interaction of at least 3 ligand molecules with nucleic acid. Nature of the spectral changes indicates possible intercalation mechanism of planar cation Bch molecule between nitrogen bases areas of helical tRNA sections. In this case, Bch and tRNA interaction identifies with this intercalator and DNA connection, described above[42].

Thus, the mentioned data show availability of use the alkylated DNA preparations of different derivation, modified by multifunctional alkylating agents, in particular – thioteph as antitumor drugs

1.2. The role of Amitozyn, an antitumoral and immunomodulatory preparation, in the tumor growth correction system.

For over fifty years this preparation has been used independently and in many complexes for malignant tumors treatment. The clinical test of amitozyn, conducted in 1967-1968 in former USSR, proved its high medicinal properties in tumors of larynx, neck of womb, prostate, ovaries, pancreas and mammary gland, urinary bladder, melanoblastoma etc., as well as in non-malignant growths (polyps, papillomas, adenomas, fibromiomas). These data were supported by clinical tests of amitozyn, conducted in 1998-2001 according to the Ministry of Health of Ukraine on the basis of the Oncology and medical radiology research Institute of the Academy of Medical Sciences of Ukraine. Antimicrobial action of amitozyn was determined on

staphylococcus mutant 209 UV-2 and UV-3 , intestinal strain O-111 and Bacillus dysenteriae sonnei.

The toxicity of amitozyn is several times lower than of some of its components – the sums of greater celandine alkaloids and thiophosphamide. And high antitumor activity of amitozyn in the experiment (Table 5) was proved in its clinical test on patients with fatal forms of malignant tumors (Table 6). Complete clinical recovery was observed in 17-18% cases, whereas observed contraction of tumors and life prolongation constituted 75% cases. Complications common to most antitumor preparations such as sanguification inhibition, dyspeptic effects, baldness and others were not observed. At the same time characteristics of immunogram normalized and specific cancerous antigens disappeared or decreased dramatically. Inherited resistance effect in rats race cured with amitozyn from Heren cancer was determined.

Amitozyn has been tested in industrial conditions at battery farms for birds viral laryngotracheitis treatment and also experimentally for pigs viral enteritis. Its high specific activity and economic effectiveness for veterinary have been proved.

Amitozyn was proved to possess antiviral action in myeloblastosis and birds viral laryngotracheitis, lymphatic leukemia in baboons and grippal pneumonia and herpesviral meningitis in mice. Also, together with doctors, we have determined its high effectiveness in viral papilloma, hepatitis, enteritis and arthritis.

Table 5. Spectrum of antitumour action of amitozyn on experimental tumors of rodents (5 hypodermic injections of 100 mg/kg every other day)

Tumor strain	Rodents group	Day of the treatment beginning	Tumor mass	Therapeutic effect	
				% T	Ief
Sarcoma-37		- 6^{th}	1,46 ± 0,03 0,20 ± 0,02 p<0,01	75,5	4,08
Lymphoma NK/Ly	Control Experiment	- 2^{nd}	0,078 ± 0,099 0,25 ± 0,024 p<0,001	67,25	3,12
Ehrlich's carcinoma	Control Experiment	- 4^{th}	0,80 ± 0,11 0,31 ± 0,018 p<0,05	61,25	2,58
Lymphosarcoma LIO-1	Control Experiment	- 2^{nd}	1,57 ± 0,14 0,66 ± 0,14 p<0,01	57,96	2,38
Melanoma Harding-Passie	Control Experiment	- 6^{th}	0,056±0,017 0,026±0,009 p<0,02	52,60	2,15
Hemocytoblastosis of Tailor-rats	Control Experiment	- 2^{nd}	18,1 ± 1,7 0	100	

Table 6. Overall result of amitozyn treatment of patients with head and neck malignant tumors. (survival, %)

Survival	Patients group	
	Control group	Main group
2-year	52,2 ± 4,2	91,9±3,5*
3-year	47,0 ± 5,0	70,1±5,0*
5-year	40,2 ± 5,2	66,1±6,6*

1.2.1. Connective tissue systemic disease

In collaboration with O.S. Abrahamovich, a doctor from Lviv, amitozyn was proved to possess a high medicinal activity in viral infectious polyarticular rheumatoid arthritis and did not inhibit sanguification and immunity in patients but even increased them mobilizing the organism. It is the first preparation of a new class of phytolytic substances – products of alkylation of amines with various actions.[43]

Abrahamovich has worked out the method of complex amitozyn treatment of connective tissue illnesses. Such treatment is based on specificifical changes in joints

synovium under infectious nonspecific evolution polyarthritis. Amitozyn is a perspective preparation in case of all diseases and states that are accompanied by connective tissue excrescence and immune system dysfunction.

The main infectious polyarthritis manifestations [IP] – are inflammatory joints changes that, unlike classical chronic inflammation, characterized by clinical coursing stability and ability to "self-sustenance". The presence of immune gamma globulin 7 and 19 in the synovial membrane, the complement utilization and the lymph-plasmocytic infiltrations existence indicate that synovial membrane processes have all typical immunologic inflammation patterns.

Morphologically synovium inflammatory changes at IP are hyperplastic process as a consequence of which villous excrescence form and ramify, at that tissue villus are sharply vascularized and infiltrated by lymphoid and plasmatic cells, which form along vessels lymphoid and plasma cell infiltrations of a follicle type.

Gamma-globulins – normal and pathologic (immune gamma globulins of the rheumatoid factor type) produced only by lymphoid tissue cells (plasmatic). It is naturally that their redundancy in the IP of the affected joints at III is a source of the pathologic immune globulins constant formation, and its surgical removal (sinovectomy) or by conservative way with use of antineoplastic (cytostatic) preparations is completely justified and scientifically valid.

One of the arguments of such opinion and amitozyn use at IP correctness is an experience of the domestic and foreign researchers, that in certain cases after total removal of the pathologically changed synovium (with presence of the dense plasma cell infiltration and its depositing of the rheumatoid factor in the membrane), achieved a considerable improvement of the clinical course as a whole and even recovery, while use of the local and general corticosteroid therapy resulted only in decreasing of the inflammatory processes acuity in the affected joint.

Surgeons, based on this fact, offer to conduct total sinovectomy with the aim of removal the lesion focus that is the main source of pathologic immunoglobulin.

Thus, the radical cure experience after total sinovectomy conduction proves not only modern opinion concerning pathogenesis of the infectious non-specific polyarthritis (infectional arthritis) appropriateness, but also maintains correctness and validity of a new pathogenetically proved method of the bloodless radical treatment of this severe disease with use of antineoplastic agent amitozyn.

The offered method has a character of partial etiopathogenetic therapy and is a method, more radical and effective comparing to the other ones, and can be compared only to the radical surgical operation – sinovectomy, widely recommended by the Research Institute of Rheumatism and Central Research Institute of Traumatic and Orthopaedic Surgery, but in general the patients refuse this operation.

Clinical practice showed that infectional arthritis patients' treatment with glucocorticoids at this stage is not completely justified as an independent method of treatment, because it doesn't give stable effect, sharply inhibits body resistance and at durable use brings on hypercorticism side effects.

After scientific substantiation and elaboration of the method, we first used amitozyn as immunomodulator and antiviral preparation in complex treatment of the serious forms of connective tissue systemic disease (CTSD): rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic dermatosclerosis (SDS), systemic vasculitis (SV), and systemic dermatosis – rosacea disease (RD) of the third and fourth stages. Further researches of amitozyn mechanism of action proved its evident antiviral, immunomodulatory, antiserotonin effects on malignant cells and pathologically changed synovial membranes that confirmed our conception appropriateness in the complex treatment of CTSD.

122 patients of the examined group were observed and treated (women – 100, men – 22, from the age of 21-56 years old, among them 79 – with RA, 11 – with SLE, 12 – with SDS, 9 – with SV, 11—with RD) with antecedent anamnesis beginning from 1 to 10 years. amitozyn at dose 0.05 g was introduced intramuscular

in a day, 20 injections per course. Along with this course, seriously ill patients with RA had the extra intra-articular introductions from 25 mg to 50 mg depending on affected joint sizes 1 time in 10 days – 5 injections per course. The first course was conducted stationary, the next 5-6 courses with one month breaks – on an outpatient basis. The treatment efficiency was evaluated directly after the ending of the 1st course and during 30 years observation after the end of several courses. Clinical-functional, laboratorial-biochemical and immunological criteria and also remissions duration and work capacity indexes of the treated patients were used. It was determined that all the AM treated patients had positive short and long-term treatment results: excellent effectiveness had 2.08 %, good one – 79.17 %, satisfactory – 18.75 %, unsatisfactory and lethal results were not registered.

In control group (the analogous examined group of 106 patients that were treated without amitozyn use) were the same indexes: 0.91%, 68.04%, 27.85%, 2.74%, and 0.46% correspondingly. Stable clinical-morphologic remission lasted on average 14.9 ± 1.1 years of the patients with RA, 12.7 ± 3.7 years – with SLE, 9.6 ± 1.2 years – with SDS, 11.5 ± 1.5 years – with SV, and 10.7 ± 1.3 years – with RD that allowed in most cases to reduce dose or to cancel nonsteroid antiinflammatory preparations and glucocorticoids. Work capacity was saved in 79.17 % cases of the treated patients.

The above proves high amitozyn effectiveness and allow recommending its use in complex treatment of the serious forms of connective tissue systemic disease.

1.2.2. Mechanism of amitozyn action

To study the mechanism of action of amitozyn, the experiments have been performed with Department of General Surgery, Bohomolets National Medical University, Kavetsky Institute of experimental pathology, oncology and radio-biology of National Academy of Sciences of Ukraine on classical experimental subcutaneous transplantable tumors: Gu-rin's carcinoma and sarcoma 45 on rats. [44] Tumor transplantation and treatment were conducted according to commonly-accepted methods. Investigations were aimed at determination of the concentration of

malonic dialdehyde (final product of lipid peroxidation as markers of free radical particles formation) in both tumor and normal tissues, the concentration of reduced glutathione that characterizes the ability of the tissue to react upon deleterious for the organism factors (for instance, free radical particles) and neutralize them, it can also be used as an indicator of a tumor reaction to antitumor influence; the activity of glutathione-S- transferase that is involved in the neutralization of hazardous substances and metabolism products. Also, a tumor reaction to the preparation has been defined through the determination of a tissue bioenergetics level using ^{31}P NMR spectroscopy. The given above biochemical indicators of a tissue metabolism are rather characteristic for determining any antitumor agents both in experiment and in clinic. It was strongly shown that antitumor preparations or substances, which are studied as promising antitumor agents, lead to the reduction of concentration of reduced glutathione in tumor and the activity of glutathione-S- transferase. They also cause a dramatic decrease in the level of a tumor bioenergetics potential. The outlined above indicators are being used in clinic for the determination of typical signs of a positive tumor response to chemo- and radio-therapy, treatment effectiveness and its prognosis. The concentrations of malonic dialdehyde, reduced glutathione, the activity of glutathione-S- transferase were determined according to conventional biochemical methods. The signals ^{31}P were registered by NMR spectrometer 300 (Varian Mercury 300, USA). The data obtained were statistically processed.

The influence of the used independently preparation as well as combined with hyperglycemia, local and general hyperthermia and in a complex with chemotherapeutical preparation platydiam has been studied.Artificial hyperglycemia does not level the anticancer effect of amitozyn, at the same time we cannot state that when combined with artificial hyperglycemia the effect of the preparation was enhanced though in some cases we observed a strong enhancement of amitozyn action on epithelial neoplasms. We suggest applying amitozyn in an experiment with animals at dose 20.0 mg/kg mass, administration intravenously every day or every other day.

The studies on a combined application of amitozyn and platydiam have proved to offer greatest promise (Table 7).When combined with amitozyn, platydiam has been found to increase its action on the background of almost absolute absence of the toxicity of platydiam action in rats with epithelial neoplasms. At this dosage change factor was 2.0.

Table 7. Results of the treatment of rats with Gu-rin's carcinoma with amitozyn (20mg/kg.) and platydiam

Treatment	Tumor size before treatment (cm^3)	Tumor growth inhibition, (%)	Animals cured (%)	Average life (days)
Control (3)	1.0	--	--	28 (16-38)
Amitozyn + platydiam (8)	1.0	100 complete tumor regression – 75%	***	***
Platydiam (6)	0.7	90 complete tumor regression – 0	0	21 (16-44)

Notes
***- given mean values, deviation of parameters is given in brackets; TGT – tumor growth time (time during which tumor reaches 25 cm^3), TGIT – tumor growth inhibition time (difference in time when tumor reaches 25 cm^3 in control and experiment), AC- animals cured, AL – average life of rats starting from day of tumor inoculation.
Amitozyn was administered at 20 mg/kg of mass weight 60 min before platydiam every day, total injections – 10, platydiam was administered 3 times in both groups; platydiam with amitozyn administered on day 1,4,7 of treatment; total number of animals is indicated in brackets.

The action of amitozyn has been observed to greatly increase when it was combined with local microwave hyperthermia in the treatment of rats with sarcoma45.

We have established that the concentration of malonic dialdehyde (MDA) in sarcoma 45 showed no increase after amitozyn administration. This indicates the absence of lipid peroxidation activation which can evidence the absence of free radicals formation in tumor tissue under the administration of amitozyn into a tumor bearing organism. These conclusions are valid for normal tissues as well.

The content of reduced glutathione was somewhat increased. This indicates the involvement of glutathione system in the neutralization of some metabolites but not the products of free radical oxidation. Special attention should be given to an increase

in reduced glutathione content in kidneys after amitozyn introduction. This may be an indication of some toxic effect on kidneys.

On the whole the data arrived at allow to conclude that the preparation does not activate lipid oxidation and it does not induce free radicals formation, while it can induce a certain toxic effect on kidneys. Therefore, based on the data on the content of MDA and reduced glutathione in tumor, the conclusion about a direct cytostatic action of amitozyn at given dosage cannot be made.

The administration of amitozyn was found to result in an increase in the content of MDA in Gu-rin's tumor that indicates lipid peroxidation activation. Of special interest is a similar effect of amitozyn observed in kidneys.

The content of reduced glutathione has been found to reduce in tumor located in the liver as well. It can be an indication to the activation of the system of glutathione, which serves as a cell protection system against various deleterious metabolites, including the products of lipid peroxidation. The validity of our assumption is supported by the absence of changes in the content of reduced glutathione in kidneys on the background of an increase in MDA concentration. On the whole these results suggest a direct action of amitozyn on Gu-rin's carcinoma, which manifests itself in a certain cytostatic effect, about which one can judge on the basis of changes in MDA and reduced glutathione content. We should seek for the proofs in direct experiments on the determination of a cytostatic effect of amitozyn on tumor cells. Also, it is noteworthy that in contrast to sarcoma 45, Gu-rin's carcinoma is epithelial neoplasm, which may suggest different mechanism of amitozyn action on cancerous neoplasms and sarcomas.

Amitozyn exerts a certain anticancer action under a rather prolonged administration. With regard to quantitative characteristics this effect can be considered moderate, though in some cases a complete tumor regression was observed. It is of great importance that this effect had a more pronounced character on connective tissue tumors

It has also been found that amitozyn has no inhibitory action on the bioenergetics of muscular tissue. At the same time certain changes of bioenergetics level take place in tumor after the introduction of amitozyn. On the whole they can be characterised as a moderate reduction in bioenergetics level, which at the same time cannot be unambiguously attributable to an inhibitory effect of amitozyn on the energy maintenance of a tumor cell functioning under the administration of amitozyn at given dosage and regimens.

The data obtained allow us to make an assumption that amitozyn antitumor effect is based first of all on immune modulatory effects which may turn out to be non-classical. We can also assume that amitozyn influences vitally important systems of tumor, inhibiting their functioning, which leads to a considerable decrease in tumor vital activity, delaying its growth and removing a tumor toxic effect on the organism. On the basis of the experiments with animals in which the retardation of tumor development was observed after amitozyn treatment was initiated, but tumor did not disappear completely, whereas the general condition was rather satisfactory and so they lived much longer than the control ones, we can make a suggestion that amitozyn exerts a certain biological effect both on tumor and a tumor bearing organism, which leads to a long coexistence of tumor and the organism or to the organism living with tumor. The possibility of this phenomenon and its therapeutic significance were pointed out by M. fon Ardene (1980). The mechanism of this state still needs understanding but its therapeutic value could be of great importance for the treatment of patients with extensive tumors and metastases with amitozyn.

1.2.3. Amitozyn in pancreatic cancer treatment

In the developed countries, pancreas cancer ranks fifth of all the tumors and second of digestive system tumors. Common disease incidence is 2 to 3 % of common oncological one, and the mortality rate is 4 to 5 %. Compared to other tumor localizations, the results on pancreatic cancer treatment are among the worst. Thus, half of the patients die in the period of 4 to 6 months after being diagnosed, and a five year survival is never more than 3% [45].

The absence of early_pathognomonic symptoms of pancreatic cancer explains its late diagnostics even when jaundice has been found in 80,6% patients, which to great extent limits the potentialities of surgical and chemotherapeutical options [46,47].

In Bohomolets National Medical University and Kyiv Center for liver, biliary tracts and pancreas surgery the elaboration and assessment of the application of preparation amitozyn in patients with pancreatic cancer complicated with jaundice was carried out by O.I.Dronov, Ya.M. Susak, E.A. Kryuchina.[48]

Their experimental investigations were made after the patients being operated on hepaticojejunostomy, in some cases, in the combination with pancreatojejunostomy and gastroenterostomy. After the morphological verification of the diagnosis, in 45 patients with pancreas cancer (experimental group), complicated with jaundice, the course of treatment with amitozyn was initiated intraoperatively or starting from day 3 in the postoperative period. In the case when the patients refused chemotherapy or when there were no indications for such therapy, the courses were conducted at a two months interval. Amitozyn was prescribed to stage III and IV patients. The preparation was administered by intravenous injection at 25 mg. in 15 ml. physiologic saline every other day until the total dose reached $\sum$=250 mg. Local negative manifestations were not revealed at the administration of the preparation. In 33 (73,3%) patients the 2 stage temperature reaction was observed. The temperature rose 3±1 h after the administration, remained steady for 6±2 h and afterwards dropped without any analgesics. In the course of therapy the patients showed no signs of intoxication. All the patients received the first course of amitozyn therapy in clinic. Afterwards the therapy was conducted on an outpatient basis with a permanent observation of a clinical picture, control of hematologic indexes and oncomarkers (CEA, CA 19-9). The control group consisted of 33 patients with pancreas cancer complicated with jaundice of about the same age, tumor development, severity rate and surgical operations conducted, who for various reasons did not receive chemotherapy.

At amitozyn application no negative biochemical and hematologic disorders in experimental and control groups of patients were revealed.

After the course of amitozyn treatment only the number of leucocytes increased with certainty ($p<0,05$) and mainly due to lymphocytes. Blood biochemical indexes were studied in the patients who received amitozyn in an early post-surgical period and in those of a control group. The biochemical examination indexes of the experimental group patients did not differ from those of the patients of the control group, which proves the experimental data concerning the non-toxic action of therapeutic doses of amitozyn.

Of special interest is a pronounced immunomodulatory action of amitozyn in its application at therapeutic doses .

Positive changes in the immunogram were registered in 38 (84,4%) patients who received amitozyn. It have been determined that when an immunogram index was within the norm or near normal limits , it did not change essentially after the course of treatment. And vise versa – the more the immonogram index deviated from the norm, the more it was observed to get normalized after treatment. Positive changes in immunological indexes were registered after the first and second courses of treatment. After that statistically reliable changes in immunological parameters were not noted.

The dynamics of changes of some oncomarkers was statistically reliable. The oncomarkers parameters tended to reduction during the courses of treatment. The studies of CA19-9 level demonstrated its actual reduction after first and second courses of treatment. This was well consistent with the amelioration of the patients' general condition and the absence of ultrasound diagnostics and computer tomography signs of oncologic disease progression. However, after the third course of treatment CA19-9 indexes were not informative. CEA value had a general tendency to reduction in the course of treatment, prior and after particular courses of therapy the changes of its value were not registered.

At the terminal stage of disease on the background of a pronounced progression of oncological disease the induces in 28 (62,2%) patients had normal or reduced values, which indicates the absolute absence of information about them during this period.

The lifetime of the experimental group patients with non resection pancreas cancer was 10,5 months, which is 3,5 months more than in the control group ($p<0,05$).

So, observations showed that in therapeutic dose amitozyn reveals no toxicity and so can be used for hyperbilirubinemia and at the symptoms of liver impairment. In the analysis of clinical symptoms of disease and data of laboratory and biochemical analyses no negative local and general reactions at amitozyn application were registered. At the same time the changes of values of main induces of immunogram revealed an immunomodulatory action of amitozyn that is important for liver impairment. The positive dynamics of CA 19-9 has proved a carcinostatic action of amitozyn. Patients life prolongation with non resection pancreas cancer on average 3,5 months ($p<0,05$) is the most important amitozyn cure rate.

Pancreas ductal adenocarcinoma is among the first ten most common causes of cancer mortality A great deal of publications on medicinal preparations of Chelidonium majus L alkaloids, amitozyn in particular, indicates that this preparation is accumulated in ontologically transformed tissues [40.] which furthered the research on therapeutic pathomorphism after it has been used on patients with pancreas cancer.

The study of amitozyn effect on therapeutic pathomorphism of pancreas cancer was conducted by O.I.Dronov, Ya.M. Susak and E.R. Deneka on the clinical base of Kiev centre for liver, bile duct and pancreas surgery, in the Department of General Surgery №1, Bohomolets National Medical University on 16 patients who underwent treatment [49]. Therapeutic pathomorphism was detected by comparing the histological material, obtained after the first operation and that after the second operation or at the section. The histological preparations, treated in a standard way, were stained with hematoxylin and eosin, trichrom according to Mason,

mucopolysaccharides in sections were revealed by PAS-reaction and mucous substances by mucicarmine.

Of all the patients, 12 of which had a second surgery on account of duodenal obstruction in the period of 5±2 months and in 4 more cases a fatal outcome was observed. The patients received 3±1 course of treatment with amitozyn. The preparation was administered daily intravenously at 25 mg, total dosage per a course was ∑=259mg intravenously.

The interval between courses was approximately 1,5 months. The changes observed in the patients were of the same character which gave some grounds to conclude about certain peculiarities of pathomorphism signs.

Therapeutic pathomorphism of pancreas cancer under the effect of amitozyn was observed on both tissue and cellular level.

Therefore, the therapeutic pathomorphism of pancreas cancer under the influence of Chelidonium majus L. alkaloids is a positive and objective phenomenon that has its properties and peculiarities. There is no doubt that like any other pathomorphism, the phenomena, have a paraspecific character. At the same time, the recurrence, systematic and cognizable character of the changes that have been morphologically investigated here, allows to make conclusions about the carcinostatic effect of amitozyn on pancreas cancer.

1.2.4. Liver cancer

Primary liver cancer is a rather rarely encountered disease. According to different statistics, its incidence rate varies from 0,2 to 3 % of all cancer cases. Males aged from 50 to 65 are most susceptible to the disease. As a rule, patients with liver cancer are referred to incurable and are to undergo symptomatic therapy. Average lifetime is 3 years in operable cancers. Five-year survival rate averages 20%. With regard to inoperable tumors average lifetime is 4 months after diagnosis. Chemotherapeutical preparations administered intravenously practically have no

effect. The administration of preparations into hepatic artery has given somewhat better results.

The case of treatment of hepatocellular adenocarcinoma with amitozyn was described by O.I. Dronov, Ya.M. Susak, R.S. Tsymbalyuk from the Department of General Surgery №1, Bohomolets National Medical University in Kiev[50].

The 14 ears alld patient with revealed tumor in the right part of the liver (the tumor measured 10,0x10,0cm., located in 6-7-8 segments). refused chemotherapy.and underwent a preoperational course of carcinostatic immunomodulating therapy with amitozyn with a total dosage 125 mg., combined with an intensive vitamin-therap The diagnose: primary liver cancer $T_3N_0M_0$ III-A stage, II clinical group.

The patient was made right hemihepatectomy. The tumor occupied a whole right part of the liver, measured 10x12x7cm, non-uniform, with signs of infiltrating growth. The tumor was removed within the boundaries of healthy issue.

During a month in the postoperative period the patient received a course of amitozyn therapy at a total dosage 375 mg.

During a yaer the patient received two more courses of a carcinostatic therapy with amitozyn at 250 mg each one.

Next year an ultrasound examination revealed an infiltrate in the right part of the liver (recurrent tumor),. During two monthes the patient received a course of amitozyn therapy at a total dosage 500 mg. After the treatment had been carried out, an ultrasound examination and computer tomography revealed no signs of recurrence and metastases.

The following two years the patient underwent amitozyn treatment twice a year at a course dose 250 mg.

Next year the patient showed signs of partial duodenal obstruction.

After the course of cytostatic immunomodulating therapy with amitozyn at a total dosage 500 mg, the signs of partial duodenal obstruction disappeared.

The computer tomography diagnostics did not reveal retro peritoneum space infiltration.

So, during five year the patient felt satisfactory, had no complaints, her weight stabilized, treatment courses toxic clinical and laboratory signs were not revealed. At present Karnovsky index is 100 %.

This clinical case demonstrates the effectiveness of the preparation in treating hepatocellular carcinoma after a combined therapy with amitozyn and its possible application as an alternative option to a routine chemotherapy.

1.2.5. Skin melanoma

Melanoma is the tumor of a low-sensitivity to cytostatic agents. In tests with experimental melanoma B-16 on the mice, it was revealed antimetastatic and less carcinostatic effect of the celandine preparations [51]. In vitro, in vivo and in clinical researches, were shown its immunomodulatory and carcinostatic properties on the most forms of human tumors, including melanoma [54, 40].

The case of treatment of the skin melanoma with rectum metastasis using celandine preparations was obserwed byYa.M. Susak with collaborators on the Department of General Surgery №1, Bohomolets National Medical University in Kiev.

At initial examination of patients with melanoma 20% of them have metastases: local (15%) or remote (5%). The other patients (15% of cases) have subsequent dissemination of tumor: 1/5 of the patients – in the soft tissues (lymph nodes, skin, and subcutaneous fat), 4/5 of the patients have visceral metastases (lungs, liver, cerebrum and bones) [52]. In average, the survival index is 4-6 months and only 6% live for 5 years and longer [53- 55].

A 68 years old patient was operated on account of face melanoma (operation – excision of the face skin melanoma). Histologic conclusion: skin melanoma. Six month later at patient examination, on the rectum posterior-dexter wall, directly next to sphincter muscle of anus was revealed a gibbous mulberry like dark purple formation, 5x4 cm size, on a wide base with necrosis site of 2x1 cm. It extends in a dexter ischiorectal area and in a groin posterior wall. A biopsy procedure was done. Pathohistologic conclusion: melanoma with necroses

and inflammation.

An operation was done – a double-barrel sigmostoma application. At a postoperative period infusion, hemostatic and anesthetic therapy was conducted; cardiac glycosides, B group vitamins, antibiotics, proserin were introduced. Postoperative period was without complications.

In a month after operation: patient underwent a therapy with interferon-α_{2a}but it was ineffective.

Then the patient relatives and herself decided to ask for amitozyn preparation therapy which was started concurrently with vitamin therapy (A, E, C vitamins), enterosorption, with use of dietotherapy for oncological patients. Total dosage of amitozyn preparation was 250 mg per each course.

In a month after initiation of amitozyn treatment it was detected partial involution of tumor, in ten months after initiation of amitozyn treatment it was revealed complete disappearance of the metastases, Clinical result confirms experimental research data with melanoma B12 and proves antimetastatic effect of this preparation.

Amitozyn therapy was not accompanied by such toxic effects as: barf, alopecia, oto- and nephrotoxicity, the therapy was better carried by the patient (toxicity level in accordance with Worldwide Organization of Health Protection is – 0). This fact allows us to improve life quality of the patient, to hasten her social adaptation – to accomplish sigmostoma closure.

The present clinical case demonstrates the effectiveness of the preparation in treatment of a skin melanoma and complete involution of tumor metastases after an amitozyn therapy and its possible application as an alternative option to a routine chemotherapy (toxic action not revealed, high life quality of the patients, social adaptation). There is a necessity of the following elaboration to ascertain a mechanism of the celandine preparation effect in skin melanoma treatment.

1.2.6. Afterhistory of the oncological patients treatment with amitozyn

According to the information of the International Union of Fight against Cancer about 80% of the human malignant neoplasms are the long-term results of

environmental factors negative influence, individual way of life and also smoking and alcohol abuse.

After Chernobyl disaster the oncopathology incidence of the region's population increased on 10% comparing to a period before the disaster.

Depending on disease localization the maximal growth registered among thyroid gland cancer patients 4.8 times.

Among female population, growth of the breast malignant neoplasms 2.6 times, neck of uterus – 1.3 times and among male population, growth of the prostate malignant tumors 2.4 times, mouth cavity 1.6 and urinary bladder 1.7 times are registered.

Drastic melanoma incidence 9.1% increasing and metastatic neck lesion without initial focus on 25.8% are observed too.

Scientific medicine is not always effective in oncological disease treatment – there are frequent complications after surgical treatment, adverse reaction to chemical preparations, to radiotherapy and to the other treatment methods that does not allow achieving desired results.

All over the world about 1500 new antineoplastic drugs are tested annually, nevertheless, not more then one preparation per year is introduced in the clinical practice.

These and other problems constrain to seek out the new more effective low toxic drugs. Amitozyn acts as an activating regulator of the immune system and is an effective antitumor preparation. It enhances the immune reactions on malignant cells and has no mutagenic, teratogenic and carcinogenic properties. Thus, the immunomodulatory effect is achieved that brings to the immune status normalization, especially evident in the cases of pathologic changes [56].

Amitozyn treatment of the oncologic patients has been conducted at Zhitomir regional oncologic dispensary (ZROD) since 1965. The aim of research was studying the short-term and long-term results and also frequency of cancer metastasis relapse

during combined Amitozyn treatment depending on the stage of disease, anatomical growth and tumor localization.

At amitozyn use in the treatment of disseminated malignant head and neck tumors, metastatic affection of the neck without primary focus (60 patients had been treated) the complications were not observed. [57].

Two-years survival index, after combined treatment conduction: radiotherapy + poly chemotherapy (the first group of the patients) was (52,2 ± 4,2)%, three-years index – (47±5)%, five-years – (40,2 ± 5,2)%. Before one year – 8 (25%) patients died. Recurrence, cancer metastases were revealed at 7 (22%) patients, among them at the period from 6 months up to 2 years. Two-years survival index, after combined treatment conduction: radiotherapy + Amitozyn (the second group) was (82,2 ± 4,4)%, three-years index – (70,1±5)%, five-years – (50,3 ± 5,4)%. Before one year – 4 (13,3%) patients died. Recurrence, cancer metastases were revealed at 5 (8,1%) patients, among them at the period from 8 months up to 2 years.

Wide use of amitozyn in present-day oncological patients' treatment will give an opportunity to improve long-term results of all age groups patients' survival.

Use of the preparation in combined treatment prevents from negative complications appearance. For detailed study of antineoplastic agents and their derivatives, it is necessary to continue researches. The long-term results prove the preparation use expediency in the present-day oncological patients' treatment.

1.2.7. Complex treatment of cancer

The aim of chemotherapy is to achieve the maximum antitumor effect and not to damage the living functions of the organism. The most of chemotherapeutical drugs possess pronounced hemato-, cardio- and nephrotoxicity. Amitozyn belongs to a new group of antiblastic substances, greater celandine alkaloids and thiophosphoric acid derivatives, that have no immunosuppressive action, but is capable to modulate immune response.

In the experimental study of amitozyn antitumor action, was determined that amitozyn at concentration 1mg/ml (at 24h exposure) showed a considerable cytotoxic effect on cells of ovary teratoblastoma of human (cells of RA-1line). The preparation penetrates into tumor cells first localizing near the nucleus and then causing their destruction and death [58].

The results of National Cancer Institute have shown malignocytolytic action of Amitozyn against tumor lines tested. The drug was found to cause G2/M arrest and apoptosis in tumor cells [59].

The analysis of cellular cycle of peripheral lymphocytes in healthy volunteers revealed no differences in the level of apoptosis cells, which explains the selective action of the drug. *In vivo* experiments proved that even long-time administration (6 months and more) of amitozyn caused no severe toxic effects.

Joint efforts of a team of authors showed that the treatment of living cells with Am reversibly perturbs the microtubule cytoskeleton, provoking a dose-dependent cell arrest in the M phase. Am changed the dynamics of tubulin polymerization in vitro, promoted the appearance of aberrant mitotic phenotypes in HeLa cells and induced apoptosis by the activation of caspase-9, caspase-3 and PARP, without inducing DNA breaks. Amitozyn treatment of HeLa cells induced changes in the phosphorylation of the growth suppressor pRb that coincided with maximum mitotic index. The dose-dependent and reversible anti-proliferative effect of Am was observed in several transformed cell lines. Importantly, the drug was also efficient against multidrug-resistant, paclitaxel-resistant or p53-deficient cells. These results open the way to further pre-clinical evaluation of Amitozyn [60].

Up to date pancreatic cancer remains one of the most aggressive and resistant to chemotherapy cancer diseases. Median survival time is 4,6 months, less than 10 % of pancreatic cancer patients survive up to 1 year after being diagnosed. The results obtained in controlled randomized clinical studies, carried out at university clinics of Kyiv and Ulm, have shown the efficiency of Amitozyn application for pancreatic cancer patients. These results are also presented in Langenbeck’s Archives of

Surgery. Median survival time was 5,2 months had the patients, treated with gemcitabin (1000 mg/m^2), In studies performed in Kyiv on 21 patients, who received Amitozyn , life quality (evaluated according to pain syndrome intensity, analgesics intake and Karnovsky index) remained higher throughout the study [61].

Colorectal cancer with liver metastases is currently considered to be incurable disease. However, new intraarterial therapy protocols have allowed to prolong the survival of such patients up to 25,7 months. At the Department of General Surgery (Bohomolets National Medical University) headed by Prof. Zemskov we started innovative methods for cancer treatment using greater celandine preparations: intra-arterial administration of amitozyn, including intraoperative administration. Also, we optimized the schemes of neo-adjuvant and adjuvant therapy with greater celandine preparations. Considering pronounced malignotoxic action of both preparations, the possibility of their administration during operation, when there is a high probability of cancer cells spread, is the real breakthrough in cancer treatment. The data concerning the survival of patients with metastatic liver cancer with initial localization in bowels, kidney, and mammary gland, when they were treated intraarterially with amitozyn, is partially presented at international congresses [62].

The data concerning the combination of celendine preparations with intermittent androgenic blockade in prostate cancer arouse considerable interest at the International Anticancer Congress, conducted in 2005 in Paris. This mode of treatment resulted in objective tumor response in 85% of patients and median value of PSA reduction was 84% one month after a course of treatment and 95 % in 3 months [63-65].

Modern oncology has only a few anticancer drugs with low toxicity. The data on clinical application of amitozyn give grounds to confirm the efficiency in the treatment of some cancers with minimal damaging action on healthy organs and tissues.

1.2.8. Inhibition of plant tumors growth

Representatives of different classes of animal organisms (nematodes, insects), primitive fungi, as well as bacteria and viruses effect plants in various ways, disrupting and changing the development of cells, tissues and organs, thus leading to tumor formation. Among the factors that cause genetic formation of tumors on plants we distinguish mutations of plant genes or the presence of oncogene sequences of agro bacteria in plant genome in the process of bacterial gene transfer in evolution [66].

Plant tumors, accompanied by tissue proliferation, are frequently occurred in nature. Among them of special interest are tumors, produced by phyto pathogenic bacterium Agrobacterium tumefaciens, which has Ti-plasmid, responsible for "coronated gal" or bacterial cancer. Plasmid initiates the process, but further it can proceed in its absence [66 -67].

Plant tumors of this line have the following general properties peculiar to malignant neoplasms of animals and humans: autonomous non-regulated growth, transplantability and aggressiveness. These tumors are able to grow in culture medium in the absence of growth phytohormones, necessary for growing normal issues. Therefore, they are used in experiments, aimed at defining certain fundamental biological concepts that provide the understanding of tumorous processes in general, as well as for studying antitumor action of some preparations [67-69].

We have performed studies on plant tumors, induced with Agrobacterium tumefaciens, with the aim of defining antitumor action of preparations amitozyn and amitozynoberamid offered for clinical test [70].

The study was performed on the tumor model of Kalanchoe diagremontiana and tomato line "Ukrainian salt-tolerant", induced with high-virulent strain Agrobacterium tumefaciens 8628. For experiments we took samples of Kalanchoe diagremontiana and tomato plants of the same age, height and thickness of stem. Agrobacterium was infected into a leaf blade or by decapitation method. For this

purpose Kalanchoe plants with 5-7 leaves were decapitated with scalpel. After 15-30 min., 48 hours' suspension of agrobacteria was applied onto the section surface at $1x10^8$ cells dose.

The plants of "Ukrainian salt-tolerant" tomato line were inoculated with agrobacteria at fruiting and the beginning of ripening stage. The plants were kept at 25-28 C throughout the experimental period.

The changes in "coronated gal" tumors weight and the determination of antitumor activities of amitozyn and amitozynoberamid were studied on the tumors of Kalanchoe plant. For control the tumors were treated with water. The size of tumors was measured every 5-6 days. Their volume was calculated by the formula:

$$p = ¶ \text{ а в с } / 4$$

where a- width, b-length, c- height of a tumor.

Since the relative weight of tumor issue approximates unit, a tumor weight quantitatively equals its volume.

In order to define the inhibitory action of antitumor preparations on plant tumors, their solutions at 0,1 mkg/ml were applied as applications at 1-2 ml, depending on a tumor size, onto the tumors, beginning with day 15 after agrobacteria inoculation.

According to the literature, first symptoms of tumors on plants at induced infection became evident on day 10-30 as small swellings and humps of white or light-green color on a stem or leaf. [67]. Similar phenomenon was observed in our experiments. According to our data, the kinetic curve of tumor growth on a Kalanchoe stem is of S-like shape. (Fig.6). Kinetic measurements show that the rate of tumor development of Kalanchoe, induced with agrobacterium, first increases, and after 30 days it comes to a halt, probably due to some limiting factors. This evidences that tumors in plants develop according to the same kinetic laws as tumors in animals and humans [67, 71, 72].

After 3-time treatment of tumors in tomatoes with amitozyn we first observed the necrosis of tumor surface cells. By day 20 after the treatment dark sports became apparent on tomato tumors (necrosis), and by day 60 the tumor necrotized completely. After the tumor necrosis the tomatoes grew normally and bore fruit.

The necrosis of surface cells of tumors in Kalanchoe became apparent after 4-time treatment, and by day 70 a necrotic spot appeared.

We observed no complete necrosis on secondary tumors of the plants that were not treated with the preparation, but their growth rate slowed down and a slight necrosis of surface cells appeared.

Studying the inhibitory action of amitozyn we found that tumor regression was observed starting from day 6 after the treatment with preparation. Tumor mean weight decreased from 22,0 ±6,35g to 13,11± 4,91g during the period of amitozyn action (29 days), and after the same period of amitozynoberamid action on tumors in Kalanchoe, it reduced from 32,05 ± 6,12g to 22,4±4,91g. It is seen from Fig. 2 that the kinetic curve representing changes in tumors weight treated with amitozyn is similar to that of amitozynoberamid. In control (water) we first observed a slight increase in tumor weight, which got stabilized after 3-time treatment. (Figure 7).

The study of antitumor action of amitozyn and amitozynoberamid on Kalanchoe plant model we determined that both preparations at concentration 0,1mg/ml (during 29 days) inhibit a tumor growth, induced with Agrobacterium tumefaciens, with no influence upon a plant growth.

Amitozyn at concentration 0,1mg/ml on tomato tumors was found to result in a complete necrosis of “coronated gal” tumors during 60 days.

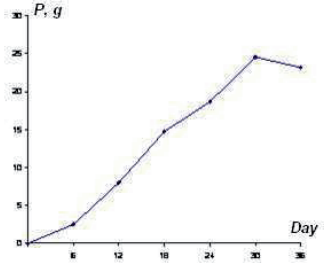


Fig.6 Kinetic curve of the weight growth of tumors induced with Agrobacterium tumefocieus 8628 on Kalanchoe daigremontiana plants

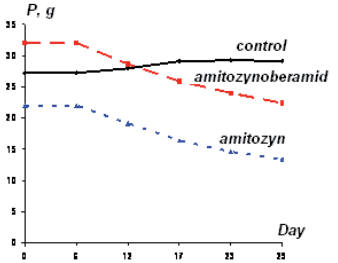


Fig.7. Kinetic curves of the changes in tumors weight infected with A. tumefacieus 8628 under the effect of antitumor preparations

The data support the advisability of pursuing research on the use of these preparations in industrial scale.

II. Antiviral, antitumor, antimicrobial and immune correcting izatizon preparation

Unification of achievements in biological, chemical and physical spheres of science promotes the progress of chemotherapeutical researches, directed to improve the existing and to develop new effective preparations with antiviral action. The arsenal of these preparations increases from year to year. At the same time, quick growth of infectious diseases that have already considered as the deleted ones is observed on all continents of our planet. Also appear new ones, among which viral diseases occupy the main place. Development of structure-directed drugs became an integral part of the modern search of drugs and investigation of the molecular recognition between the three-dimensional structural targets.

Nowadays humanity revalues the negative influence of viruses on the humankind and the visual environment of plants and animals. Character of viruses as a class of obligate intracellular and even genetic parasites determined main difficulties in creation of effective chemotherapeutical preparations for the treatment and prevention of viral infections. Main target for viral infections chemotherapy is to create effective antiviral preparations on the base of components that selectively and specifically inhibit viral reproduction and do not harm the vital activity of cell and the whole organism. Izatizon is one of such home complex preparations with antiviral, antibacterial and antitumoral activities and with immunomotropical qualities. It is a 2% solution of 1-metyl-izatin β-tiosemicarbazon in the universal solvent.

It is determined that izatizon increases the number of cells that express CD 69 antigen, induces T- and B-lymphocytes proliferation, stimulates pre-T-lymphocytes differentiation, enhances T-lymphocytes functional activity, including their capability to product lymphokines. It also stimulates natural killers' activity, metabolic and phagocytic activity of macrophages and IL-1 synthesis, increases bactericidal action of blood serum. This means that it enhances nonspecific factors of the immune system.

The beginning of the XX-th century was marked with the viral epidemic of “Spaniard” that took away many lives. Flu outbreaks on a global scale have been repeated regularly since that time. The virus with the transformed antigen structure provoked them. Serious consequences provoke viral encephalitis, hepatitis and poliomyelitis outbreaks. At the same time, viral pestilence strikes poultry, hogs, cattle, useful insects and fish. More often, the vegetative world becomes the subject of viral attacks that lead to the great economic losses for both backward and developed countries.

The alteration of the creatures living sphere has resulted in the natural resistance drop to the negative influence of environment and large extension of already known and new viral infections and cancerous growths and leucosis. AIDS occupies the special place among them. Present situation demands, along with the traditional methods of prevention and treating of viral diseases with different vaccines and serums, to form the new direction in this viral struggle with the help of medications, but the development of this promising direction delays because of the insufficient specificity and wideness of the therapeutic action of new antiviral preparations. These defects are connected, on the one hand, with the viruses that in their development depend on the metabolism intimate mechanisms in the cell-master that serve as obligate parasites and, on the other hand, with the immunosuppressive virus influence on the affected organism.

Therefore, the success in the fight with viral infections is possible only if we use preparations with high specificity of antiviral activity and their parallel immunoregulatory effect on the organism – the host. Modification products of natural materials and their analogs have such characteristics. A number of products that are the results of alkylations of natural materials and their components and analogs, obtained after many years of work directed on modification, selection and creating of new antitumoral, antiviral and antimicrobial preparations serve as a confirmation of this conception.

Izatizon was determined as the most perspective among new biologically active preparations with given characteristics that had been explored. A line of officinal forms and compositions and several prospective and functional analogs were obtained on its basis. It should be noted a wide spectrum of antiviral action of izatizon. This is an original preparation elaborated by A.I.Potopalskiy and L.V.Lozyuk. [73]

Herpes virus, mixovirus, specifically flu viruses of A and A-2, herpes labialis, herpes zoster, Marek's disease, infectious laryngotracheitis, bronchopneumonia of horses and calves, ectromelia virus, variolovaccine virus, Venezuelan encephalomyelitis virus of horses, enteritis virus of hogs, enteroviruses of swimming birds are all sensitive to izatizon. Izatizon effect during Marek's disease depends on the multiplicity of injections and directional dose that is it influences therapeutically the organism infected with viral tumor. Izatizon has an abscopal effect on the in vivo virus reproduction and reduces sickness rate during the experimental contamination and in the industrial conditions even without vaccination. The preparation is destructive for DNA- and RNA-containing viruses of insects, plants, fishes and men including AIDS virus as well.

On numerous experimental models wide dose preparation's spectrum and application scheme was established. Therapeutic and preventive effects directly depend on the concentration and application method. Izatizon shows 80-100% protective effect if we compare it with the other well-known medical preparations in appropriate application conditions.

Izatizon inhibits viral reproduction with simultaneous increasing of the specific and nonspecific resistance that is it displays immunoregulating activity. Here the weigh of animals and birds and their productivity (egg-laying qualities, shear of wool, etc.) are raise. Izatizon parallel with high specific activity belongs to the group of the substances with low-grade toxic characteristics.

Izatizon only in completely toxic doses shows intoxication in the experiments with animals (white mice, rats, cats, dogs). LD_{50} of izatizon for mice under internal

injection is 11,4 - 31,4 mg/kg; for rats 13,9 - 45,0 mg/kg; its enteral introduction for rats was equal to 133 - 319,0 mg/kg; for mice – 25 - 47,0 mg/kg. Injection of total lethal preparation doses to rats leaded to the weakly marked destructive alterations of liver, lungs and heart.

Izatizon has the capability to reduce muscle tone of the bowel and to raise its sensitivity to acetylcholine and barium chloride. The preparation does not inhibit blood formation and does not have cumulative characteristics.

During its application in the inductive phase of immunogenesis the preparation alleviates the anaphylactic shock procession, insignificantly inhibits phagocytic leukocytes activity and cytolytic characteristics of blood serum, stimulates the interferon formation. Even durational application of therapeutic concentrations did not lead to the toxic displays. 10- and 30- multiple preparation application 1/10-1/16 LD_{50} did not provoke pathomorphological changes.

Izatizon is not toxic in the recommended doses. Method and schemes of its application do not allow the overdose, as even in case of high doses and amplified schemes of treatment the side effects were not noticed. Even if continually breathe in the preparation during 10 days no toxic effects were provoked.

Examination results of izatizon medicinal forms and its structural and functional analogs, virotiazolizin preparation particularly, show great perspectives in this direction.

Virus reproduction inhibition is shown onto 2,5 lg during preventive and medical application. We see this from the researches held on the model of viral transmissible gastroenteritis of hogs (swine kidney cells).

Izatizon was used in the combination with generally accepted treatment methods during the surgical pathology of different ethiology of cattle cubs.

These researches have shown that the preparation provides the recovery and recommended as an antiphlogistic and antiseptic mean and for productivity and natural resistance elevation as well.

Izatizon medicinal forms and its analogs are very perspective against viruses of beneficial insects (oat and silkworms, etc.), plants (X and Y potato virus, tobacco mosaics virus, etc.) and against viruses of commercial fish.

Given data, authenticate the wide spectrum of izatizon antiviral activity. Clinical tests have confirmed experimental results and permitted to recommend izatizon for the wide clinical application.

The preparation is approbated and widely used in Ukraine and Russia. During last 10 years, preceding the USSR collapse, izatizon was successfully studied in poultry farming and cattle-breeding complexes of Hungary, Bulgaria and Syria. Then this work was stopped because of the money lack.

Izatizon is rewarded with the silver medal of VDNH USSR and Ukraine, international diploma of II degree and silver medal on the 70-th exhibition-fair "Omek-85" devoted to the agricultural and food industry (Budapest, august 1985).

2.1. Structural and Conformational Properties of Methisazone – the main functional part of izatizon

Changes in environmental conditions caused a decrease of living creatures natural resistance to harmful environmental factors and the mass distribution of already known and new viral, microbial-viral, fungal infections and cancer

Iizatizon is a drug of a new generation, which combines the antiviral activity and immunotropic action, and also has some antitumor properties. It is proved experimentally that the drug affects both viruses and cellular mechanisms of the immune system [74]. Ability of izatizon to stimulate activity of reparative DNA synthesis in cells has been found in herpes virus and adenovirus model systems. Ability of the drug to inhibit thymidine kinase of herpes virus and adenovirus activity resulting in inhibition of virus virulence on the early stages of the infectious process has been found. Izatizon therapeutic action under herpes and adenovirus infections and potential usefulness of the drug in AIDS has been found as well 74. Obtained data showed us the convincing importance of such drug, especially nowadays, when all over the Earth it is observed a sharp increase of the number of viral and immuno-

agressive diseases that were considered missing and the appearance of new ones, among which significant place is occupied by diseases caused by viruses. Izatizon also is active against DNA and RNA-containing viruses and has expressed immunomodulatory properties [75.]

High antiviral activity of thiosemycarbazone derivatives can be connected with the action of their active molecules that we tried to examine. A wide range of biological activity of izatizon is based on conformational structure of the main components of this drug - methisazone molecule, a thiosemicarbazone derivative, that contain isatin - and depends on solvent properties and microenvironment. Methisazone is an antiviral drug that works by inhibiting mRNA and protein synthesis, especially it has been used to provide short-term protection against smallpox [75, 76] methisazone may provide protection later in the incubation period, by inhibition of virus multiplication 76 and was found to be effective as prophylaxis for variola minor (alastrim), where contacts were not vaccinated at a significance level 77, but it is still unclear whether or not the drug exerts any suppressive effect on the immunologic response to vaccination at a time when vaccination would have no effect [76].

However, information about conformational properties of methisazone [78, 79] and prototropic tautomerism of isatin [80] – the physico-chemical characteristics that are the basis of their biological activity – remains restricted.

We used quantum chemical methods of investigation at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory that was successfully applied for the similar tasks and objects to characterize conformational diversity of methisazone molecule and its main related compound – isatin, that are the base for it's biological activity.

The optimized geometry and harmonic vibrational frequencies of studied molecules were calculated using the three-parameter hybrid B3LYP density-functional model 0, providing accurate normal mode frequencies 0 and geometries of several aromatic systems86, 0, that agree well with according experimental data, with

the 6-311++G(d,p) basis set *in vacuo*, followed by single point electronic energy calculations for the optimized molecular structures at the correlated MP2 0 level with the 6-311++G(2df,pd) basis set to consider electronic correlation effects as accurately as possible. The absence of imaginary vibrational frequencies proved that energy-minimized structures perfectly correspond to the local minima of the potential energy landscape.

The Gibbs free energy G values for all structures were obtained at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory in the following way:

$$G=E_{el}+E_{corr},$$

where MP2/6-311++G(2df,pd) level of theory was used for calculation of the electronic energy E_{el}, while B3LYP/6-311++G(d,p) – of thermal correction E_{corr} and entropy contribution.

All of the quantum chemical calculations were performed using the Gaussian 03 program package 0.

Bader's theory of atoms in molecules (AIM) was employed to analyze electron density 0. The topology of the electron density was analyzed using program package AIMAll 0 with all the default options. The presence of a bond critical point 0, namely the so-called (3,-1) point, and a bond path between hydrogen donor and acceptor, as well as the positive value of the Laplacian at this bond critical point, were considered as necessary conditions for H-bond and van der Waals contact formation. The H-bond and van der Waals contact energies were evaluated by the empirical formula 0 based on the electron density distribution at the critical point (3,-1) of the H-bond:

$$E=0.5\cdot V(r),$$

where V(r) is the value of a local potential energy at a bond critical point.

Wave functions were obtained at the level of theory used for geometry optimization.

Prototropic tautomerism of the isatin

Qualitative stereochemical analysis of isatin – initial substance for obtaining the main part of the drug izatizon – the methisazone molecule, indicates that it has five molecular prototropic tautomers (Fig. 8) - one diketo form (I) and four (II-V) enol forms. Prototropic tautomerism can be considered as a functionally important form of structural variability, which, of course, should be involved in various biochemical processes and provide functional diversity.

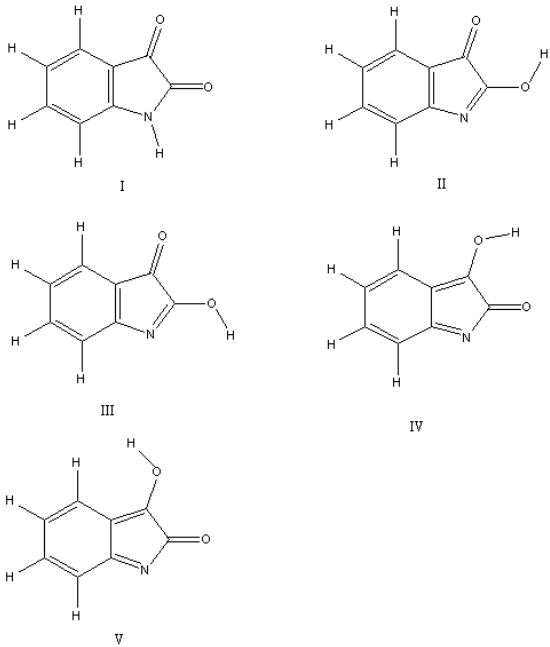


Fig. 8 Complete family of isatin prototropic molecular tautomers

Concerning the relative Gibbs free energy of all isatin prototropic tautomers is evident that among all of them diketo form **I** is the global minimum, whereas the free energies of other ones are considerably higher – the energy difference between the most favorable enol tautomer is about 11 kcal / mol. According to our calculations, all of them without any exceptions are planar (symmetry C_s) structures – some small

deviations from planarity are much lower than zero (at T → 0) amplitude of non-planar deformation vibrations.

Going the logical route, exactly diketo form **I** should be observed in polar solvent due to its considerable stabilization, since it possesses a significant dipole moment (6.38 D), that markedly higher than that for the most energetically favorable enol tautomers **II** and **III** - 2.62 and 2.88 D, accordingly (see Table 7a).

Table 7a. Relative gibbs free energies δg (kcal/mol) and dipole moments M (D) of Isatin Tautomers

Tautomer (see Fig. 1)	ΔG	μ
I	0.00	6.38
II	11.74	2.62
III	12.01	2.88
IV	34.15	4.97
V	39.73	7.31

It should be noted that isatin molecule has quite "labile" structure with regard to nonplanar deformation. This can be clearly indicated by the presence of two low lying fundamental vibrational modes (95.7 and 137.5 cm^{-1}) in tautomer **I**, which corresponds to out-of-plane deformations.

Conformational capacity of methisazone as a key to understanding its biological activity

We first studied the structural features of methisazone molecule and determined its ability to have different conformations.

Structural formula of methisazone molecule - the main constituent part of izatizon - is presented at the Fig. 9.

Fig. 9. Structural formula of the methisazone molecule and numeration of its atoms

For the first time it has been detected that hypersurface of the Gibbs free energy of the methisazone molecule has eleven minima - one is global, whereas ten of them are local. They correspond to three plane-symmetric conformations (one of which is basic) and four substantially nonplanar (symmetry C_s) pairs of mirror-symmetric conformations:

IV,**V** (C8C7N7N10=±179.2°; C7N7N10C10=$_{\mp}$70.0°; N7N10C10N11=±11.4°; N10C10N11H$_1$=$_{\mp}$17.7°; NCNH$_2$=$_{\mp}$175.2°),

VI,**VII** (C8C7N7N10=±12.4°; C7N7N10C10=±43.7°; N7N10C10N11=$_{\mp}$27.7°; N10C10N11H$_1$=$_{\mp}$30.8°; N10C10N11H$_2$=$_{\mp}$178.5°),

VII,**IX** (C8C7N7N10=±176.0°; C7N7N10C10=±175.0°; N7N10C10N11=±167.8°; N10C10N11H$_1$=$_{\mp}$177.4°; N10C10N11H$_2$=$_{\mp}$35.3°) та

X,**XI** (C8C7N7N10=±13.9°; C7N7N10C10=±56.9°; N7N10C10N11=±179.7°; N10C10N11H$_1$=±34.2°; N10C10N11H$_2$=±177.3°) (Fig. 10).

Relative Gibbs free energies of the conformations lie in the range: 0.00 - 16.11 kcal/mol (Table 8). From Table 8 it is evident that various conformers have very different values of dipole moments that arranged in a sequence: $\mu_{X,XI}$ (2.67) < μ_{III} (4.27) < $\mu_{VI,VII}$ (4.73) < μ_{II} (5.03) < μ_{I} (5.61) < $\mu_{IV,V}$ (6.51) < $\mu_{VIII,IX}$ (7.15 D). The most polar ones are the main **I** (5.61 D), **IV,V** (6.51 D) and **VIII, IX** (7.15 D) conformers. The smallest one belongs to the pair of nonsymmetrical conformers (**X,XI**) with the highest energy. These values of the dipole moments indicate that during transition

from a vacuum to the polar environment, particularly during the incorporation in the DNA double helix, methisazone conformers will be stabilized in the same order.

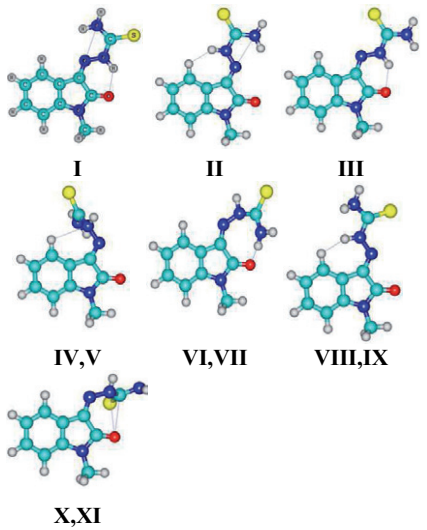


Fig. 10. Conformers of the methisazone molecule, obtained at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. Dashed lines show specific intramolecular contacts. Only one conformer is represented for mirror-symmetric pairs IV,V; VI,VII; VIII,IX and X,XI

It has been found that methisazon conformers stabilized by intramolecular H-bonds, dihydrogen bonds and van der Waals contacts; their electron-topological, structural and energetic characteristics are given in Table 8. It is worth mentioning that energetic characteristics of these structures well correlate with their electron-topological parameters varying from minimal for H-bond C6H…N10 (in conformers **IV,V**) (ρ=0.008 a.u., $\Delta\rho$=0.032 a.u., E=1.58 kcal/mol) to maximal for H-bond N11H…O8 (in conformers **VI,VII**) (ρ=0.049 a.u., $\Delta\rho$=0.148 a.u., E=14.78 kcal/mol) (see Table 8) values. Methyl group has the same orientation in all cases without any exceptions – one of its CH-bonds and C8O8 bond are co-planar.

A characteristic feature of the main conformation **I** is that it is stabilized by intramolecular H-bond N7H...O8 (E = 5.34 kcal/mol) and van der Waals contact N7...N11 (E = 4.42 kcal/mol). Conformers **II** and **VIII,IX** are stabilized by quite

weak so-called dihydrogen bonds C6H...HN10 (E = 1.92 kcal/mol) and C6H...HN10 (E = 2.29 kcal/mol), accordingly. Also, van der Waals contact is localized with the energy of 4.54 kcal/mol in molecule **II**. Conformers **IV,V** are stabilized by H-bond C6H...N10 (E = 1.58 kcal/mol). Energies of the H-bonds N10H...O8 and N11H...O8 localized in conformers **III** and **VI, VII** accordingly differ almost twice: 7.07 and 14.78 kcal/mol, accordingly. In the mirror-symmetric conformers **X,XI** revealed two van der Waals contacts O8...N10 (E = 2.25 kcal/mol) and O8...C10 (E = 2.17 kcal/mol).

Table 8. Electron-topological, structural and energetical characteristics of specific intramolecular contacts in methisazon conformers

Conformer (see Fig. 3)	Specific contacts AH…B, AH….HB and A…B	ρ^a	$\Delta\rho^b$	$d_{A...B}{}^c$	$d_{H...B}{}^d$	$\angle$AH .. B^e	E^f	ΔG^g	μ^h
I	N10H…O8	0.023	0.087	2.633	-	-	5.34	0.00	5.61
	N7…N11	0.019	0.087	2.786	-	-	4.42		
II	N7…N11	0.019	0.089	2.628	-	-	4.54	4.59	5.03
	C6H…HN10	0.011	0.039	1.931	1.931	144.3	1.92		
III	N10H…O8	0.029	0.104	2.755	1.925	135.9	7.07	6.35	4.27
IV, V	C6H…N10	0.008	0.032	3.216	2.701	108.8	1.58	14.76	6.51
VI, VII	C6H…N10	0.049	0.148	2.693	1.677	168.6	14.78	15.62	4.73
VIII, IX	N11H…O8	0.013	0.046	1.881	1.881	147.8	2.29	16.04	7.15
X, XI	O8...N10	0.010	0.040	2.954	-	-	2.25	16.11	2.67
	O8...C10	0.010	0.037	2.957	-	-	2.17		

[a] The electron density value at the BCP, a.u. [b] The Laplacian of electron density value at the BCP, a.u.; [c] Distance between A and B atoms, Å ; [d] Distance between H and B atoms, Å; [e] H-bond angle, degree; [f] Bond energy calculated by Espinosa-Molins-Lecomte method 0, kcal/mol; [g]Gibbs free energy of the conformer, kcal/mol; [h] Dipole moment of the conformer, D.

Destabilizing factors, in particular, repulsion of free electron pairs of neighboring atoms of sulfur, nitrogen and oxygen, most completely are presented in the energetically most unstable conformations **X,XI**. Their highest relative energy (16.4 kcal/mol) caused by exactly these circumstances.

Our attention attracts structural and dynamical feature of methisazone molecule – its structural "lability" confirmed by the presence of low lying torsion vibrational modes 46.1, 53.5, 77.8 cm^{-1} in the most energetically favorable conformer **I**.

A comprehensive conformational analysis of the methisazone and complete family of the molecular prototropic tautomers of isatin have been performed by means of *ab initio* calculations at the MP2/6-311++G (2df,pd)//DFT B3LYP/6-

311++G(d,p) level of theory. In general, specific intramolecular contacts – H-bonds NH…O, NH…O, CH…N; dihydrogen bond CH…HN and van der Waals contacts N…N, N…O and C…O were detected by performing electron density topological analysis for all 11 conformers of methisazone and their energies were estimated by Espinosa-Molins-Lecomte formula. Electron-topological, structural and energetic characteristics for all of these contacts are presented. It is established that the most energetically favorable prototropic tautomer of isatin is diketo form with a large energetic gap between other tautomers.

It is established taking into consideration modern investigations that exactly this property together with conformational abilities and also the possibility to form intermolecular H-bonds underlie diverse biological activities of methisazone and izatizon. We can assume that molecule can convert from the most energetically favorable to the high energy conformation that is biologically active, exactly due to the presence of specific solvent in the composition of izatizon[93].

Analysis of recent literature data and results of our study confirm the promising development of new effective drugs based on the compounds from the thiosemykarbazon class.

2.2. Antiviral activity of izatizon

Methyl-isatihn β-tiosemicarbazon or metisazon is a compound that has a wide antiviral spectrum of action and is approved as a preparation for smallpox prevention. But it has a high toxicity. 2% solution of 1-metilizatin-β-tiosemicarbazon in universal solvent was named as izatizon. This preparation is nontoxic, and has a wide antiviral spectrum and is used with success in veterinary for curing and prevention of respiratory viral infections and Marek's disease of birds.

During the study of molecular-biological mechanisms of izatizon antiviral action human adenoviruses of 1 and 2 serotypes were used. Cytotoxicity of the preparations was determined on adenovirus infected cells with the help of the method of fluorescence microscopy including fluorochromic evaluation of acridine orange

fixed cells. From this we can predetermine about the morphology of cell and simultaneously reveal in it DNA and RNA.

For testing of viral hexone synthesis (main albumen of virus capsule) immune-enzyme assay was used with MCAb to hexone of the 1 type of human adenovirus, rabbit antiserum to adenovirus of 6 type and conjugated with peroxidase of antibody against rabbit immunoglobulin. For cloning of the gene VAI RNA HindIII-fragment (6231 -11555 np according to Ad2 map) was cut out and cloned in consisting of plasmid pUC19 (pAdH5.3). On the third stage with the help of cutting out the X-bal-fragment we received plasmid pVA224 that contains the whole copy of VAI RNA gene.

Obtained data testify that there are no essential distinctions between izatizon and metisazon in vitro.

We have checked out antiviral activity of izatizon on different cell lines: Hela, Vero and HEp-2. We found that izatison stops the viral reproduction (in 50% of infected cells and hexone synthesis level) only on HEp-2cell line. Recombinant human interferon β-2 (r-IFN) had no antiviral activity in concentration 2000 units/ml, but stimulated antiviral activity of methisazone and izatizon. It displayed in strong decrease of cytocidal activity of virus and in two-fold decrease synthesis of capsule albumen. The decrease of synthesis of the viral hexone we observed in Ad1- infected cells of HEp-2 line treated with izatizon. It is necessary to mention that antiviral effect was observed only when izatizon, methisazon and interferon were applied on the early stage of viral infection – up to 8 hours after infection and there was no antiviral effect when the preparations were used later. In that way, antiviral action of izatizon and methisazone needs the presence of interferon in a medium from the one hand and from the other hand - an early activation of viral genes.

Izatizon influences the induction of interferon synthesis only in Ad1 infected HEp-2 line cells. After infection, synthesis of interferon was low till 8 hours. Than this synthesis was rising and then lowering after 12-14 hours. Cytocidal effect appeared only after 96 hours. Both methisazon and izatizon don’t stimulate synthesis

of interferon neither in vitro nor in vivo and haven't straight influence on the transmission. This shows that the preparation can influence the transcription of early viral genes and as a result, it modifies an expression of interferon in HEp2 cells.

It was detected that the ability of adenovirus to induct β-interferon synthesis in the infected cells in vitro determined an antiviral activity of izatizon. An insertion of exogenous interferon didn't influence infected adenovirus cells, but provoked an antiviral effect only in the presence of izatizon.

Study of the molecular mechanism of viral stability transgression to interferon has shown that izatizon blocks an activation of transcription of early adenovirus genes that delaying the beginning of replication and synthesis VAI RNA (the last one determines stability of adenovirus to interferon activity). The resistance of virus to interferon sharply decreases and this leads to antivirus effect.

A toxicity of izatizon on HEp-2 cell line doesn't differ from that one indicated in previous researches. It is not excluded that used Hela cell line genetically insensitive to the preparations of this type. Previously printed information points on such a possibility.

Cytomorfological analysis of fixed cell samples show that the action of izatizon connected with the delay of the promotion of viral infection, cessation of the reproduction of virus on early stages of the process.

It has been detected that methisazone and izatizon effects concerned with the interferon presence in the medium or with its induction by virus in infected cells. Yet adenoviruses are persistent to the interferon activity because they have, like many other viruses, specific protection system. Mentioned viral persistence possibly caused by synthesis of big quantity of VAI RNA. This low-molecular RNA is formed by the transcription of corresponding gene with cell RNA polimerase III on the late stage of adenoviral reproduction and it can block protein kinase activity of P1/elF2 dependent protein kinase that activates by interferon and little quantity of dcRNA. Protein kinase phosphorylates the factor of initiation elF2, blocking the translation and provoking polysome disintegration in infected cell. With the help of VAI RNA virus

blocks protein kinase, and the translation of viral albumen goes with equal speed both with presence of interferon and without it.

Izatizon in combination with interferon blocks the synthesis of adenoviral hexone. Izatizon doesn't influenced the translation by itself, so we supposed that izatizon somehow inhibited the viral protection against interferon. As the viral replication detains in the presence of izatizon and VAI RNA transcribes mainly after the beginning of virus replication, exactly here may be a trouble with VAI RNA synthesis. Indeed, in the infected cells, treated with antiviral concentrations of izatizon synthesis of VAI RNA begins with couple hour delay. As a result, its level in 4 - 5 times lower in the experiment than in control. Nozern hybridization data also confirm the results received with the help of dot analysis.

Izatizon antiadenoviral activity can depend on genetic characters of test-cell culture.

Mechanism of izatizon action consists in delay of expression of early viral genes, replications and synthesis of VAI RNA, this conduced to quick weakening of viral protection regarding to interferon. Thus, the viral gene is a target for izatizon.

2.3. Perspective directions of izatizon application

2.3.1. Izatizon application in the veterinary medicine

Modern methods and technologies of farming demand a constant promotion of medical-preventive work quality. The existing methods and means of the treatment and prevention are imperfect, not always of high-performance and they mainly influenced only the disease etiological factor. Very important moment for the veterinary practice is the complete action of new methods and means, wide spectrum of their therapeutic influence.

Izatizon is the preparation of this type, developed and patented by the candidate of biological science Lozyuk L.V. and by the candidate of medical science Potopalskiy A.I. Izatizon belongs to the group of non toxic pharmacological substances. It doesn't have cancerogenic, teratogenic and embryotoxic effects,

cumulative characteristics are absent as well and also it doesn't inhibit hematosis. Izatizon has a wide range of therapeutic influence due to the antiviral, antiphlogistic, bacteriostatic, fungistatic, analgesic, radio- and cryoprotector and immunomodulatory activity.

For the first time izatizon was approved in the poultry farming in 1978 in the Moscow veterinary academy and in the Leningrad National State Institute of fowl diseases. Izatizon and its medical forms minute study was held in DNSKI of veterinary preparations and fodder additions, Beekeeping Institute of the Russian Federation, National Agrarian University in Bila Cerkva and IMBG NASU. At present izatizon is registered as antiviral, antimicrobial and antiphlogistic preparation with wide activity spectrum. Interesting results were obtained by Izdepskiy V.Y., Rublenko M.V. and others regarding izatizon practical application with hogs, cows and rabbits. (recommendations, 1997)

Pathogenesis complicacy of the inflammatory process urges the use of multiple-factor action preparations. Izatizon is one of them. We have approved it on all kinds of farming animals, dogs and cats. The preparation has to be injected directly to the purulent area or used with the help of bandages and drainages, during festering wounds, necrotic suppurative sores, after phlegmon and abscess autopsy and their ablution with antiseptic liquids. Wet paper is applied during subcutaneous wounds. If the wound is deep gauze or adsorbent drainage, wetted in the izatizon, has to be put inside of the wound and fixed by the provisional stitch. The bondage or drainage must be changed every 24-48 hours. The swelling of the wound borders decreases and they become movable in 1 or 2 days. The outflow of the purulent necrotic mass becomes stronger, pink small-grained granulation tissue appears under it [95].

Suppurating process of septic wounds under the izatizon influence entails by the decrease of the infection level. The researches show the bacterial-static izatizon characteristics regarding *St. epidermis* and *E. coli*. But *B. corrugantus* are not sensorial to it.

Preparation application with hogs, cattle and sheeps is the most significant.

Izatizon application along with fast and full wound cleansing provides defect closure by the tender granulation tissue with elastic newly generated collagen. We have to admit that due to this the epithelization process happens earlier and there is the possibility of putting secondary solid suture.

Deep and detailed pathogenetic substantiation of izatizon application during suppurating arthritis that is very difficult for the medical treatment and leads to the essential economic losses has been elaborated. The existing therapy methods of this pathology are ineffective because of their unilateral actions – antiseptic or antibacterial.

Izatizon is used locally as wet bondage that must be changed every 1-2 days, on the early stages of the disease during the period of serofibrinous and fibrinogenous exudation. Quite often izatizon application leads to the recovering already on the early stages of the inflammation in the joint.

Izatizon must be injected directly into the joint area as a wet gauze bandage along with the surgical operation and antiseptic joint cleaning with during septic arthritis.

During osteoarthritis izatizon clinical effect exhibits in the decrease or elimination of purulent-resorptive fever signs, regional phlebit and trombophlebitis decrease. Necrotic tissues in the joint area easily segrerate and resolve that is the proteolytic reaction activation in the pathologic focus area.

Animals recover from purulent arthritis in 3-3,5 weeks if we treat them with izatizon, while if we use traditional methods of the treatment recovery will be observed only in 1,5-2 months.

Fast gaining of positive result, decongestion, cleaning of infected tissues from necrotic suppurative dentritis in 4-5 days with active wound surfaces granulation – these are the typical medicinal izatizon effects during necrotic suppurative processes.

Izatizon bondages give soft anesthetic and apparent antiphlogistic effect during strains, aseptic bursitis, tendovaginitis and arthritis. These bondages also stabilize

blood circulation and lymphokinesia in the injury area, normalize metabolism in the colagen structures and prevent chronization process, contribute to the fast renovation of the lost limb function.

Izatizon application during obstetric-gynecologic pathology.

Izatizon as a complex action preparation is very perspective for medical treatment of cattle obstetric and gynecologic pathology.

Very often, the reason of animal barrenness is in different endometritis clinical forms. According to our data, endometritis is being registered with 10-72% of cows on the live farming of Kyiv region.

The treatment of this pathology is rather difficult, laborious and not always effective.

Izatizon in the dose of 50 ml. was injected to the womb during postnatal endometritis. 10ml. of 10% Novocaine solution must be injected intra-abdominally in 10-15 min. before manipulation. Repeated injections of preparation have been carried out every 48 hours till complete recovery of the animal. Wide application of this method on Vasylkiv, Fastiv, Bila Cerkva and Pereyaslov-Khmelnitskiy live farming has shown that the treatment duration is 6,7+0,3 days with 3,5+0,1 of medical procedures. Generally adopted means – ASD-2, ichthyol, folliculin, estrophan and novocaine – give the indexes regarding treatment duration from 7,7+0,3 to 9,5+0,5 days. Erythrogenesis, leucopoiesis and endocrine gland function were stabilizing along with clinical improvement of animal condition.

Izatizon application gives the possibility to reach the 93,5% insemination level of recovered cows with 26,5+5,2 days of unproductive duration. Whereas the traditional methods have the indexes of 53,8-80% and 41-48 days respectively. Medical effect of izatizon and novocaine intra-abdominal injection combined application can be achieved due to the increase of organism safety adaptive reactions, blood circulation and lymphokinesia improvement in the womb tissues, metabolic processes normalization that exhibits in pain decrease, increase of myometrium

contraction function, enhancement of exudation and acceleration of womb involution processes.

So, izatizon is a medical system where all ingredients are active. Each of them controls its personal inflammation factor: excessive aquation, necrosis, infection inhibition, pain syndrome, evacuation and infection inhibition on the initial phase, regeneration process stimulation on the second phase of the inflammation process.

Successful treatment of patients, in modern humane and veterinary medicines, is possible only due to complex therapy taking into account aetiology, pathogenesis and disease symptoms. This process needs the applying of complex preparations with wide action spectrum to which izatizon belongs. [96] dimetylsulfoxid (demixid) and polietylenglikol (tvin) with molecular weigh 400 that unite at the same time antiviral, antibacterial, antifungal, antiphlogistic and immunemodulatory action are in the izatizon's formula.

Its effectiveness and wide action spectrum is possible due to the increasing of penetration into the tissues and organs that leads to the inhibition of intracellular viral reproduction and subtend bacterial infection [75]. At the same time even its long application as aerosols, skin and mucous membrane greasing, bondages, compresses, peroral and rectal introduction doesn't have cumulative characteristics and doesn't invoke toxic manifestations. It doesn't have irritable actions and doesn't inhibit hematosis. Having immunostimulatory characteristics izatizon increases organism resistance, provides high antiphlogistic, antihistaminic and medical-preventive effects [97].

Its rewrite for veterinary was made in 1999. A manual for application was approved by Verbyckiy P.I. the Head Government Veterinary Inspector of the Ministry of Agrarian Policy of Ukraine on 10.04.02 № 15-14/105.

Taking into account izatizon wide action spectrum we applied it during various animal diseases.

We have conducted scientific and productive researches of izatizon application in complex with basic treatment (used in farming) to study its effectiveness in

complex therapy. We applied izatizon during acute clinical course of catarrhal bronchopneumonia of cattle cubs in Chernivtsi, Kyiv, Sumy and Poltava regions. During these researches bull calves of 1,5-2 months old were divided in several groups [98].

Calves of one (control) group were treated with methods that are in farming practice (basic treatment) through application of antiallergenic preparations, antibiotics of wide activity spectrum and sulfanamide. The calves of the other group besides basic treatment additionally received izatizon per rectum in dose of 0,075 ml. of the preparation on 1 kg. of the body weigh (complex treatment) with the help of microclyster twice a day during five days. We made this procedure with the help of syringe without a needle with nozzle made from the polyethylene tube 5 centimeters in length. In 30 minutes prior to the administration it is necessary to make a cleansing enema with warm water.

We have determined that calves that hadn't been treated with izatizon had severer clinical course, treatment course was 3-4 days longer in average and their growth rate decreased comparing with that of complex treatment. Ill calves of the control groups gained clinical recovery on 10-12 days in average while calves that had been treated with izatizon recovered on 6-8 days. Moreover there was an obligatory slaughter of calves and their every day body weigh losses.

Izatizon was also successfully applied during calves treatment from infectious rinotracheitis (lung form) as an aerosol which was received with the help of SAG under the 4 atmosphere pressure on the expectation of 3-4 ml. for 1 m^3 of the house during the exposition 40-50 minutes once a day during 3-4 days.

From our point of view positive izatizon influence in the complex treatment of calves acute catarrhal bronchopneumonia is possible due to the stimulation of cell and humoral protective factors of the organism with pernicious influence on the pathogen microflora that is located in the respiratory tracts of infected calves.

High izatizon therapeutic effectiveness, comparing with traditional treatment methods, is admitted during cow therapy infected with different forms of endometritis, mastitis, vaginitis, cervicitis and vestibulitis.

During the endometritis 20% izatizon solution brovamast was injected to the womb in the dose of 20 ml. with the help of polystyrene dropper for rectozervical insemination of farm animals, connected with the syringe with rubber tube. Preparation was applied 2-4 times with the 24 hour interval, depending of the inflammation.

During cow and goat serous-catarrhal and purulent-catarrhal mastitis 10% izatizon solution on the 10% enroflox was injected internally-cisternal in the dose of 10 ml. after the previous milking of the infected part with its following massage from the bottom to the top till its tela.

During the pustular vulvo – vaginitis and other vaginitis, cervicitis and vestibulitis forms, tampon was wetted with 20% izatizon solution on the physiologic saline and introduced into the vagina cranial part with the help of forceps once a day during 3-4 days.

During horse melanoma 10% izatizon solution on 0,5% of novocaine solution was injected under the tumor basis three times a day in the dose of 5-10 ml. with the interval of 48 hours. Tumors disappeared during 14-18 days and the scars oat corn size formed instead of them, which in 2-3 weeks were covered with horsehair.

In cases of the skin surface affection wounds were smeared with izatizon or covered with gauze serviette sodden in izatizon. Than this napkin was fixed with the help of bondage and in a few days tissue granulation growth was observed that equally covered the whole wound surface. In case of need serviettes were changed every 24 hours.

During tissues injuries contamination and pathogen microflora entry to the wound and its abscess, during purulent-necrotic sores after conducting of corresponding surgical treatment of skin defected areas and neighboring tissues, izatizon was injected directly to the wound chamber and also gauze drain was used

sodden with izatizon that was changed every 24-48 hours. This leaded to the significant exudation increase of purulent-necrotic masses and rapid wound clearance. As a result its edges became movable and swelling decreased fast. After multiple izatizon application tender small-grained granulation tissue appears. After this wound was closed with stitches without following relapses of suppurative inflammation that gave the possibility to decrease significantly duration time of different processes in the skin and neighboring tissues.

Izatizon is recommended for clinical studies in the medical practice during people treatment from mass viral and infectious diseases (including AIDS), incisive illnesses of respiratory tracts, diseases of oral, pectoral and cardiac chamber, diseases of skin, gland and mucous tunic, brain affections, neuritis and neuralgia, miesitis, otitis, tumor and pretumor diseases, etc.

In the human medicine observations of volunteers ill with relapse forms of stomach ulcer and duodenum have proved izatizon high cure activity and availability during its enteral and rectal application.

So, obtained results testify to high therapeutic effectiveness of izatizon as a preparation of complex action during various inflammatory processes of respiratory system, obstetric and gynaecological cattle pathologies, during various diseases of skin and neighboring tissues of different animals and during horse melanoma treatment. This preparation has excellent prospects in veterinary and human medicine.

2.3.2. Beekeeping

Beta-tiosemicarbazons of izatin with different substitutes are very perspective. They have found their application in the chemoprophylaxis and viral disease treatment. β-tiosemicarbazons N-methyl- and N-ethylizatin are the most active among them. Their antiviral effect is resulted from the action on RNA- polyribosome and DNA- polyribosome nuclease complex, expressed by viral reproduction inhibition and immunomodulating activity.

We have studied molecular mechanisms of these compounds, and found out that they were very effective and have a wide influence spectrum due to increased penetration into the organs and tissues. This leads to the inhibition of viral intracellular reproduction and bacterial infection.

Izatin β-tiosemicarbazon decreases reproduction activity of smallpox vaccine virus, it also inhibits rhinovirus and flu virus reproduction. It is determined that Izatin β-tiosemicarbazon activates alkalotic DNKase and inhibits acid DNKase. Here it completely decreases reproduction of smallpox virus, flu A, parainfluenza of the 3-d type, arboviruses and adenoviruses.

We have also determined the antiviral action of tiosemicarbazon class compounds concerning to RNA-containing viruses. Tiosemicarbazon and marboran derivatives delay viral reproduction of different groups: poliomyelitis, rhinoviruses, mixoviruses and paramyxoviruses.

New izatizon medicinal forms. Izatoniy and lozeval were used for the treatment of mixed viral-bacterial infections.

It is very convenient and profitable to use new forms like aerosol, but at the same time, they can be added into the water and food. In case of stomach infections an alimentary introduction of prolonged action substances is favorable. For this purpose consistent medicinal forms of prolonged action (izalact, lactizet) were created. These forms do not yield to the liquid ones, getting into the organism through alimentary tract their advantage is that they don't provoke stressful situations, their production technology is cheap and they are the albuminous and vitaminous food supplements at the same time. This is very important for beekeeping where alimentary way of medicine introducing is the main and albuminous and vitaminous additions are very favorable during the absence or breaks in nectar providing in hive, what is observed on the Caucasus and in the foothills of the Carpathian Mountains.

Prevention and treatment of bee viral diseases. Viral diseases usually affect bees in the period of intensive family growth, when weather and seasonal factors lead to the lack of albuminous and vitaminous components of larvae that grow. Viruses that have affected their organisms invoke death on different stages of the germination.

Bees quickly find sick and dead larvae and throw them away from the honeycombs. We have found, during our examinations, that heterogeneity of the brood is typical for such families. Chronic clinical course is characterized by the large larvae mortality after the closure of the honeycombs. There are little islands of closed honeycombs with dead larvae inside among the majority of empty honeycombs from which young bees have hatched. They can be easily removed with the pincer. They hang as a liquid sacks on the tip of the pincer. Evolution of sick families stops. Viral paralysis affects adult bees. They suffer from the paralysis of muscles, stems, wings. Bees move along the circle, fall down from the combs to the bottom of the hive and die in convulsions. The whole bee families die if not treated.

Till the izatizon appearance and application viral disease prevention in Krasnopol's DVPG was possible only due to large expanses: extra nutrition with the adding of coniferous essential oils (coniferous natural extract, 2 grams per 1 litre of the syrup) was used largely during the month (in spring even during 1,5-2 months). Antibiotics of wide action spectrum (tetracycline, oxytetracycline, tetrachloride, etc.) or thymol (1 l. per litre of the syrup) were also used for the prevention. Antibiotics in such quantity contaminate bee's nest for a long time, get into the honey through nutrition and decrease its marketable and consumer qualities. Moreover antibiotics price has risen. Neat coniferous essential oil or coniferous extract become more expansive and scarce from year to year.

IZATIZON – is a complex preparation, N- Methyl-isatihn β-tiosemicarbazon, dimethyl sulfoxide and polyethylene glycol-400 are its components. Izatizon in the aerosol form was studied for prevention and treatment of bee viral diseases. Research and production pedigree farm of bee raising DVPG "Krasnopolyanske", scientific

production association “Dobrodeya”, RF NSI of beekeeping, joint-stock company “Biostim” and Institute of health promotion and rebirth of people of Ukraine have conducted combined researches. Positive results were obtained during izatizon application in aerosol form on 5 ml. for a honeycomb with bees. These honeycombs were double or triple sprayed with the interval of 2-3 days for viral disease prevention. Sprays were carried out if the temperature was not lower than 18°C.

IZATONIY – it is an izatizon medicinal form with wide antiviral and antibacterial action spectrum. This preparation is recommended for veterinary application during mixed infections. It inhibits RNA and DNA-viruses, and also, displays mucolytic and antibacterial effect (resolution № 432-3 from 07.01.89, approved by the Veterinary central administrative board of Russia). Izatoniy comprise N- Methyl-isatihn β-tiosemicarbazon, 1,2- ethylenebisamoniy dichloride, dimethyl sulfoxide and polyethylene glycol-400.

Izatoniy has been studied since august 1993 during mixed viral-bacterial infections in the suburban bee-gardens and also in the bee-garden №3 DVPG “Krasnopolyanske” (150 bee families). This treatment was carried out in the same way like with izatizon. The only distinction was that it was solved with water (1:50). The frame combs were widen up to 2,5-3 sm. before spraying, then they were returned to the normal condition exactly after the treatment. Medicinal effect, in all cases, occurred after the second application with the 48-72 hours interval. The preparation consumption was 15 ml for every beehive (0,3 ml of neat preparation). Trial treatments were put into practice on the half of researched bee-gardens, on the other half – regular scheduled prevention works. Bees were fed with sugar syrup with oxytetracycline 500 000 units for 1 litre, counting on 150-200 ml of the solution for a beehive (2 times with 5-7 days interval). 2 ml of the coniferous extract were added to such syrup.

Izatoniy application forwards bee multiplication. There were no disease signs found during control verifications in June and at the end of August in bee families treated with izatoniy. Capped brood was without omissions and in average each

frame was 300-400 combs larger than untreated one's. In the tentative groups larvae afterripening was on 5-8% higher than in untreated one's.

Beekeepers received in 12-15 times more production (queen bees, larval food, honey) from the tentative groups than untreated could give according to the results of the 1993-1996 seasons. Heterogeneity of the brood was observed in the bee-garden №31 in the village Kozachiy Brid in Adler's area. (antibiotics were ineffective). This bee-garden, during all the time of its existence, fulfilled the production plan at the beginning of June but not in August. The plan was fulfilled due to queens, larva food and bee comb frames production.

So, izatoniy application not only prevents infections but also stimulates evolution of bee families.

IZALAKT, LAKTIZET – consistent forms of prolonged action with the whole milk application instead of albuminous and vitaminious additions and N- Methyl-isatihn β-tiosemicarbazon and 1,2- ethylenebisamoniy dichloride carrier. 10 gr. of any preparation have to be solved in the syrup and fed in 50-s ml for each hive during 2-3 days along the week if the signs of mixed viral-bacterial diseases appear. You can repeat treatment course if necessary.

Application of all or some separate components of this complex (depending on the concrete conditions) is the best way to enrich bee vital activity and productivity[99].

Good honey yield depends on food supply with different natural and cultivated meliferous plants. We advise for all beekeepers to keep "beekeeper's carpet", that consists of new honey plants. These are fabulous annotinous and perennial flower plants: esculent, pabular, technical, medicinal and fancy. All these plants are originated in the Institute of Molecular Biology and Genetics NAS of Ukraine and in the Institute of health promotion and rebirth of people of Ukraine by method of molecular modification of hereditary apparatus. That's why these plants are resistant to droughts, frost-killing, salinity, etc. The most valuable of them are: Echinacea "Polisska beauty", Phytolacca "Polisska Fascicle", cyanosis "Polisska blue",

elecampane “Polisskiy Giant”, kvagista, ground-cherry, kavbuz, delphinium, pilotweed, giant hyssop and others.

Ascophereose or chalky brood is a regular larvae fungal disease strongly dependent on the family physical condition and dependent on this bee immunity to the given disease. The reason that has led to such a wide dispersion of this disease during last 10 years is still unknown.

Cleaning bees, while scrubbing combs from dead mummy-larvae, gnaw out sticked remains from the comb walls. This leads to the fungal littering of bee alimentary system. This has a big influence on the disease spreading among bees. This disease propagates the whole family.

Specialists recommend, in case of sickness, not only to change frames with the new one’s but also to change queen bee that is considered to be the host or a carrier of the disease along the hive. Maybe there is a reason in this, because queen bee is always in contact, including alimentary, with bees and can be a host or a carrier of the infection.

Ascophereose propagates much rarely in the strong and healthy families than in weak one’s. This disease propagates best among the quenelles drone families and drone broods where fixed temperature is absent in the brood area. Climatic conditions distress factor for brood evolution and dependent on this larvae immunity is the deciding factor in the dispersion of this disease. Decrease and increase cycling of the humidity and temperature in the bee nest, food inflow drop to the hive and dependent on this larvae feeding leads to their immunity decrease. Different viruses, inflammatory and fungus infections, which beekeeper does not even know about, influence this, because they are always hard to diagnose. They could be imperceptible visually, but they have a strong influence on the immunity decrease.

In 1999 because of late spring frosts and drought and dependent on this sudden oscillation of food inflow – all these factors caused the askosferoz dispersion. Beekeepers had to use different medicines against ascophereose, however result was not long lasting, in most cases, and the disease restored in a while. Inflow of the

strong forage to the hive has a very good influence on the treatment results fixation that every beekeeper may observe in his bee-garden. Bee family in the presence of good forage regenerates and disease decelerate or disappear.

Old frames, especially infected one's, are infection hosts (fungal spores), because bees, gnawing out old cocoons from sick brood and ill larvae remains, become again infected restoring for disease. Another place of the disease preservation is the hive bottom. A large number of small specks with spores fall down to the bottom, which bees gnaw out from combs. If beekeeper sweeps out the hive bottom by throwing away the honeycomb bag (crude net) this does not mean that, he has disinfected the hive because these small specks remain in it.

Why strong forage influences treatment process so well? Probably because bees fill up empty infected combs with honey and loose contact with their internal infected surface. A queen bee move to propagate to the newly built combs and the situation improves until autumn. However, in spring bees, having used all the food from dark combs, begin to clean them up from cocoon remains to enlarge the nest, where queen bee propagates, and contact again with the infection. Ascophereose restores. Ascophereose expansion forwarded by humidity, instability of spring weather and beekeeper's interference, which helps to cool the open brood, as well. Autumn and spring bee robberies in the bee gardens have a sufficiently big influence on the disease dispersion. These stealings happen in the infected bee families in most cases. Infection spreads, during these thefts, along the whole bee garden. This is not only ascophereose but also helomyzid flies, vaarotoz and other unknown diseases. We are surprising, at the same time, why ascophereose is spreading so well today?

As far back spring 1999 the Institute of Health Promotion and Rebirth of people of Ukraine offered to conduct in Stryy additional researches to study the influence of the new, in beekeeping, veterinary preparation called "Izatizon" on the ascophereose. Preparation instruction tells us that Izatizon increases immunity and is very effective against viral, microbial and some fungal infections, has an antitumoral action. Aerosol treatment of bees was recommended to cure ascophereose.

The treatments were conducted according to the suggested method that gave us encouraging results. Better result was obtained when izatizon was added to the food. Ascophereose disappeared for a very long time after such, not mentioned in the instruction, procedure in spite of the application of the large number of old frames in the bee garden. This procedure of medicine adding to the food also was tested many times with families that had not been treated with aerosol. The results of the treatment were encouraging because izatizon price is slight in the comparison with the other preparations, its result is very good, and it does not need a lot of time and efforts during large bee-gardens treatment. The preparation does not affect honey quality and is not harmful for bees and human organism.

We have to mention that during bee application of izatizon with nutrition it disinfects food tracts of nurse bees that make forage for larvae. In such a way, they guarantee food decontamination[100].

Probably open brood (larvae) becomes infected with ascophereose during nutritional contacts with nurse bees and having treated nurse bees, we disinfect larvae, increasing their immunity.

The researches have shown that the most effective was izatizon complex treatment of the families against ascophereose and other viral-inflammatory diseases where both aerosol treatment and preparation adding to the food were conducted. Here we have only to calculate, on the scientific level, the scheme of these treatments to guarantee the absolute health of bees and to minimize effective dozes.

During the parallel treatment of different bee families Izatizon was the most effective and the cheapest, that is very important for a beekeeper, among the other preparations of chemical and natural composition like nystatin, onions, garlic and scoping. The other preparation advantage is that it can be used for prevention of healthy families without fear to harm honey quality.

Therefore we see that bee family over one or two weeks becomes completely clean from infected larvae in the combs during izatizon application and you do not have to change the queen bee.

Combs, that were infected, during the treatment have to be marked and, as far as possible, expropriated for remelting over the season.

Probably izatizon having treated viral diseases, invisible for a beekeeper, increases the immunity of bee families and decreases to the minimum the possibility of ascophereose development that parasitizes on the open brood.

During preventive treatments, it is enough to add the preparation to the food. During ascophereose after the treatment, frames and hives have to be additionally treated with izatizon aerosol fine-dyspersated solution.

To fight effectively with the infection beekeeper, as a doctor, has to understand the ways of the disease spread, the factors that lead to the disease and the treatment method influence on the bee curing and guarantee his own and honey consumer's safety and the purity of the production from harmful preparations.

Beekeeping in modern market conditions is impossible without high profitability of bee-gardens. The complex of different conditions creates high profitability. Bee health occupies far from the last place among them. Healthy bees – this is one of the conditions for a good wintering, fast spring development and high honey yield.

Izatizon preparation was tested during 1997-1999 and 2005 in the bee-garden of the Carpathian department of the Institute of Health Promotion and Rebirth of people of Ukraine (IRRPU)

Institute's bee-garden was formed, during previous years, from different families with various diseases. First, we faced European helomyzid flies in 1997. Traditional treatment with antibiotics is difficult, expansive and ineffective.

Izatizon was used for the treatment of sick family as it was used in the veterinary as an antiviral preparation[101]. Brood frames were removed from the ill family and all the other frames, hive walls and bees were sprayed, two times in three days, with izatizon solution with sugar syrup addition. This solution is based on 0.3 ml. of pure preparation for 15 ml. of the solution for a one-bee frame. Taking into consideration the hive, for 10-frame family – 200 ml. of the solution and 4 ml. of

pure preparation. Helomyzid flies were not observed more in this family, but frankly speaking, the family has lost its progress rate and did not give any benefit in the given season. The conclusion is simple: beekeepers have to prevent diseases instead of the late treatment.

Ascophereose appeared in the bee-garden in 1998. The treatment was held in spring (the end of April). All the families – both sick and healthy were treated with izatizon solution two times. For 1 litre of 20% sugar syrup 20 ml. of the preparation was added. The quantity of infected larvae decreased but still the disease did not pass.

The disease visualized itself slightly and only in particular families during the summer period. The each family condition was recorded in the journal and the conclusions were made later. Wintering has passed successfully.

In spring of 1999, ascophereose again appeared in two families of 30 and in spite of the further treatment, these families have not recovered. The conclusion was made in 2000, and at that time the only thought was that izatizon is not enough effective against ascophereose. Families entered in winter without izatizon prevention treatments, were feed with converse sugar syrup and were some another delays (in September). The results were unfavorable. All provines and small families (6-7 frames) have survived until the spring flight but they could not fly around and died. The other families, even strong one, had a big deviation and ascophereose has appeared again.

The decision was made to change the method of izatizon treatment. Water solution spraying in spring is ineffective. Medicinal- albuminous paste was prepared:

Fluid honey – 1 litre;
Sugar-powder – 7,5 kg;
Milled pollen 0,9 kg;
Izatizon – 50 ml;
Water – 0,5 litre.

Izatizon first was dissolved with water and the pollen was mixed with sugar. 300-350 grams of pollen or 1,5-1,6 ml. of izatizon were feed to the families. Nearly 0,3 ml preparation was applied for a frame. Two families have not received the paste; they were control examples. These were families of the medium strength and disease

condition during the last year. Another one family has not eaten the paste because of bad condition and in the end died as well. For the rest of the families the results were more then satisfactory.

Ascophereose was not observed during the whole season in the families that have eaten medicinal paste. We can say this for sure because bee pollen was collected using tank that was placed in the hive. All bee problems were apparent in the tank trays, which were controlled every other day. There were not any infected larvae!

In addition, we have to mention that some families contacted with ill families and instruments and were not infected by ascophereose. One of the queen bees from those families was taken from bee farm in Mukachevo.

Prevention from ascophereose and foulbrood with izatizon is more effective than curing.

The most effective izatizon application against foulbrood and askoferoz is making of medicinal paste with it. The paste is better to give in early spring until the brood hatching. In this case, bees, that will feed larvae, will certainly eat some doze of the medicines before. If the paste is eaten faster than two weeks, it is necessary to give again the same paste portion or to feed 30 ml. of izatizon for a family with not less than 1 litre of sugar syrup, honeyed solution or converse sugar (if natural conditions allow feeding with fluid syrup).

Autumn prevention will insure bees from bad winter and whims of early spring. For this 3-4 ml. of izatizon must be added in one of the last syrup portions during feeding for winter for a family.

All these recommendations do not replace but supplement all the other measures that must be done for beekeeping during the autumn-spring period.

2.3.3. Some results of izatizon application on human volunteers in clinic

Izatizon was used in complex in the stomatology practice[102]. According to the research results we assumed that this preparation has to be on point position in the prophylaxis and treatment of many stomatological diseases. Izatizon showed itself as

a preparation of the wide activity spectrum with the significant curing effect. This effect was achieved due to combination of antiseptic, anti-inflammatory, antifungal, antivirus and keratoplastical characteristics. Izatizon gives a significant economical effect: decreasing the treatment time, it is handy in use (water solutions, spreads easily cope with other medications), does not lead to the complications, allergies, reduces the number of visits to the doctor. No contraindications are shown.

Treatment of periodontitis. Classical medications did not give us positive results in some cases of the treatment of the exacerbation of chronic periodontitis. Thus the tooth did not sustain the closure, edema, pain during the percussion, palpation in the projection zone of the root appeared. In such case during 3 visits wick drain with izatizon was placed in a well dilated canal, every other day this wick drain was changed and the tooth was stopped (provisional filling). There were no complaints during the next visit. During the examination we have determined that the root canals are clean, percussion and palpation are negative. This gave us the possibility to fill the root canals with cariosan or with other materials and to put a permanent . All this process took up three visits to the dentist.

Under the acute condition of chronic periodontitis and cystogranulomas, izatizon, as a paste was inserted into the granuloma. During the next visit the paste was removed from the root canal and it was filled with cariosan. There were no complications. The excess of the paste resolved in future.

Method of treatment of the exacerbation chronic periodontitis with izatizon. During the first visit to the dentist the tooth was opened for the outflow of the exudation**.** Home recommendations: to rinse the mouth with soda solution (1 tea-spoon of baking soda for a glass of warm boiled water).

3 days later the medical treatment of root canals with aseptic solutions was carried out. Root canals were widen with the instruments if needed. Then wick drain with izatizon was placed into the root canals and the tooth was filled with dentine-paste.

The next day the dentist changed wick drains and closed the tooth for a week (provisional filling).

After the finishing of the control term, if the complaints and inflammation signs (percussion negative, absence of edema, exudation excreta and pain) were absent, the root canals were filled, cavity liner and permanent stopping were placed. There were no complications during the year.

Treatment of the acute deep caries and traumatic pulpitis. Taking in consideration the antiseptic qualities of izatizon and its antiphlogistic effect, we concluded that it deserves to be introduced to the medicinal pastes for the treatment of pungent deep caries and traumatic pulpitis. It is recommended to put izatizon to the bottom of the carious cavity or accidentally opened pulp horn. The paste was prepared extempore on the zinc oxide basis. After the finishing of the control period, in case of the absence of complaints, the provisional filling was removed up to 2/3, then the liner and permanent stopping were put.

Treatment of simple gingivitis. In case of simple gingivitis there were no patient complaints after the elimination of the disease cause and one-fold10 min. izatizon application.

In more complicated situations izatizon was introduced into the paste formulation to obtain more effective result. It was applied to the alveolar appendix under the paraffin bondage in combination with other medications. (vitamins A, E, immunomodulators, etc.).

Treatment of hypertrophic gingivitis. 12 patients with infectious periodontitis together with symptomatic hypertrophic gingivitis or with one of the gingivitis forms (catarrhal or hypertrophic) have been treated by izatizon.

The results of the treatment give us the possibility to make the conclusion that given preparation has a high medicinal activity. Two mechanisms of action have the

decisive meaning in the izatizon therapeutic effect: antiphlogistic and growth-inhibiting effect of the granulation tissue. Here is the clinical example.

Patient K. Diagnosis: generalized periodontitis, I-II degree, acute clinical course, chronic hypertrophic gingivitis of the I-II degree, mixed form.

Fairly: gingival papilla of both jaws hypertrophied up to I and I-II degrees, swelled, with light bluish shade, they are bleeding a bit while light intubation. Over- and subgingival dental sediments are present in low quantity. The depth of paradental recesses is 3-5 mm. Tooth mobility – I degree. There is a bracket system on the upper-jaw that makes impossible the efficient oral hygiene. Moreover, low tooth coronas forward the contact of teeth with jaws. The microflora of paradental recesses is mixed. Protozoan is the outnumber (7-12 trychomonals in the visual field), fungi are presented in low quantity.

The doctor made the irrigation of the oral cavity with furacillin picked away dental sediments, made an application and lutemacia of pastes (boric ointment, trichopol, galascorbin, furazolidon) to the paradental accesses. All these procedures took 3 visits. As a result, the swell and the bleed disappeared, the bluish shade of papilla decreased, but the papilla hypertrophy persisted on the same rate. The clinic covering of some corona parts decreased for about 1 mm.

Later on five treatment sessions were held with the use of izatizon. Forms of the usage: preparation introduction to the paradental recesses with the help of wick drains or liquid ointment installation (the preparation was compounded with zinc oxide). Then a wound was dressed for 10-60 min.

As a result, the papilla hypertrophy decreased up to 1-2 mm, depending of the area (frontal or lateral). The better result was obtained after the use of the greater granulation number. There were no side effects after the izatizon application.

The given information proves the high multifarious effectiveness of izatizon in the stomatology.

Izatizon was used during the period of 1998-2005 in National academy of state administration under the President of Ukraine, Kyiv, for the treatment of acute and

chronic tonsillitis, antritis, sinusitis, bronchitis, flu, acute viral respiratory diseases, stomatitis, neuritis, neuralgia, arthritis, skin injuries and burns, vulvovaginitis, cervical erosion, genitals inflammatory processes.[103] A doze for each patient was selected individually that varied from 0,5 to 1 ml. of Izatizon according to the famous Foll-Sarchuk procedure. 316 patients were treated. From obtained data, we see the high effectiveness of the treatment. There was one case of ineffective treatment because of the organism reaction on Izatizon in the form of dermatitis. The way of Izatizon penetration was individual taking in consideration the diagnosis and clinical course of the patient: by rinsing, plugs, microclysters, skin compresses, on lesion focuses. The age of examined patients was of 1,5 to 67 years.

During flues and sinusitis the preparation was instilled to the nose, rinsed the throat. Medics rubbed Izatizon in the neck area of patients and did inhalations in case of bronchitis. They rubbed it into the skin of ill site and pain projection areas covering with gauze or polyethylene film during neuritis, neuralgias and arthritis. Pain and swells decreased for two hours.

Our researches indicate that Izatizon is a preparation of emergency care that suits to every family, first-aid post and polyclinic and also for emergency station.

2.3.4. Treatment of male uresis system under viral and inflammatory diseases

Medical collective of the urological department of the Road hospital №2 of the Stryy station use preparation “izatizon” for prostate and urethra inflammatory diseases treatment and also they use it like antiviral and antitumoral preparation during the last 5 years.

Chronic prostatitis is one of the most diffuse diseases of the male urinary and sexual systems. Information about its treatment has been well known since ancient times but still we cannot answer exactly on all the questions of the disease pathogenesis and its treatment. The search of new means for prostate inflammatory disease therapy is one of the main tasks of modern urology.

Izatizon is one of the most perspective preparations that is able to increase specific and nonspecific resistance indexes during immune status violations and possesses antiseptic and antiphlogistic characteristics. Preparation action is based on good penetration through demytilsulfoxide (izatizon component) mucous membranes and transferring of other components (methisazone, twin) to the organism tissues. Izatizon components possess antiviral, antiphlogistic, antihistaminic, analgesic, antimicrobial and fibrinolytic characteristics. Moreover, the preparation is able to restore antimicrobial antibiotic activity against resistant or weakly sensible to it bacteria strains.

Izatizon tests were held in practice in the urological department of the road hospital №2 of the Sryy station.[104] The research program includes gathering of complaints, anamnesis, physical examination, clinical analysis, prostate secreta examination, instrumental examination: ultrasonic diagnostics and cystoscopy. The preparation was used as a microclyster 30-50% of water solution or together with 10 ml. 2% of lidocain, once or twice a day during ten days.

The preparation was used for the treatment of 40 patients with acute attack of chronic prostatitis in complex with regular therapy. Except this, another (control) group (30 men) was observed. They were treated during the acute attack of chronic prostatitis only with habitual antiphlogistic therapy. Then the results of both groups were compared.

Improvement of health, symptomatology decrease, clinical analysis improvement were observed on the 4-6 day of the therapy in the patients group which was treated with izatizon, while in the control group the same result was observed on the average 7-10 day of the treatment. Relying on this it became possible to reduce antibacterial preparation application together with izatizon to 7-10 days. Antibiotic therapy average course in the control group was 14 days.

While the patients examination during the whole year, repeated prostatitis exacerbations were observed three times (3 persons) in the first group, that was 7,5%, and five times (5 persons) in the second group, that was 16,7%. Izatizon

microclysters were used to treat the disease relapses in the first patients group, their condition became stabilized and in the control group, we had to use antibiotic therapy.

Among preparation side effects, the most common is burning feeling in the application area. The addition of 2%-6-10 ml. of lidocaine solution was successfully used against this defect. It removed or reduced significantly side effects and did not influence the treatment course.

Except izatizon application for prostatitis treatment, it was also widely used against pointed condyloma. We have treatment experience of 10 patients and the obtained results were good. Izatizon was applied as applique on condyloma three times a day as a result candylomas of six patients have disappeared and have significantly decreased on the rest of the patients.

Based on previously mentioned we can claim about positive effect of “izatizon” application during prostate inflammatory disease treatment and pointed condylomas. It also decreases antibacterial preparation usage, prolongs relapse-free clinical course and it can be introduce for public application.

2.3.5. Eye viral diseases.

Izatizon was applied on an outpatient basis in form of 25% oil solution (one part of the preparation solved in the three parts of refined oil). Application method – ready solution instillation in twos drops to the affected bulbar conjunctiva. Periodicity is 6-8 times a day[105].

Patient group comprised 35 persons, mainly of the middle age with viral eye pathology. 55 eyes were treated in this group. Five patients, from the whole quantity, asked for help after insufficient treatment by the regular antiviral preparations. In one case we have observed and treated a person with viral inflammation of the right Gasser's ganglion and viral keratoconjunctivitis of the right eye. Izatizon was prescribed him for the eye treatment according to the above mentioned scheme and we also recommended compresses and greasing with Izatizon in the eruption area on the forehead. As a result we have observed condition improvement in two days with

further epithelization without keratoleukoma and significant eruption decrease on the skin. This treatment has finished with full recovery.

In case of ulcerous viral keratitis (one case was determined) the treatment was carried out with Izatizon without cryoapplication. Recovery has come without eye nebula. Once we have observed the relapse of treated eye (later we found out that this patient has violated the medical treatment schedule). Two patients were away so we weren't able to study them till the end of the treatment process, but we have already registered their condition amelioration on the third day since the beginning of Izatizon cure. One patient has refused from the preparation installation after the first try over strong pain he felt.

Practice has shown that treatment effectiveness depends on the viral form of nebula or conjunctiva and also on the period of patient visit. Persons that appealed late for medical aid (on 3-5 day since the disease started) and the inflammation process was in acme their treatment period was longer than usual.

Improvements came generally in 3,5 days after the cure start. Average disable duration – 7,5 days, pain syndrome disappearance – from 1 to 2 days, after the full recovery visual acuity restored till the initial level. Keratoleukoma wasn't observed after the recovery. Our experience shows that for the full medical course patient uses 3,0-5,0 ml of prepared preparation. During the eye viral diseases we have applied Izatizon as a basic preparation together with furacilin (1:5000) and sulfacetamide (30%) solutions in two drops 6-5 times a day.

During izatizon treatment the allergic manifestations haven't been noticed. The main preparation defect that our patients indicate as well as author who has tried it himself, is the strong burning during the first several seconds after instillation.

According to the literature, such preparations are used to cure viral diseases in ophthalmology: iduxoridin, phlorenal, tebrophen, bonaphton, poliakrylamid.

Iduxoridin-5 iodine-2-desoxsiuridin (IDU) – it enters into the DNA and leads to the base albumen creation that for it's part interrupts viral replication; influences actively on the pox and herpes viruses. In case of dendriform keratitis, if IDU is

applied as eye medicated film than cornea epithelization occurs in 4-10 days period. If the preparation is applies as drops this process takes from 7 to 15 days. Recovery during superficial keratitis comes in 60-90% of cases and during stromal – in 20-30%. Along with this, IDU has some negative characteristics:

- It ruins at room temperature and becomes toxic;
- It has frank cytostatic action on the cornea epithelium;
- It during its application viral persistence to the preparation forms with comparative ease;
- It has high effectiveness only in cases of superficial viral keratitis;
- It has low effectiveness or ineffective in cases of deep keratitis and uveitis;
- It has toxic and allergic action on the cornea (acute allergic conjunctivitis, filamentary keratitis, microanus, stromal cornea ulcer).

For these reasons it is not recommended to apply preparation more than 10 days without a break.

Florenal-bisulfate junction 2-florenonilglioxal. Exploitation form: 0,25-0,5% covering, 0,1% eye drops, 0,1% solution for underconjuctive injection. Florenal is characterized by high activity regarding flu pathogens. Ointment application effectiveness nearly corresponds to IDU. 71 patients from 73 with superficial keratitis have recovered. Average treatment length of ocular herpes during dendriform keratitis is about 14,2 days, during stromal keratitis – 22,1 days. Florenal therapeutic action enchancement is observed in complex with polyacrylamide which is an interferon inductor.

Tebrofen – 3,5,3`-5`-tetrabrom-2,4,2`,4`-tetraoxidifenil – shows high activity regarding flu viruses. It is insoluble with water and is used as 0,35 or 0,5% eye ointment. Tebrofen is effective only on the disease initial stages. It is useless during stromal keratitis. Tebrofen treatment scheme: eyes ointment is put to the conjunctival sac 3-4 times a day. Recovering is observed on the 12-th day.

Bonafton – 6-brom-1,2-oilkhenolin– shows high activity regarding herpes virus, it is used as 0,05% eye ointment. During bonafton treatment the recovery comes on the average 11,5-7,5 days. Patients had epitheliopation effects after 2-3 weeks of preparation application and they disappeared after its cessation. Treatment effectiveness has significantly risen during combined oral application.

In view of significant toxicity bonafton is recommended to apply during the grave viral process forms.

Poliakrylamid – more active during the superficial and ineffective during keratitis deep forms and is characterized by low toxicity and good tolerance.

Table 9. Comparative table of the effectiveness of Izatizon and traditional preparations

Preparation name	Improvements since the beginning of the treatment, days	recovery		Warnings
		Day from the beginning of the treatment	Percent from all patients	
+ IDU	4	10	Superficial process – 60-90% Stromal – 30%	To use not more than 10 days
+Floreal	3-4	14,2-superficial 22,1-stromal	71%	-
+Tebrofen	2-3	12-superficial	-	-
+PAA	7	10,4-superficial	-	-
+Bonafton	3-4	11,5-7,5	-	epitheliopathy
Izatizon	3,3	7,5	92%	-

From the above stated comparative review of popular antiviral ophthalmologic preparations (according to the literature data) and results received during the Izatizon viral eye pathology it is possible to admit such positive moments in favour of izatizon as:

1) preparation stability under application conditions;
2) absence of allergic manifistation;
3) no intoxication during the treatment;
4) evident medical effect in short terms (3,3 days) from the beginning of the treatment, weakening of the pain syndrome in 1-2 days;
5) relatively short average index of working capacity loss - 7,5 days;
6) all patients have reproduction of visual acuity to the prior indexes;

7) after recovery cornea epithelization passed without corneal caligo;
8) strong remission is observed after recovery;
9) small draught of ready preparation for the treatment course: 3,0-5,0 ml. 25% of Izatizon oil solution;
10) preparation long exposure on the mucoid and cornea due to its oil base.

As the defect of izatizon patients mention painful feelings during couple of seconds after preparation instillation to the conjunctival sac.

2.3.6. Effect of Izatizon on the plant productivity

Considering the present ecological conditions it is necessary to think about the problem of the organism protection from different diseases. As a result, of ecological crisis human and also plant and animal organisms became weak and their immunity level is comparatively low. This makes us weak to different harmful factors: bacteria, viruses.

Viruses are the biggest threat for us because they provoke most of serious, almost incurable illnesses that except not only acute clinical course but chronic course as well that leads to the infection complications and tumor growth. Moreover, it is very difficult to treat virus diseases because very often antiviral preparations kill not only infected cells but also they are very toxic for whole our organism. The study of new preparations that will help organism in the struggle against viral diseases is of current importance.

During two years the research was made to determine izatizon influence on plant productivity and plant persistence to the viral diseases during vegetative period. For these research the plants were chose, derived by method of purposeful non vectorial hereditary information transfer from one plant to another. . It is a hybrid of corn and rye called “Zhytnyah”. This is a plant of winter-annual type growth period and has some common characteristics with parent plants – corn and diploid rye. “Zhytnyah” was treated with izatizon by steep before sowing. After a time first harvest was obtained.

Such statistic results were consisted as balancing, comparison of phenotypic characteristics (ear length, segment quantity, awn length, seed quantity in the ear, seed weigh in the ear) control (meaning plants that weren't treated with izatizon) with characteristics received in the first generation (each characteristic sample – 100).

Phenotypic characteristics of plants treated with izatizon vary in the intervals: ear length from 5,0 – 10,5 sm.; awn length from 2,5 – 7,0 sm.; segment quantity from 12 – 30; seed quantity from 7 – 30 sm.; seed weigh from 190-800 mg. average ear length – 7,675, awn length – 4,173, segment quantity – 19,63, seed quantity – 18,65, seed weigh – 415,3.

Phenotypic characteristics of control plants (that weren't treated with izatizon) vary in the intervals: ear length from 3-7,8 sm.; awn length from 1-6 sm., segment quantity from 6-18 items, seed quantity from 5-18 sm., seed weigh from 100-470 mg. Average ear length – 4,798, awn length – 2,944, segment quantity – 10,9, seed quantity – 10,26, seed weigh – 229. Statistic calculation data are given in the Table 10.

Table 10. **Phenotypic characteristics**

	Izatizon				Divostim		
Indication name	Indication Average meaning	Average from averages	Average quadratic		Indication Average meaning	Average from averages	Average quadratic
Ear length	7,675	8,056	1,195		4,798	4,917	1,098
Awn length	4,173	5,421	0,842		2,944	3,520	1,296
Segment quantity	19,630	20,937	4,340		10,900	12,550	3,154
Seed quantity	18,650	18,364	4,842		10,260	10,820	3,042
Seed weigh	415,300	410,753	112,234		229,000	298,920	81,786

These data show that izatizon is very advisable for farms because it adapts plants to the environment unfavorable conditions, has antiviral, antibacterial and immunomodulating influence that leads to the harvest increase.

Given results were obtained under the accurate validity of preparation concentration and seed treatment technology.

Based on these researches we consider it necessary to use izatizon in farming for greater harvests receiving

Izatizon is a new generation drug that combines the antiviral activity and immunotropic action revealing also anticancer properties, especially in case of melanoblastoma. It is experimentally proved that izatizon affects both viruses and cellular mechanisms of the immune system. In model systems of herpes virus and adenovirus we detected that izatizon has an ability to stimulate the reparative DNA synthesis. [106] We discovered the drug ability to inhibit the thymidine kinase activity of the herpes virus and adenovirus that results in inhibition of virus at the early stages of infection.

We found the izatizon curative effect upon herpes virus and adenoviral infections and its availability at AIDS, tuberculosis and hepatitis C. Our data show convincingly a significance of this drug, especially nowadays, when all the continents of the earth witness a dramatic increase in the number of viral and immune aggressive diseases that were considered disappeared as well as appearance of new ones, among which the viral diseases make an essential part.

Izatizon is active against DNA-and RNA-containing viruses, it also has pronounced immunomodulatory properties [107]. Moreover, izatizon is an activator of nonspecific resistance factors via its stimulating effect on the metabolic and phagocytic functions of macrophages, and the influence on natural killer cells activity and synthesis of lysozyme.

A number of modern biotechnologies were elaborated: targeted improvement of hereditary information of biological objects; environmental cleansing by application of the plants able to assimilate atmospheric nitrogen instead of expensive fertilizers; obtaining good crop capacity of plants on highly saline soils; increase of productivity of beneficial insects, fishes, birds, animals; a method to control agrobacterial cancer of plants [108].

III. Modern molecular-genetic aspects of human and environmental sanitation.

3.1. Effects of exogenous nucleic acids application

Insects are good models for morphogenetic processes study. With this aim, along with drosophila, is used the silkmoth where an interconnection of genetic and morphologic changes in ontogenesis was investigated on maximal detailing level. This fact enables a regularity comparison of the nucleic acids exchange in the insect embryogenesis along with genetic material accumulating and existing genetic information realization that is very important in the theoretical aspect.

On the beginning of the ontogenesis, the prevailing number of events in the growing embryo is automatically regulated that is provided with the stepwise use of accumulated and anew made structural information and reserve materials along with distinct related intracellular processes and cell-cell interactions. It is obvious that on the early ontogenesis stages the molecular mechanisms, which have a decisive importance for realizing the mature organisms' characteristic, perform. Naturally, leading hand in this process belongs to the structural nucleic acids metabolism and their particularities.

On the basis of the common purpose, concluded in searching of the rational methods and means of increasing silkmoth productivity, the next technological particularities of this program realization were solved: selection of the optimal compounds – biostimulants, methods of their administration into insect's organism, and stage – that would have biologically based and rational effect[109].

In the program of long-term researches we used the silkmoth industrial hybrid, grown from a grain of Mirgorod grain factory. As the productivity stimulants were used uracil ($C_4H_4H_2O_2$) (URL), or methyluracil (MT) or 5-piperidine-methylene-methyluracil (BES-221). These compounds were used for treatment the silkmoth eggs during spring reactivation on the 2-5 day of their growth that corresponds to the periods of the mouthparts and thoracic legs appearance on the frontal metameres and blastokinesis. Furthermore, on the fifth stage of caterpillar age were used nucleic

acids – native yeast RNA, purified by the Institute of Molecular Biology and Genetics method (RN), RNA, modified by thiophosphamide (RNT) and RNA, modified by cyclophosphamide (RNC). Simultaneously from the same grain were grown caterpillars without conduction of any treatment (control), and with use of better analogue.

It is determined that the silk capsule mass of cocoons, grown according to the original technology, exceeded the similar index of the better analogue on 32 and 40%. The native and modified preparations of RNA predecessors, URL, MT and BES-221 in the offered technology were used on embryonal stage of the silkmoth growth with the purpose of their growth intensification and biomass accumulation. The effect of RNA predecessors in the range of 0,10-0,02% concentrations showed high effectiveness. Higher or lower concentrations cause considerable silkmoth productivity decrease. Besides, the obtained results testify the efficiency of stimulating on the initial stage of the grain growth including the fifth day.

In Table 11 illustrated the results of an impact of grain treatment duration on the silkmoth productivity indexes. Evidently, the optimal exposition is 1h-1.45h duration of the grain treatment. The result of a grain treatment by such preparations as URL, MT and BES-221 is a considerable intensification of the caterpillars' growing process and accumulation of their biomass, which partially transforms into the silk, and partially remains in the pupa body. High index of the pupae mass testifies about an existence of the plastic and energy material reserves in the silkmoth organism, which can be to a certain extent transformed into the silk thread protein.

The next constituent of the silkmoth growing technology is the caterpillars' forage treatment by the water solutions of the native and modified yeast RNA, RN, RNT and RNC in a first half of the fifth caterpillars' age. The use of these biostimulants specified by the included substances that efficiently transform caterpillars' biomass to target product – silk thread of the cocoons capsules.

Table 11. Productivity of the silkmoth, grown from the grain after applying the RNA predecessors on the third incubation day at different effect duration

Variant	Treatment exposition, minutes	Males, mass, mg/% before the control			Issue of the silk raw material, %	Males, mass, mg/% before the control			Issue of the silk raw material, %
		Cocoon	Pupa	Capsule		Cocoon	Pupa	Capsule	
Control (water)	-	1835	1524	311±7	16,95	1520	1218	302±8	19,87
MT, concentration 0,02%	60	2118 115,4	1707 112,0	411±12* 132,2	19,40 +2,45	1797 118,2	1394 114,4	403±12* 133,6	22,43* +2,56
	105	2883 113,5	1677 110,0	406±13* 130,7	19,50 +2,54	1779 117,0	1391 114,2	388±11* 128,4	21,81* +1,94

Note: here and below, the indexes of the silk capsule size, pointed out by asterisks, statistically may exceed the control indexes.

Use of the stimulants only in a first half of the fifth caterpillars' age specified by the increasing of the cocoons' comparative silk-bearing feature after treatment during this period, otherwise, by more effective transformation of the protein resources into silk raw material on a caterpillar stage. A treatment in the later periods allows only insignificant proportional increasing of the both component parts – cocoon-pupa and silk capsule. The materials of the Tables 11 and 12 indicate a high specificity of the preparations effect that exhibits in increasing of a silk issue on 4-7% comparing to the control variant. Such an automatic directedness of metabolism processes on mainly silk protein formation is a reason of the pupae mass decreasing on 5-10% in most cases that testifies about using of some protein reserves on transforming into the silk capsule cocoon proteins.

Thus, a high effectiveness of each group of used native and modified DNA, RNA is demonstrated. It is essential to underline that these developments concerning the beneficial silkmoth growing technologies are original, have no other analogues and protected by the whole range of the author's certificates and patents.

Table 12. The native and modified yeast RNA effect on the silkmoth productivity after treatment in first half of the fifth caterpillar age

Variant	Treatment exposition, minutes	Males, mass, mg/% before the control			Issue of the silk raw material, %	Males, mass, mg/% before the control			Issue of the silk raw material, %
		Cocoon	Pupa	Capsule		Cocoon	Pupa	Capsule	
Control (water)	-	2022	1673	349±11	17,26	1468	1174	294±9	20,03
RN	0,040	20,60 101,9	1597 95,4	463±10* 132,8	22,48 +5,25	1436 97,8	1042 88,7	394±8* 133,9	27,44* +7,41
	0,008	2161 100,9	1682 100,5	479,9* 137,2	22,17 +4,91	1487 101,3	1113 94,8	374±6* 127,4	25,15* +5,12
RNC	0,040	2040 100,9	1585 99,7	455±9* 130,4	2230 +5,04	1521 103,6	1115 95,0	406±11* 138,0	26,70* +6,67
	0,008	2158 106,7	1691 101,1	467±10* 133,8	21,64 +4,38	1567 106,7	1169 99,6	398±9* 135,6	25,40* +5,37
RNT	0,040	2123 105,0	1641 98,1	482±8* 138,1	2270 +5,44	1542 105,0	1126 95,9	416±9* 141,4	26,97* +6,94
	0,008	2118 104,7	1665 99,5	453±13* 129,9	21,39 +4,13	1531 104,3	1148 97,9	382±10* 130,1	24,95* +4,92

The silk raw material, obtained from the silkmoth cocoons, specified by the unique characteristics like high strength, moisture resistance, hygienic feature and thereby may be successfully used in the textile industry, medicine and radio electronics.

After breeding of the original monovoltine species of the silkmoth – Poliskyi tasar – biologic form, acclimatized and adapted to the industrial growing on the territory of Ukraine and other regions of the European part, there is a real possibility for development of a new field of the national economy – forest sericulture.

In the National Agrarian University together with the Institute of Molecular Biology and Genetics of NAS of Ukraine, scientifically based and tested technology that has no other analogues, relates to higher category and concerns usage of such compounds and preparations as: uracil ($C_4H_4H_2O_2$) (URL), methyluracil (MT) and 5-piperidine-methylene-6-methyluracil (BES-221) in the silkmoth culture growing. These compounds were used for directed treatment of the silkmoth eggs of Poliskyi tasar species, obtained from Kivercivskiy centre. Furthermore, as the stimulants, used on the fifth stage of the caterpillars' age, were applied native yeast RNA, purified according to the method of IMBG (RN), modified by thiophosphamide (RNT) and RNA, modified by cyclophosphamide (RNC).

To implement the technology we took three portions of silkmoth grain, the mass of each grain is 1 g, and treated with 0,1% solution of MT, URL and BES-221 over 1 min. 45 sec. on the third day of the incubation. The caterpillars of these groups on a first half of the fifth age were fed with forage treated by 0,04% water solution of RN, RNT and RNC preparations. The cocoons, grown according to this technology, had the mass exceeding the silk capsule of the control variant of cocoon on 55-59% at 40-42% - of the better analogue index. It is significant, that the obtained positive result exceeded all known native and foreign analogues on considerable amount. Until now, in the sericulture were no other similar developments.

The RNA predecessors reveal the highest effect over the range of 0,10-0,02% concentrations. Higher or lower concentrations conduce to decreasing of the effectiveness. Analysing the results of the silkmoth productivity after the grain treatment in different days of its growing, it is significant that treatment was not conducted in a first day because of mass grain ovipositing in the first too days. The experimental data indicate the effectiveness of treatment on the first periods of grain growing, including a fifth day. Later on, the results sharply decrease.

In Table 13 illustrated the results of an impact of GRAIN treatment duration on the silkmoth productivity indexes. Evidently, the optimal exposition is 1h-1.45h duration of the grain treatment. At these regimens, the silk capsule exceeded the control variant on 40,3-47,2%. It is significant, that this result obtained after the effective regulation of the protein mass redistribution in favour of the silk capsule. Herein is the specificity and uniqueness of the nucleotides effect.

The result of a grain treatment by such preparations as URL, MT and BES-221 is a considerable intensification of the caterpillar growing process and biomass accumulation. A component part of the technology is the caterpillars' forage treatment by the water solutions of the native and modified yeast RNA, RN, RNT and RNC in a first half of the fifth caterpillars' age. The use of these stimulants specified by the included substances that efficiently transform caterpillars' biomass to target

product – silk thread of the cocoons capsules. This phenomenon illustrated in the Table 14.

Table 13. Productivity of the silkmoth, grown from the grain after applying the RNA predecessors' directive effect

Variant	Treatment exposition, minutes	Females, mass, mg/% before the control			Issue of the silk raw material, %	Females, mass, mg/% before the control			Issue of the silk raw material, %
		Cocoon	Pupa	Capsule		Cocoon	Pupa	Capsule	
Control	-	5704	5268	436±16	7,64	4430	4054	376±13	8,48
MT, concentration 0,02%	60	7211 126,4	6619 125,6	592±22* 135,7	8,21 +0,57	5285 119,3	4748 117,1	537±18* 142,9	10,16* +1,68
	105	7124 124,9	6512 123,6	612±21 140,3*	8,59 +0,95	5247 118,4	4694 115,8	553±19* 147,2	10,54* +2,06

Note: here and below, the indexes of the silk capsule size, pointed out by asterisks, statistically may exceed the control indexes.

Table 14. The native and modified yeast RNA effect on the silkmoth productivity

Variant	Concentration, %	Females, mass, mg/% before the control			Issue of the silk raw material, %	Females, mass, mg/% before the control			Issue of the silk raw material, %
		Cocoon	Pupa	Capsule		Cocoon	Pupa	Capsule	
Control (water)	-	5704	5268	436±16	7,64	4430	4054	376±13	8,48
RN	0,040	5659 99,2	5002 94,9	657±11* 150,6	11,61 +3,97	4432 100,0	3878 95,6	554±19* 147,4	12,50* +4,02
	0,008	5760 100,9	5116 97,1	644±14* 147,7	11,18 +3,54	4539 102,5	3977 98,1	562±22* 149,4	12,38* +3,90
RNC	0,040	5702 100,0	5052 95,9	650±12* 149,2	11,40 +3,76	4247 95,9	3711 91,5	536±16* 142,6	12,62* +4,14
	0,008	5916 103,7	5210 100,0	646±12* 148	10,92 +3,28	4360 98,4	3808 93,9	552,16* 146,7	12,66* +4,18
RNT	0,040	5966 104,6	5339 101,3	627±14* 143,8	10,51 +2,87	4299 97,1	3775 93,1	524±12* 139,4	12,99* +4,51
	0,008	5588 101,1	5247 99,6	634±19* 145,4	10,78 +3,14	4523 102,1	3964 97,8	559±18* 148,7	12,36* +3,88

Use of the stimulants only in a first half of the fifth caterpillars' age specified by the increasing of the cocoons' comparative silk-bearing feature after treatment during this period, otherwise, by more effective transformation of the protein resources into silk raw material on a caterpillar stage. A treatment in the later period of an embryonal development allows only insignificant proportional increasing of the both component parts – cocoon-pupa and silk capsule. Illustrative data indicate a high specificity of the preparations effect that exhibits in increasing of a silk issue on 4-7% comparing to the control variant.

It is essential to underline that discovered phenomenon of the nucleic acids effect and their modifications, has a universal effect on the insects, and probably on the other fauna representatives. We obtained the similar data concerning a silkworm, silkmoth-pests of the cultural plants, insects-entomophages. Taking into account reasonable prices of the preparations and obvious problem of the own high-quality silk raw material in Ukraine, this technologies are much in demand and partially actualized.

The grains, caterpillars, pupas and silkmoth larvas are widely used as an experimental stuff in biochemical, genetic and other researches. This fact can be explained by the whole range of particularities of the silkmoth's experiments that profitably differ this specimen from other laboratory animals – rapid digenesis, frequent recurrence of the histolysis and histogenesis processes in the development cycle, growing conditions are simple enough, possibility of working with large quantity of the specimens, practical value.

Using the silkmoth as an experimental specimen, such important biophysical problems, as the ways of nitrogen assimilation, mechanism of the protein biosynthesis, regularity of amino acids, carbohydrates and fats exchange, chemistry and biochemistry of hormones, biochemistry of ontogenesis, correlation of proteins and carbohydrates exchange, biochemical aspects of the organism and its environment interconnection and others, are worked out.[110]

The silkmoth is particularly useful for researches directed on studies of the silkmoth organism response on directive effect of the highly specific compounds – morphogens, especially exogenous nucleic acids. As a result – it is an important practical solving of the problem how to increase the total vitality and producing capacity level of the specimen. The peptide bonds synthesis scale and intensity of their formation in the silkmoth organism are exceptionally larger then in other biological specimens. In the silk gland cells, only two studied and different proteins (fibrion and sericin) are synthesized, that allows to study different novel approaches concerning mechanism of the nucleic acids effect on protein bodies biosynthesis.

Moreover, silk protein synthesis characterizes by the unique amino acid compound, and is accompanied by intensive trophic, tissular proteins and amino acids rearrangement in the first periods of the silkmoth growing. Similar effects are observed in the moult and metamorphosis processes.

As the productivity stimulants, native and modified by thiophosphamide and cyclophosphamide DNA and RNA, and 1-ethoxisilotran, as the best of known analogues of the silkmoth productivity, were used.

The nucleic acids and migugene intromission to the silkmoth organism were done by treating mulberry leaves with working solutions of preparation. The counts and removal of specimens, that died according standard methods, were systematically conducted.

The investigations determined, that the age dynamic of the caterpillars' mass, grown using exogenous nucleic acids, brings to the effect of stimulation of the growth metabolism and silkmoth germination. The maximum effect obtained in the variant with DNA modified by thiophosphamide and native yeasty RNA using. The maximum of caterpillars' mass increasing comparing to control was 20,7.

The protein and hemocytes content in hemolymph of the 5^{th} age caterpillar listed in the table. It was determined considerable increasing of the protein and hemocytes in the caterpillars of those variants, where the preparation on DNA source were used, that correlates with high protein in hemolymph specimens of these variants, and as a result, reflects metabolism intensity and high level of the protein reserves in organism.

The growth of the silk gland, determined by matching of the silk gland mass and the caterpillar body mass showed that along with proportional growth of the both indexes, specimens of the experimental variants comparing to control have increase of the silk gland weight. This particularity, together with total increase of the silk gland mass, determines possibility of larger quantity of the silk secreting.

As a result of exogenous nucleic acids effect, the dynamics of the cell composition of the silkmoth caterpillar hemolymph undergoes the changes, that

characterizes increasing of the mature, phagocytized hemocytes (granulocytes) with simultaneous decreasing the number of the young forms (plasmocytes) during the total amount of cell elements oscillation. In the beginning, the largest decreasing of the hemocytes number (57,7% concerning the control) changes into short-term increasing (to 136,6%) with following decreasing of their content and with gradual norm approximation. The disintegrated, died cells number, that considerably increases (maximum to four times), testifies the progress of the pathologic process in organism. The morphologic structural failures of hemocytes are universal too and reveal in the pathologic vacuolization of hemocytes cytoplasm, that accompanied by cytomembrane rupture and their death.

The quantitative and qualitative composition changes of the free amino acids of the silkmoth caterpillar hemolymph content, as a result of exogenous nucleic acids effect, lie in the gradual increasing of the amino acids total content, achieving its maximum on the 10^{th} day, and then gradually stabilizes to norm (Table 15). It is significant and very important for understanding the metabolism intensification mechanism of the experimental caterpillars – amino acids appear, that were not detected in control specimens, - first, praline and methionine, than tyrosine and valine. The aspartic acid was not detected, alanin disappears. Further, high level of amino acid content in caterpillar hemolymth is observed until the term of cocoon formation.

Thus, the exogenous nucleic acids stimulate the growth, germination and viability of the silkmoth. The hemolymth response on acids effect appears in increasing of the protein concentration and hemocytes number after the exchange process intensification.

The domestic and world experience shows that stable functioning of agroindustrial complex depends on successful phytosanitary problem-solving. It is determined by a high level of agricultural production wastes from hazardous organisms. Wide and not always scientifically based pesticides use often brings to the

whole range of undesirable consequences. Except for environmental pollution, pesticides are the reason of the mass plant pollinators' entomophags death.

One way of the pesticides' use in planting capacity reducing is working out and introducing of the plant protection biological method [111]. The development of biomethod is effective for solving the problem of preservation and rehabilitation of the human being and environment. Validating of the production means and methods, and use of entomophags are conducted in the scientific institutions of the various country regions.

Table 15. The protein and hemocytes content in hemolymph of the silkmoth caterpillar, grown using nucleic acids

Variants	The protein number, %	td	The hemocytes number		td
			spec./ mm^3	%	
Deoxyribonucleic acid (DNA)	12,8±0,6	2,6	6609±417	160,7	2,2
DNA modified by thiophosphamide (DNT)	13,3±0,7	3,4	6512±621	158,3	2,1
DNA modified by cyclosphamide (DNC)	12,1±0,5	2,2	6492±426	157,8	2,0
Native yeasty RNA	13,1±0,7	3,1	6721±510	163,4	2,4
Modified RNA (RN)	12,0±0,4	2,2	6395±380	155,5	0,5
RNA modified by thiophosphamide (RNT)	11,8±0,5	1,8	6257±324	152,1	- 0,3
RNA modified by cyclosphamide (RNC)	11,6±0,4	1,7	6312±306	153,5	- 0,1
1-ethoxisilotran (best analogue)	10,8±0,5	-	6304±224	153,3	-
Control	10,4±0,3	-	4112±286	100,0	-

Note: The value of td counted for each preparation comparing to the best analogue.

The specimens of Trichogramma family – is the basic method of the biologic number control of many lepidopterous pests. They are represented exceptionally by the parasites of insect eggs. In biomethod practice trichogramma is used by mass breeding and issue into natural environment. Nevertheless, the parasite use is often unstable and varies in the wide range. A progress in trichogramma use is possible due to the laboratorial growing conditions rationalization and optimization. A long term trichogramma breeding on Angoumois grain moth eggs leads to parasites parameters deterioration – imago's lifetime, its motion activity and search capacity. There are number of new technical decisions concerning their growing on silkmoth eggs (Drozda, 1977; 1991). However, in any case, the researches concerning trichogramma productivity increasing are conducted with use of different compounds and methods of the directive effect.

We studied a possibility of e-DNA effect on the specimens' population of insects, important in agricultural sense to increase their productivity. We also studied a possibility of native and modified nucleic acids use in *Trichogramma pintoi Voeg* laboratorial growing regulations. Trichogramma was derived from the codling moth infested eggs exposed in the Kyiv region's apple-tree garden. In laboratorial conditions trichogramma was grown in the optimal hydrothermal regimens and photoperiod on *Sitotroga cerealella Oliv*. eggs. In researches we used native and alkyled by thiophosphamide or cyclophosphamide DNA (DNT and DNC correspondingly) in different concentrations. The water solutions of preparations were mixed with honey and fed up the imago parasite. In each research variant were 30 even-aged parasite females, and their age was within a few hours. These trichogramma females on 4 days completion were transplanted to the other test tubes with a new portion of eggs and feeding up. Such procedure was conducted until the females' natural death. Obtained results were estimated according to the accepted indexes. Distinctions probability in accordance with prolificacy index of females was determined by control (honey feeding up) with t-criterion use[112].

We studied a possible effect of spontaneous mutagenesis as a result of native and modified DNA effect on trichogramma populations. We also determined the mutagenesis phenomenon in a wide range of concentrations – from 0,0001 to 0,1%. It was analyzed 2510 parasite specimens. The visible mutations were found only in 0,71% of trichogramma population.

We also studied an influence of native and modified DNA in different concentrations on the trichogramma productivity main indexes. The results illustrated in the table. Evidently, the trichogramma's imago feeding up had a considerable effect on all the indexes. The population of parasites that was grown without feeding up (control-2) exhibits insignificant reproductive potential, vitality and lifetime. Honey feeding up led to indexes increasing. Including in a diet DNA preparations in the range of 0,001-0,0001% essentially increased the parasites' females reproductive potential and other indexes. The particularities of trichogramma biology consist in the intensity reproduction realization in the first half of females' life with one typical peak. The specificity of preparations' effect in the 0,001-0,0001% concentrations, probably, is in clear forming of several reproduction peaks at the end of females' life that was one of the influence factors. In the optimal concentration regime, it was observed a statistically admissible increasing of females' prolificacy. Therefore, one of the significant indexes of DNA preparations' effects is that females' reproductive period duration was similar to their natural lifetime (Table 16).

Table 16. Influence of the different native and modified DNA concentrations on trichogramma productivity

Preparations	Concent-ration, %	Eggs, oviposited by one female,		% infesting females	Persistence, %	Imago's lifetime, days	
		Number of specimens	Td*			Max.	Min
DNA	0,1	32,9±7	-0,8	72,1	70,3	8	4
	0,01	49,6±3	+1,7	20,2	84,4	10	6
	0,001	60,3±5	+5,2	91,8	89,7	12	8
	0,0001	59,7±4	+4,8	93,2	90,3	15	10
DNT	0,1	37,3±5	-0,7	68,5	66,2	9	7
	0,01	57,8±4	+3,8	90,3	89,4	12	10
	0,001	64,6±4	+4,9	89,7	91,2	14	11
	0,0001	62,9±5	+3,7	91,4	87,4	14	12
DNC	0,1	41,2±4	+0,3	66,9	59,4	10	7
	0,01	66,2±5	+5,1	89,7	87,7	16	12
	0,001	62,5±4	+4,7	91,6	89,2	18	15
	0,0001	60,3±5	+3,2	90,3	86,4	15	11
Control-1, honey feeding up	-	40,2±3	+2,8	80,4	85,3	12	6
Control-2, without feeding up	-	29,1±3	-	66,5	70,4	6	3

* Note: The value of td counted for each preparation comparing to the control - 1.

The researches testified that DNA preparations determine several trichogramma's effects types, in particular, participate in the directed metabolism regulation of imago females. The existence of the clear additional oviposition peaks testifies participation of the preparations in the forming processes and oocytes' ripening. The researches testified that established phenomenon of native and modified DNA stimulant effect may be used in biolaboratories for breeding the Trichogramma family entomophags specimens. As our researches testified, the last ones are the composite part of the integrated fruits and vegetables protection systems, and their realization facilitates the agrocenosises ecological situation improvement and environmental sanitation.

A study of the possibilities and perspectives of the mass laboratorial entomophages implantation, with following entomophages' colonization into

agrocenosises, still remains an important concept of the biological shielding of the cultivated plants. Exactly this method intensifies the natural regulatory factors, weakened by different reasons, and fixes the artificially settled entomophages as a component part of the agrocenosises. In the context of this problem is a matter of no less significance – mass growing of the entomophages host-insects populations. Among them, are the prevailing species – an Angoumois grain moth Sitotroga cerealella Oliv., the main host-species for growing in biolaboratories the species of *Trichogramma* family – a primary instrument of the biologic control of the lepidopterous pests complex. All efforts of the scientists and manufacturers focused on obtaining of the highly viable trichogramma's populations, although, it is obvious that trichogramma's technological and biological characteristics basically depend on a physiological state of the Angoumois grain moth – as trichogramma develops in the Angoumois grain moth eggs in biolaboratories. There are only several methods and local measures directed on the Angoumois grain moth culture stabilization. It is well known, there is quite a number of unsolved problems.

The collective researches of the National Agrarian University and the Institute of Molecular Biology and Genetics of NAS of Ukraine allowed to conduct a detailed analysis of the problem essence, to draw up the primary tasks, to work out modern methods of research, to select the most effective biostimulants, to carry out the series of experiments and to formulate objective results, scientific conclusions and production recommendations.

As all other populations of the laboratorial insect cultures, Angoumois grain moth, after continuous mass laboratorial breeding, gradually lost the highly viable population characteristics that cause unstable or weak affection of the grain and drastic decrease of the females breeding potential. Except for decrease of the breeding potential, oviposited eggs are physiologically weak and do not satisfy the linear dimension standards. As a result, trichogramma is not able to affect them or the parasite's breed characterized by a low level of vitality, motion and searching activity.

Angoumois grain moth eggs used for maintenance the uterine culture, were treated over a period of two hours by water solution of native or modified by thiophosphamide or cyclophosphamide deoxyribonucleic acids (DNA, DNT, DNC correspondingly) preparations, in a different concentration. Simultaneously, Angoumois grain moth eggs were treated by antiseptic nibiol (5-nitrox) according to the known technology.

The main development, productivity and vitality indexes of an Angoumois grain moth, grown from these eggs described in the Table 17.

It is determined that preparations, described above, testify a considerable stimulant effect, expressed in the pupae mass increasing, and as a result, the females breeding potential and vitality increasing. An optimal range of the reactant concentrations is within 0,500-0,005%. It is important, that statistically probable increasing of the test indexes is not only comparing to control, but to better analogue too.

Use of the offered compounds in an offered technology allows stably increase an Angoumois grain moth vitality and breeding potential. Except for this, achievement of a positive effect in the known technology is accompanied by severe conditions of a treatment terms: during only one day after an oviposition, that is difficult to keep in the mass production conditions of the biolaboratories, because of a high level of the population heterogeneity. The technology, we offered, considerably simplify operating processes of the preparations' use.

Table 17. The indexes an Angoumois grain moth growth after embryos' treatment by the water solutions of native and modified DNA

Preparation	Concentration, %	The average mass of females, %	Number of the eggs, oviposited by one female, specimens	Vitality, %
DNA	1,000	6,39±0,19	31,8±1,5	67,7
	0,500	7,58±0,21*	43,7±1,6*	79,6*
	0,005	7,49±0,42*	42,9±1,4*	77,9*
	0,001	6,62±0,37	33,6±1,9	68,8
DNT	1,000	6,40±0,18	30,9±1,3	69,0
	0,500	7,64±0,24*	42,5±1,2*	80,7*
	0,005	7,50±0,30*	44,3±1,7*	78,5*
	0,001	6,66±0,31	32, ±1,5	66,9
DNC	1,000	6,19±0,36	31,4±1,3	67,2
	0,500	7,88±0,36*	43,1±1,9*	81,2*
	0,005	7,72±0,41*	44,8±2,0*	79,0*
	0,001	6,54±0,33	31,7±1,2	66,5
Nibiol (5-nitrox)	-	6,72±0,18	36,8±1,2	69,4
CONTROL (without treatment)	-	6,05±0,14	33,4±1,2	65,5

Note: above and below, the indexes, pointed out by asterisks, statistically may exceed the indexes of the better analogue.

In the Table 18 has given the dependence data of a stimulant influence on an Angoumois grain moth depending on a period and exposition. A maximum result achieved in the range of 2 - 3 hours. Under the condition of a long-term influence (4 hours) exhibits the inhibition of the Angoumois grain moth growing effect.

Table 18. The comparative indexes of an Angoumois grain moth productivity after nucleotides treatment

The indexes, compared, Angoumois grain moth productivity	Result, obtained by Angoumois grain moth growing		Positive result of the original technology use comparing to known technology
	Offered technology	Known technology	
Range of the directive effect on eggs	First-fifth days	Only the first incubation day	Absence of the time limitation
Range of the acting concentrations, %	0,500-0,005	2,50-5,00	Reduction of the overdosage probability, preparations consumption 5-50 times less
Toxicological characteristic	Serial issue	Nibiol-chemical fungicide, harmful for health	Preparations and technology harmless for people and insects
Mass of the female pupae, mg	7,65	6,75	+0,9
Prolificacy, number of the eggs, oviposited by one female, specimens	47,20	36,90	+10,30
Vitality, %	79,6	69,1	+10,5
Technological effectiveness in use	High level of the entomophages vitality	Lower entomophages vitality	+15-25

The overall positive result of the original technology shows an obvious advantage over an existing one. Low preparation consumption rate and usability characterize the offered technology as a production contributing one.

Thus, we offer the accomplished scientific research, grounded and tested. Its general idea – is an intensification of the biological control of the phytophages' number in agrocenosises, and, accordingly, the chemical pesticides expenses reduction and environmental sanitation.

Based on nucleic acids and their components, some stimulants of plant growth have been recently developed. The methods of native and modified nucleic acids application for modification of the genetic apparatus of plants are under development [13].

Genetic effects of alkylated DNA were determined and some new varieties and forms of rye, millet and tomatoes with valuable qualities have been obtained. A special sort of fodder lupine with increased protein content «Industrial» and pumpkin Kavbuz «Zdorovyaha», rye «Drevlyanske», tomato «Ukrainian», Echinacea «Woodland Beauty» are registered in the State Register of plants of Ukraine and protected by copyright certificates of Ukraine. A pilot study on modified products of alkaloids and their analogues and derivatives with a wide range of biological activity is carried out. These results are used to improve human health and the environment.

A technology of obtaining new forms of plants with desired properties is created using the approach of structural modification of molecules-carriers of hereditary information (DNA and RNA).

The conformity to natural laws of the hereditary changes, were induced by using of exogenous DNA (e-DNA) preparations to obtain of the plants new forms, have been analyzed. The complex and directional character of such changes was revealed and the outlook is shown to use of exogenous DNAs for the accelerated obtaining of the promising kinds of agricultural, medicinal and ornamental plants. The hypothetical mechanism of the e-DNA action on plant heredity was proposed.

The preparation of the exogenic DNAs purified from animals and plants have been used in the concentrations ranging from 100 mcl/mg to 400 mcl/mg to obtaining the new forms of the rye. Germinating seeds of the winter habit rye cultivar Jitomirskaya were treated by the solutions of DNAs. The new forms of plants with spring habit have been obtained, that possessing the complex of the usefull features. The possible mechanism of plant heredity exchanging by using the preparations of exogenic DNAs have been proposed.

Homeobox genes as a possible targets of the action f the exogenous DNAs. 2. The conformity to natural laws of the action of the exogenous DNAs while selecting of the decreased of height diploide rye

While acting of the preparations of the plant’s and animal’s exogenous DNAs on the germinating seeds of the cultivar of the winter diploide rye Zhytomyrska, the

dominant monogenic mutation of decreasing of plant height was induced. Simuntaneously with decreasing of plant height, the complex of alterations, underlying the crop yield improvement in the rye, was observed in the lines of plants to be obtained.

Medicinal plants. The new kinds of medicinal plants are obtained by the original technolody of using of the exogenous DNA preparations. Such plants are possessing of stronger adaptation possibility and productivity of biologically active compounds, having pharmaceutical activity. Among the new kinds of plants are the echinacea purple cultivar Poliskka Beauty, the new forms of blue gigant gissope Saltresistant, greenvalerian polemonium Polis′ka Blakyt, calendula Nagaistra and the new cultivar of fild pumpkin Kavbuz Zdorov′yaga. The perspectivity of the elaborated in our laboratory tecnology have been proved for adaptation of medicinal, ornamental and agricultural plants to growing in the new environment and to the enhancement of their productivity.

Resistance to the substrate salinity of both Ukrainian salt-resistant tomato cultivars, derived through e-DNT (exogenous DNA modified with thiophosphamide) treatment, and lines generated on their basis via laboratory and vegetative techniques, have been studied.

Statistically significant excess in terms of resistance to the substrate salinity with the marine salt, chloride and sulphate salts versus the control samples was demonstrated.

The original technology of obtaining the tobacco plants possessing the complex of selectively useful features (accelerated development, high productivity, and resistance to complex salinization) has been elaborated, and inheritance of the physiologic and biochemic peculiarities of such plants has been investigated.[113] To get the important and selective changes of yellow-leaved tobacco Krupnolistny 20 (KR 20, Large-leaved 20) cultivar, the native and alkylated by thiophosphamide DNA of salt-tolerant nightshade (*Solanum nigrum L.*) and DNA of pCAMVNEO and pTi8628 plasmids have been used. Its useful advantage is the provision of a wider

change range and larger output of changed viable plants. The changes obtained by DNA action on tobacco and other plants exploited in technology elaboration have been analysed, and possible mechanism of plant heredity change by exogenic DNAs has been proposed.

The chlorophylls and carotenoids content has been investigated in first and second generations (T_1 and T_2) of opium poppy (*Papaver somniferum L.*) plants obtained after the treatment by the salt-tolerant black nightshade and *pTi8628* DNAs preparations. The increased chlorophylls content during efflorescence of the T_2 plants was determined by the changes in the leaf senescence regulation systems.

A wide application of these achievements has a considerable economic and social effect, in particular, for environmental recovery, obtaining intense yield on highly saline and nitrogen-depleted soils, as well as during the hydroponics growing with the use of seawater without its desalting. The created hybrids impress even professional selectionists: kvagista (molecular hybrid of haricot and cabbage) is a unique edible and feed culture up to three meters high with high protein content; kavbuz (molecular hybrid of pumpkin and watermelon) – the largest in the world berry of more than 60 kg of weight, it promotes removal of heavy metals and radionuclides from the organism; Kiziris (molecular hybrid of cornel and barberry); Alycos (molecular hybrid of cherry-plum and apricot).

The high viral and immunomodulating effect of amitozyn and izatizon was proved in 1992–1994 according to the program of the National AIDS Committee established by the President of Ukraine. However, these unique results have not been implemented yet. Thus, the regulatory action of the new substances have been defined on the molecular, cellular, organ, and systems levels that allows to recommend them for the molecular genetic rebirth of people and environment.

We offer the completed scientific elaborations, Complex methods of molecular-genetic recovery of human and environment to concerned teams for collaborative implementation: – Having no analogues in world practice antiviral, antimicrobial and antitumour preparations «Izatizon», «Izatitoniy», «Amitozyn»,

«Amitozynoberamid» with a high economic effect when using in medicinal care, veterinary medicine, crop production;– New varieties of cereals with high productivity on nitrogen-depleted and highly saline soils (wheat, rye, oat,millet, barley, corn, sorghum, rice), resistant to drought, bacterial and mycotic infections; – New types of pumpkin for introduction to farming (kavbuz, kavbudek, enriched in sugars, fructose in particular, carotene and oil); – New varieties and forms of medical plants, which have immunomodulating, bactericidal and anti-inflammatory effects (Echinacea, viper's bugloss, elecampane, thermopsis, phytolacca);

Salt-resistant and drought-resistant forms of plants,tomatoes of «Ukrainian Salt Tolerant» variety in particular;

Technology of cereals and vegetable cultures seed treatment, and treatment of mushrooms, which increases the productivity by 20–40 %;

Technology of beneficial insects productivity increase in beekeeping, production of oakworm and silkworm,*etc*.

The proposed biopreparations are not harmful and provide the increase in beneficial insects productivity by 1.5–2 times;

Technology of creation of new forms of plants with modified properties (creation of frost-, salt-,drought- resistant forms, transformation of winter forms of crops to spring crops);

Technology of fishery and sea-farming productivity increase by 20–40 %;

Technology of diagnostics, prevention and treatment of bacterial cancer of plants (fruit cultures, grape, vegetable cultures) by using original, ecologically harmless preparations.

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