HEMORHEOLOGY: A SURVEY

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I – INTRODUCTION

Blood is the medium for the delivery of oxygen and essential material to and removal of waste products from all the cells of the body. Oxygen is transported in the blood by the tetramic protein hemoglobin in red blood cells (RBC_s). Hemoglobin sensing the physiological oxygen gradient in tissues exploits conformation changes to bring local blood flow in the capillary networks into line with oxygen requirements of the body cells. The delivery of glucose, lipids, hormones, enzymes, salts and vitamins by te blood and removal of carbon dioxide and toxic metabolic products which is accompanied also by transferred chemical, pressure and thermal signals, plays equally a key role for the functional status of the cells. These physiological functions of blood are performed when blood and its components are adapted to their tasks. The change of molecular architecture of blood components due to pathological disturbances affect the matching of blood properties to the physiological functions. For instance, in narrow capillaries, the passage of the RBC_s and other blood formed elements is very sensitive to perfusion pressure as well as to their deformability and aggregability. Pressure reduction and/or incrased rigidity of the formed blood elements may cause a capillary plugging. The capillary plugging can easily become intensified and lead to irrevesible vascular occulusion.

The effect of the disturbances can be studied from the point of view of what factors and components of blood are involved in clinical manifestations, what biochemical phase is defective, etc... At the same time, the determination of flow aspects of the defect by hemorheological tests may be equally significant for the purpose of screening diagnostic cases as well as analysis of therapy. In comparison

to biochemical tests, the hemorheological tests can be very time sparing pointing out the type of defect within a few minutes after withdrawal of blood specimen in a way that further determination can specify details.

The properties of the matter affecting deformation and flow are called rheological properties and the science dealing with the way how the matter is deformed and flows when forces are applied to it is called rheology. The name is derived from the Greek verb *rheo*, to flow. In principle, the rheology includes everything dealing with flow behaviour. However, in practice, rheology has usually been restricted to the study of the relationships between force and deformation and flow of liquid and solid materials.

The word hemorheology was introducted by A.L. Copley in a survey on rheology of blood in 1952. He defined the term as follows «hemorheology is concerned with the deformation and flow properties of cellular and plasmatic components of blood in macroscopic, microscopic and submicroscopic dimensions, and with the rheological properties of the vessel structure which directly comes in contact with blood». This definition was adopted at the Foundation Meeting of the International Society of Hemorheology in Reykjavik in 1966. Additionally, A.L. Copley and G. Seaman widened this definition in the sense that: «hemorheology is also the study of the interaction of blood or its components and the vascular system with added foreign materials, such as drugs, plasma expanders, or prosthetic devices. Thus, hemorheology is the study of how the blood and the blood vessels can function and interact as parts of the living organism».

Hemorheology has developed on basis of cumulative speculations that the change of rheological properties of blood and its components might be the fundamental cause of many cardiovascular diseases. The large number of performed clinical studies testify this assumption. Basically, two main areas have been explored: pathologies with hematological origin (e.g., paraproteinemia, leukemias, hemolytic anemias, polyglobulinias, polycythemia, plasma cell dyscrasis, sickle cell disease) and cardiovascular pathologies associated with the risk factors of thrombosis and atherosclerosis (e.g., myocardial infarction, hypertension, strokes, venous and arterial diseases, diabetes). Thus hemorheology becomes recognised as a field of research and clinical approach for diagnostic, therapeutic and prognostic measures.

The modern history of hemorheology began a very long time ago, with the curiosity of man to understand the motion of the blood. However, until the discovery of the connection between the arteries carrying blood away from the heart and the veins returning blood to the heart by William Harvey (1578-1657), the relation between organisation and function of the circulatory system was not fully realised.

The first experimental observation of hemorheological significance was performed by A. van Leeuwenhoek in 1674. Baker, in his comment to his observation, stated in 1743: «an observation of Mr Leeuwenhoek is very well worth regarding – he took notice, that when he was greatly disordered,

the globules of his blood appeared hard and rigid, but grew softer and more viable as his health returned».

The foundation of modern hemorheology was laid by J.M.L. Poiseuille and Robin Fâhraeus (1888-1968) through her investigations of the viscosity of blood in glass capillaries. Among the rather few reports of original research his founding pertaining to the RBC_s sedimentation rate and rouleaux formation of RBC_s became clinically useful.

Behind the experimental investigations, started to understand fundamental mechanisms of hemorheological changes, had been cumulative clinical findings about disturbed circulation. G. Andral was one of the first who wrote in 1843: «People with an increased quantity of globules in the blood may have peculiar symptoms, such as vertigo and dizziness». F. Gaisböck's original description in 1905 was an association between hypertension and raised red cell count. In 1911 Burton-Opitz suggested: «A permanent increase in the viscosity might affect not only the condition of the heart, but also that of other organs. Thus, it seemed probable that one of the causes of cardiac hypertrophy might lie in the increased peripheral resistance caused solely by a greater «thickness» of the blood. He concluded: «such considerations clearly showed at an early date that the viscosity of the blood must have been an important clinical bearing».

The first testing of rheological properties of the pathological whole blood was reported by A.L. Copley in 1941. This report on blood properties of hemophilic and heparinezed blood and studies on viscosity, thixotropy and age-hardening of blood secured with different anticoagulants, from healthy subjects, created an impact that ultimately led to hemorheology becoming an organised life science.

In the definition of hemorheology, Copley implied that the blood and the vessel wall are entity which he named the vessel-blood organ. The vessel-blood organ penetrates all other organs, similar to the nervous system. He proposed that the interface between the two portions of the vessel-blood organ is controlled by the endothelial fibrin(ogen) lining.

In 1947, H. Hartert introduced the thromboelastography, which was a hemorheological technique to measure the rate of blood coagulation and rheological behaviour of blood clots without destroying its structure. Thromboelastography became accepted as a clinical hemorheological test. It is employed for screening of preoperative patients and of diagnostic cases with coagulation defects.

The clinical aspects of hemorheology were systematically investigated by numerous authors in collaborative studies with hematologists, cardiologists, vascular surgeons, renal physicians, oncologists.

A strong impectus to the progress of hemorheology was given by the publication of an international jounal named *Biorheology – An international Journal* in 1962. Since the sixties, the hemorheology is practised more and more by many biomedical scientists, physicians and surgeons and a number of papers on different aspects of this field is published. The works of S. Chien, S. Witte, H. Schmid-Schön-

bein, J.F. Stoltz, J. Dormandy and S. Oka, among others, decisively affected the growth of hemorheology. With the aim to serve as an aid in the practice in various clinical fields, a journal, named *Clinical Hemorheology*, was founded in 1981. This journal was renamed *Clinical Hemorheology and Microcirculation* in 1997 in order to follow recent trends of the clinical research in the field, the terminal blood vessels as the anatomical ad functional most important areas of hemorheology.

II – BLOOD VISCOSITY AND MODELS

Blood is a fluid suspension of cells in plasma. In human blood, the plasma is about 90% water by weight, containing 7% plasma proteins, 1% inorganic and 1% other organic substances. The cellular constituents are essentially erythrocytes. The erythrocytes are numbered about 5 millions/mm^3, white cells vary from 5000 to 8000/mm^3 and platelets from 250 000 to 300 000/mm^3. Each constituent of the blood contributes to its viscosity directly and also through the interaction of plasma and cellular constituents. The volume concentration of erythrocytes in health varies in human beings from 35 to 50% and can further increase in hypoxic environment such as living in the mountains. The averaged (mean ± S.D) dimensions of human erythrocytes are: diameter 7.65 ± 0.02 μm, minimum thickness 1.44 ± 0.01 μm, maximum thickness 2,84 ± 0.01 μm, volume 97.91± 0.41 μm^3, and surface area 29.95 ± 0.40 μm^2.

For a Newtonian fluid, which obeys Poiseuille equation, a linear pressure-flow rate curve passing through the origin is find and there exists an unique coefficient of viscosity (at a constant temperature). However in view of the non-linearity of the pressure-flow rate curce of blood it is not possible to define such unique coefficient of viscosity and the most commonly calculated parameter by using Poiseuille equation is known as the apparent viscosity and is denoted as η_a. This is calculated based on the measured values of the pressure gradient and flow rate through a rigid circular cylindrical tube. If η_o denotes the viscosity of plasma, which is generally Newtonian, then the ration of η_a / η_o is defined as the relatie viscosity, and is usually denoted as the η_r . The unit of the apparent viscosity is the Pascal-second (Pa.s) or if this is too large milli-Pascal-second (mPa.s) or centi-Poise (cP) which are interchangeable units, each being equal to 0.001 Ns/m^2. The apparent viscosity could be calculated over any flow regime as long as this is calculated by a formula that is known to work for a homogeneous Newtonian fluid.

The apparent viscosity, as determined under steady flow conditions, depends on the shear rate, hematocrit, temperature, and plasma viscosity. In addition to this under oscillating flow conditions, this parameter is further influenced by the frequency and amplitude of the measurement system. To analyse the influence of these, several mathematical models have been developed.

2-1 Variability of blood viscosity

a) Effect of shear stress-shear rate variation on viscosity

Over the large range of variability of shear stress, the respective shear rate could be determined. Haynes, by using a capillary viscometer, showed that the non-linearity of the pressure--flow relationship exists over a large range of hematocrit. These measurement are also carried out by the Couette type of viscometer. The added advantage of this viscometer over the capillary one is that the measurements could be carried out at very low shear rates. Merrill et al. showed a non-linear variation over a large range of shear rate and shear stress. The data obtained by these measurements show a straight line fit with the Casson's equation.

$$\sigma^{1/2} + \sigma_y^{1/2} + b^{1/2}\gamma^{1/2} \qquad (2.1)$$

where α and γ are the shear stress and shear rate, respectively, σ_y denotes the yield stress of the order of a 5 mPa and b is a constant. The yield stress, obtained by extrapolation of the shear stress-shear rate curve to zero shear rate as an intercept on the shear stress axis, has been found to be of significant value under various experimental and clinical conditions.

There is a critical hematocrit value Hc (running from 1.3 to 6.5 %) below which there is no yield stress. Above this Hc, the cube root of yield stress is dependent on the quantity H-Hc, where H = actual hematocrit value.

$$\sigma_y = A(H - H_c) \qquad (2.2)$$

where A is a constant equal to 0.008.

b) Effect of cell volume fraction on viscosity

The particles increase the viscosity of the hosting fluid in which they are dispersed because they disturb the mutual sliding of fluid layers in this fluid. Einstein established the first equation for the viscosity of suspension of solid particles in terms of viscosity of the continuous phase and fraction of particles given as,

$$\eta_{susp} = \eta_o + \eta_o KH = \eta_o (1 + KH) \qquad (2.3)$$

where the shape factor K for spherical particles and dilute suspension is equal to 2.5, H denotes volume concentration of particles. When dilute suspensions of rigid non-spherical particles are studied, the value of K is larger than 2.5, which can be explained by the larger volume swept by the asymetric particles than the spheres. In emulsions, due to interaction of particles, the Einstein's equation is further modified by a factor T that depends upon the viscosity of both the dispersed phase η_i and the continuous phase η_o,

$$\eta_{susp} = \eta_o (1 + KH) \qquad (2.4)$$

The term

$$T = \frac{\eta_i / \eta_o + 0.4}{\eta_i / \eta_o + 1.0} \qquad (2.5)$$

is referred to as Taylors's factor. The relative viscosity is,

$$\eta_r = \frac{\eta_{susp}}{\eta_o} = 1 + KH \; \frac{\eta_i / \eta_o + 0.4}{\eta_i / \eta_o + 1.0} \qquad (2.6)$$

The red cell membrane exhibits viscous behaviour in addition to elastic properties. Oldroyd considered the suspension behaviour of spherical drop which are surrounded by a film with viscoelastic behaviour. With modification, the equation (2.6) becomes,

$$\eta_r = 1 + KH\,\frac{(\eta_i/\eta_m)/\eta_o + 0.4}{(\eta_i/\eta_m)/\eta_o + 1.0} \qquad (2.7)$$

where η_m is function of the rheological properties of the film or membrane. Thus the membrane contribution has to be considered for calculation of relative viscosity what is generally ignored.

c) Effect of shear rate on viscosity

Since plasma is a Newtonian fluid, the non-Newtonian characteristic of the blood is developed due to suspension of the cells in this medium. The blood contains aggregates which are formed due to interaction of erythrocytes with plasma proteins such as fibrinogen and globulin. In blood at rest, all erythrocytes form large aggregates, leading to its viscoelastic behaviour.

The formed structure at rest is very sensitive to flow conditions. When the shear rate increases, blood aggregates tend to break, leading to the reduction of viscosity. With further increase of shear rate the aggregates are completely broken up. Hence the contribution due to aggregation process is reduced to a minimum. Thereafter, with further increase in shear rate, individual erythrocytes are deformed till these are fully aligned in the flow direction. Thus offering a minimum resistance to flow. Under these conditions the blood apparent viscosity is reduced to a minimum.

d) Effect of hematocrit on blood viscosity

The fluidity of blood in large vessels depends on the hematocrit and decreases with the increase of hematocrit but surprisingly never to zero even at a hematocrit value of higher than 95% when the cells are densely packed. At 50% volume concentration, the rigid spheres cease to flow, whereas, erythrocyte suspension exhibits the minimum relative viscosity compared to all other suspensions. The deoxygenated erythrocytes of sickle cell anaemia exhibit the higher viscosity at all concentrations compared to that of normal erythrocytes.

As the RBC_s concentration is the main determinant of the blood viscosity, the evaluation of other determinants require the elimination of its effect. This can be achieved by measuring viscosity of blood samples at a standard hematocrit or by calculation it from the viscosity at the measured hematocrit. Due to linear relationship between the logarithm of blood viscosity and the hematocrit at a given shear rate, the viscosity at a standard 45% hematocrit can be calculated by,

$$\eta_r(45) = [\eta_r(H)]^{45/h} \qquad (2.8)$$

where η_r is the relative viscosity defined as the ratio of blood viscosity to that of plasma η/η_o.

H and 45 denotes the native and standard hematocrit, respectively.

III – MICRORHEOLOGICAL PARAMETER OF BLOOD

3-1 Deformability of erythrocytes

Blood is a non-homogeneous system composed of deformable cells suspended in plasma. While the white blood cells are very stiff owing to viscoelastic behaviour of their cytoplasm, the red blood cells are much more flexible and are easily deformed in flow, not only in small vessels but also in large vessels. During this process, the cells are completely adapted to flow by a continuous process of membrane rolling around the cytoplasm whereby both the cytoplasm and membrane undergo deformation in a more or less elongated shapes. In this process, these cells keep a stationary orientation, the membrane rotates like the tread of a tank. This motion of the membrane is associated with periodic changes in curvature and in-plane shearing of the membrane constituents. Deformability is therefore crucial for a normal cell to function normally under in vivo circulation and experimental conditions. The erythrocyte is ideally suited for this purpose due to the absence of nucleus, the low cytoplasmic viscosity, the viscoelasticity of its membrane and high ratio of surface area to volume, giving an excess area of about 40 %. Thus the deformation of blood cells in response to circulating forces depends on extrinsic factors related to cell geometry (shape, volume and surface area of membrane) and intrinsic structural factors (elastic and viscous properties of cell membrane, cytoplasm and active contractile systems). Some of the membrane parameters as obtained by various authors are given in Table I.

a) Determinants of Erythrocyte Deformability

Erythrocytes geometry

The major advantage of the biconcave shape is the favourable surface area to volume relation for cell deformation and for exchange of metabolic products across the membrane. The normal red cell has a surface area about 30-40 % in excess of a sphere of an equal volume. The relation between surface area A and volume V can be expressed as a dimensionless parameter such as $A/V^{2/3}$, sphericity index $4.236\ V^{2/3}/A$ and surface index $A/4.836\ V^{2/3}$. The sphericity index, is unity for spheres and 0.7 for normal human red cells. The concave shape of the erythrocytes corresponds to a minimum of bending energy stored in a closed membrane. The shape probably exists rarely in the living circula-

Table I – Erythrocyte membrane parameters as obtained by micropipette techniques

Parameter	Range of Mean Values
Membrane compressibility modulus	0.1-1.0 Pa
Membrane shear elastic modulus	$(4.0\text{-}65)10^{-6}$ Pa
Membrane extensional viscosity	$(0.5\text{-}1.0)10^{-6}$ Pa.s
Membrane bending modulus	$(1.1\text{-}1.8)\ 10^{-15}$ Pa
Membrane bending viscosity	$(2.5\text{-}8.5)10^{-16}$ Pa.s

tion because of the acting of high shear stresses causing cell deformation which is further enhanced by the high cell concentration and in the capillaries by the smaller diameter of the vessel. Alteration in resting shape, when secondary to a congenital abnormality, is often associated with the loss of deformability. Acquired changes adversely affect the deformability or erythrocytes.

Internal viscosity of erythrocytes

There are two parameters contributing to the internal viscosity of the erythrocytes : a) the mean cell hemoglobin concentration (MCHC) and b) the structural constituents of the erythrocyte membrane. The MCHC when it rises, this decreases the deformability of erythrocytes. This has been observed in diseases the deformability of erythrocytes. This has been observed in diseases like sickle cell anemia. The primary disorders leading to loss of cell potassium and water, resulting in the increase in MCHC, finally decrease the deformability of erythrocytes. The MCHC can be reduced from 33% to any desired level by suspending in hypotonic medium which leads to transformation into spheres resulting in decreased deformability of erythrocytes. In a hypotonic solution of 217 mosmol, the cell volume is increased 74% but the surface area is increased only 7%. At 131 mosmol the volume is increased 74% but the surface area is increased only 7%. At 131 mosmol the red cell is spherical and it is on the verge of hemolysis.

Nakao et al. and Weed et al. had shown the dependence of adenosine triphosphate (ATP) generation on the maintenance of cell shape and deformability. The reduction in ATP is associated with the shape change which could further be contributing to the gain in calcium and loss of potassium and water. Thus causing an increase in cytoplasmic viscosity and decreasing the deformability

Influence of Vessel Size

Due to shear deformation of erythrocytes, these cells can pass through channels as small as 2.9 μm in diameter. These erythrocytes due to shear forces during flow through capillary tubes of different diameters undergo deformation. But the axis of symmetry is not present and the final shape can more adequately be described as a slipper rather than parachute. This final shape is the result of an intracellular displacement of hemoglobin solution with the increase of hematocrit and the more efficient packing due to deformation of erythrocytes when a "*sipper*" like arrangement takes place. This type of glow is impossible for relatively rigid nucleated cells. Such deformations have also been observed in various *in vivo* vessels of varying diameters. In capillary blood flow, at each branching, the direction of movement of an erythrocyte is influenced by the size of the vessels, the deformability of the cell and the ration of the velocities in the branches. Elongation, orientation and membrane tan-trading are the mechanisms behind red cell flexibility in micro-

vessels for axial migration whereas artificially rigidified red cells as well as other cells migrate too but less swiftly.

The deformability of erythrocyte plays an important role in white cell margination in the immediate post capillary venules. The deformed erythrocytes are packed up behind a slowly moving white cell. Such a phenomenon might be called "red cell under-velocity", a fact necessarily leading to a reversal of the Fahraeus effect, resulting in the increase of local hematocrit. The erythrocytes overtake the creeping white cell immediately as these enter the diverging venular channel. In doing so, under the influence of steep pressure gradient, they displace the white cells towards the venular wall where this may become adherent.

b) Molecular Basis of Erythrocyte Deformability

The basic structure of the RBC membrane is consisting of two layers of phospholipid molecules (bilayer) with their hydrophilic heads facing outward and hydrophobic tails locking into each other in the interior by hydrophobic forces. This is also consisting of the intrinsic and extrinsic proteins. The intrinsic proteins are integral part of the lipid bilayer of phospholipids. The skeletal proteins, a network of proteins consisting of spectrin, actin, protein 4.1 and adducin. Spectrin, is composed of two non identical subunits (α spectrin and β-spectrin) connected side to side to forme a heterodimer. These can further form tetramers of length 200 nm which dominate the membrane skeleton. The red cell actin, is a highly uniform filament of length 35 mm. The spectrin-actin interaction are generally stabilised. Thus a combination of lipid bilayer and proteins provide a specialised permeability barrier functions and integrity to the erythrocyte membrane, respectively by protein 4.1. The linkage to the membrane is achieved by ankrin. The second linkage between skeletal proteins and bilayer is through interaction of the integral protein glycoprotein C with the skeletal component protein 4.1.

A conceptualised view of reversible deformation of the membrane with a change in geometric shape but at constant surface area includes the rearrangement of skeletal network. In this process certain spectrin molecules becomes unfolded and extended while others are more compressed and folded. With the increased deformation, the spectrin molecules attain their maximal linear extension. This is the limit of reversible deformation. Beyond this point with the further increase in surface area with the application of force the breaking of the functional complexes may take place. When red cells are exposed to a force that extends surface area, the failure at the weakest protein bonding may take place. Thus a combination of lipid bilayer and proteins provide specialised permeability barrier functions and integrity to the erythrocyte membrane, respectively.

The erythrocyte deformability, an important hemorheological parameter contributing to the exchange of metabolic products with the tissue envi-

ronment, is thus attributed to the various constituents of the membrane and hemoglobin. Any variation in these may result in the impaired functioning of erythrocytes.

3.2. Aggregation of Erythrocytes

Anthony Van Leeuwenhoek gave the first accurate description of the aggregation of red blood cells and their disaggregation in a letter to the Royal Society: "On concerning the circulation and stagnation of blood in tadpoles", which was published in 1702. He described this process despite optical limitations, that blood in blood vessels "did in a manner stagnate, inasmuch that one could discern no separated parts in the blood, for it did appear there to be one even red colour". From his description it was clear that blood cells were not coagulated but aggregated. Fahraeus showed that the red cell aggregation in the form of rouleaux formation may facilitate the blood flow. Thus red cell rouleaux are reversible cellular clumps or aggragates which can occur physiologically. There is another term referring to such a phenomenon-agglutination, which is irreversible aggregation.

In normal blood flow especially in the arteries, the erythrocytes allow themselves to be carried along by the axial current and interactions are consequently reduced to a minimum. Due to some changes in the rheological properties, the erythrocytes may loose their capacity to adapt to flow, leading to aggregation of cells. The erythrocyte hyper-aggregation may lead to the following local consequences :

a) decrease in blood flow rate particularly in post-capillary vessels,
b) decrease of the volume concentration of erythrocytes in capillaries,
c) change in erythrocyte deformability and other rheological parameters,
d) increase in peripheral resistance,
e) tendancy to develop venous thrombosis,
f) damage to tissues as a result of anoxia.

Erythrocyte aggregation as a reversible dynamic phenomenon can be observed both in vitro and in vivo and is found to be responsible for much of the increase in viscosity at low shear rates. The aggregation combined with yield stress of blood are expected to reduce blood flow compared to that of non aggregating system. Combined with other cellular constituents, white cells and platelets, the plasma constituents contribute significantly to the aggregation process. Thus aggregation of erythrocytes is a result of the interaction of erythrocyte membrane and plasma proteins such as fibrinogen and globulins.

a) Physicochemical Mechanism of Erythrocyte Aggregation

Under normal circumstances the membrane possesses an overall negative charge contributed by all of the N-acetylneuraminic acid (sialic acid). All the sialic acid is external to the barrier since neuraminidase which does not enter the red cell can remove this charge completely. By this, a repulsive electrostatic force exists be-

tween cells. Opposing this influence, there are short range attractive forces due to electrodynamic phenomenon or van der Waal's forces. Thus the net effect on cellular adhesion is due to balance between these forces and the cellular separation. Under normal physiological conditions at normal pH and ionic strength, the range of attractive forces is so small that the glycocalyces of adjacent cells interfere before adhesive forces act significantly in cellular repulsion. This is the reason why the erythrocytes suspended in physiological saline do note aggregate.

In the aggregation process of erythrocytes, the plasma lipids do note make any contribution, whereas, the presence of fibrinogen and certain globulin fractions are necessary, which can be adsorbed on the surface of the erythrocytes. Brooks has shown that the adsorption of dextran, a polysaccharide, to the red cell surface is linearly related to the concentration in the suspending medium. The affinity of the red cell surface for this neutral polymer dextran which is adsorbed by van der Waal's force or hydrogen bonding is much less than that for positively charged polylysine, which is adsorbed to the negative charge group on the red cell surface.

The aggregation energy to erythrocytes, E_b, is provided by the macromolecular bridging of their surfaces, whereas, the disagregation energy while at rest results from the presence of sialic acid on the cell surface. The applied shear stress contributing as mechanical stress (E_s) would also tend to disaggregate the cells. Therefore, the net aggregation energy, E, at the interface between adjacent cells depends on the balance between the aggregation energy, E_b, the repulsion energy, E_e and the mechanical energy, E_s. The net aggregation energy per unit area of cell interaction, E (in ergs/cm), can be expressed as:

$$E = (Eb - Ee - Es)/Ai \qquad (3.1)$$

where A_i is the area of the interface between the aggregated cells. Under resting conditions $E_s = 0$, then the net aggregation energy per unit area at the interface between adjacent cells is.

$$E_{s=0} (E_b - E_e)/A_i \qquad (3.2)$$

Another model which explains the aggregation of erythrocytes is the depletion induced model. This is based on the concept that the surfaces of two cells are close enough so that the regions of depletion start to overlap to each other. This mechanism takes place in the presence of polymers which are effective without surface adsorption of macromolecules. This model also explains some of the mechanisms associated with aggregation of erythrocytes such as in the presence of weakly adsorbed proteins like fibrinogen as well as dextran. The high mobility of erythrocytes in concentrated dextran solutions, as observed by Brooks, is further explained as a consequence of a macromolecules depletion layer near the cell surface.

b) Mechanism of Shear Disaggregation of Erythrocytes

With the increase in shear rate a breaking up of large aggregate into

smaller ones takes place. Between shear rates of 5.8 and 46 s^{-1} each doubling of the shear rate resulted in the reduction of aggregate size by about 50% and vice versa. This relationship is valid only down to aggregate sizes of diameter 15-30 μm. This change in aggregate size is manifested by the shear thinning behaviour of blood leading to a decrease in blood viscosity with increasing flow. In as shear flow the disaggregation of rouleaux presents a complex problem involving the fluid flow, the stresses evolution and resulting deformation of cells. The work of separation per unit area may be significantly greater than the chemical affinity which promotes aggregation.

In a multicell rouleaux, a great advantage is derived for separation of an interior cell compared to that and end cell since the area over which the shear stress acts is significantly higher. This process occurs when fluid stresses are tensile with respect to the bonded area while maintaining the surface area of the cell constant. In contrast to this, the shear forces could induce a tank treading motion of the red cell membrane leading to disaggregation at low stresses compared to that of tensile forces. Chien et al. have carried out the analysis of disaggregation mechanism under oscillatory shear stress. They showed that erythrocytes in a doublet separate from each other by rolling rather than sliding of the sheared cell.

c) In vivo Erythrocyte Aggregation

The shear stresses in normal circulation are too high to allow appearance of erythrocyte aggregates in the arterioles and capillaries but these do form in venules. If the hematocrit in the venules is low, these aggregates are not likely to exert any significant influence on blood flow, since the increase in blood viscosity caused by them is negligible. Qualitative intravital observations have shown that aggregate form during shear stress deficiency, i.e., with the decrease of overall pressure. Due to long residence time in post-capillary vessels this can become the cause of prolonged stasis if associated with high hematocrit. The situation could be reverted by elevating the arterial pressure or perfusing with non-aggregating low hematocrit blood. Infusion with plasma and various dextrans in rats showed that the erythrocyte arteriolar velocity in the right cremaster muscle was significantly decreased with Dx 70 and Dx 400, and increased with plasma and Dx 40.

Due to limited access to in vivo vessels, the analysis of aggregation mechanism of erythrocytes is carried out in tubes placed in various configuration. At shear rates below 100 s^{-1}, a very slight increase in apparent viscosity was observed in vertical tubes, whereas, at shear rates below 2 s^{-1}, a more prominent decrease was noted. This reduction in viscosity is attributed to the axial accumulation of erythrocyte aggregates resulting in the formation of cell-free plasma sleeve around the wall. The progressive decrease of shear rate leads to further reduction in blood viscosity.

In contrast to vertical configuration, he tubes placed in horizontal and inclined positions show a different pattern of measured viscosity. The gravitational force acts at a different angle relative to

the direction of flow, leading to sedimentation of erythrocytes and aggregates formation. This changes the erythrocyte distribution profile to an asymmetric one. The viscosity tends to increase at low shear rates compared to that as measured in vertical tubes. Murata and Secomb carried out an analysis by developing a simple theoretical model. The blood flow through vertical tube consisting of central aggregated core surrounded by plasma was considered. The results obtained by this analysis are in good agreement with that as obtained by Reinke et al.

These studies show that the aggregation of erythrocytes is a complex dynamic process contributed by cellular and plasma factors. Some progress has been achieved and this has strengthened our understanding of this process. More data, especially from in vivo studies are required to make these analyses highly relevant.

IV – HEMORHEOLOGY OF ENDOTHELIAL CELLS

Vascular endothelial cells form a monocellular layer on all blood circulation vessel walls and their total estimated mass is approximately 1.5 kg. Their properties are not identical in all parts of the body and they acquire specificities that differentiate them according to the anatomical site, which makes their study all the more difficult. The role of endothelial cells is among to control the hemodynamics of the circulatory system through various metabolic activities affecting homeostasis, vascular tonus, blood fluidity, coagulating properties and blood cells adhesion. Recent studies have underlined the crucial role of local blood flow conditions on its properties.

In microcirculation, the endothelial cells in the venules are particularly active and constitute the physiological site of liquid exchange (permeability) and above all cellular transit. During critical ischemia, the post-capillary venules are deeply involved. Lastly, the properties of endothelial cells may be impaired in a number of diseases as atherosclerosis, hypertension, inflammation and metabolic diseases.

4.1- Two Functional States of Endothelial Cells

The endothelium is normally antithrombotic and anti-adhesive, to ensure "blood fluidity". During aggressions, frequently multifactorial (agonists and/or cytokines), the endothelium can reverse its functions by expressing stored material or by slower involvement of genes that until then had been repressed. There is a dual determinism: to stop bleeding by microvessel obliteration induced by cellular transit, triggered by inflammation chemoattraction. If these effects initially appear to be beneficial, they become deleterious in the course of ischemia.

Anti-thrombotic Action: The antithrombotic and "fluidifying" action of endothelial cells is due to three types of properties which are :

a) vaso-regulating properties: they are controlled by the release of vasomotor balance components as vasoconstricting endothelin on one hand

and prostacyclin (PG12) and vasodilatory nitrite oxide (NO) on the other,

b) anti-thrombotic properties: the cells express proteoglycans on their surface, including some negative-charge, plasminogen, sulfate glycoaminaoglycans (heparane-sulfate) and they secrete plasminogen tissular activator (t-PA) and tissular factor inhibitor. One of the fundamental actions of the endothelium in that area is the production and expression of thrombomodulin, a thrombin receptor. That protein promotes the formation of a complex which activates Protein C. That function is a major anticoagulant, controlling continual thrombin generation at the sub-endothelium and blood cell interface. The endothelium also exerts anticoagulant properties by other channels, such as the capture and degradation of thrombogenous substances (ADP, 5-HTP) and through the effect of active products on platelets,

c) anti-adhesive properties: in an inactive state the endothelial cells express adhesion receptors VCAM-1 and in an active state, they express ICAM-1 and 2.

Thrombotic and Adhesive Action: During inflammation in particular, the endothelial cell properties may be reversed. With regard to vasomotricity, the balance is often maintained to the benefit of vasodilation since the agonists are not produced and PGI2 synthesis by phospholipase A2 activation is increased. Variations in local shear stresses may modify the secretion of vasomotor substances. The coagulating compound in this case thrombomodulin is under-regulated and no longer appears on the endothelium surface. In addition, the cells express tissular factor that can bind to plasma factor VIIa. Hence thrombin production may occur all the more easily as factors I and X possess binding sites on the endothelium. Regarding fibrinolysis, PAI-1, an inhibitor of plasminogen activator of endothelial origin, is increased and t-PA is under-regulated in gene expression.

The evolution of the process of leukocyte adhesion manifests itself in several successive waves of receptor expression. The various mechanisms by which leukocytes adhere to the endothelium have recently been elucidated. A few minutes after the beginning of the activation process, P--selectin (140 kD MW) is translocated from the Palade body to the cell surface, in a position to bind leukocytes by sialid oligosaccharide residues related to the sLex group. The determinant factors of this phenomenon are histamine, bradykinine, thrombin and above all oxygenated free radicals which occur during episodes of reinfusion with oxygenated blood. Secondly, gene activation promotes E-selectin and ICAM-1 expression. Here, cytokines IL-1 and TNF-α are the main determinants, to which inflammation produtes are added (endotoxins or LPS-type derivatives, C5aC9 complex). A receptor (VCAM-1) is also expressed specifically by monocytes, eosinophils and lymphocytes.

From a kinetics standpoint, leukocyte adhesion is divided into "rolling" adhesion (partly due to L-Selectin) and interaction with endothelium se-

lectins P and E and stable adhesion. After binding, leukocytes are activated by the endothelial cells because it produces PAF-acether that comes in contact. The leukocyte then releases L-selectin and expresses the β integrins in order to bind to the ICAM-1 complex and migrate toward the extravascular domain.

4.2- Rheological Properties

With respect to the interface position of endothelial cells, the specification of their mechanical properties is particularly relevant. However, a lot of studies have investigated morphological modifications under the flow but a very few studies have been focused on stress action and deformation within the cell. Thus only one study conducted *in vitro* with confocal microscopy, during induced deformations and monitoring the motion of internalised latex beads, has shown that endothelial cells behaved like an elastic isotropic material. However, deformation is less marked in the nucleus vicinity, indicating its greater rigidity. It was also shown that endothelial cells were motile in the direction of the flow, indifferently upstream or downstream.

4.3- Response to Local Flow

The influence of local hemodynamic conditions on endothelial cells (EC) has raised increased interest over the recent years. Various studies on the subject have tried to clarify the physiology and modifications of EC during vascular diseases. Endothelial cells react to hemodynamic forces by modifying their morphology and metabolism. The morphological changes may include elongation and orientation of endothelial cells parallel to the flow direction as well as an actin filament rearrangement, responsible for cellular mobility and adhesion. The metabolic changes mainly include increased prostacyclin synthesis, plasminogen tissular activator expression, differential regulation of adhesion molecule and proto-oncogene expression and ion transfer (K^+, Ca^{++}). Shear stresses also stimulate endothelial cell proliferation and migration. It is also important to consider the condition of cells when the local flow conditions are modified. Indeed, it has been shown that confluent endothelial cells (forming a continuous monolayer) do not react in the same manner as isolated cells. One of the major differences between endothelial cell behaviour is that *in vitro* the cell renewal rate is higher than *in vivo*. That rate is reduced when shear stress exceeds 30 dyne/cm^2 in laminar flow. The endothelial cell co-culture in contact with smooth muscle cells is thought to support this cellular renewal rate. Also, in *in vivo* studies many interactions are evoked between two types of cells. Although the studies conducted were able to demonstrate the different types of cellular response to extraneous forces, the underlying mechanisms are far from being totally understood.

4.4- Mechano–Transduction – Mecanobiology

The endothelial cell response to

mechanical stimuli practically involves all the mechanisms linked to cellular growth and metabolism. Thus, ionic channels, sensitive to membrane streching, adenylate cyclase, protein kynase C have their activity modified in response to mechanical stress. According to Wang et al. and Kuchan et al., surface mechano-receptors exist, linked to the cytoskeleton. The cytoskeleton alterations induced by shear stress would disturb cellular equilibrium and so modify the activity of receptors and trans-membrane channels. Also the mechanism of gene expression is influenced by flow as well as production of endothelin 1 (ET-1), plasminogen tissular activator, NO production, PDGF γ and β, growth factors, thrombomodulin and adhesion molecules. An initial molecular model of the regulation steps of gene expression under stress was considered by Malek et al. In this model the stress activates mechano-sensitive structures, including the cytoskeleton, adhesion foci, mechano-sensitive channels and thus modifies membrane receptor activity. This initial step is then applied to the second messengers like intracellular Ca^{++}, protein kinase C (PKC), cyclic AMP (cAMP), cyclic GMP, etc., which in turn are disrupted. Such deep changes in the balance of the secondary messengers will then after the activation status of the DNA binding factors.

Various types of response of endothelial cells to the mechanical stress have been described. They are restricted to specific sequences of the DNA chain. For instance, the GAGACC sequence controls the response of plasminogen tissular activator (t-PA), of NO synthetase, ICAM-1, etc. These effects will produce time-dependent responses of functional molecules synthesised by endothelial cells. So the endothelial cell response according to the type of stress could be classified into three classes:

1) type I: early and transient increase (c-fos, c-jun, c-myc, PDGF);

2) type II: continuous increase mRNA expression (t-PA, NOS, ICAM-1);

3) type III: two-phase regulation: increase during the first two hours followed by a continuous decrease until the 12th hour (ET-1, PDGF- β, TM, VCAM-1).

Many References Are Given In:

- Born G, Schwartz CJ. Vascular endothelium (physiology, pathology and therapeutic opportunities. Schattauer (STUTTGART, NY) 1997, 391 pp.
- Ehrly A. Therapeutic hemorheology. Springer Verlag (Berlin) 1991-296 pp.
- Lowe GDO. Clinical blood rheology (multi authors books). CRC Press INC (Boca Raton-USA) 1988, 1-288 pp and 2000-2-245 pp.
- Stoltz JF. Hemorheologie and agregation erythrocytaire. Ed Med Int (PARIS) 1994, 4-264 pp.
- Stoltz JF, Singh M, Riha P. Hemorheology in practice. Monography Biomedical and Health Research-IOS Press (Amsterdam) 1999-128 pp.