MICROMECHANICAL METHODS TO DETERMINE HAEMORHEOLOGICAL PARAMETERS

DIRK SEIFFGE, D. V. M.
Hoechst AG, Werk Albert. 6200 Wiesbaden 12. P.O. Box 129101. Fed. Rep. of Germany

SUMMARY

The fluidity of blood in microcirculation is mainly determined by the micromechanical properties of its corpuscular components and its fluid, the plasma. Disorders in their rheological abilities lead to diminished perfusion of the microcirculation and cause a decrease in its nutritive function. Numerous measuring systems have been developed for diagnosing changed flow properties and especially for detecting the responsible rheological parameters. An essential condition is the reliability and reproducibility of each method. The viscosity or fluidity of blood is described by the law of

HAGEN-POISEUILLE: $\eta\,\text{blood} = \Delta p \frac{1}{\dot{V}_{\text{blood}}} \times \frac{\pi r^4}{8l}$

The responsible microrheological parameter and some of the most suitable methods for determination as follows: 1. Hematocrit 2. Temperature 3. Plasma viscosity (capillary viscometer) 3. Platelet aggregation 4. Leucozyte flexibility 5. Erythrocyte deformability (filtration systems, e.g.: Filtrometer MF 4, Single Erythrocyte Rigidometer and 6. Erythrocyte aggregation (Miniaggregometer, rheoscope). Additionally it is necessary to get information about some hematological and biochemical parameters of the blood, e.g.: plasma proteins, pH-values, osmolarity, blood gases and electrolytes. All methods should be performed under defined conditions to achieve highly reproducible and reliable results.

For a long time the flow behavior of the formed elements of the blood has frequently been neglected in respect to cardiovascular control of healthy and diseased. Blood perfusion in the arteries down to the metarterioles and in the venous system is mainly regulated by the action of the driving pressure (heart function) and the smooth muscles of the vessel wall. But there is no explanation by the same mechanism for the arterial and venous capillaries, which diverge as thin-walled, nonmuscular channels. While, previously, only diameter changes and pressure abnormalities were considered we now have to accept that in the mammalian circulatory and respiratory system, not only the transport of the blood in arteries and veins but even more so the distribution of the blood into, and its passage through the terminal ramification of the vasculature is greatly influenced by the rheological behavior of the blood cells, primarily the erythrocytes, but also the granulocytes.[1]

The exceptional fluidity of the red blood cells (RBC) is demonstrated on Fig. 1. It shows the unique adaptation of RBC in their typical slipper or bullet shape to flow within narrow capillaries. This shape change from biconcave to slipper shape is important for their unhindered passage through the narrow gaps of the microcirculation.

From the fluid dynamic point of view, a minimal blood volume ($\dot{V}$blood) is essential for the oxygen delivery to the parenchyma and for the removal of the metabolites and is described by the law of OHM

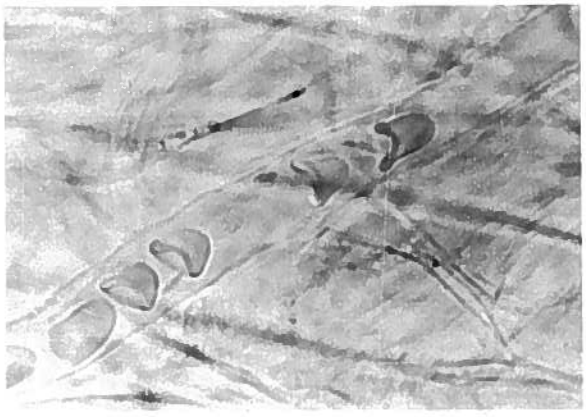

Figure 1: Adaptation of red blood cells in their typical slipper or bullet shape to flow in narrow capillaries; objective: Zeiss Planapo 63/1.4 oil immersion.

$\dot{V}\text{blood} = \frac{\Delta p}{R}$ Δp = pressure gradient over the vascular system, R = flow resistance

The pressure gradient is provided by the pumping of the heart, the law of HAGEN-POISEUILLE approximately describes the flow resistance:

$R = \frac{8\,l}{r^4}\,\eta\,\text{blood}$ l = lenght of the flow tube, r = radius of the circular flow tube, ηblood = apparent whole blood viscosity

The flow resistance can be affected by change in vessel diameter and by change in apparent whole blood viscosity, vessel length may remain constant. Flow mechanically, the blood flow volume, according the HAGEN-POISEUILLE's law, depends on:

$$\dot{V}\text{blood} = \frac{\Delta p}{l} \times \frac{r^4}{\eta\text{blood}} \times \frac{\pi}{8}$$

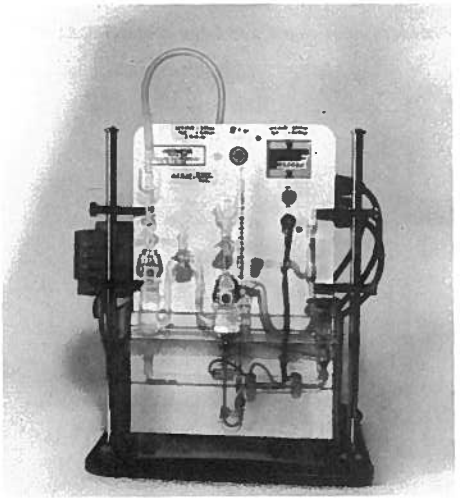

Figure 2: Coulter Harkness Viscometer for the quantification of plasma viscosity. The passage time of plasma under a constant pressure gradient through a glass capillary (diameter: 0.5 mm) is measured with the help of an electrical impedance method.

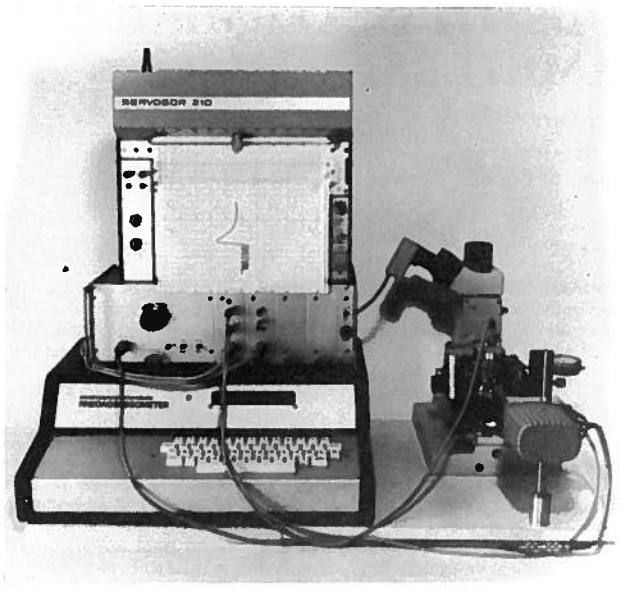

Figure 3: Microcomputer controlled rheoaggregometer (rheoscope) for the measurement of red blood cell aggregation.

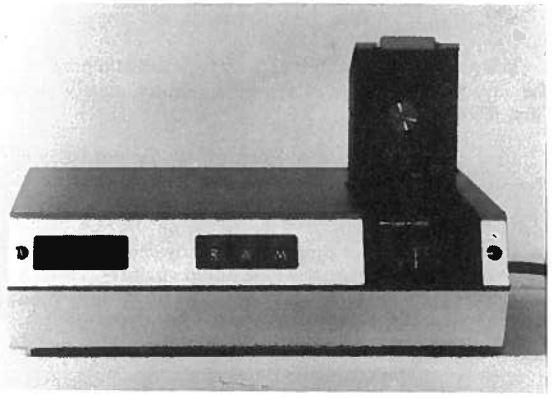

Figure 4: Automated Mini-Aggregometer (rheoscope) for the measurement of red blood cell aggregation.

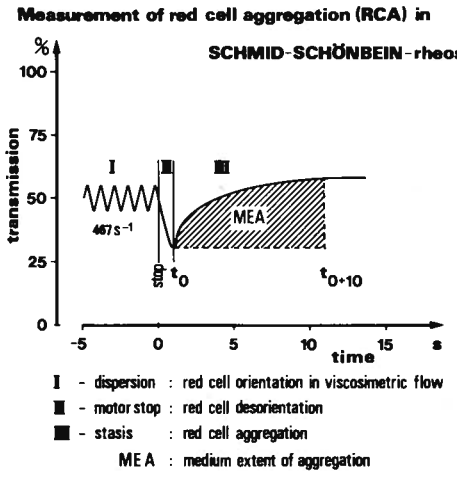

Figure 5: Measuring principle in the rheoscope: RBC dispersed and orientated in a viscometric flow and RBC-aggregation is measured after flow stop within a definite time of 10 s by changes in light-transmission. Results are expressed as medium extent of RBC-aggregation (MEA).

The micromechanical parameters responsible for the apparent whole blood viscosity (ηblood) are as flollows:

1) temperature
2) haematocrit value
3) plasma viscosity
4) RBC aggregation
5) RBC deformability
6) platelet aggregation
7) leucocyte deformability

Haemodynamically speaking, blood is a concentrated dispersion of blood cells in an isotonic protein solution, and the variable apparent viscosity of blood reflects the disturbance of plasma proteins, haematocrit value, red cell deformability and red cell aggregation on the fluidity of plasma.

It therefore seems reasonable that each factor involved in the fluidity of blood should be determined separately.

For the quantification of the flow behaviour of blood the marginal flow mechanical circumstances must be defined and should be measured at T = 37 °C:

Haematocrit

The heamatocrit represents an indirect haemorheological parameter and may be quantified for example with the help of the centrifuge method (radiating particles) or any impedance measurement (Cell Counter). It was first demonstrated by CHIEN [2] that apparent blood viscosity increases overpropontionally with increasing haematocrit. Thus, haematocrit has to be considered as an important factor in the fluidity of blood.

Plasma viscosity

The quantification of the viscosity of the NEWTONIAN fluid plasma is conducted in a rotational or better in a capillary viscometer.[3, 4] Fig. 2 shows a Coulter-Harkness-Viscometer, which measures plasma viscosity in a capillary with the help of an electrical impedance method. The degree of the plasma viscosity depends mainly on the nature and concentration of macromolecular proteins solved in the plasma.

Erythrocyte aggregation

FAHRAEUS [5] recognized that erythrocyte aggregation is a physiological behaviour of blood at low shear rates and the influence of aggregation on the RBC sedimentation rate has been extensively investigated and standardized for clinical practice.[6, 7]

Erythrocyte aggregation means a reversible adhesion of red blood cells to one another in the presence of macromolecular proteins like fibrinogen, α_2-macroglobulin or immunglobulin M. Furthermore, it depends on the shear forces present.

The physiological configuration of erythrocyte aggregation is called rouleaux, the pathophysiological one is designated as clods. KLOSE [8] has introduced the rheoscope for the quantitative evaluation of red cell aggregation (Fig. 3). The newly developed Mini-Aggregometer (9, Messers Myrenne, Roetgen, FRG) facilitates the measuring procedure and estimates directly the medium extent of red cell aggregation (Fig. 4). The measuring principle is demonstrated in Fig. 5. Red blood cells placed in a transparent measuring chamber (cone/plate configuration) were orientated in a viscometric flow at 467 s^{-1}. After flow stop the changes in the intensity of light transmission are recorded within a definite time (10 s) and converted into a dimensionless quantity.

Erythrocyte deformability

Since more than 20 years red blood cell deformability has been videly investigated and a great number of different methodological approaches have been proposed for it's determination: including filtration through filter membranes or micropores,[10, 11, 12, 13] ectacytometry [14] or micropipette-technique.[15, 16] It shows that red blood cell deformability most probably represents a haemorheological parameter, which is very difficult to determine. A schematic plotting of the relationship between «deformability», «deformation», and «fluidity» is given in Fig. 6.[17] Erythrocyte deformability

is influenced by various physico-chemical, plasmatic, and pharmacological factors.

The erythrocyte deformability depends mainly on three variables.

1. The *cytoplasmatic viscosity* is affected by the temperature, the composition and the interaction of the suspended particles.
2. The *membrane stiffness* depends on the composition of the membrane and the nature and concentration of attached components.
3. The *favourable surface* ($\sim 140\ \mu m^2$) — *to-volume* ($\sim 90\ \mu m^3$) *ratio* is stipulated by the evolution of the cell and affected by the metabolic situation of the blood.

The quantification of the erythrocyte deformability can most suitably be assesed in the flow channels which simulate capillaries of the natural microcirculation, e.g. multiple pore or single pore filter systems. Fig. 7 shows the microcomputer-controlled Filtrometer MF 4 (Messrs, Roetgen, FRG), which measures the flow curve of a red blood cell suspension through a filter membrane (Nuclepore Corp. or Mynipore) with defined pore geometry.[18, 19] The measuring tube is U-shaped, RBC are placed in one side, and the filter is inserted at the collateral side the U-shaped tube (Fig. 8). The decreasing pressure gradient initially comes to $\Delta p = 30$ mm H_2O. Different points of the slope of the red blood cell flow curve through the filter are taken for the quantification of the RBC deformability. The Single Erythrocyte Rigidometer (SER) represends a further and highly specified development for the quantification of RBC-filtrability.[20] The SER measures the passage times(ms) of individual erythrocytes through a single pore in a synthetic membrane (Fig. 9). The pore in the membrane simulates a true capillary with a preselected and estimated geometry. At a low and constant driving pressure gradient ($\Delta p = 7$ mm H_2O) the passage of the RBC's is measured by an electrical data detection device (Fig. 10). A microprocessor unit calculates a histogram or a distribution curve of all passage times of 250 erythrocytes (taken as one population) and moreover determines the medium passage time and the passage time of the «slowest» 10 % of the whole RBC population.

Leucocyte deformability, in principle, can be determined using the same filter equipment as for the erythrocytes. For the quantification of platelet aggregation the author refers to the routine laboratory equipment used, e.g. platelet aggregometer according to BORN.[21]

The above mentioned and roughly described methods to quantify haemorheological parameters of the blood do represent a selection only out of a number of numerous systems. The relevance of any haemorheological determination strictly depends on the methods used, but up to now there are no standard suspensions available for the calibration of the measuring system. All data elaborated do only represent a relative information in respect to the own

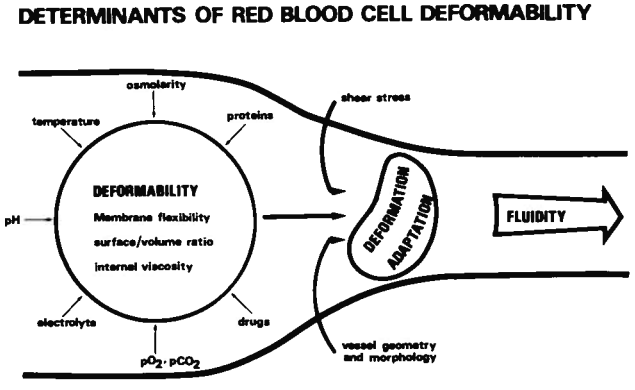

Figure 6: Relation between red blood cell «deformability», «deformation», and «fluidity», which is influenced by various physico-chemical, plasmatic-metabolic, and pharmacological factors (17).

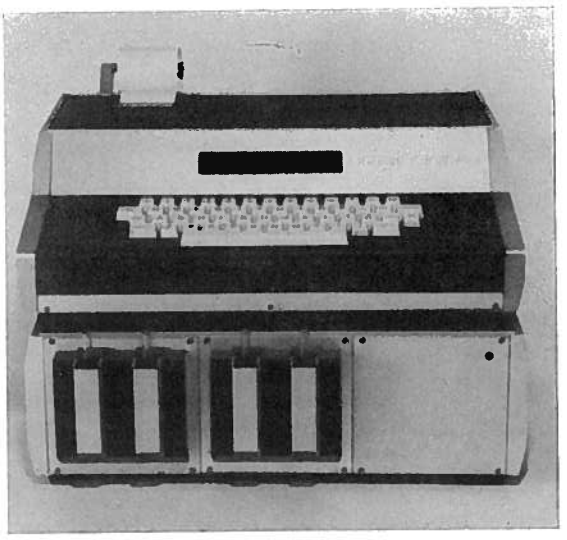

Figure 7: The microprocessor controlled Filtrometer MF 4 measures RBC flow rates through various kinds of filters.

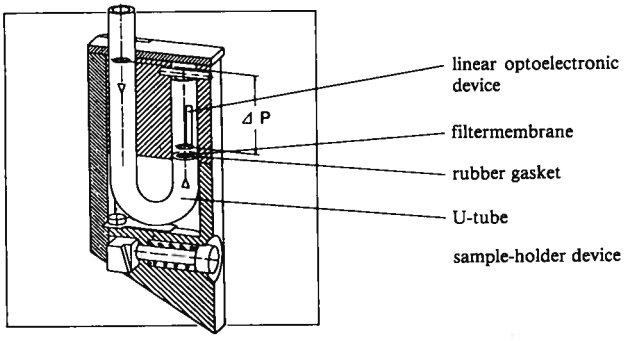

Figure 8: Schematic sketch of the sample holder device of the Filtrometer MF 4.

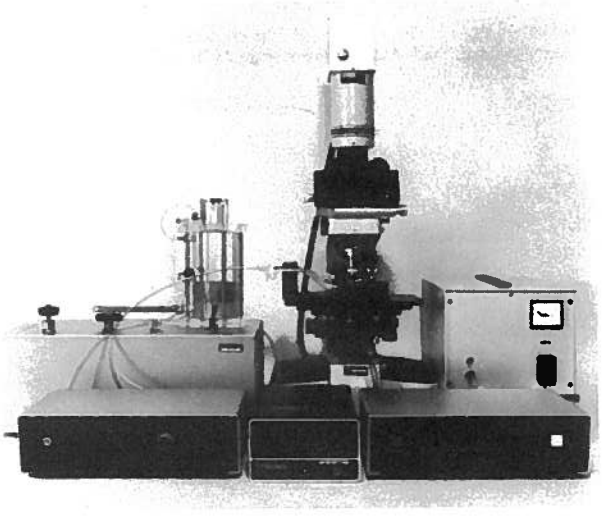

Figure 9: The Single Erythrocyte Ridigometer (SER) measures the passage times of individual RBC-s through a single pore membrane. The measuring chamber is mounted on a microscope stage and combined with a pressure generator (TSJ Calibrator, mod. 1125) and gauge (Mensor, No. 1877), and a microprocessor controlled electrical data detection device.

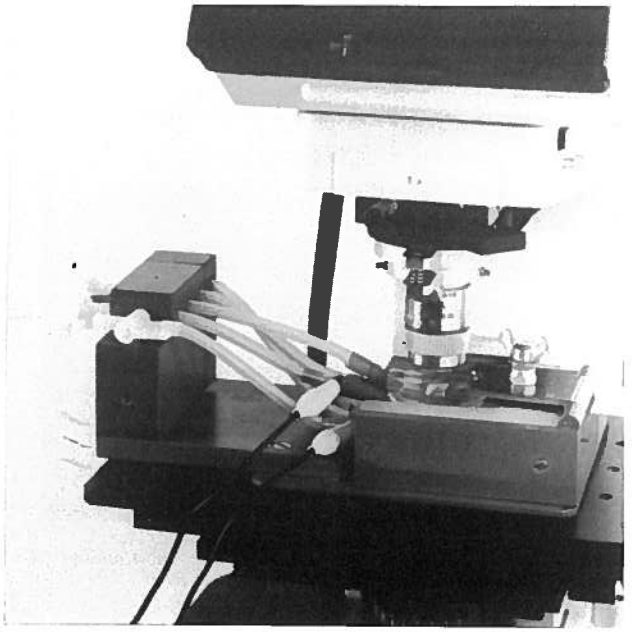

Figure 10: Measuring chamber of the SER; showing inlet and outlet tubes, party of the electrical data detection device, transparent membrane chamber, and microscope stage.

control. In conclusion, apparent whole blood viscosity, erythrocyte aggregation and deformability are physically difficult to be defined; but great progress in the view of methodical equipment has been obtained during the recent years. The reliability and reproducibility of any test system can be improved by taking into consideration that basic haemorheological relations should be obeyed and by the standardisation of the whole test-procedure.

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