STATE OF THE ART LECTURE

HEMORHEOLOGY: PATHOPHYSIOLOGICAL SIGNIFICANCE

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SUMMARY

In order to understand blood circulation, knowledge of the rheological properties of blood is required. However the characteristic parameters which must be considered differ according to the circulatory region investigated. A distinction must therefore be made between the macrorheological parameters (viscosity or viscoelasticity of blood) and the microrheological parameters (aggregation or cells deformability). In normal blood erythrocytes constitute the largest percentage of blood cells and thus hematocrit is a major parameter of blood viscosity. The presence of cells increases blood viscosity because the cells cause a greater energy dissipation during flow than for plasma alone. This increase is dependent not only on the cell concentration but also on the hydrodynamic interactions of the cells during flow (aggregation, deformation). At low shear stresses the cells aggregate and form a three dimensional structure (rouleaux). An increase in shear stress causes the deformation and orientation of RBC in the flow, thus leading to a decrease in blood viscosity. Pathological variations in these factors and the clinical symptoms they produce form the *hyperviscosity syndromes*. Considered from this general angle the etiology of hyperviscosity syndromes can be (a) an increased in plasma proteins levels or the appearance of monoclonal proteins (b) the increase of blood cells (c) the change in R.B.C. rheological properties (internal viscosity or membrane viscoelasticity) (d) the excessive aggregating tendency of the erythrocytes (rouleaux or aggregates) and perhaps that of platelets. From a hemodynamic viewpoint the hyperviscosity syndrome may lead to a slowing down and even complete cessation of local circulation and consequently favor ischemia

Although Aristote suggested that the field of physics should include not only inanimate objects, but also livins beings, the mechanics of blood flow is a fairly recent area of study. Harvey 1578-1658) undertook the first major studies and in 1628 published a volume on *the movements of the heart and of blood.* Other famous names mark the history of blood flow mechanics: Malpighi, Satorio, Boyle, Young and, of cours, Poiseuille (1799-1869) who formulated the relationship between the drop in pressure and the flow rate of a viscous fluid before showing that this law did not apply to blood. This brief reminder of the past would not be complete without mentioning the names of Fick, Kortweg and Lamb. In spite of the considerable amount of work undertaken, it has been difficult to apply rheological concepts to blood because of the fluid's specific properties and its flow caracteristics. And yet it is precisely these characteristics that make blood such an interesting research topic for the rheologist, not only for the purpose of improving understanding of certain physiopathological phenomena, but also for the original theoretical problems involved.

Unfortunately, results known to be well-founded in conventional fluid mechanics have often been applied rather too hastily to hemodynamics without even checking the validity of the provisional hypotheses. The following factors must indeed be taken into account:

- the specific nature of the fluid which is a concentrate cell suspension with complex characteristics (membrane viscoelasticity, large deformation) and liable to strong interactions.[1, 2, 3]
- blood vessel characteristics (deformable by nature)
- the nature of blood flow, mainly non-stationary.

Finally, apart from the characteristics of the fluid, the vessel walls and the nature of blood flow, other physicochemical factors that influence blood's behaviour must also be taken into account (exchange phenomena both within the fluid and on the vascular walls: these phenomena are particularly emphasized by the non-stationary nature of blood flow and the complex 'in vivo' control mechanisms and physiological regulation mechanisms).

These factors can lead to blood flow with a structure that is fundamentally different from that of conventional fluids (stagnant zones at vascular bifurcations, non-homogeneity of the medium in microcirculation, etc.). In order to study these various aspects. This paper is divided into four parts:

1. hemomechanics and physiology of blood circulation;
2. rheological characteristics of blood and red blood cells;
3. classification of hyperviscosity syndromes;
4. Hemorheology in clinical practice: present techniques.

1. HEMOMECHANICS AND PHYSIOLOGY OF BLOOD CIRCULATION

During blood circulation, blood flows from the high pressure area (arterial circulation) to the low pressure area (venous circulation) after flowing through the thousands of vessels that make up microcirculation. The flow rate in the vessels is determined by the difference in presure at the extremities of the network (approx. 100 mm Hg). In most cases, the vessels are simple in structure as they irrigate only one capillary network, but sometimes a more complicated network is observed (Kidney, Lung. etc.).

Mall's observations on dogs (1888) give a semi-quantitative idea of the vascular network (Table 1). The figure shows that for each branch on the arterial side both the number of blood vessels and their total cross-section increases. Accordingly, the capillaries have a total cross-section 7 to 800 times greater than that of the aorta although the area of one capillary is very small (approximately 5.10^{-5} cm^2). Moreover, there is no doubt that in the zones known as microcirculation, blood can no longer be considered as a continuous fluid and consequently the cells' intrinsic rheological properties (mainly the red blood cells) are fundamental in these areas. If we refer to table 1 it can also be noted that the veins, veinlets and capillaries together contain most of blood volume; this is also true in man (Table 2). As shown in table 2, venous circulation (pulmonary of systemic) is a large reservoir for blood circulation and the ratio of systemic vesels volume to pulmonary vessels volume is approximately 2 to 1 (constant ratio in the healthy subject). However, vessel volume varies considerably with pressure and the variations are much greater in the veins than in the arteries. Accordingly, the blood volume in the veins can increase two-fold or more when pressure by just a few mmHg.

As pressure increases, the veins become more and more cylindrical. For example, an increase in pressure of 25 cm H_2O results in a 300 % increase in the volume of the vena cava, whereas to achieve the same variation in the aorta requires an increase in pressure some 12 to 15 times higher.

It is therefore easy to appreciate that the rheological properties of blood and the vascular wall have a leading role to play.

From a hemorheological point of view, to acquire some idea of the flow rates in the various areas of blood circulation, characteristic values such as mean velocity Vi and the Reynolds number are determined. The first parameter can be established on the basis of the principle of conservation of mass: $Vi = Qc/Si$ where Qc = mean flow rate (cardiac flow), Si = total cross-section of the area considered. The mean results calculated for blood circulation are given in Figure 3. Undoubtedly, apart from the case of the aorta and the vena cava, where total flow rate can be obtained at any given

TABLE 1 **Vascular network in dog (from Mall 1888)**

Kind of vessel	Diameter (mm)	No	Total cross-sectional area (cm^2)	Lenght (cm)	Total volume (cm^3)	
Aorta	10	1	0.8	40	30	
Large arteries	3	40	3.0	20	60	
Main artery branches	1	600	5.0	10	50	
Terminal branches	0.6	1 800	5.0	1	25	
Arterioles	0.02	40 000 000	125	0.2	25	
Capillaries	0.008	1 200 000 000	600	0.1	60	
Venules	0.03	80 000 000	570	0.2	110	
Terminal veins	1.5	1 800	30	1	30	740
Main venous branches	2.4	600	27	10	270	
Large veins	6.0	40	11	20	220	
Vena cava	12.5	1	1.2	40	50	
					930	

moment, there is no uniform velocity distribution in the other blood vessels, and in particular in microcirculation.

The Reynolds number can be calculated on the basis of mean velocity in a blood vessel, vessel diameter and blood's kinematic velocity ($Re = VD/\nu$) According to Sutera, taking $\nu = 0.035$ cm^2/sec, the Reynolds number varies from 0.01 in the capillaries to 4500 in the aorta (Table 3). Obviously, this calculation is very approximate. In particular, it takes into account an apparent kinetic viscosity of 0.035 cm^2/sec, but disregards true local apparent viscosity. This value is most probably underestimated in the case of slow blood flow such as venous blood flow.

A final mechanical parameter is also often mentioned for characterising blood flow in a vessel: velocity gradient (or shear rate) on the vessel wall. This parameter is generally calculated using a parabolic velocity profile ($\dot{\gamma}_w = 8\ V/D$) (Table 3). Here too, the value is a rough approximation because blood does not behave like a Newtonian fluid and it is difficult to presuppose a parabolic velocity profile for all blood vessels, particularly in slow blood flow (Fig. 1).

2. RHEOLOGICAL CHARACTERISTICS OF BLOOD AND RED BLOOD CELLS

2.1. General rheological characteristics

Blood viscosity and viscoelasticity

It has now been fully proved that blood behaves like a non-Newtonian fluid and the difference compared with conventional fluids can be observed:

- during transient or periodic flow rates where there is a probable yield shear stress and variable viscosity depending on shear rate;
- during transient or periodic flow rates where memory effects appear in various forms (dephasing between stress and shear rate at simple shear during pulsed flow rate, overshoot or relaxation phenomena on sudden changes in kinematic flow rates, etc.).

TABLE 2 **Distribution of blood in human circulation (from Bazett)**

Pulmonary	Volume (ml)	Volume Systemic	(ml)
Pulmonary arteries	400	Aorta	100
Pulmonary capillaries	60	Systemic arteries	450
Venules	140	Systemic capillaries	300
Pulmonary veins	700	Venules	200
		Systemic veins	2050
Total pulmonary system	1300	Total systemic vessels	3100

Heart 250 ml — Unaccounted 550 ml

(Probably extra blood in reservoirs of liver and spleen)

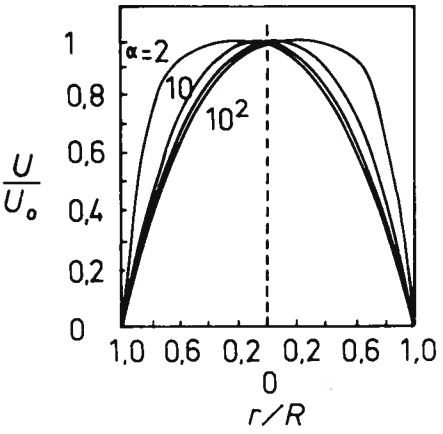

Figure 1: Theoretical velocity profile for a Casson'Fluid for different values of $\tau w/\tau o$ - (τo = 0.0225 dyn/cm^2) (from Stoltz et col.).

The following factors are revealed when apparent viscosity is displayed as a function of shear rate: high viscosity at low shear rates, primarily due to the formation of *red blood cell rouleaux.* Viscosity decreases rapidly and becomes almost constant at high shear rates. The knowledge that viscosity is high at low shear rates is extremely important factor in pathological circumstances with low flow rates (eg. venous blood flow with stasis).

Blood viscosity is dependent on the following main factors:[4, 5, 6, 7, 8, 9, 10, 11]

- cell volume concentration (a parameter which is similar to the conventional hematocrit value);
- red blood cells' mechanical properties, influenced by a number of factors, the most important of which will be detailed below.
- Plasma viscosity: among the main plasma proteins we should mention fibrinogen and albumin, which are known to have an adverse effect on blood rheology.

Studies on the rheological properties of blood during transient or periodic flow are more recent. However, due to the actual nature of 'in vivo' blood flow, blood is submitted to periodic flow imposed by cardiac pulsation.

Further, the geometrical complexity of the blood circulation system results in areas where significant accelerations in blood flow occur and consequently varied transient flow rates exist. It is for this reason that several authors have undertaken experimental investigations into the various transient or pulsed flow rates. The results obtained appear to indicate that during these types of flow rates blood exhibits viscoelastic and thixotropic properties (as long as $\dot{\gamma} < 5$ to 10 sec^{-1}).[12, 13, 14, 15]

The origin of these viscoelastic and thixotropic properties is to be found in the rheological and physical properties of the main cellular constituent of blood: the red blood cell. Indeed, apart from specific rheological properties, the blood cell also has the property of being able to form a three-dimensional network of aggregates, known as as 'rouleaux'. These structures have been objectified using light back-scattering techniques or by direct visualisation (Fig. 2). The rouleaux form a network in the plasma and each cell conveys to the corresponding rouleau elastic properties that become superimposed on the viscous properties of the plasma and hemoglobin contained in the cell. In normal blood at rest aggregation is pronounced and progressive destruction occurs under the effect of viscous friction forces that appear on the surface of the erythrocyte during blood flow. Accordingly, at low shear rates, the network made up of rouleaux and plasma, combines the conditions required for the elastic and viscous behaviour which occurs in transient or pulsed blood flow rates. On the contrary, the progressive reconstitution of the red blood cell network during gradual decrease in shear stress reveals thixotropic phenomena. At higher shear rates ($\dot{\gamma} < 5$ to 10 sec^{-1}) the viscous forces predominate over the forces due to mutual intercellular attraction, the rouleaux are destroyed and any viscoelastic properties that may remain must be attributed to the individual behaviour of the cell.

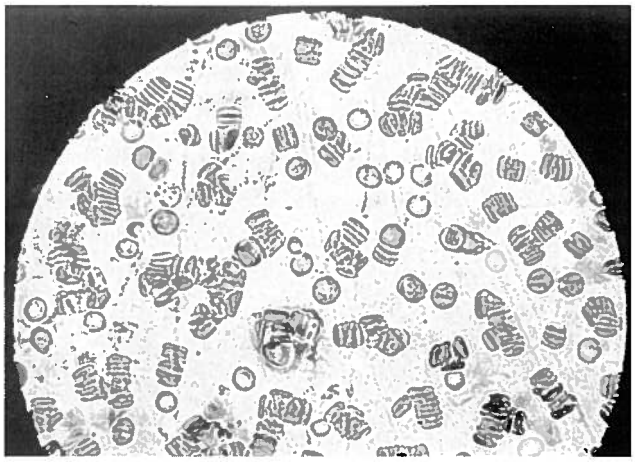

Figure 2: Formation of *Rouleaux* in normal blood.

It is easy to understand why modelisation of blood viscoelasticity and thixotropy is so difficult. One of the most frequently used methods for studying this property consists of subjecting the fluid to one-dimensional pulsed flow with «ω» pulsations and observing the frequency response through variations in complex viscosity (η^*).[16, 17, 18] On the basis of the η^* (ω) curves. Thurston suggests accounting for the variations in these values by introducing a behaviour law corresponding to superimposing Maxwell models in parallel. In collacoration with M. Lucius we have tried to extend Carreau's theory on fluids for polymers in solution by applying in to blood, taking into account the reversible deformation properties of the rouleaux.[19] Using this general approach, the notion of temporary rupture of the links between the rouleaux chains can be introduced and it is possible to proceed form the purely viscoelastic stage to the stage combining the viscoelastic and thixotropic effects generally observed for blood.

2.2. Erythrocyte rheology

The extent of erythrocyte deformation will depend on the outside forces that act on the cell as well as on the intrinsic properties of the red blood cell. The outside forces are made up to the stresses produced by the flow on the membrane; in vivo this will in fact consist of plasma viscosity and blood flow conditions.
The intrinsic deformability of the erythrocyte is dependent on the structure of the membrane and the protein network it contains. Among these proteins, a fundamental role must be ascribed to spetrin and actin. Spectrin can combine with actin while remaining attached to the membrane through certain proteins, the best identified of which is ankyrin (Fig. 3).

TABLE 3 **Mechanical characteristics of the vascular bed in Man**

	Diameter (cm)	Blood velocity (cm/sec.)	Shear rate at the vessel wall (sec^{-1})	Reynold's Number ($\gamma = 0.035$ cm^2/sec)
Aorta	1.6 - 3.2	60*	180	4500
Large arteries	0.2 - 0.6	20-50*	700	400
Arterioles	0.004	0.5+	1000 - 1500	0.05
Capillaries	0.0005 - 0.001	0.05 - 0.1+	800	0.01**
Terminal veins	0.005 - 0.01	0.2 - 0.4+	320	0.06
Large veins	0.5 - 1.0	15 - 20+	200	400
Vena Cava	2.0	10 - 15+	50	700

* Maximal values. + Mean values γ of plasma 0.015 cm^2/sec.

In parallel, with the biochemical parameters, three major microrheological parameters have been characterised: internal viscosity, surface/volume ratio, and the membrane's viscoelastic and molecular properties. Each of these parameters is connected with the above-mentioned biochemical parameters.

a) Internal viscosity (n_i)

The inside of the red blood cell is made up of hemoglobin in solution. This fluid 'confined' within the erythrocyte can be described as a Newtonian fluid.[20]

b) Geometrical factors (surface/volume ratio)

Numerous theoretical and experimental investigations have attempted to account for the specific shape of the red blood cell. It appears that the erythrocyte's shape is the result of a complex equilibrium between a very large number of parameters such as surface tension, membrane thickness, cytoskeleton structure, hydrostatic pressure through the membrane, and surface charge.[21, 22, 23, 24]

From a quantitative viewpoint, the red blood cell is generally considered to have a surface area (S) of approximately 140 μm^2 for a volume (V) of 90 μm^3, which allows deformations with a constant volume to be obtained. The volume/surface ratio is known as the Sphericity Index (S_i). It is to be noted that $S_i = 1$ in the case of a sphere, but is equal to 0.7 in the case of a normal erythrocyte. Any change in the sphericity index results in a change in the cell's deformation capacities.

c) Rheological properties of the erythrocyte membrane

The elastic behaviour of the red blood cell can be ascribed to the properties and specific structure of the cell membrane. However, the published results reveal a wide scatter in the values observed. This contradiction can be explained by the different experimental conditions used.[25, 26, 27]

According to Skalak three types of elastic deformation can be distinguished:

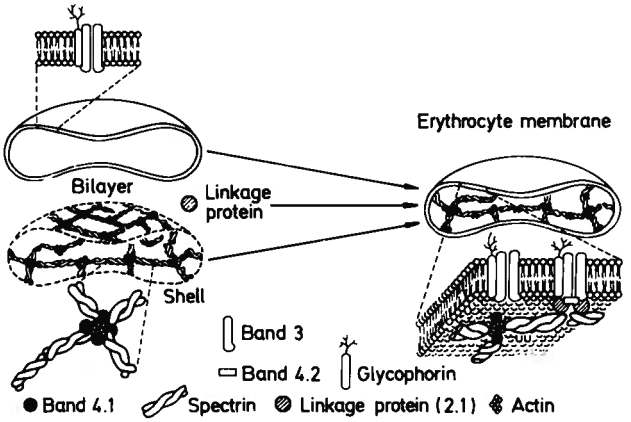

Figure 3: A simplistic diagram of the organization of the erythrocyte membrane and its constituent structural components is presented. The components are not drawn to scale and component interactions are drawn from the current views of erythrocyte protein organization.

— the stretching of the membrane isotropically as in the later stages of spherting;
— shearing of nonuniform stretching at constant area. The elastic modules in this case is very low but very large deformation can be applied and recovered elastically;
— bending deformations which change the curvature of the cell and require bending moment to be applied.

The red blood cell membrane has also viscous behaviour associated with each of the three types of elastic deformation. However, very few studies have as yet been undertaken in this field and accurate assessments of these parameters are still hypothetical.

Apart from visco-elastic properties, the erythrocyte membrane also appears to have a plastic component. Some authors have made allusions to an alteration in this plastic component being responsible for the formation of poikilocytes. Certain observations also mention thixotropic behavior. However, these results must be viewed with caution.

One of the consequences of the erythrocyte's microrheological properties is the existence of a perpetual tank tread motion of the membrane round the hemoglobin. This motion, which has been visualized by Fisher et al. allows membrane stresses to be transmitted and thus increases transfers.[28] The tank tread motion in the membrane, is characterised by a single frequency irrespective of the internal viscosity/suspending medium viscosity ratio.[29, 30]

3. THE HYPERVISCOSITY SYNDROMES

3.1. Definition and etiology of hyperviscosity syndromes [6, 31, 32, 33]

Pathological variations in blood rheological parameters and the numerous clinical symptoms they produce form the «hyperviscosity syndromes». The term «hyperviscosity» was originally used for characterising the plasma hyperviscosity observed during macroglobulinemia and it is only recently that the chapter covering hyperviscosity syndromes has been enlarged to describe the syndromes as a state in which the increased blood viscosity and increase in flow resistance must be considered as the result of the rheological behaviour of blood taken as a whole (plasma and blood cells). Considered form this general angle, the etiology of hyperviscosity syndromes can be:

a) the increase in the number of blood cells;
b) an increase in total plasma protein levels, or the appearance of a monoclonal protein
c) the increase in the erythrocyte's internal viscosity
d) the changes in the erythrocyte's viscoelastic properties
e) the excessive aggregating tendency of the erythrocyte (formation of erythrocyte rouleaux and barely dissociable aggregates) and perhaps that of the platelets.

From a hemodynamic viewpoint, the hyperviscosty syndrome may lead to a slowing down and even complete cessation of local blood circulation and consequently favor ischemia.

3.2. Hyperviscosity syndromes and hematological disorders [34, 35, 36]

a) *Hyperviscosity syndromes in polyglobulia*

Primary polyglobulia defined as a proliferation of erythrocytes, results in a red blood cell count of over 6 millions/mm3 and may or may not be associated with a proliferation of other cells and splenomegalia. Secondary polyglobulia, on the other hand, which is also defined as an increase in erythrocyte count, compensates for an anoxic condition. From a hemodynamic point, it is generally accepted that hyperviscosity reduces cardiac output.

b) *Hyperviscosity syndromes in hyperproteinemia and dysproteinemia*

The hyperviscosity syndrome in hyper and dysproteinemia result in an abnormal formation of erythrocyte rouleaux. In this case, the hyperviscosity is due to the plasma. The plasma viscosity of the latter may reach values several times higher than that of normal plasma. The erythrocyte aggregates form more easily as illustrated by the acceleration in sedimentation rate.

These hyperviscosity syndromes can be subdivided into several sub-groups defined by the presence or absence of abnormally high molecular weight proteins or by the tendency to form erythrocyte aggregates.

As a general rule, it may be assumed that the increase in viscosity of the serum of patients affected by monoclonal gammapathy stems predominantly from quantitative protein disorders (increase in total proteins and their immunoglobulin fractions), rather than from the biochemical and structural nature of the paraprotein in question.

In addition to monoclonal gammapathy, hyperviscosity syndromes can also be observed in the presence of cryoglobulins and cryofibrinogen.

c) *Hyperviscosity syndromes and internal viscosity* [20, 37, 38, 39, 40]

Normal visco-elastic behaviour in the red blood cell is apparently dependent on the relatives state of fluidity of the hemoglobin contained in the cell and on the preservation of the membrane/ /cytoplasm relationship. It has been calculated that at a cellular hemoglobin concentration of 34%, the tetramer lie just 10 Å apart. In spite of the tiny amount of space, it is remarkable to note that in their normal state the molecules are able to rotate freely. It is, however, obvious that such harmony can oly be maintained as long as the tetramerous structure is normal and the cell environment remains constant. For example, the mere fact of suspending normal red blood cells in a hypertonic medium results in an increase in internal viscosity.

Under these conditions it is easy to realise that such serious changes as those responsible for the formation of sickle-shaped cells or the precipitation of unstable hemoglobin in the form of Heintz bodies will lead to a disturbance in red blood cell deformability.

Drepanocytosis: Drepanocytosis or hemolytic anemia with sickle-shaped erythrocytes is the most serious of all hemoglobin disorders. The mutation that affects the primary structure of the α sequence results in hemoglobin precipitating in the form of a gel when partial oxygen pressure decreases (HB-SS). As a result, the erythrocyte's rheological properties are considerably impaired: the sickle-shaped erythrocytes lose their elastic properties which results dresults in a considerable increase in viscosity. The very marked increase in internal viscosity can be compared with the transformation of a fluid particles emulsion into a rigid particles suspension. In vivo the rigid cells block capillaries and the resulting hypoxia further intensifies the formation of sickled cells. This is most probably the explanation for the origin of the multiple cases of thrombosis that occur during the disease.

Unstable hemoglobin: An increasing number of patients with intra-erythrocyte inclusions (Heinz bodies) also suffer from slight to more pronounced hemolytic anemia.

Heinz bodies are caused by an unstable hemoglobin in the erythrocyte, which is inclined to precipitate in vivo. However the presence of Heinz bodies is not a specific indication of unstable hemoglobins, since they are also observed in case of alpha-thalassemia and metabolic disorders involving phosphate pentose shunts.

Betathalassemia: In homozygote patients, the symptoms are not limited to cell shape, but also involve hemoglobin fluidity (Tillmann et al).

The increase in internal viscosity is due to an excess of relatively insoluble β sequences present in the patient's red blood cells. In heterozygote patients there are fewer rigid erythrocytes. The loss of deformability is, in this case, solely due to pathological cell shapes. According to Tillmann, in some patients suffering from homozygous betathalassemia, not only the patient's own erythrocyte, but also transfused red blood cells can very quickly be damaged in an often hypertrophied spleen.

Other types of hemoglobinoses: Other types of hemoglobinoses are probably capable of causing some extent of impairment in internal viscosity. One exemple is the relatively rare case of hemoglobinoses C in which abnormal aggregation and polymerisation cause some modification in the membrane/cytoplasm relationship and an increase in internal viscosity.

d) *Hyperviscosity syndromes connected with a deficiency of the membrane*

Hereditary spherocytosis: Hereditary spherocytosis is the only type of congenital hemolytic anemia that has been diagnosed on the

basis of a rheological criterion: the presence of spherocytes and reduced resistance of the red blood cells to hypotonic solutions. The disorder may involve only a part of the erythrocyte population resulting in an osmotic fragility curve that reveals early initial hemolysis. All authors agree that spherocytes are more rigid and much less deformable.

Hereditary elliptocytosis: A very low percentage of oval or elliptic erythrocytes are present in normal blood, whereas in hereditary elliptocytosis a very rare congenital disease, more than 50 % of the cells are elongated in shape. In the symptomatic form of the disease, both osmotic fragility and autohemolysis are increased.

Other types

Secondary membrane anomalies: Congenital acanthocytosis and the Zieve syndrome in alcoholics are two examples of hyperhemolysis caused by disorders in the normal renewal of membrane phospholipids and cholesterol with plasma.

Membrane and lipid disturbances: The level of membrane fatty acids varies considerably according to the populations studied and probably reflects eating habits. This can be accounted for by the constant exchange processes that intervene between membrane lipids and plasma. The experiments carried out by Butkus et al. on dogs fed with a medium-sequence saturated fatty acid and cholesterol rich diet seem to indicate that after 12 months alterations will have occurred in the distribution of membrane phospholipids resulting in a increase in osmotic fragility. In a different field, Copper and Jandl reveal that the increase in bile salt levels during hepatitis can cause cholesterol accumulation in the erythrocyte membrane, which also induces disturbances in the rheological behavior of these cells.

Paroxystic nocturnal hemoglobinurio: Marchiafava-Micheli disease, or parozystic nocturnal hemoglobinuria, is an exceptional and extremely complex acquired corpuscular anemia. From a rheological viewpoint, it is caracterised by red blood cells that become fragile when incubated in fresh acidified plasma (Ham and Dacie test) or in a low ionic strength sucrose solution.

Auto-immune hemolytic anemia: Rheological studies on auto-immune hemolytic anemia show that the erythrocytes that carry incomplete auto-antibodies on their membranes undergo changes in their rheological properties resulting in greater osmotic fragility.

3.3. Hyperviscosity Syndromes in Degenerative Cardiovascular diseases and Atherosclerosis risk factors [41]

a) Hyperviscosity and degenerative cardiovascular diseases

Heart failure and myocardial infarction: Researches have long been aware of the existence of rheological changes during heart failure. As early as 1962, Wasilewski showed that blood hyperviscosity was one of the characteristics of cardiovascular diseases. In parallel, Mayer observed an increase in plasma viscosity during heart failure and confirmed the results obtained by Murakami et al., whereas Rosenblatt et al found no variation in the same type of patients. According to Kallio et al blood hyperviscosity is higher in patients admitted to hospital for myocardial infarction than for angor with no necrosis. We were able to show that the rheological modifications observed in patients admitted to the hospital with pre-infarction or a threat syndrome (typical electrocardiograph modifications with no necrosis wave or enzyme movement) were more pronounced in patients who ultimately developed myocardial necrosis than in patients whose condition developed favorable and stabilised. Moreover, the analysis of the hemorheological profile and development in the patients studies suggested that the disturbances observed were not merely secondary to myocardial ischemia, but almost certainly play some part in setting off and aggravating the disease. Schmid-Schönbein and well observed rouleaux dispersion which occurred only at high shear rate values during the acute phase of infarction, whereas in normal subjects rouleaux dispersion occurs at about 50 sec^{-1}

Changes in the rheological properties of the blood and an increase in fibrinogenemia during the acute stage of myocardial infarction are fully confirmed. Ditzel et al; have shown that there is a correlation between the increase in fibrinogen and α_2 globulin and the increase in blood viscosity. Kung Min Jan et al have studied how blood viscosity develops during the first 21 days of myocardial infarction. During the first 3 days, blood hyperviscosity is correlated with hematocrit value, but from the third day onwards when hematocrit returns to normal values, blood hyperviscosity progresses and is correlated with the increase in fibrinogen and α_2-globulin and increased erythrocyte aggregation. The authors also found that the higher the patient's blood viscosity on admission, the greater the risk of complications. Moreover, Dintenfass and Forbes have shown that the serum transaminase levels, which indicate the extent of myocardial necrosis, were related to the increase in blood viscosity.

Fewer studies have been carried out on blood viscosity in patients suffering from chronic heart disease. However, Lake and Dintenfass have shown that during effort trials the extent of ST displacement was correlated with blood viscosity, plasma viscosity, and fibrinogen/albumin ratio. They concluded that when interpreting repolarisation troubles in patients with heart failure, blood viscosity, which is a factor of myocardial ischemia, should also be taken into account.

Peripheral arterial disease: The plasma and blood viscosity are increased in peripheral arterial disease (Stormer, Dormandy, Stoltz et al). In parallel, the fibrinogen and globulin levels are also augmented, whereas albumin levels are reduced.[42] There is a significant correlation between fibrinogen level and plasma viscosity. These results confirm those obtained by Schmid-Schönbein et al who showed that there was an increase in erythrocyte aggregation in atheromatous patients. Moreover, Dormandy et al have shown that the blood hyperviscosity syndrome was present in vascular disease patients with intermittent claudication and that blood viscosity was higher in patients with decubitus pain than in subjects suffering from no pain at rest. These authors assume that the increase in blood viscosity may in fact be a determining cause of claudication. Dormandy et al have also shown that there is a connection between the progressive deterioration of the peripheral circulation and the initial levels of blood viscosity and fibrinogen. Accordingly, the patients whose condition worsened had initial fibrinogenemia that was almost twice the normal average; in contrast, in patients who improved, the initial fibrinogen level was less than 4 g/l.[43]

With regard to microrheology, mention could also be made of the problem of an increase in the erythrocyte's internal viscosity caused by polyinsaturated fatty acid depletion.

Cerebrovascular failure: Swank was one of the first authors to observe an increase in plasma and blood viscosity during cerebral vascular disease; the viscosity decreased following several months on a low lipid diet. Subsequently, the role played by blood viscosity in the pathogenesis of cerebral ischemia was shown by Aksyantsev and by Haggendal and Norback. These results have been confirmed by the work carried out by Eisenberg et al: who found blood hyperviscosity in patients with recent cerebral vascular attack. Moreover, there was also a sight increase in hematocrit and a high increase in fibrinogen level. In this case, there was better correlation between viscosity and hematocrit than between viscosity and fibrinogen. Hyperviscosity with low hematocrit, however, is associated with a very high fibrinogen level. It has been noted that the increase in viscosity is particularly noticeable at low shear rates. Moreover, during transient vascular accidents, it appears that the increase only exists at low shear rates. Fedine et al. also revealed an increase in viscosity as early as the first day after an ischemic accident.

In contrast, very few investigations have been devoted to the duration of hyperviscosity in cerebral vascular accidents. According to Fedine, viscosity following ischemia increases from the first to the third day, then decreases after á week and becomes normalised after two weeks. In parallel, Gottstein et al. reported that numerous vasodilatators did not alter cerebral flow rate, but that flow rate was connected with blood viscosity: a decrease in blood viscosity resulted in a significant improvement in cerebral circulation. However, the efects observed could possibly be partly attributed to the decrease in hematocrit. In another study, Arabinar et al. found parallel variations in viscosity in both the arterial blood and the peripheral venous blood and observed negative correlation between cerebral flow and arterial blood viscosity measured at low shear rates. These two observations were made both on subjects with cerebral vascular disease and on control subjects. No change in plasma

viscosity was revealed. Given the importance of the capillary bed in cerebral blood circulation, it may therefore be possible that small changes in the size of the blood vessels or in blood viscosity are enough to produce a disproportionate change in the resistance to blood flow, because of the inversion of the «Fahraeus Linqist effect»,[44] which corresponds to a certain critical capillary radius (Dintenfass).

b) Hyperviscosity syndromes and atherosclerosis risk factors

Diabetes [45]: The problems set by the pathogenesis of diabetic micro-angiopathy are numerous and it is a well known fact that the normalisation of blood glucose levels using appropriate treatment does not usually prevent degenerative vascular lesions from developing. With regard to changes in blood's rheological properties during diabetes, the following observations have been described:

a) an increase in plasma or serum viscosity [46]

b) an increase in blood viscosity generally accompanied by a parallel increase in fibrinogen and α_1 and α_2 globulin levels.[47, 48, 49, 50, 51] In a study on 448 diabetics, we did confirm that there was an increase in plasma and blood viscosity at low shear rates, but we found no significant difference based on either the complications present or the duration of the disease. In contrast, continuous blood sugar control led to a reduction in viscosity and control of the hemodynamic parameters in patients undergoing artificial pancreas treatment. Moreover, in certain cases these changes appear to accompany hemodynamic changes observed during diabetes [52, 53, 54]

c) a decrease in erythrocyte deformability assessed by the micropipette test,[55] or in blood filterability, possibly accompanied by a change in globular ATP concentration, although this is still open to discussion

d) an important increase in erythrocyte aggregation. This phenomenon appears to be connected in part with the increase in plasma globulins and fibrinogen and in lipoprotein levels.[56] (Fig 4)

Skovborg et al. have also shown that the 20% increase in blood viscosity in diabetics as compared with normal subjects, was caused by the increase in erythrocyte aggregation which was significantly correlated with the increase in fibrinogen and globulin levels. The increase in erythrocyte aggregation was connected with a decrease in vascular flow in the postcapillary vessels. Ditzel has shown, in a study on 145 young diabetics, that there was a connection between the degree of erythrocyte aggregation and that of the microangiopathy. Dintenfass confirmed this result and showed that diabetics with severe vascular complications have higher hematocrit values and fibrinogen levels and a faster sedimentation rate than diabetics with no or few complications. This increase in erythrocyte aggregation could be the explanation for the reduced blood thixotropy observed in transient flow.[50] Hart et al have shown that there was a connection between the ocurrence of vascular complications and the increase in fibrinogen levels and that the death rate was higher when fibrinogen was above 3 g/l at the start of the disease

e) an increase in the viscosity of white and red artificial thrombi in diabetics with severe vascular complications. The increase in the thrombus disintegration rate is a source of microembolism. These results should be compared with those obtained by Isogai et al regarding plasma viscoelasticity during coagulation and with those obtained by Rathbowe et al.

Hyperlipoproteinemia: Newman and Twin described an increase in blood viscosity with no change in sedimentation rate during experimental hypercholesterolemia in dogs, but few investigations have been carried out on this problem in man. Bottiger et al showed that the sedimentation rate was higher in hyperlipidemia patients than in the normolipidemic control group. Moreover during exercise the authors observed that ischemic signs were significantly more frequent in hyperlipoproteinemic subjects with an increased sedimentation rate, than in subjects with a sedimentation rate below 25 mm or in normolipidemic subjects. However plasma lipids do not give a straight forward explanation for the acceleration in sedimentation rate and the authors speculated as to whether or not the increase in sedimentation rate may be connected to the rheological properties of the blood. This hypothesis concords with our results obtained on a population made up of Type IIA, IIB and IV hyperlipoproteinemia patients who all had increased plasma and blood viscosity. In a study on arteritic patients, Stormer et al observed that 80% of the patients had increased plasma lipoproteins and that there was strong correlation between blood viscosity and cholesterolemia. These changes could be due to microrheological factors as a result of the changes in erythrocyte or even platelet *membrane microviscosity*. The connection between blood hyperviscosity and hyperlipoproteinemia may be important and could perhaps explain results of prospective studies. At some time during the development of atherosclerosis lesions there may be a stage when blood hyperviscosity becomes an important factor of prognosis.

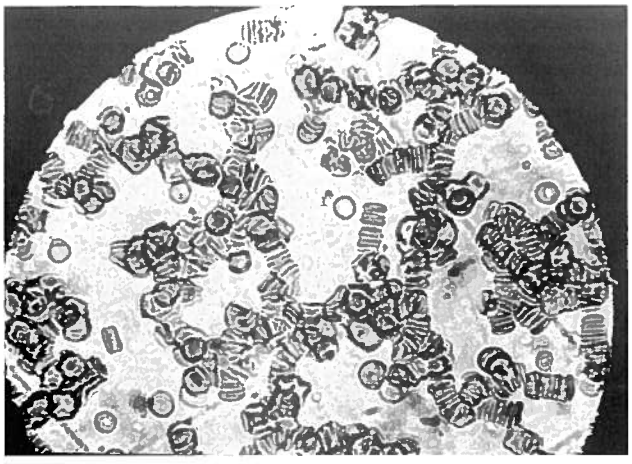

Figure 4: Erythrocyte aggregation in a diabetic patient.

Hypertension [57]: Hypertension is caused by very varied factors. The increase in pressure is usually explained by an increase in vascular resistance caused by a constriction of the arterioles. Linton et al have suggested that high arterial pressure increases the small blood vessels' permeability to fibrinogen, resulting in a change in parietal properties and a risk of erythrocyte fragmentation (hemolysis). As early as 1965 Tubblin et al mentioned the connection between blood hyperviscosity and high blood pressure and showed that there was a positive correlation between these two parameters. In this case plasma viscosity and hematocrit were abnormally high. Mesmer et al have also revealed the relationship between the increase in blood viscosity and the increase in peripheral resistances. In parallel, an increase in screen filtration pressure was observed by Hissen and Swank in connection with platelet and leucocyte aggregation.

Dintenfass and Bauer have studied the artificial formation of white thrombus (platelets) or red thrombus (platelets/erythrocytes in hypertensive subjects and observed that there was a difference in apparent viscosity of the thrombi, formation and degradation rates of the thrombi in the group of hypertensive subjects with vascular complications as compared with the group with no complications. In this case, the increase in viscosity of the thrombi could be an explanation for the persistence of arteriolar thrombi and the increase in their degradation rate could be the cause of microembolisms. Moreover, Dintenfass observed an increase in internal erythrocyte viscosity in hypertensive subjects. Dintenfass also emphasized the importance of rheological factors (increased blood viscosity, increase in erythrocyte rigidity, presence of thrombi degradation products, and platelet aggregates, etc.) which all may intervene both in the genesis of idiopathic hypertension and in the appearance of vascular complications.

It may be assumed that the excessive increase in peripheral resistances is related to the increase in blood viscosity or with a change in the rheological behaviour of one of the blood's components. This could lead to a change in the characteristics of the pressure/flow rate relationship. Indeed, because of the non-Newtonian properties of blood, a slight change in arterial pressure can lead to high variations in flow rate.

Stress: The role of stress in the pathogenesis of myocardial ischemia was emphasized by Olivier et al (1956) and has been confirmed by numerous epidemiological studies, which have also shown that stress results in increased catecholamine production. In parallel, it has been shown in healthy subjects that, emotional stress causes an increase in systolic and diastolic arterial pressure, and increase in heart rate and hypersecretion of adrenaline and noradrenaline. Blood viscosity increases during stress in man.[58] The same is true in animals. Schneider et al have shown that blood hyperviscosity occurs in man within seven to twelve minutes after adrenaline injection, with values returning to normal an hour later. It should also be noted that the hematocrit increases following injection of sympathomimetic amines. The use of cyclopropane, which is a sympathomimetic anesthetic, also results in hyperviscosity. It can therefore be assumed that increased catecholamine release is responsible for the blood hyperviscosity that accompanies stress. Stress also causes an increase in tissue osmolarity which is capable of altering internal erythrocyte viscosity. It is probable, however, that other factors such as tendency to platelet aggregation also contribute. It has been shown during exercise, stress or catecholamine injection, there is an increase in the number in the number of blood platelets, which are more adhesive and likely to aggregate presumably because they are newly formed.

Smoking: Tobacco has been shown to be a high cardiovascular risk factor in numerous epidemiological studies. Studies on the effects of cigarette smoking have revealed three important factors: carbon monoxide inhalation, catecholamine release and cutaneous vasoconstriction; the release of adrenaline and noradrenaline caused by nicotine results in an increase in arterial pressure, cardiac output and platelet aggregation.

Chmiel et al revealed that rheological changes are induced by smoking. Similar results were obtained by Cloarec et al. Blood hyperviscosity in smokers appears to be more pronounced immediately after smoking. Dintenfass studied these changes in 125 male volunteers, aged between 45 and 55, with similar body weight and average cholesterolemia of 2.25 g/l. Compared with non-smoker control subjects, smoking produced a significant change in hematocrit, fibrinogen, albumin/ fibrinogen ratio, plasma viscosity, blood viscosity and erythrocyte aggregation. The increase in fibrinogen could be due to the fact that smoking can lead to an increase in protein synthesis. It is also possible that the carbon monoxide poisoning caused by smoking displaces the oxyhemoglobin dissociation curve, thus leading to tissue hypoxia, which reduces erythrocyte deformability and increases blood viscosity and erythrocyte aggregation.

c) Hyperviscosity in various pathological circumstances

The study of the various hyperviscosity syndromes reveals the wide diversity that exists and the lack of accurate and totally convincing data available in some cases. The list of diseases causing this type of modification is far from exhaustive. Indeed, hyperviscosity syndromes can also be encountered in various pathological circumstances: inflammatory syndromes, collagenosis, parasitosis, vasomotor syndromes of the Raynaud type, shock burns, toxemia during pregnancy, fatty embolism neoplasia, etc. Unfortunately, in all these phenomena the exact origin of the rheological disturbances has still not been clearly analysed.

d) Therapeutical aspects

With regard to the therapeutical aspects, some classifications of available therapy can be made on the basis of the various etiologies suggested above.[59] It is necessary, however, to clearly distinguish between vasoactive medication, which may have an indirect hemorheological effect and other types of medical treatment which may have a more direct action. Treatment of polyglobulia and improvenent of hemodynamic factors using hemodilution are obviously effective when hyperviscosity is caused by changes in hematocrit. Plasma exchange is the logical treatment for plasma hyperviscosity. As regards rouleaux formation and red blood cells aggregates, a certain number of dispersing substances are available, but it is sometimes difficult to distinguish between the effects of dilution and the more specific effects. Diluted albumin and certain plasma substitutes are worth mentioning. The problem of platelet antiaggregating substances should be examined separately. Concerning microrheological actions, a distinction can be made between substances acting at the cell membrane, substances that act on hemoglobin and the Hb-O_2 connection and substances that appear to have a general effect on deformability (rutosides, isoxsuprine, pentoxyfilline, naftidrofuryl, suloctidil, buflomedil, cinnarizine, flunarizine, fludarene etc.) There is no doubt that there are numerous substances that should now be examined from a hemorheological point of view; this should provide further investigations into their action on hemorheological factors.

4. HEMORHEOLOGY IN CLINICAL PRACTICE: PRESENT TECHNIQUES

No clinical progress can be achieved without reliable investigation techniques for measuring a given parameter. Unfortunately though we have to admit that results obtained using non-specific techniques are sometimes used to construct physio-pathological theories. Over the past few years, in particular, a considerable number of microrheological modifications have been described in various clinical fields; although most of these observations are no doubt related to objective, phenomena, some are quite definitely connected with artefacts in the techniques applied.

In clinical hemorheology, several types of techniques will have to be used in order to acquire knowledge regarding the rheological properties of blood and blood components. These techniques can be divided into three main groups: macroscopic (methods for studying viscosity and viscoelasticity, microscopic (rouleaux, red blood cell or platelet aggregates and red blood cell deformability) and molecular (membrane spectroscopy, surface charge etc.).

4.1. Macroscopic rheological techniques

Because of blood's non-Newtonian properties, blood viscosity measurements must be carried out using viscometers that are capable of measuring viscosity values at variable shear rates. Two types of viscometer are available for clinical investigations: rotary viscometers or, capillary viscometers with a variable pressure system.

a) Rotary viscometers

The earliest viscometer used in clinical hemorheology is undoubtedly the Brookfield plan-cone viscometer. Unfortunately, although this instrument has a fairly wide range of shear rates (1.15 to 230 sec^{-1}) it lacks accuracy for use on a routine basis. As more sensitive systems became available, interest in the Brookfield viscometer decreased. Among the most widely used viscometers in Europe are: the Weissenberg rheogoniometer, the Contraves viscometers (in particular the LS30) and the Couette type viscometer manufactured by the firm Heraeus (Biovisco).

Although these viscometers can be used for routine measurements in the hospital, they do nevertheless have a certain number of disadvantages that often discourage the biologist. To start with, the instruments are expensive (costing over $ 25 000), the controls are detailed and must be adjusted frequently. A new generation of viscometers with set shear stresses (instead of shear rate) will no doubt be easier to use and be considerably less expensive. (De Deer viscometer, ferro-fluid viscometer) [60]

In order to draw conclusions regarding the rheological behaviour of blood, the results are expressed by tracing the shear stress (τ)/ shear rate ($\dot{\gamma}$) curve. However, as in clinical practice it is preferable to standardise the form in which results are expressed, we suggest using the calculation of apparent viscosity (η_a) at specific shear rate values on blood samples at patient's hematocrit and at corrected hematocrit (40 or 45 % for example).

b) Capillary viscometers

Newtonian or non-Newtonian fluids can be characterised using capillary viscometers by measuring flow rate Q at for different pressures P. In the case of Newtonian fluids, Poiseuille's formula supplies the viscosity value immediately. For non-Newtonian fluids, an apparent viscosity value can be calculated or — applying certain hypotheses — the rheological law can be extrapolated by tracing the variation in $R \cdot \Delta P/2 L$ as a function of $\frac{Q}{\pi R^3}$. At the present

time there is no easy-to-use method available for measurements in clinical hemorheology, except for plasma.

4.2. Microrheological techniques

a) Rouleaux formation: kinetic aggregation and disaggregation thresholds, and size

As discussed earlier, red blood cell aggregation in undoubtedly the major factor accounting for the non-Newtonian nature of blood at low shear rate values. Various methods have been put forward for studying this phenomenon; we shall mention three techniques that can be used for routine clinical measurements. Of these three techniques, optical methods are undoubtedly the most advanced at the present time. The light transmitted or back scattered by flowing blood varies depending on flow velocity and when these red blood cells are suspended in an artificial non-aggregating medium the variations in optical density or light back-scatter are mainly due to red blood cell orientation and deformation. It is then a question of connecting flow conditions with the state of cell aggregation.

Some authors have developed viscometric systems for studying mean rouleaux size according to the shear stresses applied. The systems are simple and are made up of a viscometer and a laser which lit the RBC suspension.[61] On the basis of the back-scattered light intensity or transmitted light intensity, these methods provide an assessment of mean rouleaux size at the applied shear rate (or a mean aggregation index). These systems are still at the prototype stage although models are being adapted for industrial purposes.

Another method consists of observing the rouleaux directly in a viscometric system (rheoscope). Using this method, that was first described by Schmid-Schönbein, it is possible to define, under experimental conditions, the mechanical threshold for red blood cell association and dissociation. In the near future digital image processing should provide the means of quantifying and defining structure parameters (aggregation threshold, disaggregation threshold, kinetic parameters)

A final possible technique consists of studying the reflection of ultrasound waves. Indeed, the amount of energy back-scattered by particles in suspension depends on the number and the size of the particles; the frequency used and, of course, the type of medium and type of particles.

b) *Red blood cell microrheological techniques*

Among the numerous methods available for approaching red blood cell microrheological properties, very few provide a quantitative approach that can be applied on a routine basis. Among the standard techniques we have chosen four that can be used for clinical investigations.

Direct observation: mainly involving the technique described above for blood rouleaux and consisting of directly observing the phenomena in a viscometric system (rheoscope). In this case, the red blood cells are suspended in a high viscosity medium and the shape of the deformed cells as well as the tank tread motion of the membrane are observed directly.[28]

Optical method: a recent technique based on laser light scattering has been put forward by Bessis et al (Ektacytometer-Technicon). The red blood cells are subjected to shear stress in the gap of a co-axial cylinder viscometer and cell deformability is assessed using the light scattering image in the small angles of an HE-NE laser passing through the RBC suspension.[62]

Under normal shear stress conditions, an elongated image is observed. At the present time, the results are expressed semi-quantitatively by studying variations in the axes of the diffraction image. Alternative techniques based on the same principle have been undertaken using plan-cone viscometers.

Filterability techniques[63]: Ever since the work undertaken by Teitel and by Gregersen et al a large number of authors have suggested using red blood cell filterability tests for approaching cell deformability. Over the past few years a considerable amount of research has been carried out using the technique known as the *whole blood technique* put forward by Reid et al. Unfortunately, this technique is highly approximate and unreliable as weel as being difficult to interpret in terms of red blood cell microrheology. During the past few years as a result of discussions conducted by an international Committee, various more specific, standardised methods have evolved and are now available on the market: the 'Filtrometer' derived from Teitel's research, the 'Erythrometer' (constant flow rate method used with 3 to 5 μ filters) and the 'Hemorheometer' (almost constant pressure method with 5 μ filters).

These various techniques have the advantage of providing the users with a standardised method and a means of calculating a red blood cell filterability index. As these techniques become more widely used in laboratories carrying out clinical or pharmacological

TABLE 4 **Techniques available for use in clinical hemorheology**

Type of instrument	Name of instrument	Manufacturer of distributor	Approximate price (in $)
Viscometers	— Wells Brookfield	Brookfield Stoughton (USA)	8 000
	— Rheogoniometer (Bio-Rheo)	San Gamo (G.B.)	60 000 - 80 000
	— LS 30	Contraves - Zurich (CH)	25 000 to 35 000
	— CV900/LV 100 System	Heraeus France (Les Ullis)	30 000
	— CS Rheometer (De Deer)	Ets Chauvin-Tassin (France)	15 000
	— Silenus Viscometer	Silenus Instruments (Victoria, Australia)	17 000
	— Sieglass-McKelvey capillary rheometer	I-Mass Inc. (USA)	25 000 - 35 000
	— Ferrofluid Couette viscometer	Sefam - Nancy (to be commercialised in 1985)	9 000
Rouleaux Investigations	— Rheoscope with video and photographic system	Myrenne GmbH (Aix-la-Chapelle-GFR)	16 000
	— Red blood cell aggregameter (cone-plan)	Myrenne GmbH (Aix-la-Chapelle) (GFR)	3 000
	— Red blood cell rheoaggregameter (Couette)	Sefam - Nancy (Commercialisation in 1985)	8 000
Red blood cell deformability and filterability	— Micropipette with integrator and video		6 000 - 10 000
	— Ektacytometer	Technicon (France)	35 000
	— Filtrometer	Myrenne GmbH (Aix-la-Chapelle)	5 000
	— Erythrometer (also capable of measuring plasma viscosity)	Sefam - Nancy	3 500 - 4 500 (depending of options)
	— Hemorheometer	IMH (France)	5 000
	— Single Pore	Myrenne GmbH (Aix la Chapelle) (GFR)	over 12 000
Spectroscopic techniques	— RPE	Numerous instruments are available for these two spectroscopic techniques	over 40 000 (7 500 - 60 000)
	— Fluorescence polarization		
	— Fluofluidimeter F1	Sefam - Nancy (France)	8 000
	— Electrophoremeter (surface charge)	Sefam - Nancy (France)	25 000

studies, it will, in the future, be possible to anticipate the possibility of several laboratories working together on a same project.

4.3. Molecular rheology: Spectroscopic methods

In order to understand rheological phenomena, knowledge must be available regarding the exchange and interaction mechanisms that take place on the molecular scale. A large amount of information can be obtained using spectroscopic techniques. The methods used must be capable of locating the position of the molecules, either by direct indentification or by using probes distributed within the system. All the spectroscopic methods available for use in molecular hemorheology involve the interaction phenomena of electromagnetic radiation.[64, 65, 56, 67]

Among the available methods, the most widely used in hemorheology are those involving either radiation (fluorescence, circular dichroism) or hertzian waves (mainly RPE).

As biological media are not usually directly suitable for spectroscopic studies, molecular probes have to be used. These probes consist of molecules chosen for their spectroscopic response qualities (spin markers, fluorescence markers). These methods used in conjunction with other techniques will, in the future, provide better understanding of rheological phenomena in the molecule. Specific instruments for carrying out routine testing at a faily low cost (e.g. SEFAM'S S1 fluo-fluidimeter) are now at the industrial stage.

4.4 Financial aspect

As seen above the methods available for use in hemorheology are extremely varied. The cost of some of these methods remains high (Table 4) (particularly in the case of conventional viscometers) and laboratories involved in clinical hemorheology must make a judicious choice of at least one method for each parameter investigated (viscosity, blood rouleaux, deformability or filterability, spectroscopy). The data given in the figure indicate that by choosing the least expensive systems it is possible to dispose of the basic equipment for 4 techniques at a cost of approximately $ 30 000.

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