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Hemostasis disorders

Hemostasis

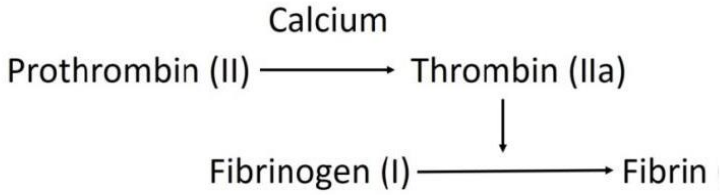
- **Hemostasis** is the process by which bleeding is stopped after an injury by the formation of a clot, while at the same time, maintaining blood in a fluid state elsewhere.

- Injury to a blood vessel results in vasoconstriction and temporary platelet plug formation.

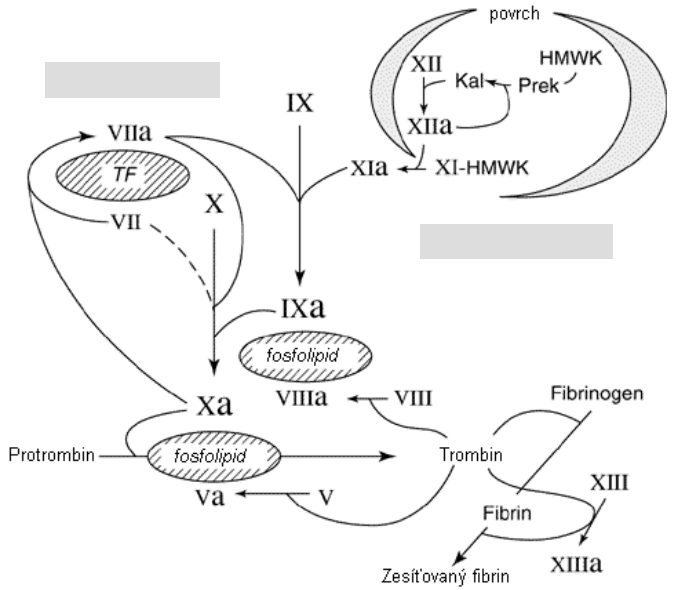
- followed by a coagulation process which arrests bleeding at the site of injury by forming a fibrin clot.

Classic theory of haemostasis - 1905

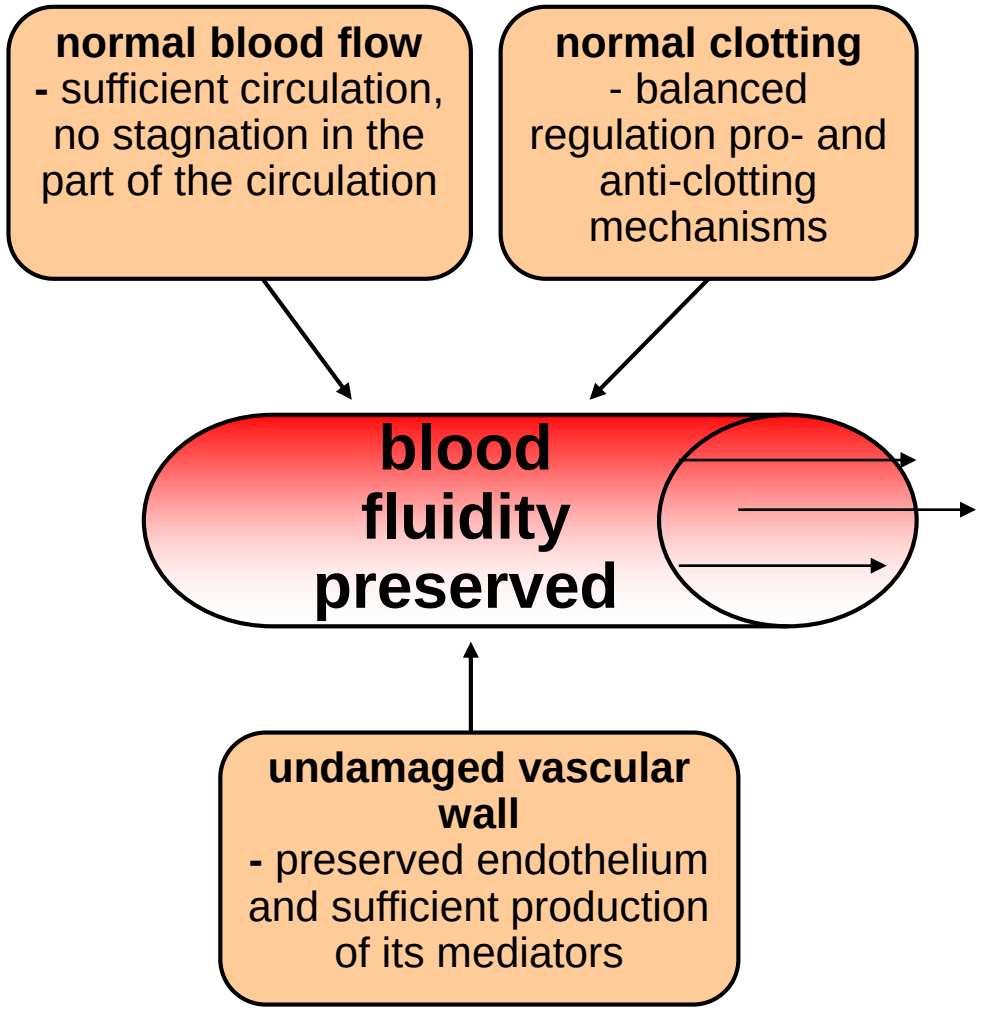
- 1860 – German pathologist Rudolf Virchow described thrombi and their tendency to embolize
- 1905 – P. Morawitz – four factor model of hemostasis



- 1964 – cascade and waterfall model of hemostasis
  - Intrinsic – all the factors required for this pathway were present in blood
    - aPTT
  - Extrinsic – requires tissue factor present in subendothelial cell membranes
    - PT

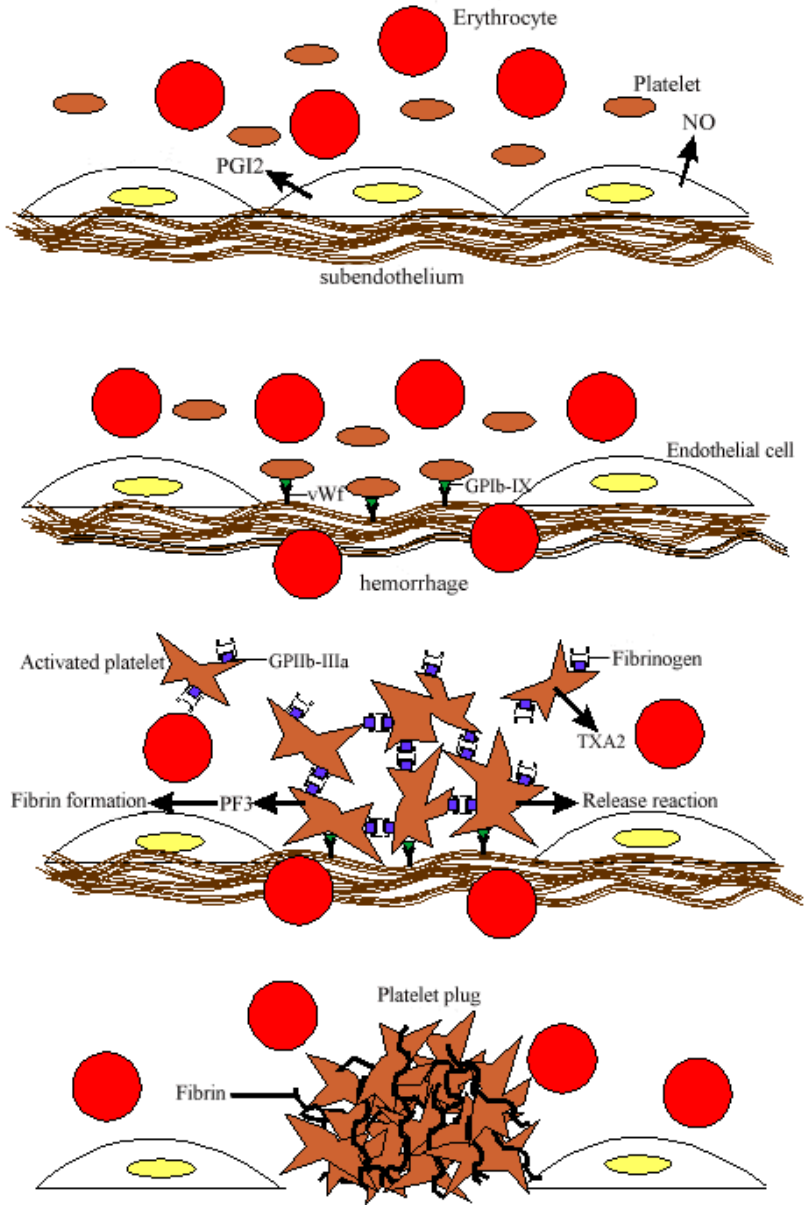


Factors of blood fluidity



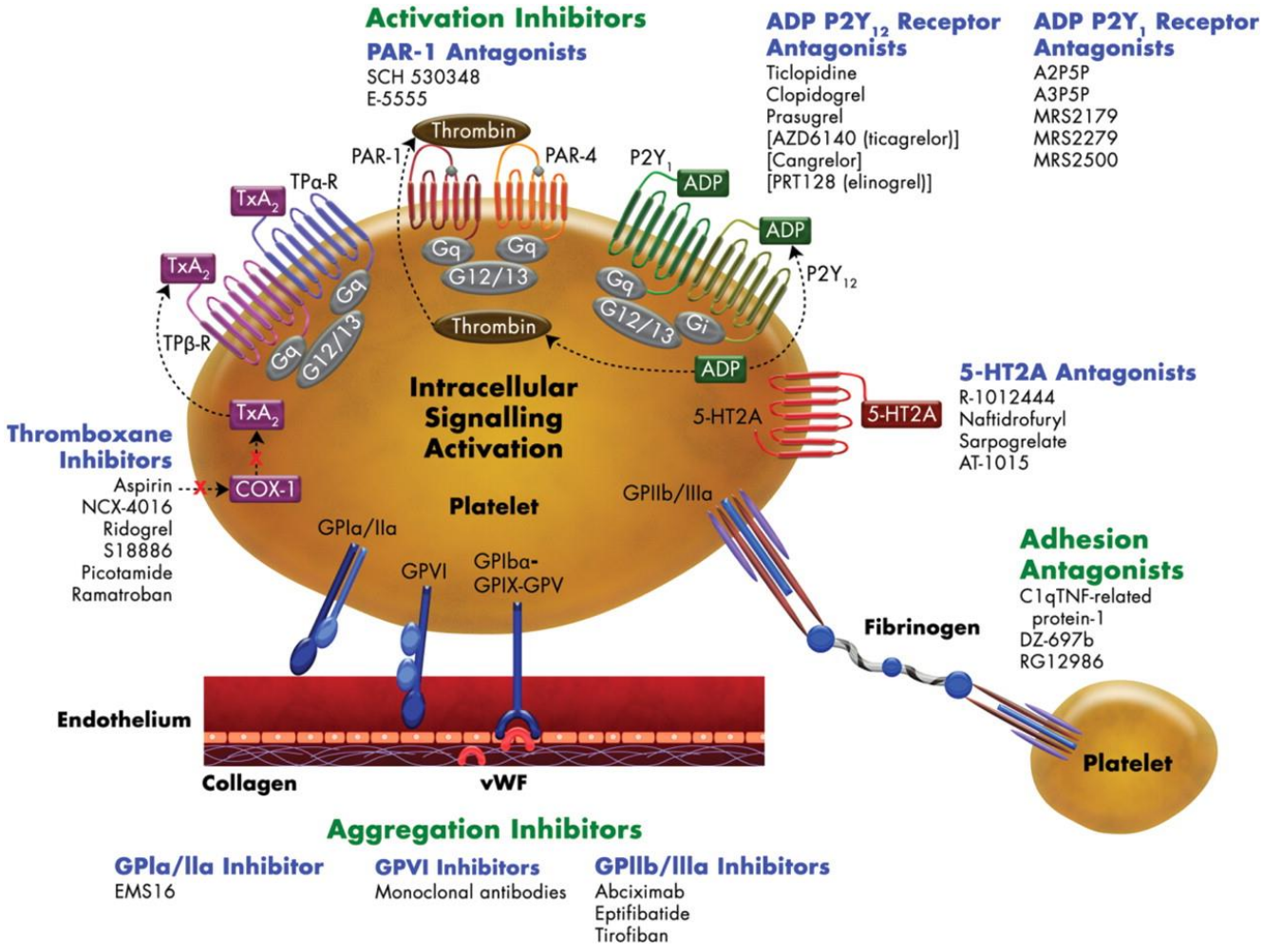
- change or failure in any of these factors (or a combination disorder) results in a failure
- physiological blood clotting (= hemostasis)
- pathological blood clotting (= thrombosis)
- increase the risk
- Spontaneous

Endothelium



- endothelium normally prevents haemostasis by secretion of platelet aggregation inhibitors and coagulation
  - NO
  - prostacyclin
  - thrombomodulin
  - heparan-sulfate
  - tPA
- when the endothelium is damaged, platelets adhere to vWf expressed on the exposed subendothelium through their receptors (GPIb-IX)
- platelets are activated and their mediators are released from the granules
  - thromboxane, PAF, ADP, serotonin → activation of other platelets (aggregation), vasoconstriction
  - Integrins expression (GPIIb/IIIa) → binding of fibrin and formation of a definitive plug
- thrombocytes also involved in the activation of secondary hemostasis

Platelet



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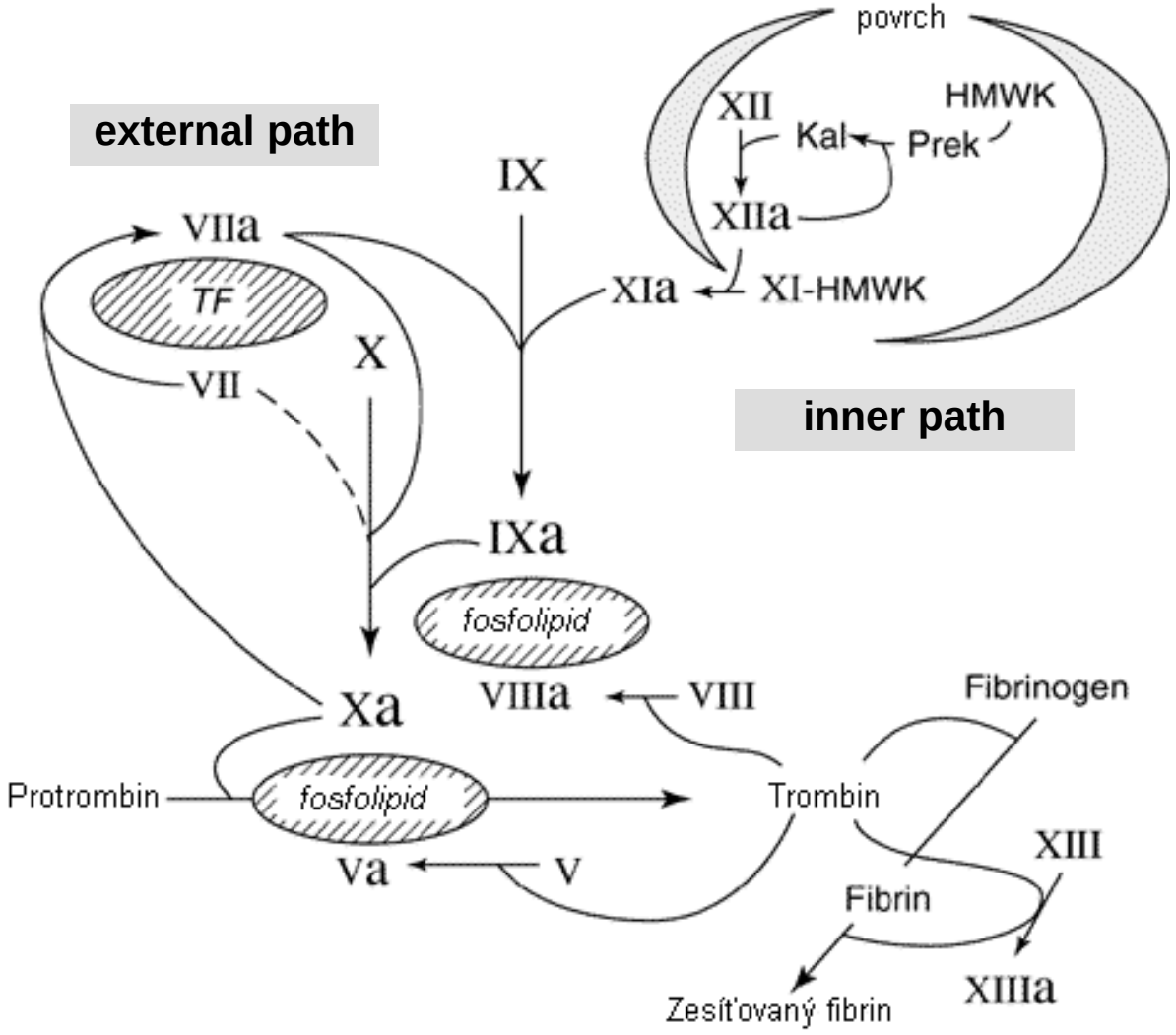
Secondary haemostasis



- 2 types of activation:
- inner patway
- occurs after HMWK contact, factors XII and XI with a negatively charged surface, e.g.
- naked collagen in the sub-endothelial layer of blood vessels
- lipoproteins (chylomikrons, VLDL)
- wall of bacteria
- external pathway
- Tissue factor (TF, fIII) released from destroid cells – f VII co-activator

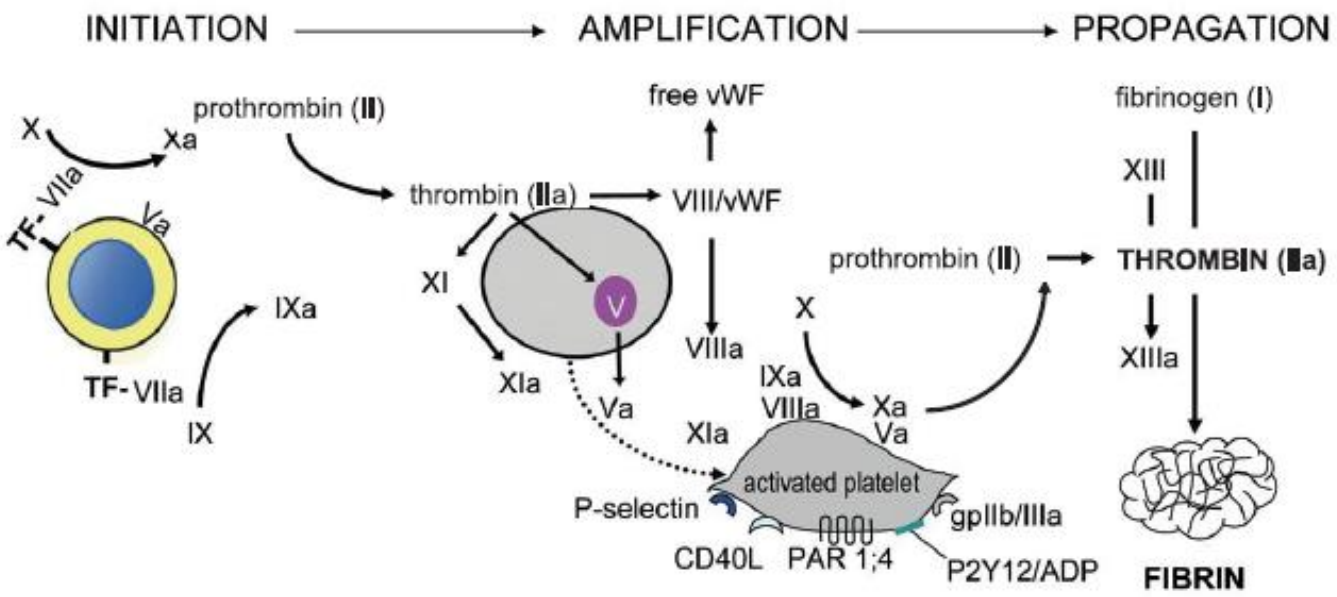
Limitations of cascade/waterfall model

- good for describing the coagulation process *in vitro*
  - selectively activated intrinsic or extrinsic pathways
- Several clinical observations:
  - Factor XII deficiency
    - do not suffer from bleeding in spite of the requirement of this factor for initiating the intrinsic pathway
    - prolonged
  - Deficiency of high molecular weight kininogen and pre-kallikrein
    - do not lead to a clinical bleeding tendency
  - Factor IX or factor VIII deficiency
    - severe bleeding even though extrinsic and common pathways are normal and should be sufficient to promote clotting
  - Deficiency of factor VII
    - also causes bleeding even though the intrinsic pathway is intact.

Cell based model

- Central point
  - Formation of thrombin from prothrombin
- Further reactions precedes
- Three phases
  - interconected
  - overlaped

- Initiation phase
- Amplification phase
- Propagation phase



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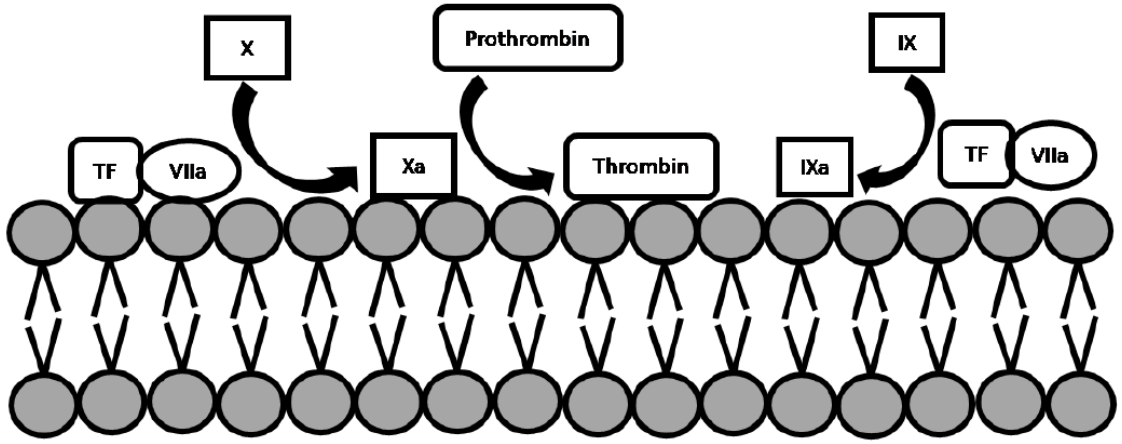
Initiation phase

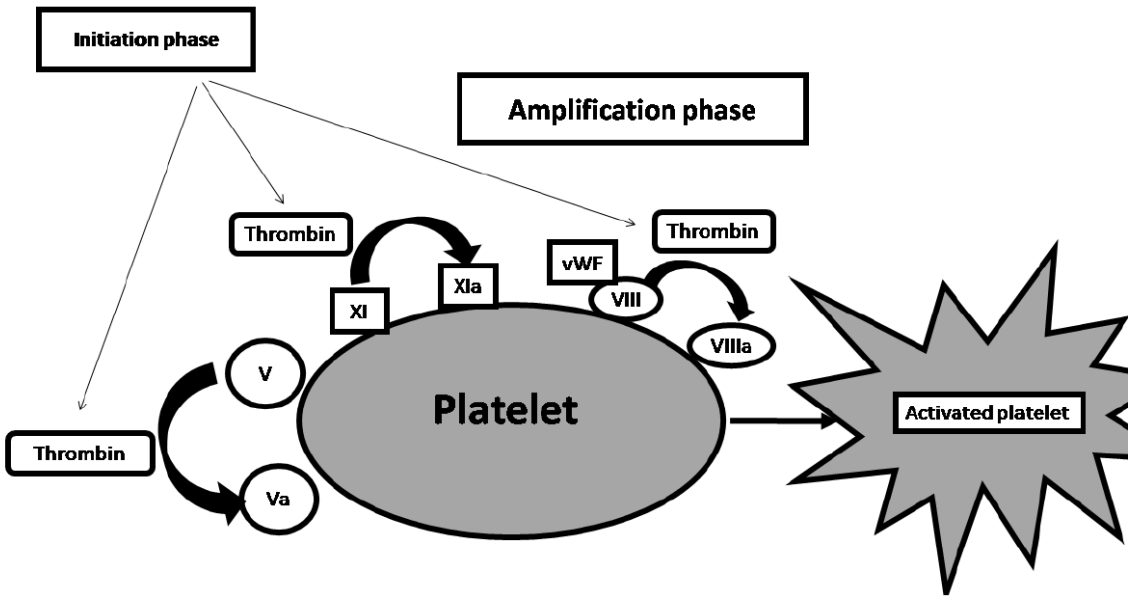
- TF – expressed only after damage
  - Subendothelial
    - Smooth muscle cells, fibroblasts, macrophages, endothelial cells
  - In circulation
    - Platelets – small amount
- On the surface of cells carrying TF
  - Contact with factor VII, activation of complex TF/VIIa
  - activation of factors X a IX
  - Conversion of prothrombin to thromb
- At this stage, only a small amount of thrombin is produced
  - Inhibitory factors
    - TFPI – inhibitory complex TFPI/Xa
    - Antitrombin III

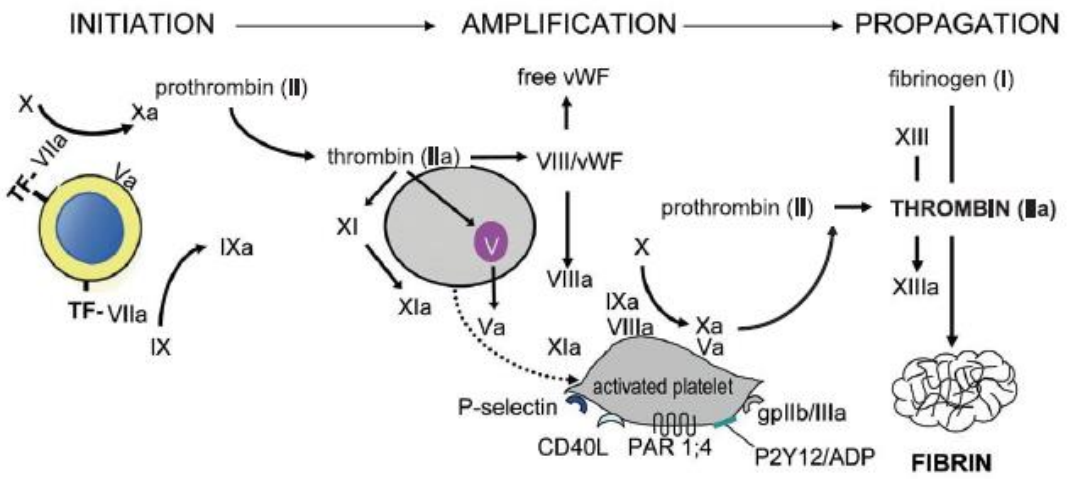


Amplification phase

- Takes place on the surface of the platelets
  - Plates adhere to vascular and extravascular structures
    - Von Willebrand factor

1) TF activate platelets
   - Activation includes
     - Irregular shape, pseudopodia (surface magnification)
     - Expression of receptors and binding sites
     - Release of serotonin, Ca, ADP, factor V, fibrinogen, PDGF, vWF
2) Activation of factor V, VIII and XI
   - Factor V is activated on the platelet surface with thrombin from the initiation phase
   - Thrombin activates factor VIII on platelets
     - vWF separates from the complex and allows for further adhesion
     - Factor VIII remains on the surface of platelets
   - Thrombin activates also factor XI





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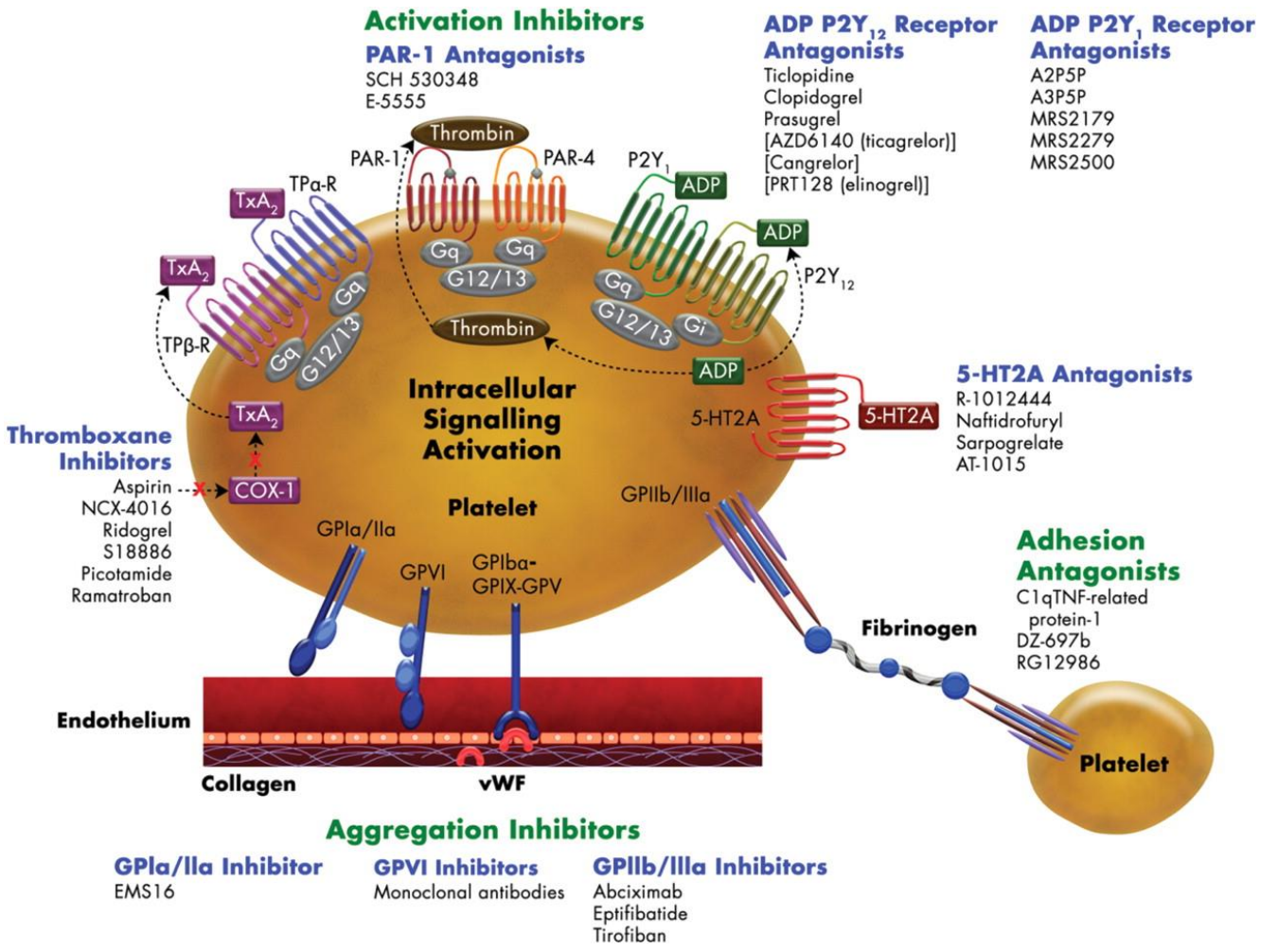
Platelet



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Platelets

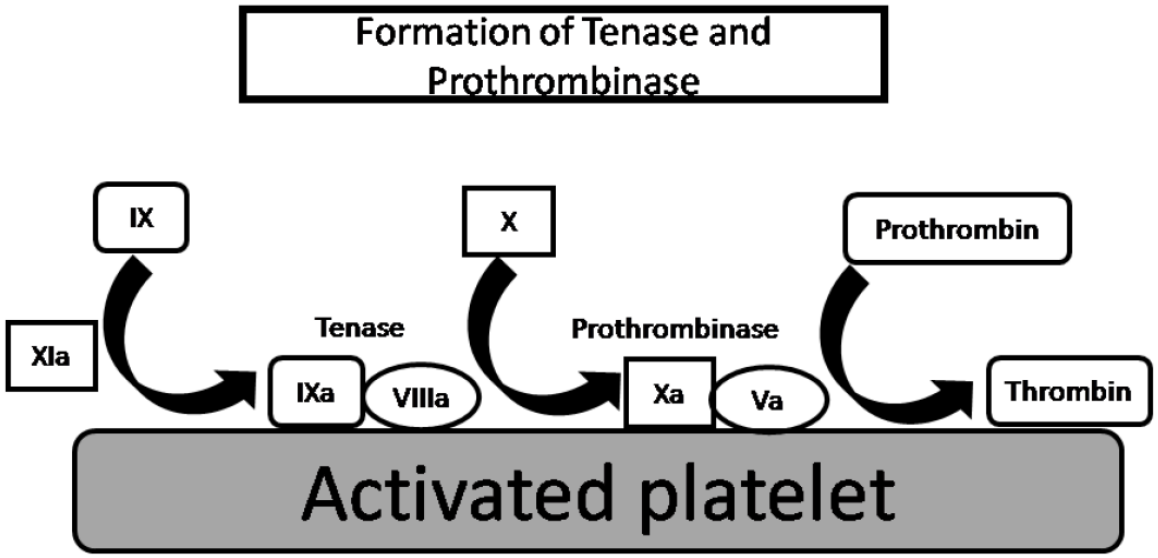
- Express **glycoprotein receptors** on membranes. Gp Ib,IIb/IIIa
- Have three types of granules
  - **Alpha granules**
    - Fibrinogen, fibronectin, factor V and VIII, PDGF, TGFb
  - **Dense bodies or delta granules**
    - ATP/ADP, ionized calcium, histamine, serotonin, epinephrine
  - **Lysosomal granules**

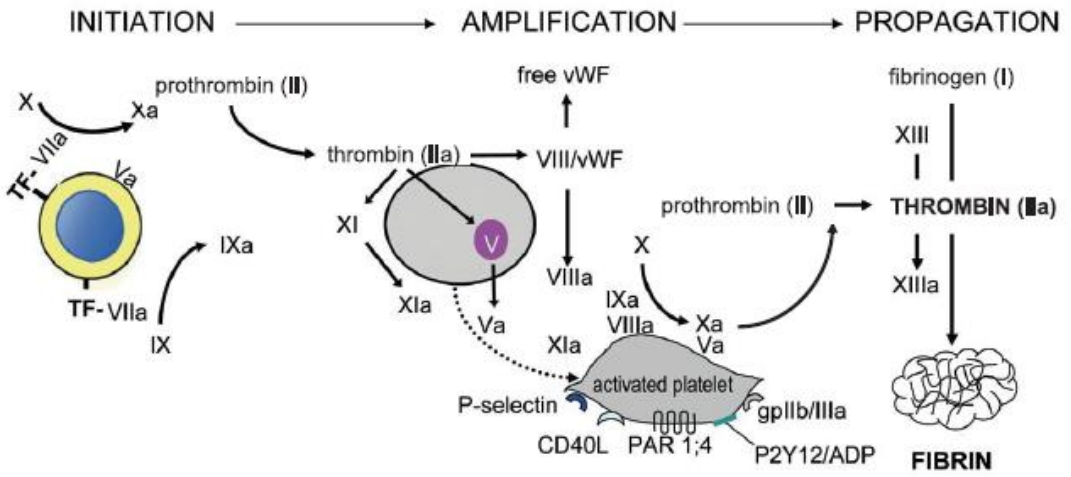
Amplification phase II

3) Tenase complex
   - Activated factor IXa binds to VIIIa – tenase complex (VIIIa/Ixa)
   - Converts factor X to active Xa
4) Prothrombinase complex
   - Activated factor Xa forms a complex with Va
5) Thrombin formation

   Prothrombinase converts prothrombin to large amounts of thrombin

   300,000 more effective than factor Xa





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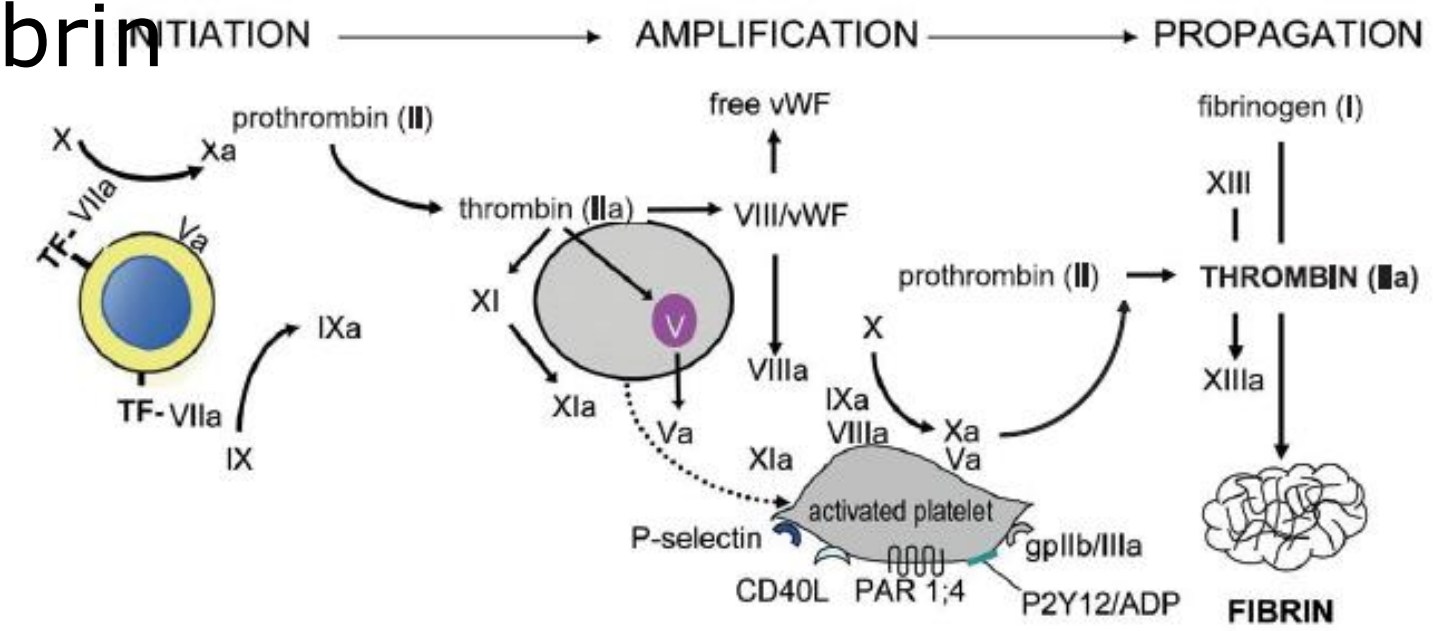
Propagation phase

- Further production of thrombin
- Thrombin converts fibrinogen to fibrin
- Creation of a stable fibrin network
  - Participation of factor XIIIa



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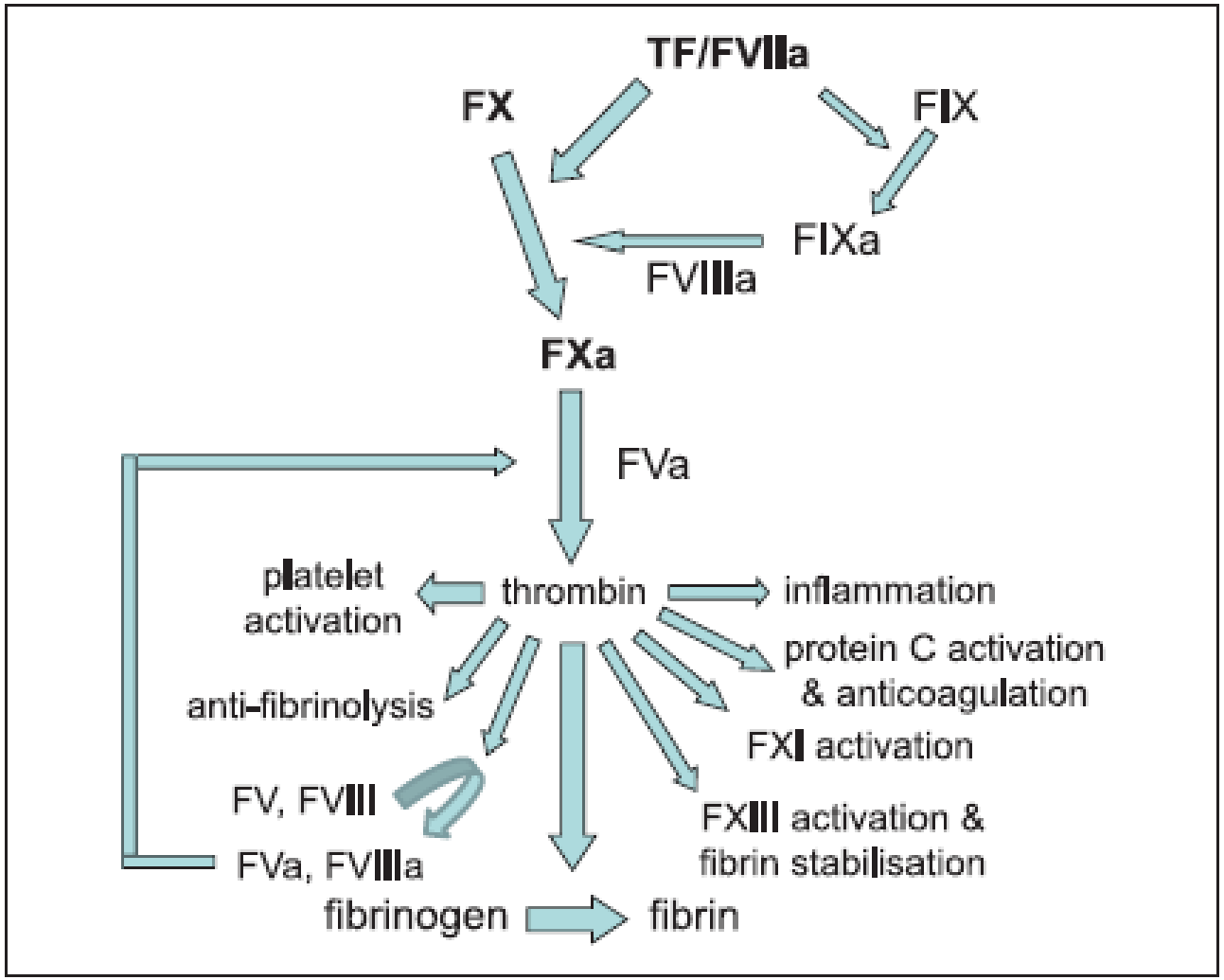
Thrombin function



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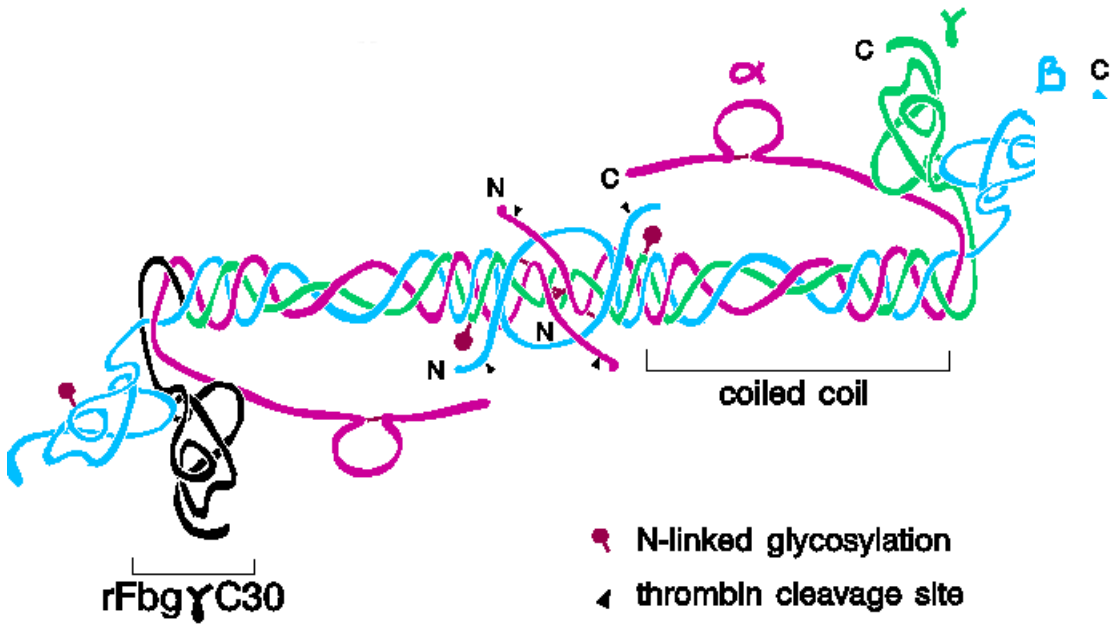
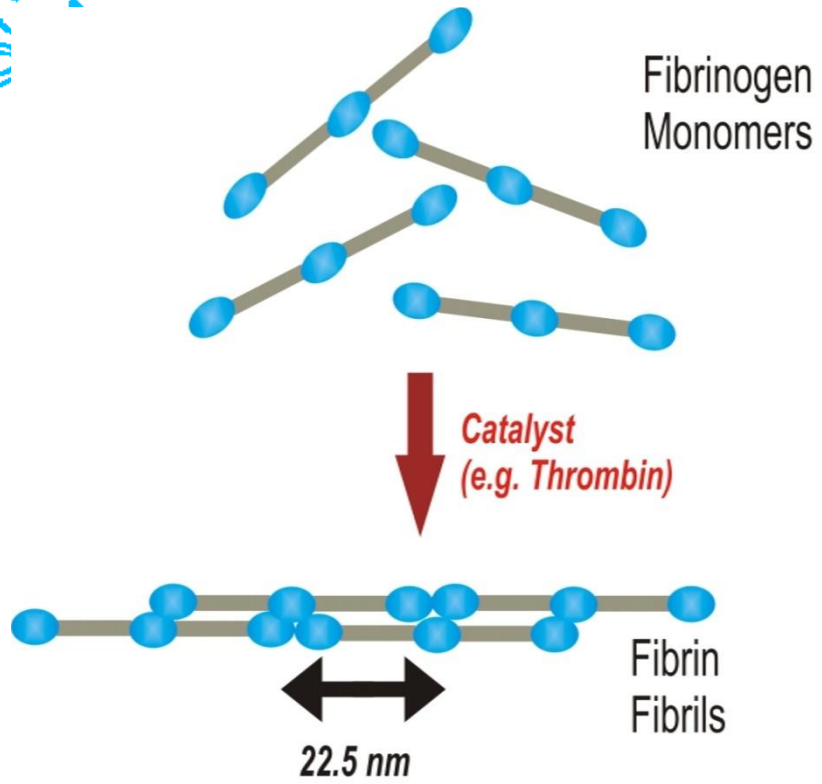
Fibrinogen - fibrin

- 3 pairs of polypeptides ([A-α][B-β][γ])2 – 340kDa
-

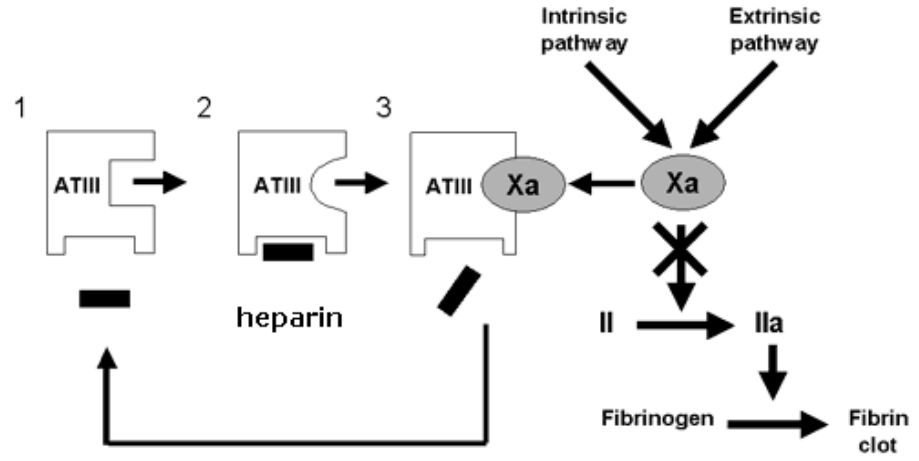


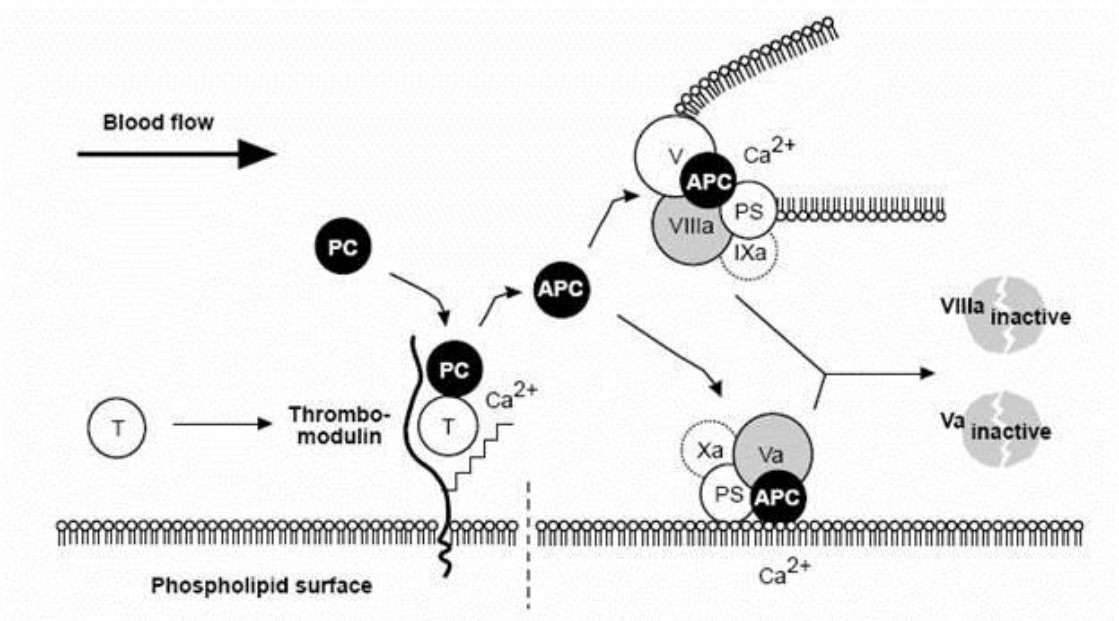


- thrombin (serine protease) breaks down fibrinopeptides A and B and generates fibrin monomers (α-β-γ)2
- monomers spontaneously aggregate and create a fibrin network
- thrombin also activates f XIII (transglutaminase), which forms transverse links between fibrin polymers

Blood clotting control mechanisms

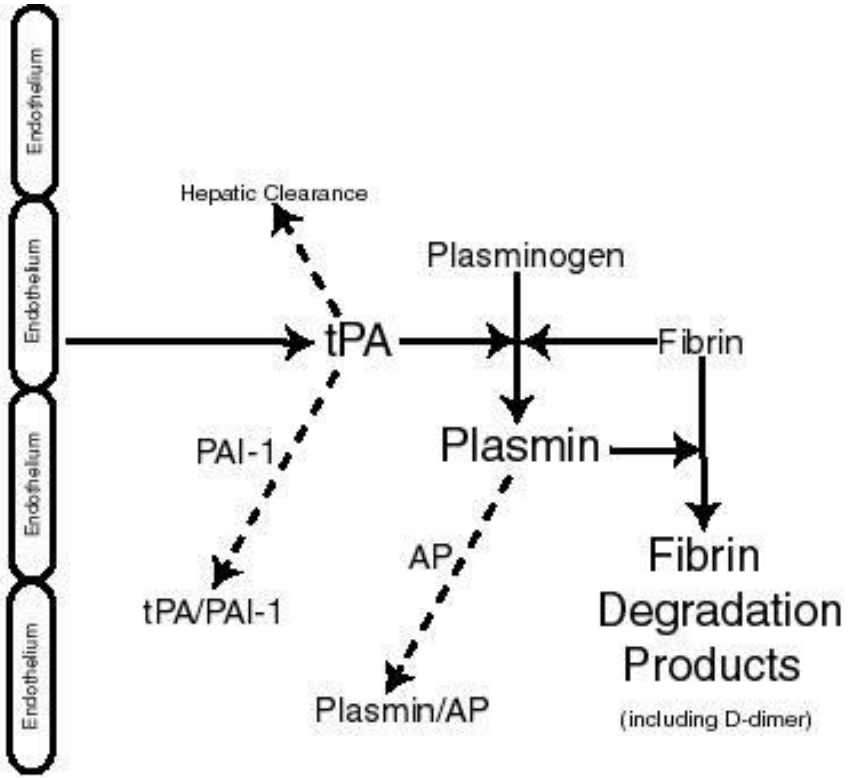
- blood flow rate
- concentration of inhibitory factors
  - (1) thrombin level control
    - antithrombin III (and heparan-sulfate)
      - ☛Inhibition of fVII, X, XI a XII
    - a2-makroglobulin
    - heparin cofactor II
    - a1-antitrypsin
  - (2) control at factor Xa level
    - protein C + thrombomodulin
    - protein S
- activity of fibrinolysis



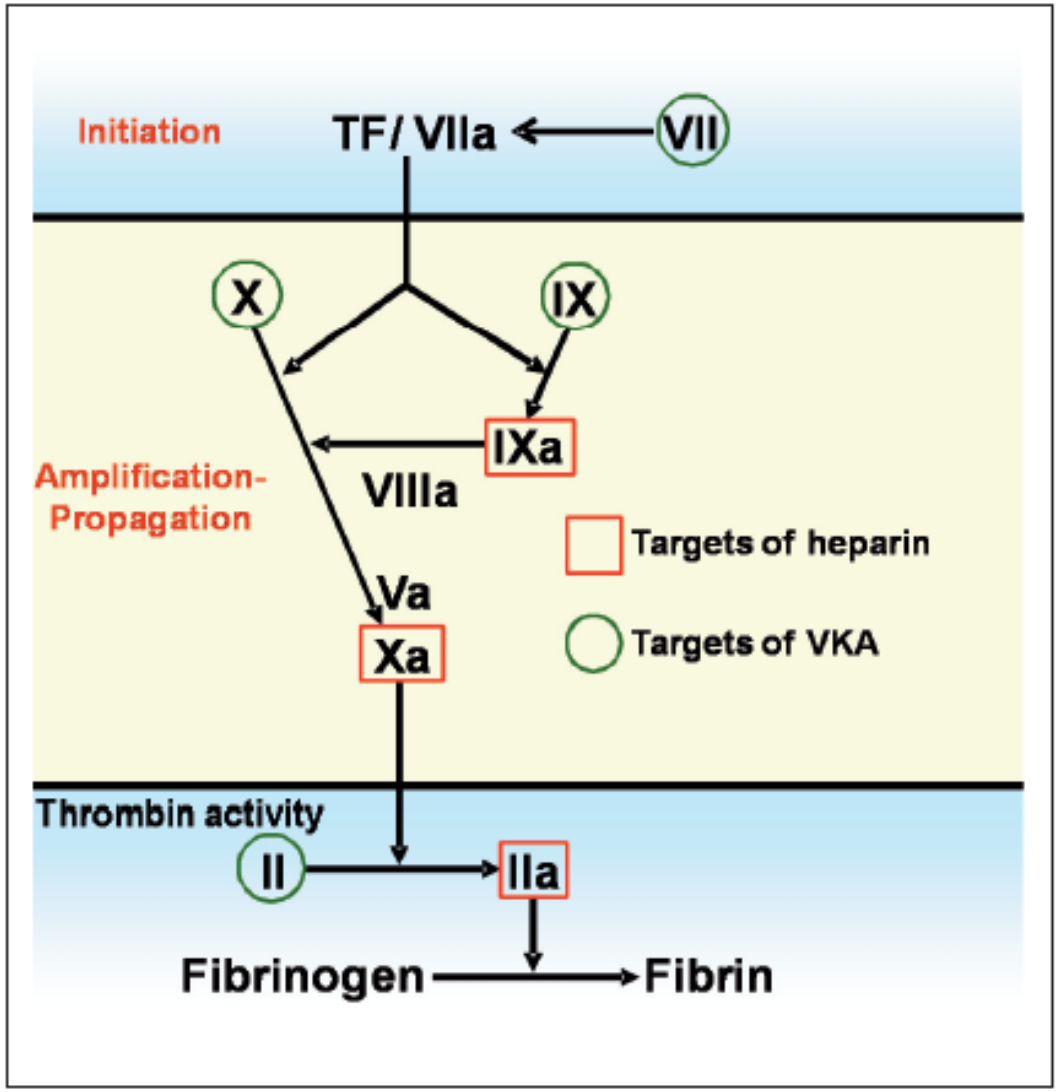


Fibrinolytic system

- plasmin (serine protease) circulates as an inactive proenzyme (plasminogen)
  - free plasmin rapidly inhibited α2-antiplasmin
- Activation of plasminogen by tPA (endothelial cells) and urokinase (epithelial cells) to plasmin
- degradation of fibrin to degradation products
- activity of tPA inhibited by PAI-1



Heparin vs. Warfarin



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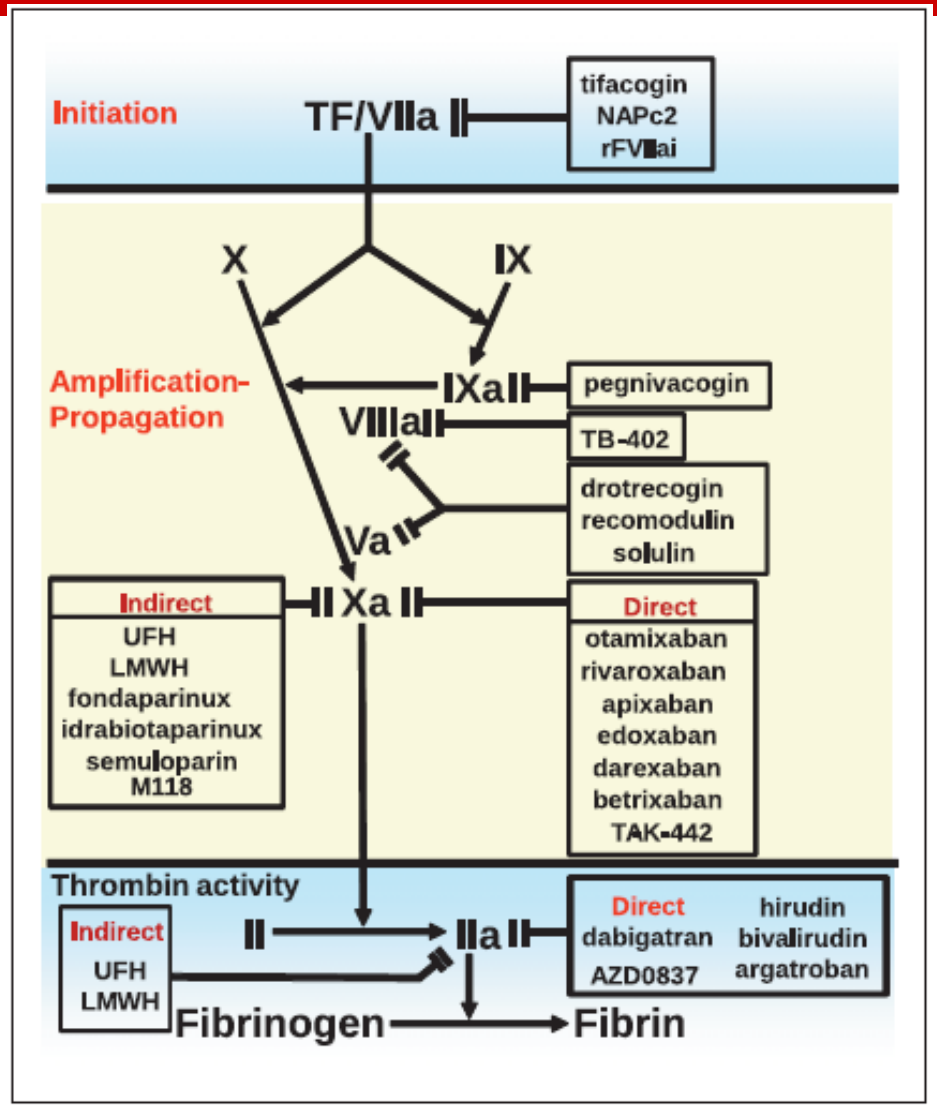
Future



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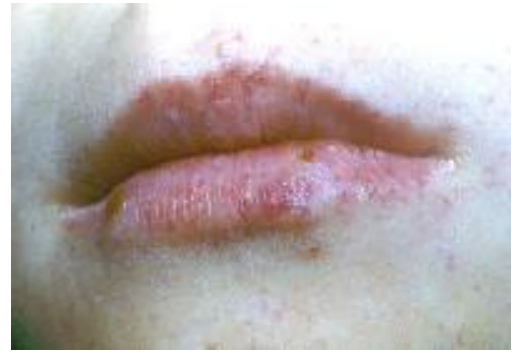
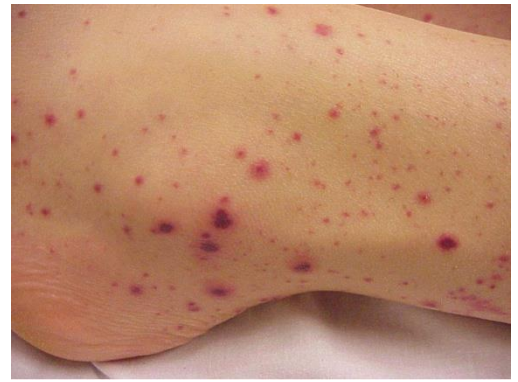
Blood clotting disorders

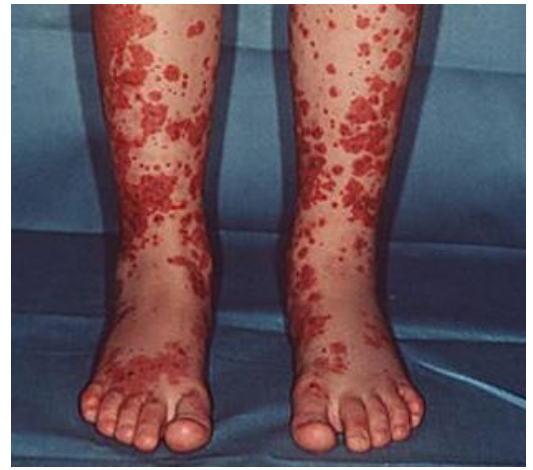
- (A) hypocoagulation conditions (bleeding diathesis)
  - primary hemostasis defect
    - vascular wall disorders (senile purpura)
    - thrombocytopenia and thrombocytopathies
    - von Willebrand disease
  - coagulopathies
    - hemophilia A and B
    - Chronic liver disease
- (B) hypercoagulation states (thrombophilia)
  - congenital
    - activated protein C resistance (APCR)
  - acquired
- (C) combined
  - Syndrome of disseminative intravascular coagulation (DIC)

Primary haemostasis defects

- symptoms: petechiae, purpura, epistaxis, bleeding from the gums or git, hematuria, menorrhagia
- (1) vascular wall disorders (vasculopathy)
  - congenital
    - telengiectasia hereditaria (m. Rendu-Osler)
      - ☛ AD, weakening of the walls of the vessels → telengiectasia (skin, mucosa, lungs, urogenital tract)
    - Ehlers-Danlos and Marphan syndrome
      - ☛ Defect of connective tissue structure (collagen)
  - acquired
    - senile purpura
    - bacterial toxins (scarlet fever, measles)
    - deficit of vit. C (scorbut)
    - immunocomplex (Henoch-Schönlein purpura)
- (2) thrombocytopenia
- (3) thrombocytopathies
- (4) von Willebrand disease

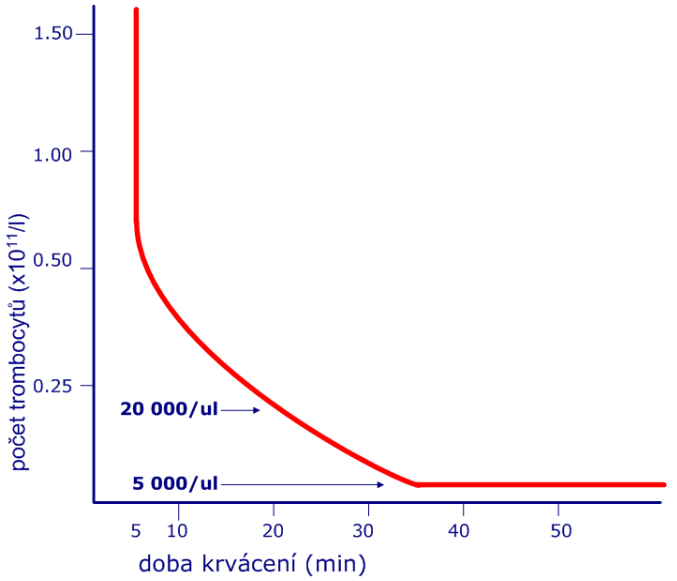


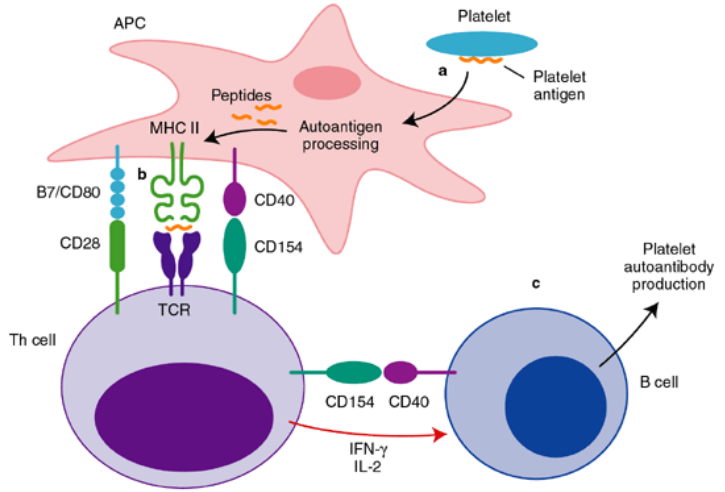




Thrombocytopenia a thrombocytopaties

- Platelet count 150 – 400 000/μl ($1.5–4\times10^{11}$/l)
- In circulation survival approx. 8-10 days
- (A) thrombocytopenia = reduction in number
  - <50 000/μl – increased risk of bleeding
  - <20 000/μl – significant risk
  - <5 000/μl – extremely high risk
  - Primary or secondary
  - Etiology
    - Reduced production
      - aplastic anemia (e.g., Fanconi's)
      - myelodysplastic syndrome
      - Myelofibrosis
      - Hereditary (e.g., May-Hegglin, Wiscott-Aldrich, Bernard-Soulier)
      - Acute leukemia
    - destruction
      - autoimmune - idiopathic thrombocytopenic purpura (ITP)
      - drugs
      - hypersplenism
    - Increased consumption
      - DIC
      - thrombotic thrombocytopenic purpura (TTP)
- (B) thrombocytopathies = impaired function
  - Aggregation and adhesion defects
    - Bernard-Soulier syndrome (disorder of receptor GPIb-IX)
    - Glanzmann thrombastenie (disorder of receptor GPIIb-IIIa)
  - Degradation disorders
    - Heřmanského-Pudlákův syndrome
    - Chédiak-Higashiho syndrome





Model of platelet autoantibody production in chronic idiopathic thrombocytopenic purpura (ITP)

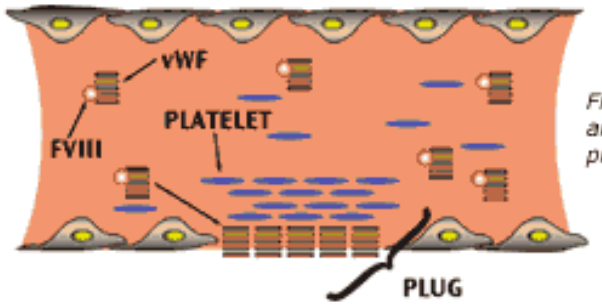
About Thrombotic Thrombocytopeneic Purpura (TTP)

- Disorder of systemic platelet aggregation in microvasculature
- Stimulus: unusually large vWf
- In children: likely to be deficiency in vWf metalloproteinase to break down vWf
- In adults: vWf metalloproteinase inhibited by autoantibodies
- Low PLT count, intravascular hemolysis, RBC fragmentation, high LDH

von Willebrand's disease

- the most common congenital coagulation disorder
- group of states leading to a reduction in plasma vWf level
  - thrombocyte adhesion disorder
  - vWf is also plasma carrier of fVIII (without vWf is unstable and rapidly degraded) → (i.e. secondary hemostasis)
- several types of vW disease
  - type 1 (~75%) – reduction of concentration in Wf
  - type 2 (~20%) – normal concentration of malfunctioning vWf
    - plate binding failure (type 2A)
    - disorder of binding to collagen subendothelial layer (type 2B)
    - fVIII transport failure (type 2N)
  - type 3 – absolute deficiency of vWf (homozygots)

Figure 1. Normal Broken Blood Vessel



First, vWF proteins from the blood line up along the broken vessel wall and attract "sticky" platelets to form a plug.

Then the platelets attract strands of fibrin to strengthen the plug and form a clot. The clot helps stop the bleeding.

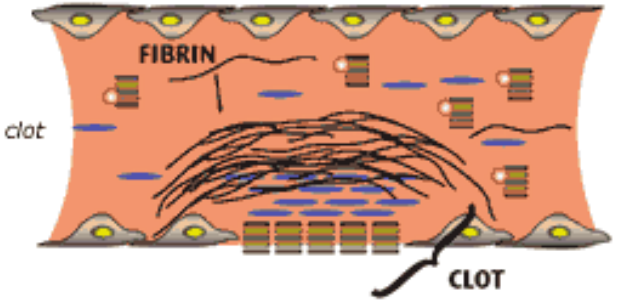
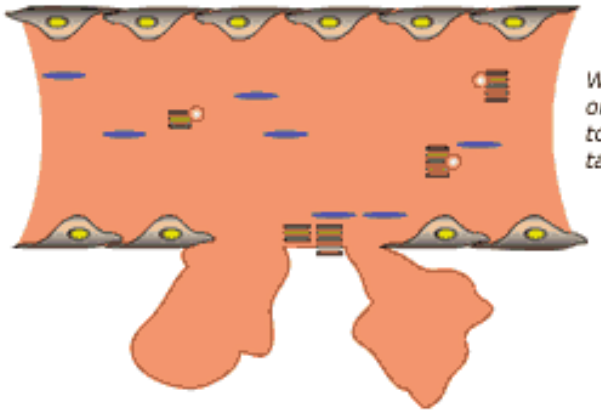


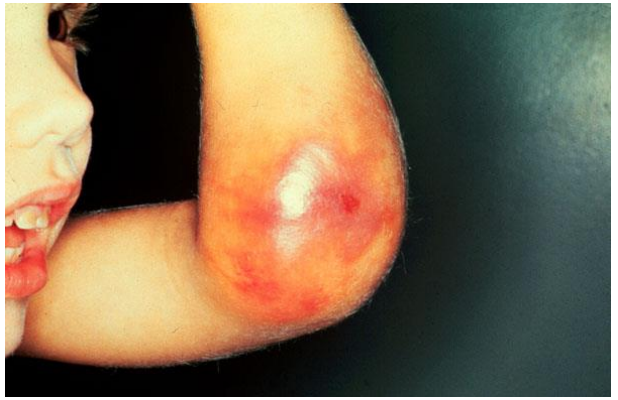
Figure 2. Broken Blood Vessel in vWD

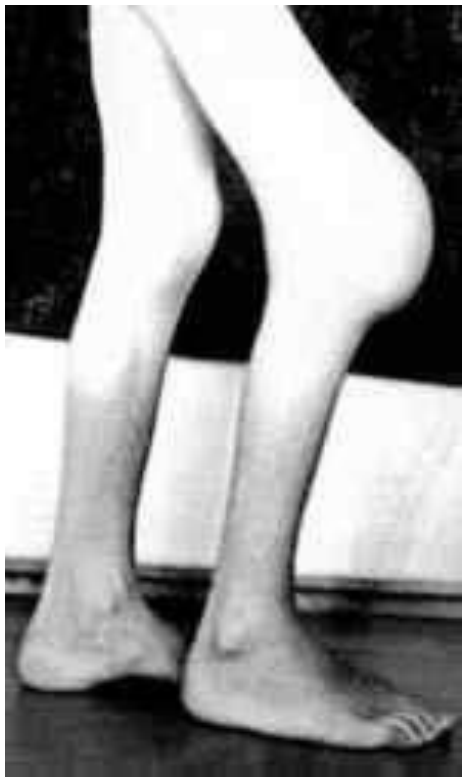


When a person has vWD, there isn't enough vWF or the vWF is damaged. The clot may take longer to form or not form properly, and bleeding make take longer to stop.

Defects of "secondary hemostasis"

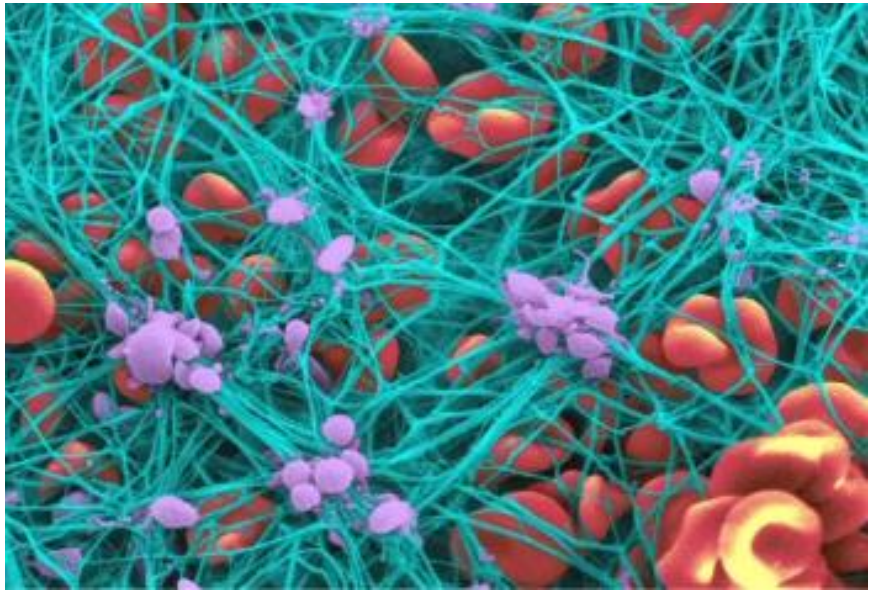
- typical tissue hemorrhage (hematomas), e.g. joints, muscles, brain, retroperitoneum, no petechiae and purpuras
- (A) congenital disorders
  - hemophilia A (Xq-linked) – defect of fVIII
    - fVIII is a cofactor of fX activation to fXa in response to catalyzed fIXa
    - reduction of concentration up to 25% of normal does not cause coagulation disorder, decrease to 25-1% mild form, <1% severe form
    - >150 point mutations in the fVIII gene – large phenotypic variability!!!
    - prevalence in the male population from 1:5,000 to 1:10,000
  - hemophilia B (Xq-linked) – defect of fIX
    - prevalence 10 times less than hemophilia A
    - >300 point mutations in the fIX gene (85% point, 3% short deletion and 12% extensive deletion)
    - defects of other factors
    - rare, mostly autosomal recessive, clinically manifest disorders only in severe deficiency
    -
      - afibrinogenemia (defect of fI)
      - hemophilia C (defect of fXI) – Ashkenazy Jews
      - Other
- (B) acquired disorders
  - hepatic insufficiency/failure
  - vitamin K deficiency (disorder of fat resorption in the intestine)
  - DIC





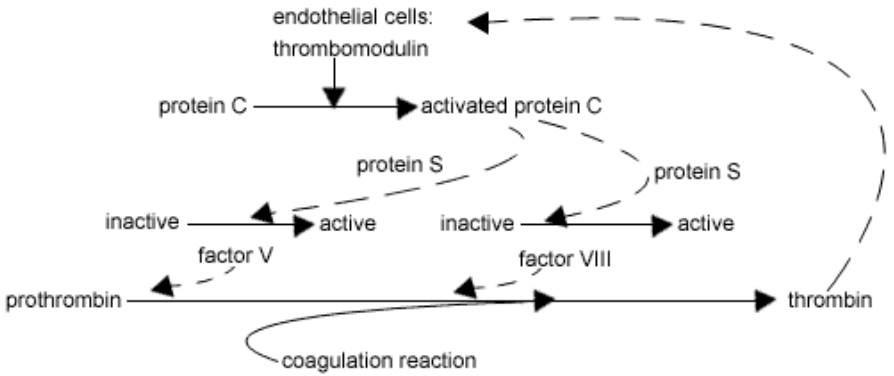
DIC (consumptive coagulopathy)

- initially excessive coagulation (thrombotic condition), then depletion of the coagulation factors (bleeding state)
- coagulation in DIC is locally unlimited and is not primarily a reaction to vessel damage
- pathogenesis
  - TF is not normally present in circulation!!!
    - endothelium or blood cells do not produce TF on their surface
    - in some pathologies TF occurs and activates factor VII
    - TF pathological resources
      - cells of other tissues – e.g. fetus cells during childbirth, extensive injuries, soaking of tumor cells during surgery, etc.
      - pathological blood elements expressing TF – e.g. in myelo- and lymphoproliferative diseases
      - pathologically activated endothelia and monocytes that begin to express TF in the membrane – e.g. endotoxin in sepsis
      - TF from erythrocyte cytoplasm released under hemolysis
- Consequences
  - Stage 1 - formation of microthrombi in microcirculation
  - ischemia to gangrene
  - Stage 2 - hypo- to afibrinogenesis,
    - bleeding into the organs
  - pathologically escalated fibrinolysis



Hypercoagulation conditions

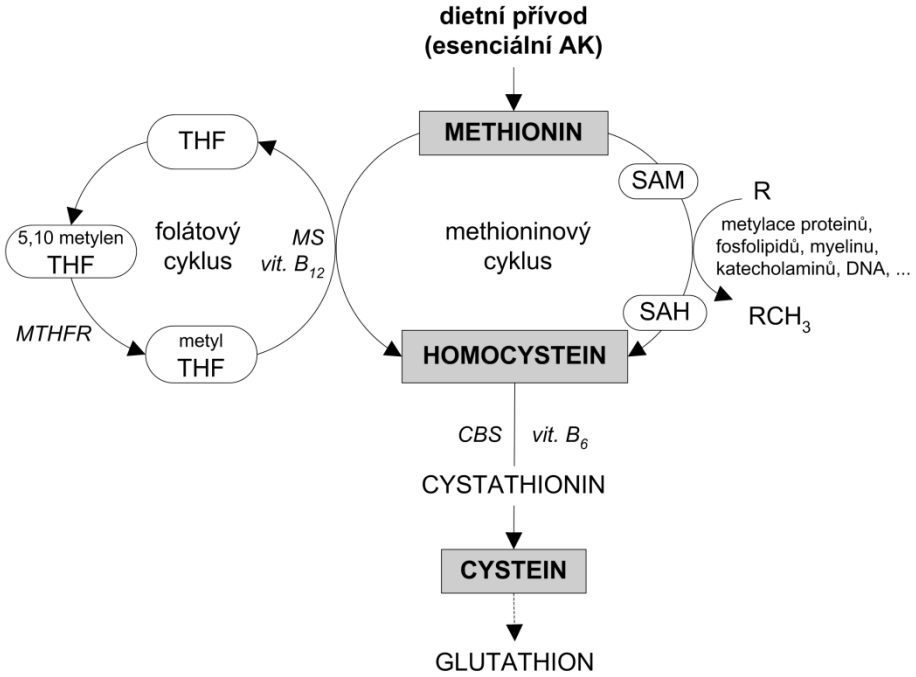
- lead to an increase in risk or even spontaneous and often repeated venous thrombosis and thromboembolism (to the lungs most often), or complications of pregnancy and infertility
- (A) congenital thrombophilia
  - (1) Disorders of the formation of clotting inhibitors
    - Defect of AT III (AR)
    - Defect of protein C and S (AD)
    - syndrome of fV resistance to activated protein C (APCR)
      - most common congenital disorder ("Leiden" mutation of fV)
    - mutation of the prothrombin gene (promotor → quantitative effect)
    - hyperhomocysteinemia (mutations in the gene for MTHFR)
    - antiphospholipid syndrome
      - Anti-cardiolipin antibodies, lupus anticoagulant, …
      - unclear pathophysiology
  - (2) fibrinolysis disorders
    - ↑LP(a)
    - ↑ PAI-1 (promotor → quantitative effect)
- (B) acquired thrombophilia
  - (1) clinical situations and complications of treatment
    - immobilization
    - hyperestrogenic conditions (pregnancy, oral contraceptives, HRT)
  - (2) Pathologies
    - Atherosclerosis
    - Obesity (↑ PAI-1)
    - Hyperviscose syndrome
      - polycytemia vera, thrombocytemia, sec. polyglobulia, gamapathy)
    - tumors
    - heart failure
    - hyperlipidemia, nephrot. syndrome
    - venous insufficiency



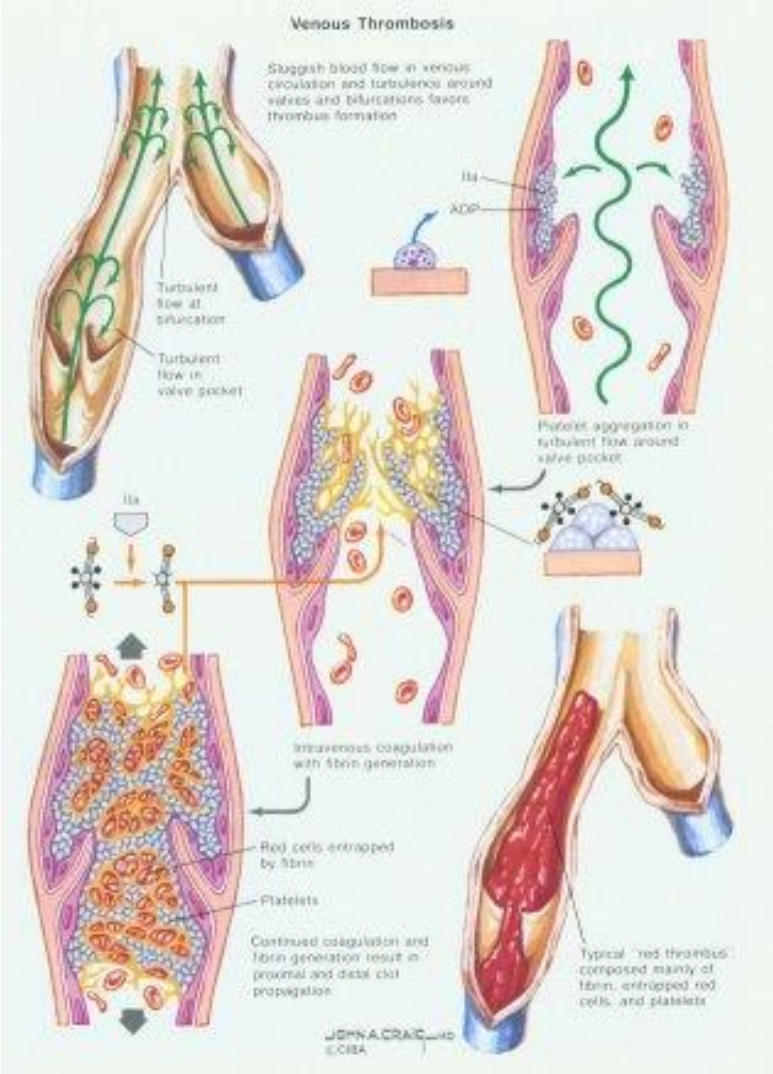
Hyperhomocysteinemia

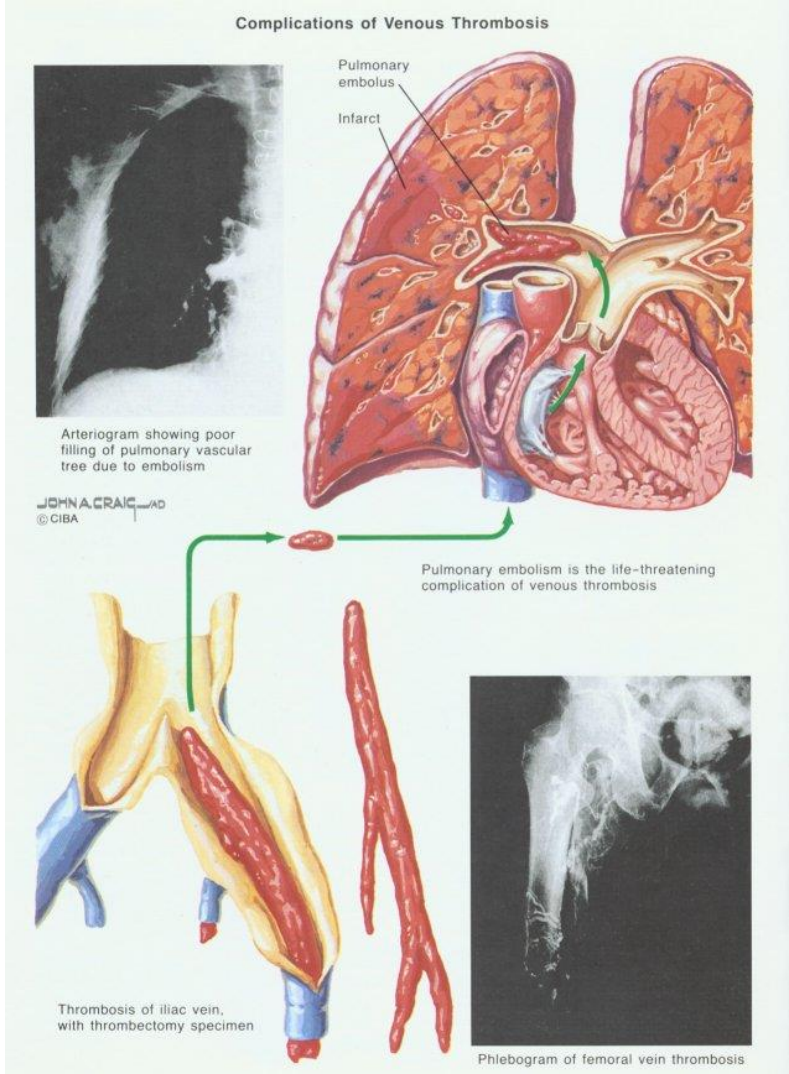
- homocysteine is an intermediate product of the transformation of methionine in the methionine cycle
  - is either further metabolized to cysteine
  - remethylated back to methionine (in the folate cycle)

- the presence of several enzymes and their cofactors (vitamins of group B, folic acid)
- the reason for the metabolism of homocysteine and subsequent HHcy may be the genetic and nutritional factors
  - mutations in enzyme-coding genes
  - decreased intake of vitamin B6, B12 and folic acid
- HHcy =pathological increase in plasma homocysteine concentrations
- HHcy is an independent risk factor for atherosclerosis and thromboembolism, fertility disorders and certain developmental and neurological abnormalities (cleft spine defects)
- homocysteine causes endothelial dysfunction and initiates apoptosis
- (A) monogenic homocystinuria
  - cystathionin-β-synthase deficiency leads to a significant elevation of plasma Hc
  - relatively rare disease
- (B) „mild hyperhomocysteinemia"
  - polymorphism in the methylenetetrahydrofolatereductase gene (MTHFR)



Deep vein thrombosis and subsequent pulmonary embolism





Understanding tests

- By mixing the examined plasma, tissue thromboplastin and calcium ions, the outer part of the coagulation cascade is triggered.
- The cascade ends with the formation of a fibrin clot.
- The result of the test is the time from mixing the mentioned substances to the formation of a clot.

Activated partial thromboplastin time (APTT)

- information on the functionality of the inner part of the coagulation cascade.
- Unlike Quick's coagulation cascade test, kaolin (Activator) triggers a negatively charged surface of an injured vessel.
- For some reactions of the inner part of the coagulation cascade (especially to activate factor X), the presence of phospholipid - platelet factor 3 (PF3) from activated platelets is required.
- The plasma used in the APTT test does not contain platelets, phospholipid should be added to the reaction. Instead of PF3, tissue phospholipid kefalin (Partial Thromboplastin) is used.
-

APTT test value

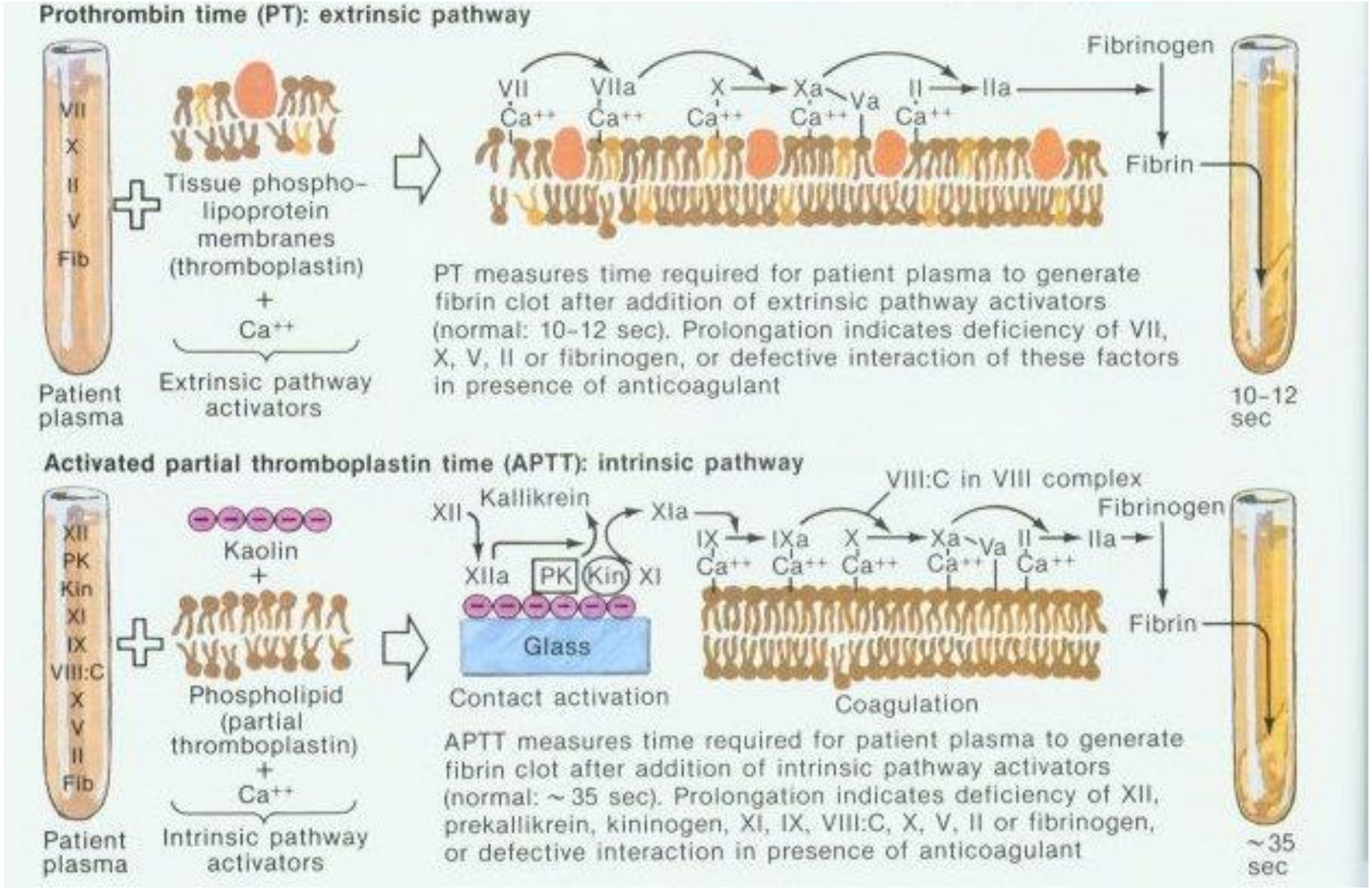
- indicates the time from the start of the coagulation cascade with calcium ions to the formation of a fibrin clot.
- Normal values range between 25-39 s.
- For example, APTT prolongation is due to
  - lack of coagulation factors of the inner part of the cascade (mainly VIII and IX),
  - heparinem therapies,
  - significant overdose of warfarin.
- In heparinized patients, the recommended APTT is 1.5 to 2.4 times the norm.

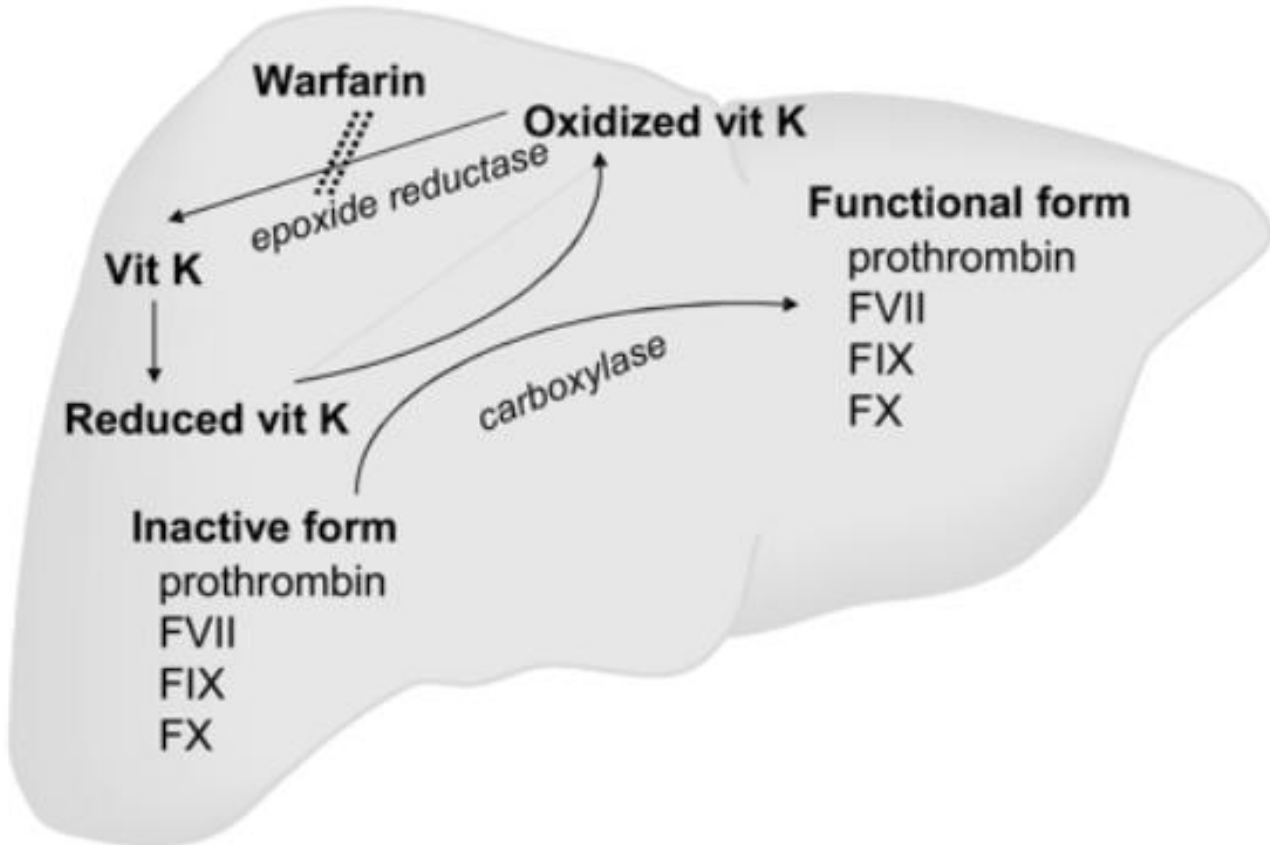
Quick test (prothrombin time, PT)

- the rate of conversion of prothrombin into thrombin by the action of tissue thromboplastin.
- Tissue thromboplastin consists of a lipoprotein component (so-called tissue factor) and a phospholipid component (also formed in tissues).
- This test determines the activity of the so-called prothrombin complex and functionality of the outer part of the coagulation cascade.

Most often used in testing the effectiveness of anticoagulant treatment of vitamin K antagonists (warfarin).

Tests


Prothrombin time (PT): extrinsic pathway
VII
X
II
V
Fib
Patient plasma
Tissue phospho-lipoprotein membranes (thromboplastin)
+
Ca++
Extrinsic pathway activators
VII Ca++
VIIa Ca++
X Ca++
Xa Ca++
Va
II Ca++
IIa
Fibrinogen
Fibrin
PT measures time required for patient plasma to generate fibrin clot after addition of extrinsic pathway activators (normal: 10–12 sec). Prolongation indicates deficiency of VII, X, V, II or fibrinogen, or defective interaction of these factors in presence of anticoagulant
10–12 sec
Activated partial thromboplastin time (APTT): intrinsic pathway
XII
PK
Kin
XI
IX
VIII:C
X
V
II
Fib
Patient plasma
Kaolin
+
Phospholipid (partial thromboplastin)
+
Ca++
Intrinsic pathway activators
XII
Kallikrein
XIa
XIIa
PK
Kin
XI
Glass
Contact activation
VIII:C in VIII complex
IX Ca++
IXa Ca++
X Ca++
Xa Ca++
Va
II Ca++
IIa
Fibrinogen
Fibrin
Coagulation
APTT measures time required for patient plasma to generate fibrin clot after addition of intrinsic pathway activators (normal: ~35 sec). Prolongation indicates deficiency of XII, prekallikrein, kininogen, XI, IX, VIII:C, X, V, II or fibrinogen, or defective interaction in presence of anticoagulant
~35 sec


Warfarin
Oxidized vit K
epoxide reductase
Vit K
Functional form
prothrombin
FVII
FIX
FX
Reduced vit K
carboxylase
Inactive form
prothrombin
FVII
FIX
FX

P450

- CYP3A4 – 50% metabolized drugs
- CYP2D6 – 20%
- CYP2C9 + CYP2C19- 15 %
- CYP2D6, CYP2C9, CYP2C19 and CYP2A6 have been demonstrated as functionally polymorphic

  - E.g. affects the metabolism of warfarin, acenocoumarol and other drugs (phenotyoin, tolbutamide, glipizide and other oral antidiabetic drugs of the type sulphonylurea).

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Cytochrome P4502C9 (CYP2C9)

- two allelic variants of the CYP2C9 gene [1, 2]
  - CYP2C9*2
    - C430T replacement in exon 3 leads to substitution $Arg^{144}Cys$
  - CYP2C9*3
    - replacement of A1075C in exon 7 leads to substitution $Ile^{359}Leu$
- CYP2C9*1 is normal in vitro, while the CYP2C9*2 variant shows less and CYP2C9*3 has significantly less enzymatic activity [3, 4]
- phenotypical manifestation is a reduction in the clearance of CYP2C9-dependent drugs

1. Stubbins MJ et al: Pharmacogenetics 1996; 6:429-329
2. Veronese ME et al: Biochem J 1993; 289:533-8
3. Rettie AE et al: Pharmacogenetics 1994; 4:39-42
4. Haining RL et al: Arch Biochem Biophys 1996; 333:447-58

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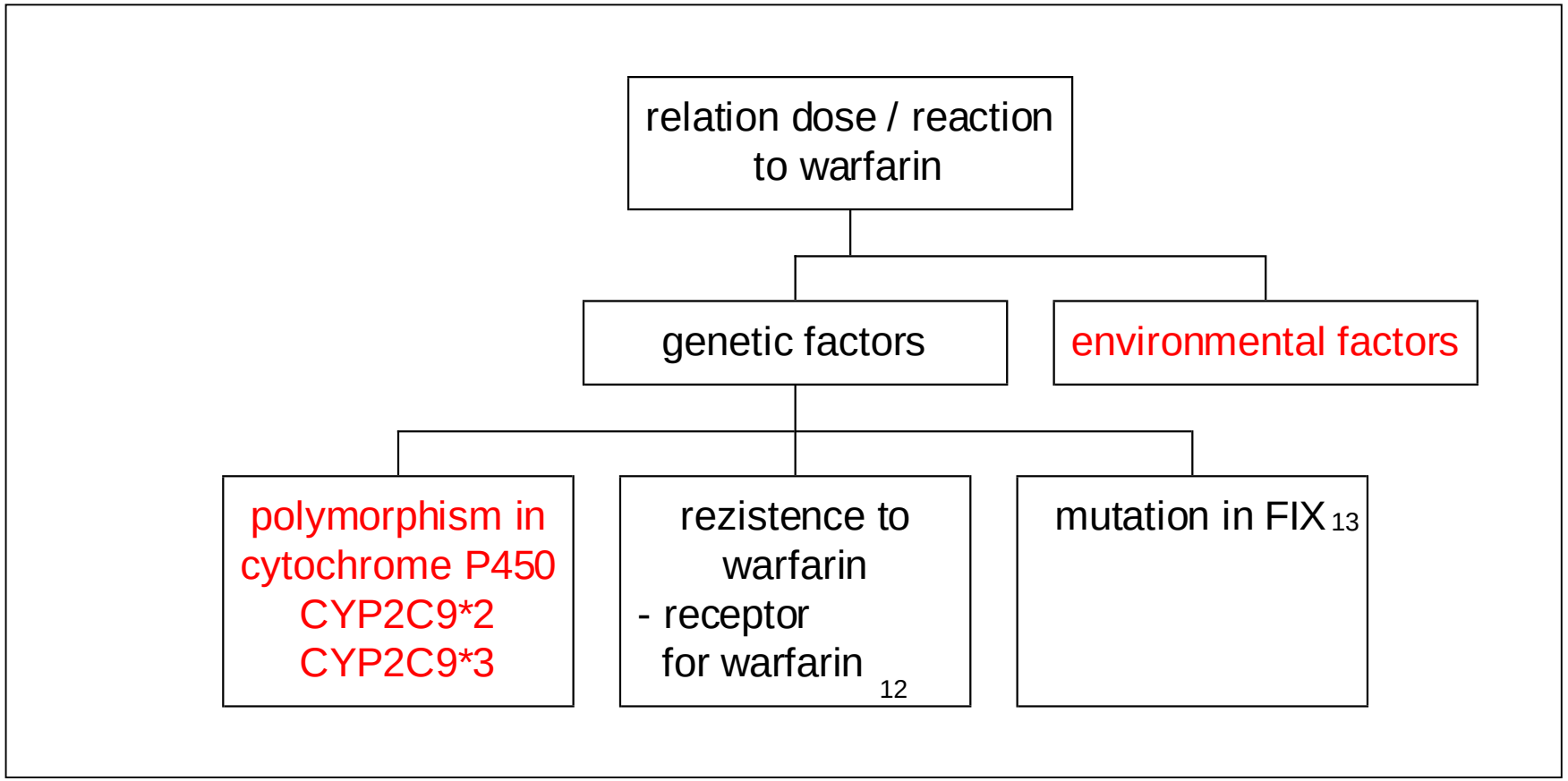
Dose/anticoagulant effect of warfarin



12. Alving BM et al: Arch Intern Med 1985; 145:499-501
13. Oldenburg J et al: Br J Hematology 1997; 98:240-4

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CYP2C9 ACTIVITY

Warfarin Dose*	Genotype
5.63 (2.56)	*1/*1
4.88 (2.57)	*1/*2
3.32 (0.94)	*1/*3
4.07 (1.48)	*2/*2
2.34 (0.35)	*2/*3
1.60 (0.81)	*3/*3

From: Higashi MK, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 287:1690-1698, 2002.

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Clinical manifestations of CYP2C9 polymorphism

- overdose at initiation of anticoagulation by standard regimens [14, 16, 17, 18, 19]
- maintenance dose required to achieve and maintain the therapeutic range of [11, 15, 16, 18, 19]
- higher risk of overdose with interactions with drugs metabolised and/or reacting with CYP2C9 [17, 21]
- instability of anticoagulant therapy [15, 16]
- prolonged anticoagulant effect after discontinuation or reduction in the dose of warfarin

14. Aithal GP et al: Lancet 1999; 353:717-9
15. Taube J et al: Blood 2000; 96:1816-9
16. Higashi HK et al: JAMA 2002; 287:1690-8
17. Verstuyft C et al: Eur J Clin Pharmacol 2003; 58:739-45

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Interaction with drugs metabolised and/or reacting with CYP2C9

17, 20, 21

Competition for substrate	Enzyme inductor	Enzyme inhibitor
ASA a většina ~~NSAID~~	rifampicin	fluvoxamin (ostatní SSRI slabí)
fenobarbital, fenytoin	fenobarbital, fenytoin	~~omeprazol~~
S-warfarin	karbamazepin	~~inhibitory HMG-CoA reduktázy~~
losartan		tolbutamid
tolbutamid		cimetidin (slabý)
sulfonamidy, dapson		~~azolová antimykotika~~ (slabá)
diazepam, tenazepam		ritonavir
fluoxetin, moclobemid		desethylamiodaron
zidovudin		

20. Topinková E et al: Postgrad Med 2002; 5:477-82

21. Naganuma M et al: J Cardiovasc Pharmacol Ther 2001; 6:636-7

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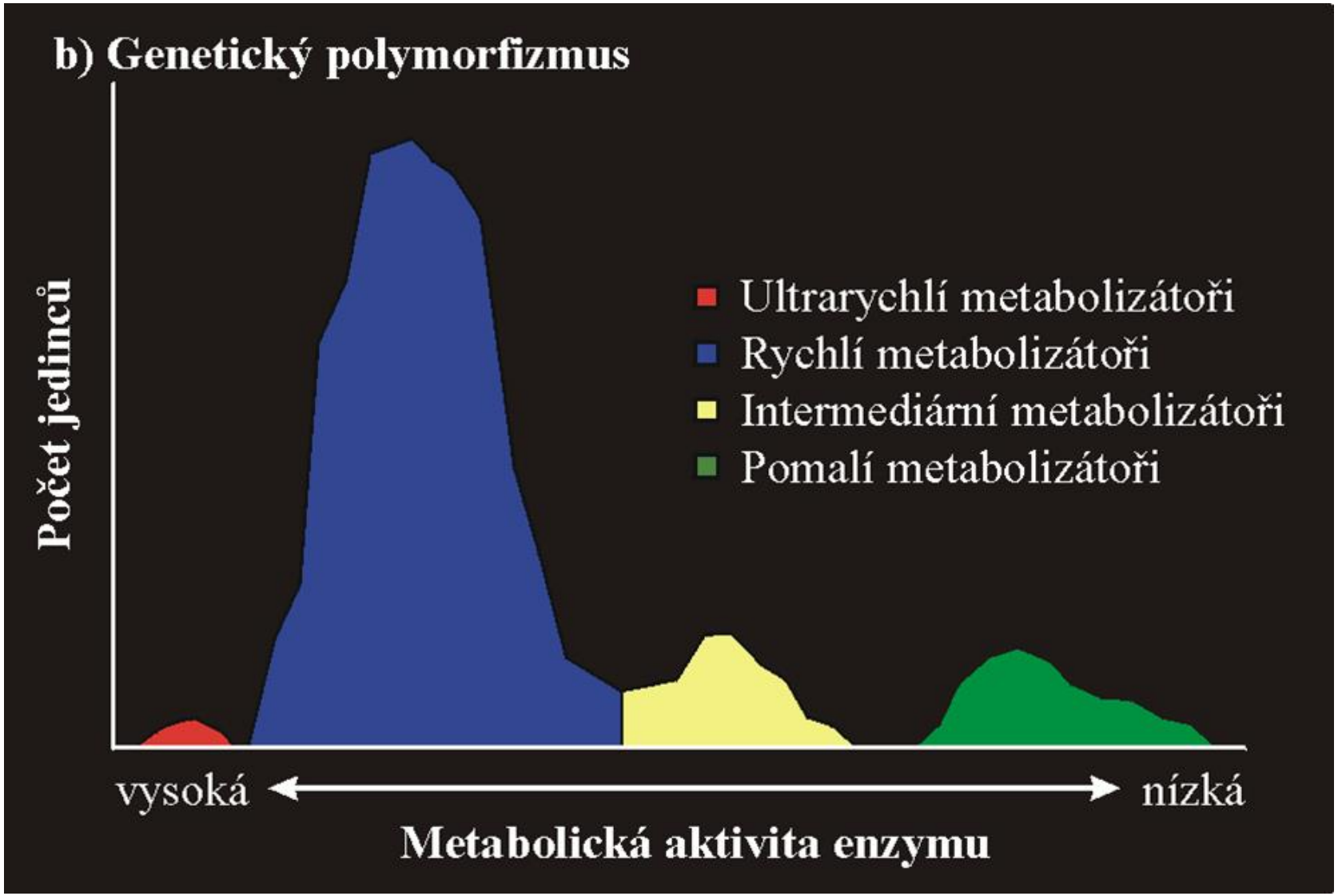
Metabolic rate

- According to the activity of the enzyme, the population can be divided into four main groups - slow metabolizers (PM), intermedial metabolizers (MS), effective metabolizers (EM) and ultra-fast metabolizers (UM).
- The majority of the Cucasian population individuals are among the so-called extensive metabolizers (EM) in which drugs are metabolized at an estimated rate.
- 5-10% of individuals are genetically identified as slow metabolizers (PM) who have slowed breakdown of metabolized substances and are at higher risk of a higher incidence of adverse reactions.
- Intermediate metabolizers (MI) are represented in 10-15% and are comparable to the PM group with long-term treatment.
- In ultrafast metabolizers (UM), metabolism takes place more intensively and does not respond clinically to normal doses of medicines and is represented in 5-10 %.


b) Genetický polymorfizmus
Počet jedinců
Ultrarychlí metabolizátoři
Rychlí metabolizátoři
Intermediární metabolizátoři
Pomalí metabolizátoři
vysoká
nízká
Metabolická aktivita enzymu

Aim of practical

- **To demonstrate the role of the following common situations in medicine (Virchow's triad)**

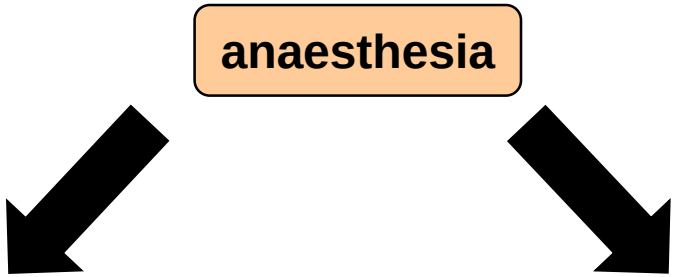
  a) blood stasis or its slower circulation,
  b) increased coagulation or decreased fibrinolytic potential,
  c) endothelial dysfunction or damage

  as precipitating factors for venous thrombosis.

- **To elucidate the effect of exogenous heparin (a homolog of endogenous antithrombin III cofactor) as a powerful anticoagulant substance with broad therapeutic and prophylactic potential in the model of experimentally induced thrombosis.**

- **To explain principles of routine coagulation tests.**

Practical part



+ heparin 4U/kg

1) preparation v. jugularis - application 2ml hypotonic solution
2) laparotomy – ligation segment of abdominal aorta and v. cava inf.
3) thoracotomy – punction of left ventricule – blood take
4) excision of ligated segment

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1) Weight of thrombus
2) aPTT

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