Haemostasis

(mechanism, diagnosis of the bleeding disorders)

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Haemostasis is one of the most important control system in the human body.

Prevents:

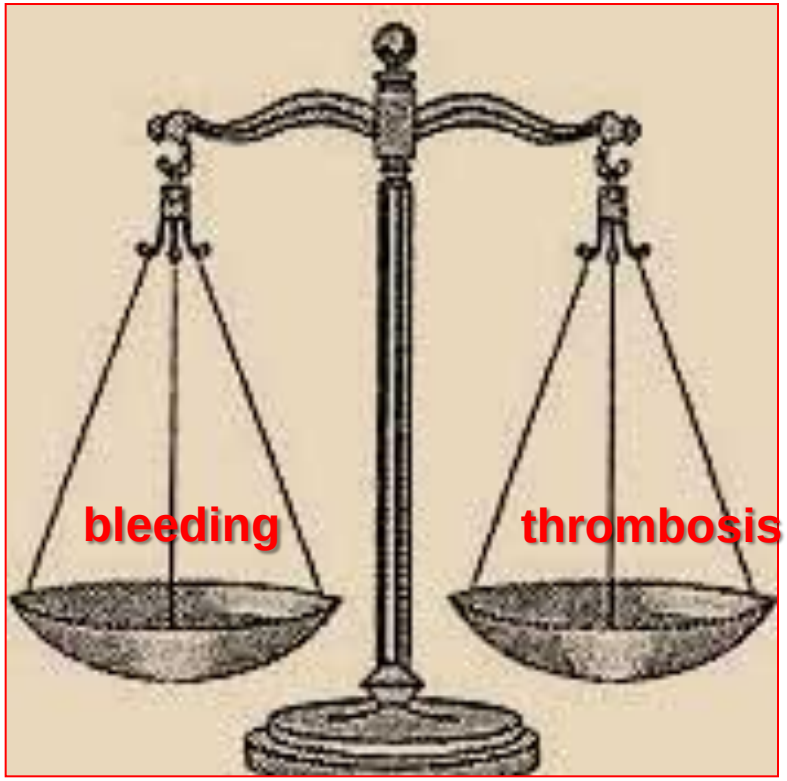
- **spontaneous haemorrhage and undue blood loss from injured vessels**

- **intravascular thrombus formation.**

Normal hemostasis

Hemostasis is tightly regulated by very complex regulatory systems.

In normal case: absence of overt bleeding/ thrombosis.



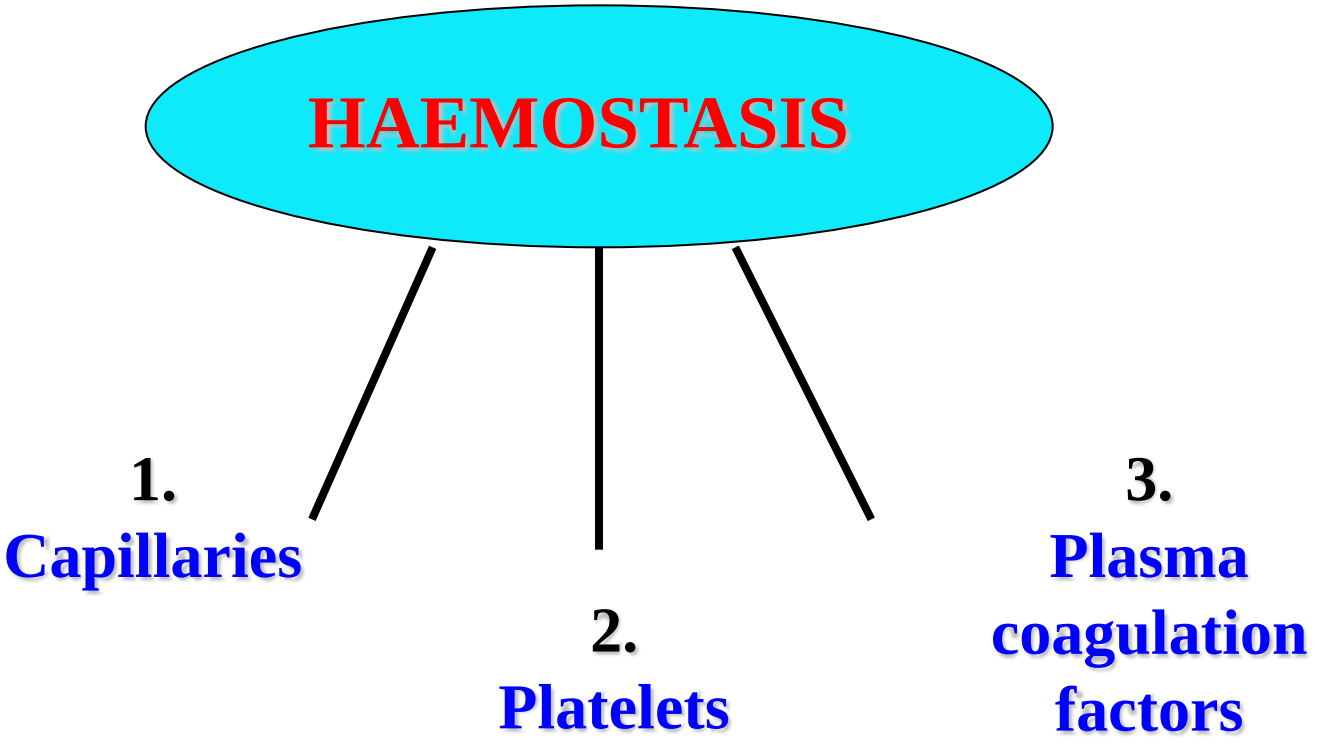
Mechanism of hemostasis

Intravascular coagulation is linked with three different systems:

1. **Blood coagulation system**
2. **Coagulation inhibitory system**
3. **Fibrinolytic system**

1.
Blood coagulation system

There are three components of blood coagulation system:

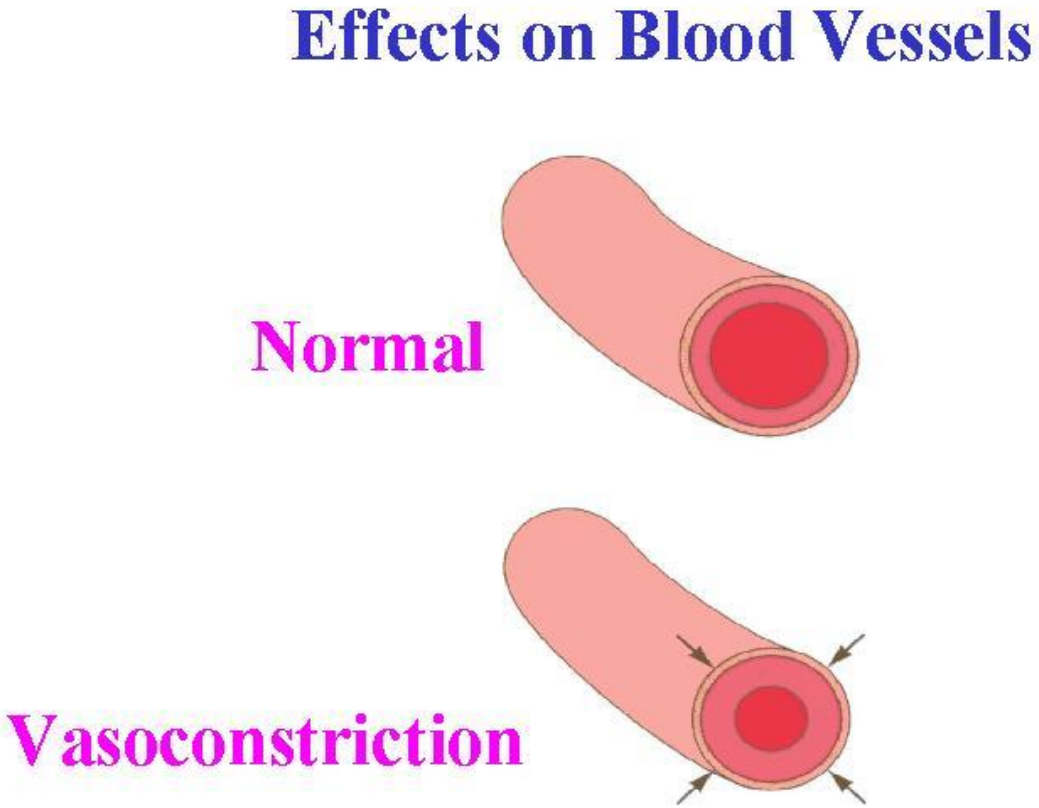


1. 2: Primary haemostasis (it is enough to stop bleeding from small injuries)

3: Secundary haemostasis (it is necessary to stop bleeding definitely)

Primary haemostasis I:

- **Capillaries and larger blood vessels react to injury by an immediate local temporary *vasoconstriction* (a reflex nervous mechanism) to reduce the amount of blood lost.**



Primary haemostasis II:

- **Platelets:**

- smallest formed elements in the blood (2-3 microns)

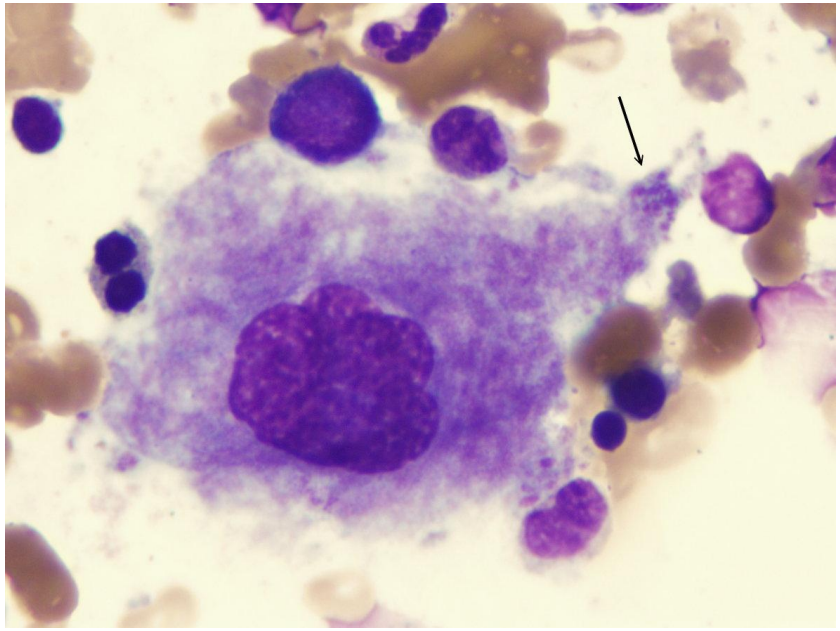
- are produced by megakaryocytes (MK) in the bone marrow

- fragments of the cytoplasm of MK-s → they have no nucleus

- half life: 8-12 days

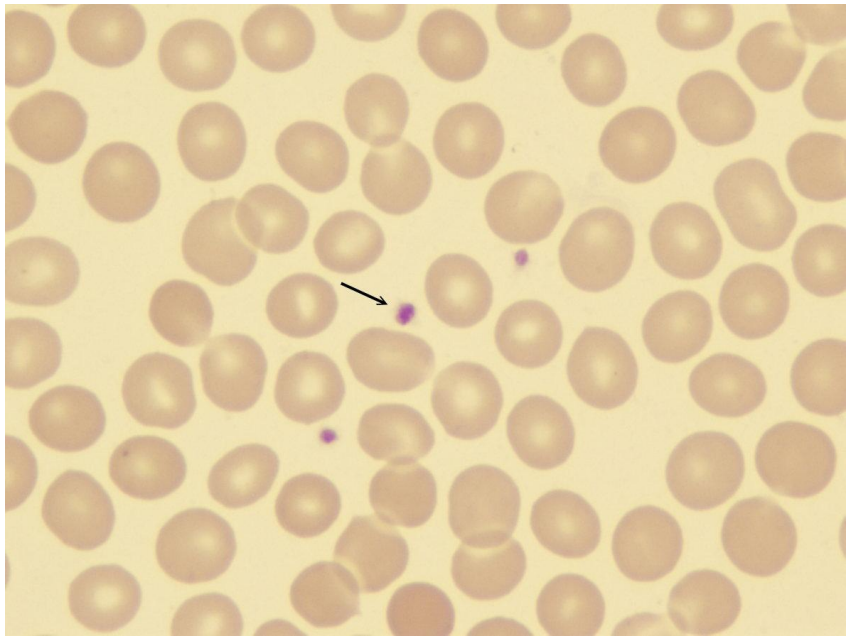
- involved in haemostasis:

- ***physically blocking endothelial disruptions***
- ***releasing active substances***
- ***providing membranes (phospholipid surface) for coagulation process***

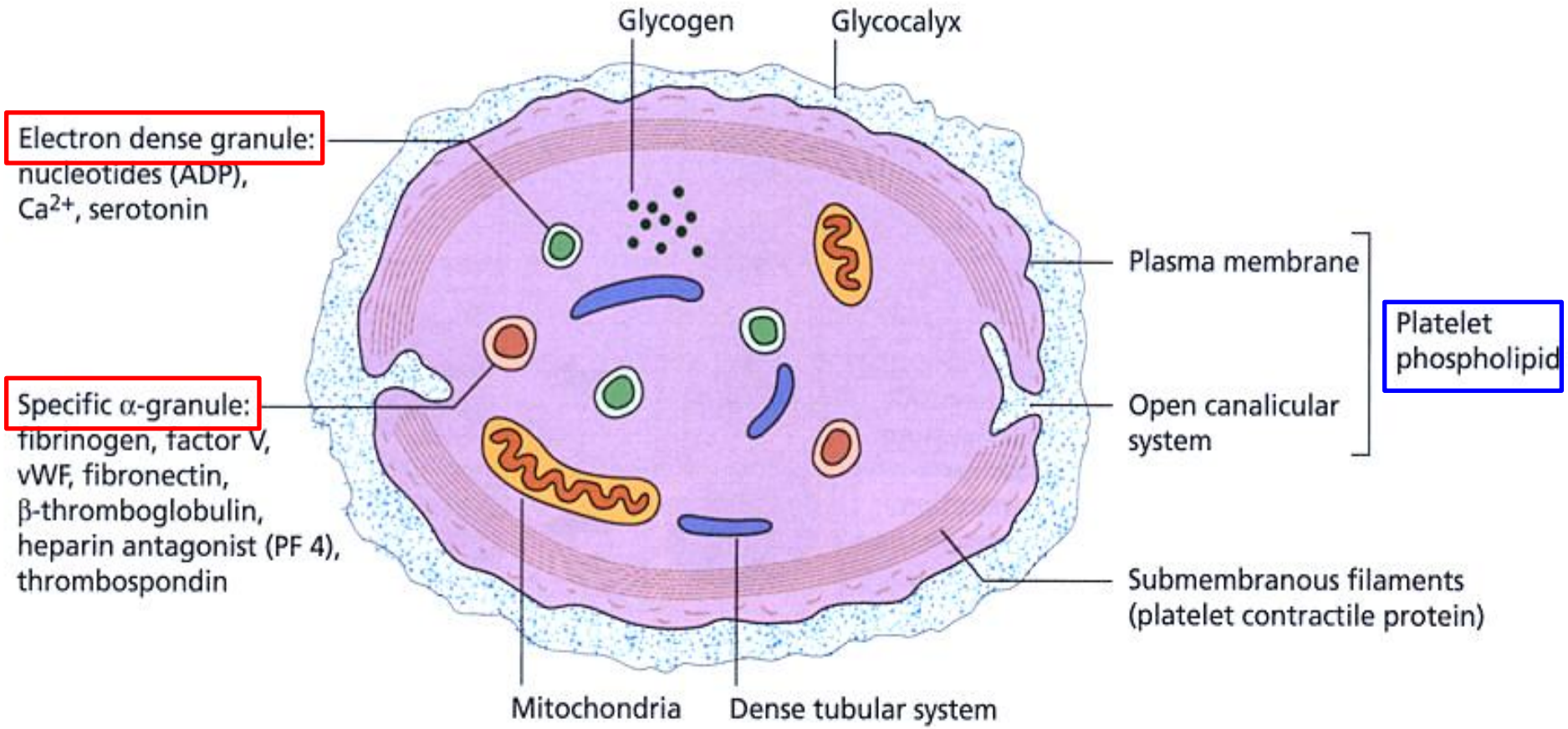


Mature MK showing platelet budding in bone marrow

Platelets in peripheral blood smear



Ultrastructure of platelets

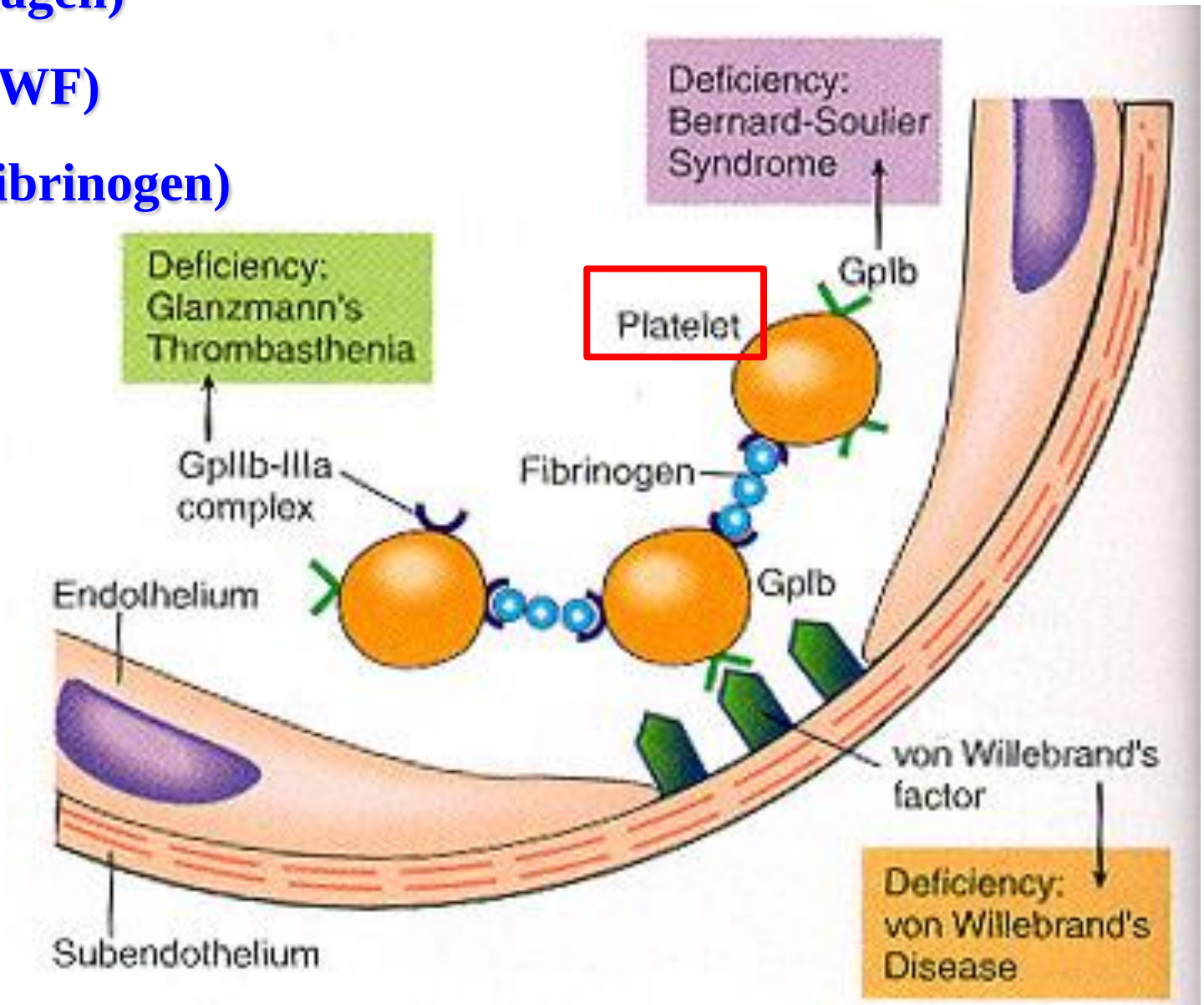


Platelet receptors:

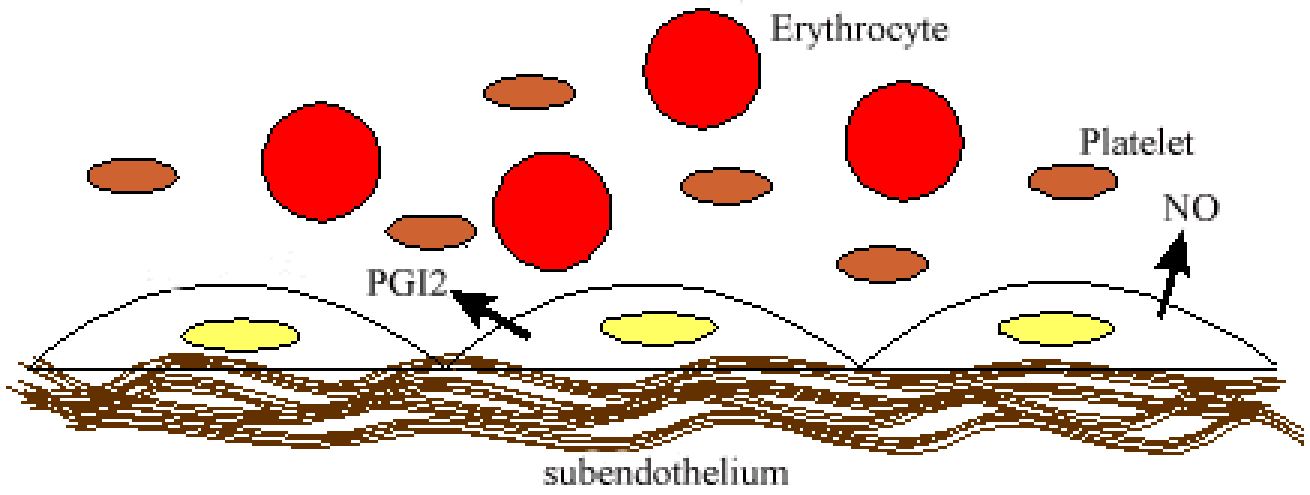
GPIa-IIa (collagen)

GPIb-IX-V (vWF)

GP IIb-IIIa (fibrinogen)

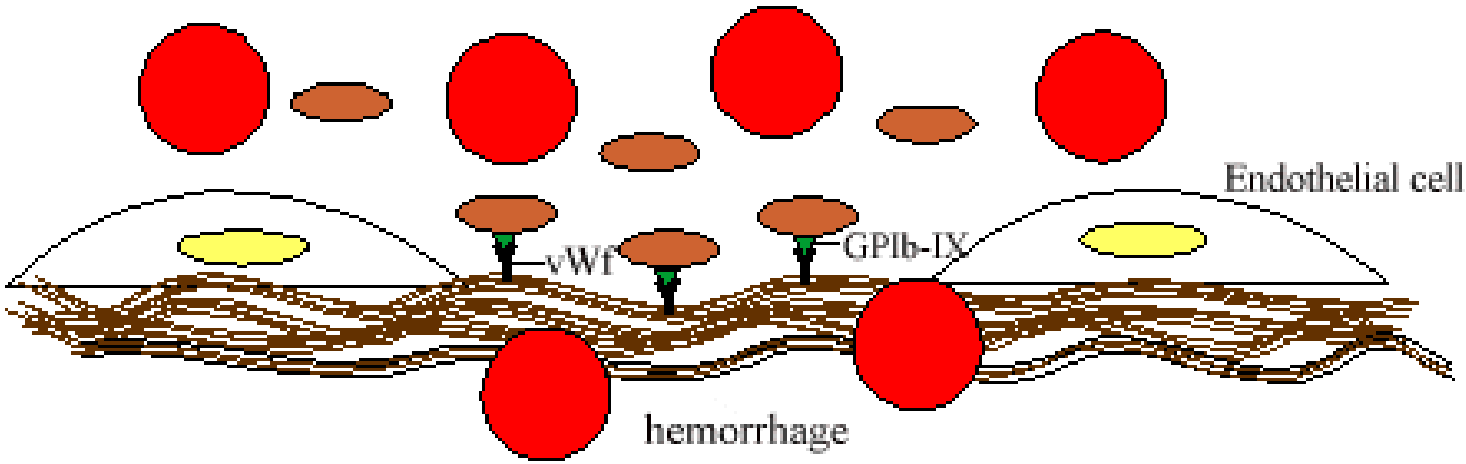


A Visual Representation of Primary Hemostasis I.



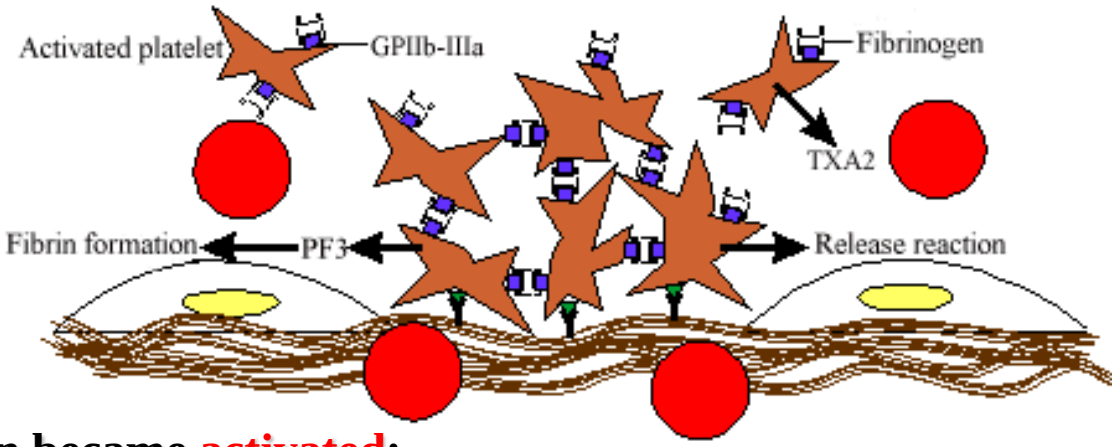
A: Under physiologic conditions, hemostasis is prevented by the *endothelium.* This provides a physical barrier and secretes platelet inhibitory products, such as prostacycline (PGI_2) and nitric oxide (NO) → platelets can't adhere to the endothelium.

A Visual Representation of Primary Hemostasis II.



B: With endothelial cell injury, *platelets* adhere to vWF in the subendothelium via the platelet membrane receptor GPIb-IX *(vWF-receptor)* *(PLATELET ADHESION).*

A Visual Representation of Primary Hemostasis III.



C: Platelets then became activated:

- they undergo a dramatic change in shape: *ACTIVATION*

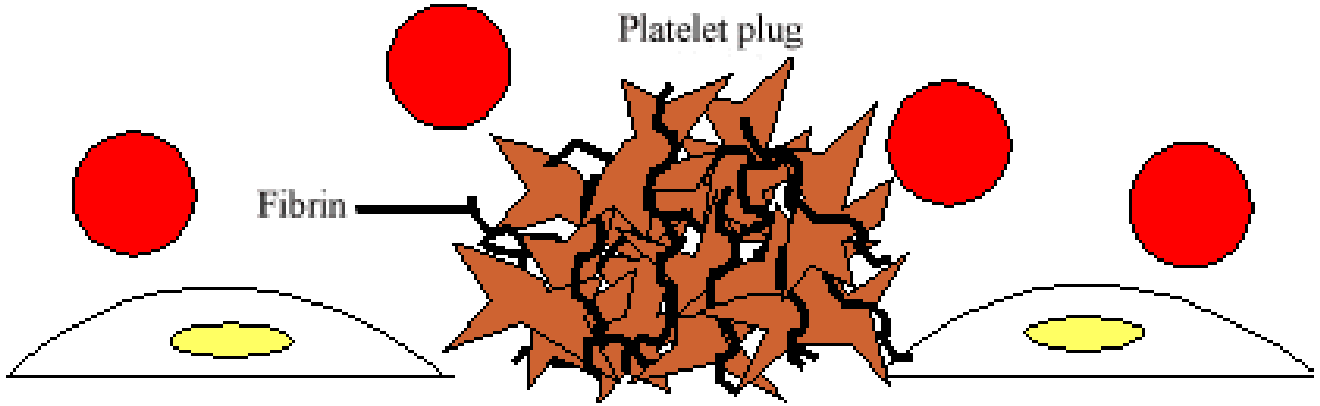
- and release contents of their granules: *RELEASE REACTION*

Other platelets are recruited to the area, resulting in a platelet aggregate: *AGGREGATION*

The platelet *membrane GPIIb-IIIa (fibrinogen receptor) will be activated*. Fibrinogen binds to this receptor, effectively crosslinking platelets *to form a* platelet plug (to „plug up" the injury).

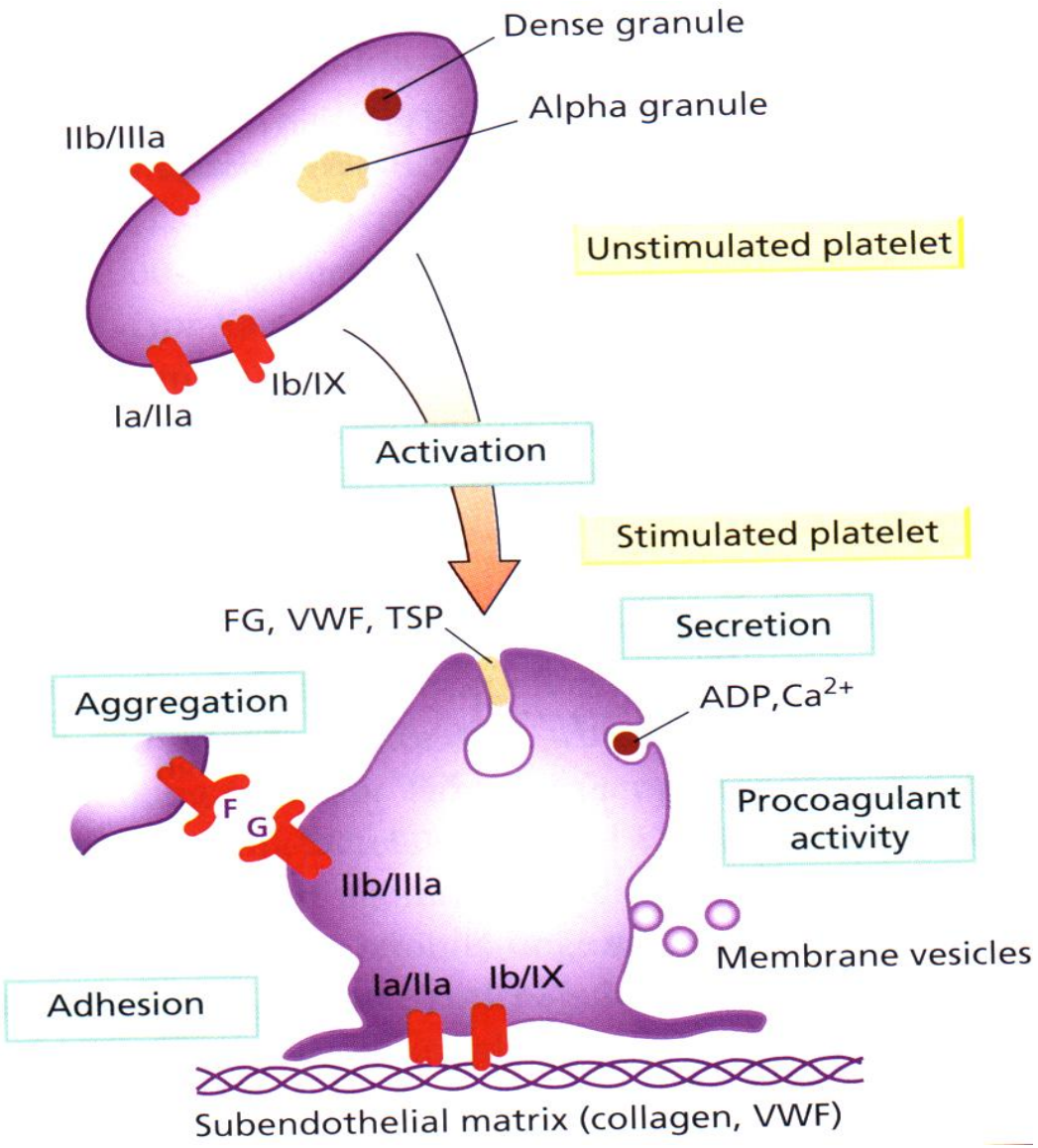
In addition, *phosphatidylserine (a phospholipid) is moved to the outer layer of the platelet membrane*. This provides the surface area for fibrin formation which resulting in stabilization of platelet plug.

A Visual Representation of Primary Hemostasis IV.

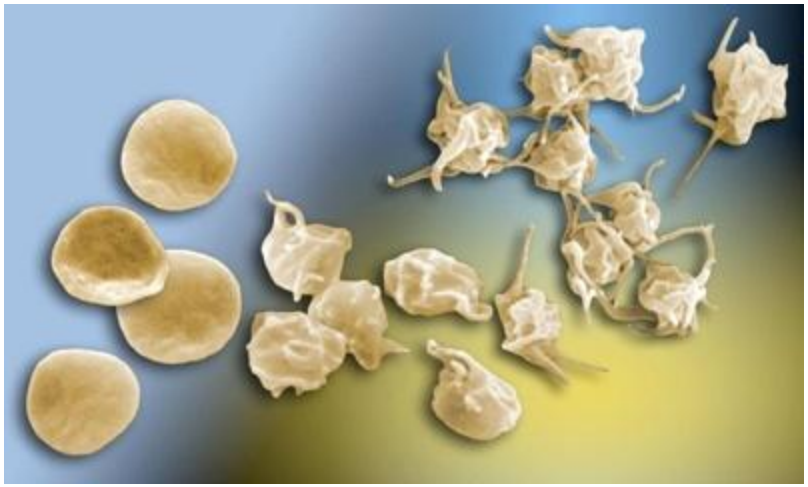


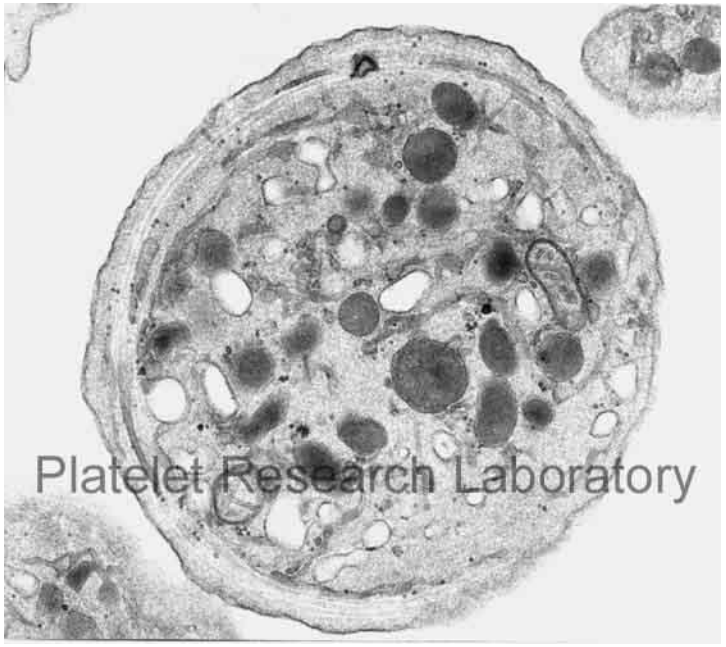
D: Fibrin is incorporated into the growing platelet plug to form a *STABLE THROMBUS*.

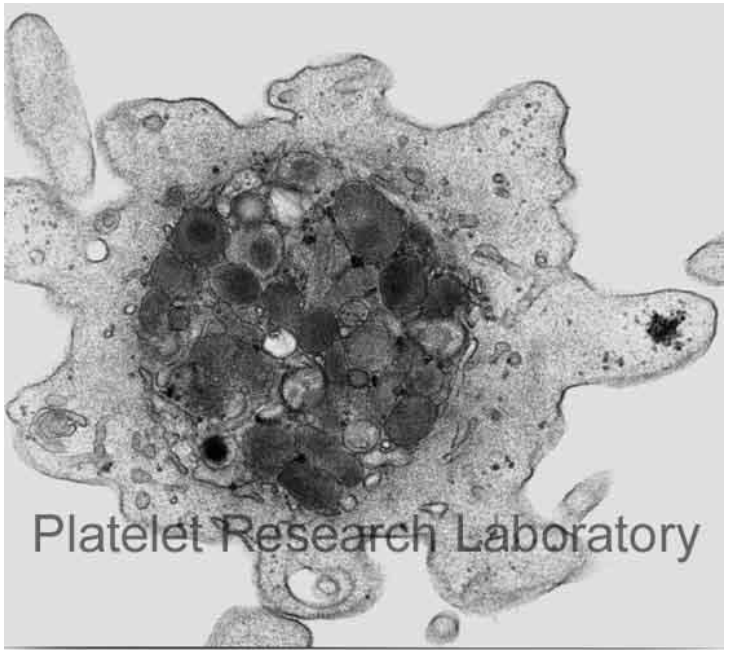
Resting and activated platelets: disc-shaped platelets turn into spiculated spheres with protruding filopedia.



Resting and activated platelets







Electron micrographs from a resting platelet (abow, x10,000), or from an activated platelet showing pseudopodia emission (below, x5000).

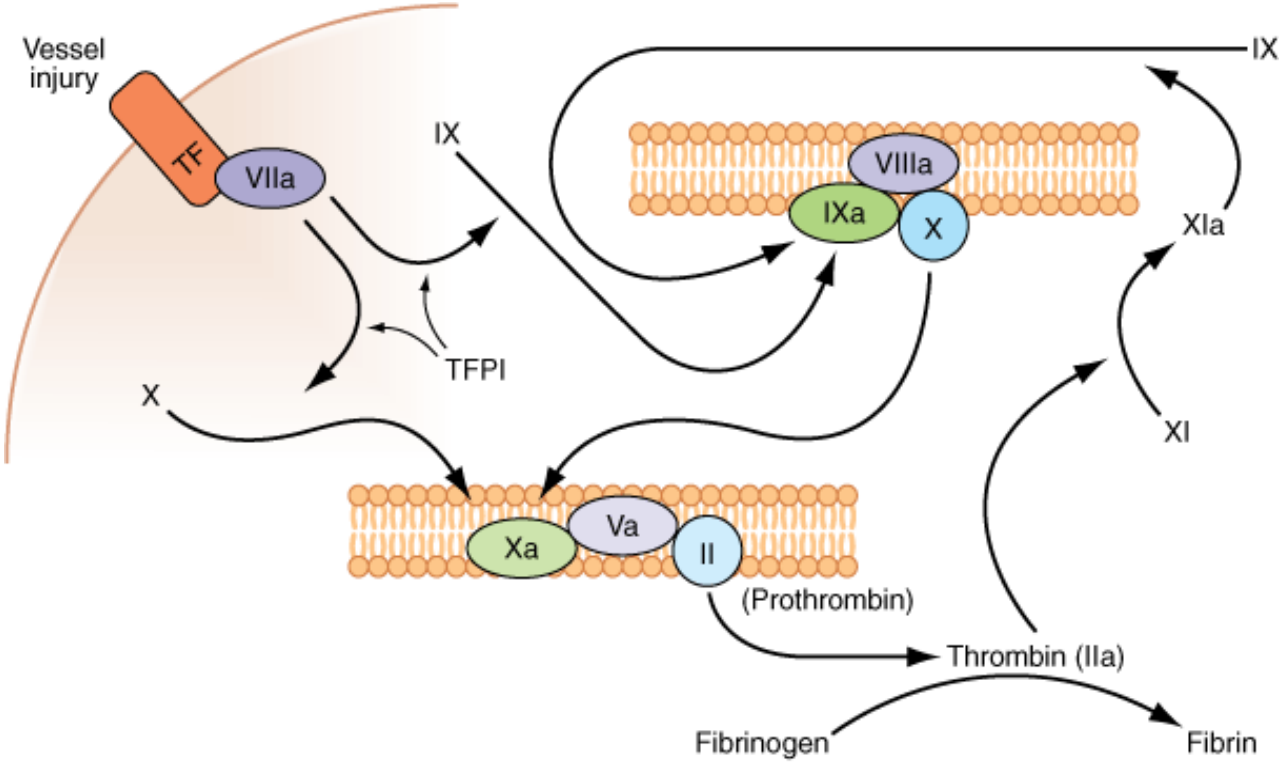
(Pictures by Dr. J. White).

Secundary haemostasis:

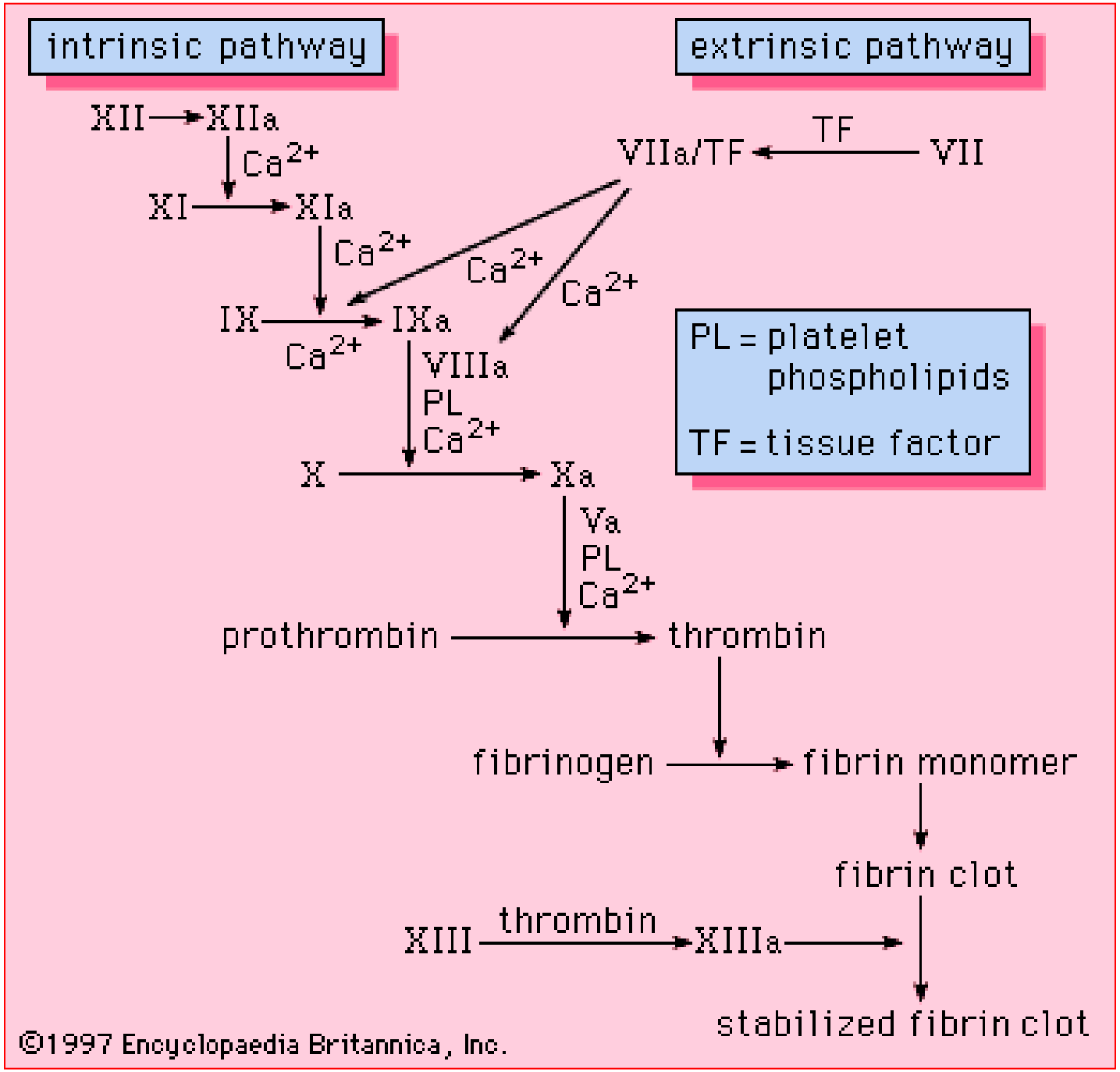
Blood coagulation factors are necessary to stop bleeding definitely.

- I: fibrinogen
- II: prothrombin
- III: tissue thromboplastin (tissue factor, TF)
- IV: Ca^{++}
- V: proaccelerin
- VI: - (= Va)
- VII: proconvertin
- VIII: antihemophilic factor (AHF)
- IX: Christmas factor (plasma thromboplastin component)
- X: Stuart factor
- XI: plasma thromboplastin antecedent (PTA)
- XII: Hageman factor (contact factor)
- XIII: fibrin stabilizing factor (Laki-Lorand factor)

Coagulation **is initiated by tissue factor (TF) exposure,** which, with factor (F)**VIIa**, activates **FIX** and **FX**, which in turn, with FVIII and FV as cofactors, respectively, results in **thrombin formation** and subsequent conversion of fibrinogen to **fibrin.** *Thrombin activates FXI, FVIII, and FV, amplifying the coagulation signal*. Coagulation requires **calcium** and takes place **on phospholipid surfaces**, usually the activated platelet membrane.



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com


intrinsic pathway
extrinsic pathway
XII → XIIa
Ca2+
XI → XIa
Ca2+
IX → IXa
Ca2+
VIIIa
PL
Ca2+
X → Xa
Va
PL
Ca2+
prothrombin → thrombin
fibrinogen → fibrin monomer
fibrin clot
XIII thrombin → XIIIa
stabilized fibrin clot
VIIa/TF ← TF — VII
Ca2+
Ca2+
PL = platelet phospholipids
TF = tissue factor
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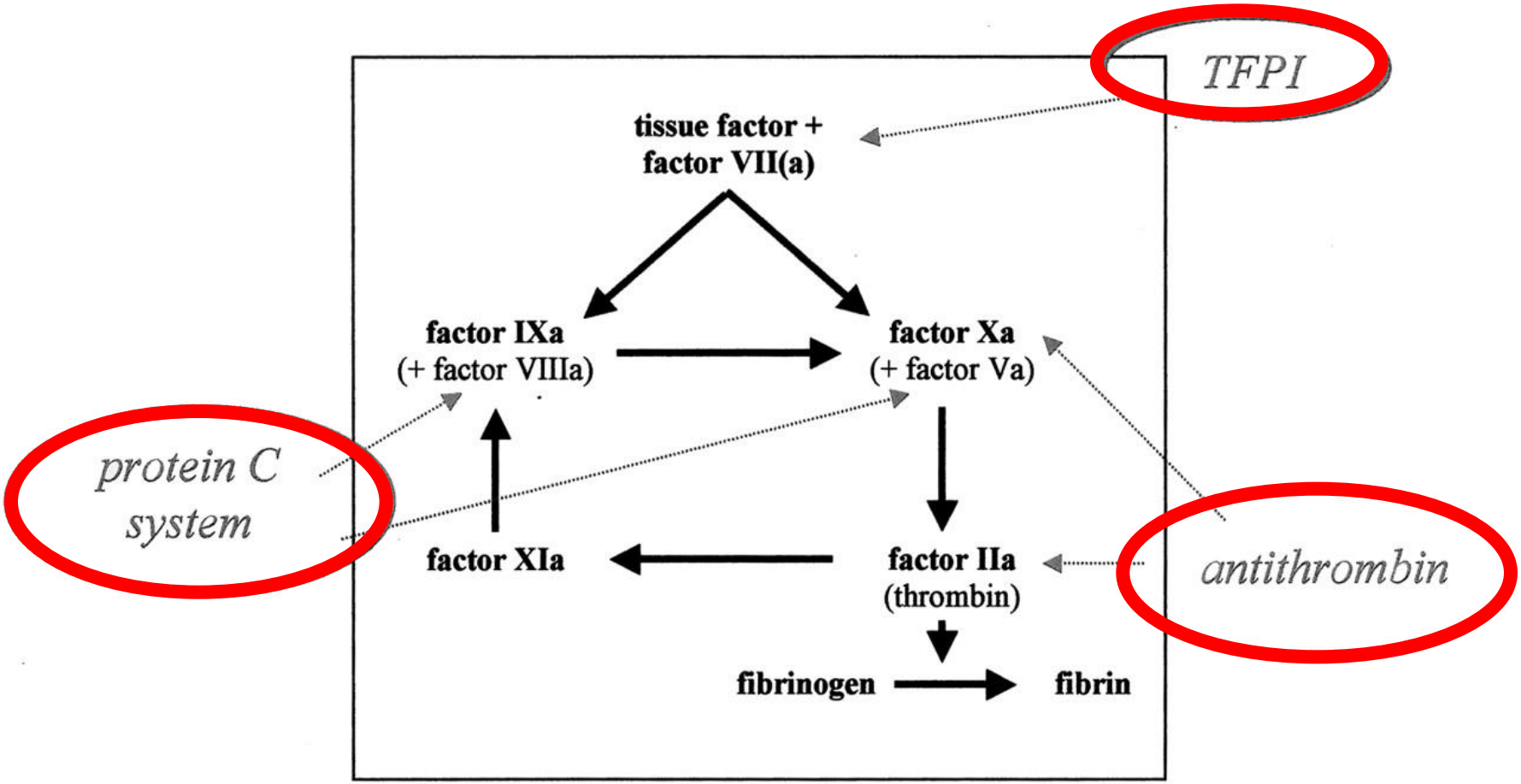
2.
Coagulation inhibitory system

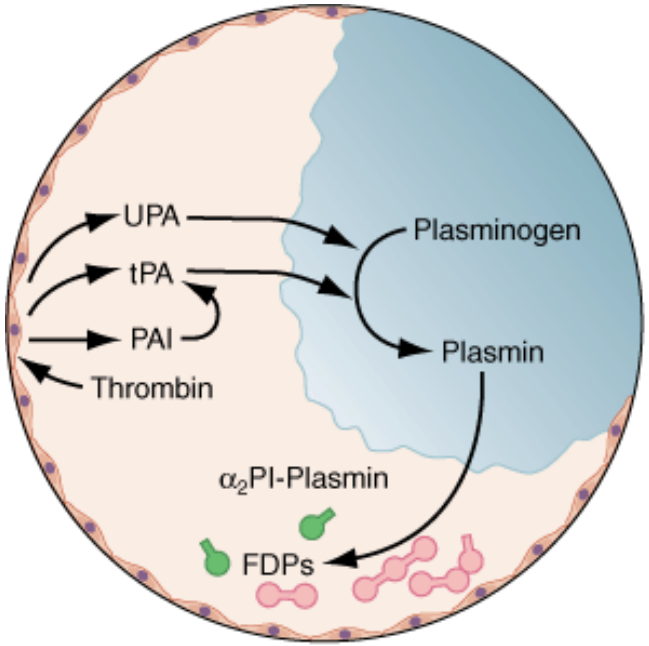

TFPI
tissue factor +
factor VII(a)
factor IXa
(+ factor VIIIa)
factor Xa
(+ factor Va)
protein C
system
factor XIa
factor IIa
(thrombin)
antithrombin
fibrinogen
fibrin

3.
Fibrinolytic system

Fibrinolysis

is the process wherein a *fibrin clot is broken down.* *It prevents blood clot from growing and becoming problematic*. Plasmin is the major protease enzym of the fibrinolytic system. It is produced in an inactive form, plasminogen.

Tissue plasminogen activator (tPA) is released from endothelial cells, binds the fibrin clot, and activates plasminogen to plasmin. *Excess fibrin is degraded by plasmin* to distinct degradation products (FDPs). Any free plasmin is complexed with alpha$_2$-antiplasmin (α_2Pl).



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Disorders of hemostasis

Disorders of the haemostatic mechanism are devided into three main groups:

- **I. Disorders of the vessels**
- **II. Disorders of the platelets**

} ***„purpuric diseases”***

- **III. Disorders of the blood coagulation mechanism *(„coagulopathies”)***

The investigation steps of a patient with a suspected disorder of haemostasis

- **case history**
  - **personal details:** epistaxis, easy bruising, haemarthrosis, secundary bleeding from cuts, scrapes, tooth extraction, bleeding during or after operations, meno-metrorrhagia, impaired healing or wounds
  - **family history:** bleeding symptoms in first-degree blood relatives
- **inspection (type of bleeding):** petecchiae, purpuras, haematomas, haemarthrosis
- **physical examination**
- **other known diseases**
- **drugs and medications:** anticoagulants, ASA, non steroids
- **laboratory tests**

Clinical approach to coagulation disorders

Questions to answer:

1. **Is the bleeding significant ?**
2. **Local or systemic ?**
3. **„Purpuric” or coagulation disorder ?**
4. **Inherited or acquired ?**

Certain signs and symptoms are virtually diagnostic of disordered haemostasis.

The main symptom of all diseases is the bleeding:

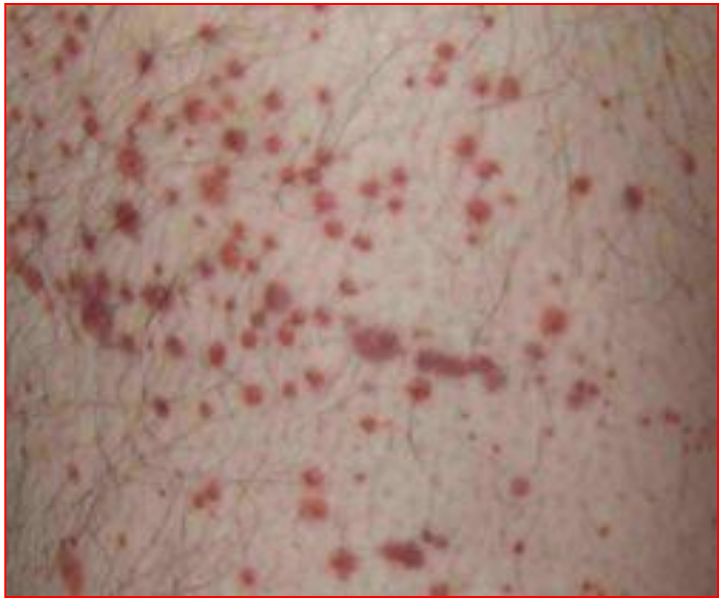
- **in the „purpuric disorders" cutaneous and mucosal bleeding usually is prominent**
- **in different types of „coagulopathies" hemarthroses, haematomas are the characteristic bleeding manifestations.**

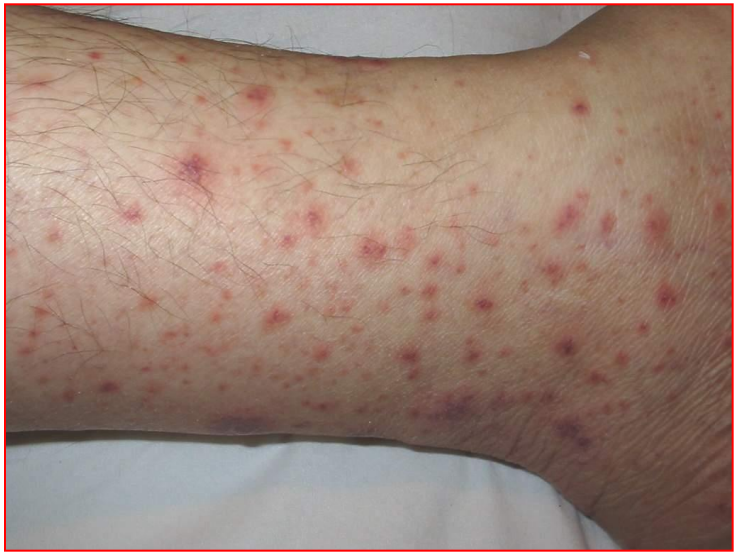
The onset of bleeding following trauma frequently is *delayed* (recur in a matter of hours)

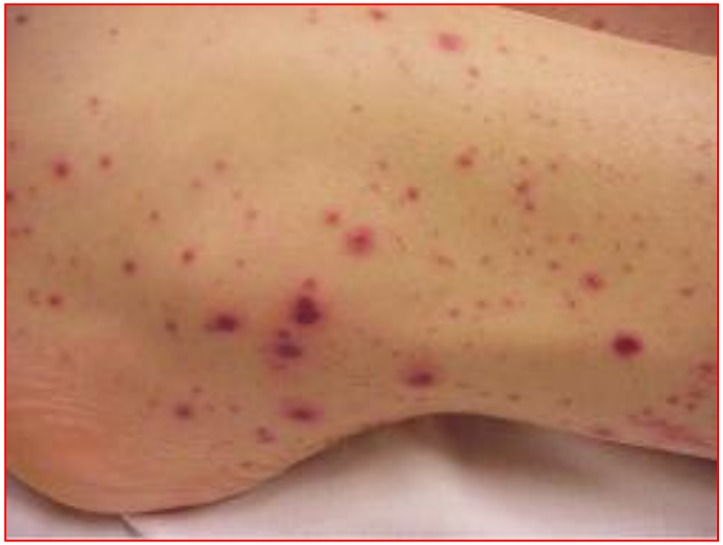
(the temporary hemostatic adequacy of the platelet plug may explain this phenomenon).

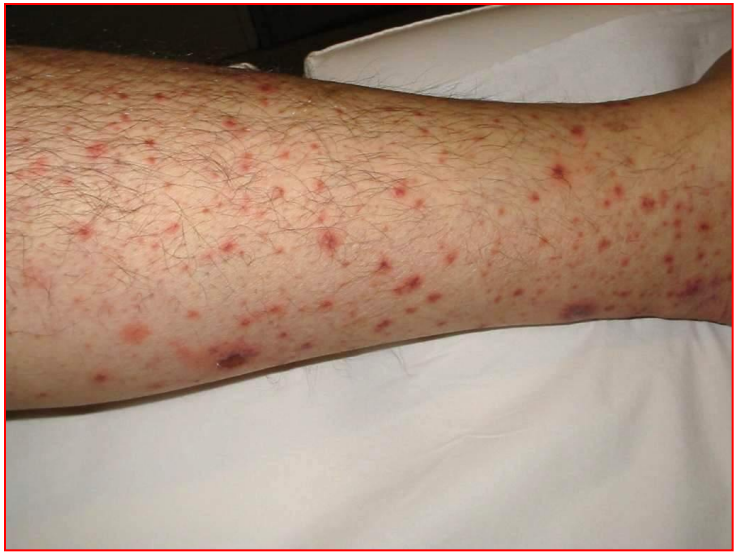
Petechiae (> 3 mm), purpuras (< 3 mm):

small capillary haemorrhages ranging from the size of a pinhead to much larger



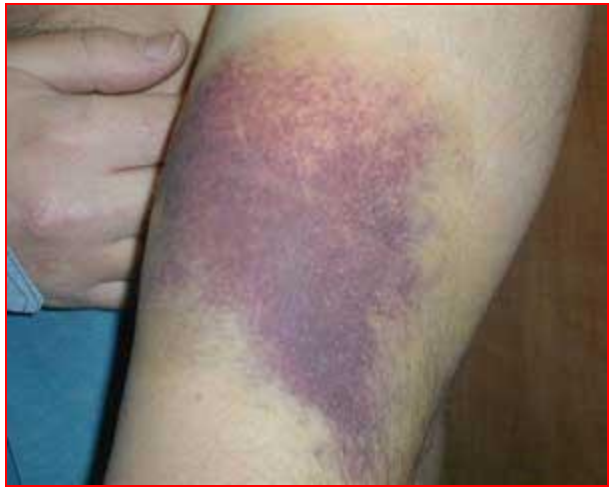


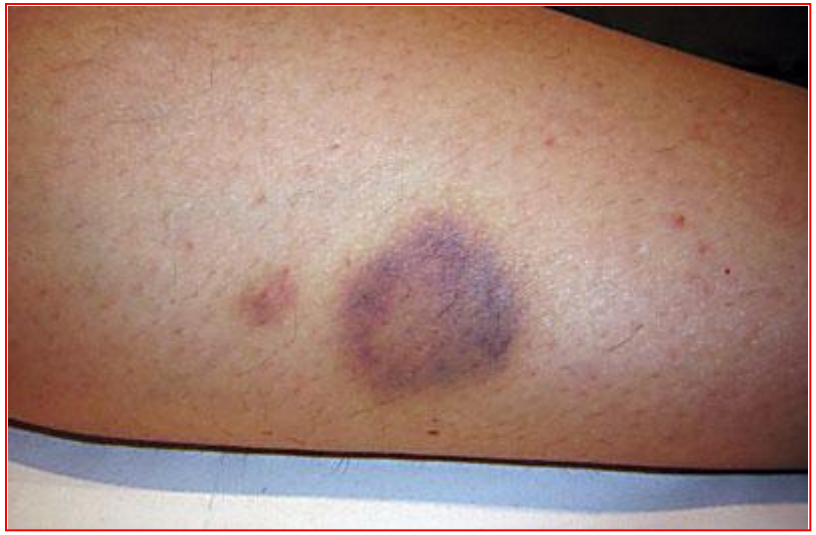


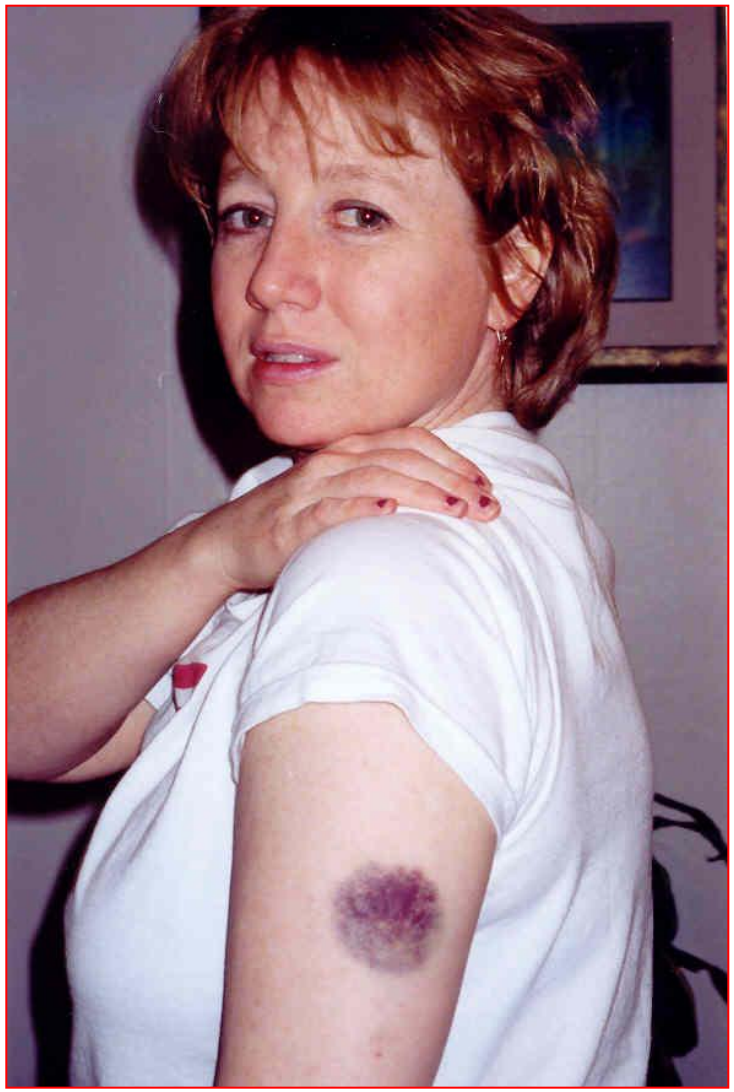


Haematomas:

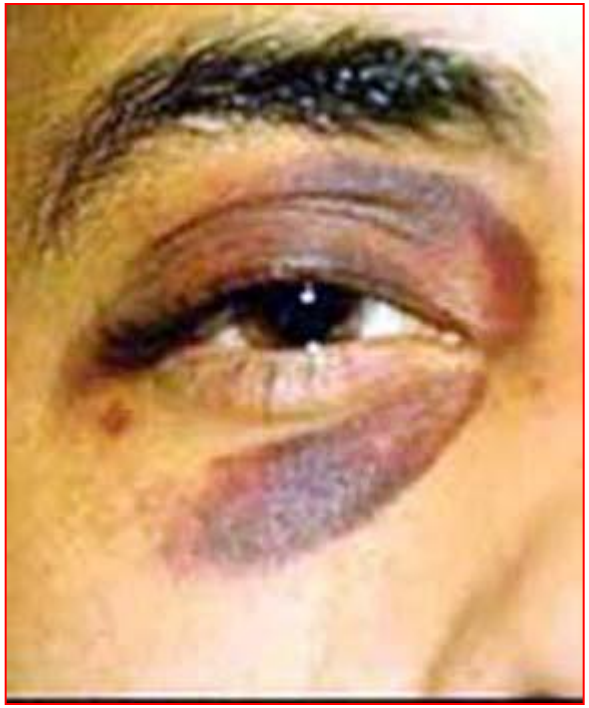
may be spontaneous (in a serious hemorrhagic disease) or may occur after trauma (in a mild hemorrhagic disease).

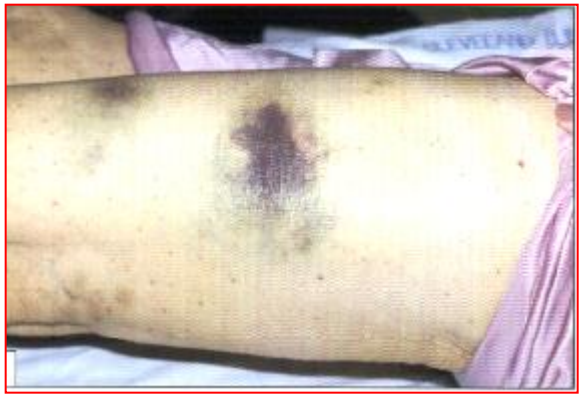


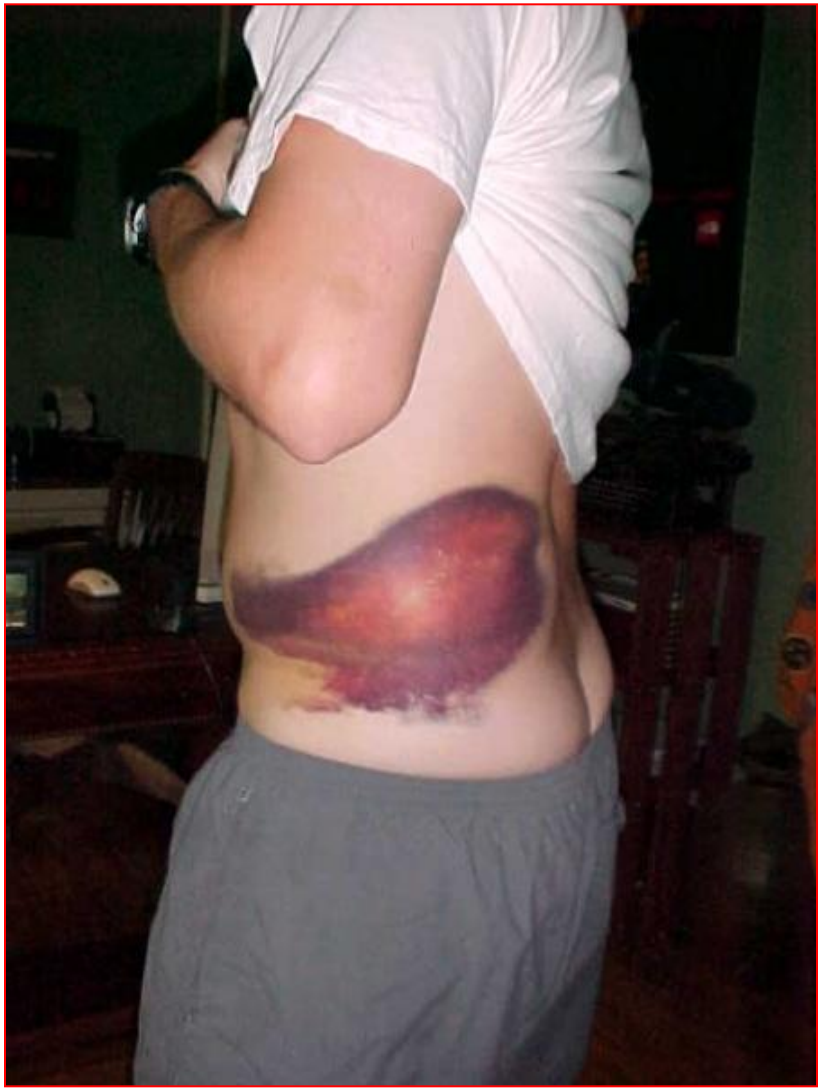




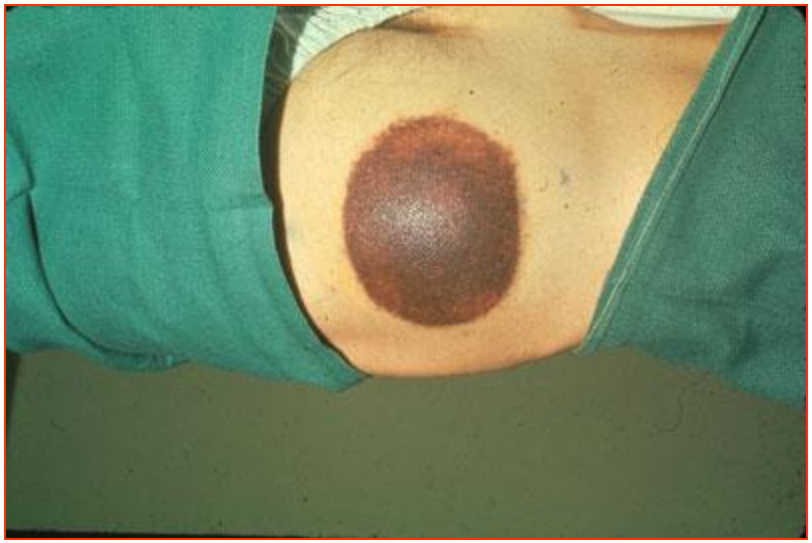
Haematomas







Remember! Intramuscular injection *may be very dangerous* to the patient with a bleeding disorder.



Venipuncture (if skilfully performed) *is without danger* (because the elasticity of the venous walls).

Laboratory Approach

Blood coagulation tests are used to find out if a patient has a bleeding disorder or not.

The results of blood tests:

1. **Demonstration of the defect**
2. **Identification of the defect(s)**
3. **Assessment of severity**
4. **Consequential studies (e. g. carrier detection)**
5. **Monitoring of treatment**

Many things can effect blood coagulation test results:

A. Patient-related factors:

hormonal changes during the menstrual cycle
use of pain killers - like aspirin
use of birth control pills or other hormone therapies
pregnancy, childbirth, or breastfeeding
blood type
intense exercise
stress

B. Technical factors:

blood coagulation tests require blood samples that have been drawn in tubes containing sodium citrate as an anticoagulant.
Skillfully performed venipuncture is necessary!

Collection of blood sample

1. **Minimum circulatory stasis**
2. **Clean venous puncture**
3. **Proper anticoagulant (sodium citrate)**
4. **Proportion of blood to anticoagulant (9:1)**
5. **Separation of plasma and storage**
6. **Effect of stress, pregnancy, drugs**
7. **Effect of Hematocrit (packed cell volume, PCV) on the proportion of plasma to anticoagulant**

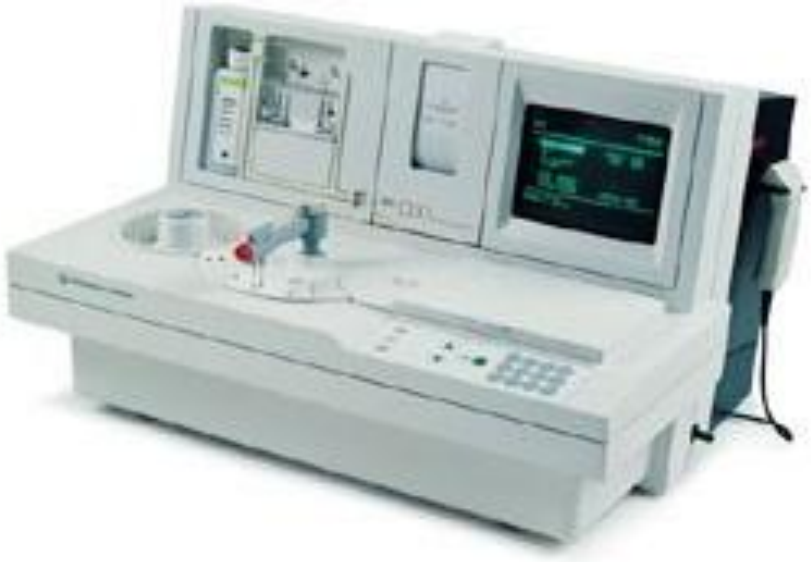
Automated blood coagulation instruments (coagulometers): measure the ability of blood to clot.

Depending on the test (e.g. PT, APTT, TT), different substances can be added to the blood plasma to trigger a clotting reaction.

The process of clotting may be monitored optically.

ACL 1000 Coagulation Analyzer:

is a fully automated, clot-end-point analyzer



Reaction tray used in optical clot detection

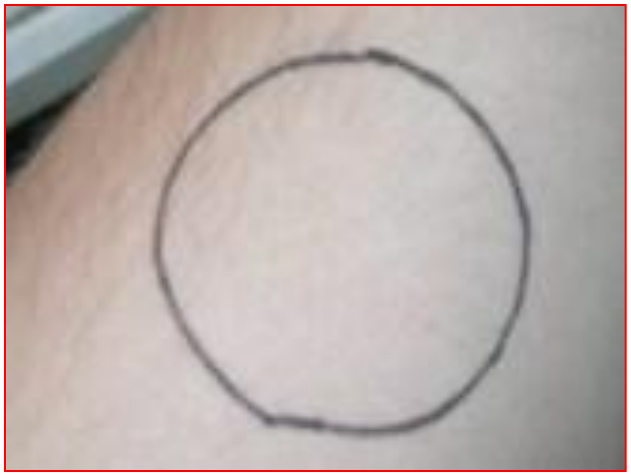
„Screening" tests of blood coagulation

- **Disorders of vessels:**
  - **Rumpel-Leede test (tourniquet test)**
- **Disorders of platelets:**
  - **Platelet count and morphology**
  - **Bleeding time (Ivy)**
- **Coagulopathies:**
  - **Whole blood clotting time**
  - **Activated partial thromboplastin time (APTT)**
  - **Prothrombin (PT, INR)**
  - **Thrombin time (TT)**

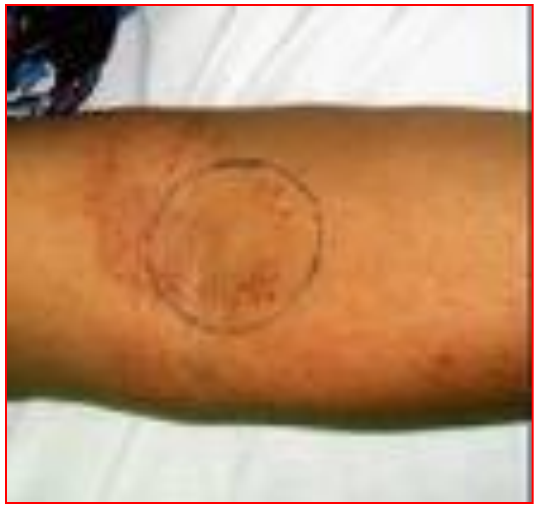
Rumpel - Leede test (Tourniquet test)

It assesses fragility of capillary walls.

***Procedure*:**
a blood pressure cuff is placed on the upper arm and inflated to a point between the systolic and diastolic blood pressure for 5 minutes.
***Positiv result*: more than 10 petecchiae.**
(It means increased capillary fragility.)



negative



positive

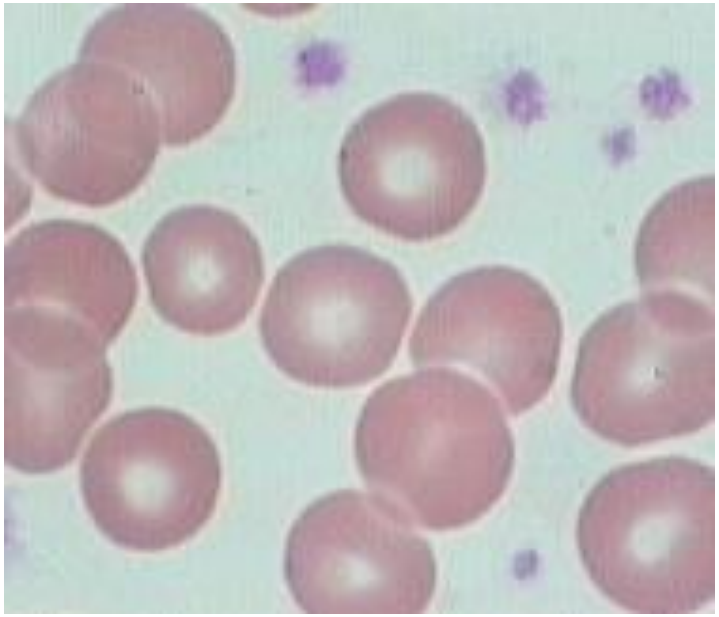
Platelet count I.

A diagnostic test that determines the number of platelets in the patient's blood.

Normal value: (100) 150-350 x10^9/l

Platelets can be observed in a direct blood smear for approximate quantity and shape.

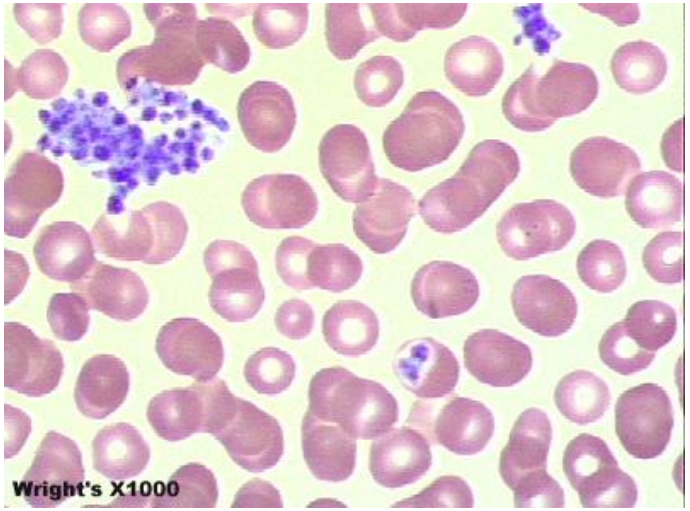
**Non-nucleated discoid cells,
2-3 microns,
blue cytoplasm containing a few azure granules.**



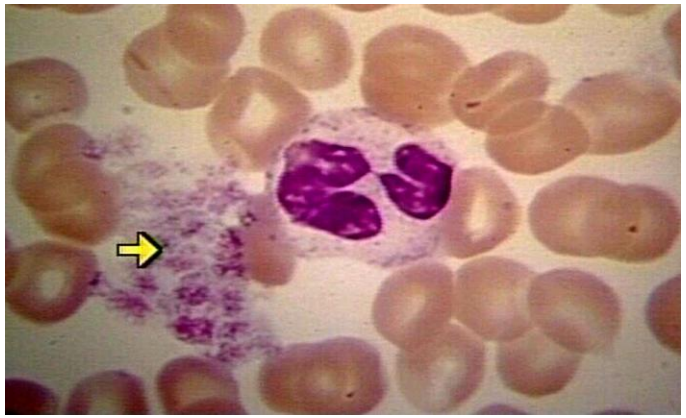
Platelet count II.

Pseudothrombocytopenia:
in vitro phenomenon:

- a false reduced number of platelet count in blood samples collected into EDTA- anticoagulant containing vials *(EDTA agglutinates platelets)*.
The correct number can be determined by blood collection in 3,8% sodium citrate.



- thrombocytopenia may be due to platelet agglutination or platelet sathelitism *(rosettes formation around neutrophils)*. No association with hemorrhagic diathesis or platelet dysfunction.



Platelet counting methods:

- **Hematocytometric (microscopic):**
  uses *phase contrast microscope* to view blood on a hemacytometer slide.

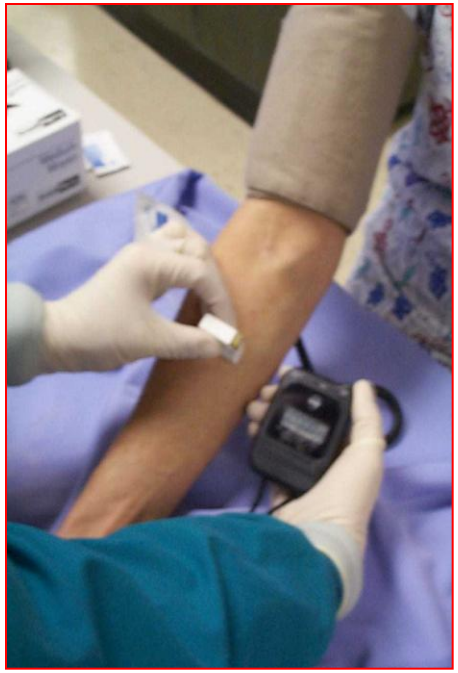
- **Electronic:**
  collected blood is diluted and counted by passing the blood through an *electronic counter*. Instrument counts only particles within the proper size range for platelets.

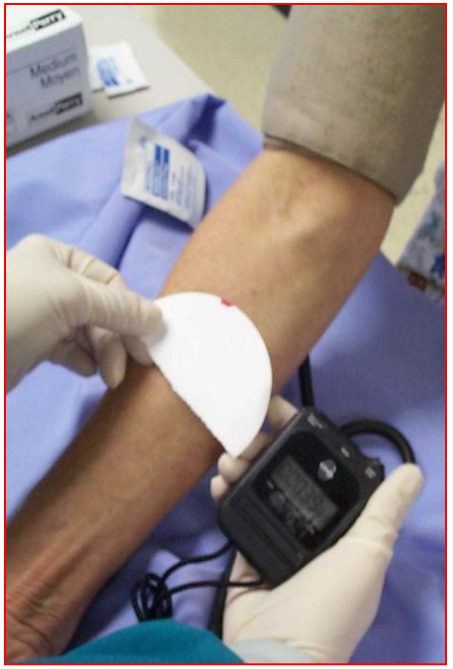
Bleeding time (Ivy)

It assesses the platelet function.

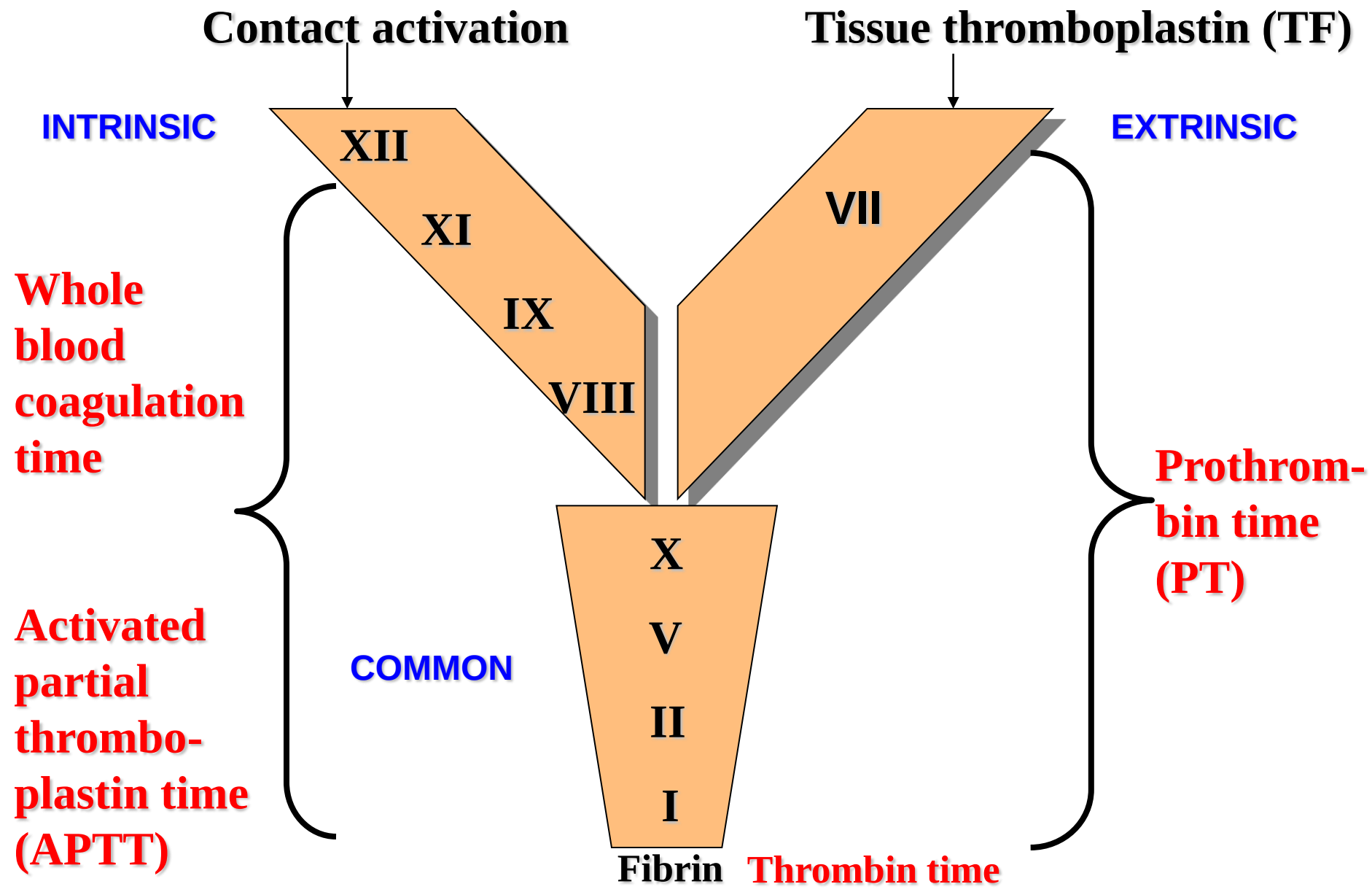
***Procedure*: cutting the underside of the patient's forearm (in an area where there is no hair or visible veins). The cut is of a standardised width (10 mm) and depth (1 mm) by an automatic device. A blood pressure cuff is placed on the upper arm, to maintain venous pressure at a special value (40 mmHg).**

The time it takes for bleeding to stop is measured.
***Normal value*: < 10 minutes.**
Prolonged:
thrombocytopenia,
thrombocytopathia,
impaired blood vessels.





Laboratory diagnosis of the coagulopathies



Prothrombin time (PT):

- Tests extrinsic and common pathways
- Thromboplastin and calcium are added to patient plasma → coagulation time depends on the factors of extrinsic and common pathways.
- Prolonged in:
  - Anticoagulant treatment (warfarin, coumarin)
  - Vitamin K deficiency (FII, FVII, FIX, FX)
  - Liver disease
  - DIC (disseminated intravascular coagulation)

Activated partial thromboplastin time (APTT):

- Tests intrinsic and common pathways
- Kaolin is added to patient plasma → coagulation time depends on the factors of intrinsic and common pathways.
- Prolonged in:
  - Heparin treatment
  - Factor deficiences (except FVII, FXIII)
  - Liver disease
  - DIC
  - Massive transfusion
  - Lupus anticoagulant

Thrombin time (TT):

- **Measures the rate of conversion of fibrinogen to fibrin upon the addition of thrombin to patient's plasma**
- **Prolonged in:**
  - **Heparin treatment**
  - **Low or abnormal fibrinogen (dysfibrinogenaemia)**
  - **DIC**

Prolonged PT/APTT:

correction test is necessary

- May be coagulation factor deficiency/inhibitor
- Test plus control plasma - 1:1 (correction test)
- Repeat PT/APTT
- > 50% correction:
  - Yes: factor deficiency
  - No: inhibitor

Diagnosis of bleeding disorders by the „screening” tests

Platelet count	Bleeding time	APTT	Prothrom-bin (PT)	Presumptive diagnosis:
Decreased	Prolonged	Norm.	Norm.	*Thrombocytopenia*
Norm.	Prolonged	Prolonged	Norm.	*von Willebrand's disease*
Norm./ increased	Prolonged	Norm.	Norm.	*Thrombocytopathia*
Norm.	Norm.	Prolonged	Norm.	*„intrinsic" pathway abnormality* (FVIII. IX. XI. XII)
Norm.	Norm.	Norm.	Prolonged	*„extrinsic"pathway abnormality* (FVII)
Norm.	Norm.	Prolonged	Prolonged	*„common" pathway abnorm.* (FI. II. V. X.)
Norm.	Norm.	Norm.	Norm.	*- /FXIII deficiency/ mild bleeding disorder*