Hemostasis and fibrinolysis – the hemostatic balance

Reference: Boron & Boulpaep Medical Physiology, 3rd Ed, Chapter 18

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Hemostasis and fibrinolysis

The normal functioning of the circulatory system depends on:
- maintenance of a normal blood fluidity and flow, and
- preservation of the blood vessel walls integrity in order to prevent blood leaking.
These requirements are fulfilled by the hemostatic and fibrinolytic processes.

Blood is normally in a liquid state inside blood vessels as long as it does not come into contact with:
- negatively charged surfaces (e.g., the collagen beneath endothelial cells) that activate an intrinsic coagulation pathway,
- tissue factors (e.g., released from damaged tissue) that activate an extrinsic coagulation pathway.

Thrombolytic/fibrinolytic pathways keep the balance of coagulation pathways by lysing blood clots/thrombus (intravascular clot).
Hemostasis and fibrinolysis involve the following components:

- Vascular / Endothelial:
  - endothelial cells
  - vascular smooth muscle

- Globular:
  - platelets,
  - erithrocytes

- Plasmatics:
  - plasmatic proteins, coagulation factors
  - plasmatic ionic Ca\(^{2+}\)
Hemostasis and fibrinolysis events

(1) local vasoconstriction to collapse vessels with an intravascular pressure below the critical closing pressure

(2) increased tissue pressure → vessel radius decrease, to diminish blood flow, decrease the hemorrhage

(3) adhesion, activation an aggregation of platelets resulting in \textit{platelet plug} formation, in the case of capillary bleeding/vessel rupture → stop the hemorrhage,

(4) coagulation or clot formation through controlled proteolysis of coagulation proteins → fibrin network stabilize the clot

(5) anticoagulant processes that prevent excessive hemostasis

(6) clot retraction and fibrinolysis that breaks up clots → vessel wall repair or fibrous organization of the clot into fibrous tissue
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(1) Local vascular constriction

Vasoconstriction raises the critical closing pressure and thus collapses vessels that have an intravascular pressure below the critical closing pressure.

Vessel constriction is also promoted by chemical byproducts of platelet plug formation and of coagulation:

1. local myogenic spasm: local *myogenic contraction of the blood* vessels is initiated by direct damage to the vascular wall.
2. local factors from platelets and endothelium: *thromboxane A2 (TXA2), serotonin (5-HT), thrombin-triggered release of endothelin 1 (ET-1) that is one of the most powerful vasoconstrictor*
3. nervous reflexes initiated by pain nerve impulses or other sensory impulses that originate from the traumatized vessel or nearby tissues.

Vascular spasm is directly related with the degree of vessel injury. The spasm can last for minutes to hours, during which time the processes of platelet plugging & blood coagulation take place.

Vasodilatation can occur in the neighboring vessels.
Hemostasis and fibrinolysis events

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(2) Increased tissue pressure

• Determined by blood extravasation into the interstitial perivascular space
• Contributes to hemostasis because it decreases transmural pressure, which is the difference between intravascular pressure and tissue pressure.
• Transmural pressure is the main determinant of blood vessel radius. Given the fourth-power relationship between flow and blood vessel radius, an increase in tissue pressure that causes radius to decrease by a factor of 2 would diminish flow by a factor of 16.

That is why pressing a finger against a small cut stop the bleeding; a tourniquet increases extravascular pressure and halt an arterial hemorrhage in a limb. Finally, surgeons routinely make use of this principle when applying hemostatic clamps to close off “bleeders.”

\[ F = \Delta P \cdot \frac{\pi r^4}{8\eta l} \]

This is the Hagen-Poiseuille equation, where \( F \) is the flow, \( \Delta P \) is the driving pressure, \( r \) is the inner radius of the tube, \( l \) is its length, and \( \eta \) is the viscosity.
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(3) Platelet functions:

**Platelet plug formation** by platelet reaction of:

- adhesion
- activation
- aggregation

Platelets have a Ca-dependent **procoagulant activity** through Platelet factor-3 (membrane phospholipid, PF-3) → F Xa and thrombin (**prothrombinase activity**)
Platelets (thrombocytes)

- produced in bone marrow by *fragmentation of megakaryocytes*

- time interval from differentiation of the stem cell to the production of platelets (*thrombocytopoiesis*) ~ 10 days

- controlled by *growth inducers* and *differentiations inducers*: thrombopoietin, IL-6, *IL-3*, Vit B12, GM-CSF (effects: ↑ in no. of megakaryocytes and ↑ in mean volume or nuclear units)

- release ~ 4,000 platelets/megakaryocyte
Platelets production

Nuclear replication

Cytoplasmic granulation

Platelets release
Platelets production

Megakaryocytes are giant cells with multiple copies of DNA in the nucleus.

The edges of the megakaryocyte break off to form cell fragments called platelets.

Stem cell

Developmental pathway

Hemocytoblast → Megakaryoblast → Promegakaryocyte → Megakaryocyte → Platelets
Platelet circulation

- Half-life in the blood: 8-12 days
- Normal count: 150,000 - 400,000 / μL
- Young platelets spend up to 36 hrs. in spleen after release from bone marrow
- Normally not active until damage occur to the vessel walls
- Eliminated from the circulation mainly by the tissue macrophage system (> than 50% in the spleen)
- Platelet membrane:
  - [glycoproteins](#) coat (repulses adherence to normal endothelium, causes adherence to injured vessel wall)
  - [phospholipids](#) that activate blood-clotting reactions
- Platelet antigens
  - specific surface AG: HPA1-5 (human platelet alloantigens)
  - also express ABO and HLA class I antigens
Platelet structure

- Disk-like shape, $\Phi = 1$-$2 \mu$m, vol=5.8 fl, colourless, without nucleus…

- Circumferential skeleton of microtubules that maintains the normal circulating discoid shape and consists in residual Golgi & ER (contain $\uparrow$[Ca], site of different enzymes, PG and TXA2 synthesis)

- The cytoplasm contains mitochondria (oxidative phosphorylation $\rightarrow$ ATP, ADP synthesis), smooth ER, lysosomes (hydrolytic enzymes), peroxisomes (catalase), fibrin-stabilizing factor (F XIII) and the following kinds of granules:
  - electron-dense granules ($Ca^{2+}$, ADP, serotonin)
  - $\alpha$-granules (heparin antagonist PF 4, vWF, PDGF, fibrinogen)
  - glycogen (anaerobic glycolysis)
Platelet structure

**Contractile protein complex system** - microfilaments: actin, myosin, fibrin, filamin, *thrombosthenin* $\rightarrow$ contraction and release of granules

**Open membrane canalicular system** facilitates the release of granules and provides a large reactive surface on which plasma coagulation proteins may be selectively absorbed

Membrane phospholipids (**platelet factor 3** - PF 3) convert: F X to F Xa and prothrombin to thrombin

Membrane glycoproteins/adhesion proteins: GPIa, GPIb, GPIIb/GPIIIa
Platelet ultrastructure

Specific α-granule: growth f. (PDGF), fibrinogen, factor V, VWF, fibronectin, β-thromboglobulin, heparin antagonist (PF4), thrombospondin

Submembraneous filaments (contractile protein)

Glycocalyx*

Peroxisome

Plasma membrane

Open canalicular system

Lysosomes

Platelet phospholipid

Mitochondria

Protein contractile system

Electron dense granule: ATP, ADP, Ca^{2+}, serotonin

Glycogen

Dense tubular system (Ca^{2+}, PG, TxA_2)
Platelet function

Platelets do not adhere to themselves / other blood cells / endothelial membranes, as long as the negative surface charge is maintained by the presence of proteoglycans (mainly heparan sulfate).

Platelet adhesion

Platelet adhesion occurs in response to:
- an increase in the shearing force at the surface of platelets or endothelial cells
- in response to vessel injury
- in response to humoral signals.

Platelet adhesion - the binding of platelets to themselves or to other components, is mediated by platelet receptors = glycoproteins (GP) in the platelet membrane.
Platelet GP receptors are integrins - integral membrane proteins (a class of matrix receptors)

Willebrand factor (vWF)
-is a glycoprotein that binds to platelet receptors Ib/IIa (Gp Ib/IIa),
-is present in the blood plasma and made by endothelial cells (stored here in the Weibel-Palade bodies) and megakaryocytes (stored in α granules of platelets).
-high shear, certain cytokines, and hypoxia trigger the release of vWF from endothelial cells.
-a breach of the endothelium exposes platelet receptors to ligands that are components of the subendothelial matrix (collagen, which binds to Gp Ia/IIa, fibronectin and laminin, both of which bind to Gp Ic/IIa).
Glycoproteins (GP) of the surface coat are important in initial events of platelet plug formation, platelet adhesion and aggregation:

- **GP Ia, GP Ia/IIa** - adhesion to collagen
- **GP Ib, IIb/IIIa** - attachment to vascular subendothelium through vWF
- **GP IIb/IIIa** - receptor for fibrinogen (platelet to platelet aggregation)
- **GP Ic/IIa** - binds fibronectin and laminin

**Von Willebrand Factor (vWF):**
- Released from endothelial cells (ECs) and platelets
- Its release from ECs is increased in stress, exercise, adrenaline infusion
Platelet activation

The binding on GP receptors of vWF, collagen, fibronectin, laminin, thrombi, etc, triggers a conformational change in the platelet receptors that initiates an intracellular signaling cascade, which leads to an exocytotic event = the release reaction or **platelet activation**.

The signal-transduction cascade involves the activation of phospholipase C and an influx of Ca2+.

Activated platelets exocytose the contents of their
-dense storage granules, (ATP, ADP, serotonin, and Ca2+).
-α granules (growth factors, vWF, clotting factor V and fibrinogen).

Activated platelets use cyclooxygenase (COX) to initiate the breakdown of arachidonic acid (AA) to thromboxane A2 (TXA2), which they release.

Platelet activation is also associated with marked cytoskeletal and morphological changes as the platelet extends first a broad lamellipodium and then many finger-like filopodia.
Actin filament dynamics and platelet activation

Activated platelet after contraction...
Platelet aggregation

- consists in irreversible fusion and fibrin embedding of platelets
- is induced/amplified by the signalling molecules released by the activated platelets:
  - ADP (binds to P2Y12 receptors on platelets),
  - serotonin
  - thromboxane A2

vWF released by activated platelets binds to the platelet receptor Gp Ib/1a, activating even more platelets and forming molecular bridges between platelets.

Platelet activation also induces a conformational change in the platelet receptor Gp IIb/IIIa, endowing it with the capacity to bind fibrinogen from blood and to form bridges between platelets, to promote the platelet plug formation.

**Antiaggregant medication:**
- Aspirin, an inhibitor of cyclooxygenase, inhibits clotting by reducing the release of thromboxane A2.
- Clopidogrel (Plavix) is an antiplatelet agent that acts by inhibiting the P2Y12 receptors on the platelet surface.
Platelet contact with collagen/damaged wall
platelet swelling/shape changes/contraction
release of granules

Adhesion to vWP/collagen

\[ \uparrow \]

Secretion of TXA$_2$, ADP ($P_{2Y12}$ platelets receptors), Ca$^{2+}$
\[ \rightarrow \] adhesion of more platelets

\[ \uparrow \]

Aggregation

\[ \rightarrow \]

Formation of platelet plug (primary haemostatic plug)

\[ \rightarrow \]

Release of PF 3 - procoagulant action \( \rightarrow \) thrombin, fibrin
Platelet plug formation (primary hemostasis)

1. Exposed collagen binds and activates platelets
2. Release of platelet factors
3. Attracts more platelets
4. Aggregate into platelet plug
   - Prevents platelet adhesion
   - Releases prostacyclin
   - Smooth muscle cells
   - Collagenin subendothelial layer

Vasoconstriction
Platelet adhesion
Platelet aggregation and release reaction
Opposing effects of PGI2 and TXA2 on AC/cAMP production/[Ca]i

Aspirin - inhibitor of cyclooxygenase, inhibits clotting by reducing the release of TXA2.
Clopidogrel - antiplatelet agent that acts by inhibiting the P2Y12 rec. on the platelet surface. ↓ adhesion & aggregation

↓Ca2+
Thrombocytolysis

Thrombocytopenia - spontaneous skin purpura and haemorrhage

- failure of platelet production
  
  drugs: chloramphenicol, penicillamine, phenylbutazone
  chemicals: benzene
  radiotherapy

- increased consumption of platelets:
  
  immune or autoimmune, drug-induced: phenacetin, rifampicin, penicillin, sulphonamides, diazepam, furosemide, tolbutamide, digitoxin;
  disseminated intravascular coagulation (DIC)
  splenomegaly (abnormal distribution/destruction of platelets)
  platelet aggregation (ristocetin, low MW heparin)
Anti-platelet Antibody (IgG)

Autoimmune thrombocytopenic purpura

Life-span of platelets reduced from 10 days to a few hours
Usually idiopathic, but also in HIV infection, etc.
Tests for platelet plug formation (primary hemostasis)

- **Bleeding time** (global test of platelet role in hemostasis) - time to stop bleeding after skin injury

- **Platelet count and Mean Platelet Volume (MPV)** - number and the uniformity of the size of platelet population

- **Platelet granule content** - electron microscopy

- **vWF assay** - measurement of the amount of vWF and its function (e.g. its interaction with platelet receptors)

- **Platelet membrane receptors/glycoproteins** - monoclonal antibodies and flow cytometry
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**(4) Hemostasis and fibrinolysis:**

Plasmatic components involved and blood clot formation

*Coagulation* is a cascade process of enzymatic reactions involving several plasma proteins (proenzymes and procofactors which are activated sequentially), lipids and ions resulting in the production of fibrin and an insoluble blood clot.

A **blood clot** is a semisolid mass composed of both platelets and fibrin, including entrapped erythrocytes, leukocytes, and serum.

A **thrombus** is an intravascular blood clot. The relative composition of thrombi varies with the site of thrombosis (i.e., thrombus formation): a higher proportion of platelets is present in clots of the arterial circulation, whereas a higher proportion of fibrin is present in clots of the venous circulation.
There is a molecular crosstalk between the processes involved in platelet plug formation and clot formation that helps coordinate hemostasis

- Platelet plug formation and blood clotting are related but distinct events that may occur in parallel or in the absence of one other.
- Activated platelets can release small amounts of some of the factors (e.g., Ca2+) that play a role in blood clotting.
- Conversely, some clotting factors (e.g., thrombin and fibrinogen) play a role in platelet plug formation.
- Fluido-coagulant balance is important, because:
  - inadequate clotting would lead to the leakage of blood from the vascular system and, ultimately, to hypovolemia;
  - overactive clotting would lead to thrombosis and cessation of blood flow
The cardiovascular system achieves the balance between an antithrombotic (anticoagulant) and a prothrombotic (procoagulant) state by a variety of components of the vascular wall and blood

- Promoting an **antithrombotic state** is normal for the endothelial cells in the vascular system.

- Promoting a **prothrombotic state** are events associated with:
  - **vascular damage**: (1) the failure of endothelial cells to produce the proper antithrombotic factors, (2) the physical removal or injury of endothelial cells, which permits the blood to come into contact with thrombogenic factors that lie beneath the endothelium.
  - **activation of platelets** by: (1) ligands that bind to platelet receptors (2) shearing forces that activate the platelets (e.g. platelets flow past artificial mechanical heart valves).
Endothelial cells and the anti- and pro-thrombotic state

**Endothelial cells (EC) produce:**

- **Von Willebrand Factor** (stored in *Weibel-Palade bodies in EC*, also synthesized in megakaryocytes and stored in platelet α-granules): *involved in platelet adhesion & aggregation*, carries Factor VIII

- **Prostacyclin (PGI$_2$):** vasodilation, *inhibit platelet adhesion & aggregation*

- **Antithrombin III (AT) & Protein C (PC) activator (thrombomodulin)** both of which inhibit coagulation

- **Tissue plasminogen activator (t-PA)** which activates fibrinolysis by activating plasminogen to plasmin.

The intact vessel wall has an important role in preventing hemostasis!
Anti- and procoagulant activities of endothelium. NO, nitric oxide; PGI₂, prostacyclin; t-PA, tissue plasminogen activator; vWF, von Willebrand factor. The thrombin receptor is also called a protease-activated receptor (PAR).
The coagulation cascade

- According to the classical view, coagulation cascade is divided into the **intrinsic, extrinsic and common pathways**. This division has been done to facilitate the understanding of *in vitro* laboratory tests, but *in vivo* however, the pathways are very closely interlinked.

- Extrinsic and intrinsic pathways are initiated by distinct mechanisms, and converge on a common pathway that generates thrombin and, ultimately, “stable” fibrin that leads to clot formation.
The coagulation cascade

The intrinsic pathway (surface contact activation) becomes activated when blood comes into contact with a negatively charged surface (e.g. in vitro - a glass test tube); occurs mainly at the membrane of activated platelets.

The extrinsic pathway is activated when blood comes in contact with damaged cell membranes; occurs mainly at a “tissue factor” that is membrane bound.

In both cases, the precipitating event triggers a chain reaction of controlled proteolysis that converts precursors (zymogens) into activated factors (serine proteases), which in turn catalyze the conversion of other precursors into other activated factors, amplifying the clotting signals.

The coagulation cascades do not occur in the fluid phase of the blood, where the concentration of coagulation factors is low.
Three essential steps for blood coagulation:

(1) rupture of the vessel or damage to the blood itself → a complex cascade of chemical reactions occurs in the blood involving coagulation factors → formation of a complex of activated substances = prothrombinase / prothrombin activator

(2) the prothrombin activator catalyzes conversion of prothrombin into thrombin. Much of the prothrombin first attaches to prothrombin receptors on the platelets already bound to the damaged tissue.

(3) thrombin acts as an enzyme to convert fibrinogen into fibrin fibers that enmesh platelets, blood cells, and plasma to form the blood clot.

The rate-limiting factor in causing blood coagulation is usually the formation of prothrombin activator and not the subsequent reactions beyond that point.
Coagulation is the series of physiological processes resulting in the arrest of bleeding.

There are four stages, all closely integrated:
1. Vascular reaction
2. Platelet reaction
3. Clot formation
4. Dissolution of the clot - fibrinolysis.
The coagulation cascade

The domain structure of the proteins of the coagulation cascade

- a signal peptide required for the translocation of the polypeptide into the endoplasmic reticulum, where the signal peptide is cleaved.

- a propeptide or γ-carboxyglutamic acid–rich domain (Gla domain) is rich in glutamic acid residues that undergo γ-carboxylation under the influence of the γ-carboxylase that requires vitamin K; is required for Ca2+ binding.

- an epidermal growth factor (EGF)-like domain has a role in forming protein complexes.

- a kringle domain is a loop structure created by several disulfide bonds that play a role in forming protein complexes and attaching the protease to its target.

- a catalytic domain confers the serine protease function to the coagulation proteins and is homologous to trypsin, chymotrypsin, and other serine proteases.

- some other domains are variable among these proteins.
Factor VII embraces tissue factor, contacting the entire length of the molecule. FVII has 4 domains strung together with flexible linkers. At the bottom is the GLA domain, which has 9 modified glutamic acids, labeled CGU. These modified amino acids have an extra carboxylic acid group that traps calcium ions. The ions interact with the membrane surface, helping FVII find tissue factor. The uppermost domain of FVII is a protein-cutting enzyme that will make the break in the factor X. This domain looks very much like other serine proteases such as trypsin and thrombin. In the middle are two small domains that assist with the recognition of tissue factor. The small molecule in green is an inhibitor that blocks the active site and thus acts as an anticoagulant that stops blood clotting (doi:10.2210/rcsb_pdb/mom_2006_3).
<table>
<thead>
<tr>
<th>NAME</th>
<th>ALTERNATE NAMES</th>
<th>PROPERTIES</th>
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<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
<td>Plasma globulin</td>
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<tr>
<td>Factor Ia</td>
<td>Fibrin</td>
<td></td>
</tr>
<tr>
<td>Factor II</td>
<td>Prothrombin</td>
<td>Plasma α₂-globulin; Synthesis in liver requires vitamin K*</td>
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<tr>
<td>Factor IIa</td>
<td>Thrombin</td>
<td>Serine protease</td>
</tr>
<tr>
<td>Factor III (cofactor)</td>
<td>Tissue factor</td>
<td>Integral membrane glycoprotein; member of type II cytokine receptor family</td>
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<td></td>
<td>Tissue thromboplastin</td>
<td>Receptor for factor VIIa</td>
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<td></td>
<td></td>
<td>Must be present in a phospholipid membrane for procoagulant activity</td>
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<tr>
<td>Factor IV</td>
<td>Ca²⁺</td>
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<tr>
<td>Factor V</td>
<td>Labile factor</td>
<td>Plasma protein synthesized by liver and stored in platelets</td>
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<td></td>
<td>Proaccelerin</td>
<td>Single-chain protein</td>
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<td></td>
<td>Accelerator globulin</td>
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<td>Factor Va (cofactor)</td>
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<td>Heterodimer held together by a single Ca²⁺ ion</td>
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<td>Highly homologous to factor VIIIa</td>
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<td>Factor VII</td>
<td>Stable factor</td>
<td>Plasma protein</td>
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<td></td>
<td>Serum prothrombin conversion accelerator (SPCA)</td>
<td>Synthesis in liver requires vitamin K*</td>
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<tr>
<td></td>
<td>Proconvertin</td>
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<td>Factor VIIa</td>
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<td>Serine protease</td>
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<td>Factor VIII</td>
<td>Antihemophilic factor (AHF)</td>
<td>Plasma protein with phospholipid-binding domain</td>
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<td>Factor VIII procoagulant component (FVIII:C)</td>
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<td>Factor VIIIa (cofactor)</td>
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<td>Factor IX</td>
<td>Christmas factor</td>
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<td>Plasma thromboplastin component (PTC)</td>
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<td>Protease</td>
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<td>Protease</td>
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<td>Procoagulant Factors</td>
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<tr>
<td>Factor XI</td>
<td>Plasma thromboplastin antecedent (PTA)</td>
<td>Plasma protein produced by megakaryocytes and stored in platelets</td>
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<td>Factor XIa</td>
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<td>Protease</td>
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<td></td>
<td></td>
<td>Disulfide-linked homodimer</td>
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<td>Factor XII</td>
<td>Hageman factor (HAF)</td>
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<tr>
<td>Factor XIIa</td>
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<td>Protease</td>
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<tr>
<td>Factor XIII</td>
<td>Fibrin-stabilizing factor (FSF)</td>
<td>Plasma protein stored in platelets</td>
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<tr>
<td>Factor XIIIa</td>
<td></td>
<td>Transglutaminase</td>
</tr>
<tr>
<td></td>
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<td>Tetramer of two A chains and two B chains</td>
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<tr>
<td>High-molecular-weight</td>
<td>HMWK</td>
<td>Plasma protein stored in platelets</td>
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<tr>
<td>kininogen</td>
<td>Fitzgerald factor</td>
<td>Kallikrein clips bradykinin from HMWK</td>
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<td>Fletcher factor</td>
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<td>von Willebrand factor</td>
<td>vWF</td>
<td>Serine protease</td>
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<td>Kallikrein clips bradykinin from HMWK</td>
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<td></td>
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<td>Plasma glycoprotein made by endothelial cells and megakaryocytes</td>
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<td></td>
<td></td>
<td>Stabilizes factor VIIIa</td>
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<td></td>
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<td>Promotes platelet adhesion and aggregation</td>
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Figure 18-12  Coagulation cascade, showing only the procoagulant factors. TF, tissue factor.
Intrinsic Pathway (Surface Contact Activation) is a cascade of protease reactions initiated by factors present within blood

- **Factor XII (Hageman factor)** is a plasma protein activated to **factor XIIa** when comes in contact with a negatively charged surface (the membrane of activated platelets or endothelial cells or glass surface *in vitro*) in the presence of HMWK.

  High molecular-weight kininogen (HMWK), a product of platelets that may be attached to the platelet membrane, serves as a cofactor and helps anchor factor XII to the charged surface. The HMWK-assisted conversion of factor XII to factor XIIa is limited in speed.

- Once a small amount of factor XIIa accumulates, this protease converts prekallikrein to **kallikrein**, with HMWK as an anchor. In turn, kallikrein accelerates, in a positive feedback, the conversion of factor XII to factor XIIa.

- Factor XIIa (anchored to HMWK) proteolytically cleaves factor XI to **factor XIa**. In turn, factor XIa (also bound to the charged surface by HMWK) proteolytically cleaves **factor IX (Christmas factor)** to factor IXa, which is a protease.

- Factor IXa (and two downstream products of the cascade, **factors Xa** and **thrombin**) proteolytically cleave factor VIII to **factor VIIIa**, a cofactor in the next reaction.

- Finally, **factors IXa and VIIIa, together with Ca2+** (which may come largely from activated platelets) and **negatively charged phospholipids**, form a trimolecular complex called **tenase**. Tenase then converts **factor X (Stuart factor)** to the active protease factor Xa, where the intrinsic and extrinsic coagulation pathways converge.
The intrinsic pathway

- One of the responses of platelets to activation is the presentation of platelet phospholipid on their surfaces, that allows the tenase complex to form.

- The role of factor VIII in this process is to act as a receptor, in the form of factor VIIIa, for factor IXa, Ca2+ and factor X.

- Factor VIIIa is termed a cofactor in the clotting cascade and is formed in the presence of minute quantities of thrombin. As the concentration of thrombin increases, factor VIIIa is ultimately cleaved by thrombin and inactivated.

- This dual action of thrombin, upon factor VIII, acts to limit the extent of tenase complex formation and thus the extent of the coagulation cascade.
Extrinsic Pathway (Tissue Factor Activation) is a cascade of protease reactions initiated by factors that are outside the vascular system.

Tissue factor (tissue thromboplastin, or factor III) is an integral membrane protein constitutively expressed by nonvascular/perivascular cells, acting as a receptor for the plasma protein factor VII. In case of an endothelial injury, factor VII comes into contact with tissue factor and activates to factor VIIa.

The tissue factor, factor VIIa, and Ca2+ form a trimolecular complex analogous to tenase, and this complex proteolytically cleaves the proenzyme factor X to factor Xa.

When factor X binds to the trimolecular complex, factor VIIa undergoes a conformational change that prevents it from dissociating from tissue factor.

Regardless of whether factor Xa arises by the intrinsic or extrinsic pathway, the coagulation cascade proceeds along the common pathway.
Common Pathway

**Factor Xa** from either the intrinsic or extrinsic pathway is the first protease of the common pathway.

Reminiscent of the conversion of factor VIII to the cofactor VIIIa in the intrinsic pathway, the downstream product thrombin clips factor V to form the **cofactor Va**. *Factor V is highly homologous to factor VIII*

Factors Xa, Va, Ca2+ and phospholipids (phosphatidylinositol and phosphatidylserine), form the **prothrombinase complex**. On the surface of activated platelets, prothrombinase acts on plasma protein **prothrombin (factor II)** to form **thrombin (factor IIa)**.

Thrombin is the central protease of the coagulation cascade responsible for proteolysis of **fibrinogen (factor I)** and releasing of **fibrin monomers** that further assemble into a **fibrin polymer**.
Prothrombin

= a plasma protein / alpha 2-globulin (68,700 da)
- 15 mg/dl in normal plasma
- formed continually by the liver in the presence of Vitamin K.
- it is an unstable protein → splits easily into smaller compounds (thrombin - 33,700 da).

Lack of vitamin K or the presence of liver disease prevent normal prothrombin formation, decrease the prothrombin level → bleeding tendency

Platelets play an important role in prothrombin conversion, as this first attaches to the its platelets’ receptors (PARs - Protease-activated receptors).
Thrombin is the central protease of the coagulation cascade, responsible for:

1. Activation of downstream components in the clotting cascade:
   - catalyze the proteolysis of fibrinogen and the formation of soluble fibrin monomers. Fibrin monomers (α, β, and γ chains) spontaneously polymerize to form a gel of fibrin polymers that traps blood cells.
   - activates factor XIII to factor XIIIa, which mediates the covalent cross-linking of the α and γ chains of fibrin polymers to form a stable fibrin mesh that is even less soluble than fibrin.

2. Positive feedback at several upstream levels of the cascade, as it catalyzes:
   - the formation of new thrombin from prothrombin
   - the formation of the cofactors Va and VIIIa.

3. Paracrine actions that influence hemostasis:
   - activate platelets through PAR-1, a protease-activated receptor (a G protein-coupled receptor).
   - causes endothelial cells to release nitric oxide (inhibit platelet aggregation and adhesion), prostaglandin I2 (PGI2), ADP, vWF, and tissue plasminogen activator.
   - combines with thrombomodulin present on endothelial cell surfaces and form a complex that activates protein C. The cofactor protein S and activated protein C degrade factors Va and VIIIa, thereby limiting their procoagulant activity.
Fibrinogen

= high-molecular-weight (MW = 340,000) plasmatic protein
- 100 to 700 mg/dl in plasma
- formed in the liver
- liver disease can decrease the concentration of circulating fibrinogen
- normally does not leak from the blood vessels into the interstitial fluids → interstitial fluids ordinarily do not coagulate (exception when capillaries permeability becomes pathologically increased)
Coagulation as a Connected Diagram:
the intrinsic and extrinsic pathway are strongly interconnected to form a network

The classical concept of independent intrinsic and extrinsic branches converging on a common pathway become nowadays obsolete.

Coagulation cascade is best conceptualized as a “connected diagram” in which the branches may interconnect in both the upstream and downstream directions:
- thrombin has multiple actions
- the trimolecular complex of [tissue factor + factor VIIa + Ca2+] of the extrinsic pathway, activates factors IX and XI of the intrinsic pathway.
- factors IXa and Xa of the intrinsic pathway can activate factor VII of the extrinsic pathway.

Clinical evidence suggests that coagulation depends largely on the extrinsic pathway. While tissue factor is normally absent from intravascular cells, inflammation can trigger peripheral blood monocytes and endothelial cells to express tissue factor, which increases the risk of coagulation (e.g. during sepsis, the tissue factor produced by circulating monocytes initiates intravascular thrombosis).
Hemostasis and fibrinolysis events

(1) vasoconstriction to collapse vessels with an intravascular pressure below the critical closing pressure

(2) increased tissue pressure $\rightarrow$ vessel radius decrease, to diminish blood flow, decrease the hemorrhage

(3) adhesion, activation an aggregation of platelets resulting in platelet plug formation, in the case of capillary bleeding/vessel rupture $\rightarrow$ stop the hemorrhage,

(4) coagulation or clot formation through controlled proteolysis of coagulation proteins $\rightarrow$ fibrin network stabilize the clot

(5) anticoagulant processes that prevent excessive hemostasis

(6) clot retraction and fibrinolysis that breaks up clots $\rightarrow$ vessel wall repair or fibrous organization of the clot into fibrous tissue
Anticoagulants keep the clotting network in check

There are important paracrine factors and anticoagulant factors, mainly of endothelial origin, that prevent hemostasis from running out of control.

**Paracrine Factors:**
- prostacyclin (PGI2)
  - promotes vasodilation and thus blood flow
  - inhibits platelet activation and thus clotting.
- nitric oxide (NO), that inhibits platelet adhesion and aggregation through cGMP.

**Anticoagulant Factors** generated by endothelial cells interfere with the clotting cascade that generates fibrin.

<table>
<thead>
<tr>
<th>NAME</th>
<th>ALTERNATE NAMES</th>
<th>PROPERTIES</th>
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</thead>
<tbody>
<tr>
<td><strong>Anticoagulant Factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor</td>
<td>TFPI</td>
<td>Protease inhibitor produced by endothelial cells GPI linked to cell membrane</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>AT III</td>
<td>Plasma protein Serine protease inhibitor, member of serpin family Inhibits factor Xa and thrombin, and probably also factors XIIa, XIa, and IXa Heparan and heparin enhance the inhibitory action</td>
</tr>
<tr>
<td>Thrombomodulin (cofactor)</td>
<td></td>
<td>Glycosaminoglycan on surface of endothelial cell Binds thrombin and promotes activation of protein C</td>
</tr>
<tr>
<td>Protein C</td>
<td>Anticoagulant protein C</td>
<td>Plasma protein Synthesis in liver requires vitamin K*</td>
</tr>
<tr>
<td></td>
<td>Autoprothrombin IIA</td>
<td>Serine protease Disulfide-linked heterodimer</td>
</tr>
<tr>
<td>Protein C&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Activated protein C</td>
<td>Plasma protein Synthesis in liver requires vitamin K* Cofactor for protein C</td>
</tr>
</tbody>
</table>
Anticoagulant Factors

1. **Tissue factor pathway inhibitor (TFPI)**
   - is a plasma protein that binds to the trimolecular complex [tissue factor + factor VIIa + Ca2+] in the extrinsic pathway and blocks the protease activity of factor VIIa.
   - is glycosylphosphatidylinositol (GPI) linked to the endothelial cell membrane, where it maintains an antithrombotic surface.

2. **Antithrombin III (AT III)** binds to and inhibits factor Xa and thrombin. The sulfated glycosaminoglycans heparan sulfate and heparin enhance the binding of AT III to factor Xa or to thrombin, thus inhibiting coagulation. Heparan sulfate is present on the external surface of most cells, including endothelial surfaces. Mast cells and basophils release heparin.

3. **Thrombomodulin** is a glycosaminoglycan product of endothelial cells, that forms a complex with thrombin, removing thrombin from the circulation and inhibiting coagulation; also binds protein C.

4. **Protein C** activates by binding to thrombomodulin-thrombin complex. Activated protein C (Ca) is a protease that, together with its cofactor protein S, inactivates the cofactors Va and VIIIa, thus inhibiting coagulation.

5. **Protein S** is the cofactor of protein C and is thus an anticoagulant.

Finally, clearance of activated clotting factors by the Kupffer cells of the liver also keeps hemostasis under control.
Heparin

- powerful anticoagulant, its concentration in the blood is normally low

- highly negatively charged conjugated polysaccharide that increases a 100x to 1000x its anticoagulant potency when it **combines with antithrombin III**
- in the presence of excess heparin, removal of free thrombin from the circulating blood by antithrombin III is almost instantaneous.

- the complex of heparin and antithrombin III removes several other activated coagulation factors in addition to thrombin: factors XII, XI, X, and IX.

-heparin is produced by many different cells of the body, but especially by the basophilic **mast cells in the pericapillary connective tissue** (> in the lungs, ~ in the liver) and by the **basophil cells of the blood**.

- used widely as a pharmacological agent in medical practice in much higher concentrations to **prevent intravascular clotting** (purified animal heparin): 0.5-1 mg/kg bw increase rapidly blood-clotting time from 6 min to ~30 min (act for 1.5 - 4 hrs); injected heparin is destroyed in the blood by an enzyme - **heparinase**
Figure 18-13  Abbreviated version of the coagulation cascade, showing the anticoagulant factors. The anticoagulant pathways are indicated in red. TF, tissue factor.
Hemostasis and fibrinolysis events

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(6) clot retraction and fibrinolysis that breaks up clots $\rightarrow$ vessel wall repair or fibrous organization of the clot into fibrous tissue
(6) Clot retraction and fibrinolysis:

Clot retraction/contraction

Within a few minutes after a clot is formed, it begins to contract through the interaction of actin and myosin in the platelets \(\rightarrow\) expresses the fluid from the clot (serum) within 20 to 60 minutes \(\rightarrow\) the edges of the broken blood vessel are pulled together

Platelets are necessary for clot retraction to occur:
- they become attached to the fibrin fibers \(\rightarrow\) bond different fibers together
- continue to release procoagulant substances -fibrin-stabilizing factor \(\rightarrow\) cross-linking bonds between adjacent fibrin fibers
- contribute directly to clot contraction by activating platelet contractile proteins (thrombosthenin, actin, and myosin molecules) \(\rightarrow\) contraction of the platelet spicules attached to the fibrin

Clot contraction is activated and accelerated by thrombin and calcium ions released from calcium stores in the mitochondria, endoplasmic reticulum, and Golgi apparatus of the platelets.
(6) Clot retraction and fibrinolysis:

Lysis of blood clots – Fibrinolysis/thrombolysis

When a clot is formed, a large amount of plasminogen is trapped in the clot along with other plasma proteins. This will not become plasmin or cause lysis of the clot until it is activated.

The process of fibrinolysis begins with the conversion of plasminogen (profibrinolysin) to plasmin (fibrinolysin, a trypsin-like proteolytic enzyme), catalyzed by one of two activators:
- tissue-type plasminogen activator (t-PA) or
- urokinase-type plasminogen activator (u-PA).
**Tissue plasminogen activator (t-PA)**

- a serine protease of endothelial origin
- converts the plasma zymogen plasminogen to the active fibrinolytic protease plasmin. The presence of fibrin greatly accelerates the conversion of plasminogen to plasmin.

**Urokinase-type plasminogen activator (u-PA)**

- present in plasma either as a single-chain protein or as the two-chain product of a proteolytic cleavage.
- converts plasminogen to the active protease plasmin, and this proteolysis requires that u-PA attach to a receptor on the cell surface called urokinase plasminogen activator receptor (u-PAR).
**Plasminogen**

- is a large, single chain glycoprotein mainly synthetized by the liver
- cleaved by t-PA at the junction between its heavy and light chains to form plasmin.

**Plasmin**

- is a serine protease that proteolytically cleaves stable fibrin to fibrin breakdown products.
- also breaks down fibrinogen, Factor V, Factor VIII, prothrombin, and Factor XII.
- also cleaves t-PA
- determines lysis of a clot by destroying many of the clotting factors → blood hypocoagulability

An important function of the plasmin system is to remove minute clots from peripheral vessels that eventually would become occluded.
<table>
<thead>
<tr>
<th>NAME</th>
<th>ALTERNATE NAME</th>
<th>PROPERTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-type plasminogen activator</td>
<td>t-PA</td>
<td>Serine protease that catalyzes hydrolysis of plasminogen at the junction between the N-terminal heavy chain and C-terminal light chain. N terminus contains two loop structures called kringles.</td>
</tr>
<tr>
<td>Urokinase-type plasminogen activator</td>
<td>u-PA</td>
<td>Serine protease</td>
</tr>
<tr>
<td>Urokinase-type plasminogen activator</td>
<td>u-PAR</td>
<td>Binds to and required for the activity of u-PA</td>
</tr>
<tr>
<td>Plasminogen</td>
<td></td>
<td>Single-chain plasma glycoprotein with large N-terminal and small C-terminal domain. N terminus contains five kringles.</td>
</tr>
<tr>
<td>Plasmin</td>
<td></td>
<td>Serine protease</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>PAI-1</td>
<td>Serpin (serine protease inhibitor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In plasma and platelets</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forms 1:1 complex with t-PA in blood</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 2</td>
<td>PAI-2</td>
<td>Serpin (serine protease inhibitor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detected only in pregnancy</td>
</tr>
<tr>
<td>α₂-antiplasmin</td>
<td>α₂-AP</td>
<td>Serpin (serine protease inhibitor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forms 1:1 complex with plasmin in blood</td>
</tr>
</tbody>
</table>
The cardiovascular system regulates fibrinolysis at several levels, using both enhancing and inhibitory mechanisms.

- **Catecholamines** and **bradykinin** increase the levels of circulating t-PA.

- **Plasminogen activator inhibitor 1 (PAI-1)** and **plasminogen activator inhibitor 2 (PAI-2)** are serine protease inhibitors (serpins) that reduce the activity of the plasminogen activators:
  - PAI-1 is produced mainly by endothelial cells and complexes with and inhibits t-PA and u-PA.
  - PAI-2 mainly inhibits u-PA; is important in pregnancy because it is produced by the placenta and may contribute to increased risk of thrombosis in pregnancy.

- **Activated protein C**, which inhibits coagulation, also inhibits PAI-1 and PAI-2, thereby facilitating fibrinolysis.

**α2-antiplasmin (α2-AP)** is a serpin that targets plasmin and is made by liver, kidney, and other tissues.

- When plasmin is not bound to fibrin (plasmin is in free solution), α2-AP complexes with and thereby readily inactivates plasmin.
- When plasmin is attached to fibrin, the inhibition by α2-AP is greatly reduced, and fibrinolysis is promoted.
Fibrinolysis

Plasminogen

Tissue plasminogen activator (TPA)
Urokinase plasminogen activator (UPA)

Inhibited by plasminogen activator inhibitor type 1 (PAI-1)

Plasmin

Degradation of factors V and VII

Inhibited by α2 antiplasmin

Fibrin

Fibrin degradation products (FDPs)

D-dimer
D-dimer E fragments
oligomers of fragments X and Y
crosslinked FDPs
XDPs

X Y D E fragments
Hemostasis overview

1. **Injury.** A blood vessel is severed. Blood and blood components (e.g., erythrocytes, white blood cells, etc.) are leaking out of the breaks.

2. **Vascular spasm.** The smooth muscle in the vessel wall contracts near the injury point, reducing blood loss.

3. **Platelet plug formation.** Platelets are activated by chemicals released from the injury site and by contact with underlying collagen. The platelets become spiked and stick to each other and the wound site.

   - Initial platelets are activated by chemicals released from the injured cells and by contact with broken collagen.
   - Bound platelets release chemicals that activate and attract other platelets.

   Forming platelet plug

   Platelets move toward source of chemical signals and bind. Platelet plug grows in size.

4. **Coagulation.** In coagulation, fibrinogen is converted to fibrin (see part b), which forms a mesh that traps more platelets and erythrocytes, producing a clot.

   Fibrin strands secure platelets and erythrocytes, effectively plugging the break.

---

(a) The general steps of clotting

(b) Fibrin synthesis cascade

**INTRINSIC PATHWAY**

- Damaged vessel wall
- Trauma to extravascular cells

**EXTRINSIC PATHWAY**

- Initial activation

**FINAL COMMON PATHWAY**

- Cross-linked fibrin clot

**Factors:**
- Inactive state
- Active state

*Diagram labels:*
- VII
- III
- X
- IX
- VIII
- XI
- XII
- IXa
- Fibrinogen
- Fibrin
- Thrombin
- Prothrombin
Cascades of the coagulation system overview

(a) Resting ECs provide natural anticoagulants (TM, AT and TFPI and ADPase) to inhibit coagulation and keep platelet activation and the coagulation cascade in check. (b) Coagulation is typically initiated by an injury to the vascular ECs, which results in the exposure of TF and collagen from the sub-endothelial tissue to the blood and the release of vWF. (c) Platelets are activated when they are exposed to TF, collagen and vWF. Activated platelets release a number of mediators (ADP, vWF) within their granules, leading to further platelet recruitment, activation, aggregation and plug formation, which is a process termed primary hemostasis. (d) The interaction between TF and factor VII initiates the extrinsic pathway. (e) The exposure of collagen to blood starts the intrinsic pathway. (f) Both the extrinsic and intrinsic pathways result in the initiation of a common pathway, which contains the cascades involved in the production of activated Factor X and thrombin and the formation of fibrin strands. (g) Fibrin strands strengthen the platelet plug and lead to the formation of a stable platelet–fibrin clot. This process is termed secondary hemostasis. (h) Kallikrein, uPA or tPA activate plasminogen to plasmin, which then degrades and reabsorbs the polymerized fibrin strands (fibrinolysis) and favor wounds healing.
Excessive Bleeding

(1) Liver diseases and/or GIT diseases and/or deficiency of bile secretion → vitamin K deficiency → prothrombin deficiency

(2) Hemophilia
- bleeding disease that occurs almost exclusively in males;
- mainly caused by an abnormality or deficiency of Factor VIII - hemophilia A (classic hemophilia), but also by Factor IX deficiency.

(3) thrombocytopenia (platelet deficiency) → thrombocytopenic purpura.
Thromboembolic Conditions in the Human Being

Thrombi (abnormal clot) and Emboli – causes:
- roughened endothelial surface of vessels caused by arteriosclerosis, infection, trauma initiate the clotting process
- very slowly blood flow through the vessels → activation of small quantities of thrombin and other procoagulants.

Use of t-PA in Treating Intravascular Clots
- Genetically engineered t-PA (tissue plasminogen activator) delivered directly to a thromboosed area through a catheter, it is effective in activating plasminogen to plasmin → dissolve intravascular clots
- if used within the first hour after thrombotic occlusion of a coronary artery, the heart is often spared serious damage.
Blood Coagulation Tests

Bleeding Time
When a sharp-pointed knife is used to pierce the tip of the finger or lobe of the ear, bleeding ordinarily lasts for 1 to 6 minutes. The time depends largely on the depth of the wound and the degree of hyperemia in the finger or ear lobe at the time of the test. Lack of any one of several of the clotting factors can prolong the bleeding time, but it is especially prolonged by lack of platelets.

Clotting Time
Method: collect blood in a chemically clean glass test tube and then to tip the tube back and forth about every 30 seconds until the blood has clotted → normal clotting time is 6 to 10 minutes.

Procedures using multiple test tubes have also been devised for determining clotting time more accurately. Unfortunately, the clotting time varies widely, depending on the method used for measuring it, so it is no longer used in many clinics.
Blood Coagulation Tests

Prothrombin Time
- gives an indication of the concentration of prothrombin in the blood
- method: blood removed from the patient is immediately oxalated so that none of the prothrombin can change into thrombin. Then, a large excess of calcium ion and tissue factor is quickly mixed with the oxalated blood
  → tissue factor activates the prothrombin-to-thrombin reaction by means of the extrinsic clotting pathway
- the time required for coagulation to take place is known as the prothrombin time. The shortness of the time is determined mainly by prothrombin concentration.
- normal prothrombin time is about 12 seconds.
**Partial thromboplastin time (PTT)**

PTT test evaluates the factors found in the intrinsic and common pathways. PTT test is used to monitor patients taking an anticlotting drug (heparin). The test is done before the first dose of heparin or whenever the dosage level is changed; and again when the heparin has reached a constant level in the blood.

The test can be done without activators, but they are usually added to shorten the clotting time, making the test more useful for monitoring heparin levels. When activators are used, the test is called activated partial thromboplastin time or APTT.

Normal results vary based on the method and activators used.

- Normal APTT - between 25-40 seconds;
- PTT results - between 60-70 seconds.

APTT results for a patient on heparin should be 1.5-2.5 times normal values. An APTT longer than 100 seconds indicates spontaneous bleeding.