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CRYSTALLOSCOPIC ESTIMATION OF ORGANISM ADAPTATION TO DIFFERENT PHYSIOLOGICAL STRESSORS

ABSTRACT

The authors assessed modification of crystallogenic properties of human blood serum action under physical exertion and psychoemotional stress. Physical exertion was modeled with PWC170 test, and examination situation was a model of psychoemotional stress. This study was performed on 35 healthy volunteers. We got blood specimens three times (without any physical and phychoemotional stresses; after physical exertion and after examination) in all volunteers. Crystallogenic properties of blood serum were tested with teziocrystalloscopic method. Results of own and initiated crystallization were estimated with special semiquantitive criteria and spectrometric analysis. It is stated that physical exertion and psychoemotional stress cause increasing of crystallogenic activity of human blood serum. This trend is more pronounced for the first impact. This is confirmed both by the results of the visual metric evaluation of crystallograms, and by the data of their spectrometric studies.

Keywords: biocrystallomics, stress factors, blood serum.

Introduction. It is known that even physiological stressors can cause significant responses from regulatory (neuro-immuno-hormonal circuit) and effector (cardiorespiratory suprasystem) systems [7, 9, 10]. At the same time, the nature of metabolic changes occurring in the body during significant physical activity and psycho-emotional stress has not been studied in detail sufficiently [2, 3, 6].

Examination stress is a strong psycho-physiological stimulus for a student with a socially determined significance and importance of the result, bringing many systems of the body out of balance for a long time, which remains after the answer to the examinator questions [1, 3, 10]. In this regard, this situation is a convenient model for assessing the emotional impact, including its influence on crystallogenic properties of the blood serum [5, 11, 12].

Currently, a number of standardized tests are known to study the physical performance of an individual [2, 7, 8]. One of the most common among them is the PWC 170 test, which allows to assess the functional reserves of the body in general and the cardiorespiratory system in particular, taking into account the age, sex and level of physical fitness of a person [2]. Moreover, the sample of Physical Working Capacity (PWC), developed by Shestrand at Karolinska University in Stockholm in the middle of XX century. In 1968 it has been recommended for determining the physical health of the person. This method (from the English Phisicsl Working Capacity - "physical performance") is to determine the power of the standard load, in which the heart rate (HR) reaches 170 beats per minute. Its standardization and predetermined the possibility of its application as a model of significant individualized physical activity.

The **aim of the study** was to evaluate the modification of crystallogenic properties of human blood serum under psycho-emotional and physical stress.

Material and methods. The assessment of crystallogenic and initiating activity of blood serum of 35 practically healthy students-volunteers (18-20 years old) before and after the influence of the stress factor, including physical activity and psychoemotional stress (passing the course exam). As a model of physical activity, the PWC 170 test was used in a variant of a veloergometric test. The power of the first and second loads was calculated from the tables taking into account anthropometric data, age and sex of the subjects [2].

To assess the impact of stress factors in all subjects, blood samples were obtained three times: in a calm state (in the intersessional period), after physical activity and immediately after the course exam. Whole blood serum was obtained by standard centrifugation. For the study of crystallogenic and initiating properties of a biological liquid was prepared microscope slide. according to the method of teziocrystalloscopy [4-6]. The basic substance in the teziographic test was 0.9% sodium chloride solution. Description of the results of own and initiated crystal formation of biological substrate was performed using a system of semi-quantitative parameters [5, 6]. The main indicators were evaluated in point scale. They were crystallizability (Cr; reflects the quantity of the crystallization – density of the crystalline elements in facies), the structure index (SI; characterized by the complexity of structureborne), the facia destruction degree (FDD; it is an indicator of the qualitative side of the process and the correctness of the formation of structures) and the clearity of the marginal zone of the micropreparation (MB).

Visual morphometry of micro specimens dried serum supplemented spectrometric study crystalloscopic and teziographic facias are verified with spectrophotometry on PowerWave XS device (USA) at wavelengths of 300, 350 and 400 nm. To eliminate the influence of the characteristics of glass on the results of spectrometric studies of biocrystals introduced a correction for the optical density of the slide material (for crystalloscopy) or a control sample as a basic substance deposited on the same glass (for teziographic test).

Statistical processing of the results was performed using the program Statistica 6.0.

Results and discussion. On the basis of the obtained results, a comparative analysis of the influence of physical activity and psycho-emotional stress on the crystallogenic and initiating properties of blood serum was performed. In particular, it was found that these physiological stressors contribute to a significant transformation of the own crystal formation of the biological fluid in question (fig. 1 and 2).

At the same time, it is important to emphasize that the direction of shifts in all the main estimates for both variants of exposure is similar, due to the universality of the stress response of the body to the stimulus, but the severity of the shift in the value of the parameters is not the same. Thus, when performing the PWC 170 test, changes in the crystalloscopic pattern of blood serum were more distinct

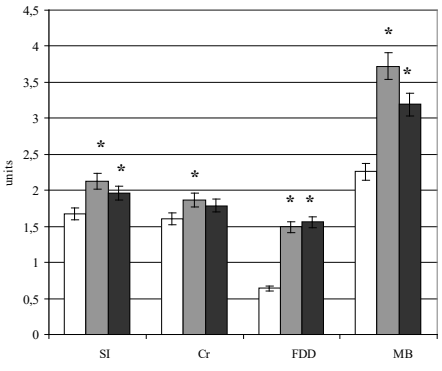

Fig. 1. Results of visuametric analysis of crystallograms of human blood serum under physical and emotional stress («*» – p<0,05 to calm state)
□ - calm state, ■ - physical exersices,
■ - emotional stress.

and included a complication of the structural organization of crystal elements (due to the predominance of dendritic crystal formations with a minimum number of single structures), an increase in their density in the facias in combination with an increase in the degree of their destruction. This was manifested in a statistically significant increase in the level of structure index, crystallizability and facia destruction degree, respectively (p<0.05). In addition, after the implementation of the veloergometric test, a significant expansion of the marginal zone of the micropreparation was observed, leading to an increase in the severity of the latter (MB), in which case the specified facias zone was wider not only compared with serum facies obtained at rest, but also under examination stress (p<0.05). Taking into account the fact that the marginal zone of the micropreparation is formed by protein macromolecules, we can assume an increase in the concentration of proteins in the biological fluid entering the blood with the intensification of metabolism in muscle tissue.

According to the results, emotional stress causes the formation of lesser shifts in the morphology of serum crystallograms (fig. 1). In particular, under examination stress there was a moderate but statistically significant tendency to complicate the organization of crystal structures (according to the structure index of facias; p<0.05), but it was less significant than during exercise. With respect to the crystallizability of the biological medium (an indicator characterizing the quantitative side of crystallogenesis) the increase in the parameter level is registered only at the trend level (p<0.1).

At emotional stress, as well as after the PWC 170 test, there was a significant expansion of the marginal zone of the micro-preparation of dehydrated blood serum (p<0.05), but in this case it was less pronounced than in physical activity. In our opinion, this may be due to the transformation of the protein spectrum of the biological fluid without a significant change in the level of total protein in the blood serum of the subjects in the pre-examination period.

The shifts are recording by the method of visuametric analysis of crystalloscopic facias were verified by their subsequent spectrometric investigation at wavelengths of 300, 350 and 400 nm. It is established that in both variants of stress the optical density of crystallograms increases, and in the case of PWC 170 load test this tendency is more distinct at all used wavelengths, statistically significantly differing both from the level of the indicator registered at rest and immediately before the exam (p<0.05). It should be noted that the increase in the optical density of facias in these cases is due to the increase in the number and complexity of the crystal elements that form the crystalloscopic picture of the biological fluid. Thus, multi-spectral data allow confirming the results parametric assessment of crystalloscopic facias at rest and in the stress response.

A comprehensive assessment of the initiating properties of blood serum in healthy individuals in the implementation of the metabolic response to physical stress and psycho-emotional stress was also carried out (fig. 3 and 4). It is revealed that, as in the case of crystallogenic activity of the biological substrate, in teziographic test under the stress-response observed an increase in the values of most visuametric parameters, but the degree of this trend varies depending on the form of the corresponding stress factor (fig. 3). In particular, according to the level of the main quantitative indicator (the tezioigraphic coefficient Q) directly after the physical activity, the increase in the initiator potential of the biofliud was recorded, as evidenced by a significant increase in this parameter (p<0.05). In contrast, in the pre-examination period no substantial changes of the density of the crystal structures in blood serum teziograms of evaluated persons is not revealed. It should be noted that the value of the criterion under consideration after physical stress was higher than before the exam (p<0.05). At the same time, both stressors contributed to the complication of the crystalline elements formed in the dried samples of biological fluid, which was indicated by a moderate increase in the level of crystallinity (p<0.05 for both cases). Similar dynamics took place in relation to the facia destruction degree as a nonspecific marker of "correctness" of crystal formation. In our opinion, the nature of changes in these parameters reflects the universal component of metabolic stress response [6, 9].

The features of the formation of the marginal zone of the micro-preparation of dehydrated blood serum under the influence of the studied stressors are generally similar to those found for the

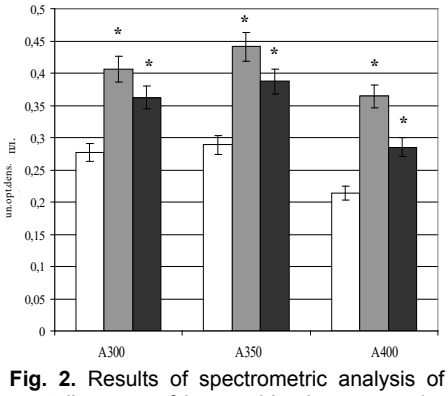

Fig. 2. Results of spectrometric analysis of crystallograms of human blood serum under physical and emotional stress («*» – p<0,05 to calm state)

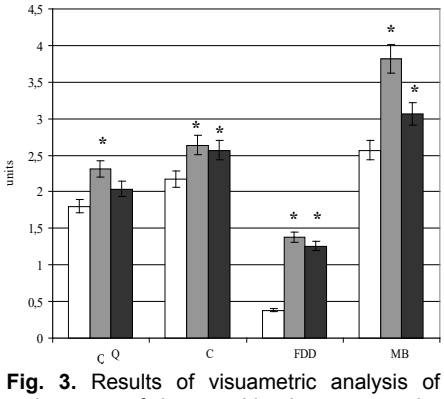

Fig. 3. Results of visuametric analysis of teziograms of human blood serum under physical and emotional stress («*» – p<0,05 to calm state).

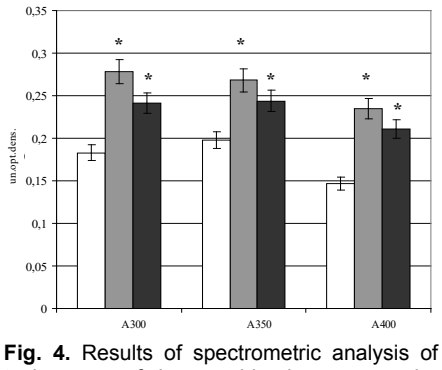

Fig. 4. Results of spectrometric analysis of teziograms of human blood serum under physical and emotional stress («*» – p<0,05 to calm state).

main teziographic coefficient, but this parameter increases statistically both after physical activity and in the pre-examination period ($p<0.05$). This allows us to assume the presence of rearrangements of serum proteome in both studied states, and when performing the PWC 170 test, these changes are more pronounced ($p<0.05$).

These shifts in the initiated crystal formation of blood serum, as in the case of crystalloscopic facias of biological fluid, are fully confirmed by the results of spectrometric studies (fig. 4). Thus, for all used wavelengths, serum tezigrams demonstrate a higher level of optical density compared to dried samples of the biological medium obtained from the same people in a calm state ($p<0.05$). At the same time at a wavelength of 300 and 400 nm the optical density of the facias of the blood serum of the subjects after exercise were significantly higher than that characteristic of the pre-exam period ($p<0.05$).

Conclusion. In whole, the study demonstrated the direction of shifts in crystallogenic and initiating properties of blood serum of students under physical activity and psycho-emotional stress, but the severity of modification varies significantly. This allows us to conclude that the teziocrystalloscopic picture of human blood serum can be an indicator of various physiological states, which is reflected in the changes in the morphology of dried micro-preparations of the biological fluid, indicating shifts in the qualitative and quantitative composition of the latter.

References

1. Дмитриева Т.Б., Воложин А.И. *Социальный стресс и психическое здоровье*. М., 2001 [Dmitrieva TB, Volozhin AI. *Social stress and mental health*. Moscow, 2001. (In Russ.)]

2. Епифанов В.А. *Лечебная физкультура и спортивная медицина*. М., 2007 [Epifanov VA. *Medical physical training and sport medicine*. Moscow, 2007. (In Russ.)]

3. Жигулина В.В. Биохимический ответ на стресс (обзор литературы). *Тверской медицинский журнал*. 2015;1:10. [Zhigulina VV. Biochemical response to stress (review). *Tverskoi Meditsinskiy Zhurnal*. 2015; 1:10. (In Russ.)]

4. Камакин Н.Ф., Мартусевич А.К. Современные подходы к кристаллоскопической идентификации состава биологических жидкостей. *Экология человека*. 2003;5:23-25. [Kamakin NF, Martusevich AK. Modern methods of crystalloscopic identification of biological fluids composition. *Ekologiya cheloveka*. 2003; 5:23-25. (In Russ.)]

5. Мартусевич А.К. *Биокристалломика в молекулярной медицине*. СПб Издательство СПбГМУ – Тверь. ООО «Издательство «Триада», 2011. [Martusevich AK. *Biocrystallomics in molecular medicine*. Saint Petersburg; Tver. Triada, 2011. (In Russ.)]

6. Мартусевич А.К., Сафарова Р.И. Информативность исследования кристаллогенеза слюны спортсменов-лыжников в прогнозировании результативности их выступления. *Вестник спортивной науки*. 2007;427-32. [Martusevich AK, Safarova RI. Informativity of saliva crystallization study of skiers in forecasting the impact of their presentations. *Vestnik sportivnoi nauki*. 2007; 4:27-32. (In Russ.)]

7. Ушаков И.Б., Орлов О.И., Баевский Р.М., Берсенев Е.Ю., Черникова А.Г. Новые технологии оценки здоровья у практически здоровых людей. *Российский физиологический журнал им. И.М. Сеченова*. 2013;99(3):313-319. [Ushakov IB, Orlov OI, Baevskii RM, Beresnev EYu, Chernikova AG.New technologies of health testing at healthy people. *Rossiiskiy fiziologicheskiy zhurnal imeni I.M. Sechenova*. 2013; 99(3):313-319. (In Russ.)]

8. Сорокин О.Г., Ушаков И.Б. Возможности и перспективы использования оценки адаптационного потенциала в практической медицине. *Экология человека*. 2005;10:11-17. [Sorokin OG, Ushakov IB. Posibilities and perspectives of the use of adaptive potential testing in practical medicine. *Ekologiya cheloveka*. 2005; 10:11-17. (In Russ.)]

9. Ушаков И.Б., Сорокин О.Г. Адаптационный потенциал человека. *Вестник РАМН*. 2004;3:8-13. [Ushakov IB, Sorokin OG. Human adaptation potential. *Vestnik RAMN*. 2004; 3:8-13. (In Russ.)]

10. Филаретова Л.П. Стресс в физиологических условиях. *Российский физиологический журнал им. И.М. Сеченова*. 2010;96(9):924-935. [Filaretova LP. Stress in physiological conditions. *Rossiiskiy fiziologicheskiy zhurnal imeni I.M. Sechenova*. 2010; 96(9):924-935. (in Russ.)]

11. Shabalin VN, Shatokhina SN, Yakovlev SA. Character of blood crystallization as an integral index of organism homeostasis. Phys. Chem. Biol. Med. 1995; 2(1): 6-9.

12. Yakhno TA, Yakhno VG, Sanin AG et al. The informative-capacity phenomenon of drying drops. IEEE Engineering in Medicine and Biology Magazine. 2004; 24(2):96-104.

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