Indian Journal of Experimental Biology
Vol. 45, January 2007, pp. 32-40

Hemorheological changes in microcirculation: Their mechanism and measurement technique

George Mchedlishvili

Microcirculation Research Center, I.Beritashcili Institute of Physiology,
Tbilisi, Georgia

Blood fluidity in the capillaries is affected significantly in diseases such as cardiac and brain infarcts, diabetic gangrene and many others. In view of the importance of physiology and pathology of capillary circulation, the hemorheological characteristics of the capillary blood flow are discussed in this article. Also, a new diagnosing technique for blood fluidity disorders is proposed. A computerized system for image analysis and determining blood rheological disorders for clinical and experimental use has also been discussed.

Keywords: Hemorheological disorders, Investigation techniques, Microcirculatory changes

Availability and state of the red cells in blood where they compose almost half of its volume and are commensurable to the capillaries diameters, represent themselves the principal factor determining the blood's specific fluidity in the most narrow micro-vessels, the capillaries. These hemorheological phenomena are most specific just for these vessels, as given by the Poiseulle'e law $Q = K(\Delta P \times D^4)/L$, where Q is the blood flow in the microvessel, ΔP is the pressure gradient along their lumen, D is the microvessels diameter, and L is their length. Accordingly, the resistance to flow is specifically high in the capillaries, leading to the pathogenesis of many essential diseases, such as the cerebral and cardiac infarcts, arterial hypertension, lower extremities gangrenes, acute inflammation in various tissues, and many others. Despite their significance for the development of these essential diseases, the pathogenic mechanism of the microcirculatory disorders remains so far to a great degree insufficiently understood. Therefore further analysis of blood flow in these vessels has been chosen as the topic of the present article.

Principal determinant of blood fluidity disorders in the capillaries

The red cells compose almost half of the circulating blood volume, while the white cells are thousands times fewer in number and much less essential from the point of view of blood fluidity in the capillaries. Therefore, it is just the red cells that represent the most significant factor determining specificities of the hemorheological properties and disorders in the micro-vessels. These vessels represent the most significant factor in the development of many micro-circulatory disorders and various human diseases since no compensation, i.e. neither collateral blood supply, nor reactive hyperemia can be efficient in this latter case.

The role of red blood cells is very significant for the hemorheological disorders, since these cells undergo specific changes inside the capillary leading to a significant rise in blood flow resistance. It is primarily the red blood cell aggregation, when these form rouleaux or are sticking together chaotically inside the microvessels lumen. This results in loss of the blood fluidity, leading to a significant slowing down of the capillary flow and development of blood flow stasis. Under these conditions the microvessels lumen is preserved, associated with sufficiently high pressure gradient inside the appropriate microvessels network.

The phenomenon of RBC aggregation causing the significant slowing-down of the blood stream up to a complete stop of flow inside the capillary lumen was first described about one-and-a-half century ago[1,2]. Since then many details of this phenomenon by the researchers have been specified[3,4]. Such a phenomenon occurs even under the conditions when the blood flow is preserved in both afferent and

Address for correspondence—
Head, Microcirculation Research Center
I. Beritashvili Institute of Physiology
14 Gotua St., 0160 Tbilisi, Georgia
E-mail: geomched@caucasus.net

efferent microvascular bifurcations, thus evidencing that the pressure gradient in the microvascular networks is still maintained. Therefore, the immediate cause of the blood flow stoppage is just the sharp increase of the local blood flow resistance inside the capillaries lumen.

By recent experiments, convincing evidence about the local intravascular RBC aggregation that produces the capillary blood stasis has been demonstrated. For this a microscopically small NaCl crystal, attached to a capillary wall with a preserved blood flow, produced immediate stoppage of a local blood flow. Subsequent wash off of the crystal regularly caused restoration of the blood flow in the appropriate microvessels. This occurred under the conditions when the blood flow remained preserved in all the afferent and efferent capillary branches, providing convincing evidence that the blood flow stoppage during the capillary stasis develops strictly locally. Under these conditions the arteriolar-venular pressure gradient certainly remained preserved. From this it was evident that the local blood stasis was immediately produced by the blood fluidity disorders inside the capillary lumen, which in turn was caused by the local RBC aggregation. Another evidence for the existence of such a mechanism is the sharp rise in resistance associated with the absence of blood outflow from a capillary as observed by cutting the microvessel close to the RBC aggregate and examined under the microscope. Both the phenomena provided evidences without any doubts during discussions by the specialists[5,6].

The red blood cells are commensurable to the blood capillary lumen and to that of the adjacent smallest arterioles and venules. Therefore, blood flowing inside all the microvessels cannot be considered as a true fluid. Under the normal and even more under the pathological conditions the capillary blood flow is regulated by the rules other than the well-known ones in the hydrodynamics and hydromechanics. Investigations on the microcirculation have demonstrated that it is just the red blood cell flow structuring that determines the blood fluidity in the microvessels[7]. The blood flow structuring is the manifestation of the blood self-organized behavior in the capillaries that is determining the blood rheological properties associated with minimum energy of the flowing blood in the microvessels lumen. Changes of the normal structuring of the erythrocytes flow is related primarily to their enhanced aggregation, reduced deformability, enhanced local hematocrit and elevated blood plasma viscosity. For efficient diagnosing of the microrheological blood properties new laboratory techniques have been developed and implemented.

Both the blood coagulation and the hemorheology are related fields of biomedical research, but they are, to a high degree, independent disciplines. These are dealing with the investigations of the properties of the blood that are different by their essence. Blood coagulation is related to origination of thrombosis, whereas, the blood fluidity in the microvessels can undergo changes, which are others than the coagulation system.

Hemorheological specificities of blood flow in the macro- and micro-vessels

For homogeneous fluids, independent of their viscous qualities, there is no principal difference in the fluidic properties during flow in tubes of various diameters. As to the blood, it can be considered as a typical fluid while flowing through larger arteries and veins, as this obeys the well-known rules of hydrodynamics. The viscoelastic properties of blood can be measured with various viscometers and the results are applicable to large arteries and veins. But these rules of classical hydrodynamics are not applicable when blood flows through capillaries, as well as in the adjacent arterioles and venules of luminal diameter less than 15-20μm. This is primarily attributed to non-homogeneous nature of blood flow in microvessels; as half of its volume is composed of cellular elements, of size larger than of the capillary lumen. These cells are invariably deformed-stretched along the microvessels, when they are advancing inside the lumen. This impedes the blood flow under the pathological conditions as the mechanical properties of erythrocytes are significantly lowered. This effect is even more pronounced during various pathologies when the erythrocyte's mechanical properties undergo significant changes due to their aggregation process inside the capillaries lumen.

The phenomenon of the erythrocyte's mechanical properties attracts much attention of the researchers and practical doctors. Several aspects related to pathologies and to the possibilities of the therapeutic effects on the blood rheological properties still remain unsolved or insufficiently analyzed. From the point of view of hemorheological disorders, in addition to the minimal size of the blood capillary lumen, peculiarity

of the structure and function of the microvessels is very significant. Primarily it is the absence of true muscular elements inside the capillary walls and of their specific vasomotor activity. Therefore, the hemorheological disorders, being of a high significance for the medical practice, are related to the peculiarities of the blood flow just in the microvessels. For better understanding of these phenomena the physical regularities of flow of the homogeneous fluids cannot be applied in these cases, since the blood, which is advancing inside the capillaries lumen is actually not a fluid in a true meaning. Hence new scientific approaches are to be found for the investigation of the blood flow and its derangements inside the microvessels.

Microvascular blood stasis: A model of rheological disorders in the capillary lumen

The problem of unexpected stoppages of blood flow in individual capillaries has attracted the attention of scientists for a long time. From the second half of the 19th century various concepts are framed on the mechanism of the blood capillary stasis Its cause was supposed to be different; a pronounced constriction of the arterial walls together with a considerable dilatation of the capillaries, pronounced concentration of the blood inside their lumen, the red blood cell aggregation, enhanced friction of the blood at the microvessels walls, etc[3]. All these concepts were not based on convincing experimental evidence and have now preserved only as a historical significance.

Since 1950s Mchedlishvilli[8] has carried out a careful experimental analysis of the possible causes of the blood stases development in the capillaries. It was, in particular, proved that the local capillary stasis develops under the conditions when the microvessels lumen does not decrease (on the contrary it has a tendency to be enlarged) and the pressure gradient inside the microvessels can be preserved or be even enhanced. During development of local capillary blood stasis the blood flow stoppage represents itself a locally developed hemorheological phenomenon due to enhanced flow resistance, exceeding significantly its original value, leading to lowering of the blood pressure inside its venous part to zero. The increased blood flow resistance inside the capillaries could be dependent in such cases only on the disturbance of the blood fluidity in the microvessels lumen, as has been demonstrated in the capillaries of transparent organs of the small laboratory animals. This evidenced that the blood stasis development is related under these conditions to the enhanced intravascular erythrocyte aggregation, observable inside the capillaries in the regions of the primary blood stasis development.

The primary blood stases originate most readily at the loci of the highest hemodynamic resistance, such as curvatures of the capillaries, where the blood flow is usually detained and the erythrocyte aggregates are readily originated and stick to the microvascular lumen. Under these conditions the aggregate size is further increased due to involvement of individual erythrocytes. In this way the loci of the primary stases originate in the capillary networks. The blood stases originate most readily during the venous blood stagnation, as well as during ischemia, although the low concentration of red blood cells in the capillary networks interferes with the aggregation of erythrocytes in ischemia. During recovery from the blood capillary stases it could be clearly seen as to how the erythrocyte aggregates slowly advance towards the venules, and as soon as they reach the larger microvascular lumen of the venules the blood flow velocity rapidly increases inside the microvessels. Thus, the analysis of the microcirculatory events in the capillary networks has clearly demonstrated that the capillary blood stasis is a purely local microcirculatory phenomenon related just to the disturbance of blood fluidity in the microvessels lumen[3,8].

Blood flow structuring as a theoretical basis of its fluidity in the microcirculation

The basic role in determining the rheological properties of blood in the microcirculation is contributed by the erythrocytes. During the normal blood flow inside the microvessels the erythrocytes are distributed in their lumen not chaotically but they advance in a certain order. This is composed primarily of the red blood cells axial flow and the parietal sheet of plasma. Such is the normal blood flow structure even in the narrowest capillaries, where the erythrocytes are usually deformed and forced in a stretched state along the microvessels lumen. The significance of the parietal plasma layer was explained in the 19th century by Poisuille. According to Poisuille parietal plasma layer creates an optimal velocity gradients between the vessels walls (where an immobile plasma layer is always present) and the

axial blood flow, which advances comparatively fast[9]. The width of the parietal plasma layer becomes decreased with slowing down of the flow and disappears during full stoppage of the flow in the microvessels lumen. During the normal flow velocity the red blood cells are always deformed inside the capillaries. To a significant degree this is dependent on the state of the microvessels' interior membranes.

Such a self-regulated advancement of erythrocytes in the microvascular lumen was identified as the "blood flow structuring", and its expression – as "the dynamic structure of the blood flow"[7,10]. Its character inside the microvessels is dependent on the sizes of both the cells and of the microvessels lumen. All the physiological and pathological phenomena of the blood flow in microvessels are primarily related to the pressure gradient and, therefore, to the red blood cell displacement in the microvessels of the terminal vascular bed. Reduction of the pressure gradient results in disturbance of the normal behavior of erythrocytes in the microvascular lumen. All the blood microrheological phenomena are very essential for the practical medicine, since it causes no doubts at present that the disorders of the blood rheological properties in the microcirculation play a key role in development of the inflammatory processes. These include the pathogenesis of widespread diseases such as the arterial hypertension[11], ischemic brain infarcts [5,7,12]; Raynaud's phenomenon[6]; diabetus mellitus[13].

The hemorheological disorders in the microcirculation possess certain peculiarities, as compared to the circulatory disturbances in the larger blood vessels. In particular, the blood flow disorders can be compensated in blood vessels due to collateral blood flow or changes of the systemic arterial pressure, especially in the cerebral circulatory bed, where a pronounced abundance of interarterial anastomoses are well developed and physiological mechanisms of compensation of various circulatory disorders are evident[14]. In the microcirculatory beds this embraces steadily even a larger amount of the capillary segments, since the local hematocrit increases in the single capillaries and the blood fluidity becomes disordered inside their lumen, thus resulting in a full arrest of the blood supply to the surrounding tissues, and decreasing the oxygen supply and the metabolic provision to the tissues. Because of an un-remittance of the capillary networks in the majority of the organs, in addition to the "rheological occlusion" the blood flow stops even in the single capillaries.

Basic laboratory indices of blood rheological disorders in microvessels

The laboratory indices of blood rheological properties of blood samples cannot naturally be fully identical with those of blood flow structuring in the living microvessels that determine the blood fluidity in the microcirculatory beds[10]. Various parts composing the blood play naturally a dissimilar role in its structuring during normal blood flow conditions and especially during various blood flow disorders in the microvascular lumen. As mentioned above, the erythrocytes, which compose about 40-45% of the blood volume (referred to as hematocrit) under the normal conditions, play most significant role. This is usually determined in the venous blood samples of patients or experimental animals by their blood centrifugation. But the value so obtained reflects only the mean hematocrit in the systemically circulating blood, which undergoes significant changes in various parts of the blood circulatory bed. The scientific discovery of the phenomenon of the local hematocrit changes[14] was approved by the. International Academy of Authors of Scientific Discoveries (No. NA-188; 15 January 1990). As to the other cellular elements of the blood, primarily the leukocytes, may have significant role only in special cases, for instance, when they are adhered to the walls of the smallest veins in the inflammatory foci. The role of thrombocytes in the changes of blood rheological properties outside the process of the thrombi formation is insignificant because of their very small sizes and a high deformability in the flow. The basic factors, which may cause disturbance of the blood rheological properties in the microcirculation during various pathologies are: (a) enhanced RBC aggregation; (b) lowered RBC deformability; (c) high local hematocrit; and (d) enhanced blood plasma viscosity[10].

The enhanced RBC aggregation is certainly the most significant factor disturbing the blood flow normal structuring and, therefore, the blood rheological properties in microvessels[5]. It was described as an example of the blood stasis in single capillaries[12] and the same has been demonstrated in recent studies.

It is necessary to distinguish in such cases the notions of "aggregation", a phenomenon of red blood cell specific pasting together and of the "aggregability", i.e. the ability to form aggregates under certain conditions. It is known that the term

"aggregation" was first proposed by Fahraeus[15] for forming specific rouleaux of the erythrocytes, as it has been used till now by the majority of authors. At a certain period of time there was an attempt to distinguish it from the English term "sludge" when the erythrocytes stick to each other chaotically and form blocks of various sizes and forms[16]. However, these two phenomena are actually identical, since there is no convincing evidence that the nature of these two kinds of erythrocytes pasting together is different (unlike the erythrocyte agglutination, dependent on a specific interaction of antibodies and antigens on the red cell surfaces). The majority of hemorheologists consider the adhesive macromolecules (such as fibrinogen, immunoglobulin, and others) associated with the same mechanism, which is different only by the intensity[17]. As has been shown by Fahraeus[15], the force in-between the erythrocytes can be quite different.

The enhanced intracapillary erythrocyte aggregation is accompanied by an increase of local hematocrit in appropriate microvessels, and this may promote appearance of secondary stasis in the microvascular networks. Under the conditions when there is a steep pressure gradient and fast blood flow velocity, the erythrocyte aggregation cannot be disturbed by itself. Then blood flow even promotes the axial shift of erythrocytes and enlargement of the parietal plasmatic layer[18].

The mechanism of the enhanced intracapillary erythrocyte aggregation was investigated usually in experiments *in vitro*. It was established that the aggregation is related to the availability of high molecular, as well as structurally anisometric protein molecules of the fibrinogen type, which combine the membranes of individual erythrocytes in the blood plasma[17,19]. Similar effects with high molecular dextrans have been observed. It was also demonstrated that different adhesive molecules combine the erythrocytes by various ways[20].

During various pathologies, such as arterial hypertension and ischemic brain infarct, the RBC aggregation is significantly enhanced[11,21]. Therefore the hyperfibrinogenemia is well-reasonably considered as a significant risk-factor that can cause appropriate clinical disturbances during these diseases[19]. Such a pathological mechanism may, in all probability, be responsible for the hyperfibrinogenemia in single capillaries and cause, in turn, the local increase of intravascular aggregation of erythrocytes due to abnormal increase of water outflow and of the transmitting of the low-molecular proteins from the microvessels into the surrounding tissues[8]. An analogous phenomenon is also evident during acute inflammatory processes when blood stases develop in many capillaries[3]. In general the effects of local hemorheological disturbances leading to development of blood stases in singular microvessels may be even more pronounced than the systemic enhancement of erythrocyte aggregation, which is usually found in the venous blood during various pathologies.

The enhanced rigidity (the lowered deformability) of the red blood cell membranes may also be a factor that disturbs the normal structuring of the red blood cells in microvessels. However convincing experimental evidence for the significance of this factor in the disturbances of blood rheological properties during various pathologies are still restricted. This was clearly shown only during pronounced decrease of the erythrocytes deformability caused artificially by diamide in experimental animals[22]. The effect of lowered deformability of erythrocytes on microvascular blood flow was shown in diabetic patients[23]. These data evidence that the lowered deformability of red blood cell is a factor that disturbs the rheological properties of blood in the microcirculation much more significantly than their aggregability.

The raised blood plasma viscosity is usually related to enhancement of high molecular compounds, in particular, fibrinogen, cholesterol, immunoglobulins, and gamma-globulins in the blood plasma[19]. The high blood plasma viscosity can disturb the blood structuring in narrow capillaries, especially in the brain cortex, where in addition to axial flow of the significantly deformed erythrocytes there is only a narrow parietal layer of blood plasma[14].

Clinical appraisal of blood rheological properties in patients

During consideration of this problem it is necessary to take into account that such parameter as blood viscosity detected with various kinds of viscometers and photometers is not well-grounded for the blood capillaries. If the notion "blood viscosity" for the blood circulation system is permissible, this is only for the blood flow analysis in larger vessels, but not for the whole circulatory bed, and especially not for the microcirculation.

Specific techniques of laboratory investigations of blood rheological properties could not naturally be the subject of the present article. Therefore only some principles, which would help to the laboratory workers to appraise various techniques in this field[7] have been covered here. Since for development of various pathologies the hemorheological disturbances are primarily significant which develop in the microcirculation, the clinical diagnostic techniques can be appraised just from this point of view. In general the clinical laboratories have applied some techniques for appraisal of the hemorheological disorders and this was at that time when the hemorheology was not been separated in an independent theoretical and practical biomedical discipline. Now these techniques require the perfection. Their choice should be made according to those criteria, which should correspond to the present-day level of knowledge about the disturbances of blood rheological disorders just in the blood microcirculation. Among the four factors mentioned above as disturbing the blood rheological properties in the microcirculation – the RBC aggregation, their deformation, the microvascular hematocrit, and blood plasma viscosity – primarily to be chosen are those, which disturb the blood rheological properties the most. It is the red blood cell aggregability, while their deformability is certainly less significant, although this problem requires further and even more careful investigation. The local hematocrit, as well as the blood plasma viscosity cannot be investigated in the appropriate microvessels in the clinical laboratories. The difficulty is related to the fact that the process of investigation of blood rheological properties is frequently related to their artificial changes and even disturbances, which is certainly not permissible.

For appraisal of the RBC aggregability many laboratory techniques were proposed, the majority of which were, unfortunately, very complex and possessed other drawbacks as well. These were often: (a) not direct, and did not evaluate the phenomena to be studied, but rather their indirect manifestations, as, for instance, erythrocyte sedimentation velocity instead of their aggregation as such; (b) many of them were not directly related to factors, which are actually to be studied (e.g., when transmission of light through the erythrocytes are appraised instead of their aggregation as such); (c) they are often not sensitive and therefore the changes that should be evaluated are not sufficiently pronounced or even remain in general not detected. In such cases the appraisal of specific rheological changes of blood are not only of full value, but in general not sufficiently valued. The techniques used by the author for investigation of the erythrocyte aggregability[24,25] have certain features and are free from many drawbacks, typically as in other investigation techniques.

We present below the principal criteria, which can be most helpful for a correct choice and for successful application of the investigation techniques of blood rheological properties in the clinical laboratories, as well as for the animal experiments. On the basis of author's experience the features of the techniques should be in particular as follows:

1. The investigation procedure should be optimal, i.e. the red blood cells should remain in their natural environment, the own blood plasma, and minimally touched by the non-natural surroundings. Such procedures as their washing and addition of anticoagulants or active substances should be completely excluded or be minimized during the investigation.
2. The laboratory techniques should be maximally direct, i.e. these should evaluate the RBC aggregation as such, and not the side effects like their sedimentation rate or transmission of light, or other rays transmission through the blood. The number of the aggregated and non-aggregated erythrocytes should be presented directly and not as indirect data.
3. The actual changes should be presented quantitatively for each hemorheological parameter, while the obtained numbers should reflect the sense of the parameter being investigated (i.e., the number of the red cells in the aggregates). For comparison of data obtained from various patients and in various laboratories the stress applied to the erythrocytes under study should be standardized.
4. The technique applied should be maximally sensitive from the point of view of the evaluation of the indices of blood rheological properties being investigated. The investigation results should not be dependent on the erythrocytes environments.

In general, for the clinical use it is not sufficient to choose correctly the parameters of the blood rheological properties to be studied in the patients. Only such investigation techniques should be applied, which would provide the direct, quantitative and comparable data about the specific hemorheological properties in the patients. Various manifestations of these properties, especially in the microcirculation, are sufficiently complex and therefore they should be investigated only by using appropriate techniques. Otherwise errors may originate during the investigation, which are hardly detected and make the results not only unreliable but also not comparable.

The problems of blood rheological disorders in the microcirculation, i.e. in the blood vessels being narrower than 15-20 μm, have a very high significance for a better understanding of the blood circulation under various physiological and even more so under the pathological conditions. Therefore, for a better understanding of these problems the basic and the applied investigation of the microcirculation should be carried out not in the artificial tubes but in the living microvessels, where the conditions of the blood flow and their disturbances are to a significant degree specific.

Identification of the basic factors responsible for the normal blood flow structuring and for the respective hemorheological disorders in the microvessels have a great practical significance. This provides the evaluation of specific changes, which are responsible for hemorheologic disturbances and determine their disorders in the patient's blood. The single factors producing these disturbances were investigated formerly without a sufficient clear understanding of the specific effects on blood rheological disorders, which may be in evidence in the microcirculatory beds. The present article is aimed to create for the biomedical specialists a clear image of the complex behavior of the blood structural elements in the microvascular networks, which can be to a sufficient degree independent in individual blood vessels and may produce specific circulatory disturbances of blood supply to appropriate portions of organs and tissues, thus contributing in the development of the most significant human pathologies.

Improved diagnostic technique for the analysis of hemorheological disorders in the capillaries

Now, with RBC aggregability emerging as most significant factor, responsible for the blood flow disorders inside the capillary blood vessels, it is very essential to apply such diagnostic techniques that would diagnose properly the actual hemorheological disorders in the patients suffering from such diseases where the microcirculation has been disturbed. This would also play a significant role in development of disorders of the blood supply to the appropriate tissues such as the brain, the myocardium during development of their infarcts, the lower extremities during foot gangrene, etc. Unfortunately these techniques were so far insufficiently reliable, since they were often indirect or not quantitative (as the erythrocyte sedimentation rate or the Myrenne aggregometry, etc.). Fortunately, such diagnostic techniques have been significantly improved and applied in laboratory and clinical studies in the recent decades[24,26].

The laboratory technique for investigation of the RBC aggregability in the human and animal blood samples identified as the "Georgian technique" introduced at the beginning of the 1990s[27], provides medical workers and researchers with a direct microscopic, quantitative data on this most essential index of blood rheological properties in the microcirculation. The investigation procedure comprises the following steps: (a) sampling of venous blood (ca. 4 ml), (b) its centrifuging to obtain blood plasma, (c) dilution in own plasma of blood, (d) introducing into a glass chamber, (e) standard mixing of the suspension, and (f) image analysis for qualification of the erythrocyte aggregability.

The erythrocyte aggregability index reflects the actual tendency of red cells to form aggregates of various shapes and sizes in their own plasma. It shows the relationship between the total areas of aggregated and non-aggregated RBC, and represents the most intelligible presentation of red blood cell tendency to form aggregates under the effect of intrinsic factors.

An improved computerized system is developed for evaluation of the erythrocyte aggregability index, identified by the author as the "Georgian index". It is based on IBM compatible personal computer. For analysis of the images original software has been developed, which enables to differentiate erythrocyte aggregates according to various optical density of the red cells and plasma. The system is easily adaptable for using with any brands of IBM compatible PC's and does not require any special training of the personnel. The system readily allows copying of the image, as well as permits printing out the results of the analysis.

This technique has the following advantages:

1) It is easy to use and does not require special training of the personnel;
2) The technique is direct, it provides immediate microscopic visualization, as well as quantitative analysis of the RBC aggregation;
3) Just the erythrocyte aggregability is evaluated, and not its side effects (sedimentation rate, light transmission, etc., as determined by other commonly used methods);
4) The procedure is optimal: the red cells remain in their natural environment, their own blood plasma;
5) The shear stress of blood samples under study is standardized;
6) Shape of the aggregated erythrocytes is quantitatively presented at its direct visualization;
7) Artificial effects caused by washing of the RBCs, addition of anticoagulants and other active substances during the procedure are excluded;
8) The technique does not require standardization of hematocrit in the blood samples under study;
9) Possible slips and abnormal effects are minimized, and if present, could be easily revealed;
10) The technique is about twice as sensitive as other techniques commonly applied for the same purpose;
11) Room temperature conditions do not disturb the results of the investigation;
12) Admissible time span for performing investigation in the chamber is at least 5-6 hr;
13) The results obtained in different laboratories are comparable and independent of the specific equipment applied.

The procedure is reliable: Perennial investigations have shown that when they are conducted reliably, the RBC aggregability index may vary insignificantly under the following conditions: (a) sequential testing of 4-6 vision fields in the same chamber, (b) the same blood samples in two different chambers, (c) within several hours of residence of the blood in a chamber, (d) with hematocrit alterations from about 30 to 50, at a temperature of 15º-40º C for the same blood samples.

Conclusion

Thorough analysis of various immediate causes of the local blood flow resistance is primarily the intracapillary erythrocyte aggregation. This was convincingly proven by the author's experimental and clinical research[12]. This was further proved that it was the intravascular erythrocyte pasting together that cause a drastic increase of local blood flow resistance up to its complete stop under such specific conditions. Another argument for the key role of the intracapillary erythrocyte aggregation in the increase of the intracapillary blood flow resistance, is a sharp increase of arteriolar-venular blood flow advancement of the red cells after reaching the larger, venous segments of the microvascular networks.

References

1 Jones AW, Content and fluxes of electrolytes, in *Handbook of physiology*". Sec. 2: *Cardiovascular physiology*; edited by D. F. Bohr, A.P. Somlyo and H.V.Sparks (Am Physiol. Soc., Bethesda, Maryland) Vol 2, 1987, 253.

2 Lister P, On the early stages of inflammation, *Edinburg Med J*, 3 (1957) 656.

3 Mchedlishvili G, *Capillary circulation* (Georgian Academy of Sciences, Tbilisi) 1958, 187.

4 Mchedlishvili G I, *Blood microcirculation* (Nauka, Leningrad) 1989, 296.

5 Mchedlishvili G & Maeda N, Blood flow structure related to red cell flow: A determinant of blood fluidity in narrow microvessels. *Japan J. Physiol*., 51 (2001) 19.

6 Mchedlishvili G, Mantskava M & Pargalava N, Arteriolar resistance and hemorheological disorders related to Raynaud's phenomenon, *Microvasc Res*, 62 (2001) 190.

7 Mchedlishvili G, Disturbed blood flow structuring as critical factor of hemorheological disorders in microcirculation, *Clin. Hemorheol. Microcirc,* 19 (1998) 315.

8 Mchedlishvili G, Investigation of the mechanism of the capillary stasis, *Trans I.Beritashvili Institute Physiology*, 9 (1953) 279.

9 Pappenheimer R. Contribution to microvascular research of Jean Leonard Marie Poiseuill, in: *Handbook of physiology,* Sect. 2: *The cardiovascular system, 4: Microcirculation,* Part 1, edited by E.M. Renkin & C.Ch. Michel, (Am. Physiol. Soc. Bethesda, MD), 1994, 1.

10 Mchedlishvili G, Dynamic structure of blood flow in microvessels, *Microcirc Endoth Lymphatics*, 7 (1991) 3.

11 Tsinamdzvrishvili B, Beritashvili N & Mchedlishvili G, Further insight into blood rheological disturbances in essential hypertension, *Clin. Hemorheol*, 13 (1995) 697.

12 Mchedlishvili G, Microvascular stasis caused by deranged blood flow structure in microvessels, in. *Microcirculatory stasis in the brain,* edited by M. Tomita, G Mchedlishvili, W.I.Rosemblum, W.-D.Heiss & Y Fucuuchi, (Excerpta Medica, Amsterdam), 1993, 11.

13 Mantskava M, Pargalava N & Mchedlishvili G, Direct beneficial effect of insulin on blood rheological disorders in the microcirculation, *Clin. Hemorh Microcirc*, 30 (2004) 431.

14 Mchedlishvili G, Role of cerebral microcirculation in secondary brain damage, in: *Mechanisms of secondary brain damage*, edited by A Baethmann, K. G. Go, & A. Unterberg. (Plenum Press, New York and London) 1986, 338.

15 Fahraeus R, The suspension stability of the blood, *Physiol. Rev,* 9 (1929) 241.

16 Knisely M H, Bloch E H, Elliot T S & Warner L, Sludged blood, *Science*. 106 (1947) 431.
17 London M, The role of blood rheology in regulating blood pressure, *Clin Hemorheol. Microcirc,* 17 (1997) 93.
18 Soutani M, Suzuki Y, Tateishi N & Maeda N, Quantitative evaluation of flow dynamics of erythrocytes in microvessels: Influence of erythrocyte aggregation. *Am. J. Physiol,* (Heart Circulation Physiology) H268 (1995) 1959.
19 Ernst E. Fibrinogen, a cardiovascular risk factor, *Clin. Hemorheo*l, 12 (1992) 805.
20 Maeda N & Shiga T, Red cell aggregation, due to interactions with plasma proteins *J. Blood Rheology*, 7 (1993) 3.
21 Mchedlishvili G, Attempt to score various techniques for examination of the erythrocyte aggregability in blood, *Clin. Hemorheol*, 16 (1996) 193.
22 Driessen G K, Fishher T M, Haest C W M, Inhoffen W.& Schmid-Schoenbein H, Flow behaviour of rigid red blood cells in the microcirculation. *Int. J. Microcirc. Clin. Experim,* 3 (1984) 197.
23 Schmid-Schoenbein H & Teitel P, *In vitro* assessment of "covertly abnormal" blood rheology: Critical appraisal of presently available microrheological methodology. A review focusing on diabetic retinopathy as a possible consequence of rheological occlusion. *Clin. Hemorheol,* 7 (1987) 203.
24 Mchedlishvili G, Beritashvili N, Lominadze D & Tsinamdzgvrishvili B, Technique for direct and quantitative evaluation of erythrocyte aggregability in blood samples. *Biorheology*, 30 (1993) 153.
25 Mchedlishvili G, Red blood cell aggregability in blood Criteria for scoring the techiques, *Clin. Hemorheol. Microcirc*, 18 (1998) 161.
26 Mchedlishvili G, Shakarishvili R, Aloeva M & Momtselidze N Elaborated - "Georgian index" of erythrocyte aggregability characterizing the microrheological disorders associated with brain infarct, *Clin. Hemorheol*, 15 (1995) 783.
27 Urdulashvili T, Momtselidze N, Mantskava M, Narsia N & Mchedlishvili G, Hemorheological disorders and arteriolar resistance during ischemic heart disease. *Clin. Hemorheol. Microcir*. 30 (2004) 399.