HEMORHEOLOGY & BIOPHYSICAL PROPERTIES OF BLOOD CELLS

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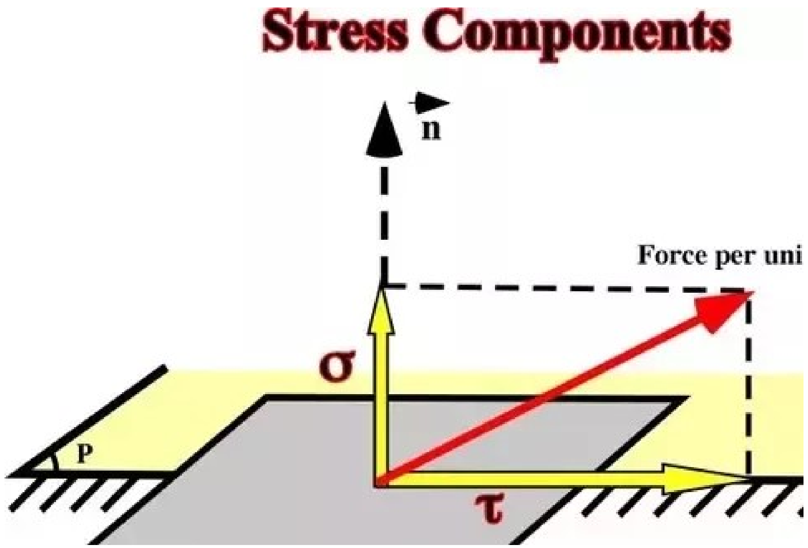
Basic Aspects of Rheology

- ***Rheology*** is the scientific field that **deals with the flow and deformation behavior of materials** under consideration **being solids or fluids**, including ***liquids and gases.***

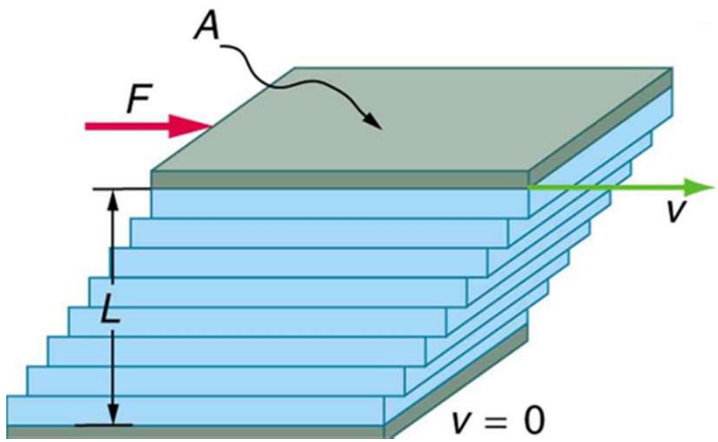
- ***Deformation*** can be defined as the **relative displacement of material points within the body**.
  - An object or body is said to have deformed **if its shape or size is altered due to the action of appropriate forces.**
  - **If the degree of deformation changes continuously with respect to time, the body is considered to be flowing.**

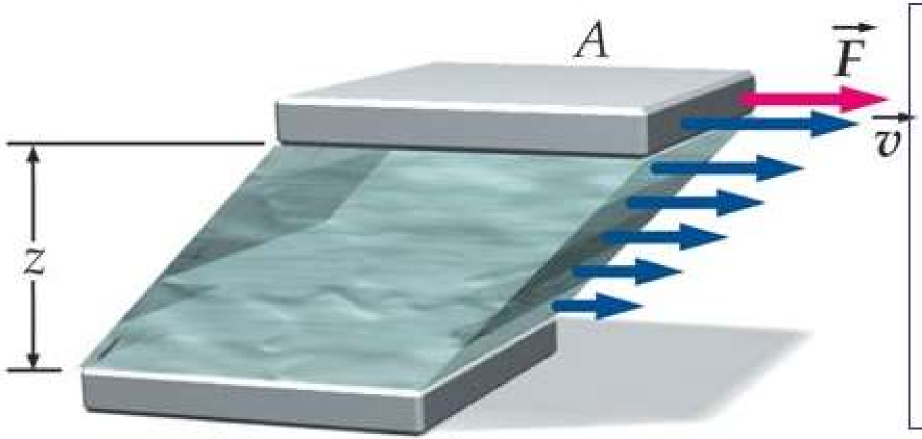
In understanding the degree of deformation (or flow) of a material, the force applied per unit area must be considered.

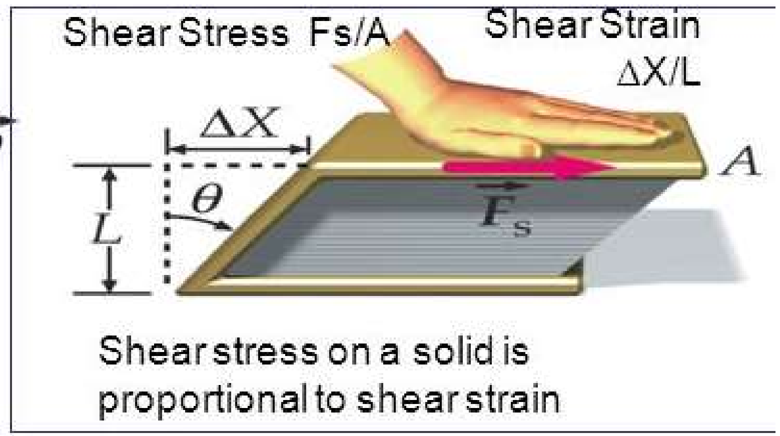
- The deforming force, termed **stress**, may have several components, including:
  - **Normal stress**, the force per unit area acting perpendicular to the surface (**is defined as pressure in a fluid**).
  - **Shear stress**, **the force per unit area acting parallel to the surface.**



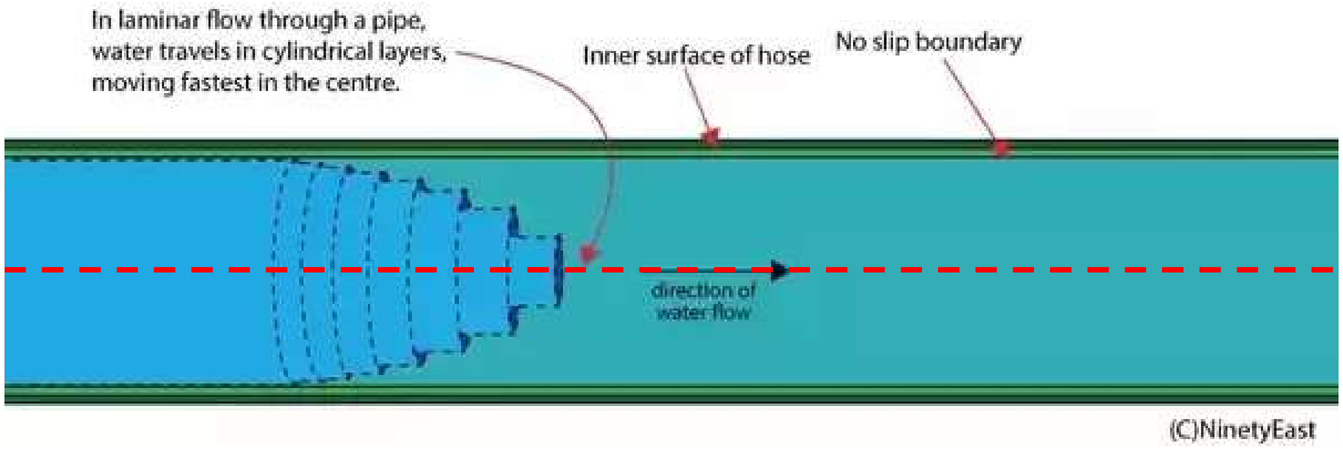
- **Shear stress results** in **shear strain (degree of deformation)**, **often termed *shear rate*, in which the layers of material** (Eg… Fluids) ***move parallel to each other*** in a progressive manner.

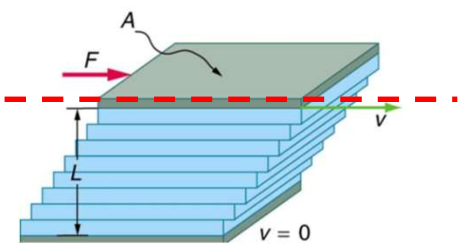




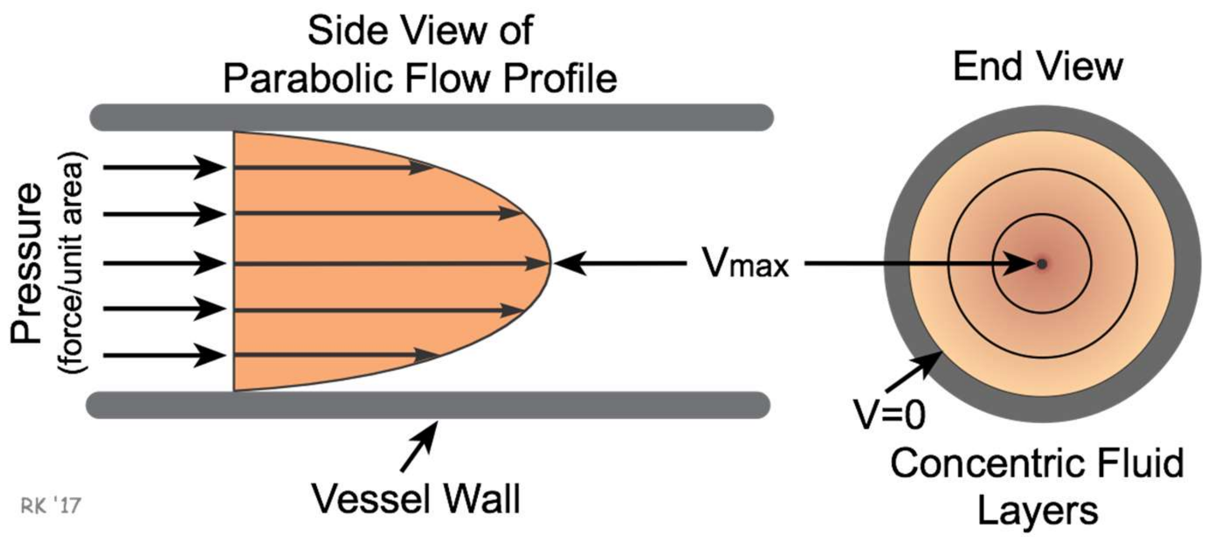


- **Fluid mechanics have revealed that:**
  - For a pipe of **constant diameter and length and for a given fluid**, ***the resistance to flow (viscosity)*** **depended on the flow conditions** within the pipe.
  - **During slow flow,** ***pressure drop (reflecting the resistance to flow)*** was **proportional to the speed of flow**.
- **Under these conditions**, it can be said that **the liquid particles move smoothly in adjacent planes (laminae) parallel to the tube wall**; this type of flow is called ***laminar flow***.





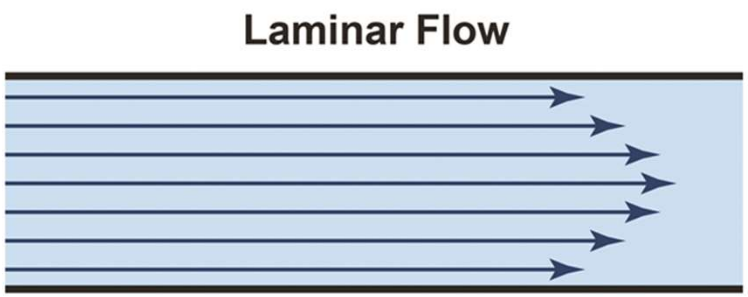
Laminar Flow

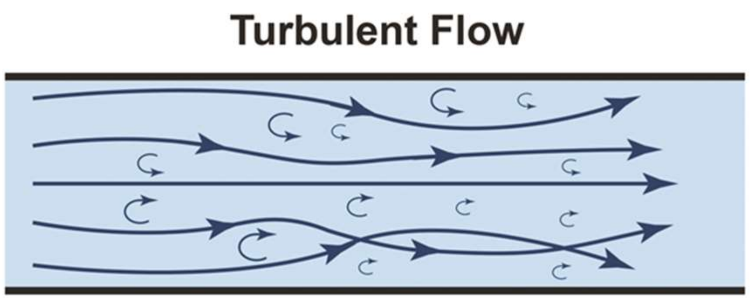


- Is characterized by concentric layers of fluid (Eg… Liquids) moving in parallel down the length of a tube.
  - **The highest velocity (V_{max}) is found in the center of the tube.**
  - **The lowest velocity (V=0) is found along the tube wall.**
- **The flow profile is parabolic once laminar flow is fully developed.**
- This occurs in long, straight blood vessels, under **steady flow conditions**.
- **Is the normal condition for blood flow in most blood vessels.**

With increasing flow rate (refers to the amount of fluid passing a given position along the length of a tube or vessel)…

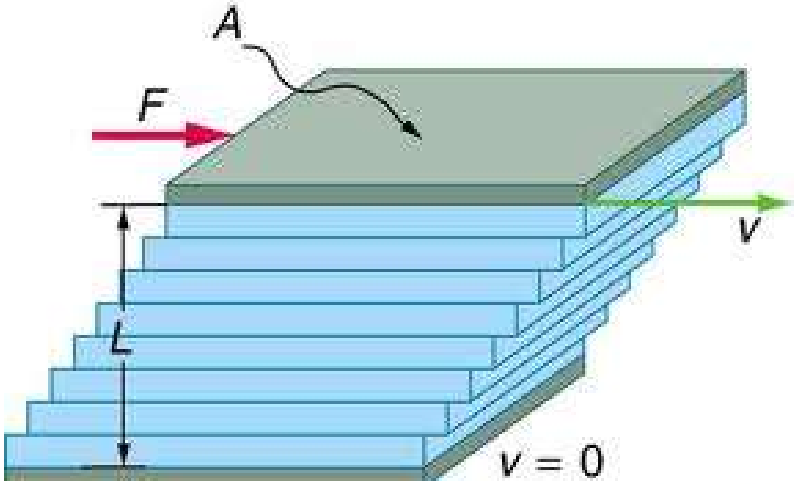
- The **fluid flow to become irregular**, with fluid moving in **swirls and irregular patterns**.
- This type of chaotic flow is termed "***turbulent***", and **the degree of turbulence increases with increasing flow rate**.
- **Under such turbulent conditions**:
  - The pressure drop is proportional to the square of the speed of flow,
  - ***Resistance to flow (viscosity)* is greater with turbulence than it is for laminar flow.**

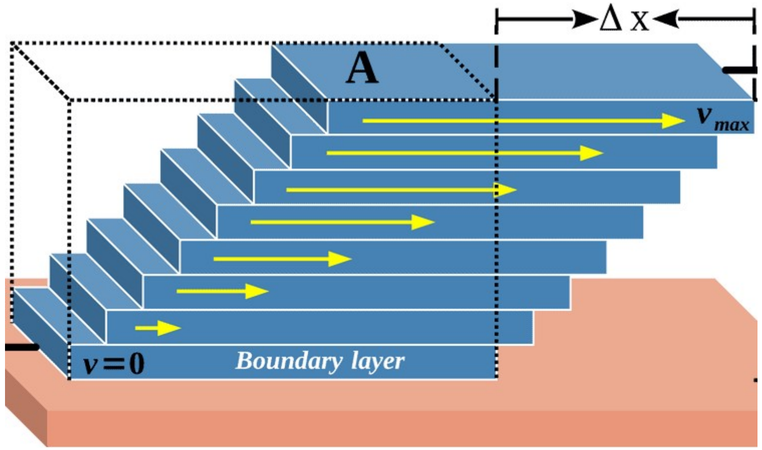


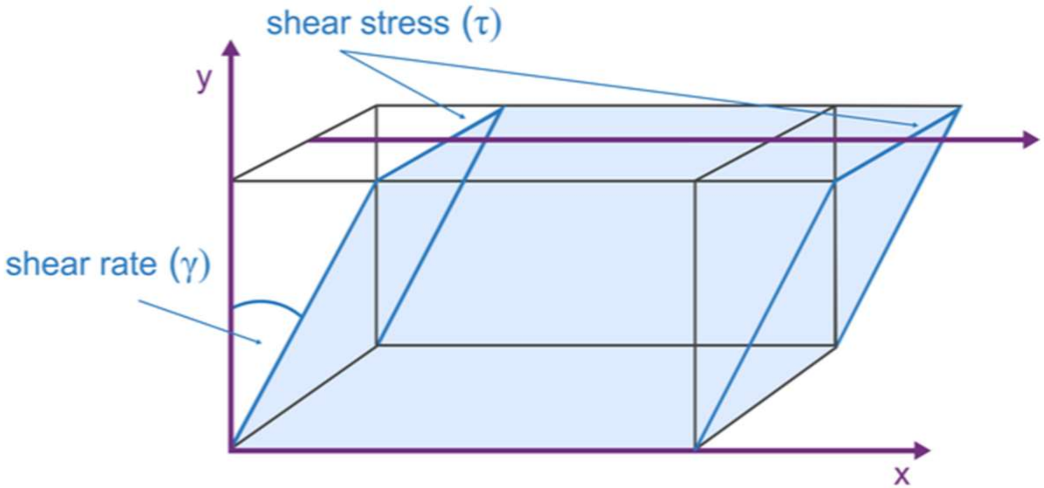


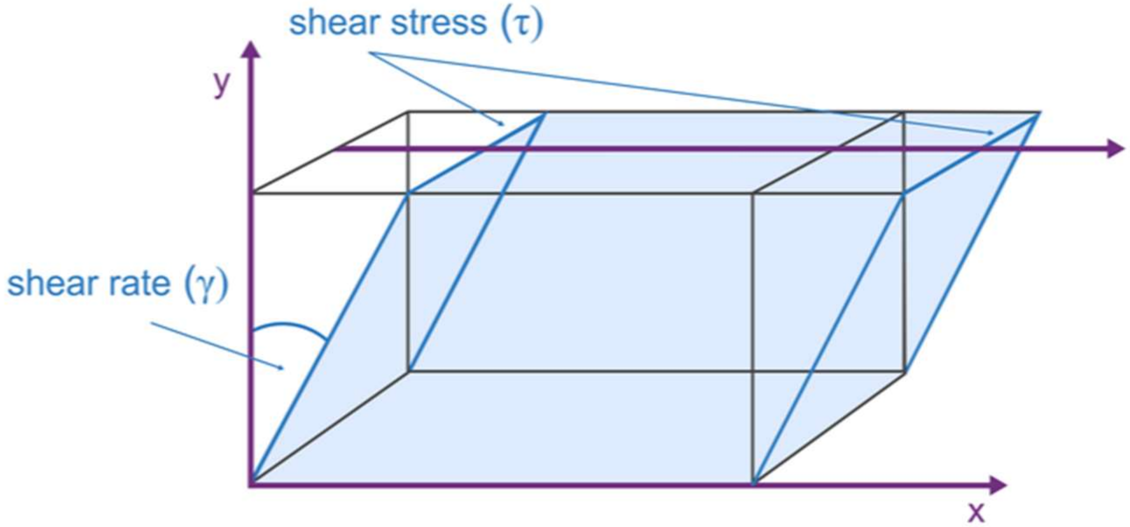
Under laminar flow conditions,

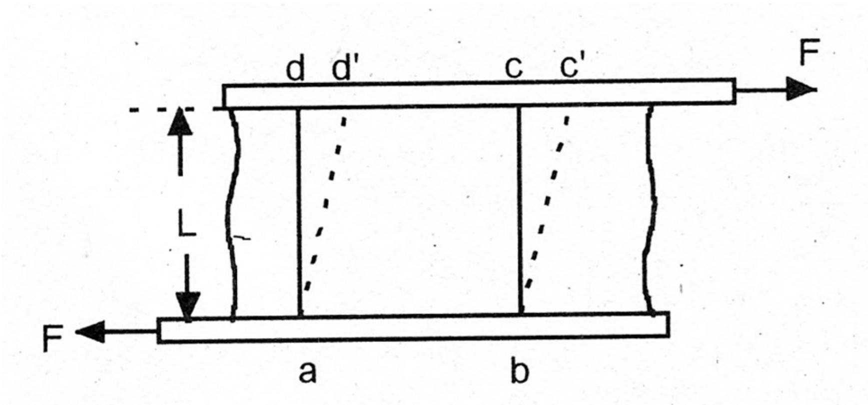
- **A shear stress – shear rate relationship is used to define the fluidity of liquids.**
  - ***Reflects the internal resistance between fluid layers (laminas)*** and thus **reflects** the ***viscosity*** of the fluid.









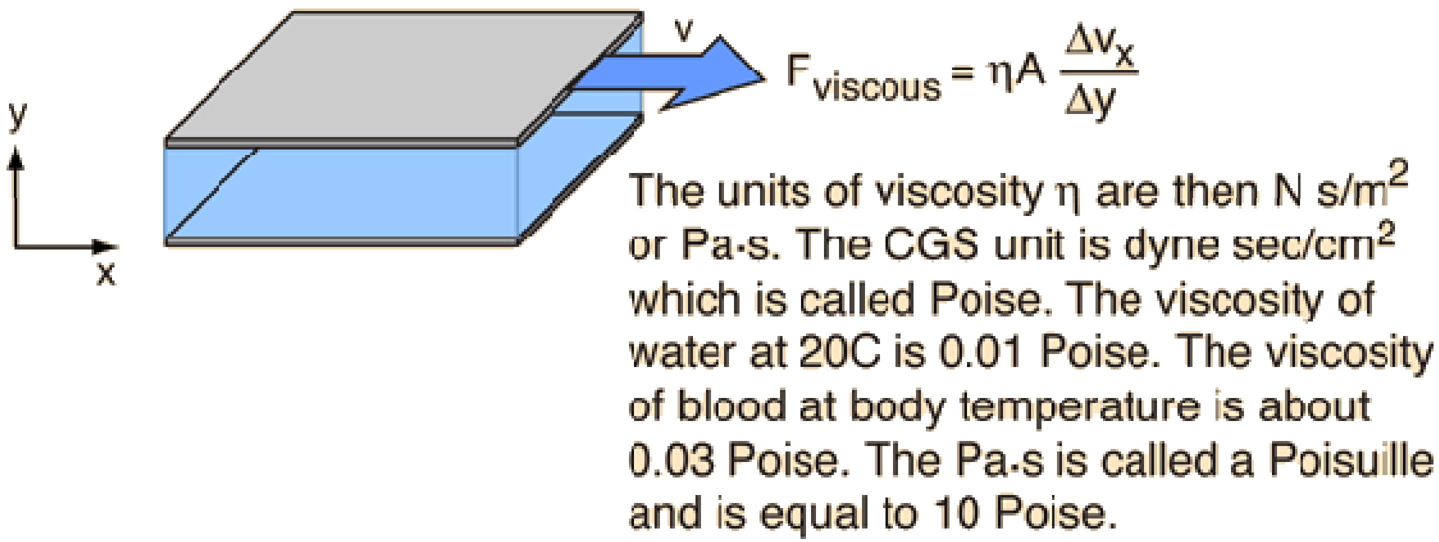


$$\frac{F}{A} = \eta \frac{1}{L} \frac{d\left(\frac{cc'}{L}\right)}{dt}$$

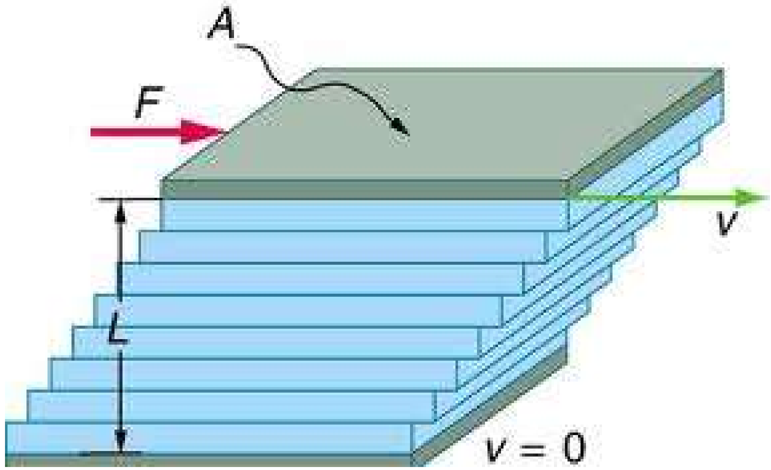
$$\frac{F}{A} = \eta \frac{v}{L}$$

Viscosity

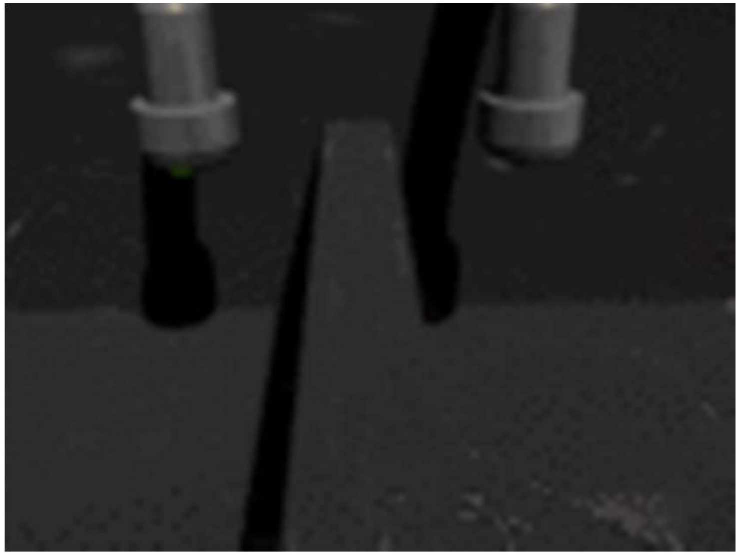
- **Under conditions of laminar flow;**
  - The **force** required to move a plate at constant speed against **the resistance of a fluid** is proportional to the **area of the plate** and to the **velocity gradient perpendicular to the plate**.
  - The constant of proportionality is called the **viscosity**.



*** Pa·s = pascal-second**



$$\frac{F}{A} = \eta \frac{v}{L}$$



$$\eta = \frac{F/A}{v/L} = \frac{shear\ stress}{velocity/distance} = \frac{N/m^2}{\frac{m}{s}/m} = \frac{N}{m^2} \cdot s = Pa \cdot s \quad (SI)$$

$$= \frac{dyn}{cm^2} \cdot s = Poise \quad (CGS)$$

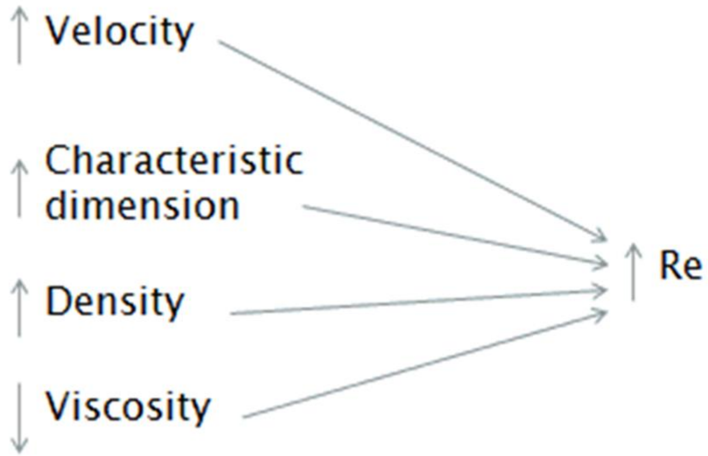
$* \; 1\, Pa \cdot s = 10\, poise$ $\qquad$ $* \; 1\, poise = 100\, cp\ (centipoise)$

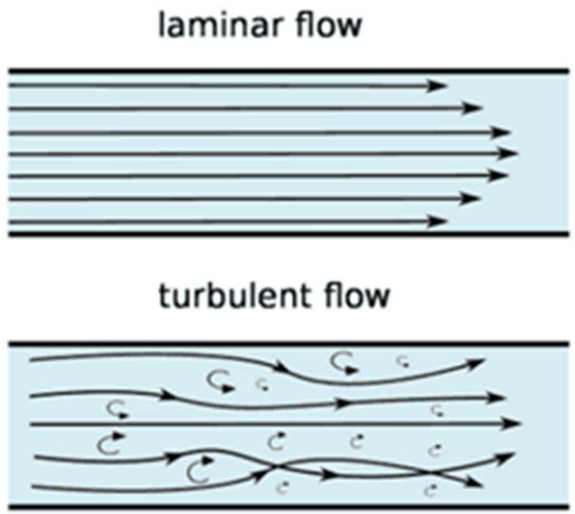
Turbulence does not begin to occur until the ***flow velocity*** becomes high enough to break **flowing laminas** apart.

Turbulence **occurs** when a critical **Reynolds number (Re)** is **exceeded**. Re is a way to predict under ideal conditions when turbulence will occur.

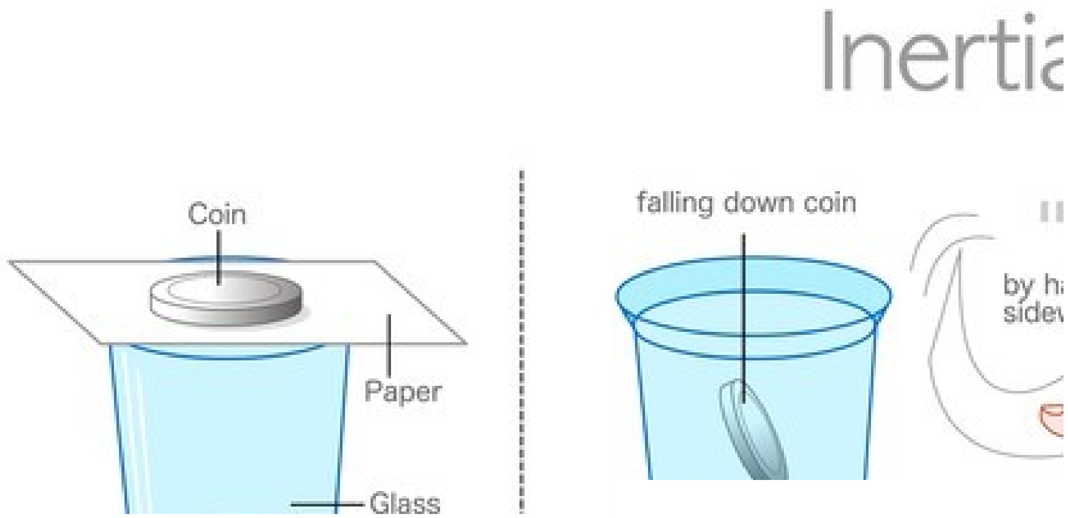
Where V = mean velocity, D = vessel diameter, ρ = blood density, and η = blood viscosity

$$Re = \frac{(\overline{V} \cdot D \cdot \rho)}{\eta}$$





It can be interpreted that when the **viscous forces are dominant** (slow flow, low Re) they are **sufficient enough to keep all the fluid particles in line**, then the flow is laminar.



Momentum Conservation

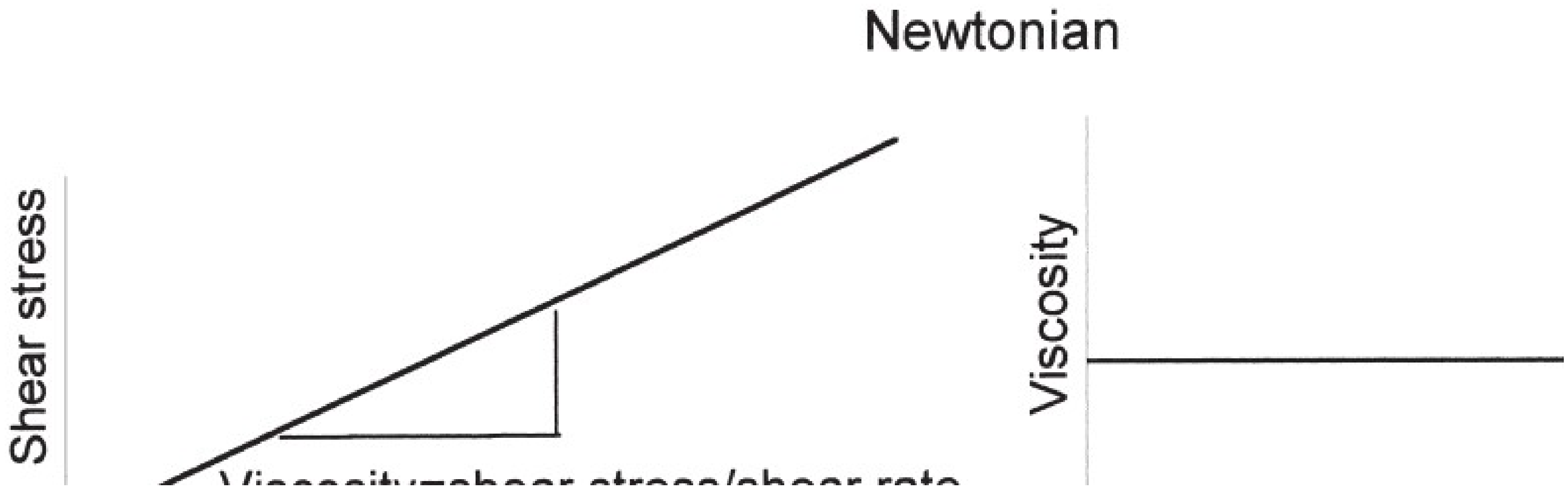
- Newton's Second Law
  - Force = Mass * Acceleration
  - Alternate method: From Reynold's theorem
- Fluid Flow
  - Force = Momentum flux + Momentum Accumulation rate

$$\sum F = \iint_{s} V \rho (V.n) dA + \frac{\partial}{\partial t} \oiiint_{Vol} V \rho d(Vol)$$

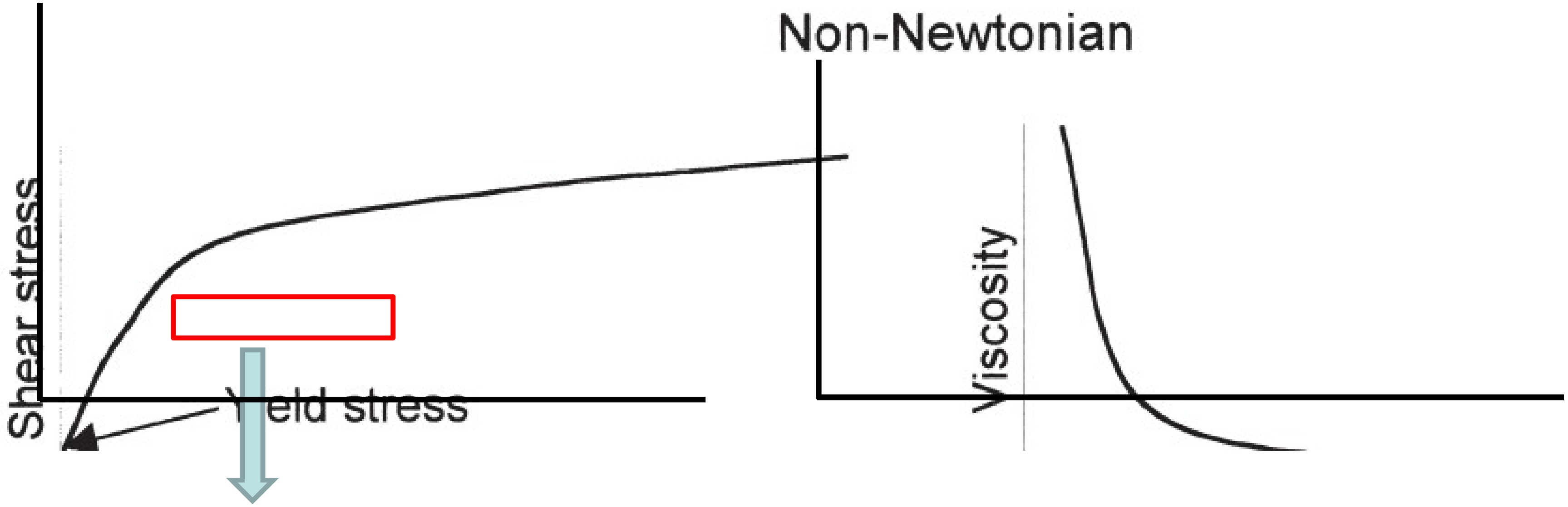
Flux   Accumulation Rate

From a rheological point of view, liquids can be divided into two main groups: **Newtonian & Non-Newtonian Liquids**

- **In Newtonian liquids**, the viscosity is **independent** of variations in shear rate or shear stress.
  - For these fluids **the slope of the shear stress–shear rate relation** is **constant over the range of shear stress examined**, and thus the ***viscosity is constant***.



- **In Non-Newtonian liquids,** the ***viscosity is not a constant*** but rather **<u>depends on</u>** the **magnitude of the shear stress or shear rate**.



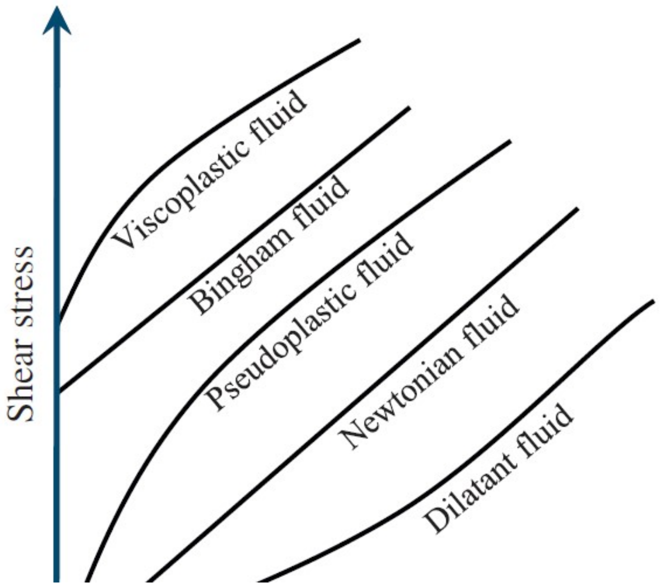
"0" shear rate <u>means</u> "**no flow**"; means "**stasis**"

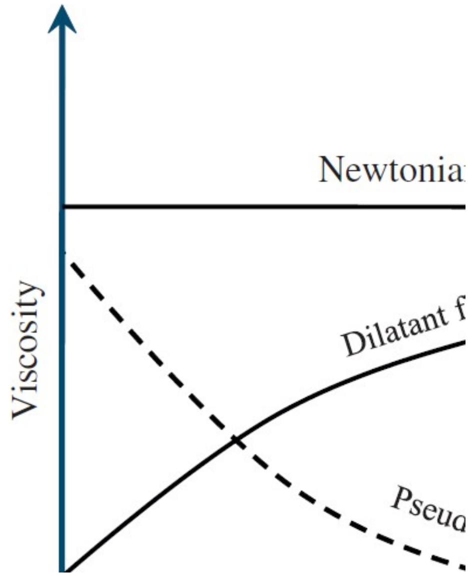
At stasis, normal blood has a ***yield stress*** of about …

Examples for Non-Newtonian Substances		
Biofluids	**Foods**	**Industry**
■ Blood (regarding shear rate) ■ Semen ■ Synovial ■ Saliva	■ Egg white ■ Chocolates ■ Dairy products (Butter, cheese, yogurts) ■ Salad dressings (Ketchup, mayonnaise, etc.) ■ Ice-cream	■ Paints ■ Lubrication oils and greases ■ Soups ■ Pharmaceutical products (Such as: Foams, creams, suspensions) ■ Shampoos and toothpaste ■ Nail polish

In fluid mechanics, three fluidic groups are categorized based on non-Newtonian fluids behavior:

- **Time-independent fluids:** are not depend on time. It means that the shear rate is a function of stress at that point and at that time.
  - **Eg…** Bingham, Pseudoplastic and Dilatant fluids.
- **Time-dependent fluids:** are complicated fluids and the viscosity depends on the duration of shearing.
  - **Eg…** Yogurt, synovial fluid and printer ink.
- **Viscoelastic fluids:** are mainly viscous but they have some elastic behaviors, especially after deformation.
  - **Eg…** Some lubricants.



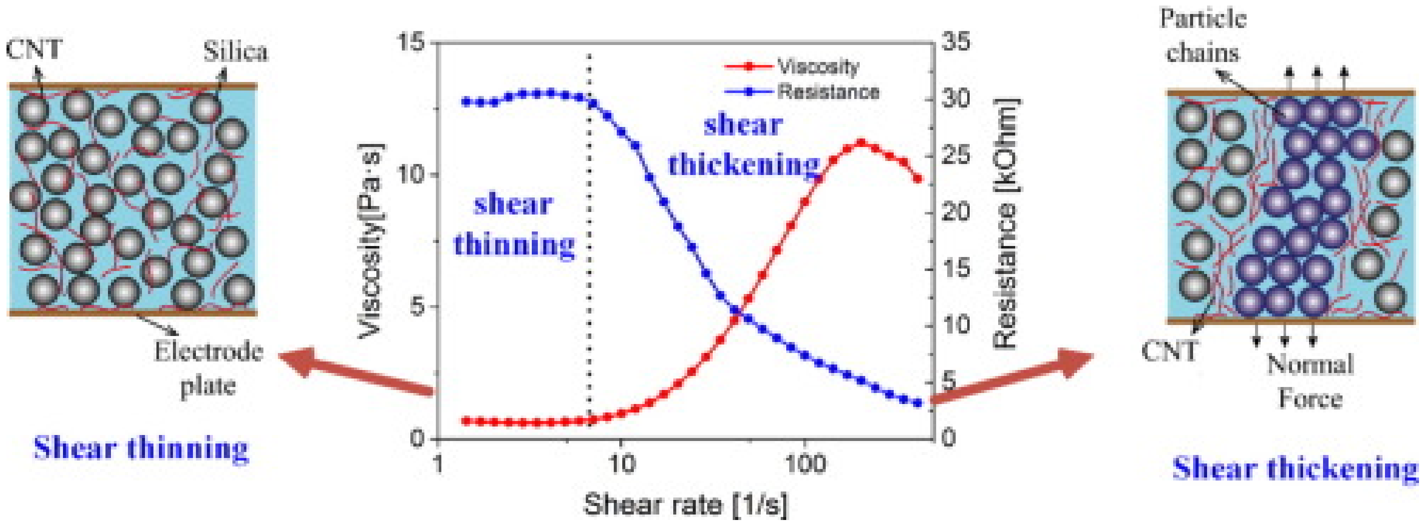


- ***Dilatant (or shear thickening)*** is described by **an increase in viscosity with increasing shear rate. Eg…** A suspension of cornstarch in water.

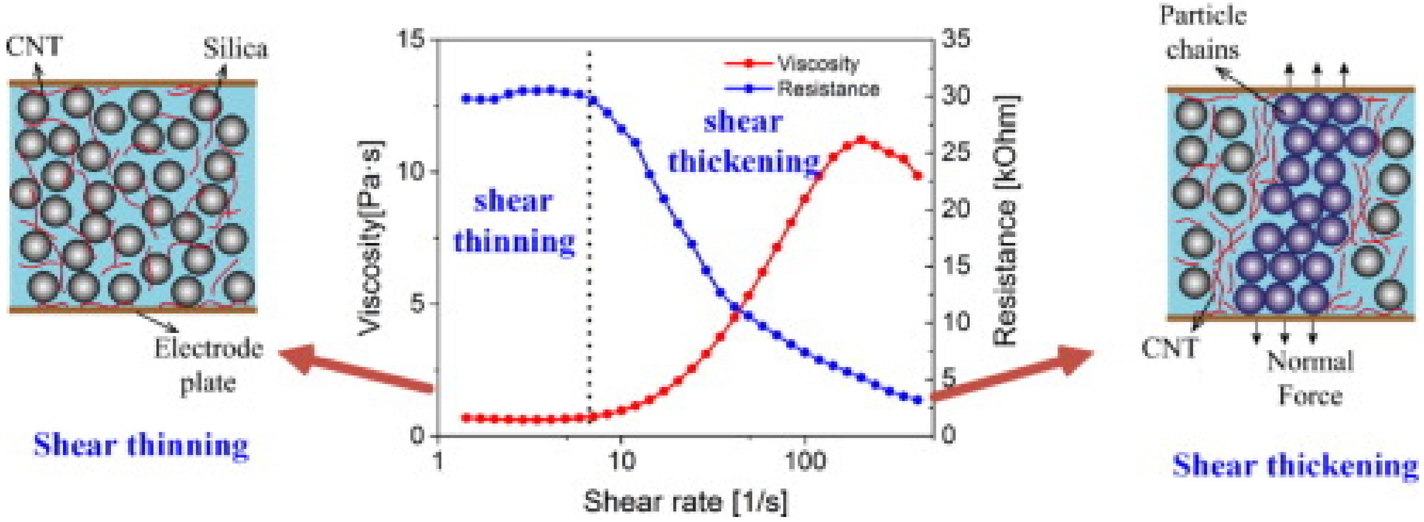
- ***Pseudoplastic (or shear thinning)*** is described by **a decrease in viscosity with increasing shear rate. Eg…** Whipped cream

- ***Viscoplastic*** is described by **the existence of a yield stress which has to be exceeded for the fluid to flow or deform. Eg…** Mayonnaise sauce.

- ***Bingham*** has **linear viscosity but a certain amount of yield stress must be applied to the fluid to begin flow or deformation. Eg…** Chocolate.



- **Shear-thickening fluids** are the fluids that **behave like a liquid when there are no forces applied and turn in a very stiff solid-like structure in presence of high shear rates.**
  - Since **this liquid-solid transition** is **the result of a rapid increase of viscosity**, these highly nonlinear fluids have been exploited for the design of damping systems and shock absorbers.
  - **Eg…** A suspension of cornstarch (mısır nişastası) in water.
  - If such a suspension is compressed quickly by hand, the suspension will turn almost solid. If releasing the pressure, the suspension will flow freely again.



- **In shear-thinning fluids, the *viscosity decreases* as the *shear rate increases*.**
- As we apply much shear stress, the shear rate of fluid increases; i.e. as we increase the shear stress further, the fluid begins to deforms more easily and shows to be less viscous.
  - **Eg… Motor oil, Ketchup, Whipped cream and**

What is Hemorheology?

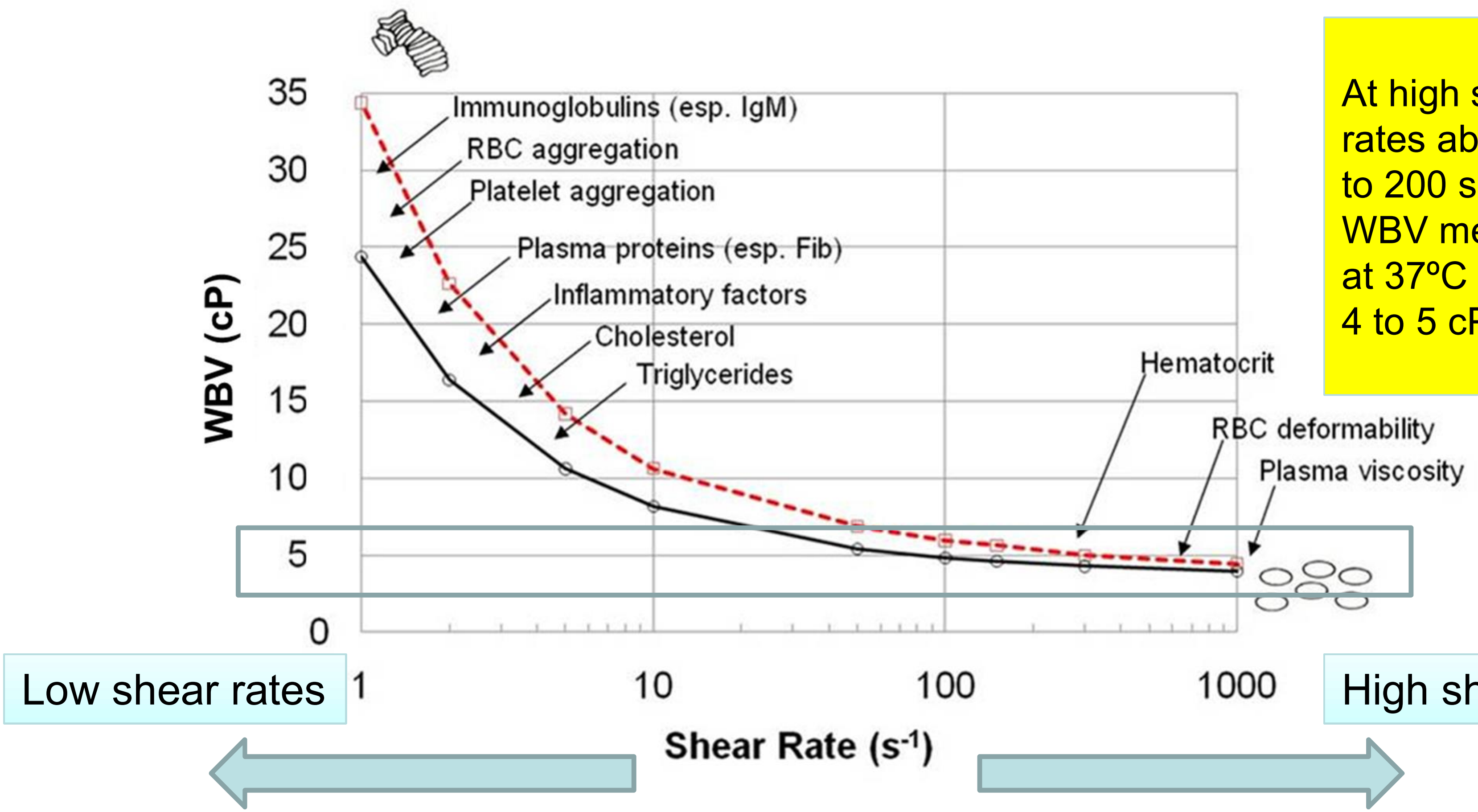
- Simply, hemorheology **deals with the flow and deformation behavior of blood and its formed elements** (i.e., RBCs, WBCs, platelets).
- The **rheological properties of blood** are of **basic science and clinical interest**.
- **Blood rheology can be altered in many disease states.**
- There is an increasing amount of clinical and experimental data clearly indicating that **the flow behavior of blood is a major determinant of proper tissue perfusion.**

Rheology of Blood

- **From a biological point of view**, blood can be considered as a tissue comprising various types of cells (i.e., RBCs, WBCs, and platelets) and a liquid intercellular material (i.e., plasma).

- **From a rheological point of view**, **blood can be thought of as a two-phase liquid;**
  - Can be **considered as a *solid-liquid suspension***, **with the cellular elements being the solid phase.**
  - Can be **considered as a *liquid-liquid emulsion* based on the liquid-like behavior of RBCs under shear.**

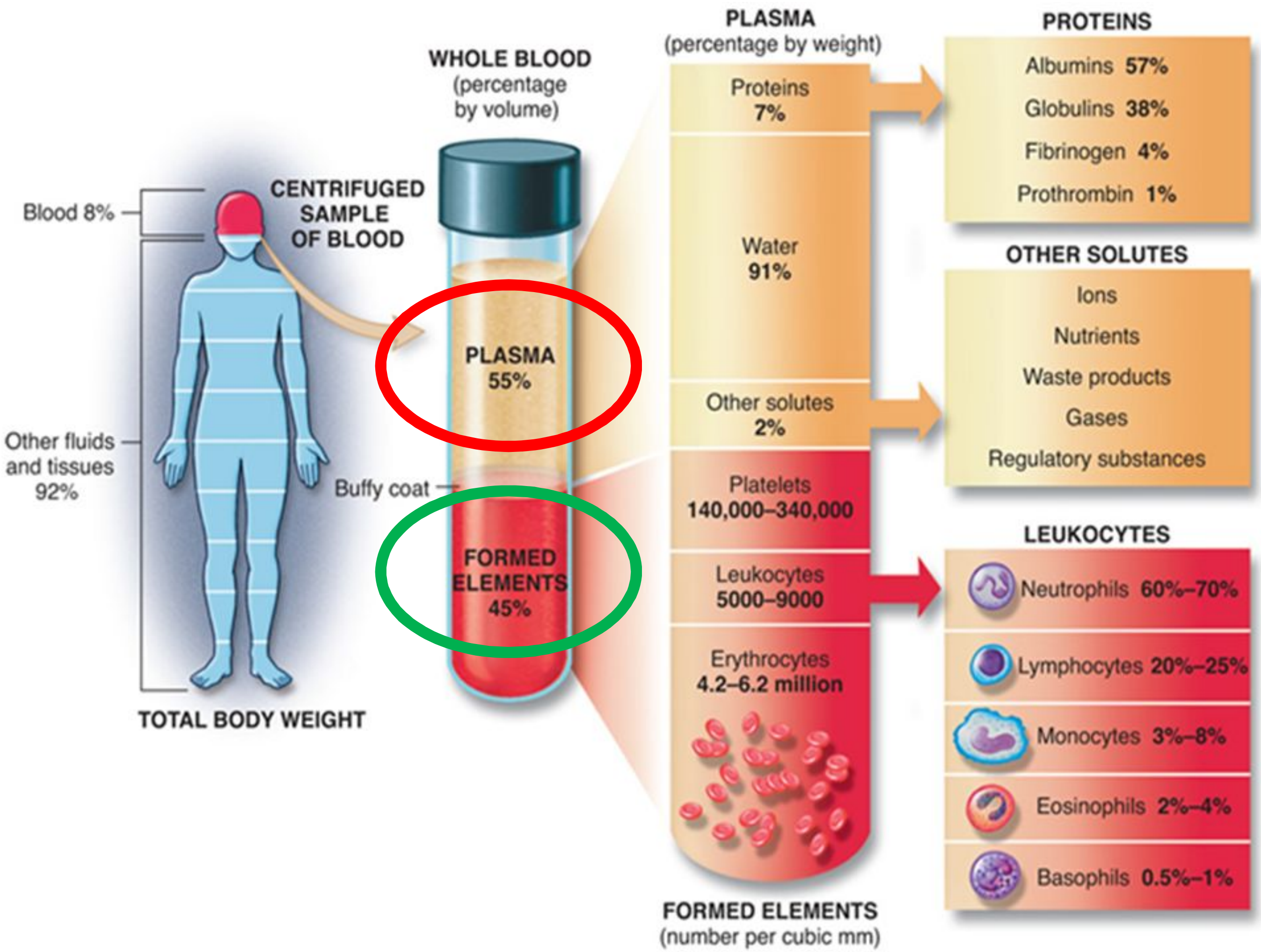
Whole (Normal) Blood Viscosity



At high shear rates above 100 to 200 s^{-1}, the WBV measured at 37°C is about 4 to 5 cP.

Viscosity curves for 2 apparently healthy males, each having HCT of 45.

Determinants of Blood Fluidity



Blood fluidity **at a given shear rate and temperature** is determined by the **rheological properties of the plasma and cellular phases and also by the volume fraction** (i.e., hematocrit) **of the cellular phase**.

Component and % of blood	Subcomponent and % of component	Type and % (where appropriate)	Site of production	Major function(s)
Plasma 46–63 percent	Water 92 percent	Fluid	Absorbed by intestinal tract or produced by metabolism	Transport medium
	Plasma proteins 7 percent	Albumin 54–60 percent	Liver	Maintain osmotic concentration, transport lipid molecules
		Globulins 35–38 percent	Alpha globulins—liver	Transport, maintain osmotic concentration
			Beta globulins—liver	Transport, maintain osmotic concentration
			Gamma globulins (immunoglobulins) —plasma cells	Immune responses
		Fibrinogen 4–7 percent	Liver	Blood clotting in hemostasis
	Regulatory proteins <1 percent	Hormones and enzymes	Various sources	Regulate various body functions
	Other solutes 1 percent	Nutrients, gases, and wastes	Absorbed by intestinal tract, exchanged in respiratory system, or produced by cells	Numerous and varied
Formed elements 37–54 percent	Erythrocytes 99 percent	Erythrocytes	Red bone marrow	Transport gases, primarily oxygen and some carbon dioxide
	Leukocytes <1 percent Platelets <1 percent	Granular leukocytes: neutrophils eosinophils basophils	Red bone marrow	Nonspecific immunity
		Agranular leukocytes: lymphocytes monocytes	Lymphocytes: bone marrow and lymphatic tissue	Lymphocytes: specific immunity
			Monocytes: red bone marrow	Monocytes: nonspecific immunity
	Platelets <1 percent		Megakaryocytes: red bone marrow	Hemostasis

Effects of Plasma on Blood Viscosity

- **Plasma** is the suspending phase for the cellular elements in blood, and thus **a change in its viscosity directly affects blood viscosity (regardless of the hematocrit and the properties of the cellular elements)**.

- The normal range of **plasma viscosity** is 1.10 – 1.35 cP at 37ºC, but **higher values** are seen in **disease states** or **after tissue injury**.

- Is a good, nonspecific indicator of disease processes.

- Is increased in pathophysiological conditions associated with **acute phase reactions (This increase is closely related to the protein content of plasma)**.

ETKINLIKLER:

Bugün
13:30 - 15:15
Hemorheology and Biophysical Properties of Blood Cells

Çarşamba
09:30 - 11:15
Introduction to Biophysics

Perşembe
13:30 - 15:15 Special Stimulus-Conduction System of Heart and Action Potential in Heart Cells

YAPILACAKLAR:

Yapılacak yok

Plasma Viscosity, gives a measure of the acute phase response;
of causes (e.g. infection, neoplasm or inflammatory disease).

Interpretation of PV Results

Plasma viscosity is a non-specific screening test used in diagnos

Results	PV range (mPa.s) Reported at 25°C
Normal Range	1.50 - 1.72
Low results	< 1.40
High results	1.75 - 2.00

Acute Phase Reactions (or Response)

- Is a systemic reaction against infection or tissue injury, which involves fever, leukocytosis, increased vascular permeability and increased serum levels of **acute phase proteins**.

Positive APPs	Negative APPs
C-reactive protein (CRP)	Albumin
Serum Amyloid A (SAA)	Transferrin
Haptoglobin (Hp)	Transthyretin
Ceruloplasmin	Retinol-binding protein
α2-Macroglobulin	**Note: Negative Acute Phase Protein "decreases" in inflammation**
α1-Acid glycoprotein (AGP)	
Fibrinogen	
Complement (C3, C4)	

- Acute phase reactants, such as fibrinogen, contribute significantly to the nonspecific increase of plasma viscosity in disease processes.

- Plasma viscosity can increase up to 5 to 6 cP in patients with abnormal protein levels such as seen in clinical states termed paraproteinemia.

Factors Affecting Plasma Viscosity

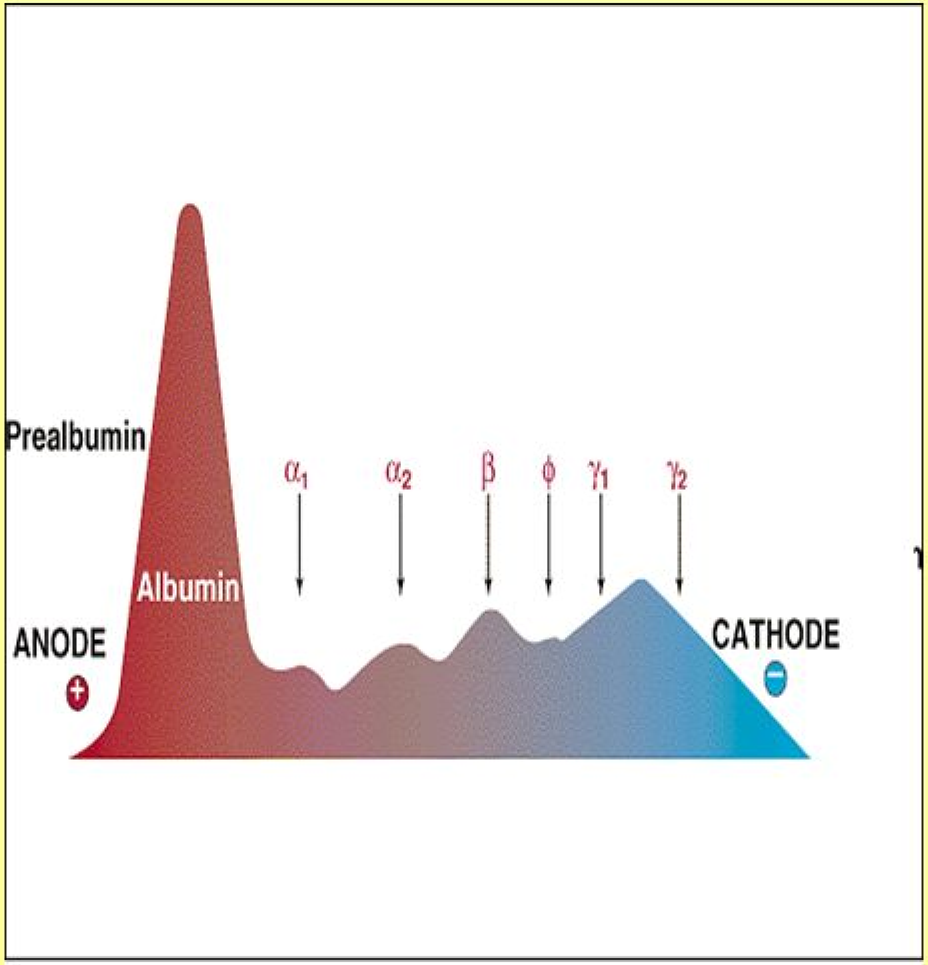
Proteins

- Are all very large, heavy molecules, make up about 7% of plasma by weight.
- Are **generally subdivided** according to their electrophoretic mobility **into** ***albumins, α-, β-, and γ-globulins groups***.
  - However, this approach masks the wide variety of different proteins that exist in the plasma **(e.g., there are carriers of lipids, metals and other factors, many immunoglobulins, clotting factors and fibrinogen).**
- Are necessary to the carriage of many vital materials, to defense against infection, to hemostasis, etc.

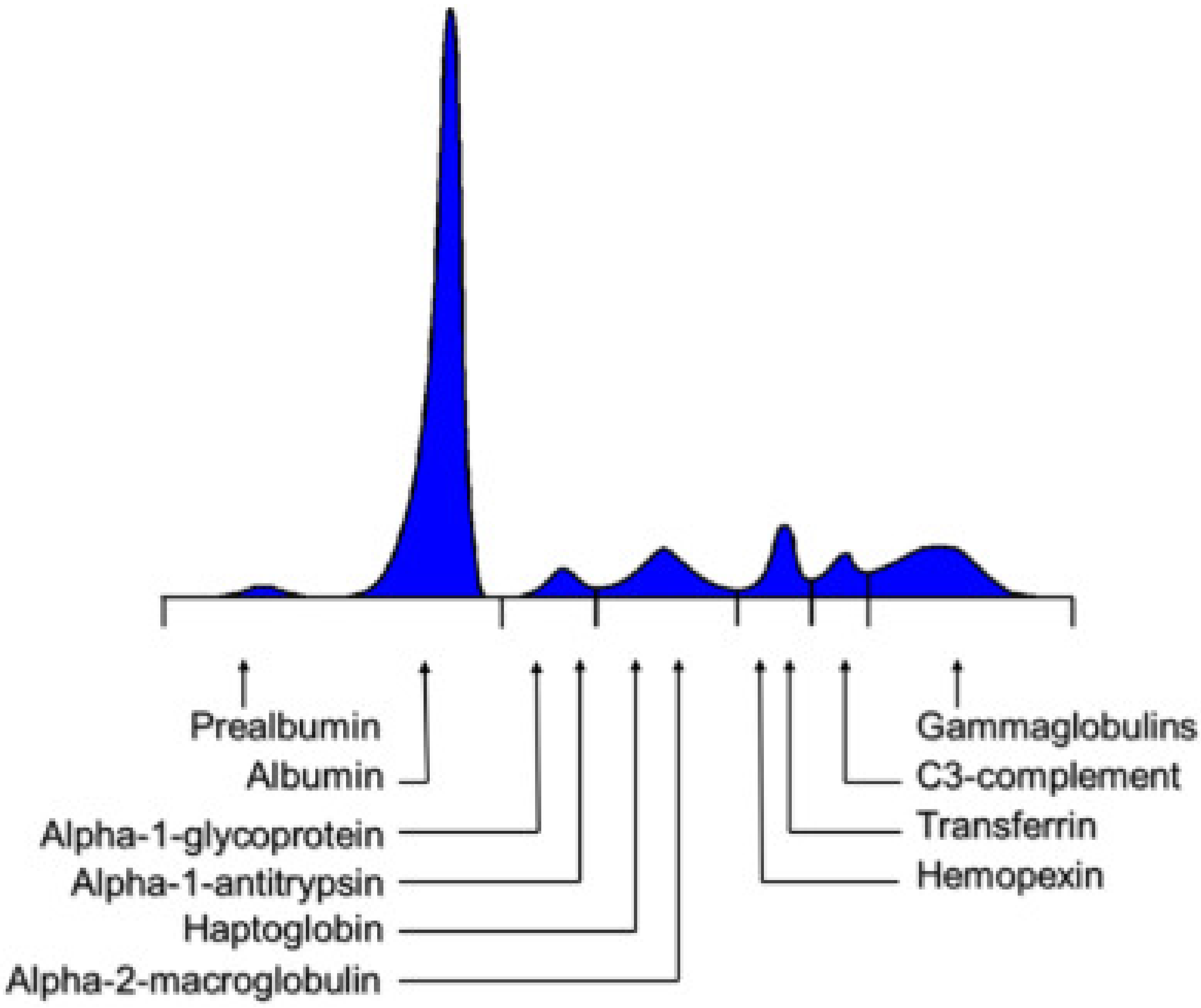
Classification of plasma proteins

- by **electrophoretic mobility**

  - prealbumins
  - albumin
  - alpha, beta and gama-globulins
  - fibrinogen



The figure is from textbook: Devlin, T. M. (editor): Textbook of Biochemistry with Clinical Correlations, 4th ed. Wiley-Liss, Inc., New York, 1997. ISBN 0-471-15451-2


Prealbumin
Albumin
Alpha-1-glycoprotein
Alpha-1-antitrypsin
Haptoglobin
Alpha-2-macroglobulin
Gammaglobulins
C3-complement
Transferrin
Hemopexin

Fractions of plasma proteins

Fraction	Rel. Amount (%)	C (g/L)
Albumins: Albumin, pre-albumin (transthyretin)	52 – 58	34 – 50
α_1-globulins: Thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein	2.4 – 4.4	2 – 4
α_2-globulins: Haptoglobin, macroglobulin, ceruloplasmin	6.1 – 10.1	5 - 9
β-globulins: Transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system	8.5 – 14.5	6 – 11
γ-globulins: IgG, IgM, IgA, IgD, IgE	10 – 21	8 – 15

- **Proteins are hemorheologically important for two reasons:**
  - **First**, because of their **relatively high concentration**, **their large size and often asymmetrical shapes**, they have ***a large effect on the viscosity of plasma***.
    - The normal range of plasma viscosity is 1.10 – 1.35 cP at 37ºC, while the water's is 0.69 cP at the same temperature. The difference between them is due almost entirely to the plasma proteins.
  - **Secondly**, ***fibrinogen*** **cause RBCs to stick loosely together** in characteristic face-to-face aggregates, **like piles of coins**, called ***rouleaux***.
    - From a hemorheological point of view **rouleaux formation is important** because it **causes the viscosity of blood to be very dependent on the shear rate** to which it is exposed.
    - Thus **the viscosity of normal blood is high at low shear rate**, but steadily ***falls as the shear rate increases*** **and** ***the shear forces increasingly disperse the rouleaux*** **(phenomenon is known as shear thinning).**

Factors Affecting Plasma Viscosity

Ions

Table 1.8 Approximate Major Cations, Anions and Proteins of the Intracellular and Extracellular (Blood Plasma and Interstitium) Fluids

Substance	Blood Plasma	Interstitium	Intracellular
Na^{+} (mM)	142	145	15
Ca^{++} (mM)	2.4	1.2	0.0001
K^{+} (mM)	4.4	4.5	120
Mg^{++} (mM)	0.9	0.55	18
Cl^{-} (mM)	102	116	20
HCO_3^{-} (mM)	22	25	16
HPO_4^{2-} (mM)	1.4	0.8	0.7
Proteins (g/dL)	7	1	30

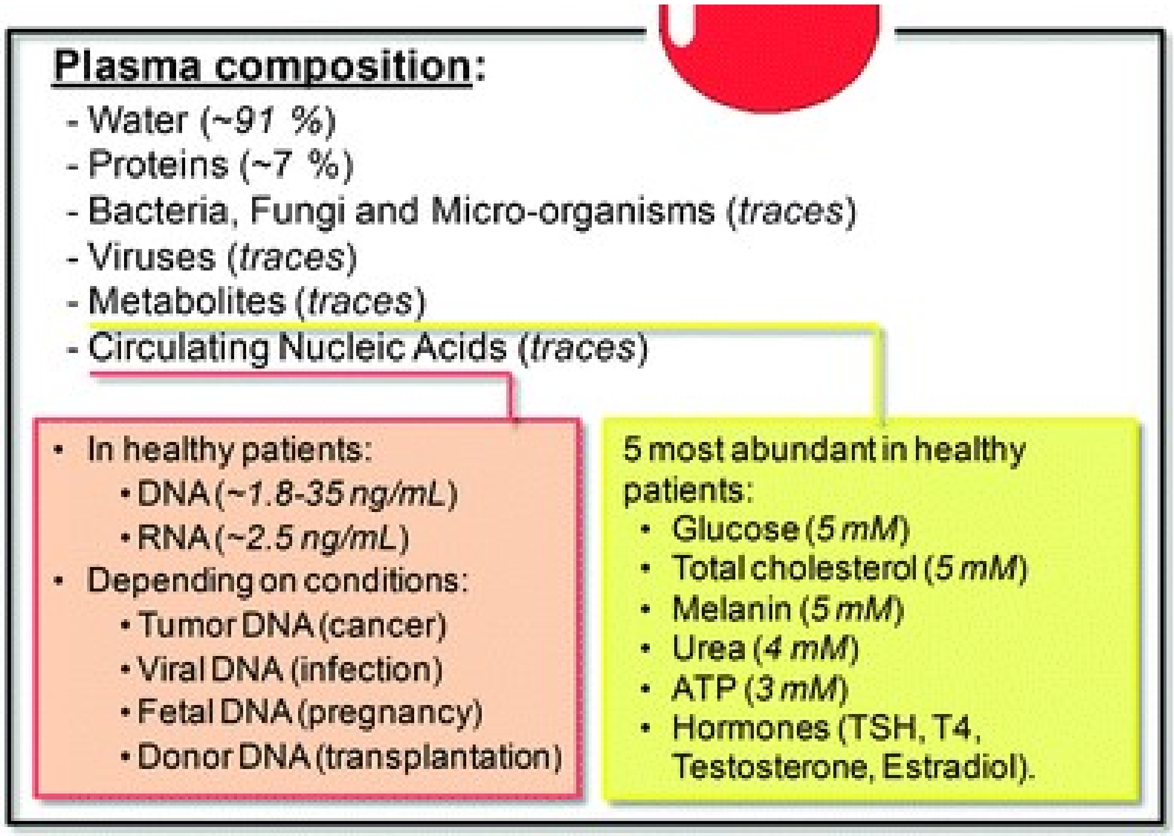
- By far the most concentrated of the cations is Na+ and so it is the most potent from an osmotic point of view.

- For this reason amongst others, it is necessary that **Na** concentration be tightly controlled.
  - In the healthy human the concentration is maintained in the normal range of 135 to 145mM.
- From a hemorheological standpoint this is very important:
  - **If Na+ concentration rises or falls outside this range, RBCs shrink or swell**,
  - **With this volume change** having a **profound influence on RBCs mechanical properties**,
  - **Hence, on RBCs' effect on blood viscosity.**

- The other particularly important ion from a hemorheological point of view is the **anion HCO_3^-**.
  - HCO_3^- concentration has a normal range of 24 to 30mM.
- Its importance lies in:
  - It is being **one of the factors** involved in **controlling blood pH** and in maintaining it in the very narrow normal range of 7.35 to 7.45 which is vital to normal bodily function.
- **If the pH deviates far from the normal range**, it ***has deleterious effects on the mechanical properties of RBCs*** and hence ***on their viscometric effects.***

Factors Affecting Plasma Viscosity

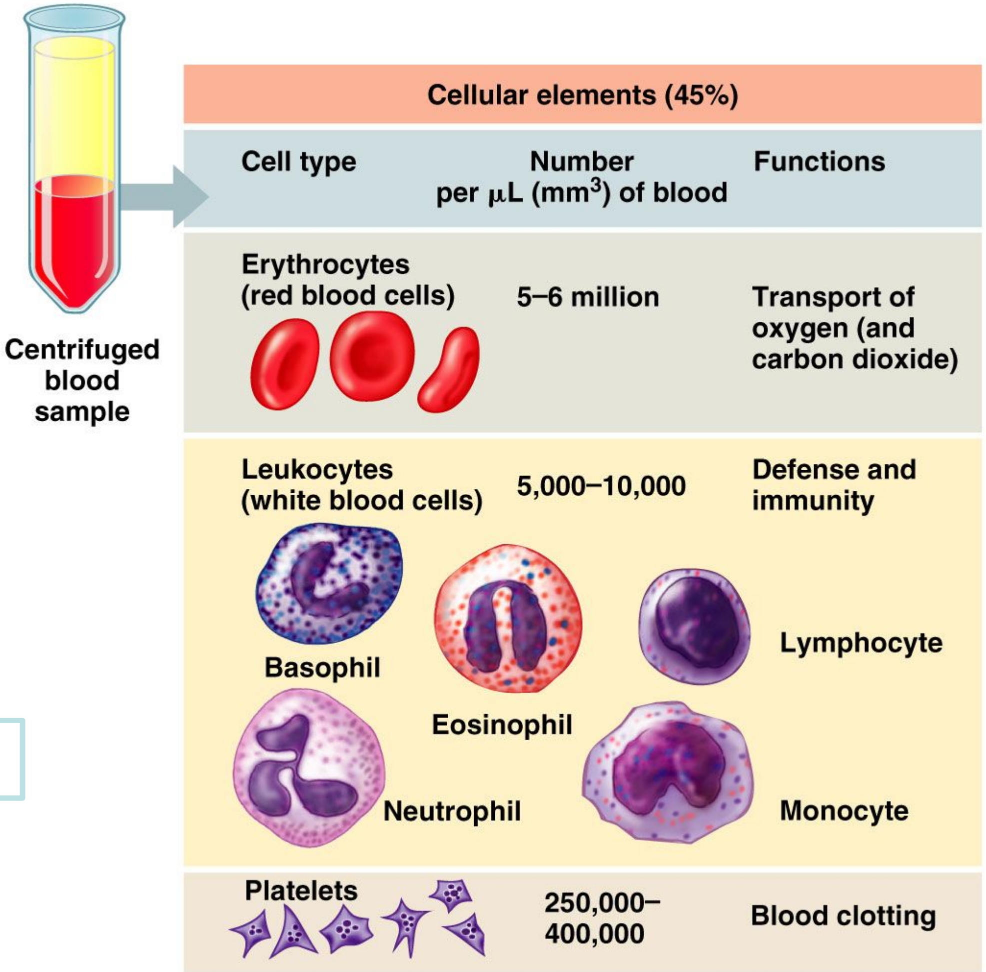
Metabolic Molecules



- They have relatively **minor** hemorheological effect.

Effects of Formed Elements on Blood Viscosity

Erythrocytes (RBCs)

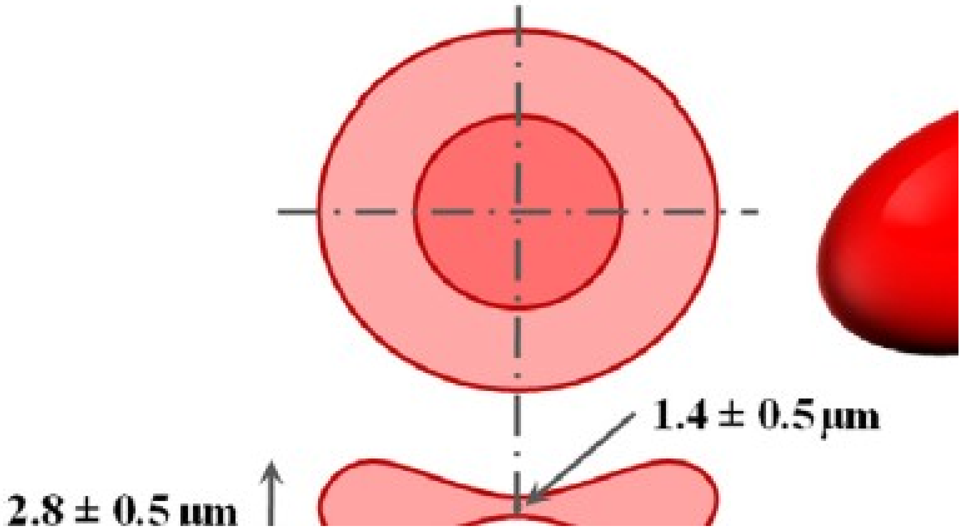


Main reason

high concentration

Only about 1%

Physical properties of the RBCs



1. Have an **unusual shape**, biconcave discs, and so **have the capacity to align with the direction of flow**.

2. Are **extremely flexible** and so will **deform** and extend **under shear forces** **(if the shear forces are high enough to slightly deform these cells)**.

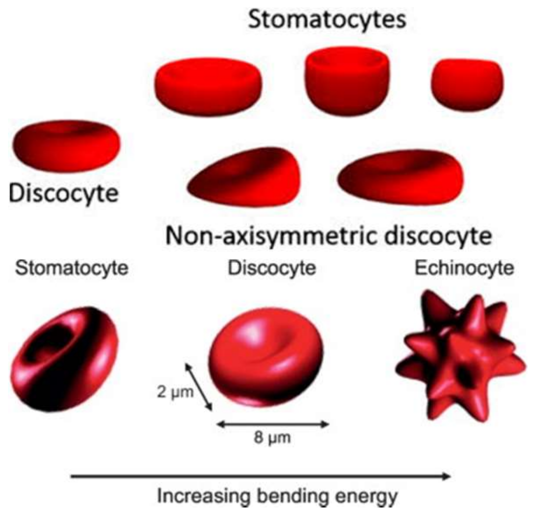
3. Have the tendency to **adhere together loosely**, as **rouleaux**, **under the influence of plasma proteins** (***especially fibrinogen***).

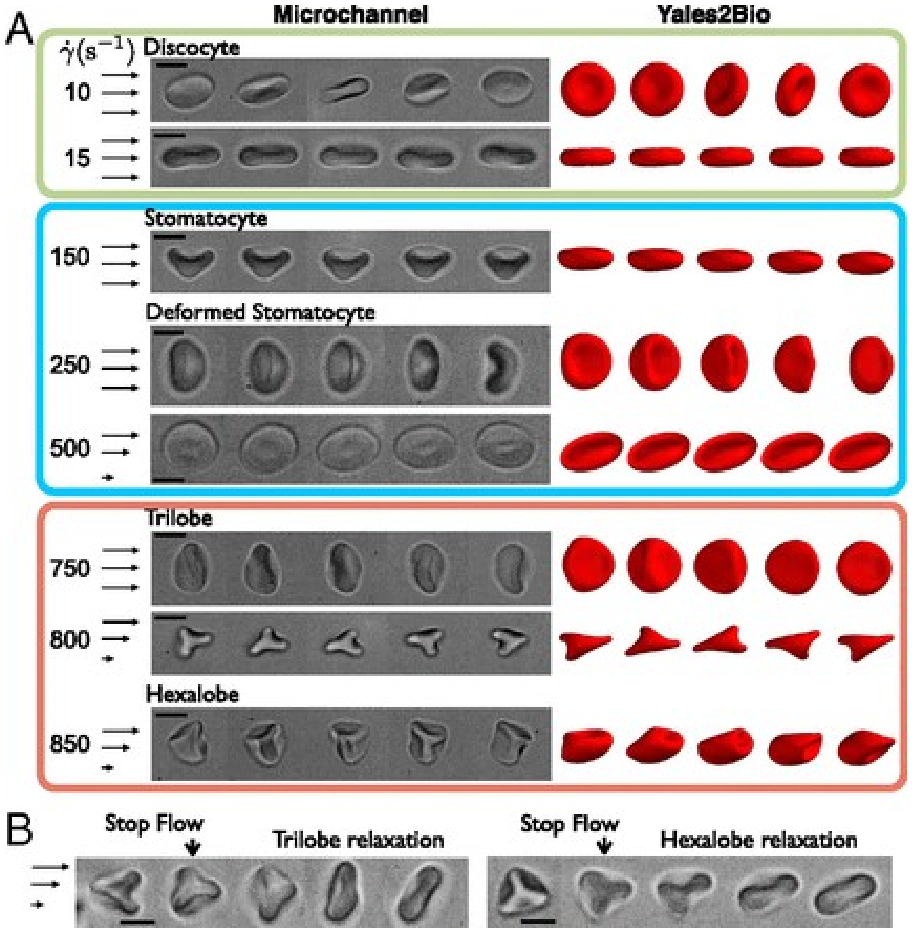
4. **Contain** a **hemoglobin** solution of high concentration (normal range 32 to 36 g/dl) **which has an effect on the speed** with which they can deform under shear forces.

Two main rheological features of RBCs

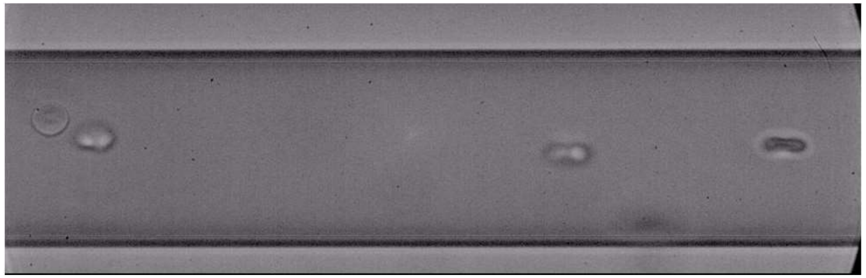
RBC Deformation

RBC Aggregation

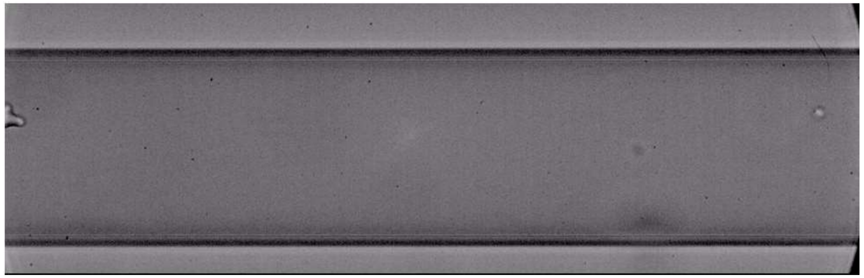




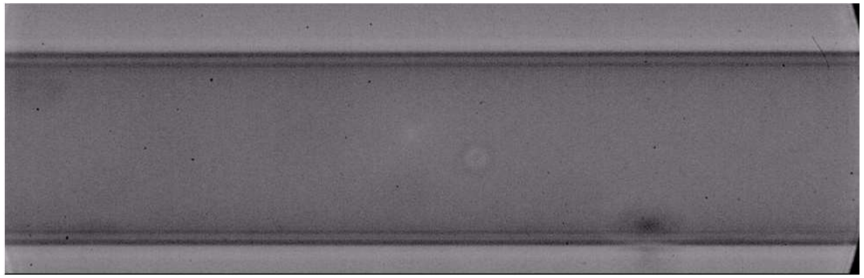
Relaxation of a hexalobe RBC after stopping the flow in a round glass capillary with a diameter of 50 µm. Video was initially acquired with a high-speed camera at 1,500 fps and is displayed here at the frame rate of 100 fps.



Folded stomatocyte with a vacillating-breathing motion in flow at $\dot{\gamma}$ = 500 s^{-1}. Video was initially acquired with a high-speed camera at 3,500 frames per second (fps) in a round glass capillary with a diameter of 50 µm and is displayed here at the frame rate of 7 fps.



Side view of a trilobe RBC rotating in flow at $\dot{\gamma}$ = 800 s^{-1}. Video was initially acquired with a high-speed camera at 4,000 fps in a round glass capillary with a diameter of 50 µm and is displayed here at the frame rate of 7 fps.



Hexalobe rotating in flow at $\dot{\gamma}$ = 850 s^{-1}. Video was initially acquired with a high-speed camera at 5,000 fps in a round glass capillary with a diameter of 50 µm and is displayed here at the frame rate of 7 fps.

RBC Deformation (Deformability)

- **RBCs** respond to applied forces by **extensive changes of their shape**, with the **degree of deformation under a given force** is known as ***RBC deformability***.
- **Blood flows only because the RBCs are deformable and can be reoriented to slide on the low viscosity plasma.**
- RBCs **behave as elastic bodies**, and thus the **shape change is reversible** when the deforming forces are removed.
- The **RBC membrane with underlying cytoskeleton**, is the structured element that **primarily determines the cell's dynamic mechanical behavior**.
- The **lipid bilayer of the membrane** is ***purely viscous*** and ***makes almost no contribution*** to the elastic behavior of the RBCs membrane.

Table 1. Rheological properties of normal adult RBC

Parameter	Typical Value	Notes
Mean cell volume	90 fl	
Membrane surface area	~140 μm^2	There is about 40% excess surfa required to enclose the cell volu Allows changes in shape withou the membrane
Membrane surface viscosity	0.7 μPa.s.m	This value is much greater than viscosity of a simple lipid bilay conversion to a bulk viscosity c dividing by the membrane thick then also much greater than the viscosity
Cytoplasmic viscosity	6.4 mPa.s	Highly dependent on mean cell concentration
Membrane shear elastic modulus	6.0 μN/m	The gradient of a stress/strain cu undergoes linear stretching; pro the membrane skeleton; confers elastic behaviour on RBC
Bending elastic modulus	1.6 $\times 10^{-19}$ N.m	Determines e.g., resistance to fc of elongation
Elastic modulus for area compressibility*	300-600 mN/m	High value reflects great resista to area dilation; only approx. 2% occur before RBC rupture
Time for shape recovery	0.12 s	Determined by ratio of membra

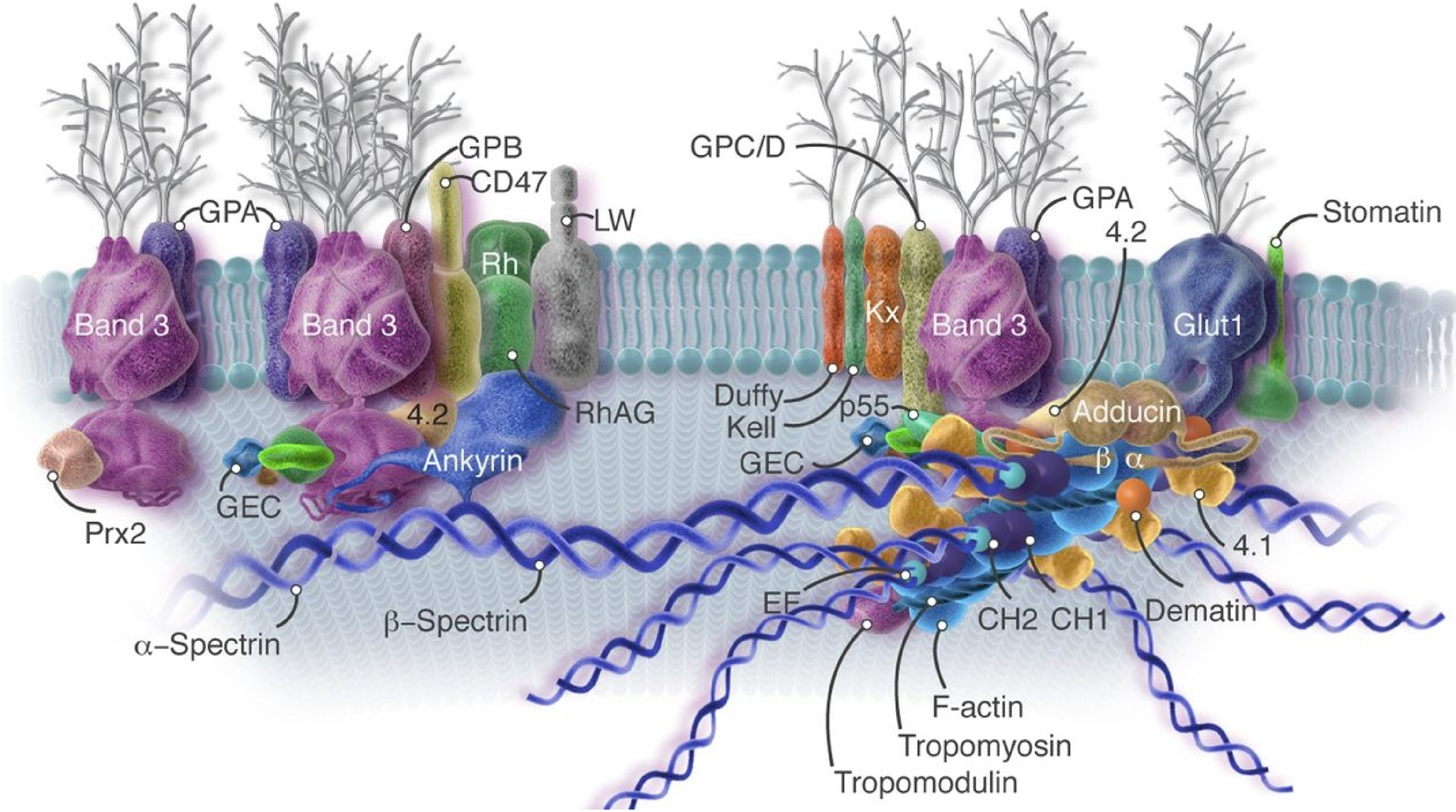
- In addition to membrane elastic and viscous properties as **determinants of RBC deformability**, ***two additional factors*** also **contribute to** this cellular property:
  - The ***cytoplasmic viscosity of RBCs***, which in **normal RBCs is solely determined by** the **hemoglobin concentration**.
  - The **biconcave-discoid geometry**, which **provides excess area for the contained volume** and thus **enables shape changes without increasing the surface area of the membrane**.

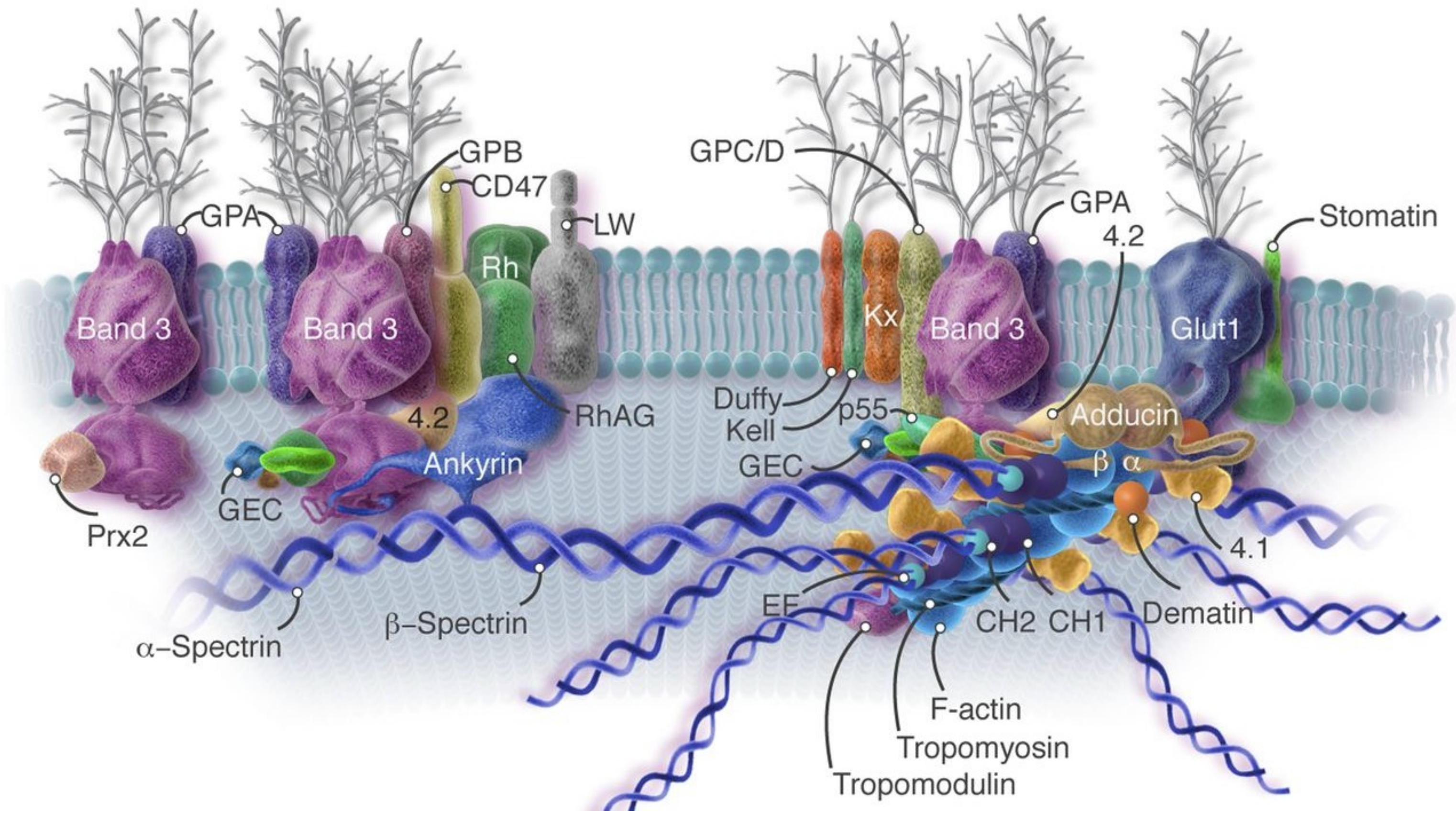
- **An increase of surface area** is necessary **in order to deform a sphere**, yet the ***RBC membrane is extremely resistant to area increases***.

- The ***degree of hydration*** of the cell is thus **an important determinant of its surface area–volume relationship**:

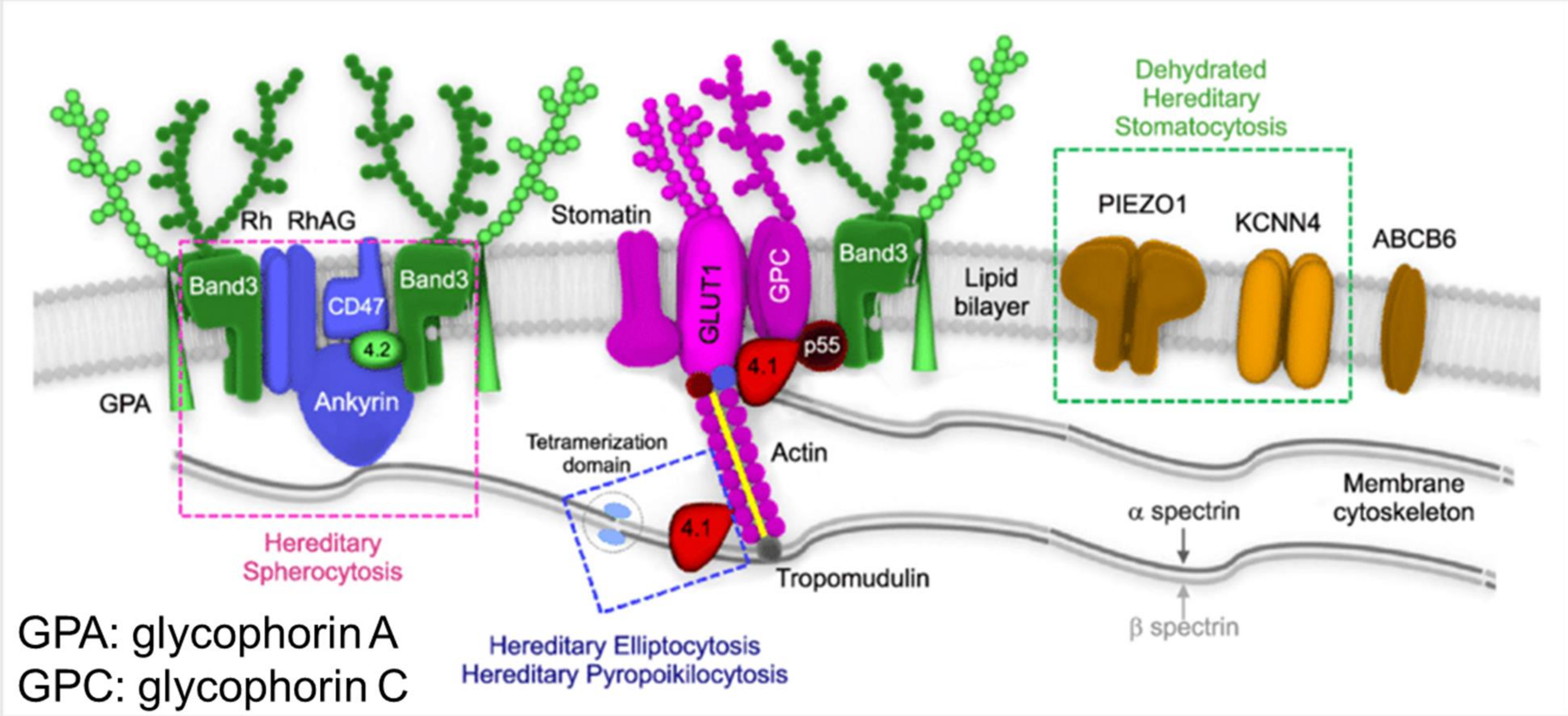
  - **If** ***RBCs are overhydrated***, their **volume will increase**, whereas **their surface area remains unchanged,** ***thereby reducing cell deformability*****.**

  - **Conversely**, the **cytosolic concentration of hemoglobin**, and **hence the cytosolic viscosity**, is **increased** when cells are ***underhydrated***, thereby also leading to ***reduced cell deformability***.

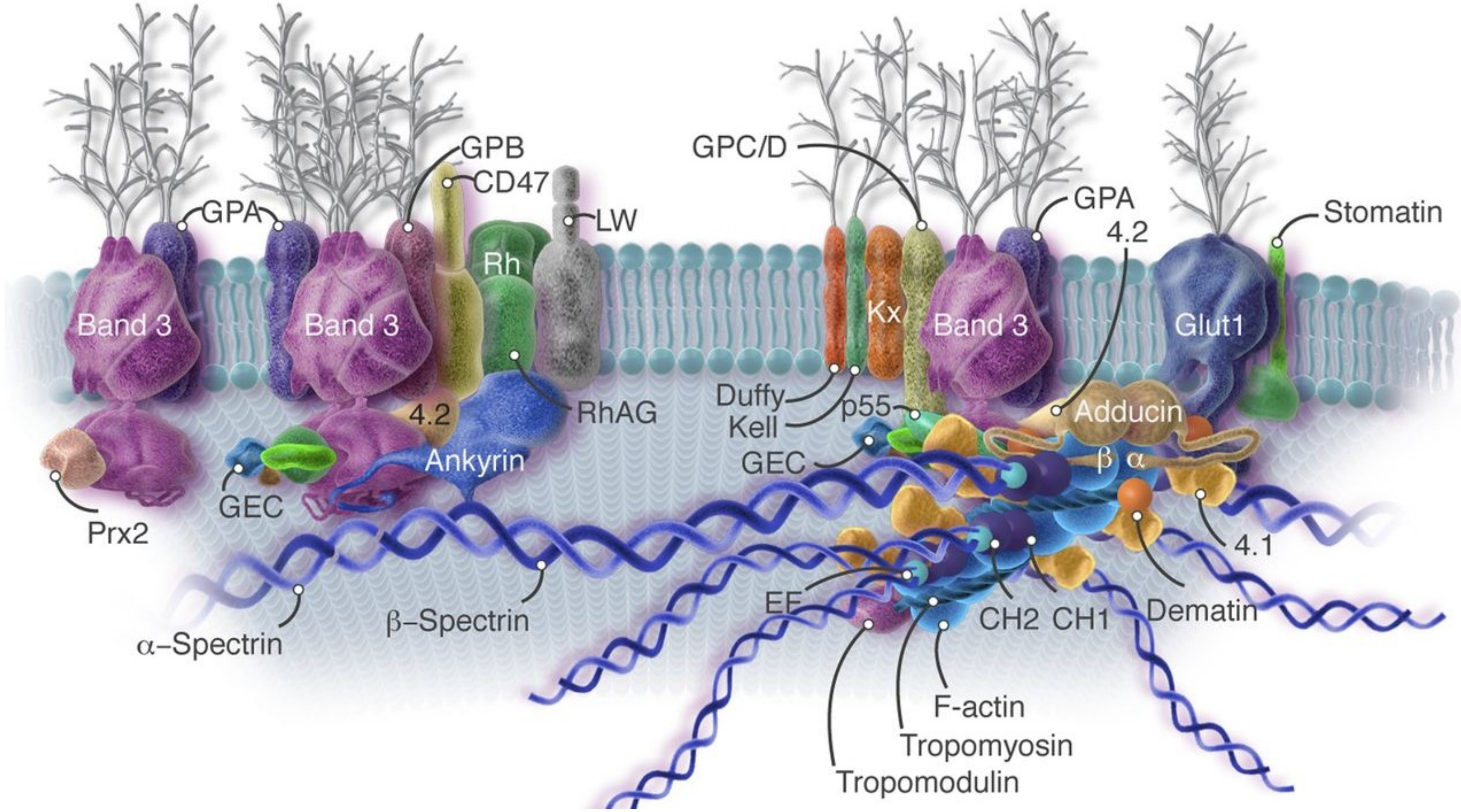

GPB
CD47
LW
GPA
Rh
Band 3
Band 3
4.2
RhAG
Ankyrin
GEC
Prx2
α–Spectrin
β–Spectrin
GPC/D
GPA
Stomatin
4.2
Kx
Band 3
Glut1
Duffy
Kell
p55
GEC
Adducin
β α
4.1
EF
CH2
CH1
Dematin
F-actin
Tropomyosin
Tropomodulin



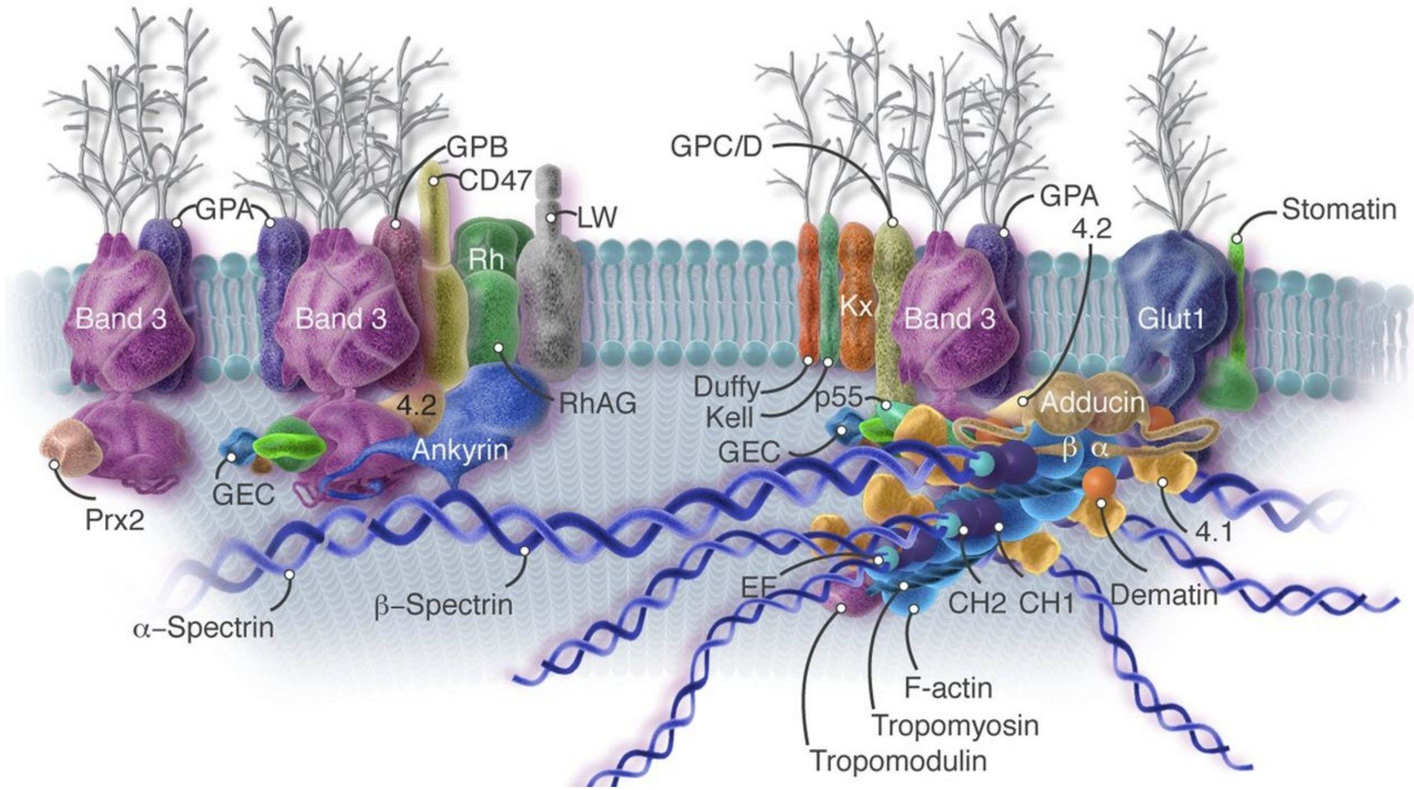
- By using several techniques, more than 300 proteins at the RBC membrane cytoskeleton have been identified.
- RBC membrane cytoskeleton is **a network of proteins lying just beneath the cell membrane**, including **actin, ankyrin, proteins 4.1, 4.2, and 4.9, p55, and adducin** with the protein **spectrin the most important component of this network.**



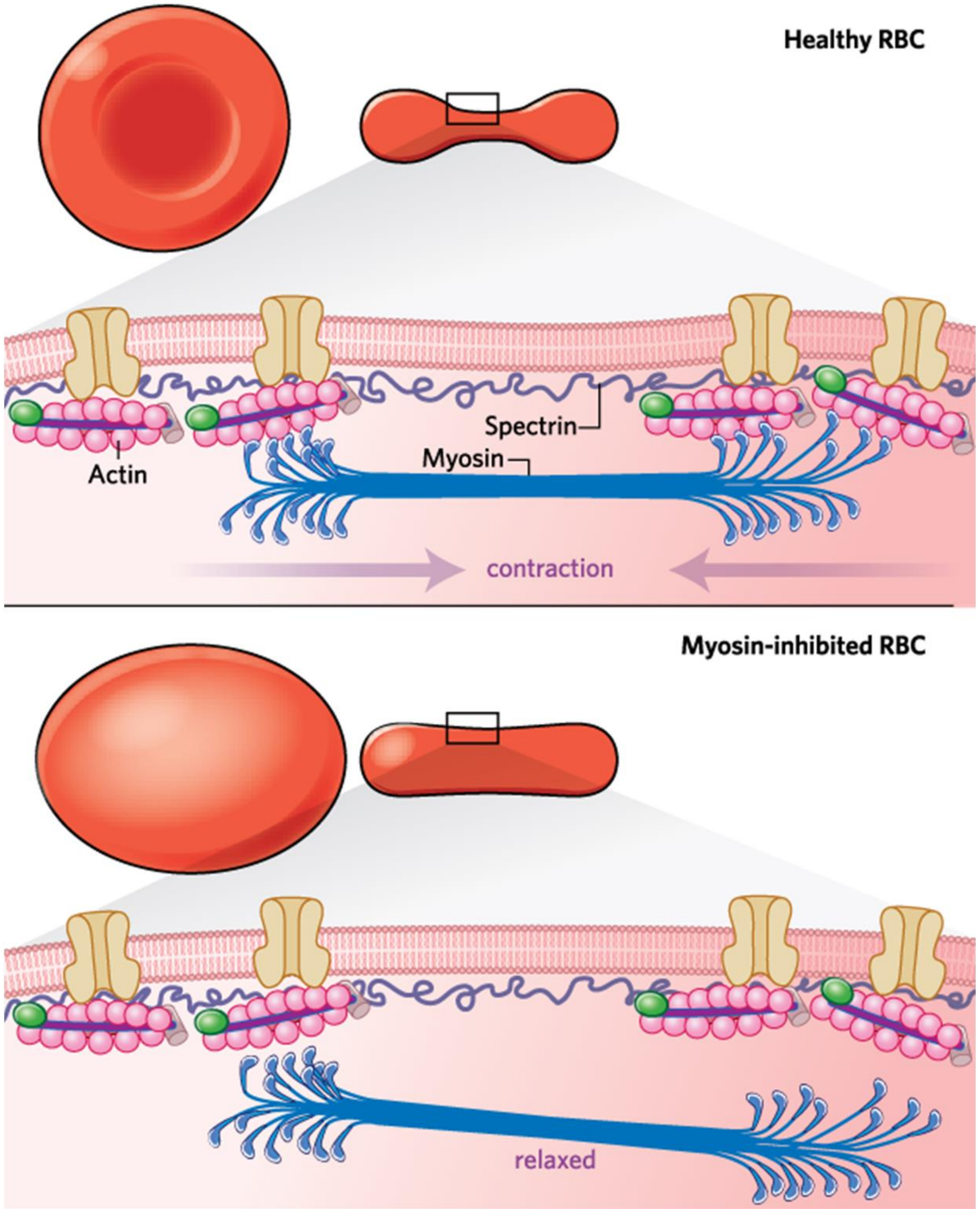
- A highly ordered arrangement of tetramers of **α and β spectrin interconnection with actin and protein 4.1**, and **bonds to the overlying RBC membrane via band 3 and glycophorin C (GPC) provide the structural integrity of the RBC and underpin its ability to repeatedly deform in the microcirculation**.



- The **stability of the membrane skeleton** ***is influenced not only*** by the primary sequence of its component proteins but also by the **levels of protein phosphorylation**.
- In addition to these interactions, ***spectrin and protein 4.1 engage in*** a number of other **protein-protein interactions** that **affect cellular mechanical properties.**



- ***Spectrin binds to adducin and ankyrin*** as well as a number of kinases. (**E.g… cAMP-dependent protein kinase and protein kinase C**)
- **Protein 4.1 binds to membrane proteins Band 3, GPC, and p55,** a palmitoylated membrane protein, and cytoplasmic calcium regulating proteins such as **calmodulin**.
- These interactions in turn **influence both the elasticity and mechanical stability** of the **RBCs membrane**.



Healthy RBC
Spectrin
Myosin
Actin
contraction
Myosin-inhibited RBC
relaxed

RBC Aggregation (Aggregability)

- ***RBC aggregation*** is the process of RBC forming linear and three-dimensional aggregates (aka: ***rouleaux***).
  - This process is reversible such that aggregates will reform at stasis upon the removal of external forces.
- ***RBC aggregability*** reflects the intrinsic tendency of RBC to form aggregates.
- **RBC aggregation** is **determined by the balance** between the **forces promoting aggregation** and **those forces opposing aggregation**.

Aggregating Forces

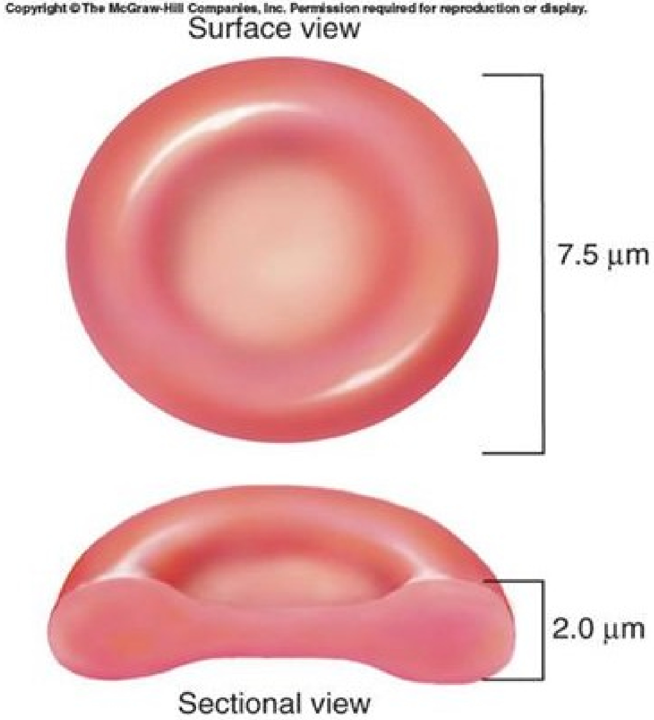
- Biconcave-Discoid Shape
- Hematocrit Effect
- Plasma Proteins
- Dextrans
- Cellular Factors (age and density of RBCs)
- Subject-to-Subject Variations

Disaggregating forces

- Shear Forces
- Surface Charge Density
- Membrane Strain

Aggregating Forces

- **Biconcave-Discoid Shape**
  - Is an **important feature** for **rouleaux formation**, and **any divergence** from this shape leads to **deviations** from normal **aggregation behavior**.
  - ***Even slight changes*** **in shape due to osmotic pressure** (i.e., increase or decrease of cell volume) or **free-radical attack** ***may significantly affect RBC aggregation***.



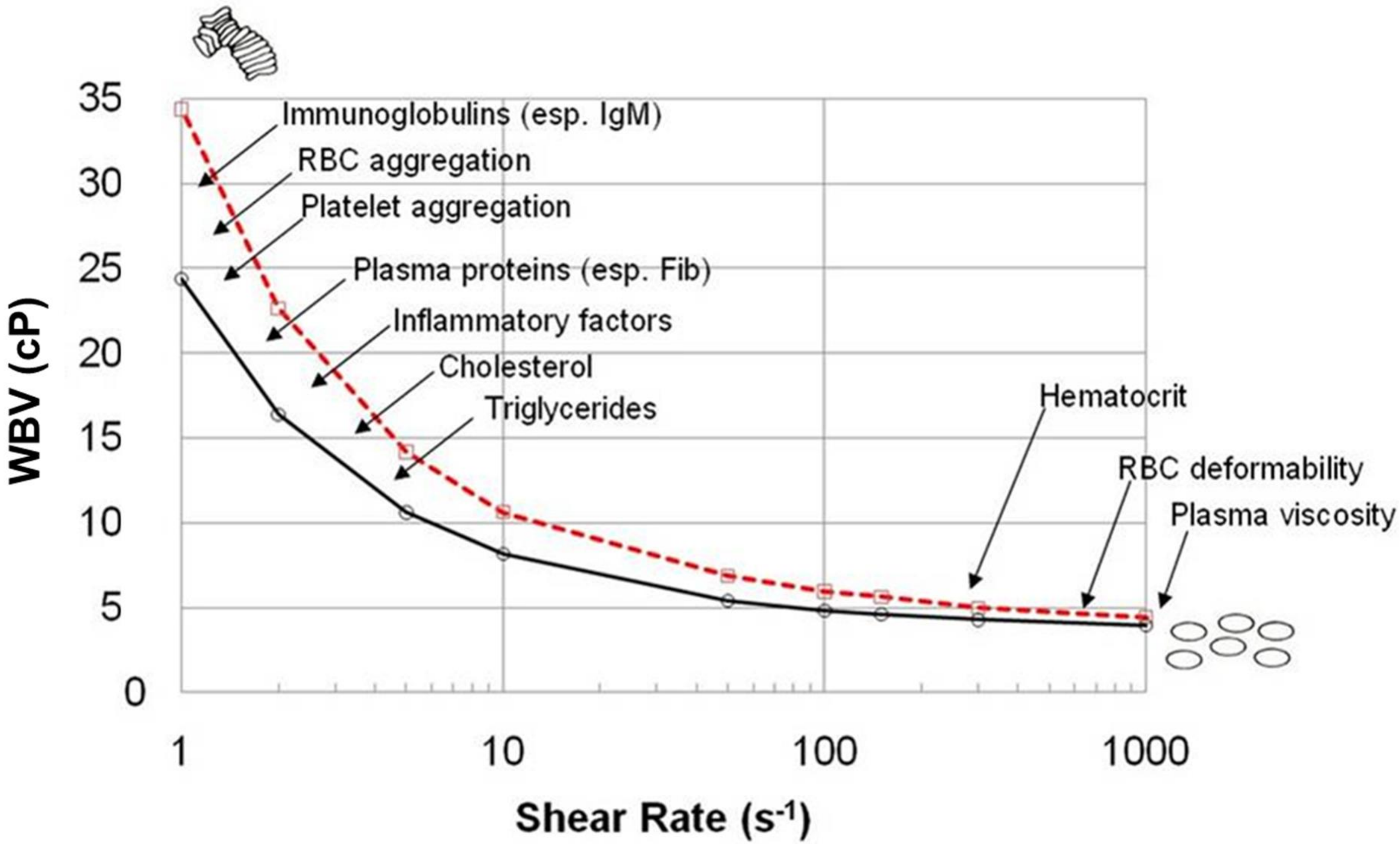
Aggregating Forces

- **Plasma Proteins**
  - ***Fibrinogen*** is generally accepted to be the most **potent plasma protein** for **inducing RBC aggregation**.
  - The physiological plasma concentration is **~150–300 mg/dl** but higher levels are possible.
  - It is an acute phase protein in that its production and plasma levels are enhanced in several diseases such as cardiovascular disease or any form of inflammation.
  - **Fibrinogen affects essentially all aspects of RBC aggregation (e.g., aggregate size, yield stress, viscosity of red cell suspensions, and erythrocyte sedimentation rate)**.

TABLE 2.1
Effects of Various Plasma Proteins on RBC Aggregation[a]

Plasma Protein	M_m (kDa)	Plasma concentration (mg/ml)	Effects on Aggregation
Fibrinogen	340	1.5–3.0	↑
Immunoglobulin G	150	8	↑, no effect
Immunoglobulin M	900	1.5	↑
C-reactive protein	25	<0.01	↑
Transferrin	80	2.0–3.6	no effect
Haptoglobin	38	0.5–2.5	↑, no effect
Ceruloplasmin	151	0.2–0.4	no effect
α_1-acid glycoprotein	40	0.5–1.0	↑, no effect
Albumin	66	20–50	↓,↑, no effect

Plasma concentrations are normal, nonpathological concentrations; ↑: promotes aggregation; ↓: inhibits aggregation. Note that for some proteins different effects on aggregation have been reported.



Viscosity curves for 2 apparently healthy males, each having HCT of 45.

Aggregating Forces

- **Dextran**
  - The **aggregation of RBCs** is **not only induced** by several plasma proteins but can also be **affected by various polymers and macromolecules** that are **not naturally found in plasma**.
  - Is a neutral polyglucose, **above** a **molecular mass of 40 kDa**, **(E.g… Dextran 150 and Dextran T100)** ***induces RBC aggregation*****.**
    - If the molecular mass is below this threshold, dextran can either have no effect on RBC aggregation or can reduce or inhibit rouleaux formation in the presence of other aggregating agents.
  - **Dextran and its derivatives** are used in the manufacture of **blood plasma extenders**, **heparin substitutes** for anticoagulant therapy, **cosmetics (as a moisturizer and a thickener)** and other products (**Bakery products, ice cream, frozen and dried foods, etc…)**

Aggregating Forces

- **Hematocrit Effect**
  - The **volume fraction of RBC in a suspension** termed as the ***hematocrit***, (expressed as either volume percent (%) or as a fraction (liter/liter)), is **an important determinant of the aggregation process**.
  - ***Cells must come in contact to form aggregates.***
  - ***Aggregation*** **begins** when the random movement of **RBC brings them into** ***close proximity*** and obviously the ***frequency of close contacts increases hematocrit***.
  - The ***time course of RBC aggregation*** becomes ***faster*** as the ***hematocrit*** is ***increased.***

Hematocrit as a Determinant of Whole-Blood Viscosity

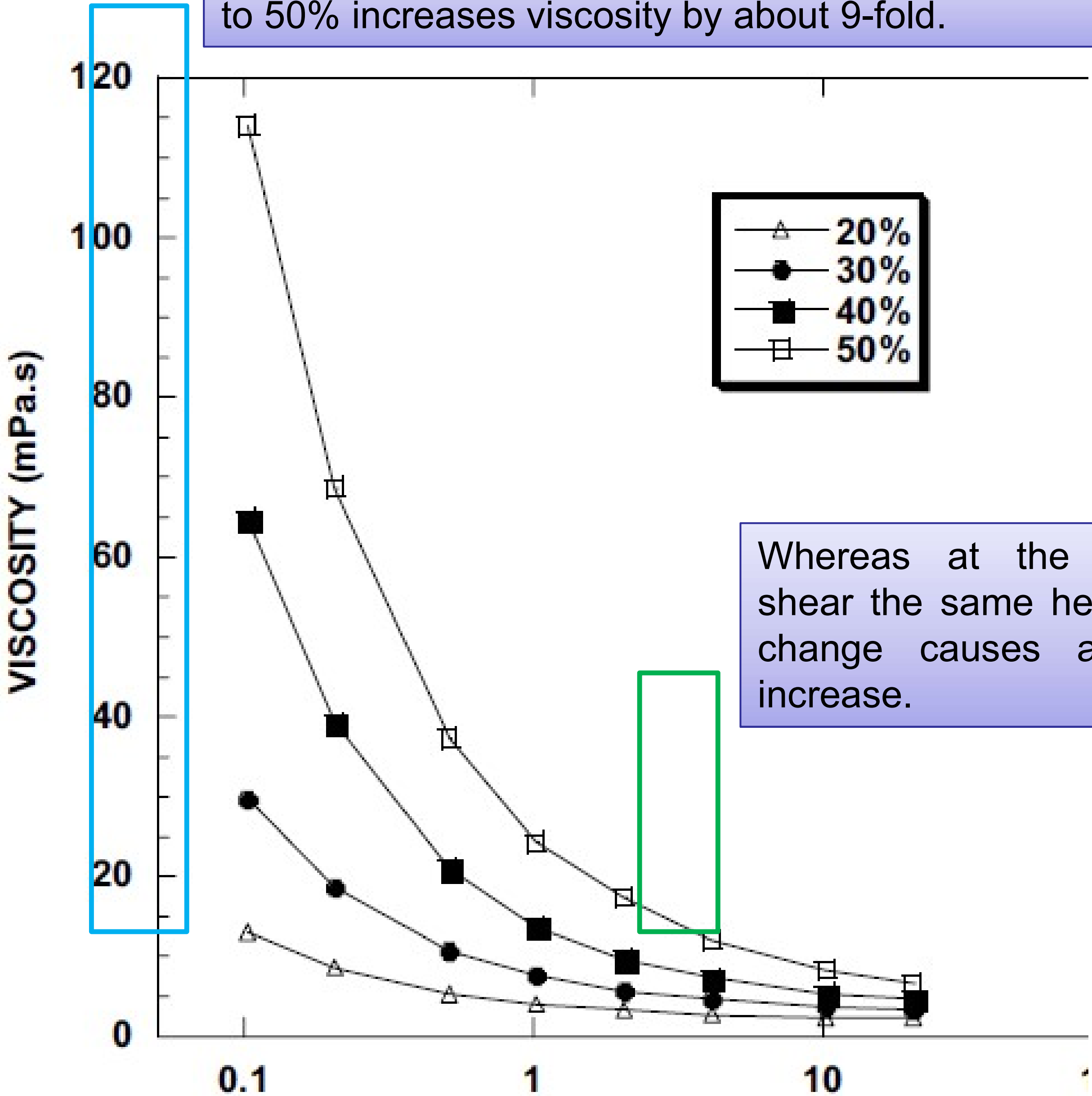
- ***WBV is strongly dependent on hematocrit.***
- The relationship between blood viscosity and hematocrit is approximately of the form:

$$\log(\text{viscosity}) = k_1 + k_2(\text{hematocrit})$$

(where k1 and k2 are shear rate dependent)

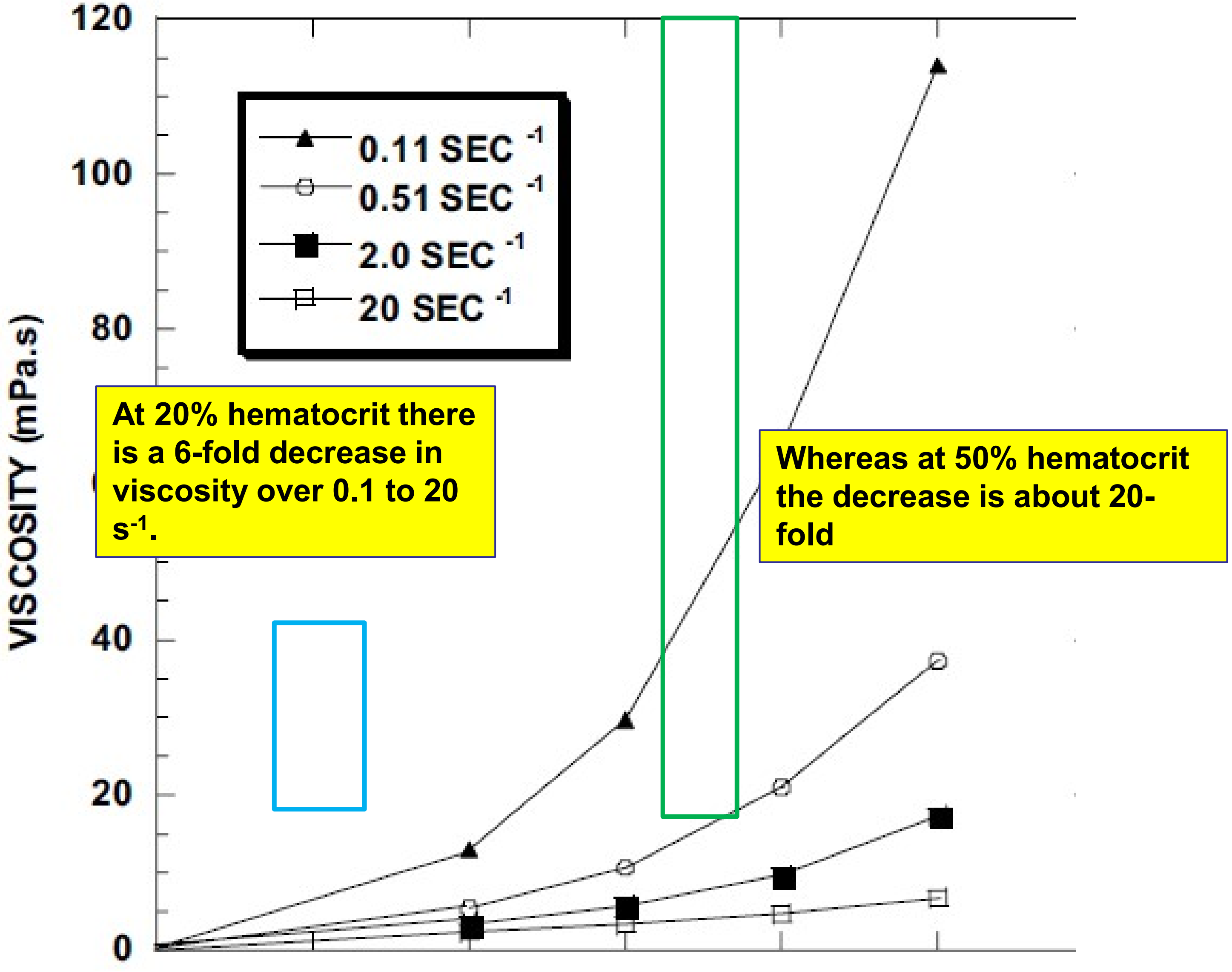
- Typical values for the **viscosity of healthy blood** at **shear rates of 0.277 s^{-1} and 128.5 s^{-1}** are:
  - 39 ± 4 and 4.3 ± 0.2 mPa.s respectively for females
  - 48 ± 6 and 4.7 ± 0.2 mPa.s respectively for males.
- The gender differences are, of course, due to the **lower hematocrit** that is normal for females.

At the lowest shear rate, a change in hematocrit from 20% to 50% increases viscosity by about 9-fold.



Whereas at the highest shear the same hematocrit change causes a 3-fold increase.

Deviation from Newtonian behavior is also hematocrit dependent



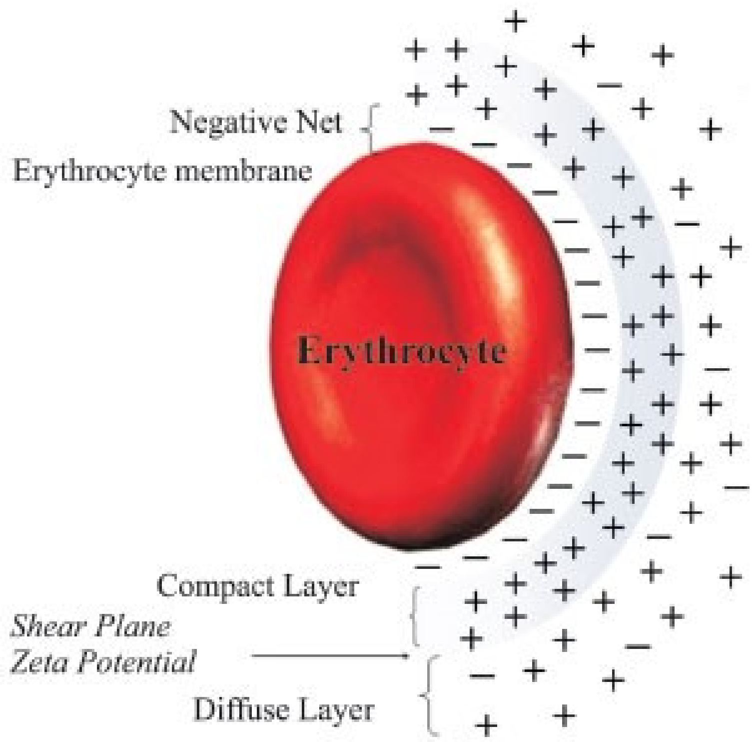
Aggregating Forces	Disaggregating forces
• Biconcave-Discoid Shape • Hematocrit Effect • Plasma Proteins • Dextrans • Cellular Factors (age and density of RBCs) • Subject-to-Subject Variations	• Shear Forces • Surface Charge Density • Membrane Strain

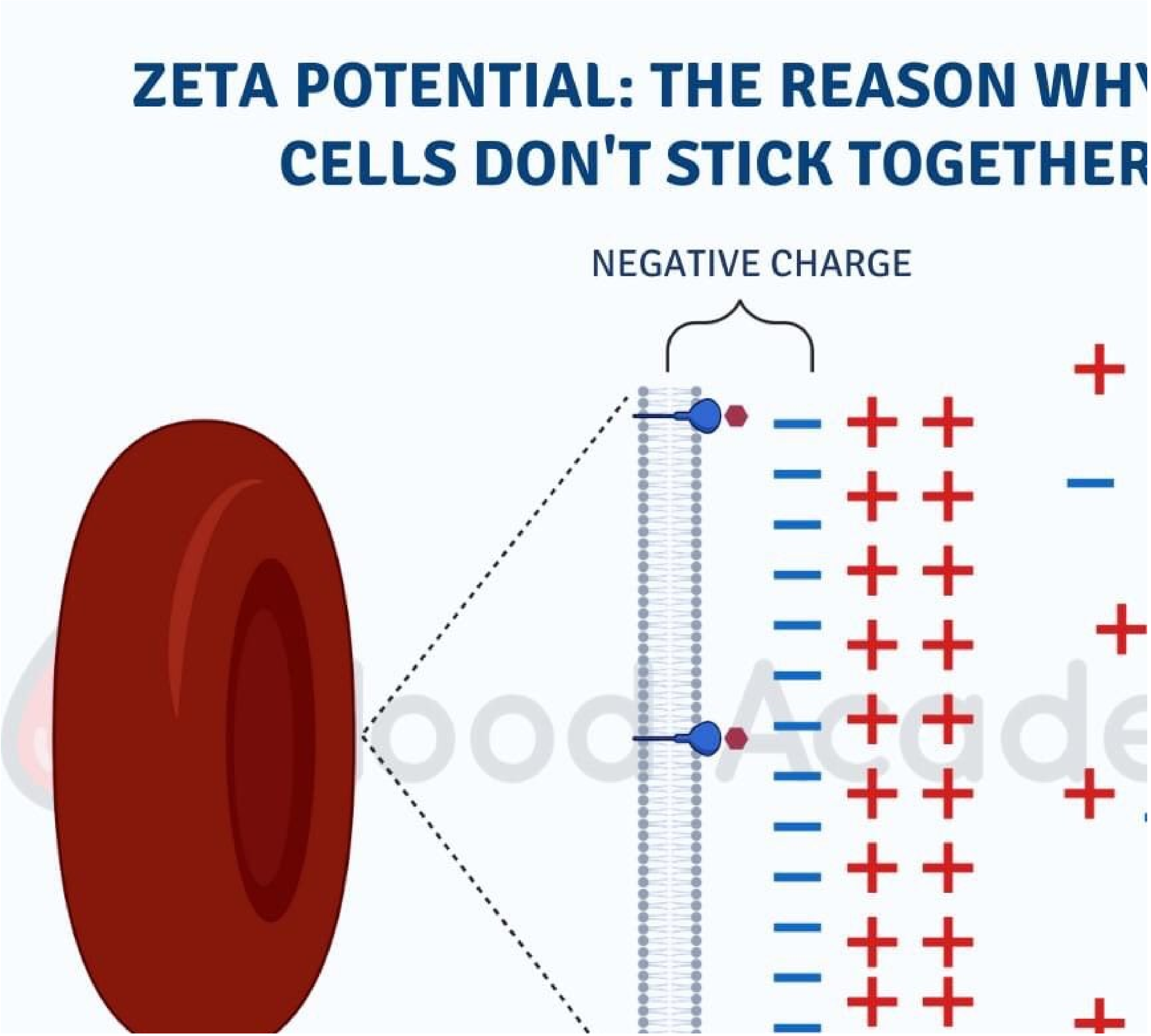
Disaggregating Forces

- **Shear Forces**
  - Arise due to mechanical forces such as shear during flow through a tube.
  - **Shear forces prevent rouleaux formation** or ***disperse existing aggregates***, and thus there is **an inverse relationship** between the **magnitude of shear forces** and the **size of RBC aggregates**.
  - **In normal**, non-pathologic blood, **aggregates** usually **are dispersed** when they **are subjected to** ***low shear rates of 20 – 40*** s^{-1}.

Disaggregating Forces

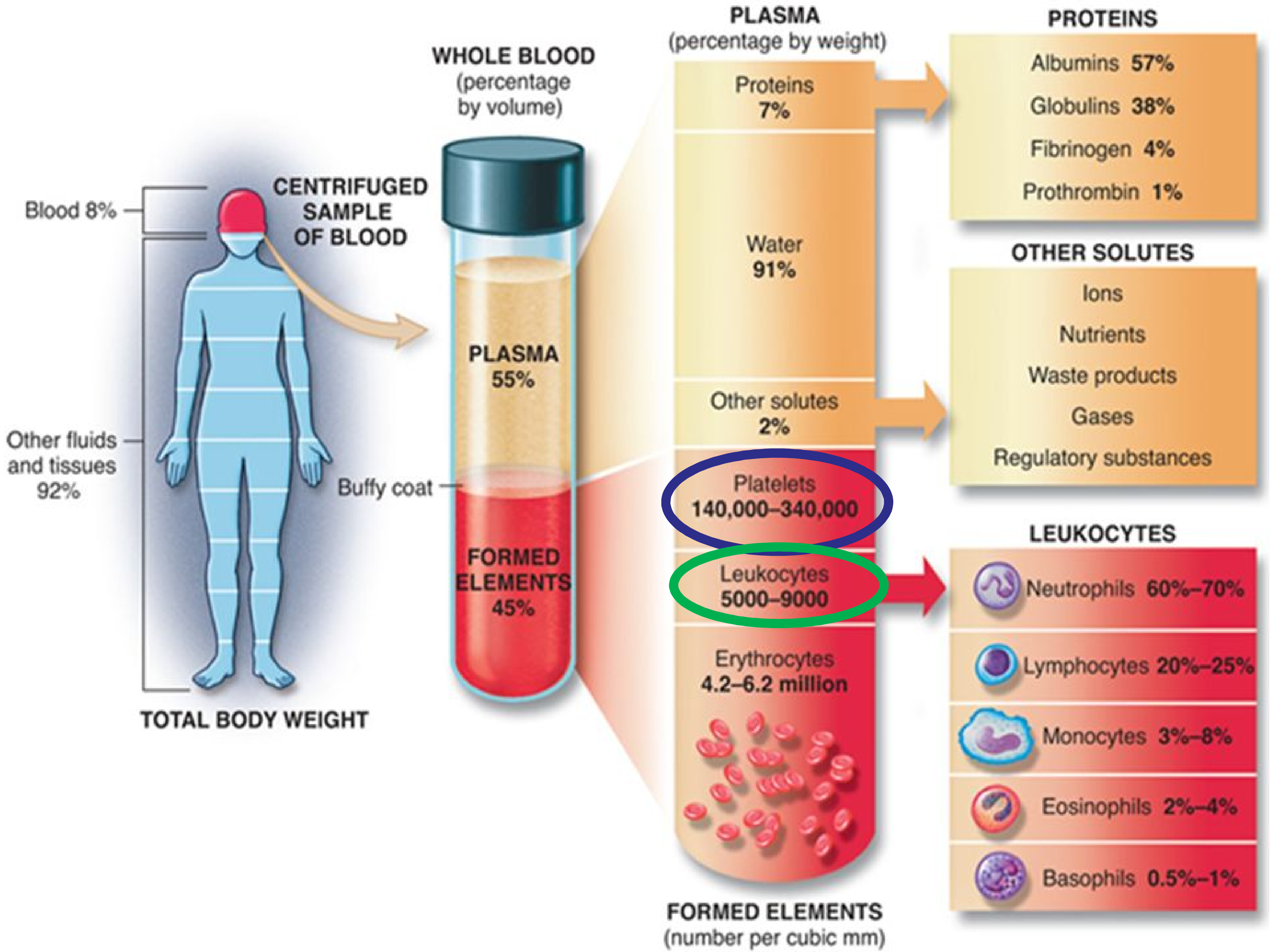
- **Surface Charge Density**
  - *RBC possess* a net **negative surface charge**, mainly due to the *sialic acid residues* on the **membrane surface**.
  - Therefore, there is a **repulsive electrostatic force** *pushing individual RBC apart from each other.*
  - This force clearly *opposes rouleaux formation.*




ZETA POTENTIAL: THE REASON WHY
CELLS DON'T STICK TOGETHER
NEGATIVE CHARGE

Disaggregating Forces

- **Membrane Strain**
  - **RBCs need to deform to allow an area of close contact** between cells and hence **to allow aggregation**; ***membrane strain opposes this deformation.***
  - **Impaired RBCs deformability** leads to ***decreased RBC aggregation.***
  - **RBCs membrane** can ***withstand a finite strain***, beyond which it ***ruptures***. The classical **yield areal strain of 2–4% for RBCs** is generally accepted for a quasi-static strain.



Blood 8%
Other fluids and tissues 92%
TOTAL BODY WEIGHT
CENTRIFUGED SAMPLE OF BLOOD
WHOLE BLOOD (percentage by volume)
PLASMA 55%
Buffy coat
FORMED ELEMENTS 45%
PLASMA (percentage by weight)
Proteins 7%
Water 91%
Other solutes 2%
Platelets 140,000–340,000
Leukocytes 5000–9000
Erythrocytes 4.2–6.2 million
FORMED ELEMENTS (number per cubic mm)
PROTEINS
Albumins 57%
Globulins 38%
Fibrinogen 4%
Prothrombin 1%
OTHER SOLUTES
Ions
Nutrients
Waste products
Gases
Regulatory substances
LEUKOCYTES
Neutrophils 60%–70%
Lymphocytes 20%–25%
Monocytes 3%–8%
Eosinophils 2%–4%
Basophils 0.5%–1%

Leukocytes (WBCs)

Table 1.6 Approximate Percentages of the
Types

WBC Type	**Percen**
Neutrophils	62.
Eosinophils	2.
Basophils	0.

- **WBCs have very little role** to play in ***determining the normal WBV*** because **their** ***volume concentration*** is **so much smaller** than that of the **RBCs**. **(If their volume fraction is not abnormally elevated)**
- ***WBCs play a major role*** as a **determinant of flow in the microcirculation**.

Thrombocytes (Platelets)

- From a hemorheological point of view the **platelets are of little importance.**
  - The reason for this finding is that they are **much smaller than either the RBCs or WBCs**, having diameters of the order of 2-3 μm; their **overall volume in normal blood** is ***even less*** than that associated with the **WBCs**.
- The consequence is that ***they neither influence whole blood viscosity nor microvascular resistance to any significant degree.***

Point of Comparison	Red blood cells	White blood cells		Platelets
		Granulocytes and monocytes	Lymphocytes	
Origin	red bone marrow	red bone marrow	thymus, red bone marrow	red bone marrow, lungs
Cells present per mm^3 of blood (approximate)	5 500 000 (male) 4 500 000 (female)	6000	2000	250 000
Relative size	small (8 μm diameter)	largest (up to 25 μm)	large (10 μm)	smallest (2 μm)
Function	to carry oxygen and carbon dioxide to and from cells	to engulf foreign particles	to play a role in the formation of antibodies (defence function)	to play a role in the clotting of blood (defence function)
Life span	120 days	a few hours to a few days	unknown	2–8 days
Appearance				

Changes of Hemorheological Significance Associated with Pathology

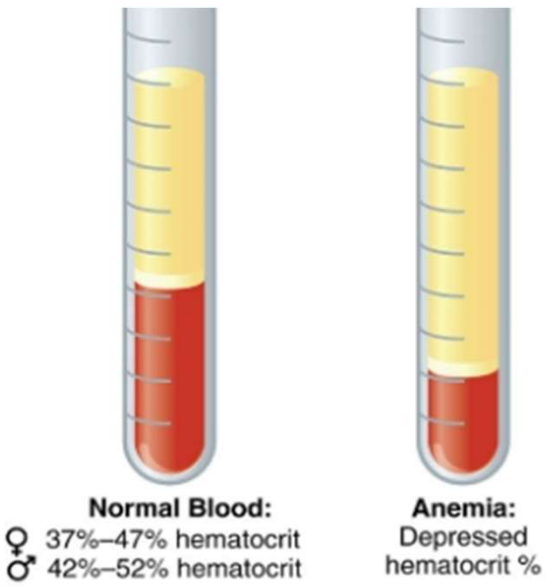
Plasma Proteins

- The plasma protein concentration is mainly responsible for the elevation of plasma viscosity above that of water.
- In ***Waldenstrom's macroglobulinaemia*** and ***multiple myeloma***:
  - **Concentration** of **large macroglobulins** **increases massively** and **has a very large effect on plasma viscosity**, often with up to **a 20-fold increase** in the viscometric effect of these proteins.
  - Many of the proteins in these conditions are abnormal and **highly rouleaugenic**.
  - The consequent large increase in the strength and size of rouleaux leads to ***markedly increased low shear rate blood viscosity***.
  - These effects have ***deleterious effects on blood flow in all vessels*** of the circulation, **especially those in the microcirculation**.

- The plasma protein with the **greatest rouleaugenic effect** is ***fibrinogen***, and hence **the relevance of plasma fibrinogen concentration increases** in association with many clinical conditions.
- **E.g…**
  - Diabetes mellitus,
  - Hypertension,
  - Pregnancy,
  - Post surgical trauma,
  - Infection, etc.
- This means that **the strength of rouleaux formation** and **the shear thinning of blood** is ***frequently elevated*** in association with these conditions.

RBCs
(Decreased or Increased Hematocrit)

- The most obvious example for this condition is ***anemia***, due to any cause, where the **lowering** of the whole blood **hemoglobin concentration** ***inevitably reduces*** the **viscous effect of the RBC mass**.
- Hemorheological problem in here is that it is often complicated by associated **changes in the individual RBCs**.

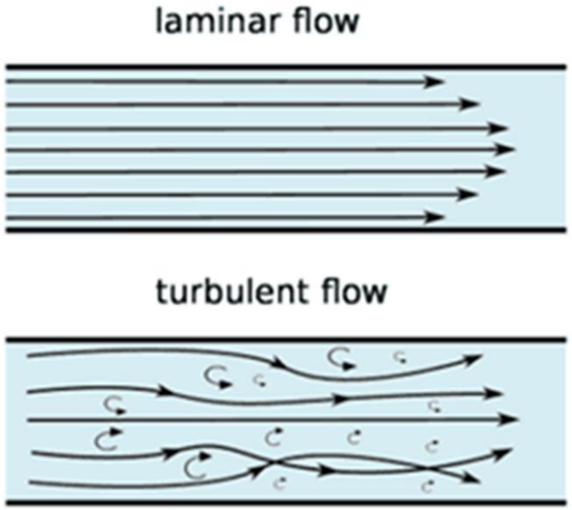


In Anemia

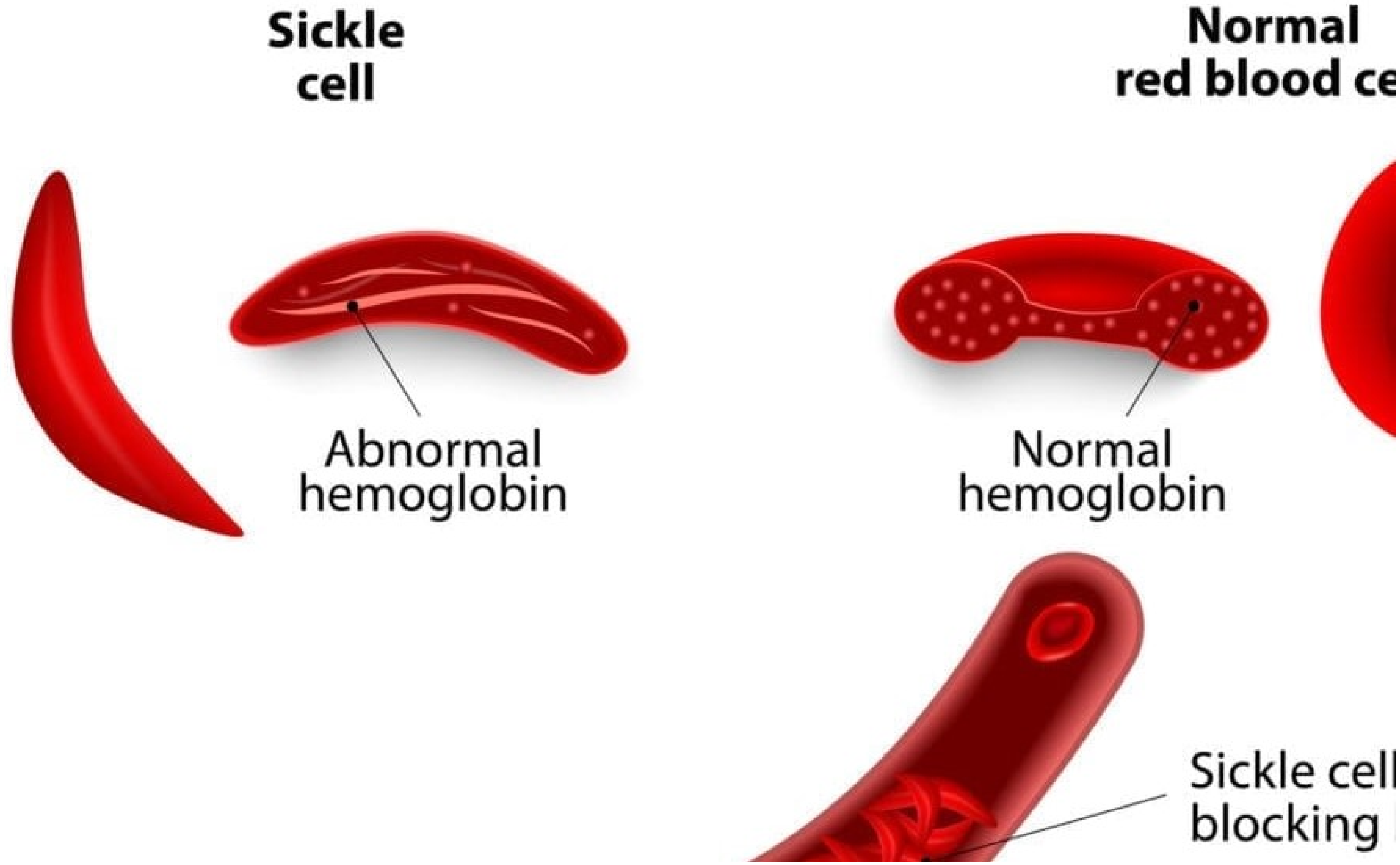
Where V = mean velocity, D = vessel diameter, ρ = blood density, and η = blood viscosity

$$Re = \frac{(\overline{V} \cdot D \cdot \rho)}{\eta}$$

↓ HCT



- In ***iron deficiency anemia*** the cells **become smaller than normal**, while in ***folic acid- or vitamin B12-deficiency anemia*** the cells **increase in size**.
- In ***thalassemia***, the cells are again often **smaller than normal** and they are also **less deformable**.
- **Such changes may have negative influences on microvascular flow.**
- ***E.g…***
  - ***Sickle cell anemia,*** where the cells sickle in low oxygen conditions, ***become quite rigid*** and have very substantial problems negotiating the **microcirculation**.
  - **Repeated sickle-unsickle cycles** during flow through the body eventually leads to their membranes ***becoming damaged and irreversibly rigid***.
  - **As a consequence**, ***the resistance to blood flow in large vessels is lower than normal***, while in **the microcirculation it can be considerably higher, even leading to stasis**.

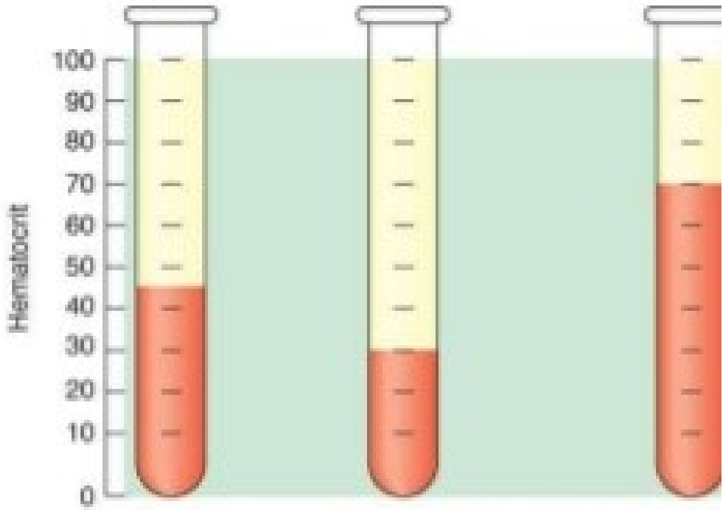


Sickle cell
Abnormal hemoglobin
Normal red blood ce
Normal hemoglobin
Sickle cell blocking

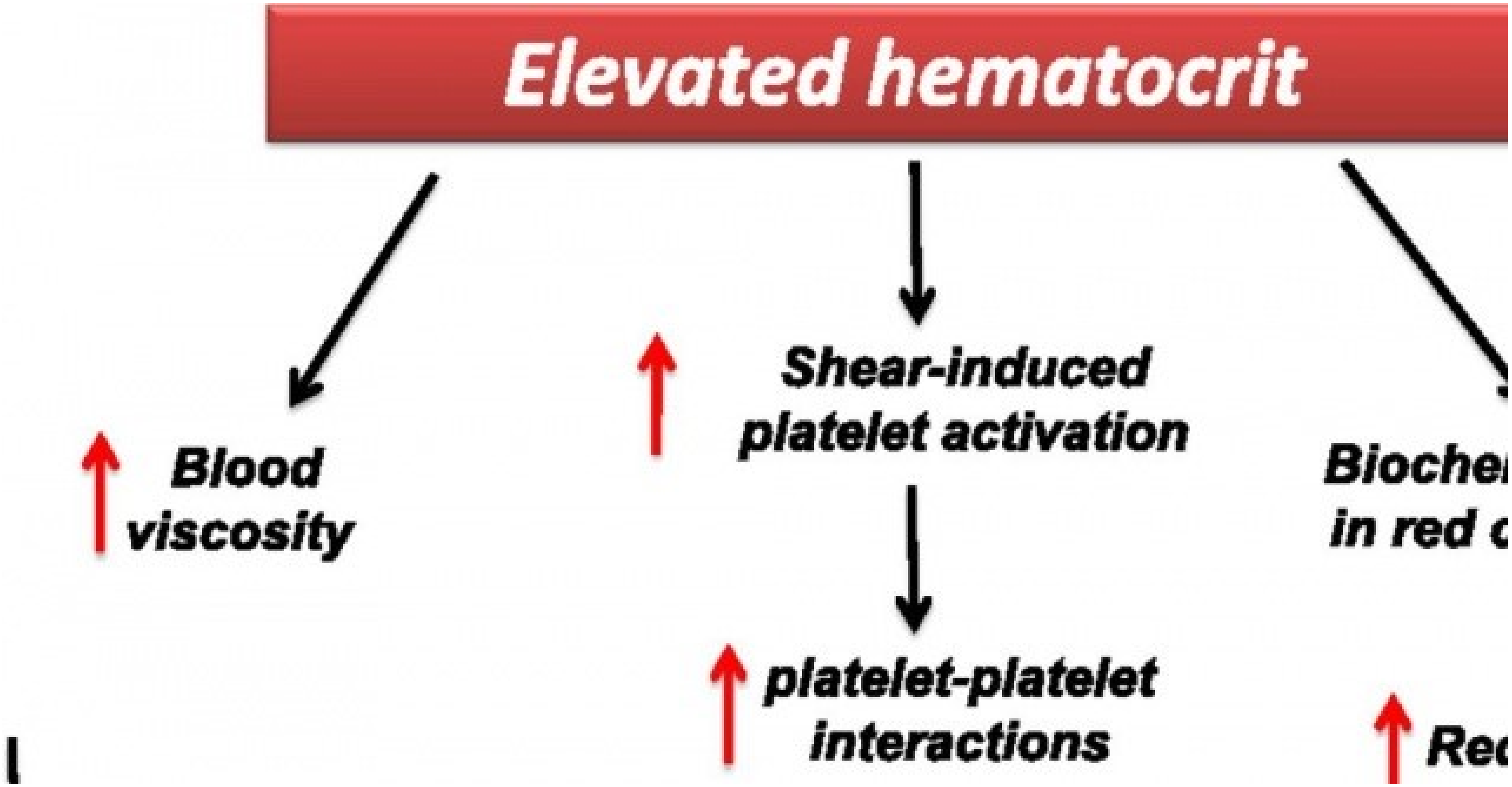
High Hematocrit Level

Some factors may cause a rise in hematocr
such as:

- Polycythemia
- Heart or kidney problems
- Intake of anabolic steroids
- Dehydration
- Diarehea
- Lung problems
- Burns



Respiratory diseases, Hypoxia, Polycythemia, etc.



WBCs

- **WBCs in the healthy individual are not of sufficient concentration to affect whole blood viscosity.**
- But, they do have a **significant resistive effect at the microcirculatory** level due to ***their relative stiffness***.
- The **WBC count** can become ***extremely high***: the most prominent are the ***classic leukemias***.
  - WBC count increase by an order of magnitude or even more.
  - Under these circumstances they **are relevant to** ***the viscosity of the whole blood*** and, because of their ***large volume concentration***, ***relative inflexibility***, ***irregular shape***, and ***viscoelasticity***.
  - **WBCs can cause** ***the viscosity to rise enormously*** and **so affect** ***large vessel flow***.

References

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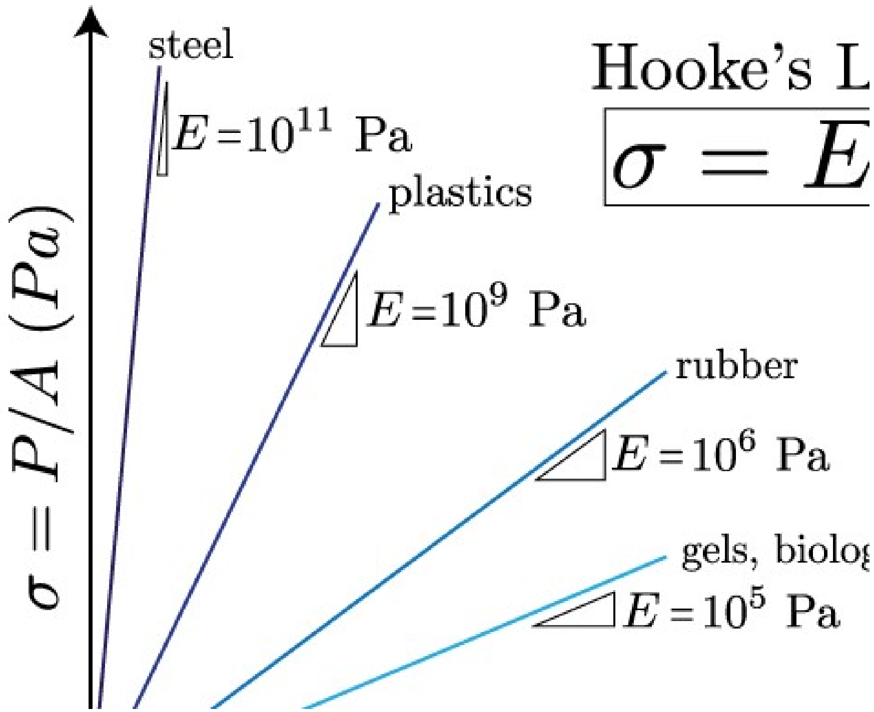
4. Chandran KB, Yoganathan AP, Rittgers SE. Biofluid Mechanics: The Human Circulation. CRC, Taylor & Francis, New York, USA, 2007.
5. Baskurt O, Neu B, Meiselman HJ. Red Blood Cell Aggregation. CRC, Taylor & Francis, New York, USA, 2016.

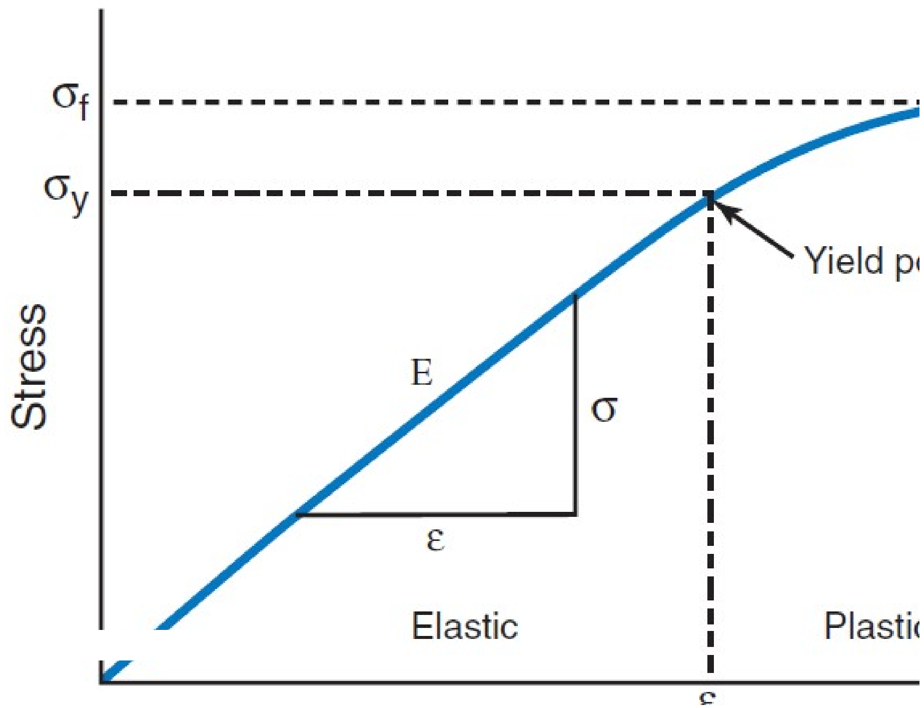
Lecture Notes

Quick Reminder

Law of Elasticity (AKA: Hooke's Law)

- Defines the linear relationship b/w stress and strain within the elastic region.

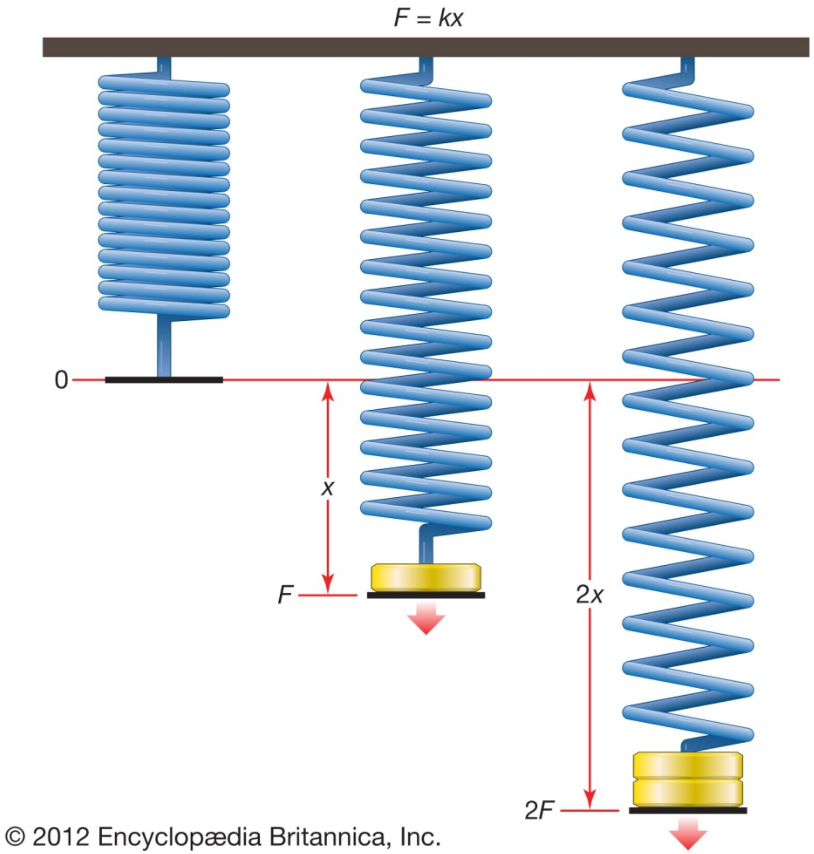


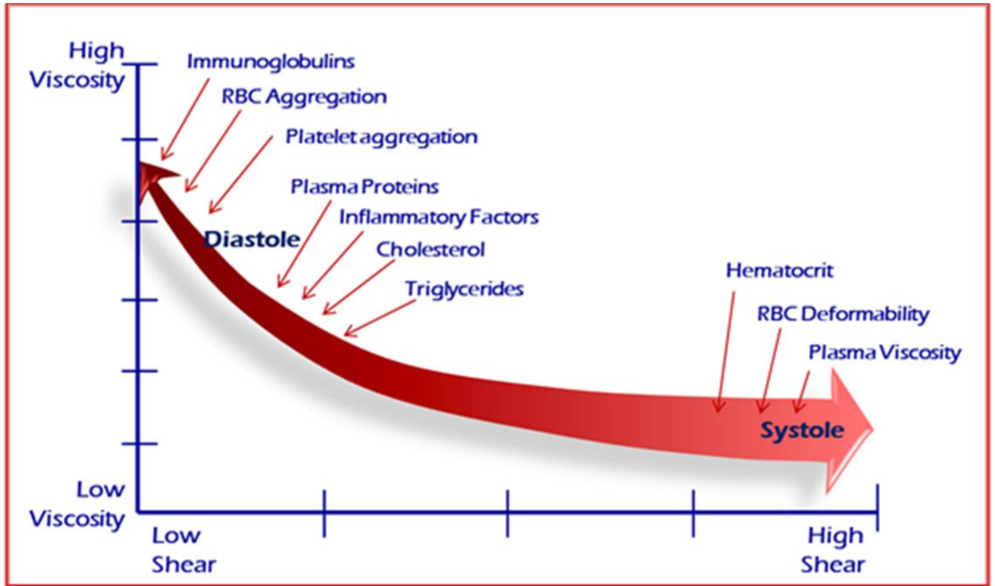


Quick Reminder

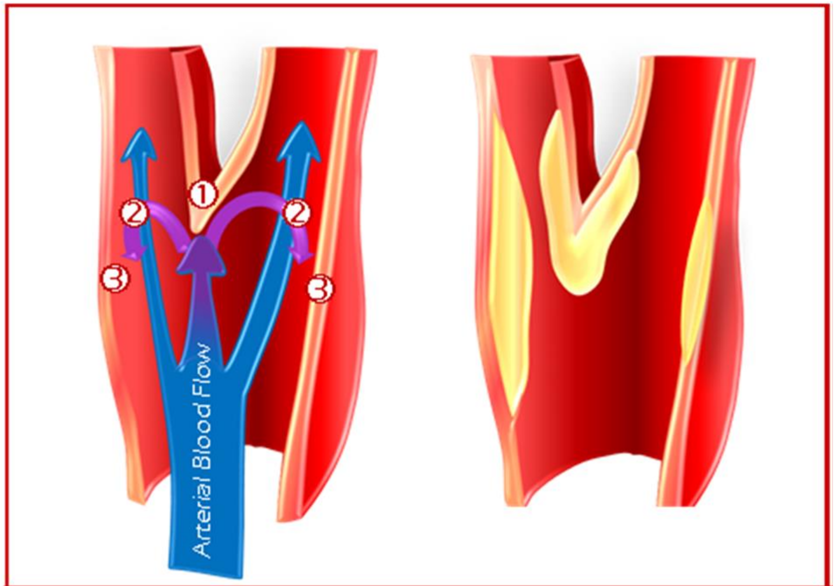
Hooke's Law

- Gives the relationship b/w the force applied to an unstretched spring and the amount the spring is stretched.





At systole, blood is flowing faster and is less viscous. At diastole, slower flowing blood is more viscous. Factors associated with cardiovascular disease impact blood viscosity at different points along the curve.



Abrasive, highly viscous blood damages the intima at a bifurcation point ❶, where turbulent flow ❷ causes further damage to lateral walls ❸. This initiates the atherosclerotic process resulting in plaque formation in these locations.

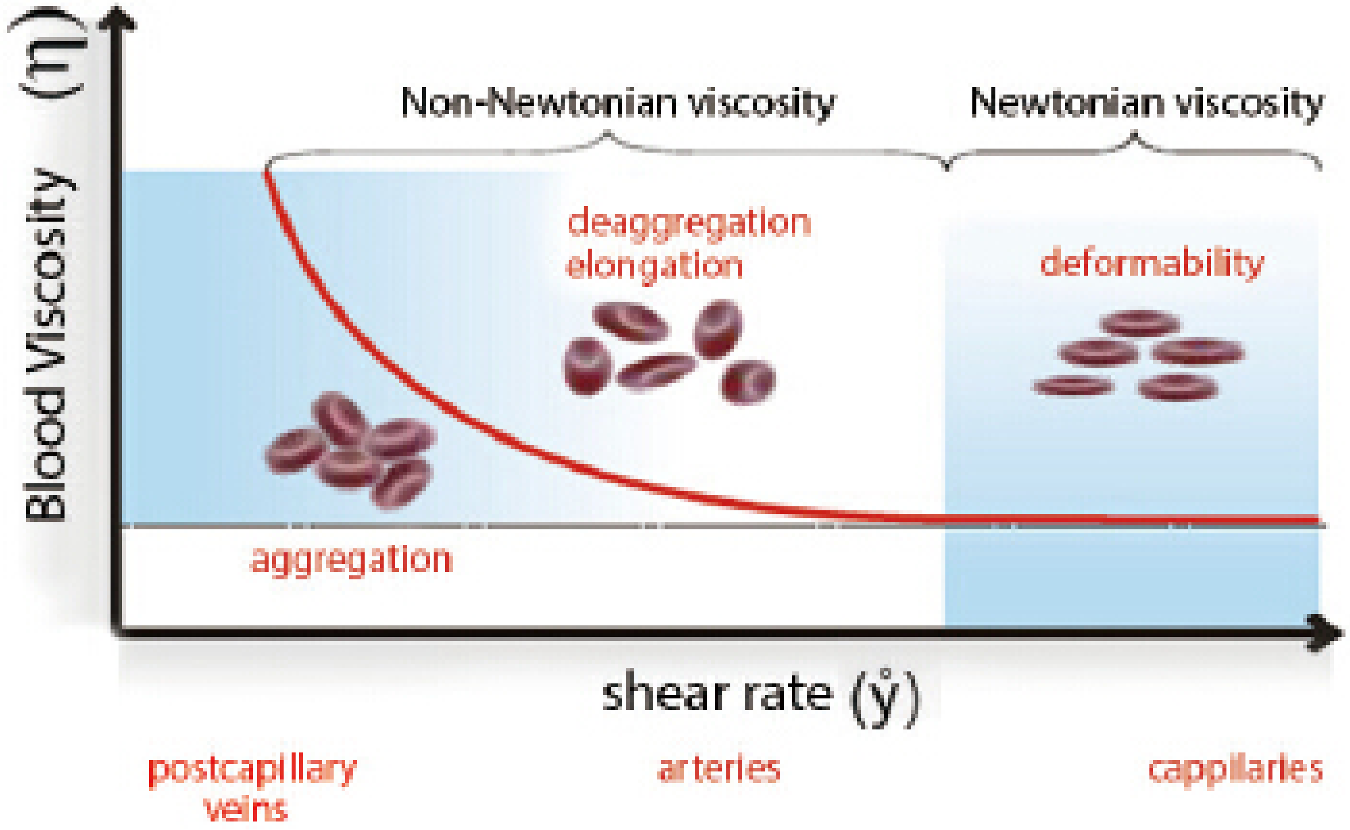


Figure 1 Shear-viscosity curve for blood.