

REVIEW

By Assoc. Dr. Sergey Nikolov Ranchev, in scientific direction 4.5. Mathematics-Biomechanics at the BAS Institute of Mechanics, according to the procedure for occupying the academic position "Associate Professor" in the field of higher education 7. Health care and sports, professional direction 7.6 Sports, specialty "Biomechanics" for the needs of the "Anatomy and Biomechanics" department at the National Sports Academy "Vasil Levski" with candidate ch. assistant professor Ivan Mirchev Ivanov, PhD.

Ch. assistant professor Ivan Mirchev Ivanov, PhD, was born on June 8, 1977. He completed secondary education in his hometown of Kazanlak. He graduated in engineering physics at the Faculty of Physics of SU "Kl. Ohridski" Sofia. After the competition, he continues his education as a full-time graduate student in Biomechanics in the Biomechanics department of the Institute of Mechanics of the BAS. He successfully defended his thesis with the topic: "Rheological properties of blood and their modeling" in 2008. He continues his research in the field of hemorheology as a researcher at the Institute of Mechanics of the BAS. Since 2014, he has been working as a teacher at the "Vasil Levski" National Sports Academy, at the "Anatomy and Biomechanics" department, where his research activities are expanding into other areas of Biomechanics. To the same department, in parallel with the participation in its scientific research activity, he leads lectures and exercises on Biomechanics to students and currently.

Those submitted for review by Ch. assistant professor Ivan Mirchev Ivanov, PhD, materials for the procedure include: Habilitation thesis - monograph "Sport and Hemoreology" in volume 154 pages and a set of 26 of his publications. They correspond and are related to the specialty and field of Higher Education announced in the procedure. The 26 publications can be conditionally divided into 7 groups according to objective and methodological features as follows: Group I- including works 1 and 2; Group II- including works 3, 4, 5, 9, 10, 11 and 12; Group III - including works 6, 7, 8, 23, 25 and 26; Group IV- including works 13 and 14; Group V - including works 15, 16, 17; Group VI- including works 18; 19 and 22; Group VII- including works 20, 21 and 24.

The monograph "Sport and Hemorheology" is structured in an Introduction and five chapters:

- Chapter I is entitled Hemorheology. It introduces the methodological apparatus of physics used in the description of chemorheological processes. The morphology and functions of blood cells are examined, as well as the main factors determining the rheological properties of blood. Newton's basic rheological law and the main models describing the rheological behavior of liquids are reviewed. The study of viscosity, as a complex characteristic of the investigation of a certain liquid, especially blood, is justified. The essence of specific biophysicochemical characteristics and processes in the blood flow is considered.
- In Chapter II Hemorheological changes during physical exertion, specific manifestations of the general regularities of the work of the cardiovascular and circulatory system of the body during physical exertion in sports are examined, and in particular the changes in: biomechanical properties of red blood cells are examined; basic blood plasma components (fibrinogen, albumins, globulins) in different training regimes in sports; changes in blood vessels and blood

flow; blood volume; changes in the endothelial cells of the vascular walls; blood pressure; hypoxia; hemorheological changes and their interaction during different motor activity.

- Chapter III Anti-inflammatory and metabolic effect of regular physical exercise - examines positive results of sports, on: the overall work of the body; reduction of visceral adipose tissue, as a factor provoking inflammatory processes; increased expression of interleukin 6 (IL-6); Increase in levels of circulating hormones (cortisol and adrenaline).
- Chapter IV Sodium nitrite (E250) and zinc (Zn) modulators of the hemorheological characteristics of blood and blood cells - examines changes in blood viscosity and hematometric indicators of erythrocytes under the influence of sodium nitrite; hematological and hematometric changes in leukocytes and platelets under the influence of sodium nitrite; influence of zinc on hemorheology.
- Chapter V Conclusion An abstract summary is made, listing the main results and conclusions in the previous chapters. It is tied to tasks for further research concerning the relationship between sport and hemorheology and has a programmatic nature in a certain sense.

In general, the monograph reflects both the author's own original research and the characteristics of an overview analytical study covering over 300 sources reflecting the main ideas, approaches and results in the indicated field. For the conditions of our country, this research presented in this way has a pioneering character. The way the text is constructed is such that highly specialized mathematical training is not required to work with it. This has been achieved with excellent visualization, through a graphical representation of the nature and trends in the established quantitative assessments of the investigated relationships, processes, relationships, etc. And all this despite the fact that an extremely complex matter is presented, the product of qualitative and quantitative chemorheological studies, imposing using complex and heavy mathematical apparatus. A fact that is a certificate of the author's good pedagogical sense and approach in conveying highly specialized complex knowledge.

Publications 1 and 2 of Group I are oriented to the experimental study of the relationship of blood viscosity with the process of formation and growth of blood clots (dynamics of thrombogenesis (hemocoagulation)).

- In the conditions of the hypothesis that the blood flow is a flat stationary flow of an incompressible fluid, the increase in blood viscosity over time with an increase in the development of the coagulation process was tracked according to three parameters: duration of the entire process over time; clot initiation time and clot formation time. A directly proportional relationship between hematocrit levels and the development of the hemocoagulation process was established.
- The kinetics of clot formation is studied by changing a set of blood components - hematocrit, fibrinogen concentration and temperature of the environment in which the hemocoagulation process develops, by using viscoelastic and dielectric protocols. It was found that increasing the temperature accelerated the formation of clots, but decreased their hardness. The results of experimental rheometric and ROTEM studies of the thrombogenesis process correlate well.

Publications 3, 4, 5, 9, 10, 11 and 12 of Group II are aimed at researching the rheological and electrical properties of erythrocytes in experimental conditions (interaction with dextran, PEG, glutaraldehyde, polymer nanoparticles (macromolecules of polyacrylic acid with different

molecular weight and architecture, as well as stabilized polymer micelles with a mixed shell of PAA and polyethylene oxide blocks). The measurements were based on a Contraves Low Shear 30 rotary rheometer, a device with a data acquisition system, Rheoscan 100 software and program. Objectives set and experimental results obtained in this group of studies:

- The study presents an electrorheological method for the quantitative determination of microstructural changes in blood due to aggregation-disaggregation of red blood cells and deformation processes at different shear rates and at different local structure of the flow field. The results show that the specific electrical conductivity of blood and erythrocyte suspension is highly dependent on shear rate, hematocrit, temperature, and blood flow modes used.
- Conductivity is investigated as a function of time and shear rate, in parallel with the rheological properties of the samples in transient flow regimes with different local structure (Couette flow - in it the blood flow has anisotropic properties, as in stationary blood flow between two coaxial cylinders with an increase in the strain rate, the specific electrical conductivity increases in the direction parallel to the cylindrical shear planes and decreases across the flow. With the performed effusions, it is proved that the morphological erythrocyte characteristics influence the electrical and mechanical properties of blood.
- Mechanical and electrical properties of normal suspensions of red blood cells solidified after treatment with glutaraldehyde in isotonic saline with added dextran, polyethylene glycol, and all suspensions were adjusted to a hematocrit of 40%. The results show that the shear viscosity and conductivity of RBC suspensions in dextran and PEG are strongly influenced by flow, shear rate, concentration, cell deformability, and morphology. The method used is sensitive for studying the mechanical and electrical properties of erythrocyte suspensions, as well as for the experimental description of red blood cells.
- The calibration of the used experimental system, when measuring the viscosity, as well as the specific electrical conductivity of the analyzed liquid, is discussed. They are determined simultaneously at different strain rates and different temperatures. With the proposed calibration method, the correspondence equations between the measured quantities obtained in relative units and their real values were determined.

Publications 6, 7, 8, 23, 25 and 26 of Group III refer to hemorheological changes of hematometric characteristics of erythrocytes under the influence of toxic substances - sodium nitrite (E250); various metal salts - cadmium acetate, cobalt fluoride, etc.

- The influence on the rheological properties of blood and erythrocyte hematometric indices (hemoglobin-Hb, hematocrit-HCT, number of erythrocytes-RBC, mean cell volume of erythrocytes- MCV, volume index of heterogeneity of erythrocytes- RDV, total amount of hemoglobin in a certain volume- MCH, amount of hemoglobin in a single erythrocyte- MCHC) in the acute treatment with Sodium nitrite (NaNO_2) of rats. Sodium nitrite, also known as food additive E250, is a precursor to various organic compounds (pharmaceuticals, dyes and pesticides). The reported quantitative values of hematometric parameters in the experimental model may be a suitable marker for predicting sodium nitrite intoxication and methemoglobinemia in animals and humans. The obtained results help to clarify the influence of sodium nitrite on the morpho-functional properties of blood cells. Acute treatment with sodium nitrite produces different effects on white blood cell and platelet counts.

- The vasodilating action of nitrites is used by athletes to improve performance. The aim of the study was to investigate the influence of acute treatment with sodium nitrite on hematometric parameters of white blood cells and platelets in an experimental model in rats. The obtained results help to clarify the influence of sodium nitrite on the morpho-functional properties of blood cells. Acute treatment with NaNO_2 shows various effects on white blood cells and platelet counts, it has negative effects on immunity and blood cell aggregation.
- The influence of metal compounds: cadmium acetate $[\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}]$, cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) and sodium nitrite (NaNO_2) on the rheological properties of blood is investigated, through the factors that determine them: number and volume of blood cells, their membrane and morphological properties, etc. when treated with them in rats. Changes in the rheological properties, viscosity of the blood and plasma of the treated rodents were observed when compared to a control group of untreated rodents, depending on the species, the concentration of metal salts, the species of the rodents and the type of treatment. The observed changes in blood and plasma viscosity were confirmed morphologically by studies of the same blood samples performed at the same time.
- The study evaluated the effect of chronic treatment with cadmium (Cd) in combination with reduced and therapeutic doses of monensin on hematological parameters and its relationship with rheological characteristics in mice. Hematological parameters and erythrocyte morphology were assessed with whole blood viscosity (WBV). Cadmium treatment decreases hemoglobin and increases the volumetric index of erythrocyte heterogeneity. Addition of a high dose of monensin significantly improved erythrocyte indices in comparison. Correlations were found between WBV and RBC, Hb, Hct, MCV and RDW. The results indicate that hemorheological parameters such as WBV should be monitored in parallel with hematological parameters when monensin is administered and heavy metal intoxication is suspected.
- Changes in erythrocyte morphology induced by addition of cobalt chloride (CoCl_2) to blood at different temperatures are poorly studied. The aim of the study was to investigate the in vitro effect of CoCl_2 on erythrocyte morphology and characteristics of red blood cell suspensions after incubation at different temperatures. CoCl_2 and temperature were found to affect erythrocyte morphology and indices, probably by inducing structural, biomechanical and biochemical changes in the erythrocyte membrane.
- The effect on the hematological and hemorheological parameters of rats was investigated by acute treatment with sodium nitrite (NaNO_2) by a single intraperitoneal injection of 50 mg/kg body weight, after which the hematological and hemorheological parameters were measured over time as follows: 1 hour, 5 hours, 24 hours, 48 hours and 5, 10, 20 days after injection. Acute treatment with NaNO_2 has been found to induce significant haematological and rheological changes, therefore these should be monitored during high dose exposure or prolonged treatment with the compound.

Publications 13 and 14 of Group IV relate to a study of the stabilometric stability of the body in air pistol shooting.

- The stabilometric resistance of the shooters' body when shooting with a pneumatic pistol is investigated. The study tracks the fluctuations of the general center of gravity (CGG), while simultaneously recording the trajectory of the weapon's crosshairs when fired. A direct relationship was established between the sagittal oscillations of the OCT and the vertical track

of the sight when firing. A new quantitative criterion for evaluating this relationship has been found and defined. A quantitative criterion for "sight stability" is also proposed based on the analysis of shooting, through the Russian electronic shooting training system "SKATT". The obtained results have applications in the selection and training of young shooters, as well as in the training process and improvement of experienced shooters.

- In this study, the relationship between the kinesthetic stability of the body and the specific technique of self-control of the shooter's body when shooting is sought. A parallel recording of the balance stability of the shooter, obtained from the balancing platform, and the oscillations of the sight - through the electronic shooting training system "SCATT" was made. A comparative study was performed between the results obtained by the two methods, showing a significant correlation between the SCATT results in the straight shot position and the spatial parameters of the body.

Publications 15, 16 and 17 of Group V are co-authored by the author of this review, therefore I will allow myself to note only the subject of research and the research approach, without commenting and evaluating the results obtained. The work of synovial joints in the mode of isometric stretching was studied. The research was carried out using the following methods: direct measurement, using nuclear magnetic resonance, joint echography, as well as literature data - an overview of the solved problems and the research methods used for the purpose.

Publications 18; 19 and 22 of Group VI are essentially a hemorheological study and analysis of blood and blood cells of patients with type-2 diabetes.

- The aim of the study is to evaluate the rheological properties of blood in patients with type 2 diabetes in comparison with hemorheological parameters with a control group of healthy people. Hemorheological variables including hematocrit (Ht), fibrinogen (Fib), and whole blood viscosity (WBV) at shear rates from 0.0237 s⁻¹ to 128.5 s⁻¹. The results of this study confirm a significant increase in patients' blood viscosity (WBV) compared to controls over the indicated range of shear rates. This is due to the increased values of hematocrit, fibrinogen and erythrocytes. Rheological phenomena, such as increased aggregation and reduced deformation of erythrocytes, also contribute to increased blood viscosity, which leads to changes in the blood flow of blood vessels in diabetes.

- In the study, the disturbances in skin temperature after cold stress in patients with type 2 diabetes are monitored and to evaluate the dynamics of changes in temperature fluctuations corresponding to myogenic, neurogenic and endothelial regulation of vascular tone. Significant correlations were obtained between the increased blood viscosity over the entire range of shear rates and the values of skin temperature fluctuations in the three frequency ranges in the patient group. Spectral analysis of variations in skin vascular tone provides useful additional information on regulatory mechanisms affecting skin microcirculation.

- Given the hemorheological disorders induced by type 2 diabetes mellitus, leading to pathological changes in whole blood viscosity (WBV), changes in blood coagulation and changes in thrombogenesis, the kinetics of induced in vitro blood clots in patients and in healthy donors was investigated. Specific to this research is the application of the modern method of atomic force microscopy (AFM), which characterizes very well the blood cells and their participation in thrombogenesis in patients with diabetes mellitus. The results showed that clot

formation in patients was accelerated at the applied low shear rate. WBVs at full coagulation in patients are higher than in healthy individuals. Morphological characteristics and functional transformations of erythrocytes (RBCs) and platelets in the experimentally formed shear clots studied with ACM indicate that mainly "activated" platelets form the experimentally induced clots in the patients. Atypical erythrocytes (echinocytes, stomatocytes, target-like erythrocytes, etc.), poikilocytosis, and red blood cell anisocytosis are also seen in patient-formed clots.

Publications 20, 21 and 24 of Group VII are single studies, which have in common that they do not belong to any of the above groups.

- The first of this group of works is related to an experimental assessment of the relationship between kinematic and anthropometric parameters with the effectiveness of selected fighting techniques for a representative group of elite athletes. Parameters for the assessment are selected objective indicators of body composition (weight, water content, body fat, muscle mass, index of adipose tissue of internal organs, index of physical condition, bone mass, basic metabolic rate and metabolic age. The kinematic parameters observed in the experiment are the execution of the Gyaku-Tsuki techniques for the upper limbs, and Mae-Geri and Yoko-Geri for the lower limbs. The analysis of the relationship between the selected parameters and the evaluation of the performed techniques gives a significant relationship in karate athletes between body mass, percentage of muscle mass and bone mass and metabolic rate, as well as a negative correlation between fat mass and percent water content. The results of the study are applicable to the selection of karate athletes.

- The purpose of the research is the possibilities of ultrasonic methods for measuring and analyzing a set of characteristics of motor oils. These opportunities are related to improving: the quality of service; car service expertise; the oil film thickness information in the piston-cylinder group, ball and hydrodynamic bearings. The results include a description of ultrasonic methods for measuring the thickness of the oil film in the piston-cylinder group, ball and hydrodynamic bearings, measuring the tribological and rheological properties of the oil, assessing the oil level in the engine, measuring the temperature and the presence of air bubbles in the lube oil supply lines and crankcase, oil viscosity profile in thrust bearing. All this has to do with analysis and diagnostics of the technique in automobile and motor sports.

- The work investigates in vivo dissolution of crystalline insulin forms in the subcutaneous therapy of diabetes. Dissolution is an important step in the subsequent absorption of insulin. The dissolution rate of insulin crystals was found to depend on the composition of the flooding liquid and the type of crystals, with the effects of pH and flooding rate being less pronounced. The results of the conducted research suggest the possibility of targeted control over dissolution rates in vivo by developing specific formulations based on insulin crystals in order to personalize insulin therapy, which is a trend in the modern approach to drug therapies.

In the applicant's attached materials, contributions are listed - the result of the research activity carried out. Here they are listed in an abbreviated version:

- The statistically significant relationship between the change in the rheological and electrical properties of normal blood and blood coagulum has been experimentally proven. It has been shown that this relationship strongly depends on the strain rate, temperature and concentration of various additives in the studied blood samples (nanoparticles, Dextran, PEG).

- It has been experimentally proven that by measuring the specific electrical conductivity σ over time of a coagulating blood sample, it is possible to estimate and characterize parameters (t_d , t_i and t_s) describing the kinetics of coagulation. This confirms the diagnostic value of the measurement.
- A series of studies have shown that acute intraperitoneal injection of NaNO_2 has an opposite effect on leukocyte and platelet populations. After the treatment, the number of phagocytizing cells - monocytes and granulocytes - decreases statistically significantly, which logically leads to an increased susceptibility to infections. Hematometric indices for platelets showed a significant increase in PLT count and PCT. These results suggest that NaNO_2 affects platelet activity, altering the coagulation status of blood.
- Results of experimental studies prove that hemorheological parameters such as whole blood viscosity (WBV), plasma viscosity (PV), as well as the main hematometric indices (Hb, HCT, MCV, MCHC and others) are suitable quantitative biological markers for monitoring of nitrite intoxication in medical toxicology.
- An algorithm was created for the calibration of an experimental system for determining the specific electrical conductivity of blood, based on a LowShear Contraves rotary viscometer.
- An analytical equation was derived to calculate the actual values of the specific electrical conductivity σ of the tested sample in millisiemens per centimeter, mS/cm.
- The influence of two types of nanoparticles on the rheological and electrical properties of erythrocyte suspensions has been experimentally proven. The research conducted and the results obtained lead to the following conclusions:
 - At shear rates above 11.02 s^{-1} , pure PAA solutions show a statistically significant increase in shear viscosity compared to the micellar solution or physiologic- resulting from the interactions between the rod-like linear chains oriented along the shear flow lines;
 - Structure, concentration and shape ???are important factors affecting the rheological and electrical properties of an erythrocyte suspension. Linear PAA nanoparticles affect the rheological behavior of red blood cells to a greater extent than stabilized spherical micelles, a fact that should be considered in their biomedical application.
- The direct relationship between the shooter's balance stability and the results achieved when shooting with a pneumatic pistol has been experimentally proven.
- A new quantitative criterion for sight stability in airgun shooting based on an electronic shooting training system has been introduced.
- Physical loads of different frequency, intensity and duration cause a different hemorheological (strictly individual) response in athletes. The author's contribution in this case consists in the synthesis and structuring of changes of different nature and degree (positive or negative, strong, weak, moderate) in the biomechanical and fluid properties of blood and blood cells: erythrocyte deformability; erythrocyte aggregation; change in the concentration of plasma components: fibrinogen, albumins, testosterone, globulin, etc.; changes in blood flow (via vasodilation and change in overall blood viscosity); changes in blood volume; changes in the endothelial cells of the vascular walls; changes in blood pressure; changes resulting from tissue hypoxia; interacting, different in nature hemorheological changes.

- The candidate's monograph "SPORTS AND CHEMOREOLOGY" describes: original author's models of hemorheological changes; as well as experimental results of the candidate contributing to the successful adaptation of the training programs to improve the health status of the participants and to optimize the sports form of the elite athletes (athletes) practicing individual or collective sports.
- An experimental model was developed to study the influence of isometric stretching on intra-articular spatial characteristics of the knee joint.

I completely agree with the applicant's claims. Outside of my agreement, as evidence of these contributions, sources and scientific forums where they have been approved are indicated, 62 citations of the results obtained by researchers from national and international colleges working in the fields of biomechanics and chemorheology are attached.

In conclusion, I believe that with the presented materials and the results reflected in them for participation in the procedure for the occupation of the academic position "Docent" in the field of higher education 7. Health care and sports, professional direction 7.6 Sports, specialty "Biomechanics" Dr. Ivan Mirchev Ivanov, convincingly proves that he meets the requirements for holding this position, according to the provisions of ZRASRB, PPZRASRB, as well as the "Regulations for the acquisition of the scientific degree "Doctor of Sciences" and for holding academic positions in the National Sports Academy "Vasil Levski ". Because of which:

I recommend the respected Academic Council of the National Sports Academy "Vasil Levski" TO CHOOSE Dr. Ivan Mirchev Ivanov as "Associate Professor" in the field of higher education 7. Health care and sports, professional direction 7.6 Sports, specialty "Biomechanics" at the department "Anatomy and Biomechanics" of the National Sports Academy "Vasil Levski".

16.03.2023,

Sofia

Reviewer:.....

/ Assoc. Prof. Dr. S. Ranchev/