# Biological and Medical Fluid Mechanics I

4. Blood damage

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## 4.1.1 General remarks

- **blood damage** = non physiological change of the blood
  - → destruction of erythrocytes (RBC)
  - → destruction of thrombocytes (TC)
  - → irreversible aggregation of TC and RBC
  - → conversion of fibrinogen (fibrin etc.)
- **hemolysis = disposal of the erythrocytes**
- natural hemolysis rate: approx. 1,5 -$10^8$ RBC/min

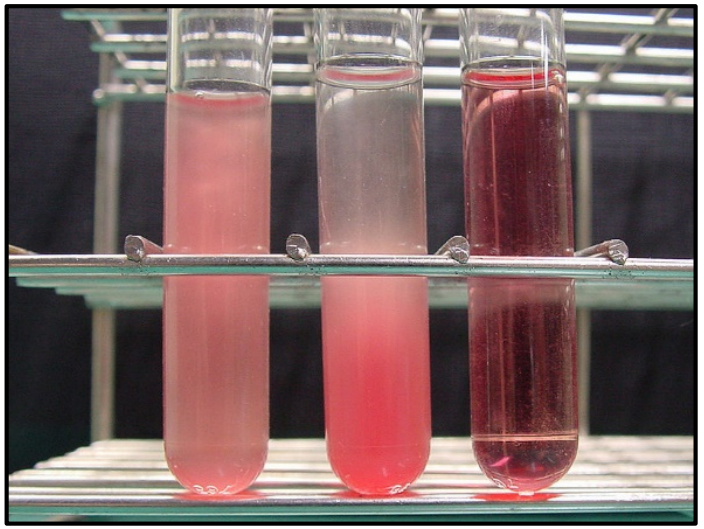

Fig. 4.1: Blood with (right) and without (left, middle) hemolysis [1]



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## 4.1.1 General remarks

- **consequences of hemolysis:**
  - → **blood deficiency** (Anemia)
  - → excess of the haptoglobin binding capacity
    - → Fe-deficiency, **load on the kidneys**
  - → excess of the disposal capacity of the liver for haptoglobin-hemoglobin-complex
    - → **free bilirubin** in blood plasma

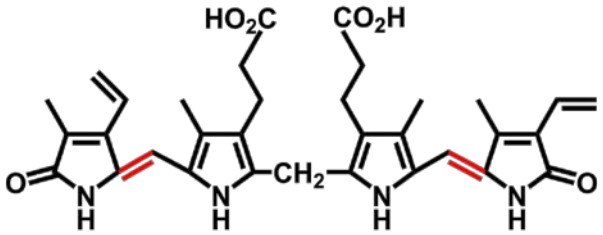


Fig. 4.2: Bilirubin (structural formula) [2]

  - → **jaundice**, **kernicterus**, **gallstone**


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## 4.1.2 Forms of hemolysis

### 1. Osmotic hemolysis:

- **hypotonic** fluid
- alteration from opaque to transparent: **ghosts**

  → ghosts in isotonic fluid are biconcave

  → ghosts in hypotonic and high concentrated Hb-solution: absorption of Hb
- consequences:

  → stretching of the membrane

  → “openings” occur in the lipid-bilayer by a stretch beyond 10%

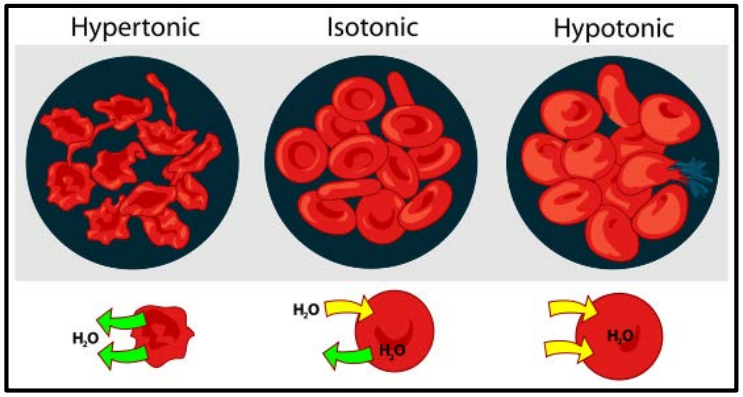


Fig. 4.3: Influence of osmotic pressure on RBC [3]

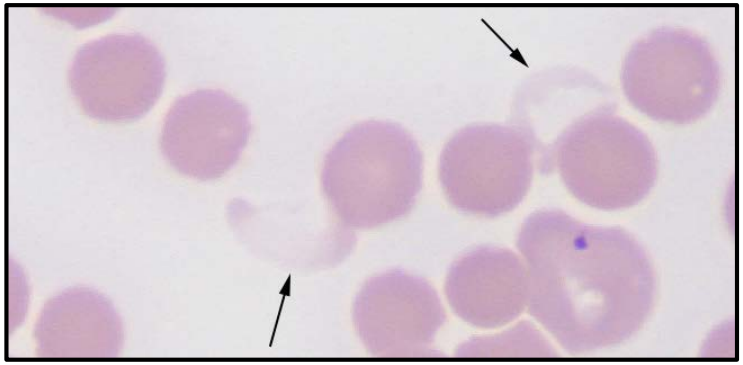

Fig. 4.4: Ghost red blood cells (arrows) [4]



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## 4.1.2 Forms of hemolysis

### 2. Chemical hemolysis:

- fat soluble materials such as **alcohol, ether, chloroform** make the membrane vulnerable to hemolysis
- adipose materials such as **cholesterol** protect the RBC membrane of hemolysis


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## 4.1.2 Forms of hemolysis

### 3. Superficial physical effects:

- carrier changes the properties of the membrane, e.g. **glass**

→ **crenation**

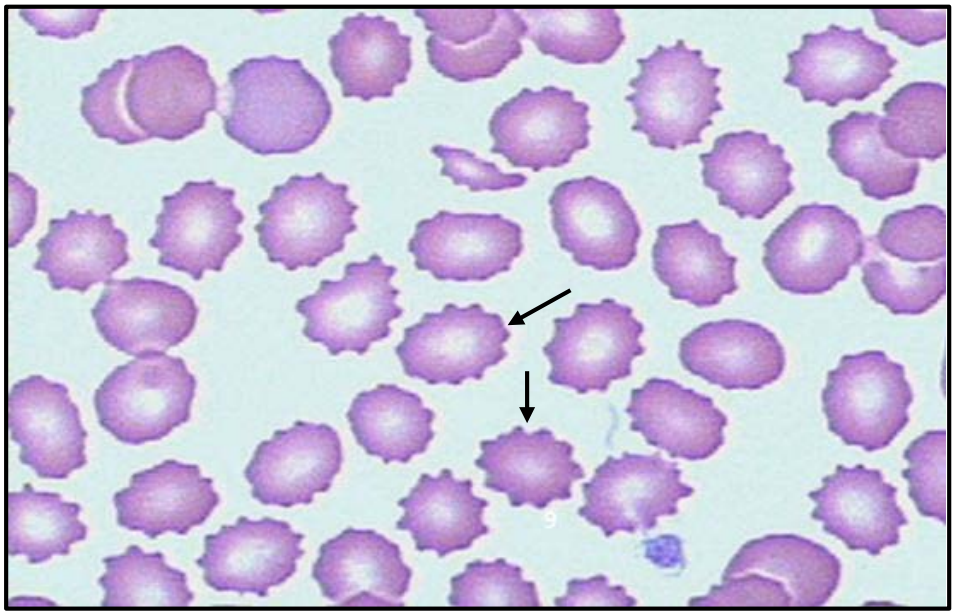

Fig. 4.5: Crenated RBC (arrows) [5]



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## 4.1.2 Forms of hemolysis

### 4. Mechanical effects:

- due to flow phenomena such as **static pressure**, **shear stresses**

→ **see next chapter: 4.1.3 *Blood damage due to flow phenomena***


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## 4.1.3 Blood damage due to flow phenomena

### Observations in medical systems

1. **Gehrmann, Loogen, Bleifeld (1964):**
   - patients with artificial heart valves

     → larger dispersed RBC-life span than normal patients
   - autologous and homologous RBCs

     → for some patients the life span of the RBC for homologous as well as for autologous RBC is strongly shortened


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## 4.1.3 Blood damage due to flow phenomena

### Observations in medical systems

2. **Pasternak:**
   - ultrafiltrating kidney with ,,high pressure rolling pump" and $\Delta p > 1$ bar in animal experiment

   → massive hemolysis

   → assumption: **blood pump causes hemolysis**

   → **causing mechanisms:**

   1. mechanical destruction due to squeezing and strange surface
      - contact with material
      - reverse flow through the gap on the valve seat
      - suture leakage

## 4.1.3 Blood damage due to flow phenomena

### Observations in medical systems

- turbulent down stream from the valve
- cavitation

2. blood pump
   - contact with material
   - tube occlusion
   - reverse stream
   - significant pressure fluctuations

## 4.1.3 Blood damage due to flow phenomena

### Hemolysis in flue dampers

→ Can flow obstacles like the artificial heart valves represent cause heightened blood damage?

1. **Experiment arrangement**
   - basic element of he hemolysis pump with aperture plates + strainer discs
   - 5 basic units (each with different throttle)
   - blood movement: 1. pulsates through inlet
     2. is pumped downward through throttle
     3. flows back

     → due to pulsating air pressure on container reflecting surface

     → due to gravity


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## 4.1.3 Blood damage due to flow phenomena

### Hemolysis in flue dampers

- control of the pulses is produced by each two light passages at the transparent standpipe
- flue damper: strainer disks and aperture plates have the same pressure loss coefficient $\zeta := 2\Delta p/\rho v^2$

  $\rightarrow$ influence of different flow fields

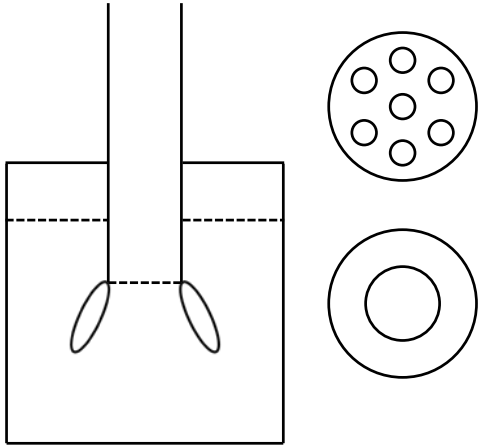

Fig. 4.6: Tank with strainer disc

- to simulate different contraction rates by the heart: pressure pulse is varied by a parallel connection of air reservoirs
- fresh, heparinized or with citrate stabilized pork blood is used

## 4.1.3 Blood damage due to flow phenomena

### Hemolysis in flue dampers

**2. Results**

- steeper pressure leads to higher blood damage

a) higher pressure loss coefficient leads for aperture plates to stronger blood damage

b) strainer discs with same pressure loss coefficients as well as the single aperture plate lead to bigger blood damage

→ the curve $\Delta PHb(\zeta)$ shows a minimum.

→ reason: influence from hole number and pressure loss superimpose

## 4.1.3 Blood damage due to flow phenomena

### Hemolysis in flue dampers

**3. Discussion**

- natural heart valves:

  → ζ = 6-10

  → δp/ δt ≈ 20000 mmWS/sec
- fine structure of the flow field plays a big role besides the pressure loss
- artificial heart valves can cause blood damage due to the flow dynamics in the prosthesis
- the described experiment procedure leads to the fundamental results, but it leaves no declaration over mechanism and damage amplitude of the single parameters like: → velocity gradient

## 4.1.3 Blood damage due to flow phenomena

### Hemolysis in flue dampers

→ shear stress

→ turbulent degree

→ pressure change

→ velocity

→ intensity of the wall interaction etc.

- all these parameters for a valve prosthesis can contribute to blood damage!

## 4.1.3 Blood damage due to flow phenomena

### Influence of static pressure

- known from literature: **negative pressure leads to hemolysis**

1. **Assumption**

→ binding capacity of the Hb is pressure dependent

→ by faster pressure decrease $O_2$ – release happens faster than the transport of $O_2$ out of the RBC

→ gas is released

→ leads to the rupture of the RBCs

2. **Experiment description**

- whole blood conserve (heparinized) in plastic test-tubes in a chamber which is periodically evacuated

## 4.1.3 Blood damage due to flow phenomena

### Influence of static pressure

- variation: → δp/ δt
  → Δp = final pressure (till boiling point)
  → drop period

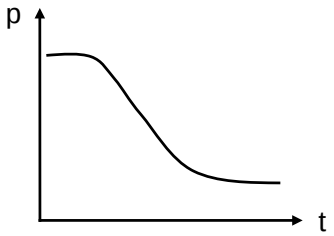


Fig. 4.7: Relation between pressure and time

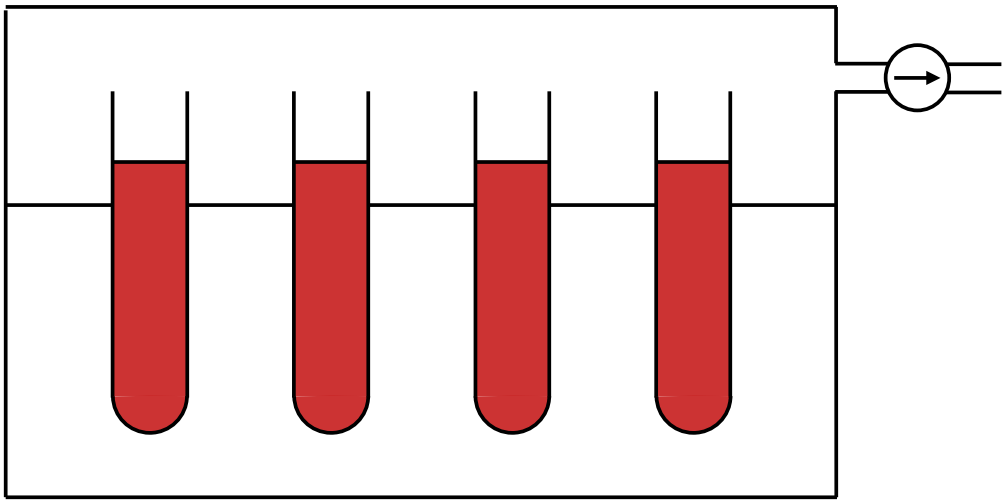

Fig. 4.8: Test tubes with blood in periodically evacuated chamber


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## 4.1.3 Blood damage due to flow phenomena

### Influence of static pressure

3. **Result**
   - no difference in the hemolysis rate in comparison to the probes which are subjected to the same handling except for the pressure drop
4. **Consequence**
   - no precautionary measures necessary to avoid the negative pressure in artificial circulation systems as long as the used boundary value is not over/under crossed

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

1. **Historical overview**
   - about 1967 world wide investigations are carried out [Blackshear, Bernstein, Leverett, Shapiro, Williams u.a.]
   - at first: investigations in modified rheometers (couette, cone-plate)
   - **results:**

     → $\tau < 1000$ dyn/cm²: wall influence dominates

     → $\tau > 1000$ dyn/cm² and load times longer than some minutes: shear stress influence prevails

     → load times $t_B \leq 0{,}27$ sec: wall influence dominates at $\tau \leq 3000$ dyn/cm² in capillary tubes ($\tau$ in the flow not defined!)


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## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

→ long load times (>sec) are of low interest for most clinical questions, e.g. roll pump gap and artificial heart valves, because the blood cells are only for fractions of seconds or even msec in domains of higher shear stress

- influence of short load time

→ assumptions of A.R.Williams: oscillating wire, J.A.Rooney: oscillating gas bubble

→ results: at load times of $10^{-3}$-$10^{-4}$ sec the critical shear stress is at 5600 dyn/cm² or even 4500 dyn/cm²

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

- **problems:**

  → flow field barely computable

  → period during which the RBCs are subjected to domains of certain shear stresses is not exactly assignable

- **further influences and studies:** Load frequency? Accumulation? Fatigue?

  → studies of Forstrom + Blackshear: Fluid-stream-measurements (jet-test)

  → advantage: no wall interactions

  → disadvantages: as previously $t_B = 10^{-3}$-$10^{-4}$ sec, $\tau_{krit}$ = 40000–60000 dyn/cm²

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

**2. Experimental determination**

- **goal:** development of a system that makes the state of the RBC observable at relative exact designation of shear stress and load period for the RBC
- **experimental setup:**
  - → micro gap canal plate
  - → photo optic production
  - → transparent plastics
  - → micro gap canal on the object table of the microscope
  - → RBC in vicinity of the cover glass

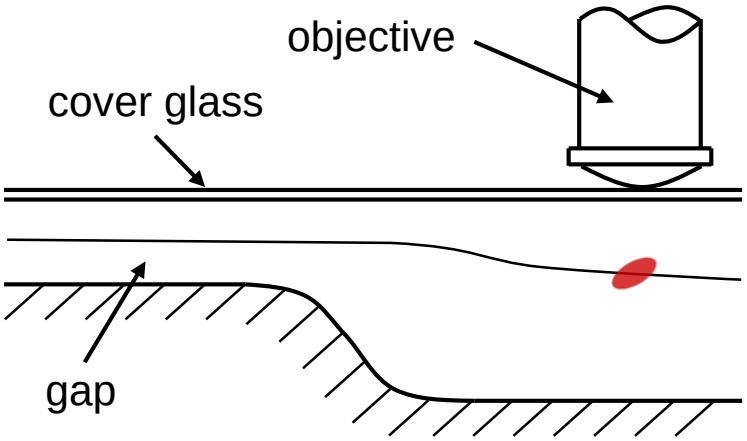


Fig. 4.8: Rotating microscopic table and counter rotation objective

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

- **execution of the test:**
  - → RBCs are suspended from finger tip in a Dextran solution ($\eta \uparrow$)
  - → Strobocin-flash gun of period of 1ms
  - → pressure measurement over catheter (filled with paraffin oil)
- **qualitative results:**
  - → deformation in the shear field (compare Tropfen, Taylor)
  - → membrane elastic
  - → after de-loading: cell membrane is too big for the remaining cell content
    - → formation of wrinkles

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

- **quantitative results:**

  → surface of the RBC remains constant

  → for cell content I it holds approximately: $I = t \cdot t_B^{0,5}$ (empiric)

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

**3. Method of resolution**

- axial flowed Couette-rheometer gap

  (Heuser, Köhler)

  \+ well defined load

  – less well defined load time

  → quantity to be measured: Phb

  → load period: $5 \cdot 10\text{-}3\ s \leq tB \leq 0{,}5\ s$

  → load intensity: $500\ dyn/cm^2 \leq t \leq 7000\ dyn/cm^2$

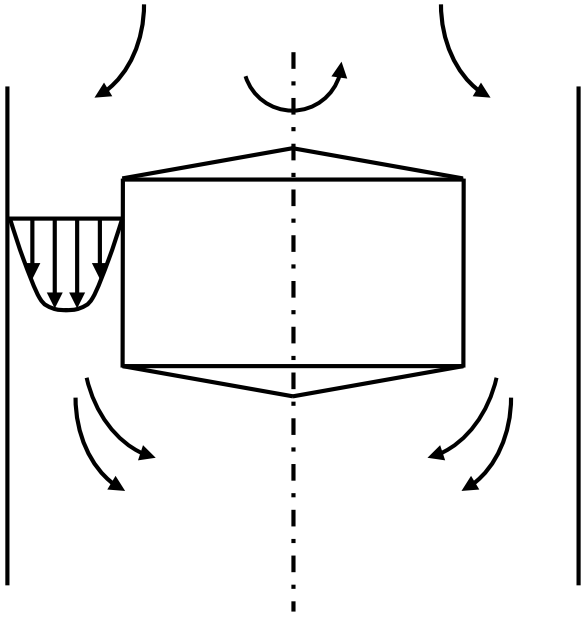

Fig. 4.10: Axial Couette-rheometer

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

- cone-plate rheometer with microscopic observation (Schmid-Schönbein)

→ result: $\tau_{krit} \approx 1500$ dyn/cm²; $t_B$= several minutes

→ observation: constriction and detachment of cell parts

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

4. **Sub lethal damage of the RBC**
   - **hypothesis:**

     → at **shear stress** ↓ and **load period** ↓ :

     respectively **membrane stress** ↓ and **membrane elongation** ↓ result
   - small pores open, which indeed don't let Hb to escape, but smaller molecules and ions:

     → $K^+$-ions

     → $Na^+$-ions

     → ATP (Adenosine-Triphosphate, energy carrier)

     → 2,3 DPG (2,3 Diphosphoglycerate, control of the $O_2$-binding capacity)

## 4.1.3 Blood damage due to flow phenomena

### Influence of shear stresses and load period

- **consequences:**

  a) increased $Na^+$-inflow:

  → swelling of the RBC → lower flexibility

  b) $K^+$-ions-discharge:

  → hardening of the membrane

  c) ATP-discharge:

  → ADP → stimulation of the platelets-aggregation (thrombocytes)

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## 4.1.4 Measurement of hemolysis

1. **Probe preparation**
   - withdrawel of blood probes
   - separation of the plasma and the blood cells (usually centrifuge)
2. **Measurement of the Hb-concentration in the plasma**
   - via photometer: Hb → Hb- cyanide (stable form)
3. **Measurement of the extinction**
   - with monochromatic light: $\lambda_1$ = 540nm and $\lambda_2$ = 680nm

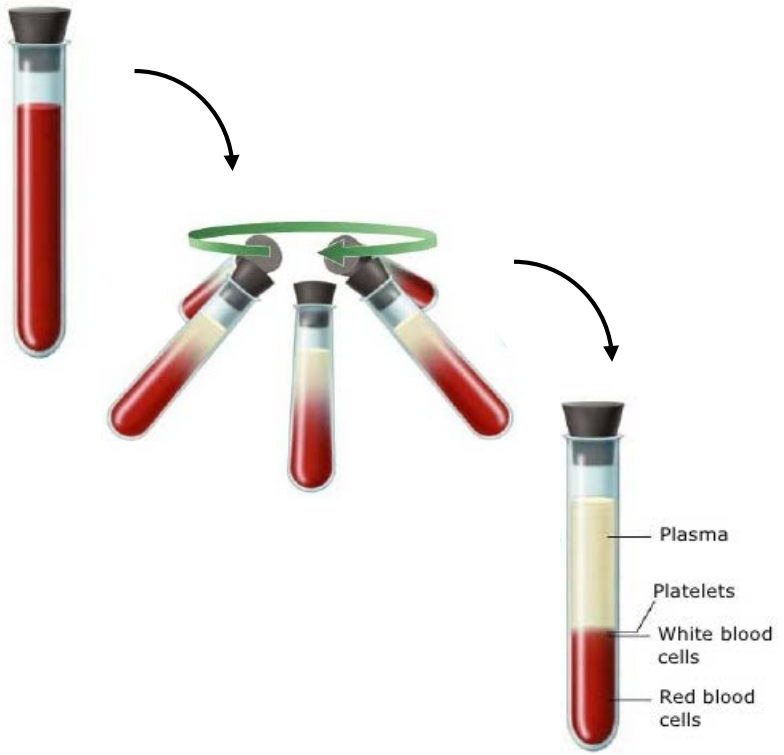


Fig. 4.11: Centrifuged blood [5]

## 4.1.4 Measurement of hemolysis

- extinction E = -lg(T) with T= transmission = $\frac{\text{reflected intensity}}{\text{incident intensity}}$
- connected to the concentration C according to Lambert-Beer's law:
  E = k·C (k = material constant)
- besides hemoglobin other substances that occur in the RBC and are sufficiently concentrated in the plasma can serve for the determination of the hemolysis degree
- examples:
  - → **ions** ($K^+$)
  - → **fats**
  - → **enzymes** (glucose- 6- phosphate- dehydrogenase, (G-6-PDH) and lactate-dehydrogenase (LDH))

## 4.1.4 Measurement of hemolysis

### Definition parameters

1. **Increase of plasma-hemoglobin concetration ΔPHb**

   [mgHb/100 ml Plasma] = PHb(t) - PHb(t0).

   → appropriate for the comparison between different flow internals in ONE test system

   → no force of expression e.g. for blood pumps, because wanted volume doesn't fit

2. **Hemolysis index IH** [mgHb/l00 ml Blood] = (released Hb)/(needed volume).

   → adequate for the comparison of different pump systems

## 4.1.4 Measurement of hemolysis

### Definition parameters

3. **Hemolysis rate Hr = IH·V̇** [mg/min] = released amount of haemoglobin /time
   → appropriate when the incident amount of Hb is of a certain interest, e.g. for kidney stress
4. **Hemolysis degree Hg** := (I0 – I)/ I0 = Volume change of the single RBC;
   → $0 \leq Hg \leq 1$
   → appropriate when measurements are not based on released content materials but on the change of the single RBC volume


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# Sources

[1] https://en.wikipedia.org/wiki/Hemolysis
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[5] https://www.slideshare.net/guestbe8f00/human-circulatory-system

# Thank you for your attention!