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Fraunhofer lines in the solar spectrum and immunodeficiency problems(*)

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Summary. — The importance of the presence of Fraunhofer lines in the solar spectrum for development of immunodeficiency has been demonstrated. *In vitro* and *in vivo* tests of illumination within Mg lines have proved the possibility to reduce the level of HIV/AIDS development.

PACS 87.50.-a – Effects of radiation and external fields on biomolecules, cells and higher organisms.

1. – Introduction

The acuteness of immunodeficiency problems is determined by not only increased scales of the relevant studies but also an active interest of mass media in the HIV/AIDS [1-30]. Extensive respective information can be found in the State Report "On the Human Health in the Russian Federation in 2002" [24], in the WHO/WMO/UNEP Report of Russia "Climate change and human health: threats and return measures. Summary" [2], in the materials of the joint meeting of the Russian Academy of Sciences and of the Russian Academy of Medical Sciences ("Science for Human Health") with the participation of the Academies of Agriculture and Arts [29], as well as in numerous materials of mass media [9, 12].

The XV International AIDS Conference held in Bangkok (Thailand) in July 2004 with nearly 20000 participants summarized the respective developments [3] and drew attention to the situation in sub-Saharan Africa where in some regions the number of HIV-infected adults exceeds 30% of total population. Only few people in developing countries who face the greatest risk of HIV infection receive key prevention services. The world needs $12 billion next year to comprehensively address the global epidemics [3]. The scale of world-wide danger requires an urgent mobilization of resources to avert it (a

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considerable progress in availability of antiretrovirus therapy (ART) has been achieved in Brazil). To reach this objective, new mechanisms of funding should be applied through the International AIDS programme supported by the World Bank and the Global Fund for combating AIDS, tuberculosis, and malaria.

Price-cutting with respect to antiretrovirus medicines has made it possible for the WHO to set the problem of providing 3 million people with this medicine in poor countries by the year 2005. Solution of the problem is hindered by the poverty of population of the African countries who are HIV-infected most of all. More organized efforts are needed in the developed countries as well.

The growing threat of HIV/AIDS propagation and respective socio-economic consequences have stimulated the development of the Joint United Nations Programme on HIV/AIDS (UNAIDS) with the participation of nine UN specialized agencies.

A 5-year President's Emergency Plan for AIDS Relief (PEPFAR) has been worked out in the USA to combat HIV/AIDS on a global scale with the total funding of 15 billion dollars [27]. Major objectives of the programme are to provide treatment of, at least, 2 million of the HIV-infected people and preventive treatment of 7 million people. According to this programme, 9 billion dollars are planned to fund respective measures in 14 countries of Africa and Caribbean region, where 50% of the HIV-infected people live. As predicted, by the year 2010, neglecting necessary measures will cause an increase of the number of the HIV-infected people up to 85 million at a level of mortality reaching 200 million people by 2020.

During two years after the previous AIDS Conference in Barcelona, more than 10 million people got HIV-infected and 6 millions died of AIDS [18]. The HIV-infected countries loose annually teachers, medical and other personnel whose number exceeds the newly-trained personnel. About 14 million children have lost either one or both parents. Only in the Asian-Pacific region the number of the HIV-infected in 2002 increased by about one million, and more than 4 millions of the HIV-infected live in India, which constitutes about 10% of its total population. Regions of the rapid AIDS propagation are Eastern Europe and Central Asia, where the number of the HIV-infected people exceeded one million (compared to 30 thousand in 1990).

The perilous level of HIV/AIDS has been reached in south-eastern Asia [3]. Except for Africa, such countries as Mjanma, Thailand, and Cambodia are characterized by the highest level of HIV-infection, where the number of the deceased may exceed those in sub-Saharan Africa by the year 2010.

From the data of Pokrovsky [20], in Russia, the HIV/AIDS acquires a dangerous scale. As of 1 December 2003, the registered number of the HIV-infected reached 260 thousand but in fact it is about one million, 90% of which are young people aged under 30. According to [1], the Russian statistics is as follows: 8% of young Russians buy drugs every day; 4.1% of prisoners are HIV-infected; 2700 children were born by HIV-infected mothers in 2003; 10% of Russians can become HIV-infected by 2010 if health services do not improve; every hundredth person in Norilsk is HIV-infected.

In the context of the materials of the Conference in Bangkok, three key aspects of the HIV/AIDS combating problem have been formulated [14]:

1) *An urgent need for medical personnel and funding*

With the medicines price-cutting, the deficit of "human resources" becomes especially acute. According to WHO estimates, only in Africa about 100 thousand of the medical personnel either died of AIDS, or emigrated, or changed their job for high-paid ones. The annual need of financing is very high, and by 2007 it will reach 20 million dollars.

Clearly, within the next 10-15 years, the developing countries will not be able to finance the measures connected with HIV/AIDS combating.

2) *Taking due measures on HIV/AIDS prophylaxis*

There is no doubt that inadequacy of such measures excludes the possibility to reduce the scale of HIV/AIDS propagation, especially in the case of population groups with a high risk to get infected and fall ill. It is necessary to develop national complex programmes on HIV/AIDS prophylaxis.

3) *Mobilizing national efforts to combat HIV/AIDS*

Efforts should be concentrated in various countries to substantiate priorities with due consideration of possibilities to use both national and international resources. In this connection, of special importance is to solve a complicated problem such as developing the AIDS vaccine [3, 5, 8]. From the time of HIV-I recognition as an etiologic AIDS factor more than 20 years ago, large-scale developments have been accomplished which led to a deeper understanding of this virus and the respective HIV-generated disease. These developments included numerous studies from the processes of virus /"host" cells interaction (at molecular level) to a complicated pathogenesis of HIV-I with the infected patients. Studies of virus and its interaction with the immune system revealed new molecular mechanisms of the human immune system's functioning, though the level of understanding such mechanisms is still inadequate.

According to UNAIDS data, at present more than 40 million people in the world have got HIV-I–infected, especially in poor countries of sub-Saharan Africa (from other data, about 50 million are HIV-infected [19]). In 2003, the number of the infected increased by 5 million (mainly due to the southern regions of Western Europe and Asia), with the daily increase by 14 thousand people (95% are representatives of the developing world). This pandemic can lead to a destabilization of the whole countries and, under the "interactive" world conditions, the scales may be global.

Emini and Koff [5] believe that an efficient way limiting the propagation of infection is to develop and apply a vaccine that can prevent from infecting new "hosts" affected by HIV. Special features of HIV-I determine, however, an extreme complexity of this problem. The point is that an initial infection is followed by a rapid *in vivo* development of the virus population which reflects an appearance of substantial viremia and long-term infectedness. Apparently, this stability testifies to an ability of virus to resist its removal by the immune system and to continuously reproduce the population. A considerable scale of intra-individual and population variability of virus make it difficult to control the development of virus and to discover an efficient vaccine. A critically important factor determining the HIV-I capability to infect a new cell of the "host" is the presence on the cells' surface of glycoprotein gp^{20} and gp^{41} interacting with antibodies. However, despite the impediments for the functioning of antiviral antibodies, the immune system can be efficient in HIV-I combating. The response of the antiviral immune system plays an important role as a factor controlling the propagation of virus. Difficulties and contradictions connected with the development of vaccine have brought forth a hot discussion on this problem in the journal "Science" [26].

As has been mentioned earlier [13], a poorly studied aspect of the HIV/AIDS problems is connected with studies of possible environmental impacts. As was shown, an important aspect of the immunodeficiency development in general and especially such dangerous diseases as HIV/AIDS and hepatite-B, C consists in the necessity to consider the role of changes in the fine structure of the solar radiation spectrum caused by the presence of

Fraunhofer lines and, in this connection, the significance of some metals in the functioning of the genetic apparatus.

2. – Impact of radiation in the magnesium lines on the process of inverse transcription and inactivation of the AIDS virus

Magnesium atoms are known to play the most important role in fermentative reactions connected with the functioning of the genetic apparatus: activated ferments ensure a transcription and replication of the genetic code. Magnesium plays an important stabilizing role in maintenance of the geometric structure of the DNA dual spiral, tertiary structure of t-RNA, etc. The magnesium atom can perform these functions only when its electronic shell is protected against external forcings, including light, in particular. As was shown earlier [4, 6], a substantial increase of the biomolecules geometry stability is ensured with inclusion in their composition of the atoms of metals whose absorption lines coincide with respective Fraunhofer lines of sunlight. This sharply decreases the probability of perturbation of electron orbits of the atoms of metals that constitute the biomolecules and control the functioning geometry of important parts of molecules (active centers) [4].

In this connection, in developing a remedy for the human immunodeficiency virus (HIV) to combat AIDS, it is necessary to take into account the structure and replicative cycle of the AIDS virus, since the virus can be affected at any stage of its life cycle, namely: to blockade the binding of the virus with the human tissue cells; to break the process of the viral RNA release from the virus' protein shell; to suppress the process of inverse transcription (information read-out from the RNA-matrix at DNA-synthesis); to break the translation block (synthesis of viral proteins); to break the process of viral proteins modification; to break the assemblage and release of new virions from a cell.

Proceeding from the above results, the process of inverse transcription was influenced considering the following. The process of inverse transcription in the AIDS virus, like in other retro-viruses, is realized with the help of the inverse transcriptase (revertase) which functions with the participation of the atoms of magnesium. In this connection, it was decided to investigate the effect of light, corresponding to electron transitions of the magnesium atom, on the stability and fermentative activity of an individual ferment (inverse transcriptase), and then, in case of a positive effect, to administer this exposure to the AIDS virus containing this ferment responsible for a very important process of inverse transcriptase in the virus' life cycle. The AIDS virus inverse transcriptase is characterized by several fermentative activities, very important for virus functioning: DNA-polymerase activity, due to which the one-chain DNA is synthesized, complementary to viral RNA; ribonuclease activity responsible for splitting the initial RNA, and besides, the inverse transcriptase synthesizes the needed second chain of DNA using the first one as a matrix.

The main objective was to study the effect of radiation in the magnesium absorption lines on the process of inverse transcription (synthesis of DNA complementary to RNA), since RNA is the genetic material for retroviruses (which is also the AIDS virus). For genes expression, responsible for synthesis of viral proteins, a DNA-copy of viral RNA should appear.

A first step at the biochemical stage of study was to extract and purify RNA. The RNA was extracted from viruses of potatoes and pea in the following way. The investigated material was homogenized, then centrifugalized to separate nuclei, and then the centrifugate was used. A depigmentation and deproteinization was made by processing

TABLE I. – *Laboratory experiments.*

Duration of DNA synthesis (min)	Inclusion of the radioactive mark into DNA (pulse/min)		
	Irradiation test (average of three readings)	Dark control (average of three readings)	Percentage of the active ferment preservation
0	1508	1900	
20	4005	5787	69
40	4384	7682	57
60	4683	9103	51
90	8704	22465	39

the centrifugate with the mixture of phenol-chloroform-isomyl-butane alcohol, then it was centrifugalized during 10 minutes at a speed of 10,000 rpm at 0°C. Then we extracted the water phase from RNA, added 3M potassium acetate (pH $\sim$ 5.5) to the final concentration 0.2M, mixed with 2.5 volume of the etalon and kept at -20°C, then centrifugalized at 200 rpm at 0°C. Then we suspended the sediment in 3M potassium acetate (pH $\sim$ 6), centrifugalized and suspended the final resulting sediment containing RNA in a 70% etalon.

After this, the RNA-complementary DNA was synthesized using the inverse transcriptase (the "Farmatsia" firm) whose activity was affected by its irradiation on magnesium lines.

The reaction mixture to synthesize DNA on the RNA matrix included the following components: T_{vis}-buffer (pH $\sim$ 8.3); MgCl and KCl solutions, solution of dithiotriethyl**;** solution of RNA-sine: solutions of desoxynucleotide triphosphates; solution of marked desoxyadenosine triphosphate; trace admixture $(dT)_{12}$, and inverse transcriptase.

The DNA synthesis was monitored through the inclusion of the marked desoxyadenosine triphosphate, reading-out the radiation counter tube and taking an autoradiograph from the agarose gel after electrophoresis of the synthesized DNA in this agarose gel. Then the DNA synthesis kinetics in sunlight was recorded during 20, 40, 60, and 90 minutes. At the same time, for control purposes, the DNA synthesis kinetics in dark conditions was recorded.

The obtained data of experiment on introduction of marked ATPh and DNA synthesized after the indicated scheme with the help of revertase are given in table I.

From the data on inclusion of the radioactive mark into DNA in its synthesis on RNA matrix with the help of inverse transcriptase and from the data on electrophoresis of the synthesized DNA in the agarose gel the conclusion can be drawn that exposure to light corresponding to electron transitions in magnesium atoms affects the *in vitro* process of inverse transcription, the ferment activity (inverse transcriptase) decreasing by more than 50%.

To study the effect on the AIDS virus of light corresponding to electron transitions in magnesium atoms, the following model system was used: human T-lymphoblasted cells infecting with the human immunodeficiency virus of type I (HIV-I), which are the etiologic cause of AIDS.

A high sensitivity of some cellular lines to HIV-infection makes it possible to use them to create a cellular analog to the processes of the human HIV-infection. The data obtained using the cell culture method are at present one of the main channels of information on the nature of the human immunodeficiency virus, since there are no

TABLE II. – *The effect of light corresponding to electron transitions in magnesium atoms on HIV-I reproduction in the cell cultures.*

Experiment	Characteristic of cells			
	MT-4		CEM	
	1	2	1	2
Control of cells (non-infected)	55.3	96.4	58.0	89.1
Control of virus (cells+virus)	20.1	29.5	38.4	73.8
Cells+virus+light (exp. 20 min)	50.4	27.3	65.6	59.9
Cells+virus+light (exp. 40 min)	40.0	81.7	54.3	83.4
Cells+virus+light (exp. 90 min)	43.7	844	64.4	834

Note: 1: number of cells ($\times 10^5$); 2: cells' vital capacity (%).

adequate animal AIDS models so far. This fact explains our choice of the system's model.

Two species of cell cultures were used in the experiments: MT-4 and CEM differing in their biologic characteristics and representing, respectively, two types of the course of viral infection—pungent (MT-4) and chronic (CEM).

As a result of HIV-infecting of the sensitive cells, after some time, visible traces of their reproduction appear: cytodestructive changes occur in the cells, syncytium forms, and the number of bioplasts starts decreasing. When the processes develop following the type of a pungent infection, these phenomena manifest themselves most vividly. Therefore in assessing the effect of light on HIV reproduction in the cell culture, special attention has been paid to changes in the biological characteristics of infected cells and a comparison has been made with the respective biological characteristics of non-infected cells.

Three series of experiments have been carried out using the following schemes:

cells MT-4 —HIV-I_f infection,

cells CEM —HIV-I infection.

The MT-4 and CEM cells were grown as a suspension in the RPMI 1640 medium with 15% of fetal serum, 100 μg/ml of hentamicine in concentration $(3–5)\cdot 10^5$ cells/ml.

The cultural liquid of the HIV H9/IIIB producing cells was used as a source of HIV-I. For this purpose, the cells were cultivated during 4-5 days in the RPMI 1640 medium with 15% fetal calf serum in the atmosphere with a 5% CO_2 content and 90% humidity. With the end of incubation, the cells were extracted by low-grade centrifugation, and the supernatant after the differential centrifugation was used to infect the cells.

In the course of experiments the viral material was exposed to different dozes of light. As the working exposures, 20, 40, and 90 minutes were chosen. After the light-exposure of HIV-samples, they were placed into panels with sensitive cell cultures. For control purposes, similar HIV-medications were used but not exposed to light. The infected cultures were incubated during 4-7 days in the atmosphere (5% CO_2, 90% humidity, 37°C). On the forth and seventh days of the experiment the number of cells and their vital activity in the panels were determined. The results obtained are listed in table II.

The data obtained show that the control (non-infected) cell cultures are characterized by a good reproduction of cells —5.5×10^6 cells/ml and a high vital capacity— 89.1 and 96.4% (for two species of cells, MT-4 and CEM, respectively).

The control case (HIV-infected cells but not exposed to light) revealed a substantial

decrease of the number of cells due to the cytopatic effect of virus —up to 29.5% in the MT-4 cells and up to 73.8% in the CEM cells.

Thus the results obtained indicate that the 40–90 min exposure of HIV to light corresponding to electron transitions in magnesium atoms substantially reduces the level of virus reproduction in the cell cultures. These data agree well with the earlier experiments on inactivation of inverse transcriptase —one of the most important ferments needed for virus reproduction.

Thus the approach under discussion has made it possible to achieve a substantial inactivation of the AIDS virus with the use of weak radiation on the lines corresponding to electron transitions in magnesium atoms. These data have served the starting point to further investigations aimed at inactivation of the AIDS virus and development of diagnostic test-systems and vaccines.

3. – New concept of AIDS treatment

As mentioned above, the development of the HIV-affecting medications has turned out to be a very difficult problem. The point is that the HIV includes its genes into the genetic material of the cell to be infected, causing thereby a long-term infection. Even if the cell does not produce viral particles, it can preserve these, as if dreaming, HIV-genes. Such a cell is not controlled by the immune system because there are no viral anti-genes on its surface. Therefore it is impossible to completely remove this virus from the organism. In this connection, the efforts of scientists have been directed towards the development of medications which would stimulate the immune system to prevent the virus from causing a disease. However, this approach cannot be sufficiently efficient, too, since the immune system cannot be infinitely stimulated.

The task should be aimed at blocking the HIV before it and its genetic material are included into DNA of the "host" cell. The only known medicine that can block one of the stages of the HIV life cycle (block the synthesis of the viral DNA of inverse transcriptase) is azidothymidine. It can relieve the cause of disease and prolong the life of the AIDS patients. However this medicine has a serious drawback —it is highly toxic, especially for marrow, and promotes the anemia development (reduces the number of red corpuscles). With its frequent taking, it can reduce the number of leucocytes and platelets.

The genetic material for HIV is known to be a RNA molecule, which, in its turn, contains five magnesium ions. Also, it is known that the inverse transcriptase ferment contains zinc ions.

Our investigations have shown that the HIV can be inactivated (weakened) by exposing it to light corresponding to electron transitions of Mg and Zn atoms. This approach realizes the HIV blockade at all its vital stages —from its inclusion into a cell to a release of new virions from the cell.

The development of efficient methods of treating the HIV-infected patents was started in 1986. During this period a theory was developed of the effect of light (in the visible) on HIV reproduction. Unique sources of light have been created and on their basis a special instrumental complex has been constructed. Our approach has been verified by numerous experiments which suggested the conclusion about the efficiency of this approach in solving the HIV/AIDS problem. The realized developments have been patented.

As an illustration, below are given results of two recent experiments carried out in the D. I. Ivanovsky Institute of Virology of the Russian Academy of Medical Sciences.

TABLE III. – *The effect on HIV of monochromatic light source No. 2 (the fifth day of the experiment).*

Solution	HIV-infection					
	Exposure to light			Control		
	1*	2**	3***	1	2	3
1:2	67	0.7	0	46	0.8	60
1:4	81	0.9	0	56	0.5	40
1:8	81	1.3	0	56	0.7	36
1:16	83	1.8	0	61	0.7	32
1:32	88	1.4	0	82	0.9	25
1:64	87	1.6	0	84	1.3	21
1:128	85	1.3	0	90	1.6	16 -
1:256	86	2.0	0	92	1.3	7
1:512	90	1.6	0	89	1.5	6
1:1024	96	1.9	0	89	1.6	0

Experimental conditions	1	2	3
Cells control	96	1.9	0

Note: 1*: vital capacity of cells; 2**: number of cells ($\times 10^6$/ml); 3***: syncytia (number/hole).

Experiment 1. Study of the virulicide efficiency of the source of monochromatic light with respect to HIV.

Viruses. The HIV-1889A culture was used from the collection of viruses of the Institute of Virology.

Cells. Human lymphoblastoid cells MT-4 from the collection of cells of the Institute of Virology. The cells were cultivated at the concentration $(3–5)\cdot 10^5$ cells/ml in the RPMI 1640 medium with the use of a 10% fetal cow serum (Sigma, USA), 100 μg of hentamicine (Pharmachim, Bulgaria). The vital capacity of the cells was tested by marking with a 0.4% solution of trypan blue (Serva, Germany).

Instrumentation. Sources of monochromatic light No. 1 and No. 2 developed by P. P. Fedchenko.

Studies were carried out in 24 holed plastic panels (Costar, USA). The HIV was exposed to monochromatic light from sources No. 1 and No. 2. Then the cells' suspension was HIV-infected and cultivated at 37°C in the 5% CO_2 atmosphere during 5-9 days till the moment of analyzing the results. The HIV reproduction in the sensitive cells was assessed by their vital capacity, virus-induced cytopatic effect (CPE), and syncytia (gigantic multinuclear cells).

The results obtained (tables III, IV) convincingly illustrate the efficiency of monochromatic light impacts manifested through reducing the HIV activity. It was found out, in particular, that the virus-induced syncytia vanished from all cultures of the illuminated virus in the cases of both five and nine days after cells' infecting.

Experiment 2. Study of the effect of the monochromatic light sources on HIV.

In this case, first, the blood of every patient (three patients) was examined for the presence of HIV and the number of lymphocytes was determined. Then every patient

TABLE IV. – *The effect on HIV of monochromatic light source No. 2 (the ninth day of the experiment).*

Solution	HIV-infection					
	Exposure to light			Control		
	1*	2**	3***	1	2	3
1:2	64	0.66	0	3	0.03	80
1:4	71	0.99	0	15	0.067	60
1:8	81	1.13	0	21	0.16	45
1:16	82	1.26	0	21	0.2	30
1:32	81	1.13	0	36	0.5	18
1:64	81	1.23	0	45	0.8	15
1:128	82	1.19	0	44	0.79	10
1:256	82	1.26	0	50	0.88	11
1:512	83	1.33	0	55	0.9	8
1:1024	85	1.39	0	60	1.1	2

Experimental conditions	1	2	3
Cells control	84	1.5	0

Note: 1*: vital capacity of cells; 2**: number of cells ($\times 10^6$/ml); 3***: syncytia (number/hole).

was exposed to light and, again, their blood was examined. The blood analyses were made with the time intervals 1, 10, and 30 days.

The results showed that after the experiment the number of lymphocytes in the peripheral blood of all three patients increased, on the average, by $0.6 \cdot 10^6$ number/ml.

When co-cultivating lymphocytes in the peripheral blood of the HIV-infected patients with HIV-infection-sensitive lymphoblastoid cells MT-4, the vital capability of the cells increased in the case of co-cultivating with lymphocytes extracted after an exposure to monochromatic light.

At present, our developments reveal the possibility to treat the HIV-infected patients by HIV-blocking at any stage of its life cycle. Treatment means the following. A patient treated with this method, practically, will never fall ill of AIDS, though he cannot, so far, be totally saved from HIV-infection. There is the possibility of holding up a decrease of lymphocytes in the organism and restricting thereby a further development of immunodeficiency.

The results obtained are the result of interdisciplinary developments carried out during 20 years. Their clinical tests and mass application are only possible based on a close cooperation of specialists in the spheres such as medicine, biochemistry, physics, and ecology. The acute character of the HIV/AIDS problem (as well as hepatite-B, C) necessitates a close cooperation of the Russian Academy of Sciences and the Russian Academy of Medical Sciences (in the context of the Joint Resolution adopted by the Academies in December 2003 [29]) and the needed financial support.

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