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building 4, Av. Peremogy, 37,
Kyiv, 03056,
National Technical University of Ukraine
"Igor Sikorsky Kyiv Polytechnic Institute"

E-mail: ibb@kpi.ua
http://ibb.kpi.ua

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Original article

AUTOMATED ASSESSMENT OF A STUDENTS CIRCULATORY SYSTEM FUNCTIONAL STATE USING MARTINE'S TEST

B.A. Voinyk[1], G.V. Borisova[1], V.S. Umanets[1]*, G.L. Boiko[1], A.V. Pavlov[2], Ie.A. Nastenko[1]

[1]Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine
[2]International Research and Training Center for Information Technologies and Systems, NAS and MES of Ukraine, Kyiv, Ukraine

*Corresponding author: 2_bytes@ukr.net

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Background. The systematic self-control of students' health state allows optimizing the educational process organization of physical education and contribute improving the functioning of body systems. One of the common methods of such observation is the functional Martine's test, which provides an opportunity to investigate the dynamics of changes in blood pressure and heart rate between the resting state and every minute for five minutes after the load. In most cases, this load characteristic sufficiently fully reflects the student's cardiovascular system state. The determination of the characteristic patterns of such states will allow offering a mechanism for assessing the functional state of the circulatory system. The regular Martine's testing will allow observing the dynamics and evaluating changes in the body during the observation period.
Objective. Creation of an automated system for assessing of a student's circulatory system functional state changes.
Methods. The students database of the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute" created during the research by the Department of Physical Education, was used for the study. To determine the functional patterns, separately for boys and girls, cluster analysis was used by the "*k*-means" method in the parameter space of the blood pressure and heart rate measured during the functional Martine's test. As a result, 7 clusters of states for young men and 8 clusters for girls were obtained. Clusters differ significantly in the nature of the response of blood pressure and heart rate to the load test. The centroids of the resulting clusters were further considered as functional patterns (the most typical representatives) of the body's response to the test physical load. The assessment of the circulatory system functional state is calculated by comparing the student test data with the previously defined functional patterns by the minimum Euclidean distance criterion. Conclusions about the functional state of the system are formed.
Results. The clusters of the circulatory system functional states in the extended space of blood pressure and heart rate parameters of functional Martine's test are obtained. The clusters correspond to the normal states and different stages of the regulatory reserves reduction of the body. The algorithm allowing to consider features of an organism functional state at essential deviation of Martine's test indicators from a certain functional pattern is developed. This admits controlling the individual level of physical activity, adapting the training program and identifying conditions requiring additional medical control.
Conclusions. As a study result, the automated system for assessing the functional state of technical university students circulatory system using Martine's test was developed. The system relates observation to one of the study sample clusters that were obtained using the "*k*-means" method. For additional information on the state of the circulatory system, the average radius of the cluster is used, since objects that are far removed from the center may have properties similar to those of a neighboring cluster. The developed system provides an opportunity for screening control the students cardiovascular system state during the educational process.

Keywords: clustering; "*k*-means" method; functional circulation pattern; minimal Euclidean distance; cluster average radius; functional Martine's test.

Introduction

Systematic self-monitoring of your health is a necessary step for improving the functioning of body's systems and supporting it in a tone. Performing physical exercises positively affects the student's body and allows performing an analysis of physical abilities. With the help of Martine's functional test, it is possible to investigate the dynamics of changes in blood pressure and pulse between the resting state and every minute for five minutes after exercise.

Since the state of physical health can vary regardless of physical activity, the regular conduct of Martine's test [1, 2] will allow observing the dynamics of changes in the body for a certain period [3–5].

The objective of the work is to create a system for assessing changes in the functional state of the circulatory system by comparing the test parameters with predefined functional blood circulation patterns based on the criterion of the minimum

Euclidean distance and forming of basic and additional conclusions about the state of the organism.

Materials and methods

The students database of the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", created in the course of research conducted by the Department of Physical Education, was used for the study.

All students had a medical certificate about the ability of taking physical education courses, as well as gave written consent to the passing of the test. Testing consisted of going through Martine's test that is, performing 20 sit-ups in 30 seconds.

Immediately before the start of the test and every minute for 5 minutes afterwards, the systolic and diastolic blood pressure and heart rate (HR) values were measured.

To determine the functional patterns (centroids of clusters), a database of 1419 students single multidimensional observations was used. The gender composition of the base is 800 men and 619 women. The dimension of clustering space of the circulatory system functional state was determined dim = 18: systolic, diastolic blood pressure and heart rate for 6 points of time Martine's test.

To formulate a formal description of the characteristics of each cluster, 353 quantitative indicators were analyzed that reflect the functional state of the circulatory system and the psychophysical state of patients.

Separately for males and females, to determine the functional patterns, that is, the ratios of the above indicators, a cluster analysis using the "*k*-means" [6] method was performed. At the preliminary stage of the research, 7 clusters were obtained for males and 8 clusters in females data, which differed significantly from each other by the nature of the responses of blood pressure (BP) and heart rate metrics to Martine's test. The centroids of the resulting clusters were further considered as functional patterns (the most typical representatives) of the body's response to the test of physical activity.

To study changes in the state of the circulatory system, the database contained data from repeated tests of students, which were performed from 2 to 6 times at different intervals. This database contained 590 observations, consisting of 268 female and 322 male students and additional 133 indicators of the psychophysical status of students.

Euclidean distance was used as a criterion for the proximity of the individual student, named below as x_j to the center c_i of a particular cluster *i*:

$$d_{i,j}^2 = \sqrt{\sum_{k=1}^{18} (c_i^{(k)} - x_j^{(k)})^2} \quad (1)$$

where $c_i^{(k)}$ is a coordinate value *k* of cluster centroid *i*, and $x_j^{(k)}$ is a coordinate value *k* of the object x_j which is the member of cluster *i*. In addition, for each cluster, a parameter is calculated, which we will call as it's average radius:

$$R_i = \frac{\sum_{j=1}^{n_i} \sqrt{\sum_{k=1}^{18} (c_i^{(k)} - x_j^{(k)})^2}}{n_i}$$

where $x_j^{(k)}$ is a coordinate value *k* of object x_j, which is a member of cluster *i*; n_i is an object quantity of cluster with number *i*.

Results

The study used a database containing blood pressure and cardiovascular measurements of students who had taken Martine's test more than once.

The reciprocal arrangement of clusters' centroids, which were used as functional patterns of response to an exercise test, are presented in Figs. 1–4 where SP and DP are systolic and diastolic blood pressure respectively. Here, each cluster centroid is represented by 6 centers corresponding to 6 time points of Martine's test.

To assess the circulatory system functional state the following algorithm was developed and applied.

1. Using proximity measure (1), we define the cluster with minimal distance to the analyzed object.

2. Using proximity measure (1), we define the cluster with subminimal distance to the analyzed object.

3. Determine whether the parameters of this test were located within the average radius of the cluster ("yes" or "no").

4. If test parameters were located within the cluster average radius, then only the information about functional and psychophysical features of the closest, cluster is displayed as result.

5. If "no", the information about functional and psychophysical features of the cluster with a subminimum distance is displayed in addition.

The flowchart of the above algorithm is shown in Fig. 5.

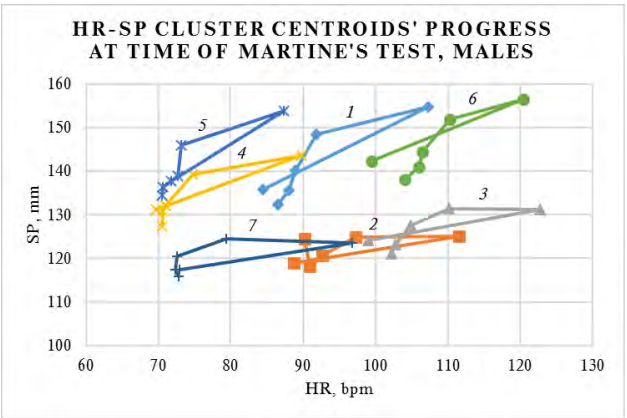

Figure 1: Changes in systolic blood pressure indicators before and within 5 min after Martine's test. Males (*1–7* – clusters *1–7* respectively)

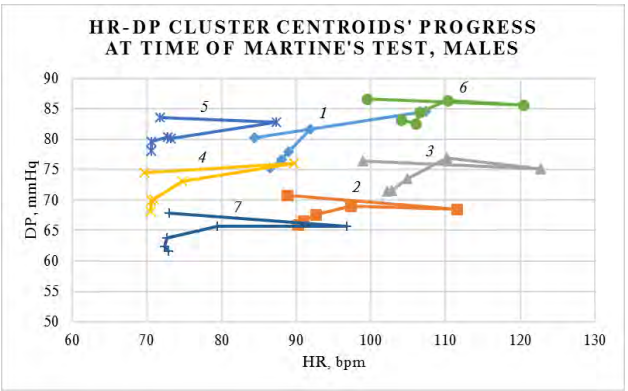

Figure 2: Changes in diastolic blood pressure measurements before and within 5 min after Martine's test. Males (*1–7* – clusters *1–7* respectively)

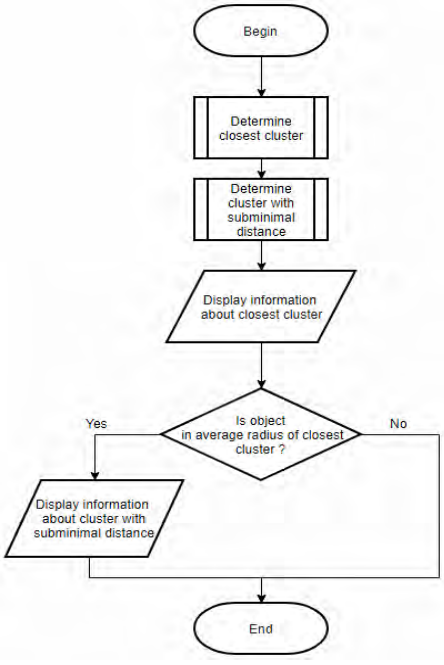

Figure 5: Flowchart of an algorithm for generating conclusions about student's current functional state

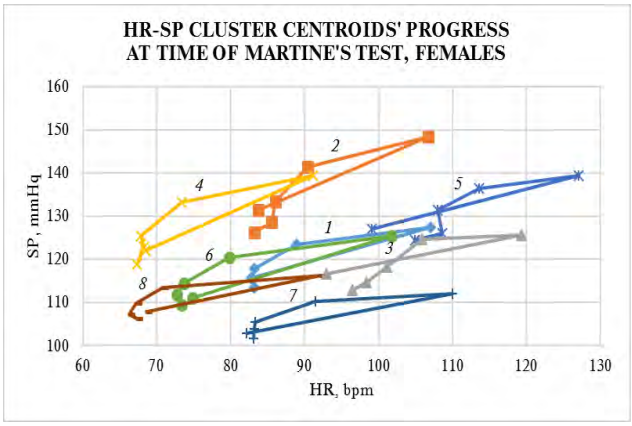

Figure 3: Changes in systolic blood pressure indicators before and within 5 min after Martine's test. Females (*1–8* – clusters *1–8* respectively)

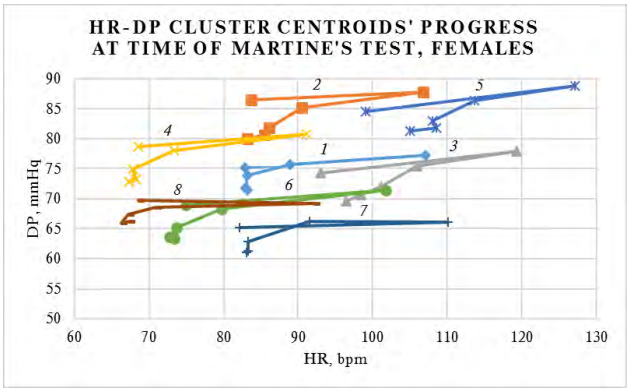

Figure 4: Changes in diastolic blood pressure indicators before and within 5 min after Martine's test. Females (*1–8* – clusters *1–8* respectively)

Discussion

Using the developed system, the results of the monitoring application of Martine's test were analyzed from 1 to 6 times in 590 observation students, which consisted of 268 female and 322 male students. Each observation corresponds to a certain code of the functional state (cluster number).

Causes of existing functional states of blood circulation were analyzed using 133 indicators of the psychophysical state of students. The values of these indicators in clusters were analyzed using a dispersion analysis [7].

Analysis of changes in these indicators is not the purpose of this work and deserves a separate consideration.

Distribution of students in clusters, presented in Tables 1, 2 allows estimating the prevalence of a certain regulatory type in student groups.

Thus, the developed algorithm allows taking into account additional possible features of the

functional state of the organism with significant deviation of the parameters of Martine's test from a certain functional pattern, that is, the cluster average radius.

Table 1. Number of students in clusters. Males

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
40	67	45	50	6	11	103

Table 2. Number of students in clusters. Females

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
58	16	35	13	8	41	51	46

The algorithm allows determining blood circulation conditions, which correspond to reduction of regulatory reserves and significant increase in blood pressure. This allows controlling the individual level of physical activity, modifying the training program and identifying conditions that require additional medical control.

In addition, the use of a system for screening control of large groups of students is possible for the identification of persons at high risk of sudden circulatory disorders.

The results of the system were compared with expert assessments. A completely acceptable (87%) match of both types of estimates is established.

Conclusions

As a study result, a system for assessing the functional state of the students cardiovascular system with the use of Martine's test was developed. The system matches observation to one of the study sample clusters that were obtained using the "*k*-means" method. For additional information on the circulatory system state, the mean radius of the cluster is used, since objects that are far removed from the center may have properties similar to those of a neighboring cluster.

The developed system provides an opportunity to control the state of the cardiovascular system as a one-time, and to conduct screening control of a group of students.

The size of the input vector (input structure) and the number of functional patterns are not fixed and can be modified in the process of improving the system.

The future studies will focus on finding larger differences between clusters to refine their characteristics. Also, the studies will focus on finding patterns of changes in the functional status of the circulatory system of students who have passed Martine's test more than once in order to identify risk groups of sudden circulatory disorders and decrease functional reserves. Further research will be aimed at determining the optimal number of functional patterns and a more detailed assessment of changes in physiological parameters.

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Б.О. Войник, Г.В. Борисова, В.С. Уманець, Г.Л. Бойко, А.В. Павлов, Є.А. Настенко

АВТОМАТИЗОВАНА ОЦІНКА ФУНКЦІОНАЛЬНОГО СТАНУ СИСТЕМИ КРОВООБІГУ СТУДЕНТІВ ІЗ ВИКОРИСТАННЯМ ПРОБИ МАРТІНЕ

Проблематика. Систематичні самостійні спостереження студентів за станом свого здоров'я дають змогу оптимізувати організацію навчального процесу фізичного виховання і сприяють поліпшенню функціонального стану систем організму. Однією з поширених методик такого спостереження є функціональна проба Мартіне, яка надає можливість досліджувати динаміку зміни

артеріального тиску і пульсу в період між станом спокою і на кожній хвилині після навантаження протягом п'яти хвилин. Вказана навантажувальна характеристика в більшості випадків досить повно відображає стан серцево-судинної системи студента. Визначення характерних патернів таких станів дасть змогу пропонувати механізм оцінки функціонального стану системи кровообігу. Регулярне проведення проби Мартіне дасть можливість спостерігати динаміку й оцінювати зміни в організмі за період спостережень.

Мета. Створення системи автоматизованої оцінки змін функціонального стану системи кровообігу студентів.

Методика реалізації. Для дослідження використовувалася база даних студентів Національного технічного університету України "Київський політехнічний інститут імені Ігоря Сікорського", створена в процесі досліджень кафедри фізичного виховання. Для визначення функціональних патернів, окремо для юнаків і дівчат, застосовувався кластерний аналіз методом "*k*-середніх" у просторі параметрів артеріальний тиск–пульс, виміряних у процесі проведення проби Мартіне. В результаті було отримано 7 кластерів станів для юнаків і 8 кластерів для дівчат. Кластери істотно відрізняються за характером реакції показників артеріального тиску і пульсу на виконання тесту. Центроїди отриманих кластерів у подальшому розглядалися як функціональні патерни (типові представники) реакції організму на тестове фізичне навантаження. Оцінка функціонального стану системи кровообігу розраховується у порівнянні показників тесту студента з раніше визначеними функціональними патернами за критерієм мінімальної евклідової відстані. Формуються висновки про стан системи.

Результати. Отримано кластери функціональних станів системи кровообігу в розширеному просторі параметрів артеріальний тиск–пульс проби Мартіне. Кластери відповідають станам норми і різним стадіям зниження регуляторних резервів організму. Розроблено алгоритм, що дає змогу враховувати особливості функціонального стану організму при суттєвому відхиленні показників проби Мартіне від певного функціонального патерну. Це дає можливість контролювати індивідуальний рівень фізичних навантажень, адаптувати програму тренувань і виявляти стани, що вимагають додаткового медичного контролю.

Висновки. Розроблено систему автоматизованої оцінки функціонального стану кровообігу студентів технічного університету із застосуванням проби Мартіне. Система відносить спостереження в один із кластерів навчальної вибірки, отриманих із використанням методу "*k*-середніх". Для додаткової інформації про стан системи кровообігу використовується середній радіус кластера, оскільки об'єкти, сильно віддалені від центра, можуть мати властивості, близькі до властивостей сусіднього кластера. Розроблена система дає змогу як здійснювати контроль за станом системи кровообігу одноразово, так і проводити скринінговий контроль групи студентів.

Ключові слова: кластеризація; метод "*k*-середніх"; функціональний патерн кровообігу; мінімальна евклідова відстань; середній радіус кластера; функціональна проба Мартіне.

Б.А. Войник, Г.В. Борисова, В.С. Уманец, Г.Л. Бойко, А.В. Павлов, Е.А. Настенко

АВТОМАТИЗИРОВАННАЯ ОЦЕНКА ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ СИСТЕМЫ КРОВООБРАЩЕНИЯ СТУДЕНТОВ С ИСПОЛЬЗОВАНИЕМ ПРОБЫ МАРТИНЕ

Проблематика. Систематические самостоятельные наблюдения студентов за состоянием своего здоровья позволяют оптимизировать организацию учебного процесса физического воспитания и способствуют улучшению функционального состоянию систем организма. Одной из распространенных методик такого наблюдения является функциональная проба Мартине, которая предоставляет возможность исследовать динамику изменения артериального давления и пульса в период между состоянием покоя и на каждой минуте после нагрузки в течение пяти минут. Указанная нагрузочная характеристика в большинстве случаев достаточно полно отражает состояние сердечно-сосудистой системы студента. Определение характерных паттернов таких состояний позволит предлагать механизм оценки функционального состояния системы кровообращения. Регулярное проведение пробы Мартине позволит наблюдать динамику и оценивать изменения в организме за период наблюдений.

Цель. Создание системы автоматизированной оценки изменений функционального состояния системы кровообращения студентов.

Методика реализации. Для исследования использовалась база данных студентов Национального технического университета Украины "Киевский политехнический институт имени Игоря Сикорского", созданная в процессе исследований кафедры физического воспитания. Для определения функциональных паттернов, отдельно для юношей и девушек, применялся кластерный анализ методом "*k*-средних" в пространстве параметров артериальное давление–пульс, измеренных в процессе проведения пробы Мартине. В результате были получены 7 кластеров состояний для юношей и 8 кластеров для девушек. Кластеры существенно отличаются по характеру реакции показателей артериального давления и пульса на выполнение теста. Центроиды полученных кластеров в дальнейшем рассматривались как функциональные паттерны (типичные представители) реакции организма на тестовую физическую нагрузку. Оценка функционального состояния системы кровообращения рассчитывается посредством сравнения показателей теста студента с ранее определенными функциональными паттернами по критерию минимального евклидового расстояния. Формируются заключения о состоянии системы.

Результаты. Получены кластеры функциональных состояний системы кровообращения в расширенном пространстве параметров артериальное давление–пульс пробы Мартине. Кластеры соответствуют состояниям нормы и различным стадиям снижения регуляторных резервов организма. Разработан алгоритм, позволяющий учитывать особенности функционального состояния организма при существенном отклонении показателей пробы Мартине от определенного функционального паттерна. Это дает возможность контролировать индивидуальный уровень физических нагрузок, адаптировать программу тренировок и выявлять состояния, требующие дополнительного медицинского контроля.

Выводы. В результате проведенного исследования была разработана система автоматизированной оценки функционального состояния кровообращения студентов технического университета с применением пробы Мартине. Система относит наблюдения в один из кластеров обучающей выборки, полученных с использованием метода "*k*-средних". Для дополнительной информации о состоянии системы кровообращения используется средний радиус кластера, поскольку объекты, сильно удаленные от центра, могут иметь свойства, близкие к свойствам соседнего кластера. Разработанная система позволяет как осуществлять контроль за состоянием системы кровообращения единовременно, так и проводить скрининговый контроль группы студентов.

Ключевые слова: кластеризация; метод "*k*-средних"; функциональный паттерн кровообращения; минимальное евклидово расстояние; средний радиус кластера; функциональная проба Мартине.

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Review

SOME OBSERVATIONS ON THE ROLE OF WATER STATES FOR BIOLOGICAL AND THERAPEUTICAL EFFECTS

M. Scalia[1]*, P. Avino[2,3], M. Sperini[1], V. Viccaro[4], A. Pisani[2], V.I. Valenzi[1,2,5]

[1]Interuniversity Center of Research for Sustainable Development, Rome, Italy
[2]International Committee for Research and Study of Environmental Factors, Belgium
[3]Istituto Nazionale Assicurazione Infortuni sul Lavoro, Rome, Italy
[4]EU.NA.M. Institute, Rome, Italy
[5]UNIGLOBUS University Assisi, Assisi, Italy

*Corresponding author: massimo.scalia@uniroma1.it

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The peculiar properties of water are recalled, among them the "memory of water", underlining the behavior of cell water, whose "ordered" structure is probably due to the endogenous electromagnetic fields generated by organelles inside the cells, such mitochondria and microtubules. Biological and clinical implications of water dynamic changes are reported, mainly based on SEP (Skin Electric Parameters) analysis and a hypothesis that has been advanced about an "action at distance" exerted by low concentration drugs. Nowadays, regarding measures of electro-cutaneous parameters our research can resort to a device of advanced electronics, APEC 300, that, beside an Electro Acupuncture operational function, but with quantitative precise values, can detect micro variations on parameters of cell aqueous solutions, and those of the water too, when the analyzed object is solicited by an external electromagnetic weak field, as well as by a drug at a very low concentration. A draft of two possible lines of research is outlined in conclusions, one depending only on ourselves and regarding "water tests" in the context of the "memory of water" experiments. By the side of EIS experiments about conductivity and impedance spectroscopy, in this article very high-resolution measures are suggested, by means of APEC 300, of an important parameter, the potential; a kind of measurements less complex and more reliable regarding the device/object interference, to which can besides easily be associated a frequency spectrum in the extremely low frequency region. In this line of research, it is the first time, for what we know, that some measurements of the potential, with its frequency spectrum, have been carried out for water without and with stimulation (the latter represented by the action of a magnetic field). This first set of measures by APEC 300 reveals the region 0–0.5 Hz as one of special interest.

Keywords: cell water; water dynamic changes; biological and clinical implications; skin electric parameters; Electro Acupuncture of Voll; APEC 300 and high resolution measures; drugs at very low concentration; potential measurements and frequency spectrum; memory of water.

Introduction

How to explain several astonishing properties of water, the substance so strictly connected to life and its multiple forms? How do play endogenous electromagnetic fields in "ordering" cell water? Starting from these questions, after a brief recalling of magnetic properties of water and its "memory", we underline the central hypothesis enhanced by Herbert Fröhlich, who developed a theory of biological coherence based on quantum interactions between dipolar constituents of biomolecules, such as those of the enzymes, membranes, organelles and the same cells; a relevant feature of this theory is exactly the special structure supposed for the biologic water (micro-trabecular structure). This kind of reasoning has been a background for many other Physicists and Doctors, whose relevant contributions are recalled and quoted in references.

Up to now, we are faced with an abundance of theoretical models, some of them are briefly reported, mainly provided by Physicists, and a relative poorness of experimental data presented by Physicians. An exception can be represented by Reinhold Voll and its Electro Acupuncture (EAV), with experiments reported in scientific literature up to day. Dr. Voll theorized, on the basis of the experience of Chinese medicine, a tight link among the values of the Skin Electric Parameters (SEP) and the health status performance; and EAV is a diagnosis method to reveal pathophysiological processes, as inflammations, pain, allergy, gastrointestinal function, etc.

Some few relevant clinic experiences are reported and commented, and, just in the perspective of a significant multiplication of rigorous experimental data, we present in the last section a research project, no more than a draft, on which is the case of spending few words. The implementation of the project is founded on a device of advanced electronics, APEC 300 that is an analyzer of electro-cutaneous parameters – *impedance*, *potential* – realized in Rome by some researchers of Bioelectromagnetism (BEM) Section of the Interuniversity Center of Research for Sustainable Development (CIRPS). This apparatus can perform EAV, with a higher resolving power and a greater reliability of results than the traditional apparatus, and can measure, among several its applications, the skin potential level as function of time, besides providing its frequency spectrum at extremely low frequencies. It is worth to underline that APEC 300 can also measure bioelectric activity of cell cultures or aqueous solutions of drugs or pharmaceutical complexes (to assess their effectiveness or dangerousness).

In the last twenty years, much attention has been devoted to the behavior of aqueous solutions of cells, enzymes or more simple organic compounds when they are solicited by a *very weak magnetic field*. Many *impedance* measures have been realized, while a little attention has been given to the *potential*, perhaps due to the scarce reliability of the available measure instruments. Our idea is to exploit the high resolving power of APEC 300 to test not only aqueous solutions impedance, but also the *potential*; not only when aqueous solutions are submitted to an external magnetic field, but *under the influence of a specific drug at a very low concentration*. The further step would be to detect, in a collaboration with Physicians, the impedance response of a patient who has been given that drug at that concentration level, to be compared with the same response in absence of the drug assumption.

A not negligible part of the article regards "water tests" linked to "water memory" experiments. EIS devices for high-resolution detection of electrochemical properties of aqueous solutions are to be managed very cautiously, not only for reliability of measure but also to avoid device/object interference. Starting just from a comparison between tap water and stimulated tap water, the possibility of a more "at hand" measure method – that of measuring the variations of potential in frequency spectrum (up to less than 100 nanoVolt) – has obtained a first translation in practice by the set of measurements where the stimulation is given by the action of an alternating magnetic field at 50 Hz frequency (126.10 μT maximum intensity). Figures and data of the experiment are presented in the Annex. The measurements have been realized by APEC 300 and the corresponding frequency spectrum shows as a region of special interest the one from 0 to 0.5 Hz. This occurrence was in somewhat manner expected due to a similar behavior recorded during the phase of improving and calibration of APEC 300, when biological objects like fruit or leaves (their skin) were used as test materials.

1. The anomalies of water

The water molecule, H_2O, consists of two hydrogen atoms linked to an oxygen atom by a polar covalent bond. The H-O link distance is 0.958 Å and the O-H-O binding angle is 104.45°. The arrangement of electrons in the molecule determines electrical asymmetry. The difference of electronegativity between O (3.44; one of the electronegative elements of the periodic table of the elements) and H (2.20) makes the water molecule a permanent electric dipole of nature that allows the formation of hydrogen bonds between atoms of H of a molecule and atoms of O of other water molecules. The spatial structure of the water molecule is of tetrahedral geometry, with the O atom at the center of the tetrahedron (Fig. 1).

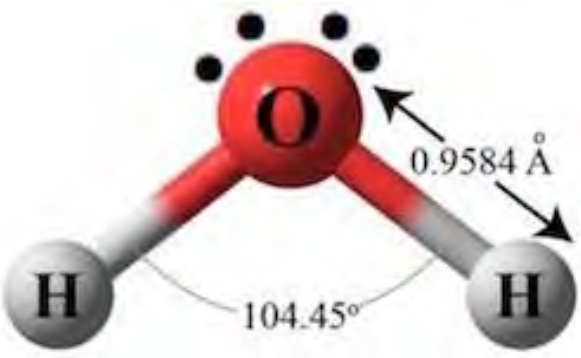

Figure 1: Spatial structure of water

All these peculiar features of water make it a unique molecule with physical and chemical properties that are still termed "abnormal" because of the inability to fully explain them even today.

Exceptional solvent, water is the life-giving molecule on Earth: it covers about 70% of the Earth's surface, accounts for 65% of a human and 90% of macromolecules present in biological systems. If the water did not exist, no chemical reac-

tions, no transport of nutrients and waste, etc. would exist. In a word, without water, there would be no life.

Within the range of environmental temperature and pressure, water is found in all three physical aggregation states: solid (ice), liquid (liquid water) and aeriform (water vapor).

Most of the models proposed to explain the peculiar properties of water start from the study of an isolated water molecule, then extend its characteristics and behaviors to the water molecules bound to it. Each model aims to predict the behavior of water in its three aggregation states and subsequently verify its compatibility with experimental chemical-physical properties.

Problems arise when, through intermolecular electrostatic attraction forces (short-range forces), a lot of water molecules are assembled to form the liquid phase together. None of the water models proposed to date, based on short-range forces, is able to describe satisfactorily ***the "abnormal" chemical-physical behavior of water***.

Density. It has been observed experimentally that the number of water molecules found in 1 cm^3 of liquid in the range 0 °C ≤ T ≤ 4 °C ***increases instead of decreasing***; water has its maximum density at T = 3.98 °C and not at 0 °C.

Viscosity. It gives a measure of the force that obstructs the sliding of the molecules with respect to each other, is linked to intermolecular forces and depends on temperature and pressure: generally decreases as temperature rises and decreases in pressure. The ***viscosity*** of the water decreases as the temperature rises, as expected, but ***increases as the pressure decreases***. In more detail: for liquid water at T < 30 °C, increasing the pressure viscosity begins to decrease; then continuing to increase the pressure the abnormal trend ceases and the viscosity begins to increase.

This is one of the characteristics of water that allows life on Earth; let's just think of surfaces of frozen water, where the ice is above and below the biological activities in the liquid water are continuing.

Surface tension. Also, this property depends on the intermolecular forces; water is characterized by a ***very high surface tension***. Even this abnormal behavior is at the basis of the existence of life; in fact, surface tension plays a key role in capillary phenomena, such as rising of lymph in blood vessels and water from the soil.

Melting point, boiling point, and specific heat. All three of ***these points have higher values than you would expect***. Generally, the smaller the molecules are and the smaller the boiling point. In theory, the water should boil at −93 °C and solidify at a temperature of only a few degrees below. Fortunately for living beings is not so.

Moreover, the specific heat of a solid is generally greater than that of the same liquid substance. Instead, when water gels, its specific heat drops to a value equal to half that of liquid water, therefore, by supplying energy to liquid water, only half of this energy is spent to raise the temperature, the remainder is stored in the mass of the liquid.

This property causes the water to absorb and give heat very slowly, and this peculiarity is also crucial for life on Earth. In fact, living beings are so protected from sudden temperature fluctuations, the oceans absorb solar energy by storing it and forming a huge energy tank, which is gradually released as heat when the warm ocean currents move slowly from the tropical regions towards the cold ones of the poles. All these mechanisms have made possible the development of life on Earth and contribute to maintaining it.

Dielectric constant. The dielectric constant for 20 °C is ***very high*** (εr = 80) compared to that of ice (εr = 5). This peculiarity allows the water to have high absorption in the frequency range from microwaves to infrared rays; to be an excellent solvent and also a dissociating agent. In the water, due to the high dielectric constant, there is a loosening of the bonds between the molecules dissolved therein and therefore thermal agitation is sufficient to ionize them; by this way, liquid *water is richer in electrolytes*, thus enabling all vital processes. Eventually, tissues and organs with high water content are in a state of ionization.

2. Magnetic properties and the "memory" of water

We below list a set of properties, on which there is a debate and are not recognized by a great part of academic scientists:

Water flowing in a pipe, sited in the magnetic field generated by the two poles of a magnet acquires some properties:

- no limestone incrustations are formed;
- dissolves the limestone incrustations that may be present;
- its surface tension decreases;
- it has a very pleasant taste, while under normal conditions is a tasteless and odorless liquid
- desalinates salty soils;
- it greatly limits the survival of microorganisms.

The most controversial property is undoubtedly the *magnetic memory of water*.

It may be useful to recall a very brief story about the scientific path that preceded that controversy, starting from the pioneering works of Giorgio Piccardi at the end of the Thirties. After studying the precipitation of various substances in water, Piccardi was convinced that different ways of precipitation were due to the action of magnetic fields. By virtue of this action, water becomes "activated" in two different configurations: "T", less viscous than normal water; "R", more viscous, with different precipitation effects [1–3]. Activation modifies also biological properties of water and, in this way, biological processes in living systems [4]. In successive experiments, aimed to avoid the limestone formation in boilers and pipes, Piccardi observed that very weak signals from external environment, mainly from solar fluctuations, were able to induce a change of state in solutions [5, 6]. In late Fifties Piccardi documented with a series of experiments the action of low frequency electromagnetic fields on water. He noted that water treated with electromagnetic fields of 10 kHz frequency was activated and showed a clear decrease in surface tension [7, 8]. It's worth to note that Piccardi was a "precursor" also with the debate that arose at the time about his researches; a full insight of his wide contributions in various scientific sectors can be found, mainly about water, in [9].

In 1988 Jacques Benveniste published, with other researchers, a paper describing the action of very high dilutions of anti-IgE antibody on the degranulation of human basophils [10], findings which seemed to support the concept of homeopathy. Since Benveniste was a recognized immunologist and head of the INSERM Unit 200 (immunology, allergy, and inflammation), the French National Institute of Health and Medical Research, the results of that paper gave rise to an international controversy. A journalist coined the term *"water memory"* because in the Benveniste's experiment the dilutions of the antibody were so high that no of its molecules remained but only molecules of water. Thus, a conclusion to draw out was that the "activation" of water, operated by anti-IgE antibody, lingered also in its absence making water biologically active. Time later, in the nineties, after studying further biological effects in presence of a magnetic field [11], Benveniste asserted that this "memory" could be digitized, transmitted, and reinserted into another sample of water, which would then contain the same active qualities as the first sample [12]. Encouraged by the public support of Brian Josephson, a physicist Nobel laureate, Benveniste continued his experiments along the same basic lines, culminating with a paper claiming the effect could be transmitted over phone lines [13]; this was followed by another paper on electronically remote-transmission [14].

Several Physicists (among them, Preparata, Del Giudice, Zhadin, Widom, Srivastava) have elaborated theories about the peculiar structures of water, which are supposed to be capable of retaining information on its conformation in a stable manner. Their model and theories have already been directly presented by one of us, or in any case reported, in "Cosmos and Biosphere" Conferences (see [15–18]).

3. Water in the cells

The cell consists mainly of water, as well as the interstitial fluid, the electrolytic solution in direct contact with cells that is responsible for their nourishment and purification. The charges at the two surfaces delimiting the cell membrane are not balanced, thus generating a difference in potential. Within a cell the interaction between mitochondria and microtubules allows the generation of an electromagnetic field that can play a key role in ***long-range communication*** and cellular organizational structure.

Starting in the late Sixties and continuing until his death in 1991, ***Herbert Fröhlich***, a famous physicist, developed a theory of biological coherence based on quantum interactions between dipolar constituents of biomolecules, such as those of the enzymes, membranes, organelles and the same cells; a relevant feature of this theory was exactly the special structure supposed for the biologic water (micro-trabecular structure). He thought that endogenous electromagnetic fields exist in all living systems and they are responsible for long-range cell communication [19]. Living systems are open, nonlinear, far from the thermodynamic equilibrium and very highly organized. He hypothesized on the basis of quantum mechanics that ***endogenous electromagnetic fields*** contribute to maintain all these characteristics. The fundamental cell organelles to argue this theory are the mitochondria and microtubules; the latter, filaments that make up the cytoskeleton, are dipolar structures subjected to oscillations and are aligned with mitochondria during the interphase. The static electric field of mitochondria allows the generation of electromagnetic field produced by microtubules, because the latter os-

cillate. As a result of the hypothesized nonlinear interactions, Frцhlich predicted the ***generation of coherent modes of excitation, representable as quantum dipole oscillations, in the microwave frequency range*** [20].

This line of reasoning is homogeneous with that of Del Giudice and Preparata [21, 22] – Del Gudice was a follower of Frцlich – and is at the base of many current hypotheses on the nature and behavior of cellular water, hereafter shortly recalled.

About 90% of cellular water is present as "bulk water", or as ordinary water. Ordinary water is located in the aqueous compartments of the cell, then in the cytosol, in the nucleus, and within the cytoplasmic organelles. The remaining 10% – perhaps also a greater percentage than that held up to now – is called "bound water" (or primary hydration, primary hydration water, ordered water). Bound water is near the surface of free ions, metabolites, macromolecules and structural components of the cell and shows different properties compared to ordinary water: higher viscosity, decreased thermal motion of molecules and different pH. In the "ordered water" there is a charge separation – let's recall the peculiar properties due to the electronegativity difference between hydrogen and oxygen – and this separation generates layers of ***coherence domains***, explainable with quantum mechanics. The coherence domains are formed around structures with hydrophilic surfaces, including microtubules and mitochondria, and, outside the cell, they can have macroscopic dimensions and properties similar to a gel. In a cell, coherence domains are formed because of electrostatic field of mitochondria and are subjected to electromagnetic fields produced by microtubules. The oscillation frequency induced in coherence domains by an electromagnetic field is lower than the frequency of that field in the vacuum; this event prevents loss by radiation outside the coherence domains. The energy of a molecule in a coherent state is lower than in the incoherent state, and this "energy gap" can be seen as generating a coherent state.

The plausibility of this framework is such that we often refer to it even if we don't know *any experimental measure that directly shows the existence of those domains.*

The existence of electromagnetic fields generated by cell components – the *endogenous electromagnetic fields* – has been detected on different living systems (see [23–25]) and their existence can be postulated in all living systems. With endogenous electromagnetic fields could be possible to explain the long-range cell communication that the only chemical communication cannot explain, because chemical communication uses short-range forces generated from chemical bonds.

4. Biological and clinical implication of water dynamics changes

Water dynamics changes are relevant in pathophysiological status? The question is actually without a clear answer while the main part of scientific and medical Community thinks and wrote that water dynamics is no stable and is dominated by Brownian motions. Generally, Philip Ball wrote: *"Water molecules associate by means of weak chemical bonds called hydrogen bonds. Although in the main they form and break on timescales of about a trillionth of a second, nonetheless they seem to offer a vague possibility that water might form clusters of molecules with specific shapes and behaviors"* [26].

The existence of clusters, coherent domains, collective phenomena in the global biological system of humans is somewhat intuitive. Billions of cell work together simultaneously and it couldn't possible without a structure of coordination and a great order inside of human body. If a somewhat mechanistic comparison is allowed, the ferromagnetic materials would not be such if they did not have highly ordered domains inside them, the Weiss regions. We can look at diseases as a factor introducing a certain degree of disorder into the human body, in this sense they cause increase in entropy in biological systems, and in their predominant medium, the water. We can conjecture that a good method to detect a disease, and how it is working, is to refer to the pathway of Chinese meridians, measuring with an electronic device the bioelectric impedance of those points of the skin, that have been individuated by a millenarian experience as a source of information on health. In fact, it is well known and the whole Voll's practice witnesses that Chinese medicine correlates those points of skin with internal organs of human body.

In fact, correspondingly to those points – about two thousand and half, of which only few on the hands – the cutaneous *resistance* is lower than in all other points of the skin, up to one order of magnitude, as it is reported by a lot of experimental data; and one can compare a known datum with the measured value he is obtaining. The first who attempted this way to perform a diagnosis was

Dr. ***Reinhold Voll*** with his EAV (Electro Acupuncture of Voll), in the Fifties. EAV is a diagnosis method still worldwide applied, with a not negligible presence in scientific literature (see, for instance, [27–30]), even though the reliability of the obtained diagnosis strongly depends on cleverness of physician, his knowledge of Chinese medicine and of the Voll's "diseases atlas".

Over last sixty years, Voll and many other scientists hypothesized that human body could be seen as an electrical system, endowed with electric circuitry, and that the values of the Skin Electric Parameters (SEP) could be connected with the health status performance and pathophysiological processes, as inflammations, pain, allergy, gastrointestinal function, etc. General measurements on meridians have highlighted in subjects, mainly affected by meteoropathies but also by other pathologies, that the electrical resistance was averagely increasing up to 43 Scale Units (SU) of SEP, corresponding to 130,000 Ω [31, 32].

In a first interpretation proposed by Reinhold Voll the *"normal"* value of the measurement was set to 50 SU, corresponding to 95,000 Ohm. This level was presented as an equilibrium value for human health, so that many efforts by doctors using EAV were devoted obtaining this goal. After the administration of a coherent therapy, we registered an average decrease in resistance at 39,000 Ω (70 SU); on the contrary, in many chronic diseases an average increase of resistance to 130,000 Ohm (43 SU) was measured; and in cancers, as well as in other critical disorders, we observed an increase of resistance up to 250,000 Ohm [33].

Another experiment, always following the EAV procedure, has been performed with twelve "homogeneous" subjects, i.e., all in a "normality" range of health state, meteosensitivity but not meteoropathic, none younger than fifty years (it was the personnel of a clinic). Each of these persons was individually subjected to three measures in sunny weather and three measures in cloudy weather; all the obtained values for the two weather conditions were reported in a table, ordered following a non decreasing trend, that is, at the first places (1,2) persons with the smaller values of SU, in the second places (3, ..., 8) those with greater SU values and so on. The three groups of measures for each of the two weather conditions, represented by dotted lines blue, red and yellow, exhibited an interesting homogeneity of behavior [34] (see Fig. 2).

In Fig. 2, in abscisses is reported the number of the volunteers that were submitted to the measures (from 1 up to 12 persons). In ordinates is represented the Scale Units, from 0 to 100, that are correlated to the resistance in Ohm (on the right of the table).

In this context a fundamental step was, in 1988, the approval in Moscow by the Minister of Health of the Voll System for studying and monitoring of Elio-biological interaction [35].

Nevertheless, after 20 years of experiences in diagnostic and therapeutic use of EAV we came to the conviction that the resistance measure has a dynamical character and the objective of therapy driven by the SEP be not the "normal" level of 50 SU, but the increase of conductivity of the skin in correspondence to the Chinese meridians (let's remind the correlation among those points and organs of human body). Increasing of conductivity in human bioelectrical system, an objective that can be achieved by means of many therapeutic tools as drugs (all kind), magnetotherapy, SPA therapy, climatotherapy etc., turned out in a success factor in many therapies.

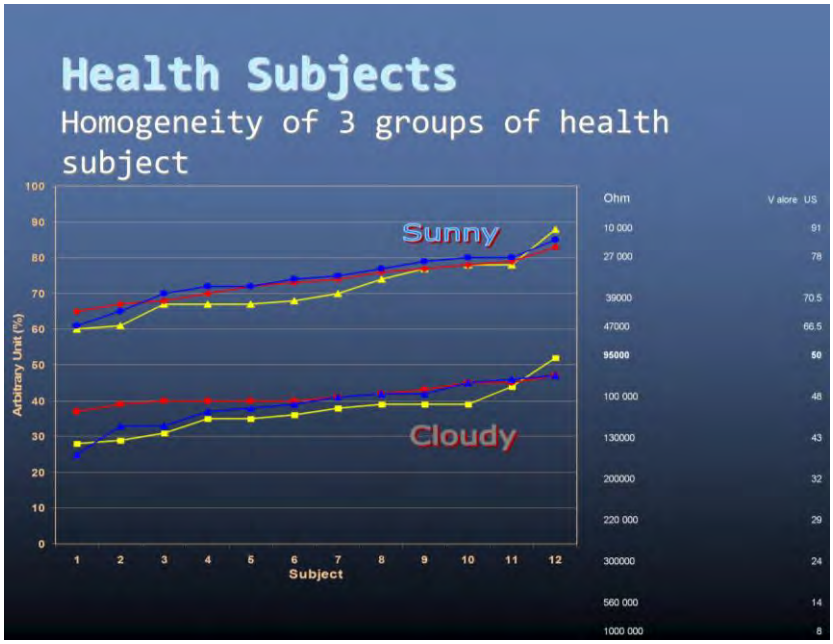

Figure 2: SEP variations under influence of weather conditions

Trying to find an explanation of those successes, many efforts have been devoted by some Italian researchers (Avino, Gigante, Grimaldi, Marinelli, Valenzi) that per-

formed over the time several experiments, only for few of which it has been reached, in the assessment of those researchers, the status worthy of a scientific referee. The latter papers will be mentioned after; immediately here, we will recall some of those results, but recurring to a simplified scheme, to be taken as *a linear approximation* of the more complex electric circuitry that underlies the models that explain how it works electroacupuncture of Voll, characterized by the *nonlinearity of skin response to solicitation*.

In this linear approximation, aimed at not to burden understanding with technicality, it is possible to give a role to a very simple but fundamental law of electricity, the ***Ohm's law***, that linearly links the physical quantities in the simplest of electric circuits:

$$V = i \cdot R,$$

where V is the difference of potential (or tension) at the ends of a conductor; R is a characteristic of the material the conductor is made of, the resistance, and i is the current intensity that flows in the conductor by virtue of the applied V. In the cases where one measures a significant decrease of R between two points of the skin, Ohm's law shows that, correspondingly, the current intensity i has increased, where V is the tension applied to those two points, like is usual when EAV measurements, for a small value of the stimulating tension V, are performed. To a greater current intensity corresponds a greater value of bioelectric power W, that, *in this simplified scheme*, is given by

$$W = V \cdot i;$$

and just looking at the influence of the sun radiation intensity, i.e., the electromagnetic power density through a surface unit orthogonal to the rays of the sun, on the values of SEP such as that are determined by EAV, it seems reasonable hypothesizing that the correlation with the increase of current i and, consequently, of bioelectrical power W provides electrophysiological parameters fundamental in the regulation of human body. On the other hand, bioelectric power in the body districts, measurable with muscular test by a dynamometer (0−100 Kg), vary with functional correlations (performance status, immunological status, muscle power, pain, inflammation, allergy, dyspnea, etc.), signaling better performance at the increase of bioelectric power.

Today we prefer to speak of *impedance*, because a pure resistance is one of the limit concepts we find in Physics; in fact, also at extremely low frequencies (less than ten Hertz) there is always a capacitive component that, together with a resistance, gives rise to electric impedance.

Good news about electro-cutaneous parameters is that we can now use a device of advanced electronics, APEC 300, with a very higher resolving power than Voll's apparatus; further, it can be used for acupuncture with the usual punctiform electrodes, as well as in other applications different from acupuncture, performed with ordinary electrodes. In this latter way one partially loses the completeness of information linked to acupuncture in Chinese points, but gains in the reliability of diagnosis when one is not expert in Chinese medicine.

Moreover, this device allows directly measure the ***skin potential*** as a function of time, a parameter strangely forgotten in many scientific reports that, instead, is very rich of information about health status. By virtue of a software installed inside the device, it can be immediately provided an analysis in frequency – the spectrum associated to the skin potential curve – that, in the region of]0.1] Hz, gives a so called "electromagnetic footprint" that we are conjecturing could characterize the health status of each body district submitted to the measure. This kind of measure is a mere registration of signals emitted by our body, like an ECG or EEG, and not a response to an electric stimulation, as happens in all measurements of impedance, with applications obviously easier than those measures that request specific approval or protocol.

The existence of bioelectric parameters measurable on the skin can suggest that an electromagnetic (e.m.) mechanism could underlie the so called pseudo-allergies to drug, food etc.: since these symptoms are the effects of drug, the ***possibility arises that the molecules of drugs could involve an e.m. action apart from the well know chemical action***; and the molecular signaling, registered by means of SEP measurements, performed comparing without/with drug cases, could play an interesting role in biological processes and in therapy. Hence, the paradigm: ***It's the dose that makes the drug***, may not be universal.

In the graphs of Fig. 3 in the ordinates is represented the value of SU and in abscisses the time of exposition of the patient to drug. In the experiment were tested 8 drugs against pain and inflammation: *nimesulide, diclofenac*, *ibuprofen piroxicam*, *naprossene*, *indometacine*, *COX2*, and *cortison*. Only one (Nimesulide) was able to improve clinical condition, in particular reduction of pain. To this

improvement was correlated a special behavior of SEP, as can be easily seen by comparing the two graphs of Fig. 3 [36]. This kind of comparison could be used for the choice of FANS, gastroprotector, antistaminic etc., on the basis of the molecular signaling of drug on SEP, and can be detected as a strong variation of resistance that, following Voll, bioelectrically modulates health status.

Fig. 3 is worthy of a particular remark: the graph on the right has been obtained not as a result of ingestion, but simply posing the chosen drug outside the body, even though at a small distance (1.5 cm). How is it possible a kind of drug action that is not an exaggeration to define as an ***"action at distance"***? An analysis of this fact implies a lot of caution, it was the hitch that obliged Einstein to a complete reformulation of classical Physics, that led to the field equations of General Relativity. Among other important things, the latter theory gives the right answer to why Earth goes around the Sun; in fact, this astronomical motion cannot be explained in terms of Newton's universal gravitation law precisely because this kind of ***immediate action***, ***at distance***, should have an infinite velocity in contrast with the postulate of light velocity as unsurpassed limit for any physical action. Thinking about it, the nimesulide of our experiment is sited at a distance like that of Earth-Sun, with respect to the scale of the cells on which it acts.

In the case of electromagnetic interactions, that undergo for what the electrostatic action is concerned the same criticism of Einstein versus gravitation law, there is another function, besides electric field **E** and magnetic field **H**: the vector potential **A**, not directly observable and characterizing the wave propagation of electromagnetic fields. Many theoretical Physicists – Bohm, Aharonov, Josephson, Puthoff, Preparata, Trukan, Widom, Srivastava, and others – have assigned to **A** a key role in ***action at distance***, not in contradiction with General Relativity; and that bacterial DNA sequences can produce electromagnetic signals [15] is another experimental example of an action at distance supported by electromagnetic fields. It is an intriguing possibility, we are working on it, that the vector potential **A** be the "director" of non chemical drug action at distance; it could theoretically explain many problems, like that represented in Fig. 3, that occur in medical practice.

All this reasoning underlines that we are in a kind of "excess" of theory and models with respect to available experimental data. It is one of our goals to begin balancing this situation, that could provoke some justified criticism, with a program of experimental measurements, some of whose are briefly sketched in the following section.

Another line of research to understand how some drug signals from water or directly by drug or food (without chemical interactions) could be able to induce change in cells is to investigate if some of those effects can be interpreted in terms of quantum coherence interactions (see sect. 3), whose behavior is represented in the following Fig. 4, just to mention it. The context of this research approach is the so-called quantum chemistry, in which we are trying to move a few steps.

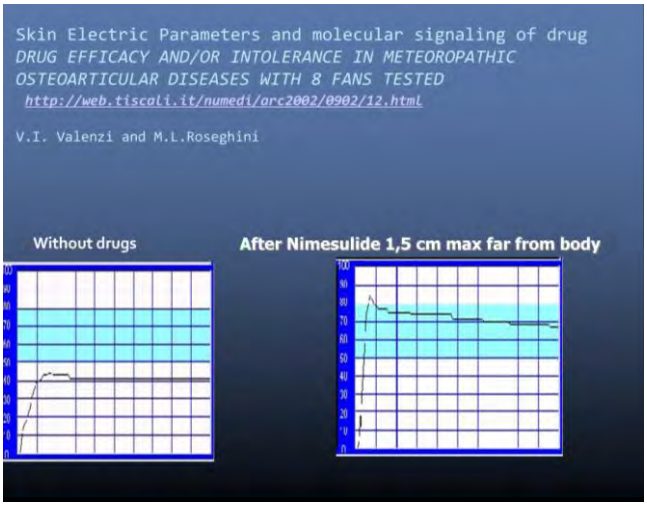

Figure 3: SEP variation in time due to molecular signaling of drug

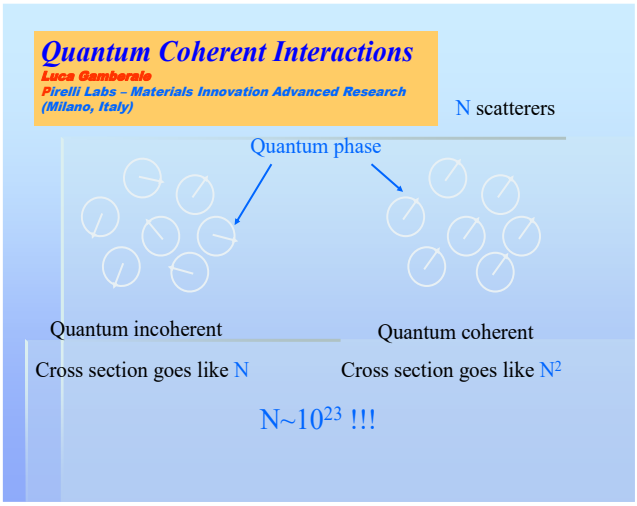

Figure 4: Different behavior between two groups of coherent and incoherent

This development could play a real role in our understanding of the critical problem of side effects of drug (more than *100,000* Americans die every year [37]), and orient medical practice approach to driven therapy by quantum chemistry in order to improve risk benefit ratio in pharmacotherapy.

Conclusions

A two-fold possibility of research is open ahead of us: clinical tests, "water tests". Let's sketch a draft.

In both cases we mainly resort to APEC 300 (see sec. 4), designed in the Bio-electromagnetism section of CIRPS and mainly aimed to measure electro-cutaneous (EC) parameters. This device is equipped with a high resolving power, able to record the smallest variations of the measured values and, then, of the investigated properties. It can also perform a measure seldom treated in literature, the ***potential***, and we like to recall that to each measure of this kind one can associate a frequency spectrum, that is a Fourier analysis of the potential, by virtue of internal software of the device. Measures realized in the phase of calibrating and testing APEC 300 have persuaded us that the frequency spectrum of potential could represent, probably in the]0−0.1] Hz region, an electromagnetic "footprint" characterizing each type of living object under measure (human body, body districts or systems, fruits, leaves of plants, cells, etc.).

About ***the first line of research*** we underline that APEC 300, in its actual configuration, can be used ***by doctors*** not only for EAV, with more reliable results than the traditional ones such as those reported in references, but also to perform precocious diagnoses through the applications of ordinary electrodes to the skin areas sensible to detect: gastrointestinal diseases [38], premonitory signals of breast cancers [39] or female genital disturbs [40]. Further experimental measures could confirm these results and extend EC analysis to other pathologies. *These researches need of a collaboration with clinical structures and doctors interested and willing to perform such kind of experiments.* Thus, we are not able, now, to schedule time and steps of research.

Another kind of clinical application is to measure the quantity and the characteristics of the overall water contained in the body, as it is possible to do by APEC 300, as a test of health status; and to remark the variations of body water under the assumption of a specific drug. This test has, probably, not to be submitted to medical approvals or bioethics committee and we are expecting *some address by clinicians to convert spot measures on consenting subjects in a possible research program.*

The ***second line of research, the "water tests"***, can be in turn subdivided into two issues:

An extremely weak "field" can act on an organic or biological system? This is a recurring problem in scientific researches and is the case of mentioning again Giorgio Piccardi, because he was afraid that many his experiments on the activation of water could be altered by "fluctuations" due to the different velocity of Earth in the different seasons, the variations of the solar magnetic activity, the "atmospheric potential" changing during the experiment. A witness of this concern is the subject of the last paper he wrote with Carmen Capel–Boute [41].

The posed question has positive and measurable answer from a stock of natural phenomena, where a solicitation of very low intensity is at the base of a response: the electroreception in elasmobranchs is activated by 1 μV; the human eye retina is capable of detecting few photons; a very low concentration from 10^{-10} M to 10^{-12} M of the aldosterone or anti-diuretic hormones is capable of saving the sodium request of the renal tubules in our body, and, not physiological, the Zhadin effect [42].

In the last twenty years, much attention has been devoted to the behavior of ***aqueous solutions*** of cells, enzymes or more simple organic compounds, when are solicited by an extremely weak magnetic alternating field, mainly in the ELF region. Our idea is to test biological or organic ***aqueous solutions*** at a very low level of concentration, measuring their electric properties like *potential* and *impedance*, not only when submitted to an external magnetic field; better, ***under the influence of a specific drug at a very low concentration***.

To this aim, we remember that the potential and impedance measures by APEC 300 maintain a rigorous sense also when not applied to the EC parameters, as, i.e., in the case of cell cultures or aqueous solutions; besides the device has an operational range that allows analyzing the micro variations of potential or impedance. A comparison between a not exposed cell culture with the treated one with a specific pharmaceutical at a very low concentration is achievable, almost immediately, in terms of the change of color of the culture through a gamma extending to the color of the cell apoptosis. Experimental mea-

sures can individuate the threshold value, if any, below which there is no difference between the two cases. The further step is to detect the impedance response of a patient who has been given that drug at that concentration level, to be compared with the same response in absence of the drug assumption.

This kind of research postulates a partnership among bio-physicists and clinicians.

"Water memory". Benveniste's biology experiments have suggested the existence of molecular-like effects without molecules ("memory of water") [10], [11]. On the same line of reasoning Luc Montagnier, in collaboration with other authors, proposes that "nanostructures" from viral or bacterial DNA are able to produce, in high aqueous dilutions, electromagnetic signals (EMS) emitting in a ULF region (500−3000 Hz); essential to this occurrence is an electromagnetic stimulation by a natural field at one of the Schuman's frequencies (starting from 7.8 Hz) or also by an artificial field at 50−60 Hz. The authors assert that the need for such stimulation witnesses a resonance phenomenon and a theoretical explanation of EMS is possible by resorting to the quantum field theory [43], [44]. Another experiment, led by Livio Giuliani, seems to confirm, at a first step, the Montagnier's results, thus supporting a new diagnostic perspective to the development of highly sensitive detection system for chronic bacterial infections in human and animal diseases, since in the genomic DNA of most pathogenic bacteria are contained sequences which are able to generate such EMS [45]. In a subsequent paper, it has been shown that the recorded EMS and nanostructures induced in water carry the DNA, by retrieval of that same DNA by classical biochemical amplification methods. Moreover, such a transduction process has also been observed in living human cells exposed to EMS irradiation [46].

These and other similar experiments recall the difficulties and specific conditions, encountered or to be satisfied, for electrochemical measurements of water and aqueous solutions. Over the last decades measures on pH, DC/AC conductivity, impedance spectroscopy have been realized for characterizing effects of "weak emissions". A particular attention has been given to EIS devices that are apparatuses able to measure conductivity and impedance spectroscopy (see [47−49]). We can remark, softly, that these latter measurements are not only complex but also that they need a lot of caution to avoid interferences of device on the specimen under measure; for instance, measurement on conductivity can drastically alter the characteristics of aqueous solutions, in such a way that hits the reliability of experimental results. Moreover, EIS "philosophy" doesn't take in account a significant parameter, the potential, on whose measure can base reliable comparisons between stimulated and not stimulated aqueous solutions.

Thus, it appears to us useful to integrate the experiments on water and aqueous solutions by directly measuring their potential and analyzing the corresponding frequency spectrum. Despite its high resolving power, APEC 300 is relatively easy to be used; measurements of potential are almost not interfering with the object under measure because are not a response to a stimulation but a detection of an endogenous property, and external noise can be downsized to a very low and quantifiable level; in fact, the device is able, in this kind of application, to distinguish noise variations of 10 nV (1 nV = $= 10^{-9}$ V), that is the same level of accuracy, or better, of other measure procedures, but much more at the hand.

Starting from a primordial question − what is the method for a physical, quantitative detection of a difference, if any and in an easy verifiable manner, between water and water that has been stimulated − we have carried out a first set of measurements of potential of tap water and treated tap water by an alternating magnetic field at 50 Hz and 126.10 μT peak intensity. The comparison between the frequency spectrum for the two cases exhibits a high reduction of the maxima of potential for the magnetized water, as one can see in the figures of the Annex, where, besides, the other experimental parameters are given.

One datum can well express the difference between the two cases: in the region]0−0.3] Hz, the mean value of the potential is 0,207 ± 0,015 mV for tap water while is 0.035 ± 0.003 mV for magnetized tap water. Beyond 0.40−0.45 Hz frequency, and up to 30 Hz, the values of the potential are very small, of the same order of the errors.

The special relevance of a so tight and ultra-low band of frequency is not so astonishing if, for instance, we look at most experiments of Heart Rate Variability (HRV), since they take place in the same frequency band.

Acknowledgments

This experimental project can be realized by the BEM Section of CIRPS in its laboratory and we hope to report some first results in next months.

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ANNEX

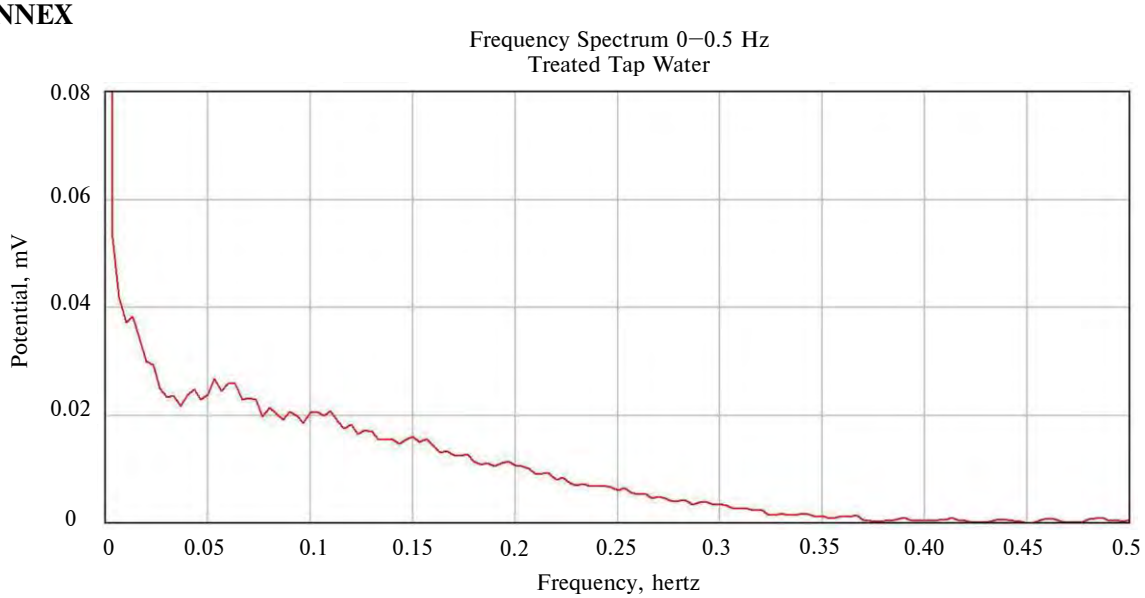

Fig. I

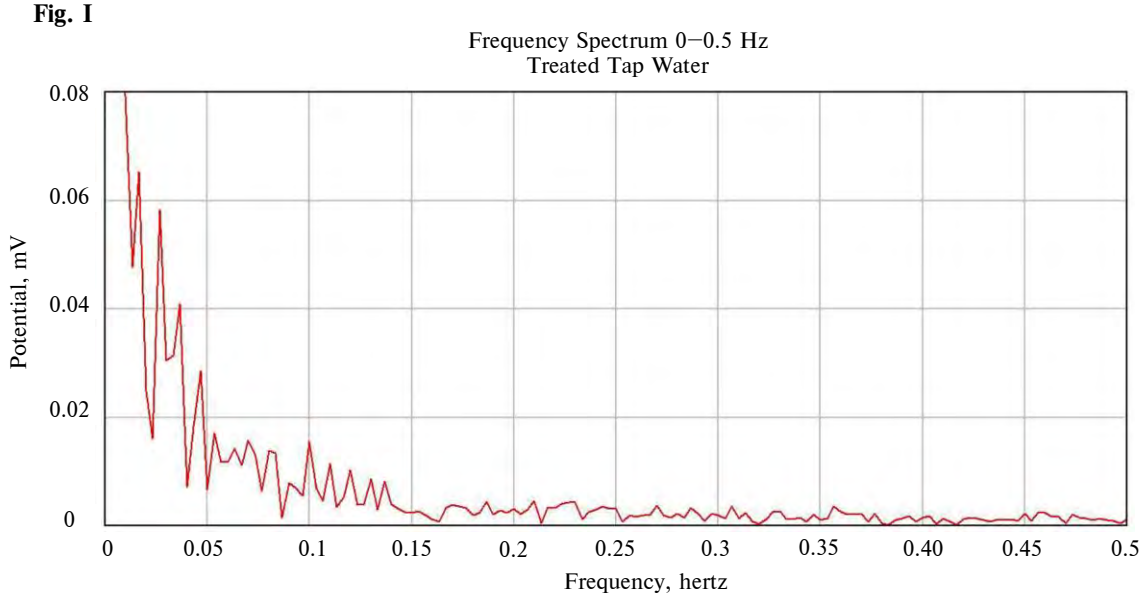

Fig. II

Five measures for each case have been performed and all measures have been realized in a "climatized" room able to maintain constant temperature and humidity; room magnetic field was less than 0,03 μT. The exposure time in order to magnetize water was 15 minutes for each measure and the peak value for the alternating magnetic field at 50 Hz was 126.10 μT. Water was contained in special small trays electrically connected, suitable also for experiments with aqueous solutions of drugs or cell cultures. Fig. I and Fig. II are representative of the five spectra obtained respectively for the two cases, and give at glance the reduction of potential for magnetized water (the scale of potential in Fig. II is one tenth of that of Fig. I); to better quantify this diminishing, the spectrum integral curve has been calculated to obtain the mean value of the potential. The values reported in the article, respectively 0.207 mV and 0.035 mV, are the mean value among the five values obtained for each case. Due to the limited number of events, the error has been calculated as the maximum semi-dispersion, respectively 0.015 mV and 0.003 mV. In Fig. II, the difference between the values of potential at 0.2 Hz and at 0.25 Hz is about 60 nV.

М. Скалья, П. Авіно, М. Сперіні, В. Віккаро, А. Пісані, В.І. Васенсі

ДЕЯКІ СПОСТЕРЕЖЕННЯ ЩОДО РОЛІ СТАНІВ ВОДИ У БІОЛОГІЧНИХ І ТЕРАПЕВТИЧНИХ ЕФЕКТАХ

Виявлені особливі властивості води, в тому числі “пам’ять води”, акцентують увагу на поведінці води в клітині, “упорядкована” структура якої, ймовірно, обумовлена ендогенними електромагнітними полями, що генеруються органелами всередині клітин, такими як мітохондрії та мікротрубочки. Повідомляється про біологічні та клінічні наслідки динамічних змін води, головним чином на основі аналізу SEP (Skin Electric Parameters), і висувається гіпотеза про “дію на відстані”, яка чиниться препаратами низької концентрації. Нині для вимірювання електрошкірних параметрів у нашому дослідженні може бути використаний пристрій сучасної електроніки APEC 300, який поряд з електроакупунктурою за Фоллем, але з точними кількісними значеннями, може виявляти мікрозміни параметрів водних розчинів клітини, а також води, коли аналізований об’єкт зазнає впливу зовнішнього електромагнітного слабкого поля, так само як і препарату за дуже низької концентрації. У висновках пропонується проект двох можливих напрямів досліджень, один із яких залежить тільки від нас самих і від “випробувань води” в контексті експериментів із “пам’яттю води”. Поряд з експериментами CEI щодо провідності та імпедансної спектроскопії, у роботі з використанням APEC 300 пропонуються дуже високочутливі вимірювання важливого параметра – потенціалу; свого роду вимірювання, менш складні та більш надійні щодо перешкод пристрою/об’єкта, який, крім того, може легко мати частотний спектр у наднизькочастотній області. В цьому напрямі досліджень уперше, наскільки нам відомо, деякі вимірювання потенціалу з його частотним спектром були проведені для води без та зі стимуляцією (остання представлена дією магнітного поля). Цей перший комплекс вимірювань APEC 300 становить особливий інтерес для діапазону 0–0,5 Гц.

Ключові слова: вода в клітині; динамічні зміни води; біологічні та клінічні наслідки; електричні параметри шкіри; електроакупунктура Фолля; APEC 300 і високочутливе вимірювання; низькі концентрації препаратів; потенційні вимірювання і частотний спектр; пам’ять води.

М. Скалья, П. Авино, М. Сперини, В. Виккаро, А. Писани, В.И. Валенси

НЕКОТОРЫЕ НАБЛЮДЕНИЯ ОТНОСИТЕЛЬНО РОЛИ СОСТОЯНИЙ ВОДЫ В БИОЛОГИЧЕСКИХ И ТЕРАПЕВТИЧЕСКИХ ЭФФЕКТАХ

Обнаруженные особенные свойства воды, в том числе “память воды”, акцентируют внимание на поведении воды в клетке, “упорядоченная” структура которой, вероятно, обусловлена эндогенными электромагнитными полями, генерируемыми органеллами внутри клеток, такими как митохондрии и микротрубочки. Сообщается о биологических и клинических последствиях динамических изменений воды, главным образом на основе анализа SEP (Skin Electric Parameters), и выдвигается гипотеза о “*действии на расстоянии*”, оказываемом препаратами низкой концентрации. В настоящее время касательно измерения электрокожных параметров наше исследование может прибегнуть к устройству современной электроники APEC 300, которое наряду с электроакупунктурой по Фоллю, но с количественными точными значениями, может обнаруживать микроизменения параметров водных растворов клетки, а также воды, когда анализируемый объект подвергается действию внешнего электромагнитного слабого поля, равно как и препарата при очень низкой концентрации. В выводах излагается проект двух возможных направлений исследований, один из которых зависит только от нас самих и от “испытаний воды” в контексте экспериментов с “памятью воды”. Наряду с экспериментами СЭИ по проводимости и импедансной спектроскопии, в работе с использованием APEC 300 предлагаются очень высокочувствительные измерения важного параметра – потенциала; своего рода измерения, менее сложные и более надежные в отношении помех устройства/объекта, который, кроме того, может легко располагать частотным спектром в сверхнизкочастотной области. В этом направлении исследований впервые, насколько нам известно, некоторые измерения потенциала с его частотным спектром были проведены для воды без и со стимуляцией (последняя представлена действием магнитного поля). Этот первый комплекс измерений APEC 300 представляет особый интерес для диапазона 0–0,5 Гц.

Ключевые слова: вода в клетке; динамические изменения воды; биологические и клинические последствия; электрические параметры кожи; электроакупунктура Фолля; APEC 300 и высокочувствительное измерение; низкие концентрации препаратов; потенциальные измерения и частотный спектр; память воды.

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Review

CURRENT SAFETY DATA OF THE COMPLEX HERBAL MEDICINE WITH SEDATIVE AND CARDIOPROTECTIVE ACTIONS

N. Gorchakova[1], E. Heimuller[2], A. Galkin[3,4]*

[1]Alexander Bogomolets National Medical University, Kyiv, Ukraine
[2]Dr. Gustav Klein GmbH & Co. KG, Germany
[3]Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine
[4]UA PRO-PHARMA LLC, Kyiv, Ukraine

*Corresponding author: alexfbt@gmail.com

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Background. The risk management plan is the one of main documents of pharmacovigilance system – a document focuses on the planning of pharmacovigilance activities and minimization of risks. As the use of herbal medicines has increased, so too have the reports of suspected toxicity and adverse events. As many herbal products on the market have not been thoroughly tested for their pharmacology and toxicology, pharmacovigilance has paramount importance in detecting unwanted reactions. One of the herbal preparations widely used to support the cardiovascular system under stress, neurocirculatory dystonia, cardiac neurosis, in the complex treatment of arterial hypertension, angina pectoris, arrhythmia is a complex medicinal product Carvelis, oral drops, solution. The active pharmaceutical ingredients of the drug are: extract from a mixture of hawthorn leaves, flowers and fruit (Crataegi folii cum flore, fructus extractum); herb extract of canine nettle (Leonuri herbae extractum); melissae herbia extract Melissae herbae extractum); valerian root extract (Valerianae radix extractum).
Objective. Critical analysis of current scientific data on the benefit/risk ratio of a complex herbal medicine with sedative and cardioprotective effects.
Results. Pathophysiological and epidemiological characteristic of following nosological forms have been analyzed: stress, neurocirculatory dystonia, cardiac neurosis, arterial hypertension, angina pectoris, arrhythmia. Current data on phytopreparation safety and estimation benefit/risk ratio have been generalized. Available clinical data were analyzed for individual phytocomponents or its combinations.
Conclusions. The prevalence, seriousness and severity of the course of all manifestations of neurocirculatory dystonia, stress, nervous excitement, heart neurosis, especially with disproportionate approaches to the treatment of these diseases, is a serious medical and social problem. Given the proven efficacy of the use of Carvelis preparation components, and also that the risks are predictable, and their occurrence can be countered, and if they occur it can be effectively suppress with other drugs, the benefit of using the phytopreparation prevails above the risks.

Keywords: pharmacovigilance; risk management plan; hawthorn; motherwort; valerian; melissa.

Introduction

Pharmacovigilance is the pharmacological science, the part of modern health care aimed at ensuring the safety of patients in the use of drugs, reducing morbidity and mortality. Marketing authorization holders are obliged to create, provide and guarantee the functioning of their own pharmacovigilance system. And it is a prerequisite for presence of medicines in the market. Now the main vector of pharmacovigilance in developed countries is the transition from reactive (case detection – and response to proactive) to proactive (planning and prevention of cases) management. There are three main documents of pharmacovigilance system, and they are focused on organization quality management, as well as on certain preparation pharmacovigilance data. The first of them is risk management plan – a document containing a detailed description of the risk management system and focuses on the planning of pharmacovigilance activities and minimization of risks. The second one is periodic safety update report (PSUR) – a document that focuses on analyzing available cumulative and interval information on the safety and efficacy of the certain medicinal product and assessing the benefit/risk ratio. And the third important document is addendum to clinical overview – a document that contains a critical analysis of the current benefit/risk profile of the certain medicinal product conducted on the basis of PSUR data, as well as on safety and efficacy data collected from the time of the first registration or last re-registration of medicinal product. Proactive

management allows you to manage the risks associated with drugs, both among the population, and in the work of pharmaceutical companies and regulators [1–4].

As the use of herbal medicines has increased, so too have the reports of suspected toxicity and adverse events. Such unwanted reactions can be due to (i) side effects (usually detectable by pharmacodynamics and often predictable); (ii) reactions occurring as a result of overdose, overduration, tolerance, dependence-addiction (detectable either by pharmacodynamics or pharmacovigilance), (iii) hypersensitivity, allergic and idiosyncratic reactions (detectable by pharmacovigilance), (iv) mid-term and long-term toxic effects including liver, renal, cardiac and neurotoxicity also genotoxicity and teratogenicity (detectable by *in vitro* and *in vivo* toxicological studies or by pharmacovigilance). As many herbal products on the market have not been thoroughly tested for their pharmacology and toxicology, pharmacovigilance has paramount importance in detecting unwanted reactions [5].

One of the herbal preparations widely used to support the cardiovascular system under stress (stress), neurocirculatory dystonia, cardiac neurosis, in the complex treatment of arterial hypertension, angina pectoris, arrhythmia is a complex medicinal product Carvelis, oral drops, solution (Dr. Gustav Klein GmbH & Co. KG, Germany). The active pharmaceutical ingredients of the drug are: extract from a mixture of hawthorn leaves, flowers and fruit (Crataegi folii cum flore, fructus extractum); herb extract of canine nettle (Leonuri herbae extractum); melissae herbia extract Melissae herbae extractum); valerian root extract (Valerianae radix extractum).

The purpose of this article is a critical analysis of current scientific data on the benefit/risk ratio of a complex herbal medicine with sedative and cardioprotective effects.

Pathophysiological and epidemiological characteristic of nosological forms for the treatment of which a phytopreparation is prescribed

Support for the cardiovascular system at nervous tension (stress). Stress is a nonspecific reaction of the body in response to the strong action of the stimulus from the outside, which exceeds the norm, as well as the corresponding reaction of the nervous system. There are the following types of stress: informational, emotional, physiological and post-traumatic. The activity of the sympathetic nervous system during the period of stress is characterized by an increase in the allocation of "stress" hormones, an increase in the frequency of cardiac contractions, blood pressure. In this case, there are organic changes in the heart, possibly arrhythmias and ischemia. Conditions are also developed for the development of thrombosis, the appearance or progression of atherosclerosis, coronary heart disease. Such a complex of physiological and pathomorphological changes leads to the development and progression of most cardiovascular diseases. There is a direct relationship between the level of stress, daily average blood pressure and heart rate [6].

The tendency to stress of different severity has any individual regardless of socio-economic status. In the United States up to 90% of the adult population is periodically under stress, 60% of them are exposed to stress factors 1-2 times a week, and 30% – every day. At the same time, in 2/3 of cases, an appeal to the doctor is due to diseases and conditions, the basis of development of which lies the effect of stress factors. The annual economic cost of treatment and rehabilitation of stress-affected people in the United States is about $ 300 billion [7].

Data for adult World Trade Center Health Registry enrollees residing in New York State on enrollment and no cardiovascular disease history (n = 46,346) were linked to a New York State hospital discharge-reporting system.

The increased risk of hospitalization among patients with cerebrovascular or cardiovascular disease was observed in women (corrective risk factor 1.32, 95% confidence interval 1.01 to 1.71) but not men (risk factor correction factor [AHR] 1.16, 95% confidence interval [CI] from 0.97 to 1.40). All patients were characterized by post-traumatic stress disorder. Among those who were evacuated from the World Trade Center or were engaged in restoration, a high level of hospitalization with cardiovascular diseases in men was, at the same time, for women, such data were unclear. Such data primarily relate to the diagnosis of "ischemic heart disease". It should be noted that stress has led to an increase in hospitalizations with cerebrovascular disorders in men, but not in women [8].

The results of the Japan Collaborative Cohort Study for the Assessment of Cancer Risk for the perception of stress were interesting. In this study, more than 70,000 patients aged 40 to 79 years participated. The exclusion criteria were: a stroke, an ischemic heart disease, or cancer in anamnesis. Women who were under severe stress had twice the

risk of death from stroke and coronary artery disease and 1.5 times higher than the risk of any cardiovascular disease. For men, this dependence was not so pronounced, but chronic stress in men often led to myocardial infarction [9].

Another authors published the results of a 20-year survival study of patients with CHD who were under constant influence of emotional stress [10]. Researchers have shown that in a group of patients who are under the influence of persistently pronounced stress, after 3 years, the survival rate is significantly lower than in the groups of patients with no stress or low intensity stress. At the same time, after 10 and 20 years of observation, this difference was more pronounced.

It should be noted that for the treatment of stress, the following drugs are used: antidepressants, neuroleptics, nootropics, tranquilizers, vitamin therapy (vitamins B and C); aromatherapy, sports, and yoga are also used [11–13].

Neurocirculatory dystonia (NCD) is a symptom of a neurogenic nature that occurs due to the influence of a number of etiological provocative factors, which is manifested in the appearance of instability of the pulse and indicators of blood pressure, cardialgia, respiratory disturbances, autonomic disorders, violations of the tone of muscle fibers and the vascular wall, and also low tolerance to the usual physical activity and the impact of stressful situations. NCD is the result of an autonomic nervous system (ANS) imbalance, so the form of NCD depends on the predominance of a certain part of the ANS (sympathetic or parasympathetic) [14].

NCD cannot be called a complete illness. Most often it is associated with a symptom, the main expression of which is a violation of the cardiovascular, nervous and respiratory systems. It is most often diagnosed in young people, who eventually experience various cardiovascular disorders. The fact that neurocirculatory dystonia is more often diagnosed in young people is due to the fact that in the younger generation there is a discrepancy between physical development and the slow formation of the neuroendocrine system. With regard to the causes of the disease, they include: intoxication, acute and chronic infections, fatigue, regular sleep deprivation, malnutrition, physical overload, frequent changes in sexual partners and stress. In addition, in some patients neurocirculatory dystonia is manifested under the influence of poor heredity. It is now taken to distinguish three types of neurocirculatory dystonia: cardiac, hypertonic and antihypertensive. If reveals are too intense and leads to constant irritability or sleep disturbances, following preparations are recommended: phenobarbital, valerian preparations, oxazepam and some other tranquilizers. Medicinal treatment may include: valerian and motherwort based preparations; tranquilizers (tofisopam); antidepressants (amitriptyline); nootropic drugs (piracetam); cerebral angiocorrectors (vinpocetine) [15].

Cardiac neurosis is a functional disorder of the cardiovascular and nervous systems. The pathogenesis is the weakening of the regulatory activity of the cerebral cortex and the associated violation of the functions of the subcortical centers of the autonomic nervous system. Often, the causes of cardiac neurosis are stress, mental health and physical strain. Stress provokes a sharp release of adrenaline, which damages the heart and the judges. An important role in violating the autonomic regulation of the cardiovascular system is the use of coffee, alcohol and smoking. The risk group of this disease includes people of all ages [16–18].

The most common symptom is pain in the sternum and in the area of the heart (with irradiation in the left arm and shoulder blade). Even with insignificant agitation, heart rhythm abnormalities (arrhythmia), a feeling of "heart fading", heartbeat attacks (tachycardia), or severe pulsation, which is felt throughout the body. Manifestation of the following symptoms also is possible: feeling of lack of air or breathlessness, raising the body temperature up to subfebrile level, labile arterial pressure, dyspeptic disorders in the form of vomiting, nausea associated with eating, wheezing, abdominal pain. General weakness, fatigue, even with insignificant physical activity, headaches, signs of asthenia, insomnia or drowsiness, sudden tides of heat or cold are also possible. There are redness of the face and chest, increased sweating, arms and legs begin to dull, paresthesias, limbs are cold to the touch; often there are symptoms such as feelings of sadness, a feeling of tension inside, shaking hands and feet, a feeling of fear of death, tearfulness [16–18].

In the acute period of treatment, the appointment of tranquilizers and beta-blockers is indicated. In the future, medical therapy is aimed at eliminating signs of heart neurosis, sedation and the elimination of unpleasant symptoms, and includes herbal preparations, a complex of vitamins and minerals (high concentration of magnesium and vitamins of group B). Literature data analysis shows that the herbs of choice are hawthorn, valerian, motherwort, passiflora, mentha, yarrow, pot marigold and some others [17, 18].

Hypertension (stage 1 and 2). Stress-induced arterial hypertension is an acute increase in blood pressure under the influence of psycho-emotional factors. It can occur in people with normal blood pressure and those with arterial hypertension [6]. This disease is a problem of young people living in cities. Experts have found that the probability of developing hypertension in rural areas is much lower. In older people, stress provokes heart rate abnormalities. At the same age, the risk of developing a heart attack and stroke increases.

In stress, following physiological parameters are changed: the frequency of cardiac contractions increases, respiration is accelerated, the tone of smooth muscles is changed (including the gastrointestinal tract), diameter of the pupils is changed, increases blood pressure and glucose concentration in the blood. All this leads to the fact that the body becomes a state of preparedness for survival in an extreme situation. But when it happens every day, the mechanisms that are responsible for the functioning of the cardiovascular system in a state of stress come to fruition, as well as pathological changes occur (in any organs associated with blood circulation regulation). All this can lead to a chronological process of increasing blood pressure [19, 20]. The constant effect of stress factors observed during the earthquake in the south of Italy led to a steady increase in blood pressure – an average of 20% increased systolic and 46% diastolic arterial pressure [21].

Angina and arrhythmia. Angina pectoris is a kind of coronary heart disease, the main symptom of which is pain syndrome. Angina pectoris is common among mature and elderly people. Both males and females are equally prone to the risk of angina, but in different age periods the incidence is different. Angina occurs due to the development of atherosclerosis in the cardiac arteries. Sudden angina attacks can be caused by excessive excitement, walking, physical activity, smoking, using a large dose of alcohol, staying in a stuffy, dusty, smoky room or in high frost. Usually, pain occurs in the pre-dawn time. Various meteorological, geomagnetic factors, high humidity, atmospheric pressure fluctuations can also provoke the appearance of pain. There are also remote risk factors that accelerate and aggravate the development of angina: diabetes mellitus, obesity, hypodynamia, frequent mental stress, stress, alcoholism, abuse of salty and greasy foods, refined carbohydrates. Symptoms are over after the use of nitroglycerin [22, 23].

Among medical plants containing antiatherosclerotic substances are corn (female inflorescence, with young silk), hawthorn flowers and fruits, rose hips, mountain arnica flowers, Jacob's-ladder roots, elderberry leaves, black chokeberry fruits, common nettle, garlic, beet, and carrots. Herbal infusions will not relieve pain; they have a prophylactic effect, so they should be used for a long time, usually in combination with other preparations [24, 25].

Jitters. Excitement is a biological process that consists of nerve impulses and activates one or another body or element. The process of excitation occurs in all organs that consist of the nervous and muscular tissue, and in the glands. A specific sign of muscle arousal is its reduction. In the nerve cells during the excitation, nerve impulses are generated, the glandular cells secrete the secretion. A specific property of excitation is the ability to pass through nerve fibers, which provides a physiological link between all systems and elements of the organism, their functional unity. The best way to suppress anxiety and nervous excitement is to use special medical herbs [6].

Current data on phytopreparation safety and estimation benefit/risk ratio

The problem of autonomic dysfunction today is one of the main problems of modern medicine. Symptoms of neurocirculatory disorders combined with different manifestations of vegetative imbalance are noted in 21–60% of all patients, including 25–30% of patients of therapists and family doctors, and in 30–50% of cardiac patients [26].

At present, it is generally accepted that neurocirculatory dystonia is an integral part of the clinical picture of neurosis, which, in turn, is the most common cause of neurocyclecular dystonia. Now many forms of neurosis, accompanied by manifestations of NCD, are considered within the framework of a wider range of pathologies – psychosomatic disorders [27].

The pathogenesis of psychosomatic diseases is complex and multifaceted. It involves important in terms of providing the optimal level of neurohumoral regulation of the structure of the central nervous system (CNS), as the hypothalamic-pituitary and limbic systems, regulatory centers of the cerebral cortex, adrenal glands, etc. However, it should be emphasized that the main role in the development of psychosomatic pathology is played by the situation of chronic stress. It is chronic stress that is one of the most important factors in the "launching" of a multi-chain mechanism for the development of psychosomatic diseases with a

predominant lesion, in turn, of the nervous or cardiovascular, digestive or other systems [28].

The most common variant of the clinical course of NCD is cardiovascular syndrome. The greatest clinical significance is NCD, which occurs with the phenomena of hypersympathicotonia, constant or crisis increase in blood pressure and severe tachycardia. The danger of this form of NCD is the increased risk of developing such forms of pathology as coronary artery disease, hypertension, arrhythmias, cerebral circulation disorder, etc. Therefore, adequate pharmacotherapy of this variant of NDC is especially relevant [29].

Correct treatment of autonomic dysfunction is possible with the administration of traditional somatic therapy (antihypertensive preparations, coronary antics, beta-blockers, proton pump inhibitors, etc.) together with neuropharmacological preparations that influence the dynamics of the clinically leading syndromes linked with the central mechanisms of dysregulation.

Psychoemotional imbalance is one of the most common manifestations of vegetative vascular dystonia; it sharply impairs patients' quality of life and ultimately contributes to an increase in the pathogenetic "vicious circle" of stress-induced CNS disorders.

Normalization not only will become a psychoemotional sphere at vegetative vascular dystonia, but also a correction of actually vegetative disorders can be achieved at the expense of appropriate therapeutic means of sedative and vegetative stabilizing action, among which the leading place is occupied with phytopreparations.

Especially popular in recent years have been combined phytopreparations that contribute to the normalization of the central regulatory mechanisms for the development of psychosomatic pathology, which stabilize the cortical subcortical relationship, the violations of which are the basis of vegetative vascular dystonia and psycho-emotional imbalance. The main advantages of herbal preparations include: the possibility of using proven standard effective combinations of biologically active substances in one dosage form; reduction of forced polypragmasia while preserving or increasing the effectiveness of treatment; improvement of compliance (for the patient and the doctor); increasing the economic availability of treatment [30–32].

The phytopreparation drug Carvelis (Dr. Gustav Klein GmbH & Co. KG, Germany) in the form of drops for oral use contains all mentioned components (based on 10 ml of solution: an extract of a mixture of hawthorn leaves, flowers and fruits – 7.125 ml; extract of motherwort herbs – 1.0 ml; melissa leaves extract – 1.0 ml; valerian root extract – 0.5 ml). The drug is indicated for the treatment of various psyche-related disorders: NCD, cardio neurosis, arterial hypertension (1–2 stage), angina (1–2 stage), stressed arrhythmias, and also as a pharmacoprophylaxis for relieving excitement, stress, and emotional lability in chronic stress.

Hawthorn. This plant contains large number of vitamins and other biologically active substances. In berries there are a number of vitamins A and K, as well as C and E, B group vitamins, saponins and flavonoids, starch and fructose, organic acids, essential oils and pectin, choline and sorbitol. Some substances in the hawthorn are extremely rarely found in other edible fruits and plants. This makes it unique in its kind. For example, ursolic acid, has an antimicrobial effect and promotes vasodilatation [33, 34].

Hawthorn preparations has a light diuretic effect, has antitumor activity and positively affects cardiac stimulation. Acids in the fruit have a positive effect on the skin, have a rejuvenating effect. Due to the use of berries, active regeneration of cells is restored. The beneficial properties of the hawthorn fruit are manifested in the cardiovascular system. Substances from the fruit expand coronary vessels, soften the work of the arteries of the brain, which increases the saturation of the body's blood oxygen. As a result, the number of dizziness and headaches decreases, the sleep is stabilized and the degree of fatigue of the body decreases during the day. It is proved such effects as hypotensive, coronarolytic, antiarrhythmic, cardioprotective. Its uniqueness is precisely in the presence of regulatory, stabilizing action in a variety of cardio neurotic manifestations (tachycardia, cardiac severity, cardialgia, lability of arterial pressure). However, the hawthorn is an effective cardiomaterial. It is characterized by pronounced antihypoxic and antioxidant effects [33–35].

Substances from hawthorn berries strengthen the blood vessels, reduces the level of harmful cholesterol, improves blood coagulation. As a result, the risk of developing atherosclerotic plaques decreases. At the recommended dosage, the contraindications are minimal. First of all, this is an individual intolerance. Caution should be taken during pregnancy and breastfeeding. Gladiolus can cause an increase in the tone of the uterus. Restrictions also apply to patients in the post-stroke period and people with severe kidney disease. Consultation with a doctor, in these cases, is necessary [36, 37].

Available data from 687 individual patients treated with quantified hawthorn extract or placebo in ten studies were pooled [38]. The study analyzed the effect of treatment on the physiological parameters of patients and the symptoms of the disease, as well as the relationship with the severity of the symptoms and sex. Reduced severity of typical symptoms (reduced tolerance to physical activity, exertional dyspnea, weakness, fatigue and palpitation) were associated with more active treatment and in patients with more severe symptoms. At the same time, there was a weak correlation between improvements in MWL, PRHP and symptoms. Accordingly, the effect of treatment of hawthorn extract on physiological results and typical symptoms was modulated according to severity. Taking into account the basic differences, the benefits were comparable in male and female patients with physical inferiority during early chronic heart failure.

A total of 14 clinical trials was screened in the Cochrane database. All studies were randomized, double-blind, and placebo-controlled using hawthorn leaves and flowers. In ten clinical trials, 855 patients with chronic heart failure (Classes I–III according to the Classification of the New York Heart Association). The results were recorded as a weighted mean difference (WMD) with a 95% confidence interval (CI). For the physiological outcome, the maximum burden of using the hawthorn extract was more significant than placebo (WMD (watts) 5.35, 95% CI 0,71 to 10.00, $P < 0.02$, $n = 380$. Exercise tolerance was significantly higher in patients receiving hawthorn extract (WMD (W/min) 122.76, 95% CI 32.74 to 212.78, $n = 98$). The pressure-heart rate indicator, as an index of oxygen consumption in the heart, also showed a positive decrease in the treatment of hawthorn extract (WMD (mm Hg)/min) –19.22, 95% CI –30.46 to –7.98, $n = 264$). Symptoms such as dyspnea and fatigue were significantly improved in the treatment of the gullet compared to placebo (WMD –5.47, 95 % CI –8.68 to –2.26, $n = 239$). No mortality and cardiac disease data were reported except for one study in which death was reported (three of them active, one in control) without providing additional information. Side effects reported were rare, mild and transient; They included nausea, dizziness, heart and gastrointestinal complaints [39].

In clinical trials conducted in EU countries, which involved 1780 patients, acute toxicity was not observed. In the European market between 1992 and 2011, about 810 million doses of dry extract of hawthorn leaves and flowers were sold [40].

Motherwort. The plant contains such useful substances as alkaloids, glycosides, polysaccharides, saponins, tannins, essential oils, vitamins. The mechanism of action of the vestibula is determined by its activating effect on the gamma-aminobutyric acid (GABA) receptors, as well as the normalization of the neurotransmitter balance – an increase in the activity of the inhibitory GABA-ergic and the weakening of the activating catecholaminergic systems of the brain due to its modulating effect on the release, reuptake and receptor binding in said systems [41].

In addition to the central sedative, mild anti-anxiety and hypnotic action, motherwort preparations have valuable in the conditions of vegetative-vascular dystonia, antispasmodic, antihypertensive and anti-anginal effects, increases cardiac output and slows down the heart rate [42].

Thus, in official medicine, motherwort is prescribed in cases of neurosis (its various forms), hysteria, myocarditis, cardiosclerosis, cardiovascular insufficiency I–II degree. In traditional medicine it is used at various nervous upsets, frightened, hypertension; it also helps to improve sleep and general well-being. There are no particularly serious contraindications, but it is not recommended to use it for hypotension. With prolonged use, it can cause an allergic reaction up to complete intolerance, but it is very rare [43, 44].

Motherwort is well known and widely used in Chinese medicine. Reviews [45, 46] give an overview of the chemical composition with an emphasis on biological activity and ethnopharmology. The authors describe the mechanisms that can be the basis of the protective effects in the cardiopulmonary bypass, for example by inhibiting intracellular active forms of oxygen (ROS) and various cellular mechanisms in preventing apoptosis. In addition, the authors say that the peculiarities of modulation of intracellular homeostasis Ca^{2+} from the side of the thistle can explain its cardiovascular protective effects.

Motherwort, an herbal substance, has been used in the EU for more than 30 years, it has been present in official pharmacopeias for more than 50 years, and continuous use in the form of herbal tea has been recorded for almost 400 years. For example, in 2008, in Lithuania, 98 thousand packets were packed with 25 ml tincture. Over 350,000 packages were sold from 2003 to March 2009 [47].

Valerian. This plant contains in its composition more than 120 chemical components, among which the most important in the clinical plan are essential oils, valipotriates, amino acids, salts of

organic acids, phenolic compounds, alkaloids, etc. [48]. The main effect of valerian is the sedation of the central nervous system. It also helps to restore emotional balance, cope with headaches, reduce feelings of anxiety and improve working capacity. Positive effect is valerian and cardiovascular system. It is prescribed for an increased heartbeat, with angina pectoris and pain in the heart. Substances contained in the root of the plant, have vasodilator effect.

In Germany, in the 1980–1990's, about 300 clinical trials of combined herbal preparations were conducted, including 10 valerian-based preparations. All valerian-containing preparations according to the criteria of evidence-based medicine were clinically effective in various forms of therapeutic and neurological pathology, especially psychosomatic diseases of the cardiological and gastroenterological profile [49].

Valerian is harmless; however, at high doses and in excess of the course of treatment, unpleasant side effects may occur in the form of reduced performance, drowsiness, lethargy, and also disorders of the function of the gastrointestinal tract. If the remedies made from this plant take longer than two months or substantially exceed recommended doses, gastrointestinal malfunction, depression, drowsiness, nausea and headache may occur. Valerian is contraindicated for people with intolerance to plant components and those suffering from enterocolitis [50, 51].

An open, prospective cohort study was conducted in 89 centers in Germany. Valerian was compared with homeopathic preparation Neurexan in 409 people (aged 18 to 75 years), suffering from insomnia. Duration of sleep and insomnia were evaluated based on daily records of patients for 14 days. Sleep quality was estimated at 28 $\pm$ 1 day. An analysis of the efficacy of therapy after 14 days of treatment showed improved sleep durations compared to baseline in both therapies. Side effects reported: headache, dizziness, paradoxical stimulation, anxiety and cardiac abnormalities [52].

A prospective, unaddressed, non-interventional, observational study was conducted in 49 nationwide centers in Germany on April 3, 2006 (included in the first patient) until December 27, 2006 (the last patient completed the study), which included 807 patients with the following symptoms: anxiety/nervousness, hyperactivity; sleep disturbance; significant phases of insomnia; night anxiety; difficulty concentrating/forgetting; fatigue; absurdity; gloom; gastrointestinal disorders; or headache/pressure using Neurexan versus various valerian extracts. There was an improvement in symptoms in both treatment groups. Compliance with the treatment was high: doctors reported "excellent" or "good" compliance for 492 (86.2%) patients taking the drug and 178 (79.5%) patients in the valerian group. In the group where patients used valerian extracts no side effects were reported [53].

The purpose of another study was to determine whether valerian-lemon balm could contribute to sleep improvement in 100 women during menopause. The study used clinical random testing compared with the control group to study the effect of an herbal supplement containing valerian-lemon balm on sleep in menopausal women aged 50–60 years. The sample method consisted of two steps: 1) a non-randomized sample of centers; 2) randomized treatment appointment in each center. The study showed that valerian-lemon balms can improve the duration and quality of sleep in women with sleep disturbances during menopause. In addition, no adverse effects were reported in the treatment process [54].

There is a high degree of use of valerian root drugs in the EU member states: according to IMS Health, the sales of valerian root in the EU in 2002 exceeded 50 million units. Almost 50% were sold in Germany. The following side effects were noted which may be related to the consumption of the valerian root: symptoms on the side of the gastrointestinal tract, such as nausea, colic in the abdomen (frequency unknown). In the EU, the traditional use of herbal substance valerian and its preparations is considered to be a positive risk group due to minimal side effects [55].

Melissa. The plant contains a large number of organic acids, saponins, flavonoids, resins, tannins, tannins, essential oils. It is possible to continue the list with such trace elements as copper, manganese, iron, potassium, selenium, zinc, magnesium, calcium, and also vitamins of groups B and C.

Amber acid, which is a part of Melissa, is one of the strongest natural antioxidants, effectively protecting cells of the brain and peripheral organs from the effects of free radicals, which are inevitable companions of chronic stress. Melissa also has the properties of a psychoemotional stabilizer and a cognitive activator (improving mood and cognitive functions, possibly through a modulating effect on the cholinergic and monoaminergic systems of the brain), and spasmolytic action [56].

Prolonged use of medication with Melissa stimulates the medication reduces arterial pressure, relieves nervous tension, which manifests itself by night shake. Melissa is a diuretic that helps to

normalize heart rhythm dysfunction, as it can quickly and effectively reduce heart rate, which increases the life span of an organism. In addition to enhancing the cardiac function, Melissa is able to control the metabolic processes of the body, enhance the functions of the liver, brain, eliminate heat, and remove bile from the body [57].

There are no strict contraindications for taking medication with Melissa. But there are some points in which its use is not recommended by doctors. These include hypotension, a work in which an increased concentration of attention is required, motor activity, rapid psychological reaction (for example, the work of the motor vehicle driver) [57, 58].

Melissa is a traditional herbal remedy for use in the indicated indications, solely on the basis of long-term use. Traditional use of Melissa is following: traditional herbal preparation for the relief of mild symptoms of mental stress and insomnia; traditional medicinal herbal preparation for the symptomatic treatment of minor complaints of gastrointestinal manifestations, including abdominal distension and flatulence [57, 58].

Complex action of phytopreparation characteristics and risk assessment. Optimization of the pharmacotherapy of vegetative-vascular dystonia and, in particular, neuro-circulatory dystonia can be achieved through the use of combined preparations containing all the above-mentioned components (hawthorn, motherwort, melissa, valerian). This seems to be all the more justified that these medical herbs are characterized by a high degree of safety. Their application in therapeutic doses, both in mono-form and in various combinations, demonstrated a minimal risk of side effects coming nearer to placebo. In essence, all the side effects of these agents are associated either with individual intolerance, or with a substantial excess of therapeutic doses and are characterized by increased sedation, drowsiness and weakness (for valerian, motherwort, and melissa) or minor dyspeptic disorders, hypotension, bradycardia (for hawthorn). Based on the dose dependence of all the main effects of these plants, the elimination of these side effects is easily achieved by reducing the dose of the preparation.

Phytopreparation can be prescribed to young people as an independent therapy for functional disorders (autonomic dysfunction syndrome), chronic fatigue syndrome, elderly patients with age-related cardiovascular changes, and decreased exercise tolerance when active therapy (antihypertensive, symptomatic treatment of CHD) is not yet shown. Phytopreparation increases the efficiency of complex therapy of arterial hypertension, coronary artery disease, heart failure as a symptomatic remedy due to the content of hawthorn. Along with regular use, it is possible to receive the preparation "on demand" in people who are prone to excessive vegetative reactions to social stimuli, with significant physical activity, accompanied by palpitations and shortness of breath, as well as with meteosensitivity [59].

Comprehensive, combined multimodal neurotropic, cardiopulmonary and vasotropic effect of the preparation – sedative, antihypertensive, antiarrhythmic, coronarolytic, cardioprotective, vegetative stabilizing. It is such an effect that is necessary for the successful treatment of various manifestations of vegetative dystonia both from the central nervous system and from the cardiovascular system, it is this combination of ingredients that allows you to carry out not just symptomatic, but pathogenetically substantiated therapy with maximum correction of disorders of the mechanisms of central regulation of autonomic functions and psycho-emotional balance. At the same time phytopreparation, in contrast to phenobarbital-containing drugs, does not cause addiction and rejection syndrome, does not show excessive sedation and sedation, can support in the process of treatment, a full level of social activity.

Despite the fact that the preparation consists of individual herbal medicines, it should be evaluated as the only active drug (the Table).

Table: Basic safety issues

Nature of the risk factors	Unfavorable (side) effects
Important identified risks	Reactions of hypersensitivity. Depression and other disorders accompanied by inhibition of the central nervous system. Pronounced arterial hypotension. Bradycardia
Important potential risks	Severe liver dysfunction
Lack of information	The use of preparation during pregnancy and breastfeeding. Application of the drug to children

Medicinal herbs that are part of the phytopreparation have long-standing traditions of use as sedative or auxiliary substances for sleep. Publications over the last 20 years, several hundred scientific studies with a participation of 5200 participants, have confirmed the positive effects that de-

teriorate the benefits of using valerian in sleep disorders [60]. Reports of the European Medicines Agency for *Leonurus cardiaca* L., herba, *Crataegus folii*, fructus spp., and *Melissa officinalis* L. recognize the traditional use of herbal medicinal products for nerve stress, nerve heart complaints and sleep disturbances [40, 47, 55, 58].

Diseases that arise in connection with the deterioration of the psycho-emotional background (the effect on the body of various stress factors, causing mood disorders and depression), dramatically reduce efficiency and cause suffering, both the patient and his relatives. Numerous studies show that they are like cardiovascular diseases becoming one of the most common pathologies. Thus, about 19 million people in the United States suffer from depression, which is approximately 10% of the total population. According to WHO, depressive disorders in 2000 accounted for 40% of all mental illnesses registered in the world. In practically all developed countries, health authorities are concerned about the current situation and are working to develop different ways to implement it. The effectiveness of modern antidepressants does not exceed 70%, and about one third of people suffering from depression, are resistant to the used drugs, which prompts intensive search for new antidepressants. Admission of psychotropic drugs not only improves the quality of life, but also prevents the development of somatic pathologies. In connection with this, a very promising direction is the use of medicinal plants as sources of combined medications. Side effects in phytotherapy occur much less often and the effect of drugs is much softer, which contributes to their use in the initial stages of the disease, and especially for prevention. Among medicines for the correction of the psycho-emotional state a special place is taken by sedative drugs that are given without a prescription, often they are used without consulting a physician [61, 62].

Conclusions

The prevalence, seriousness and severity of the course of all manifestations of neurocirculatory dystonia, stress, nervous excitement, heart neurosis, especially with disproportionate approaches to the treatment of these diseases, is a serious medical and social problem.

Given the proven efficacy of the use of Carvelis preparation components, and also that the risks are predictable, and their occurrence can be countered (if not prescribed to a group of patients who have contraindications), and if they occur it can be effectively suppress with other drugs, the benefit of using the phytopreparation prevails above the risks.

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Н.О. Горчакова, Е. Хеймуллер, О.Ю. Галкін

СУЧАСНІ ДАНІ ПРО БЕЗПЕЧНІСТЬ КОМПЛЕКСНОГО РОСЛИННОГО ПРЕПАРАТУ ІЗ СЕДАТИВНИМИ І КАРДІОПРОТЕКТОРНИМИ ВЛАСТИВОСТЯМИ

Проблематика. План управління ризиками є одним із основних документів системи фармакологічного нагляду – документ присвячений плануванню діяльності з фармаконагляду та мінімізації ризиків. Збільшення використання рослинних лікарських засобів приводить до розширення знань щодо токсичного впливу лікарських рослин та побічних ефектів. У цьому випадку фармаконагляд має надзвичайно важливе значення для виявлення й аналізу небажаних реакцій. Одним із рослинних препаратів, що широко застосовуються для підтримки серцево-судинної системи під впливом стресу, при нейроциркуляторній дистонії, неврозі серця, у комплексному лікуванні артеріальної гіпертензії, стенокардії, аритмії, є комплексний лікарський засіб Карвеліс, краплі

оральні, розчин. Активними фармацевтичними інгредієнтами препарату є екстракти із суміші листя, квітів і плодів глоду, трави собачої кропиви, листя меліси, кореня валеріани.
Мета. Критичний аналіз сучасних наукових даних про співвідношення користі та ризику комплексного фітопрепарату із седативними та кардіопротекторними властивостями.
Результати. Проаналізовано патофізіологічну та епідеміологічну характеристику таких нозологічних форм: стрес, нейроциркуляторна дистонія, невроз серця, артеріальна гіпертензія, стенокардія, аритмія. Узагальнено поточні дані щодо безпеки фітопрепарату та співвідношення користі та ризику. Доступні клінічні дані було проаналізовано для окремих фітокомпонентів або їх комбінацій.
Висновки. Поширеність, серйозність і тяжкість перебігу всіх проявів нейроциркуляторної дистонії, стресу, нервового збудження, серцевого неврозу, особливо з непропорційними підходами до лікування цих захворювань, є серйозною медико-соціальною проблемою. З огляду на підтверджену ефективність використання компонентів препарату Карвеліс, а також на те, що ризики є передбачуваними, їх виникнення може бути ефективно подолане, перевага використання фітопрепарату переважає над ризиками.

Ключові слова: фармакологічний нагляд; план управління ризиками; глід; собача кропива; валеріана; меліса.

Н.А. Горчакова, Э. Хеймуллер, А.Ю. Галкин

ТЕКУЩИЕ ДАННЫЕ ПО БЕЗОПАСНОСТИ КОМПЛЕКСНОГО РАСТИТЕЛЬНОГО ПРЕПАРАТА С СЕДАТИВНЫМИ И КАРДИОПРОТЕКТОРНЫМИ СВОЙСТВАМИ

Проблематика. План управления рисками является одним из основных документов системы фармакологического надзора – документ посвящен планированию деятельности по фармаконадзору и минимизации рисков. Увеличение использования растительных лекарственных средств приводит к расширению знаний о токсическом действии лекарственных растений и побочных эффектах. В данном случае фармаконадзор играет чрезвычайно важное значение для выявления и анализа нежелательных реакций. Одним из растительных препаратов, широко применяющихся для поддержания сердечно-сосудистой системы при стрессе, нейроциркуляторной дистонии, неврозе сердца, в комплексном лечении артериальной гипертензии, стенокардии, аритмии является комплексный препарат Карвелис, капли оральные, раствор. Действующими компонентами препарата являются экстракты из смеси листьев, цветов и плодов боярышника, травы пустырника, листьев мелиссы, корня валерианы.
Цель. Критический анализ современных научных данных о соотношении пользы и риска комплексного фитопрепарата с седативными и кардиопротекторными свойствами.
Результаты. Проанализирована патофизиологическая и эпидемиологическая характеристика следующих нозологических форм: стресс, нейроциркуляторная дистония, невроз сердца, артериальная гипертензия, стенокардия, аритмия. Обобщенны текущие данные о безопасности фитопрепарата и соотношении пользы и риска. Доступные клинические данные были проанализированы для отдельных фитокомпонентов или их комбинаций.
Выводы. Распространенность, серьезность и тяжесть течения всех проявлений нейроциркуляторной дистонии, стресса, нервного возбуждения, сердечного невроза, особенно с непропорциональными подходами к лечению этих заболеваний, являются серьезной медико-социальной проблемой. С учетом подтвержденной эффективности использования компонентов препарата Карвелис, а также того, что риски являются предсказуемыми, их возникновение может быть эффективно преодолено, преимущество использования фитопрепарата преобладает над рисками.

Ключевые слова: фармакологический надзор; план управления рисками; боярышник; пустырник; валериана; мелисса.

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Original article

TECHNOLOGICAL SOLUTION OF BIOGAS OUTPUT INCREASING AT GRAIN DISTILLERY SPENT WASH FERMENTATION

N.B. Golub, M.V. Potapova*

Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine

*Corresponding author: maryana.potapova2@gmail.com

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Background. Distillery spent wash is the main waste of the alcohol industry. This is highly concentrated wastewater, which is characterized by high chemical oxygen demand (COD) values – up to 60–120 g O_2/dm^3 – and low pH values – up to 3.7. It makes its processing a complicated task. One of the methods of its utilization is anaerobic fermentation with the production of biogas. Inert carriers for microorganism immobilization or granulation are used to intensify the process of anaerobic treatment and increase sludge concentration. Due to the high concentration of anaerobic microorganisms in granules, compared to the number of microorganisms in free-floating active sludge, the process of methanogenesis is more intensive with a high yield of biogas and a decrease in COD.
Objective. To determine the possible centres of anaerobic sludge granulation in highly concentrated waste of alcohol production to increase biogas output in the process of waste treatment.
Methods. Activated carbon, modified carbon on which surface calcium ions were precipitated, $FeCl_3$, silica gel based sorbent, talc based sorbent were used to form pellets as the centres of microorganism accumulation.
Results. Pellets have not been formed with the use of activated carbon; modified carbon with calcium ions; silica gel based sorbent and ferrum compounds. Pellets of activated sludge were received with the use of talc based sorbent – formation of initial biofilm on a carrier was observed on the third day of fermentation and then it was growing in pellets.
Conclusions. It was demonstrated that for highly concentrated waste effluents of a distillery (grain distillery spent wash), which after co-fermentation with poultry manure contain volatile fatty acids in concentration 800–2000 mg/dm^3, it is needed to use sorbents to receive microorganism pellets as a centre of granulation. Sorbents must contain donors and proton acceptors. It was established that the use of granular activated sludge for the distillery waste purification improves the efficiency of COD and Biochemical Oxygen Demand removal by 15–17%, increases biogas output by 26 ± 2% and biogas methane content by 8 ± 1%.

Keywords: granulation; biogas; distillery spent wash; fermentation; methane.

Introduction

Grain distillery spent wash (GDSW) as a highly concentrated wastewater is characterized by the high values of COD – up to 60–120 g of O_2/dm^3 and low pH values – up to 3.7. It influences a choice of methods and technological solutions of waste treatment. One of the methods of waste utilization used is an anaerobic fermentation with receiving of biogas [1]. Based on the fact that the hydrogen value influences on methane production by its producers and the optimal pH value for the methanogenesis process makes up 6.8–8.0 [2], the technology assumes a minor content of spent wash (2–3%) in the fermenter. It has an effect on the process cost effectiveness and a target product output [3].

The proposed two-stage technology [4] allows for adjusting the pH value in the first reactor due to introduction of poultry manure with the concurrent obtaining of biogas and its output increase. The process is characterized by the balance of nutrition components of microbial association and rational correlation C:N:P. Biogas is also produced from dissolved high- and low-molecular compounds during the subsequent anaerobic stage of the advanced waste treatment. But the process rate and the waste purification level depend, first of all, on the concentration of free-floating microorganisms, which varies in reactors of different type from 2 to 4 g/dm^3 [2].

Inert carriers for microorganism immobilization or granulation are used to increase sludge concentration. This results in the increase of process rate for substance destruction, the decrease of hydraulic period for waste retention in the reactor and its volume that lowers operating costs with no loss in quality of waste purification [5], [6].

The high load on the granular sludge is achieved through the improvement of sedimentation properties. It guarantees its presence in the reactor zone and makes impossible to wash out sludge with the upward water flow. The high specific activity of methanogenic bacteria (2 kg of COD/kg of dry organic substance per day, upon the load of 50 kg COD/m^3) is also conditioned by interspecific transfer of hydrogen between the pelleted colonies of acetogenic bacteria and hydrogenotrophic methanogenic archaea and their protection with pellet matrix from negative impacts resulting in the raised destruction rate of organic substances and their transformation into a desired product – biogas [6], [7].

It was shown [8] that the granulation rate and the characteristics of granular sludge depend on microorganism species composition and their ratio in pellets, substrate specificity, hydrophobicity, surface charge, and capability for extracellular polymers synthesis.

Under real-life conditions, the granulation process might be disturbed due to different factors which result in a sludge washout from the reactor, pellet destruction, reduced biogas output and efficiency of wastewater treatment. Therefore, the study of granulation process in a course of anaerobic treatment of highly concentrated wastewater with methane production is a relevant task.

The work objective is to determine the possible centres of anaerobic sludge granulation in highly concentrated waste of alcohol production to increase biogas output in the process of waste treatment.

The following tasks need to be solved to meet the objective:

– to study formation of pellets of anaerobic sludge in distillery waste using the granulation centres of different nature;

– to establish an impact of granular activated sludge on efficacy of methane production and wastewater treatment.

Materials and methods

Nowadays, there is no any accepted model or theory of pellets formation. Table 1 demonstrates the models which explain the current theories of pellets formation from microorganisms [6]–[10].

As is seen from Table 1, all the models based on physical granulation theory explain the formation of granular sludge by the effect of physical factors which are intrinsic to the biomass fermentation process with the biogas production and run in the reactor: liquid and gas flow rate, ageing of sludge and its excessive volume removal from the reactor, quantity and qualitative composition of suspended solids in initial sludge, etc.

The models, which describe granulation from the view point of physiological peculiarities of microorganism species and strains (e.g., *Methanobacterium* AZ strain, *Methanothrix* bacteria) as well as their interaction, are attributed to microbiological [6].

Within the frames of thermodynamic theory, there are four granulation stages defined: transport of microorganism cells of activated sludge to the surface of inert material or other cells; initial reversible adsorption of substrate by means of physical and chemical interaction; irreversible attachment of cells to substrate with microbial appendages or exopolymers; cell propagation and pellet development. The microorganism granulation mechanisms are also explained proceeding from thermodynamic conditions of the grain growing processes influenced by many factors such as: hydrophobicity and electrophoretic mobility, proton translocation on the surface of bacterial membranes which stimulate its activation.

The use of granular sludge is expected at Stage 2 of GDSW treatment [4]. That's why liquid fraction formed after sedimentation and release of solids, fermented (within 20 days) mixture of GDSW, poultry manure and industrial wastes of a distillery at the ratio of 1:1.7:0.06, relatively, on a dry organic substance (DOS), served as a standardized test solution [4]. The phase separation was carried out using centrifugation (centrifuge CLK-1) for 15 min at the velocity of 3000 rpm. Characteristic water values at entry to reactor 2 are given in Table 2.

The following has been used for granulation in capacity of the centres of microorganism accumulation:

– activated carbon, grade 207EA "Ecofilter" (Kyiv, Ukraine), with surface area 950–1100 m^2/g and bulk density 0.48–0.52 g/cm^3, grain size – 4.75–2.00 mm;

– modified carbon to which surface calcium ions were precipitated. Precipitation was carried out by means of keeping activated carbon in calcium chloride solution with concentration 0.05%;

– $FeCl_3$ which is often used for granulation in methane tanks during the treatment of waste effluents of breweries, yeast plants, sugar beets, and potatoes processing plants. Since hydrolysed $FeCl_3$ is produced in the form of oxo- and hydroxy-compounds, their aggregation serves as a primer for granulation;

Table 1: Theories and models of the anaerobic sludge granulation process [6]–[10]

Group	Model	Model idea
Physical and chemical theories	Model of inert cores	Anaerobic bacteria, which result in formation of initial biofilms, in particular, embryonic granules, are attached to inert micro particles by means of surfaces structures
	Selection pressure theory	A washout of dispersed sludge and a selection of heavy sludge take place depending on the rates of hydraulic load and biogas production
	The growth of colonized suspended particles	Pellets are formed by means of growing of small fraction biofilm (microorganism colonization) that was produced due to friction, or flown into a reactor with waste water
Microbiologic/ ecologic theories	Consolidation of micro-floccules of microorganisms of *Methanothrix* genus	*Methanothrix* filament serves as a matrix for cocci and coli. First, micro-floccules are formed and then they are consolidated in pellets due to surface properties and morphology of *Methanothrix* bacteria
	Capetown's model	Granulation takes place by means of *Methanobacterium* AZ, which under the conditions of high partial pressure of H_2, unlimited source of nitrogen in the form of ammonium, limited quantity of cysteine and in neutral media, releases extracellular polypeptide that binds *Methanobacterium* AZ and other bacteria in pellets
	Spaghetti model	Pellets are formed by microorganisms of *Methanothrix* genus. In the beginning, they resemble the spaghetti swirls formed by the *Methanothrix* long filaments
	Model of many layers	*Methanothrix* grouping is the centres of granulation. The layer-by-layer growth of pellets is performed with a spatial separation of various trophic groups of microorganisms: H_2-producing acetogenic bacteria and H_2-consuming bacteria
	Syntrophic micro colony model	Micro colonies, by means of syntrophic relations, may lead to the creation of consortiums and subsequent formation of pellets, because consortiums are characterized by stable conditions, co-evolution, and protection against the effects of microorganism media participating in the given consortium
	Model of three types of pellets which split volatile fatty acids (VFA)	*Methanothrix, Methanosarcina* bacteria participate in the granulation process and they form 3 types of pellets which split VFA: 1 – compact spherical pellets comprising mainly of rod-like bacteria which resemble *Methanothrix soehngenii* in short chains or separate cells; 2 – spherical pellets comprising mainly of poorly twisted filamentous bacteria which are attached to inert particles. Predominating bacteria resemble *Methanothrix soehngenii*; 3 – compact spherical pellets comprising mainly of bacteria of *Metaphosarcina* type
	Model of *Methanothrix* bundles	Pellets are formed from the aggregates of *Methanothrix* and other bacteria. By means of filaments, *Methanothrix* forms specific bundles separated by an external matrix. When bundle sizes get larger, the external matrix is deactivated, and the centre of pellets is formed. This centre is made of compact filaments of *Methanothrix*
	Model of anaerobic granulation of defined species	Pellet formation is based on the capability of bacterial species to produce dense aggregates in an anaerobic reactor and/or ensure surface binding for other bacteria, which are unable to form aggregates and pellets. It is believed that methanogenic microbes are a key species for granulation. In particular, the species such as *Methanothrix, Methanobrevibacter,* and *Methanosarcina*
Thermodynamic theories	Surface tension model	Bacteria receive the maximum possible free adhesion energy under the low or high liquid surface tension. Pellets formed under the low liquid surface tension values and which contain acytogenes around methanogenic association contribute to more stable characteristics of the process, because they are less sensitive to gas bubble adhesion and subsequent washout

Table continuation

	Four-stage model	4 stages of granulation: 1. Cells transfer to the surface of uncolonized inert material or other cells. 2. Initial reversible adsorption by means of physical and chemical force on substrate. 3. Irreversible adhesion of cells to substrate with the use of microbial appendages and/or polymers. 4. Cell propagation and pellet development
	Proton translocation – dehydration model	The (molecular) mechanism of sediment granulation is based on proton translocation activity on the surfaces of bacterial membranes. According to theory, the sludge granulation process takes four stages: 1. Dehydration of bacterial surfaces; 2. Formation of embryonic pellets; 3. Pellet maturation; 4. Post-maturation
	Crystallized core formation model	Granulation stages: 1. Growth and generation of different bacterial species; 2. Bacterial attachment or adhesion with the cells of suspended substances or other bacteria with the formation of matrix or the pellet core as a crystallization centre. 3. Seizure or integration of bacteria, microcolony growth and, finally, formation of a spherical pellet 1–5 mm in diameter
Other	Cellular automation model	Cellular automation model is defined as a spatial discrete-time system in which the state of automation is defined by a set of rules acting locally, but are applied across the system. This model is aimed at the reproduction of microbial structures under the conditions of limited substrate transfer. The substrate gradients created by local substrate consumption allow bacteria placed on the "top" to have more accessible substrates compared to those placed at the bottom. Therefore, the structure of microcolony or biofilm is related to the resource availability
	Cell-cell interconnection model	Some bacteria by virtue of intercellular interactions form a biofilm, which under certain conditions, initiates granulation
	Cluster model	The precise mechanism has not been defined yet, but it is known that the interconnection between substrate composition and/or concentration and kinetic properties of microorganisms plays a key role. There are several hypotheses available: 1. Physical aspects associated with the space limitation for microorganisms upon growing a biofilm. When divided, daughter cells occupy certain space and push away neighbouring cells. The clusters were received at this process modelling; 2. Under the conditions of substrate exhaustion in the deeper layers of biofilm or aggregate, microbes are divided actively into the upper areas only and form a new biomass. Therefore, the growth of biofilm or aggregate becomes unidirectional, and the colonies are grown as the "fingers" toward volume liquid; 3. Anaerobes produce signalling molecules from cell-to-cell, which may stimulate formation of pores and channels. These signalling molecules or their structural analogues may also be presented in waste effluents
	General four-stage model	1. Initiation of bacteria-bacteria contact or bacteria attachment to the cores by means of physical relocation; 2. Support to stable multicellular contacts by means of initial attracting forces; 3. Maturation of cell aggregations by virtue of microbial forces; 4. Formation of steady three-dimensional structure of microbial aggregate by hydrodynamic displacement forces

Table 2: Water parameters after GDSW and poultry co-fermentation

Indicator	Value
Chemical oxygen demand, COD, O_2 mg/dm^3	2800
Biological oxygen demand, BOD, O_2 mg/dm^3	1500
Suspended substances, SS, mg/dm^3	2300
Volatile fatty acids, VFA, mg/dm^3	1230
pH	7.58

– silica gel based sorbent, grain size – 2-3 mm (Institute of New Materials of Shandong Academy of Sciences, China)

– talc based sorbent, grain size – 2-3 mm (Institute of New Materials of Shandong Academy of Sciences, China).

Anaerobic sludge from a fermenter for receiving biogas from GDSW was used as inoculum in the Ecobiotechnology and Bioenergetics Department of the National Technical University "Igor Sikorsky Kyiv Polytechnic Institute". The GDSW used was provided by Chervonoslobodskyi Distillery, Kyiv Oblast.

The granulation process with the use of different centres of adsorption has been carried out in the laboratory shaking flask with heating at the temperature of 37 ± 2 °C, stirring rate is 60 rpm. Cone flasks with the gas outlet system for removal of biogas produced were used as reactors as well as they made impossible to penetrate the air to the reactor medium.

The degree of anaerobic sludge granulation was evaluated using the microscope XSP-139TP (Ulab TM, China) with 20× zoom and the energy-dispersive spectrometer (EDS) Oxford X-Max 50.

Wastewater treatment using free-floating and granular activated sludge has been carried out under the anaerobic conditions in the mesophilic mode at the temperature of 40 ± 2 °C in reactors with capacity of 1.5 dm^3, fill factor – 0.8. The substrate was stirred using paddled impeller sat the rate of 60 rpm. Each reactor was connected to the wet type gas receiver for biogas collection.

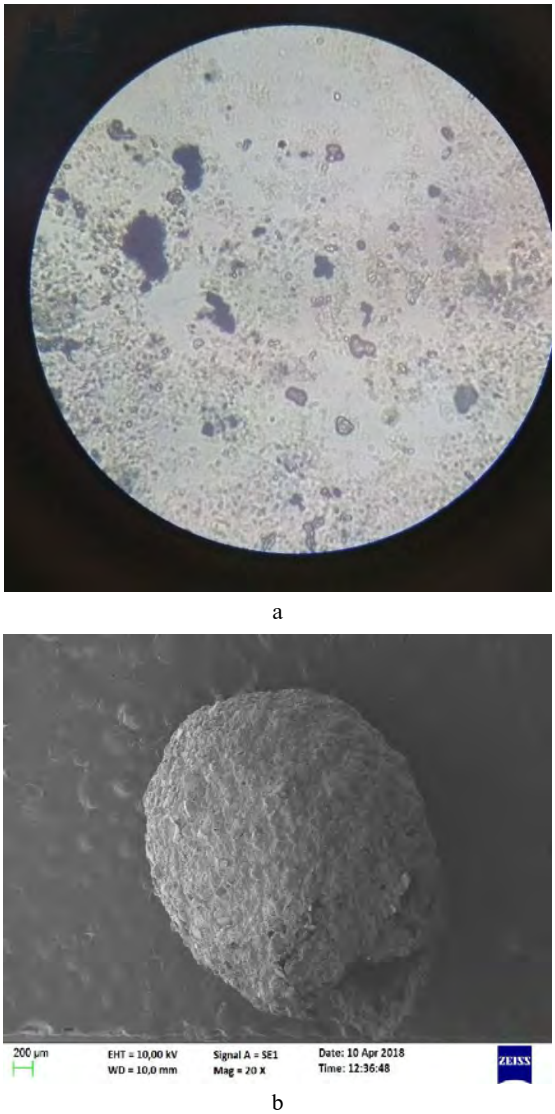
The values of BOD, COD, and SS were calculated using the standard method [11], medium pH was measured using the ion meter I–160 MI (RF) [12].

Biogas composition was determined with the gas chromatograph LHM-8-MD (RF) [13] using the standard method, VFA liquid concentration was established with the liquid chromatograph HPLC Shimadzu (Kyoto, Japan) using the standard method [14].

Results

Pellets have not been formed with the use of activated carbon, silica gel based sorbent and ferrum compounds that can be explained by specifics of GDSW composition and the microorganism association properties.

By using talc based sorbent as a granulation centre, the formation of initial biofilm on a carrier has been observed on the third day of fermentation and then it was grown in an activated sludge pellet. The obtained pellets are given in Fig. 1.



a

b

Figure 1: Anaerobic sludge before granulation (a), pellets formed on talc based sorbent (b)

Fig. 2 demonstrates the comparative characteristic of biogas output and methane content in it with the use of granular and free-floating anaerobic sludge in water treatment at Stage 2 of the GDSW

processing. The ratio of inoculate and substrate on a DOS basis is 1:5, relatively.

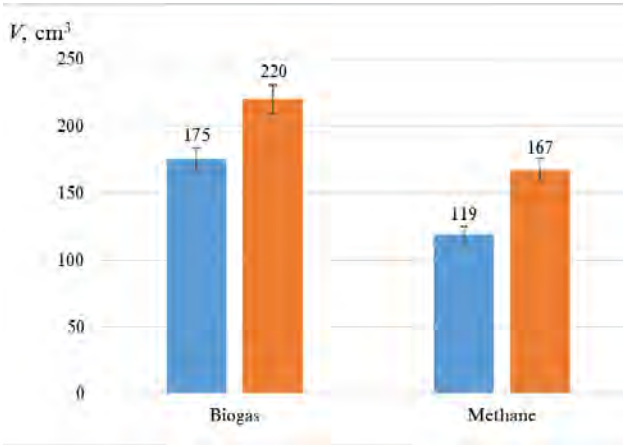


Figure 2: Biogas and methane output (V) per day with the use of free-floating (blue colour) and granular (orange colour) anaerobic sludge

The water treatment parameters at Stage 2 of distillery waste disposal per day with the initial COD content – 2800 mg/dm^3 are given in Table 3.

Table 3: Water purification degree at Stage 2 of GDSW processing

Indicator	Indicator value with the use of free-floating anaerobic sludge	Indicator value with the use of granular sludge
COD, mg/dm^3/removal efficiency, %	780 ± 46.87/ 72 ± 1.5	310 ± 43.39/ 89 ± 1.77
BOD, mg/dm^3/removal efficiency, %	400 ± 36.06/ 73 ± 2.12	180 ± 17.39/ 88 ± 1.5
SS, mg/dm^3/removal efficiency, %	600 ± 44.91/ 74 ± 2.12	200 ± 29.75/ 91 ± 1.4

Discussion

The choice of granulation centres is based on heterogeneity of their surfaces on which aggregation of suspended solids and microorganisms may have place.

Not all microorganism species interact with the inert surface of activated carbon [15], [16]. The availability of high concentration of volatile fatty acids in water also hinders the aggregation of substances and microorganisms, because they prevent neutralization of particle charges and, correspondingly, their aggregation as on carbon surface, so against each other. The presence of acids in solution explains the absence of granulation on other above mentioned sorbents. Silica gel pellets were destroyed in 7 days of being in wastewater, which confirms the studied medium aggressivity.

The aggregation of hydroxyl compounds between themselves and the formation of adsorption centres do not take place with the use of ferrum compounds and the availability of acids. Hydroxyl compounds are transformed into soluble state by reaction:

$$Fe(OH)_2^+ + CH_3COOH \rightarrow$$
$$\rightarrow 2\ CH_3COO^- + Fe^{3+} + 2H^+.$$

The carbon activation with calcium ions for the creation of activated carbon interaction centres with microorganisms did not demonstrate positive results. Perhaps, this is associated with the availability of competition between negatively charged surface of microorganisms and the anions of volatile fatty acids for the attachment centres, which reduces or makes impossible the microorganism interaction with activated carbon.

So far as the surface of talc based sorbent particle, except the extended surface (of pores), contains the oxygen and magnesium atoms, this allows for sedimentation of charged and uncharged particles. The pellets are grown due to the formation of layers which from the beginning contain nutrients in suspended particles, which precipitate on the pellet surface, and various microorganism species that are capable as to destruct particles, so to produce methane. Moreover, in the process of granulation, other species, which are not adsorbed on sorbent, are attached to the pellets due to the formation of polymeric polysaccharidic or polypeptide matrix that can be produced by *Methanobacterium* and other species [16]. As described in literature sources [6], [9], [10], [17], methanogenic bacteria that form the bedded structures, *Methanothrix soehngenii (Methanosaeta concilii), Methanosarcina spp., Methanobrevibacter*, play a special role in the formation and functioning of anaerobic sludge pellets.

The sorbent due to the availability of oxygen atoms on the surface and the bacterial cells with a negative charge of the surface leads to the formation of hydration shell. The change of a pellet surface charge and its partial dehydration take place by means of proton transfer to the membrane surface upon substrate oxidation with acetogenic bacteria. The absence of hydration shell results in hydrophobic interaction between the cells and pellet compaction. The metabolite transport between the cells facilitates this process also [18]–[20].

A spherical pellet shape is produced under the effect of hydraulic force of rising biogas bubbles. The separation of microorganism species in a pellet is determined by the velocity of nutrients transport between them.

Therefore, for wastewater with concentration of volatile fatty acids within 800−2000 mg/dm^3, it is possible to use the granulation centres of sorbents, which contain proton donors and acceptors, to form the anaerobic sludge pellets.

As is seen from Fig. 2, biogas output is higher by 26 ± 2%, if granular anaerobic sludge is used. At the same time, biogas methane content is increased by 8 ± 1%. It means the granulation of anaerobic sludge provides the possibility to increase both biogas output and biogas methane content.

As is seen from Table 3, the wastewater treatment process, with the use of granular activated sludge, is characterized by a higher removal efficiency of polluting substances. Recovery rate of organic substances is increased, and removal degree reaches 89%, which is by 17% higher compared to the use of free-floating sludge.

Conclusions

It was demonstrated that for highly concentrated waste effluents of the distillery (GDSW), which after co-fermentation with poultry manure contain volatile fatty acids in concentration 800-2000 mg/dm^3, it is necessary to use sorbents containing both proton donors and acceptors to obtain microorganism pellets in capacity of granulation centres. Talc can serve as a basic substance for such sorbents.

It was established that the use of granular activated sludge for the distillery wastewater treatment increases the removal efficiency of COD and BOD by 15−17%, increases biogas output by 26 ± 2% and biogas methane content − by 8 ± 1%.

Further study of the abiotic factors influence (temperature, pH of the medium, availability of nutrients) on the formed active sludge granules in the conditions of our technological process.

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Н.Б. Голуб, М.В. Потапова

ТЕХНОЛОГІЧНЕ РІШЕННЯ ПІДВИЩЕННЯ ВИХОДУ БІОГАЗУ ПРИ ФЕРМЕНТАЦІЇ ЗЕРНОВОЇ ПІСЛЯСПИРТОВОЇ БАРДИ

Проблематика. Основним відходом спиртової промисловості є післяспиртова барда. Вона є висококонцентрованою стічною водою та характеризується високими значеннями хімічного споживання кисню (ХСК) – до 60–120 г O_2/дм^3 – і низькими значеннями pH – до 3,7, що робить її переробку непростим завданням. Одним із використовуваних методів її утилізації є анаеробне бродіння з одержанням біогазу. Для інтенсифікації процесу анаеробного очищення сьогодні використовують методи підвищення концентрації мулу, а саме – інертні носії для іммобілізації мікроорганізмів або грануляцію. За рахунок вищої концентрації анаеробних мікроорганізмів у гранулах, порівняно з кількістю мікроорганізмів у вільноплаваючому активному мулі, процес метаногенезу відбувається більш інтенсивно з вищим виходом біогазу та зниженням ХСК.
Мета. Визначення можливих центрів грануляції анаеробного мулу у висококонцентрованих стоках спиртового виробництва для підвищення продукування біогазу в процесі їх очищення.
Методика реалізації. Для формування гранул як центри акумуляції мікроорганізмів використовували: активоване вугілля, модифіковане вугілля, на поверхню якого осаджено іони кальцію, $FeCl_3$, сорбент на основі силікагелю, сорбент на основі тальку.
Результати. За використання активованого вугілля; модифікованого вугілля з йонами кальцію; сорбенту на основі силікагелю та сполук феруму не відбувалось утворення гранул. Гранули активного мулу були одержані за використання сорбенту на основі тальку – на третю добу ферментації спостерігали утворення початкової біоплівки на носії, яка потім розросталась у гранули.
Висновки. Показано, що для висококонцентрованих стічних вод спиртзаводу (післяспиртова барда), які після коферментації з пташиним послідом містять леткі жирні кислоти у концентраціях 800–2000 мг/дм^3, для отримання гранул мікроорганізмів як центри грануляції необхідно використовувати сорбенти, що містять у своєму складі як донори, так і акцептори протонів. Встановлено, що використання гранульованого активного мулу для очищення стоків спиртзаводу збільшує ефективність видалення хімічного та біохімічного споживання кисню на 15–17 %, підвищує вихід біогазу на 26 ± 2 %, вміст метану в біогазі – на 8 ± 1 %.

Ключові слова: грануляція; біогаз; післяспиртова барда; ферментація; метан.

Н.Б. Голуб, М.В. Потапова

ТЕХНОЛОГИЧЕСКОЕ РЕШЕНИЕ ПОВЫШЕНИЯ ВЫХОДА БИОГАЗА ПРИ ФЕРМЕНТАЦИИ ЗЕРНОВОЙ ПОСЛЕСПИРТОВОЙ БАРДЫ

Проблематика. Основным отходом спиртовой промышленности является послеспиртовая барда. Это высококонцентрированная сточная вода, которая характеризуется высокими значениями химического потребления кислорода (ХПК) – до 60–120 г O_2/дм^3 – и низкими значениями pH – до 3,7, что делает ее переработку непростой задачей. Одним из методов ее утилизации является анаэробное брожение с получением биогаза. Для интенсификации процесса анаэробной очистки сегодня используют методы повышения концентрации ила, а именно – инертные носители для иммобилизации микроорганизмов или грануляцию. За счет высокой концентрации анаэробных микроорганизмов в гранулах, по сравнению с количеством микроорганизмов в свободноплавающем активном иле, процесс метаногенеза проходит более интенсивно с высоким выходом биогаза и снижением ХПК.
Цель. Определение возможных центров грануляции анаэробного ила в высококонцентрированных стоках спиртового производства для повышения выработки биогаза в процессе их очистки.
Методика реализации. Для формирования гранул как центры аккумуляции микроорганизмов использовали: активированный уголь; модифицированный уголь, на поверхность которого осаждены ионы кальция; $FeCl_3$; сорбент на основе силикагеля; сорбент на основе талька.
Результаты. При использовании активированного угля; модифицированного угля с ионами кальция; сорбента на основе силикагеля и соединений железа образования гранул не наблюдалось. Гранулы активного ила были получены при использовании сорбента на основе талька – на третьи сутки ферментации наблюдали образование начальной биопленки на носителе, которая затем разрасталась в гранулы.
Выводы. Показано, что для высококонцентрированных сточных вод спиртзавода (послеспиртовая барда), которые после коферментации с птичьим пометом содержат летучие жирные кислоты в концентрациях 800–2000 мг/дм^3, для получения гранул микроорганизмов как центры грануляции необходимо использовать сорбенты, содержащие в своем составе как доноры, так и акцепторы протонов. Установлено, что использование гранулированного активного ила для очистки стоков спиртзавода увеличивает эффективность удаления химического и биохимического потребления кислорода на 15–17 %, повышает выход биогаза на 26 ± 2 %, содержание метана в биогазе – на 8 ± 1 %.

Ключевые слова: грануляция; биогаз; послеспиртовая барда; ферментация; метан.

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Original article

OPTIMAL BODY MASSES FOR DIFFERENT OLYMPIC SPORTS

I. Kudybyn[1], I. Nesteruk[1]*, S. Pereverzyev[2], A. Redaelli[3], B. Shepetyuk[4], O. Chertov[1]

[1]Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine
[2]Johann Radon Institute for Computational and Applied Mathematics, Linz, Austria
[3]Politecnico di Milano, Milan, Italy
[4]Yuriy Fedkovych Chernivtsi National University, Chernivtsi, Ukraine

*Corresponding author: inesteruk@yahoo.com

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Background. The weight of the best athletes at different distances and in different sports can be very different. It is known that rowers, as a rule, are very massive. In order to give a chance to athletes with a small mass, the rowing federation was introduced a special category with the weight restrictions. These facts are connected with the peculiarities of the aerobic and un-aerobic activities, drag and propulsion characteristics.
Objective. In this paper, we don't try to explain the body mass differences. We will only fix them for different sports: running, swimming (free style), skiing, skating, cycling, and rowing and for different Olympic distances.
Methods. A significant amount of data on the velocity and body mass of the best athletes professionals (both female and male) in Olympic sports (running, swimming, skiing, skating, cycling and rowing) was collected and analyzed. Since the weights of the best athletes only are taken into consideration, the expected results can be treated as the most suitable (optimal) body masses for different sports and distances. In order to check, how the values of body mass and average speed on the distance fit the linear dependence, the equation of the linear regression was used. The regression coefficient and two parameters of the straight line were calculated. In order to check that the velocity of skating doesn't depend on the athlete mass, the Fisher test was used.
Results. The optimal masses of athletes for different distances and sports were calculated. In general, for the short distances, the large masses of athletes are optimal and they decrease for long distances. Exception is the results for female swimming and running, where the largest masses of athletes correspond to the medium distances. For longer distances, results show that the most performing athletes are characterized by a lower body mass, except for skating. For a fixed distance, the optimal weights of female athletes are $79.5 \pm 3.1\%$ of the weight of male athletes, at the same time they develop $89.4 \pm 1.8\%$ of the speed of men.
Conclusions. The presented statistical analysis indicates that within one distance there is no significant dependence of speed on the mass (the exception is only rowing). For each distance there are their optimal masses of athletes, which are different for different sports. The revealed facts can be used in athlete selection and training. They need also further investigations with the use of the metabolic and propulsion peculiarities.

Keywords: Olympic sports; linear regression; Fisher test; athlete selection.

Introduction

The weight of the best athletes at different distances and in different sports can be very different. E.g., the body mass of 10 000 m runner champion – Kenenisa Bekele (55 kg), [1] – is only 58.5% of mass of 100 m one – Usain Bolt (94 kg) [2]. It is known that rowers, as a rule, are very massive. In order to give a chance to athletes with a small mass, the rowing federation was introduced a special category with the weight restrictions (72.5 kg for men) and (59 kg for women) [3, 4].

These facts are connected with the peculiarities of the aerobic and un-aerobic activities. The allometry and Kleiber's law [5] may be used to explain the drastic mass differences for sprinters and stayers. For rowing and swimming (where the movement is almost neutral buoyant), the substantial part of the energy released in the body is used to overcome the drag in water, while for human running the air drag can be neglected, but significant energy is used to support the weight [6].

In this paper, we don't try to explain the body mass differences. We will only fix them for different sports: running, swimming (free style), skiing, skating, cycling and rowing (individual category without athlete weight limitations [3, 4]), and for different Olympic distances. For this purpose, the statistical information about the best male and female athletes were collected and analyzed with the use of linear regression (for every distance) and the Fisher test (for skating). Since only the weights of the best athletes will be taken into consideration, the expected results can be treated as the most appropriate (optimal) body masses for different sports and distances. For example, many men compete on a distance of 100 m, but far not all run it for

less than 10 seconds. One reason might be inappropriate weight. Therefore, we specifically use the data of athletes, who showed the best time to determine the most appropriate (optimal) body weights. The optimum weight of the body for each sport and distance is a result of natural selection. We just fix it.

Materials and Methods

Information collection. The information was collected on the official sites of sports (associations): International Association of Athletics Federations [7], International Swimming Federation [8], International Ski Federation [9], International Skating Union [10], International Cycling Union [11], International Rowing Federation [12], as well as on other sports sites in 2015–2016. The best sports achievements of athletes (male and female) over the past 3–4 years have been analyzed, as well as some of the best results achieved earlier. In total, information was collected and analyzed for 6 kinds of sports: running (8 male + 8 female distances, 692 results), swimming (free style) (5m + 5f distances, 609 results), skiing (6m + 6f distances, 553 results), skating (5m + 5f distances, 680 results), cycling (4m + 4f distances, 397 results), rowing (individual, no weight limit) (1m + 1f, 616 results (including intermediate ones)).

Linear regression. In order to check, how the values of body mass m and average speed U on the distance (collected for the fixed sport and the distance) fit the linear dependence, the equation of the linear regression m on U (the optimal straight line, minimizing the sum of squared distances between collected and theoretical points) was used [13]. The regression coefficient and two parameters of the straight line were calculated with the use of known formulas [13].

Fisher test for skating. In order to check that the velocity of skating doesn't depend on the athlete mass, two groups with smaller and larger mass were selected for every Olympic skating distance and for males and females separately. These two groups have an equal number of persons. For each group, the average velocities and body masses will be calculated. Then the Between group variability and Within group variability will be calculated according to the formulas:

$$B_{gv} = \sum_{i=1}^{m} n_i \frac{(\overline{Y_i} - \overline{Y})^2}{m-1} \quad (1)$$

where B_{gv} is Between group variability, $\overline{Y_i}$ denotes the sample mean in the i-th group, n_i is the number of observations in the i-th group, $\overline{Y}$ denotes the overall mean of the data, $m = 2$ denotes the number of groups;

$$W_{gv} = \sum_{i=1}^{m} \sum_{j=1}^{n_i} \frac{(Y_{ij} - \overline{Y_i})^2}{n-m} \quad (2)$$

where W_{gv} is the Within group variability, Y_{ij} is the j-th observation in the i-th out of m groups, n is the overall sample size [14].

We will use the F-test to check the null hypothesis that says that the average speeds do not differ for light and heavy athletes. The experimental value of the Fisher function can be calculated with the use of the formula:

$$F = \frac{B_{gv}}{W_{gv}}. \quad (3)$$

The corresponding values will be compared with the critical value $F_c(k_1, k_2)$, $k_1 = m - 1 = 1$, $k_2 = n - m$ of the Fisher function at a desired significance or confidence level α.

Results

Velocity versus weight dependences for fixed distances. The collected values of body mass and velocity for every athlete are shown by small rhombuses in Figs. 1–6 for running, swimming, skiing, skating, cycling, and rowing respectively. The boundaries of rhombuses are blue for males and red for females. The corresponding average values of the body mass and velocity are shown by greater rhombuses for every distance. The corresponding linear regression lines and regression coefficients are also shown in Figs 1–6. In the case of rowing, there is only one Olympic distance – 2000 meters. For this case, the intermediate results for 500 m and 1000 m are also represented in Fig. 6.

Figs. 1–5 demonstrate that within one distance there is no significant dependence of speed on the athlete weight. Corresponding correlation coefficients may be negative and positive but are close to zero. In the case of rowing, the speed increases with the increase of the body mass; especially for female athletes (the corresponding values of the correlation coefficient exceed 0.7).

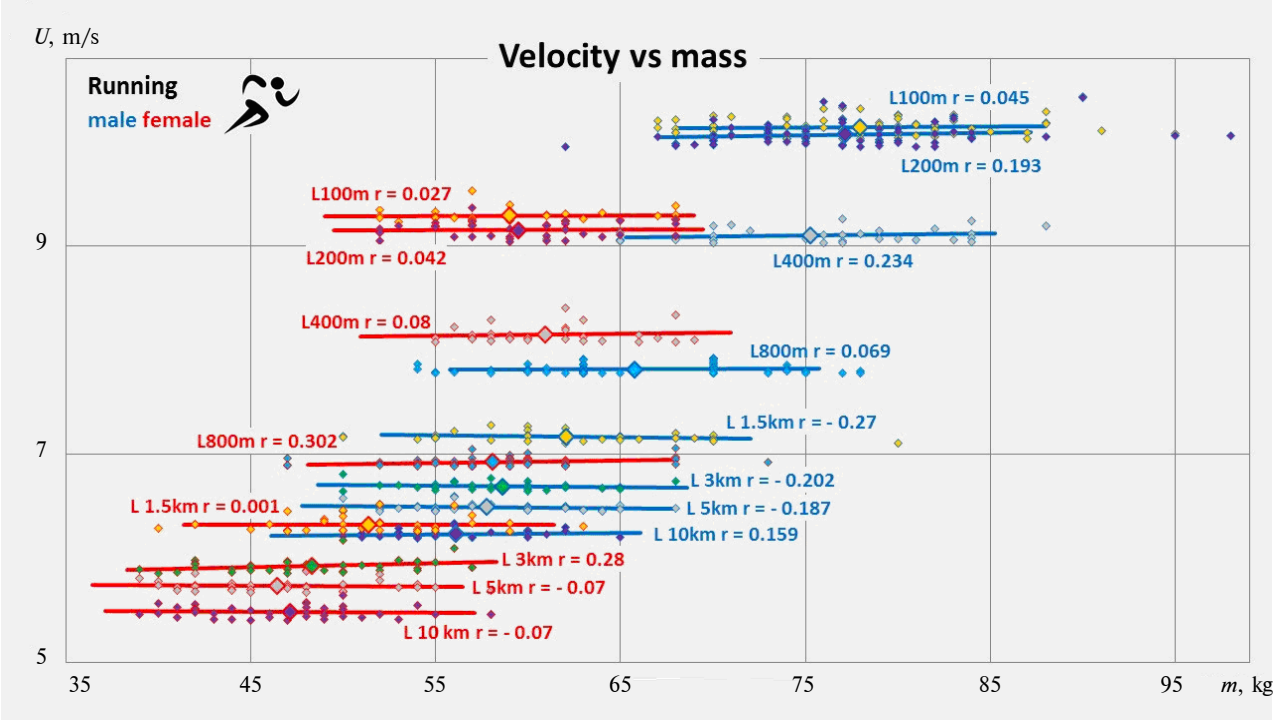

Figure 1: Running. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

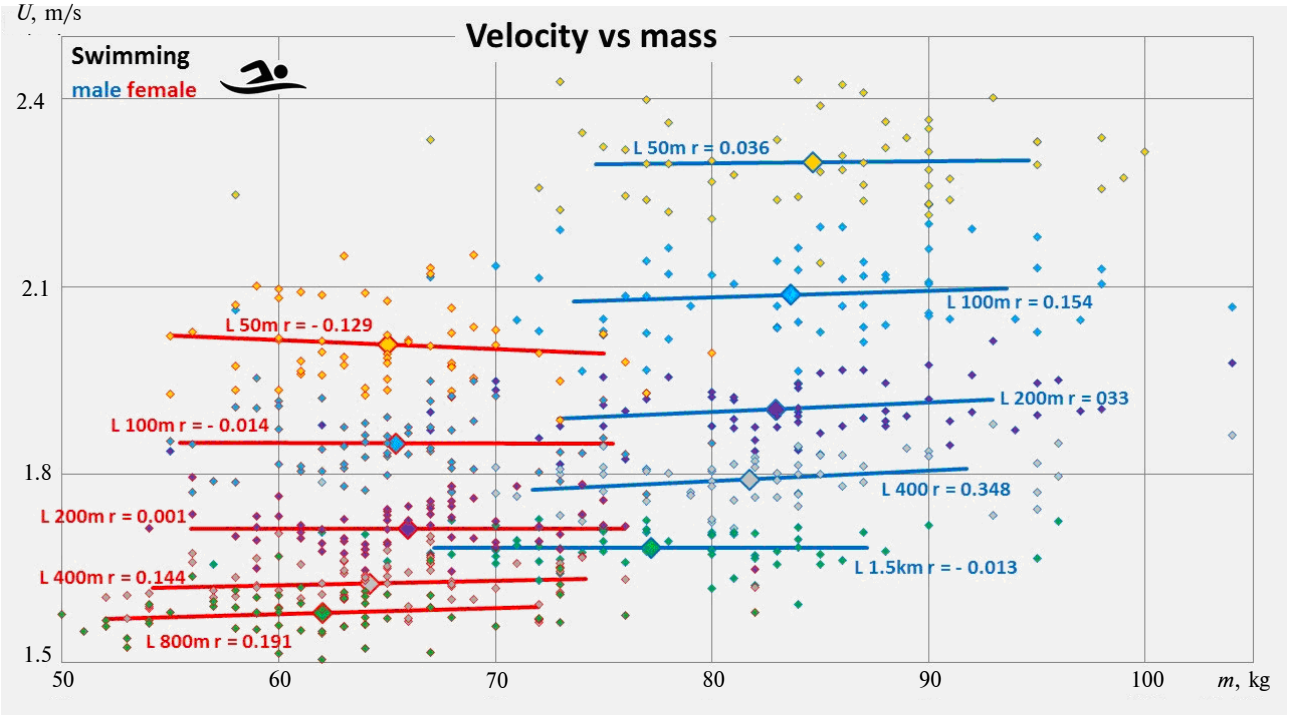

Figure 2: Swimming. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

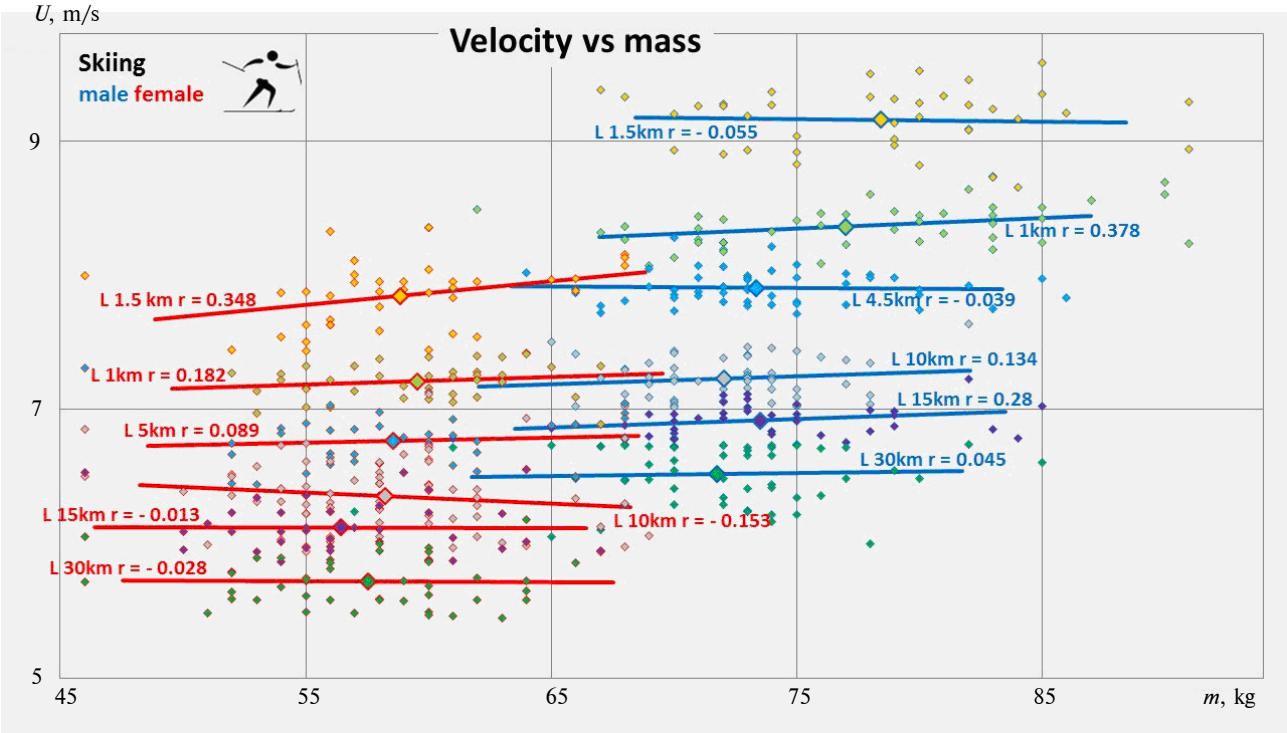

Figure 3: Skiing. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

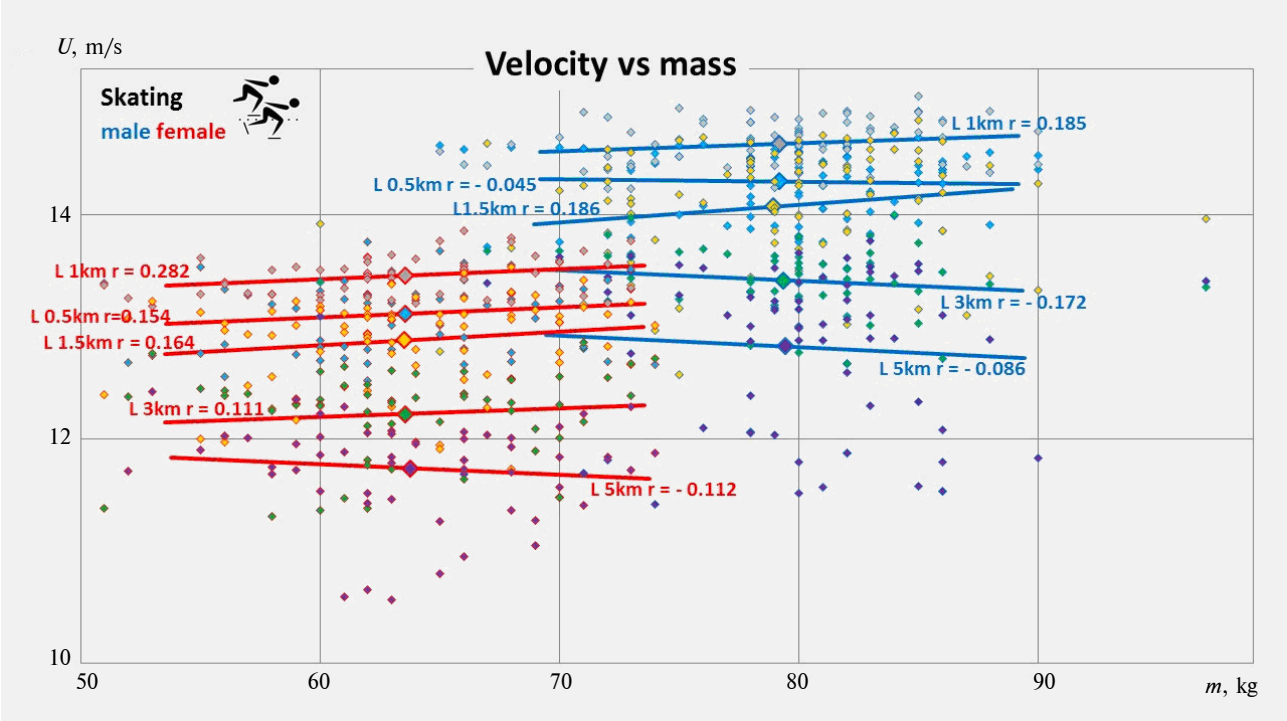

Figure 4: Skating. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

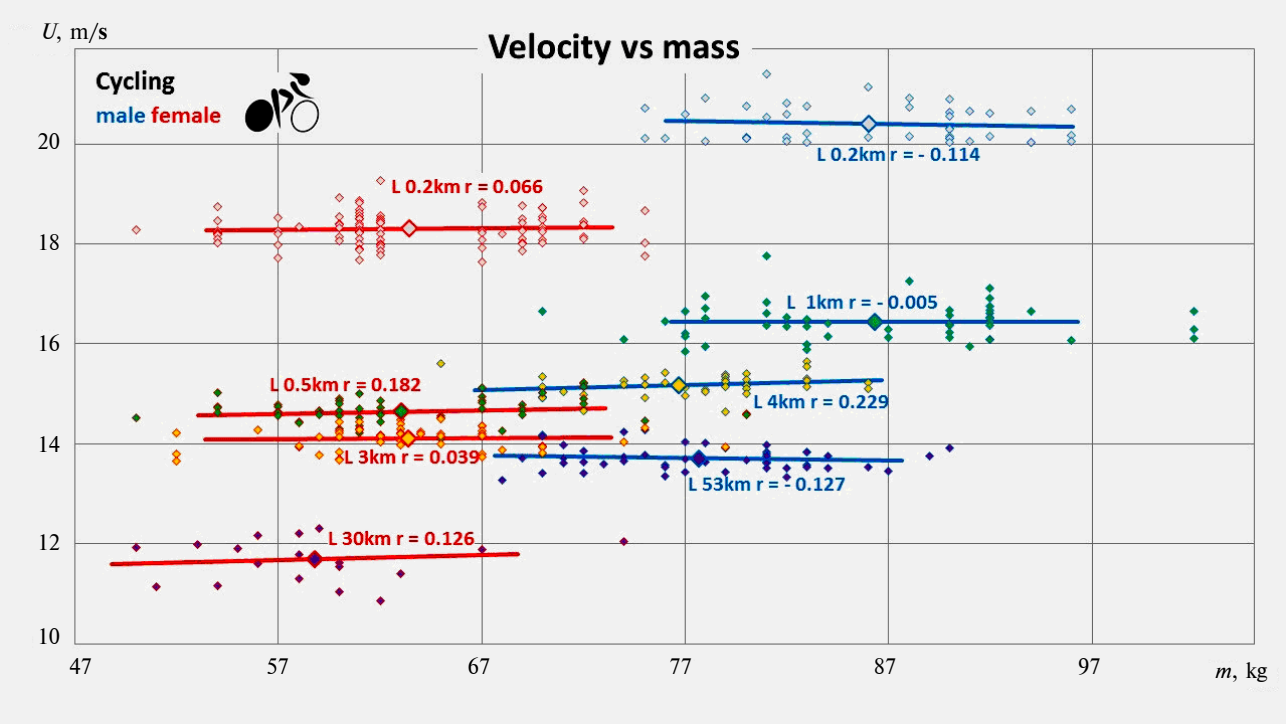

Figure 5: Cycling. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

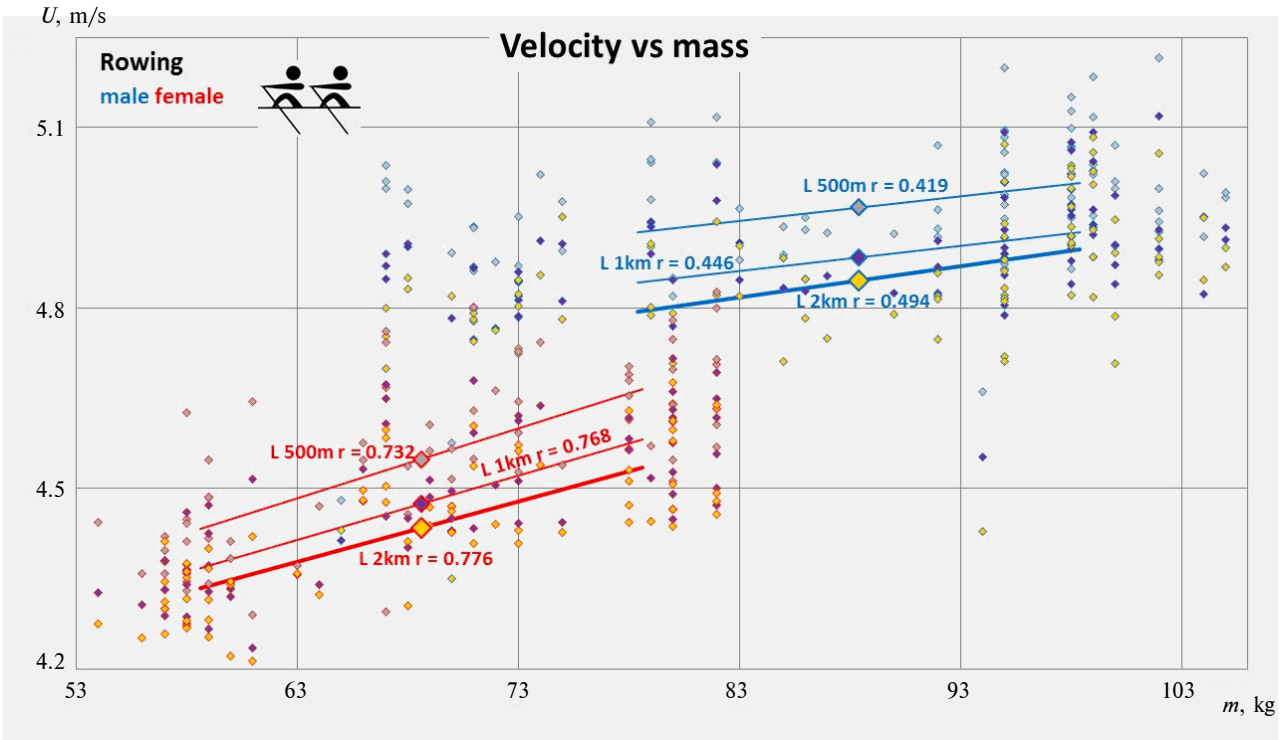

Figure 6: Rowing. The values of body mass and velocity. Small rhombuses are values for every athlete. Greater rhombuses are average values for every distance. The boundaries of rhombuses, corresponding linear regression lines and regression coefficients are blue for males and red for females

Average velocity versus average weight for different distances and sports. In spite of the weak dependence of speed on the mass at individual distances, in the case of running, swimming, cycling, and skiing, we can see an obvious reduction the average weight of the stayers in comparison with the sprinters in Figs. 1–3, 5 and in Table 1. E.g., for running the body mass of sprinters (100 m distance) is much larger than for stayers (10 km distance); the corresponding ratios are 1.25 and 1.39 for females and males respectively. For cycling the corresponding ratios are 1.08 (females) and 1.2 (males); 1.05(f) and 1.1(m) for swimming; 1.03(f) and 1.07(m) for skiing. For female athletes, the difference in body mass of sprinters and stayers is smaller in comparison with males.

For skating the difference in body mass of sprinters and stayers is practically invisible (see Figs. 4 and 7). Because the skaters are all-round (one athlete can develop high speed at different distances), this result is not surprising. To determine the dependence of velocity versus mass, a dispersion analysis for groups of light and heavy athletes for each of the Olympic skating distances will be presented in Section "Mass independence for skating".

Table 1: Average weights and speeds of athletes for different distances and sports

Sports	Distance, m	Mass, kg		Speed, m/s		% female/male at fixed distance		Duration, s	% female/male at fixed duration	
		male	female	male	female	mass	speed		mass	speed
Rowing	2000	88.39 ±12.07	68.64 ±9.04	4.85 ±0.13	4.43 ±0.12	77.7	91.5			
Cycling	200	86.05 ±6.11	63.43 ±5.84	20.40 ±0.36	18.30 ±0.32	73.7	89.7	10.93	73.8	90.1
	500		63.05 ±6.36		14.65 ±0.25			34.13	73.2	79.1
	1000	86.31 ±7.51		16.44 ±0.37						
	3000		63.38 ±5.41		14.11 ±0.23			212.66	80.1	91.1
	4000	76.66 ±4.98		15.17 ±0.22						
	29900		58.79 ±5.65		11.69 ±0.44			2557.50	76.1	82.0
	53500	77.65 ±5.60		13.71 ±0.24						
	Cycling average								**75.8**	**85.6**
Swimming	50	84.64 ±8.39	65.02 ±5.46	2.30 ±0.06	2.01 ±0.06	76.8	87.3	24.90	76.9	88.3
	100	83.63 ±8.67	65.43 ±5.43	2.09 ±0.06	1.85 ±0.05	78.2	88.6	54.05	78.3	89.5
	200	82.94 ±8.89	65.95 ±5.39	1.90 ±0.04	1.71 ±0.04	79.5	90.0	116.72	79.6	90.5
	400	81.73 ±7.80	64.19 ±6.32	1.79 ±0.04	1.63 ±0.03	78.5	90.7	245.97	78.7	90.9
	800		62.00 ±6.78		1.58 ±0.03			506.53	82.4	90.5
	1500	77.18 ±7.56		1.68 ±0.03						
	Swimming average					**78.3**	**89.2**		**79.2**	**89.9**
Skating	500	79.17 ±5.66	63.56 ±5.36	14.30 ±0.29	13.11 ±0.31	80.3	91.7	38.13	80.3	91.5
	1000	79.17 ±5.66	63.55 ±5.55	14.63 ±0.21	13.45 ±0.17	80.3	91.9	74.34	80.3	92.5
	1500	78.92 ±6.02	63.53 ±5.23	14.07 ±0.51	12.88 ±0.39	80.5	91.5	116.46	80.5	91.9
	3000	79.36 ±5.66	63.54 ±5.41	13.41 ±0.31	12.22 ±0.38	80.1	91.1	245.47	80.1	91.6
	5000	79.44 ±5.79	63.77 ±5.29	12.82 ±0.70	11.74 ±0.44	80.3	91.5	389.89	80.2	92.3
	Skating average					**80.3**	**91.6**		**80.3**	**92.0**

Table continuation

Sports	Distance, m	Mass, kg		Speed, m/s		% female/male at fixed distance		Duration, s	% female/male at fixed duration	
		male	female	male	female	mass	speed		mass	speed
Running	100	77.97 ±6.09	59.00 ±4.90	10.14 ±0.09	9.29 ±0.06	75.7	91.6	10.76	75.7	91.7
	200	77.1 ±7.90	59.49 ±4.44	10.07 ±0.10	9.15 ±0.07	77.1	90.9	21.85	77.2	91.6
	400	75.26 ±7.06	60.94 ±3.85	9.10 ±0.06	8.15 ±0.08	81.0	89.5	49.08	81.9	90.7
	800	65.75 ±6.27	58.09 ±4.70	7.82 ±0.04	6.93 ±0.04	88.4	88.6	115.50	89.0	89.5
	1500	62.06 ±6.64	51.39 ±5.07	7.17 ±0.04	6.33 ±0.07	82.8	88.2	237.12	83.3	88.9
	3000	58.61 ±4.54	48.31 ±4.86	6.69 ±0.04	5.93 ±0.06	82.4	88.6	505.98	82.6	89.0
	5000	57.77 ±4.58	46.44 ±4.88	6.49 ±0.04	5.74 ±0.05	80.4	88.4	871.45	80.7	88.8
	10000	56.10 ±3.77	47.12 ±4.64	6.24 ±0.04	5.49 ±0.05	84.0	88.0	1603.74	83.7	88.9
	Running average					**81.5**	**89.2**		**81.8**	**89.9**
Skiing	1000	76.98 ±7.27	59.54 ±4.06	8.36 ±0.15	7.21 ±0.12	77.3	86.2	138.67	76.7	82.8
	1500	78.43 ±5.88	58.84 ±4.67	9.16 ±0.23	7.85 ±0.24	75.0	85.7	191.15	75.4	86.5
	4500	73.37 ±5.07		7.91 ±0.12						
	5000		58.56 ±4.89		6.77 ±0.20			739.03	80.1	87.1
	10000	72.06 ±3.50	58.23 ±4.93	7.23 ±0.15	6.35 ±0.26	80.8	87.9	1573.92	80.4	88.8
	15000	73.53 ±5.10	56.41 ±4.44	6.92 ±0.11	6.12 ±0.21	76.7	88.5	2450.73	76.9	89.1
	30000	71.75 ±4.81	57.53 ±4.58	6.52 ±0.21	5.72 ±0.18	80.2	87.7	4601.59	79.8	89.1
	Skiing average					**78.0**	**87.2**		**78.2**	**87.2**
	Average					**79.5** ±3.13	**89.4** ±1.85		**79.4** ±3.31	**89.1** ±3.16

The average values of speed for different distances and sports are shown in Fig. 7 versus the average body mass of athletes. It can be seen that the optimal body mass is different for different sports. The heaviest athletes are successful in rowing. In sprint cycling, sprint swimming and skating, the mass of best athletes is rather smaller. The weight of skiers is usually even smaller. The somewhat chaotic nature of the dependences of the skier average speed versus average mass (see corresponding plots for male and female athletes in Fig. 7) can be explained by different profiles of distances and different weather conditions. The smallest are long distances runners. Their average weights are only 69% (f) and 63% (m) of the rower ones.

We analyzed also the ratio of the average masses of female and male athletes and ratio of average speeds for identical distances (female/male at fixed distance), see Table 1. The ratio of the average weight of female athletes to the average weight of male athletes (averaged as well for all sports and distances) is 79.5 ± 3.1%, at the same time the female athletes develop 89.4 ± 1.8% of the speed of male ones.

Average velocity versus average duration of sports activity. Fig. 8 and Table 1 illustrate the influence of the duration of a sports activity (the time spent on a distance) on the average velocity for different sports. Due to the fatigue, the speeds usually monotonously decrease with the duration increasing. Some exceptions occur only for 1 km and 1.5 km distances in skating and skiing respectively. Smaller speeds at shorter distances can be explained by the fact that athletes do not have enough time to reach the maximum speed.

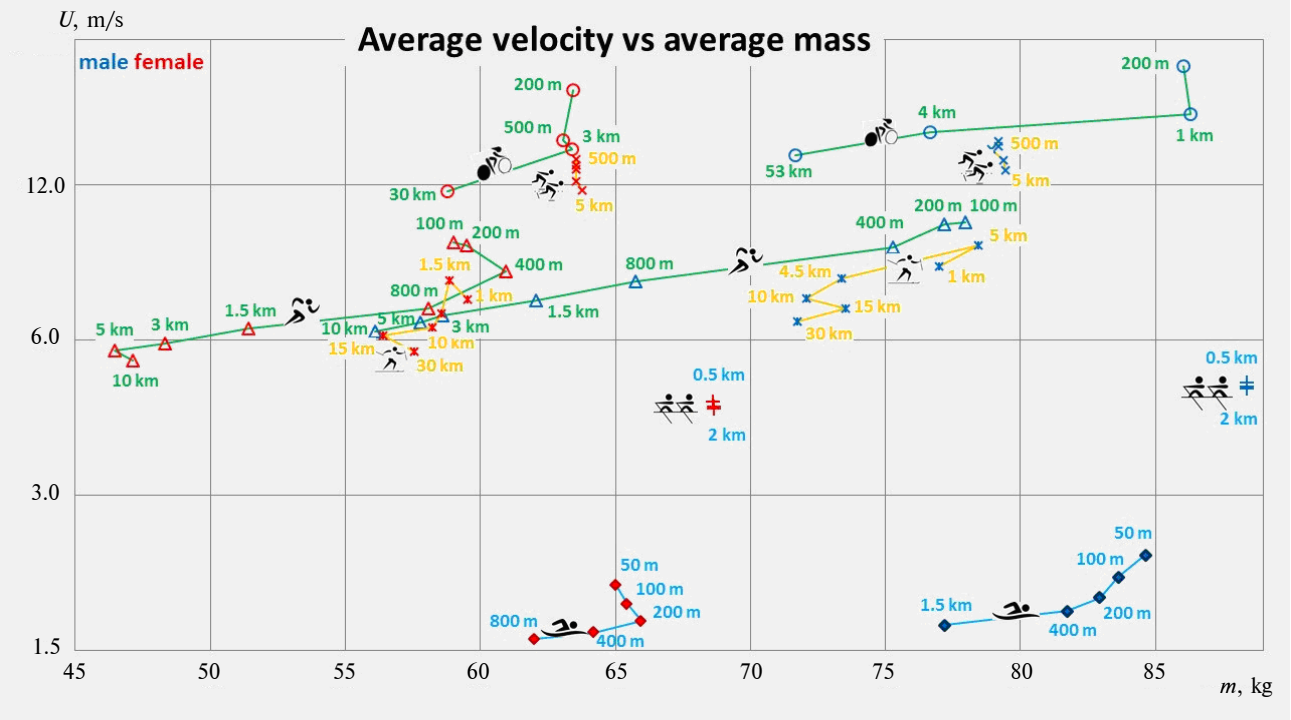

Figure 7: The average values of speed for different distances and sports versus the average body mass of athletes: triangles – running, rhombuses – swimming, snowflakes – skiing, tilted crosses – skating, circles – cycling, straight crosses – rowing. Signs are blue for males and red for females

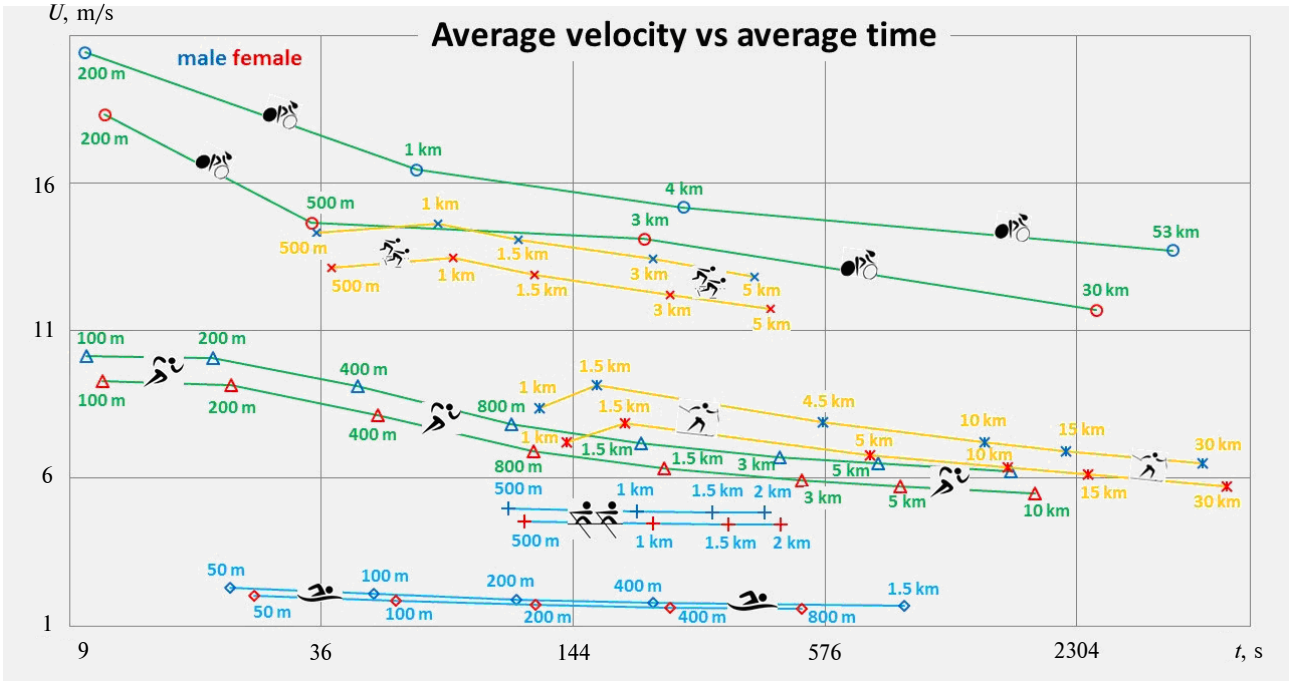

Figure 8: The influence of the duration of a sports activity (the time spent on a distance) on the average velocity for different sports: triangles – running, rhombuses – swimming, snowflakes – skiing, tilted crosses – skating, circles – cycling, straight crosses – rowing. Signs are blue for males and red for females

Table 1 also represents the ratio of the average speeds of female athletes to male ones at the fixed time of activity (female/male at fixed duration). If a value of speed at a fixed value of duration is unknown, we used linear interpolation of the results for nearest distances. It can be seen that female athletes on average develop 89.1 ± 3.2% of the speed of male ones.

Average body mass versus average time of activity. Fig. 9 and Table 1 show the average body mass versus the duration of a sports activity (the time spent on a distance) for different sports and distances. For rowers, the average mass is unchanged, since the Fig. 9 shows the intermediate results at only one distance of 2 km. As noted earlier, for skaters, the average mass varies very little. For skiers, the somewhat chaotic nature of the dependencies can be explained by different distance profiles and different weather conditions that affect the average duration of the activity. Nevertheless, for male skiers, it can be seen the tendency of weight decreasing for longer distances, in comparison with the female athletes.

Table 1 also represents the ratio of the average weight of female athletes to male ones at the fixed time of activity (female/male at fixed duration). The ratio of the average weight of female athletes to the average weight of male athletes (averaged as well for all sports and distances) is 79.4 ± 3.3%. The ratio of the average weight of women to men one among adult persons is about 83% [15, 16]), and is rather different for different countries (e.g., 73–77% for Croatia, Bangladesh, and Vietnam; 79–83% for Ethiopia, North Korea, Kuwait, England, Australia, South Korea, Germany, and Japan; 85–88% for Canada, USA, Brazil, Chile, and Ukraine; 94.5% for Russia).

Mass independence for skating. Table 2 shows the results of the F-test calculation. We ranked the results of the skating on the weight (n is the total number of results for each distance) and divided them into two groups equal in number (one group with heavier athletes and the second group with lighter athletes). Then we calculated the Between group variability (B_{gv} , see formula (1)), and Within group variability (W_{gv} , see eq. (2)) and the value of Fisher function (according to (3)). The critical values of Fisher function (Fc) are also shown in Table 2 for significance levels $\alpha = 0.05$ and $\alpha = 0.01$. The results indicate that the calculated values of the Fisher function are smaller than the critical ones (it is only one exception for male 1500 m distance

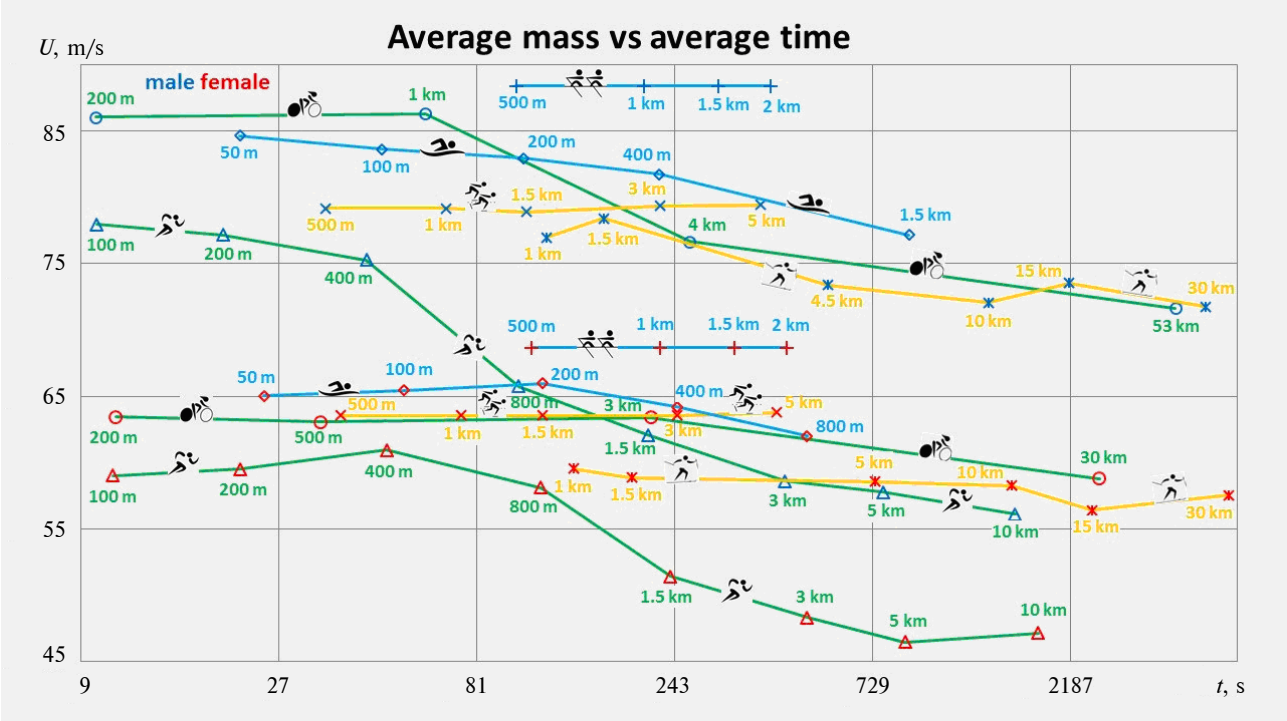

Figure 9: The average body mass versus the duration of a sports activity (the time spent on a distance) for different sports and distances: triangles – running, rhombuses – swimming, snowflakes – skiing, tilted crosses – skating, circles – cycling, straight crosses – rowing. Signs are blue for males and red for females

at the significance level $\alpha = 0.05$). Thus, we can conclude that the skaters' speed does not depend on their weight.

Discussion

Fig. 8 and Table 1 show the speed differences for different sports. The fasters athletes are cyclists. Skaters and skiers are slower. These speed differences can be explained by different friction coefficients. The runners are the slowest athletes among moving in air. Their movement is a series of jumps which is connected with rather high energy loss [6, 17]. Water sports are the lowest, since the drag in water is very high [18]. Due to the special shaped elongated shape of the boat [19], the rowers are more than twice faster.

The human running champions are approximately 3 times slower than cheetah – the fastest terrestrial animal. To estimate the efficiency of motion, a special characteristic – capacity-efficiency, C_e – was proposed in [6, 20], which indicates how much metabolic capacity (energy released in the body per unit of weight and per unit of time) is used to move the center of mass. The estimations of C_e presented in [21] yield the values 0.286 m/s (Male record, 100 m, Usain Bolt), 0.268 m/s (Female record, 100m, Florence Griffith-Joyner) and 0.319 m/s (Male record 10 km, Kenenisa Bekele). In comparison for cheetah $C_e = 0.277$ m/s [21]. Thus, the running effectiveness of our champions is comparable with one of the fastest terrestrial animal. Nevertheless, the running effectiveness of the fastest horses ($C_e = 0.467$ m/s) and kangaroos ($C_e = 0.567$ m/s) is higher [21]).

It must be noted that real maximum metabolic rate of human athletes is approximately 2.9 m/s (28 W/kg), [22]. It means that only 1/10 part of the capacity released in body is used to move the center of running body. In the case of swimming this part is much smaller. E.g., $C_e = 0.0097$ m/s [20] for fastest underwater human swimming style (dolphin kick, speed 2.7 m/s). The best fish swimmers are more than 10 times faster. E.g. the Atlantic sailfish, *Istiophorus albicans* is able to achieve the speed 30.6 m/s [20]. The highest value of $C_e = 8.4$ m/s was calculated for the juvenile Blue shark, *Prionace glauca* in [20]. Thus

Table 2: The results of F-test for male and female skating

Gender	Distance, m	Mass, kg			Velocity, m/s (average)	Average		F	B_{gv}	W_{gv}	n	Fc $\alpha = 0.05$	Fc $\alpha = 0.01$
		min	max	average		mass, kg	speed, m/s						
Male	500	65 80	80 90	74.97 83.62	14.35 14.27	79.17	14.30	1.58	0.12	0.08	74	4.0	7.1
Male	1000	65 80	80 90	74.44 83.42	14.61 14.69	79.17	14.63	3.07	0.13	0.04	72	4.0	7.1
Male	1500	60 80	80 97	74.20 83.37	13.96 14.18	78.92	14.07	4.19	1.09	0.26	92	4.0	7.1
Male	3000	60 80	80 97	75.25 83.32	13.44 13.40	79.36	13.41	0.27	0.02	0.09	56	4.1	7.3
Male	5000	60 80	80 97	75.18 83.56	12.98 12.76	79.44	12.82	1.86	0.82	0.44	68	4.0	7.1
Female	500	51 63	63 73	59.19 67.66	13.09 13.16	63.56	13.11	0.93	0.08	0.09	64	4.0	7.1
Female	1000	51 63	63 73	59.04 67.81	13.41 13.49	63.55	13.45	2.64	0.08	0.03	52	4.1	7.3
Female	1500	51 63	63 74	59.29 67.61	12.86 12.89	63.53	12.88	0.11	0.02	0.15	76	4.0	7.1
Female	3000	51 63	63 74	59.13 67.73	12.17 12.30	63.54	12.22	1.71	0.24	0.14	60	4.0	7.1
Female	5000	52 63	63 74	59.42 68.15	11.82 11.70	63.77	11.74	1.08	0.19	0.17	52	4.1	7.3

compared with the fast aquatic animals, both the speed and effectiveness of human swimming is much lower. The low drag elongated shape of the rowing boat (and may be higher propulsion coefficient) allows more than 5 times increase the efficiency of movement in water. E.g., $C_e \approx 0.051$ m/s for rowing Lightweight Men Single, best time [6].

The sprinter running speed does not substantially depend on the body mass, but increases with the increasing of the stride length [21]. The statistical analysis of fit male and female sprint runners [23] demonstrate the linear increasing of the speed versus stride length. Since the taller and heavier athletes have larger stride length, we can expect that they have the highest speed. The optimal weight of 100m male runners (approx. 78 kg, see Table 1) is larger than the average weight of adult men (74 kg). Nevertheless, the optimal weight of 100m female runners (approx. 59 kg, see Table 1) is smaller than the average weight of adult women (61 kg).

For male cycling, running and swimming the optimal body mass decreases with the increasing the duration of activity (the only exception is the cycling distance of 1km). In comparison, for female cycling, running and swimming dependence of the optimal body mass versus the duration of activity is not monotonous. This peculiarity of female sports needs further investigations.

Conclusions

A significant amount of data on the velocity and body mass of both female and male athletes professionals in Olympic sports (running, swimming, skiing, skating, cycling, and rowing) was collected.

The average values for different distances were calculated and the linear regression analysis was applied to find the relationships between the body mass and the velocity for every distance.

Within one distance there is no significant dependence of speed on the mass (except for rowing). For each distance, there are their optimal masses of athletes, which are different for different sports. This result needs further investigations and analysis.

The large masses of athletes are optimal for the short distances. For longer distances they usually decrease, but not for the female swimming and running (where the largest masses of athletes correspond to the medium distances) and not for the male and female skating (where speed is practically independent from the body mass). These facts need to be studied and explained.

For a fixed distance, the optimal masses of female athletes are 79.5 ± 3.1% of the weight of male athletes, at the same time they develop 89.4 ± 1.8% of the speed of men.

The obtained results can be also useful for selection of athletes for different sports and distances. The differences in optimal weights for different sport and distances need further investigations. They can be very complicated, since the metabolic and propulsion peculiarities, drag in water or/and air, energy waste to support the body on the ground, wheels friction drag etc. must be taken into account.

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І.Б. Кудибин, І.Г. Нестерук, С.В. Переверзєв, А. Редаеллі, Б.Д. Шепетюк, О.Р. Чертов

ОПТИМАЛЬНІ МАСИ ТІЛА ДЛЯ РІЗНИХ ОЛІМПІЙСЬКИХ ВИДІВ СПОРТУ

Проблематика. Вага кращих спортсменів на різних відстанях і в різних видах спорту може бути дуже різною. Відомо, що веслярі, як правило, дуже масивні. Для того щоб дати шанс атлетам з меншою масою тіла, федерація з веслування ввела спеціальну категорію з ваговими обмеженнями. Ці факти пов'язані з особливостями аеробної та неаеробної активності, характеристиками опору та руху.
Мета. У цій статті ми не намагаємося пояснити різницю між масами тіла спортсменів. Ми лише зафіксуємо їх для різних видів спорту (біг, плавання, лижні перегони, ковзанярський спорт, велоспорт, веслування) і для різних олімпійських дистанцій.
Методика реалізації. Ми зібрали та проаналізували значну кількість даних про швидкість та масу тіла кращих спортсменів-професіоналів (жінок та чоловіків) у олімпійських видах спорту (біг, плавання, лижні перегони, ковзанярський спорт, велоспорт, веслування). Оскільки ми беремо до уваги маси тіла тільки найкращих спортсменів, то отримані результати можуть розглядатись як найбільш сприятливі (оптимальні) маси тіла для різних видів спорту і відстаней. Для того щоб перевірити, наскільки значення маси тіла і середньої швидкості в межах однієї дистанції відповідають лінійній залежності, ми використали рівняння лінійної регресії. Розраховано коефіцієнт кореляції та параметри прямої регресії. Для того щоб перевірити припущення, що швидкість ковзанярів не залежить від маси спортсмена, ми використали тест Фішера.
Результати. Розраховано оптимальні маси спортсменів для різних відстаней у різних видах спорту. Загалом на коротких відстанях великі маси спортсменів є оптимальними, і вони зменшуються для великих відстаней. Винятком є результати для плавання та бігу серед жінок, де найбільші маси спортсменів відповідають середнім дистанціям. Для більш довгих відстаней результати показують, що найбільш успішні спортсмени характеризуються меншою масою тіла, за винятком ковзанярського спорту. Для фіксованих відстаней оптимальні маси тіла атлетів-жінок становлять 79,5 ± 3,1 % від маси тіла атлетів-чоловіків, у той же час жінки-спортсмени розвивають швидкість, яка становить 89,4 ± 1,8 % від швидкості чоловіків-спортсменів.
Висновки. Проведений статистичний аналіз свідчить, що в межах однієї дистанції немає значної залежності між швидкістю та масою тіла спортсмена (за винятком веслування). Для кожної відстані існують оптимальні маси тіла спортсменів, які є різними для різних видів спорту. Встановлені факти можуть бути використані у підборі спортсменів та їх підготовці. Вони потребують також подальших досліджень з урахуванням особливостей метаболізму та руху.

Ключові слова: олімпійський спорт; лінійна регресія; тест Фішера; підбір спортсменів.

И.Б. Кудыбин, И.Г. Нестерук, С.В. Переверзев, А. Редаелли, Б.Д. Шепетюк, О.Р. Чертов

ОПТИМАЛЬНЫЕ МАССЫ ТЕЛА ДЛЯ РАЗНЫХ ОЛИМПИЙСКИХ ВИДОВ СПОРТА

Проблематика. Вес лучших спортсменов на разных дистанциях и в разных видах спорта может сильно отличатся. Известно, что гребцы, например, довольно массивные. Для того чтобы дать шанс атлетам с меньшей массой тела, федерация гребцов ввела специальную категорию с весовыми ограничениями. Эти факты связаны з особенностями аэробной и неаэробной активности, характеристиками сопротивления и движения.

Цель. В этой статье мы не пытаемся объяснить разницу между массами тела спортсменов. Мы только определим их для разных видов спорта (бег, плавание, лыжные гонки, конькобежный спорт, велоспорт, гребля) и для разных олимпийских дистанций.

Методика реализации. Мы собрали и проанализировали большое количество данных о скорости и массе тела лучших профессиональных спортсменов (женщин и мужчин) в олимпийских видах спорта (бег, плавание, лыжные гонки, конькобежный спорт, велоспорт, гребля). Так как мы принимаем во внимание массы тела только лучших спортсменов, то полученные результаты могут рассматриваться как наиболее подходящие (оптимальные) массы тела для разных видов спорта и дистанций. Для того чтобы проверить, насколько значения массы тела и средней скорости в рамках одной дистанции соответствуют линейной зависимости, мы использовали уравнения линейной регрессии. Рассчитаны коэффициент корреляции и два параметра прямой регрессии. Для того чтобы проверить предположение, что скорость конькобежцев не зависит от массы тела спортсмена, мы использовали тест Фишера.

Результаты. Рассчитаны оптимальные массы спортсменов для разных дистанций в разных видах спорта. В общем, на коротких дистанциях оптимальными являются большие массы тела спортсменов, и они уменьшаются для более длинных дистанций. Исключение составляют результаты для плавания и бега среди женщин, где самые большие массы спортсменов соответствуют средним дистанциям. Для более длинных дистанций результаты показывают, что наиболее успешные спортсмены обладают меньшей массой тела, за исключением конькобежного спорта. Для фиксированных дистанций оптимальные массы тела атлетов-женщин составляют 79,5 ± 3,1 % от массы тела атлетов-мужчин, в то же время женщины-спортсмены развивают скорость, которая составляет 89,4 ± 1,8 % от скорости мужчин-спортсменов.

Выводы. Проведенный статистический анализ показал, что в рамках одной дистанции нет значительной зависимости между скоростью и массой тела спортсмена (за исключением гребли). Для каждой дистанции существуют оптимальные массы тела спортсменов, которые оказались разными для разных видов спорта. Полученные результаты могут быть использованы в подборе спортсменов и их подготовке. Они нуждаются также в дополнительных исследованиях с учетом особенностей метаболизма и движения.

Ключевые слова: олимпийский спорт; линейная регрессия; тест Фишера; подбор спортсменов.

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Original article

РЕАЛИЗАЦИЯ ФОТОИНДУЦИРОВАННОЙ РОСТОВОЙ АКТИВНОСТИ МАКРОМИЦЕТОВ: ВЛИЯНИЕ СПОСОБА КУЛЬТИВИРОВАНИЯ И КОНЦЕНТРАЦИИ УГЛЕРОДА И АЗОТА

Н.Л. Поединок[1]*, О.Б. Михайлова[2], Н.Н. Сергийчук[3], А.М. Негрейко[4]
[1]ДУ "Институт пищевой биотехнологии и геномики НАН Украины", Киев, Украина
[2]Институт ботаники имени Н.Г. Холодного НАН Украины, Киев, Украина
[3]Открытый международный университет развития человека "Украина", Киев, Украина
[4]Институт физики НАН Украины, Киев, Украина

*Corresponding author: n.poyedinok@gmail.com

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Проблематика. Научные основы фоторегуляции биосинтетической активности съедобных и лекарственных макромицетов.
Цель. Определение влияния способа культивирования макромицетов, концентрации углерода и азота на реализацию их фотоиндуцированной ростовой активности.
Методика реализации. Посевной мицелий *C. militaris*, *F. velutipes*, *H. erinaceus*, *L. edodes* и *P. ostreatus*, облученный низкоинтенсивным лазерным светом в разных диапазонах длин волн при энергетической дозе облучения 230 мДж/см^2, культивировали поверхностно на жидкой среде, а также глубинным способом. В качестве источника углерода использовали глюкозу, азота – пептон в различных концентрациях. Определяли накопление биомассы и эффективность потребления глюкозы.
Результаты. Доказано, что степень реализации фотоиндукции зависит от состава питательной среды и способа культивирования макромицетов. Установлено, что для получения максимального стимулирующего эффекта после низкоинтенсивного лазерного излучения следует проводить глубинное культивирование фотоактивированного посевного мицелия. Кратковременное низкоинтенсивное лазерное излучение приводит к изменению трофики макромицетов и выражается в увеличении скорости накопления биомассы и эффективности потребления глюкозы. Снижение концентрации глюкозы в среде повышает фотоиндуцированную активность посевного мицелия *C. militaris*, *G. lucidum*, *L. edodes*, *H. erinaceus* и *P. ostreatus*. Достоверного влияния концентрации азота на фотостимуляцию роста макромицетов не установлено.
Выводы. Полученные результаты являются основанием для дальнейших исследований влияния различных факторов на фотоиндукцию биосинтетической активности съедобных и лекарственных грибов с целью интенсификации технологических этапов из культивирования.

Keywords: макромицеты; низкоинтенсивный свет; облучение; фотоиндукция; ростовая активность.

Введение

Макромицеты являются источником активных компонентов, которые содержатся в плодовых телах, глубинно культивируемом мицелии и культуральной жидкости [1]. Свет наряду с другими экологическими факторами, такими как температура и влажность, влияет на жизнедеятельность практически всех видов грибов. Механизмы их фоторецепции не изучены полностью, и поэтому при разработке методов световых воздействий на грибной организм часто преобладают эмпирические подходы. Однако использование света в биотехнологии возможно даже при отсутствии информации о механизмах его действия. Уже известно, что низкоинтенсивный когерентный (лазерный) и некогерентный свет может быть эффективно использован для регуляции биосинтетической активности съедобных и лекарственных грибов [2].

Однако в настоящее время какие-либо сведения о факторах, влияющих на проявление фотоиндуцированных изменений у макромицетов, практически отсутствуют. Тем не менее их знание необходимо для эффективного использования искусственного света со строго контролируемыми параметрами в биотехнологиях культивирования макромицетов.

Авторы, проводившие исследования с микромицетами, показали, что характер среды, на которой выращивается гриб, и способ культивирования определяют, будет ли свет стимулировать или уменьшать скорость роста

грибов [3–9]. Была установлена взаимосвязь фотоиндуцированной активности ферментов *Trichoderma reesei* E.G. Simmons, участвующих в деструкции клеточной стенки растений и источников питания [9]. При рассмотрении вопросов фоторецепции и экспрессии ферментов и генов у грибов большое значение уделяется углеводному обмену. Известно, что свет может значительно ингибировать поглощение глюкозы *Aspergillus ornatus* Raper, Fennell & Tresner [10], а глюкозоамилазная активность мицелия *Aspergillus niger* Tiegh. под воздействием голубого света увеличивается более чем в 2,5 раза по сравнению с мицелием, выращенным в темноте [11]. Фоточувствительность метаболизма азота у грибов изучена довольно незначительно. Тем не менее некоторые исследователи считают, что связь между светом и азотным обменом существует [12, 13]. Обнаружено, что среди генов, контролирующих циркадные часы, есть несколько генов, участвующих в метаболизме азота [14]. Синий свет индуцирует снижение нитратредуктазной активности у *Neurospora crassa* Shear & O.B.Dodge. Это дает основания предположить, что поскольку свет влияет на метаболизм азота и углерода у грибов, то, соответственно, и концентрации источников азота и углерода могут влиять на их чувствительность к свету.

Таким образом, исходя из сказанного выше, целью работы является определение влияния способа культивирования, концентрации углерода и азота на реализацию фотоиндуцированной ростовой активности съедобных и лекарственных макромицетов.

Материалы и методы

Объектами исследований были чистые культуры макромицетов *Cordyceps militaris* (L.) Link 2029, *Flammulina velutipes* (Curtis) Singer 1883, *Hericium erinaceus* (Bull.) Pers 963, *Lentinula edodes* (Berk.) Pegler 711, *Pleurotus ostreatus* (Jacq.) P. Kumm. 1688, известные как продуценты пищевой биомассы и биологически активных веществ, из Коллекции культур шляпочных грибов Института ботаники имени Н.Г. Холодного НАН Украины (IBK) [15].

Исследования влияния способа культивирования на фотоиндуцированную ростовую активность макромицетов проводили на жидком пивном сусле (8° по Баллингу). Выращенный поверхностно на сусло-агаре в чашках Петри при температуре 25–26 °С мицелий подвергали облучению. Сразу после этого вырезали диски (5 мм в диаметре) с мицелием. В колбы Эрленмейера с 150 мл питательной среды помещали по 5 мицелиальных дисков и культивировали в стационарном или динамическом режиме на качалке (180 об/мин) при той же температуре. Длительность культивирования зависела от видовой принадлежности штаммов и их ростовых характеристик (7–14 сут). Концентрацию абсолютно сухой биомассы (а.с.б.) определяли весовым методом. Изменение ростовых показателей после облучения светом низкой интенсивности определяли в процентах по отношению к контролю.

При изучении влияния концентраций глюкозы на фотоиндуцированную ростовую активность макромицетов использовали глюкозо-пептонную среду с разными концентрациями глюкозы (10, 30 и 50 г/л и 3 г/л пептона). Влияние концентраций азота – на глюкозо-пептонной среде (30 г/л глюкозы и разные концентрации пептона – 1, 3 и 4 г/л).

Источниками когерентного видимого низкоинтенсивного лазерного излучения (НИЛИ) служили газовые лазеры: гелий-неоновый лазер ЛГН-215 с излучением на длине волны 632,8 нм (красный цвет), производства НПО “Полярон”, Львов, Украина, и аргоновый ионный лазер (модифицированная модель ЛГН-106М1 производства НПО “Плазма”, Россия), излучение на длине волны 514,5 нм (зеленый свет) и 488,0 (синий свет) нм. Лазерный луч расфокусировался линзой до размера области чашки Петри.

Плотность мощности излучения измеряли, используя цифровой оптический измеритель мощности и энергии PM-100D, Thorlabs Inc., со стандартным фотодиодным датчиком мощности S120C, рабочий диапазон 400–1100 нм. Энергетическая доза облучения, определенная как произведение плотности мощности и времени облучения, составляла 230 мДж/см^2. Световую обработку посевного материала проводили при полном отсутствии других источников света.

Для обработки полученных результатов использовали программу Excel 2007. Повторность экспериментов – 5-кратная. Статистически достоверными считались результаты исследований, в соответствии с *t*-критерием Стьюдента, при уровне значимости $p \leq 0{,}05$.

Результаты

Результаты, представленные в табл. 1, подтверждают выдвинутую нами ранее гипотезу о влиянии способа культивирования на процессы, которые определяют изменения ростовых характеристик, вызванные световыми воздействиями.

Наибольший стимулирующий эффект, полученный при использовании НИЛИ для активации роста изученных видов макромицетов, получен при глубинном культивировании. Накопление биомассы при культивировании в динамическом режиме увеличивалось, в зависимости от штамма и режима облучения, на 13 и до более чем 150 % по сравнению с культивированием в стационарном режиме.

При изучении влияния концентраций глюкозы на фотоиндуцированную ростовую активность макромицетов наибольший стимулирующий эффект после облучения для всех исследованных штаммов отмечали на среде, содержащей 10 г/л глюкозы (табл. 2). Повышение концентрации глюкозы в питательной среде приводило к снижению процента увеличения накопления биомассы по отношению к контролю (10 г/л глюкозы) у *C. militaris* – на 7,6 %, у *F. velutipes* – на 9,3 %, у *L. edodes* – на 24,4 % на среде с 30 г/л глюкозы и на 47 % на среде с 50 г/л.

Такая же закономерность наблюдалась и в экспериментах с *H. erinaceus* и *P. ostreatus*, у которых снижение фотоиндуцированной активности составляло на средах с 30 и 50 г/л глюкозы у *P. ostreatus* – 10,2 и 23,1 % и у *H. erinaceus* – 19,3 и 31,3 % и соответственно.

Критерием оценки потребления глюкозы, при культивировании облученных и контрольных штаммов на питательных средах с разной ее концентрацией, служила величина экономического коэффициента. Установлено, что эффективность потребления глюкозы во всех вариантах опыта и в контроле выше на среде с 10 г/л глюкозы (рис. 1). С экологической точки зрения, это можно объяснить адаптационной реакцией организма гриба к стрессовым факторам окружающей среды. Однако полученные нами данные позволяют утверждать, что повышение экономического эффекта у штаммов после воздействия НИЛИ значительно более выражено. Эффективность освоения субстрата

Таблица 1: Фотоиндуцированные изменения роста макромицетов при стационарном и глубинном способах культивирования на жидкой среде (% увеличения биомассы)

Вид	Длина волны, нм		
	632,8 нм (красный)	514,5 нм (зеленый)	488,0 нм (синий)
C. militaris	Стационарный режим		
	6,2 ± 0,4	1,5 ± 0,2	**15,5 ± 0,3•**
	Глубинное культивирование		
	19,6 ± 0,6*	7,7 ± 0,2*	**25,8 ± 1,2•**
F. velutipes	Стационарный режим		
	25,4 ± 0,3*	1,1 ± 0,1	**34,4 ± 1,8•**
	Глубинное культивирование		
	56,3 ± 1,7*	–11,5 ± 1,0*	**78,2 ± 1,2•**
H. erinaceus	Стационарный режим		
	39,4 ± 1,8*	32,5 ± 1,3*	**45,6 ± 5,1•**
	Глубинное культивирование		
	51,8 ± 3,4*	43,6 ± 1,8*	**68,8 ± 2,6•**
L. edodes	Стационарный режим		
	75,1 ± 1,6•	66,7 ± 2,1*	76.9 ± 1,8*
	Глубинное культивирование		
	139,8 ± 2,4•	117,6 ± 2,2*	**153,8 ± 3,1•**
P. ostreatus	Стационарный режим		
	41,9 ± 1,4•	34,6 ± 0,4*	20,4 ± 0,17*
	Глубинное культивирование		
	57,4 ± 1,0•	46,0 ± 1,5*	29,2 ± 0,17*

*Статистически достоверные различия по отношению к контролю ($p \leq 0,05$), результаты представлены как $M \pm n$, $n = 5$.

Таблица 2: Фотоиндукция ростовой активности макромицетов на средах с разной концентрацией глюкозы (а.с.б, г/л)

Концентрация глюкозы, г/л	Контроль, без облучения	632,8 нм	% увеличения а.с.б
C. militaris			
10	4,6 ± 0,1	5,8 ± 0,2*	31,0
30	6,1 ± 0,2	7,5 ± 0,3*	23,4
50	6,4 ± 0,3	7,9 ± 0,1*	23,7
F. velutipes			
10	5,2 ± 0,3	7,0 ± 0,1*	39,0
30	9,7 ± 0,4	12,4 ± 0,3*	30,1
50	10,1 ± 0,3	13,2 ± 0,4*	30,0
H. erinaceus			
10	4,4 ± 0,2	7,9 ± 0,1*	73,1
30	6,3 ± 0,3	9,6 ± 0,2*	54,0
50	8,4 ± 0,2	12,2 ± 0,2*	42,1
L. edodes			
10	4,0 ± 0,1	7,9 ± 0,2*	97,6
30	8,3 ± 0,2	14,3 ± 0,3*	73,4
50	11,1 ± 0,4	16,7 ± 0,2*	50,1
P. ostreatus			
10	5,4 ± 0,2	8,5 ± 0,2*	64,2
30	12,0 ± 0,1	18,0 ± 0,4*	54,6
50	13,8 ± 0,3	19,0 ± 0,3*	41,1

*Достоверные различия по отношению к контролю ($p \leq 0,05$), результаты представлены как $M \pm n$, $n = 5$

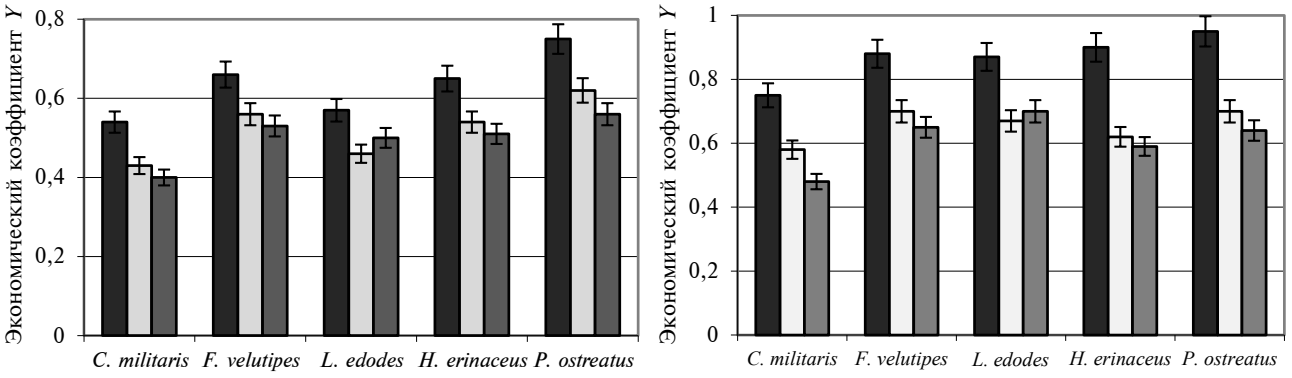

Рисунок 1: Влияние концентрации источника углерода (глюкоза) на эффективность его потребления макромицетами: (а) облученный посевной мицелий, (б) контроль, посевной мицелий без облучения: ■ – 10 г/л, □ – 30 г/л, ■ – 50 г/л

необлученными штаммами на среде с 10 г/л глюкозы выше на 10 % по сравнению с культивированием на среде с 30 г/л глюкозы, а в опыте после световой обработки на 30–45 % по сравнению со средой с 30 г/л глюкозы и на 35–56 % с 50 г/л глюкозы.

Культивирование посевного мицелия, облученного НИЛИ такой же длины волны, 632,8 нм, что и в предыдущем опыте, на ГПС с разными концентрациями пептона (1, 3 и 4 г/л) не показали достоверного влияния концентрации источника азота на фотоиндуцированную стимуляцию роста макромицетов (рис. 2). Увеличение накопления биомассы фотоактивированным мицелием, по сравнению с необлученным, было практически одинаковым в пределах вида гриба во всех вариантах опыта.

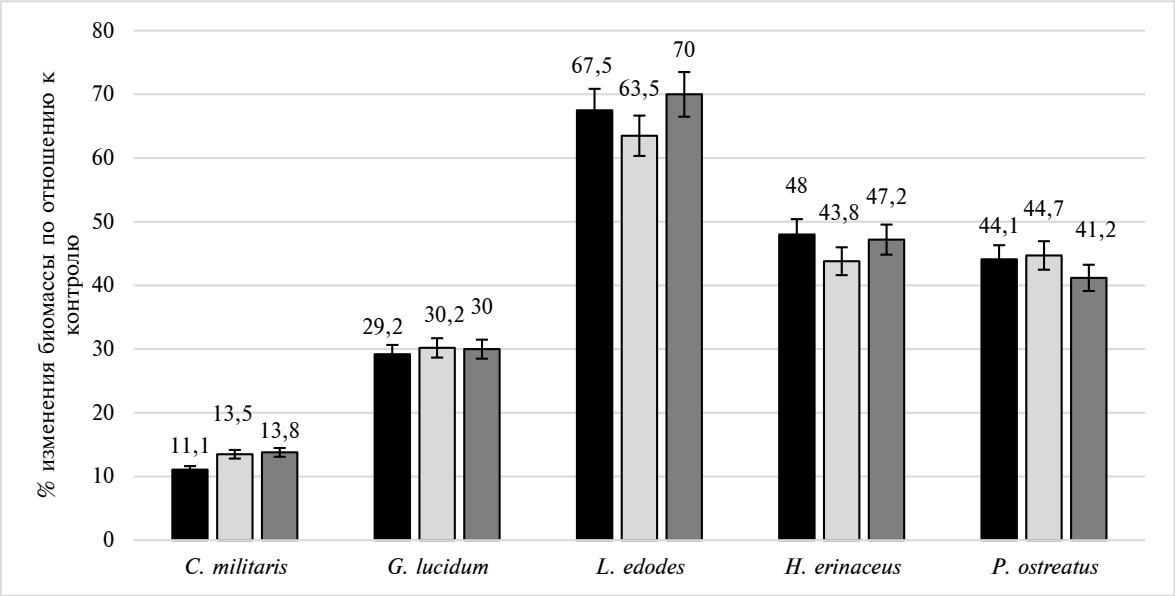

Рисунок 2: Ростовая активность макромицетов на средах с разной концентрацией пептона: ■ – 1 г/л, □ – 3 г/л, ■ – 4 г/л

Обсуждение

Изучение накопления биомассы на жидких средах при использовании посевного мицелия, облученного низкоинтенсивным лазерным светом в разных диапазонах длин волн, позволило получить объективную картину влияния способа культивирования на реализацию фотоиндуцирующего эффекта. Таким образом, наши данные и результаты исследований, полученные другими исследователями при работе с микромицетами [5, 9], свидетельствуют о том, что трансформация энергии света, поглощаемой грибными клетками, определяется последующими условиями культивирования грибов.

Установленная нами зависимость степени фотоактивации роста мицелия изученных видов грибов от концентрации углерода дала основание предположить, что НИЛИ приводит к изменению их трофики и выражается в увеличении эффективности потребления источника углерода на средах с пониженным содержанием глюкозы. Полученные данные подтвердили наше предположение (см. рис. 1).

Отсутствие достоверного влияния концентрации азота на фотоиндуцированную стимуляцию роста макромицетов тем не менее позволяет предположить, что его концентрация может влиять на индукцию НИЛИ других изменений их биологической активности, таких как синтез ферментов, полисахаридов, меланинов и пр., что не всегда коррелирует с их ростовой активностью [16–20]. Это может быть основанием для проведения исследований, направленных на изучение влияния концентраций азота на метаболизм макромицетов.

Выводы

Доказано, что степень реализации фотоиндукции зависит от состава питательной среды и способа культивирования макромицетов. Установлено, что для получения максимального стимулирующего эффекта после НИЛИ следует проводить глубинное культивирование фотоактивированного посевного мицелия.

Кратковременное НИЛИ приводит к изменению трофики макромицетов и выражается в увеличении скорости накопления биомассы и эффективности потребления глюкозы. Снижение концентрации глюкозы в среде повышает фотоиндуцированную активность посевного мицелия *C. militaris*, *F. velutipes*, *L. edodes*, *H. erinaceus* и *P. ostreatus*, что проявляется в дополнительном увеличении накопления биомассы при глубинном культивировании.

Не установлено достоверного влияния концентрации азота на фотоиндуцированную стимуляцию роста макромицетов.

Полученные результаты являются основанием для дальнейших исследований влияния различных факторов на фотоиндукцию биосинтетической активности съедобных и лекарственных грибов с целью интенсификации технологических этапов их культивирования.

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Н.Л. Поєдинок, О.Б. Михайлова, Н.М. Сергійчук, А.М. Негрійко

РЕАЛІЗАЦІЯ ФОТОІНДУКОВАНОЇ РОСТОВОЇ АКТИВНОСТІ МАКРОМІЦЕТІВ: ВПЛИВ СПОСОБУ КУЛЬТИВУВАННЯ ТА КОНЦЕНТРАЦІЇ ВУГЛЕЦЮ І АЗОТУ

Проблематика. Наукові основи фоторегуляції біосинтетичної активності їстівних і лікарських макроміцетів.
Мета. Встановлення впливу способу культивування макроміцетів, концентрації вуглецю і азоту на реалізацію їх фотоіндукованої ростової активності.
Методика реалізації. Посівний міцелій *C. militaris*, *F. velutipes*, *H. erinaceus*, *L. edodes* і *P. ostreatus*, який опромінювали низькоінтенсивним лазерним світлом за різних діапазонів довжини хвилі при енергетичній дозі опромінення 230 мДж/см^2, культивували

поверхнево на рідкому живильному середовищі, а також глибинним методом. Як джерело вуглецю використовували глюкозу, азоту – пептон у різних концентраціях. Визначали накопичення біомаси і ефективність використання глюкози.
Результати. Визначено, що ступінь реалізації фотоіндукції залежить від складу живильного середовища та способу культивування макроміцетів. Встановлено, що для отримання максимального стимулюючого ефекту після низькоінтенсивного лазерного випромінювання слід проводити глибинне культивування фотоактивованого посівного міцелію. Короткочасне низькоінтенсивне лазерне випромінювання призводить до зміни трофіки макроміцетів і виражається в збільшенні швидкості накопичення біомаси та ефективності споживання глюкози. Зниження концентрації глюкози у живильному середовищі підвищує фотоіндуковану активність посівного міцелію *C. militaris*, *G. lucidum*, *L. edodes*, *H. erinaceus* і *P. ostreatus*. Достовірного впливу концентрації азоту на фотостимуляцію росту макроміцетів не встановлено.
Висновки. Отримані результати є підставою для подальшого дослідження впливу різних чинників на фотоіндукцію біосинтетичної активності їстівних і лікарських грибів з метою інтенсифікації технологічних етапів їх культивування.

Ключові слова: макроміцети; низькоінтенсивне світло; опромінення; фотоіндукція; ростова активність.

N.L. Poyedinok, O.B. Mykhaylova, N.N. Sergiichuk, A.M. Negriyko

REALIZATION OF MACROMYCETE PHOTOINDUCED GROWTH ACTIVITY: INFLUENCE OF CULTIVATION WAYS AND THE CONCENTRATION OF CARBON AND NITROGEN

Background. Scientific principles of the biosynthetic activity photoregulation of edible and medicinal macromycetes.
Objective. The aim of the paper is to determine the influence of macromycete cultivation method, carbon and nitrogen concentration on the realization of their photoinduced growth activity.
Methods. Seed mycelium *C. militaris*, *F. velutipes*, *H. erinaceus*, *L. edodes*, and *P. ostreatus* irradiated with low-intensity laser light in different wavelength ranges at an energy dose of 230 mJ/cm^2, was cultivated superficially on a liquid medium, and also by the deep cultivation method. As carbon source glucose was used, nitrogen – peptone at various concentrations. The biomass accumulation and glucose consumption efficiency were determined.
Results. It is proved that the realization degree of the photoinduction depends on the composition of the nutrient medium and the method of macromycete cultivation. It is established that in order to obtain the maximum stimulating effect after low-intensity laser radiation, a deep cultivation of the photoactivated seed mycelium should be carried out. A short-term low-intensity laser radiation results in a change in the trophism of macromycetes and is expressed in an increase in the biomass accumulation rate and in the efficiency of glucose consumption. Reducing the glucose concentration in the medium increases the photoinduced activity of the seed mycelium *C. militaris*, *G. lucidum*, *L. edodes*, *H. erinaceus*, and *P. ostreatus*. There was no significant effect of nitrogen concentration on photoinduced stimulation of macromycete growth.
Conclusions. The results of the research are the basis for further studies of the influence of various factors on the photoinduction of the biosynthetic activity of edible and medicinal mushrooms to intensify the technological stages of their cultivation.

Keywords: macromycetes; low-intensity light; irradiation; photoinduction; growth activity.