EXAMINATION OF COAGULATION AND FIBRINOLYSIS

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I.
Physiology

Hemostasis

= The physiologic process protecting the integrity of the vascular system after tissue injury.
Bleeding is halted to minimize blood loss.

The hemostatic mechanisms include following steps:

1. Resting phase - To maintain blood in a fluid state while circulating within the vascular system
2. After injury - To arrest bleeding at the site of injury by formation of hemostatic plug
3. Restitution - To ensure the removal of the hemostatic plug when healing is complete

Hemostasis

Hemostasis is involved in

- **stress reaction**
- **inflammatory response**

Protective role ⋮ **non-specific defense mechanism**

X

Patho-genetic role ⋮ **thrombosis / embolism**

atherosclerosis

Hemostasis

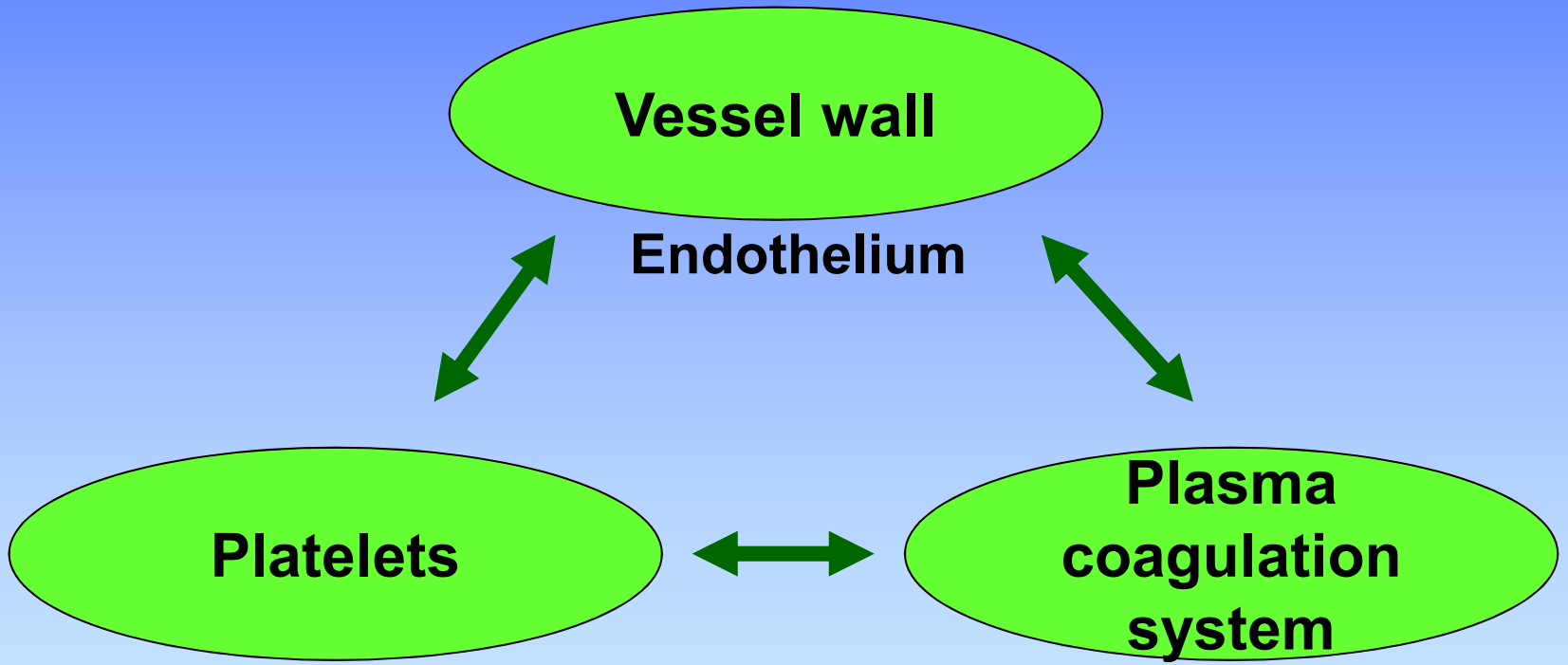
Hemostasis as a physiological process must be:

1. **Rapid**
2. **Localized**
3. **Reversible**

Inappropriate hemostasis:

- **Thrombosis / embolism**
- **DIC (disseminated intra-vascular coagulation)**
- **bleeding / blood loss**

Hemostasis

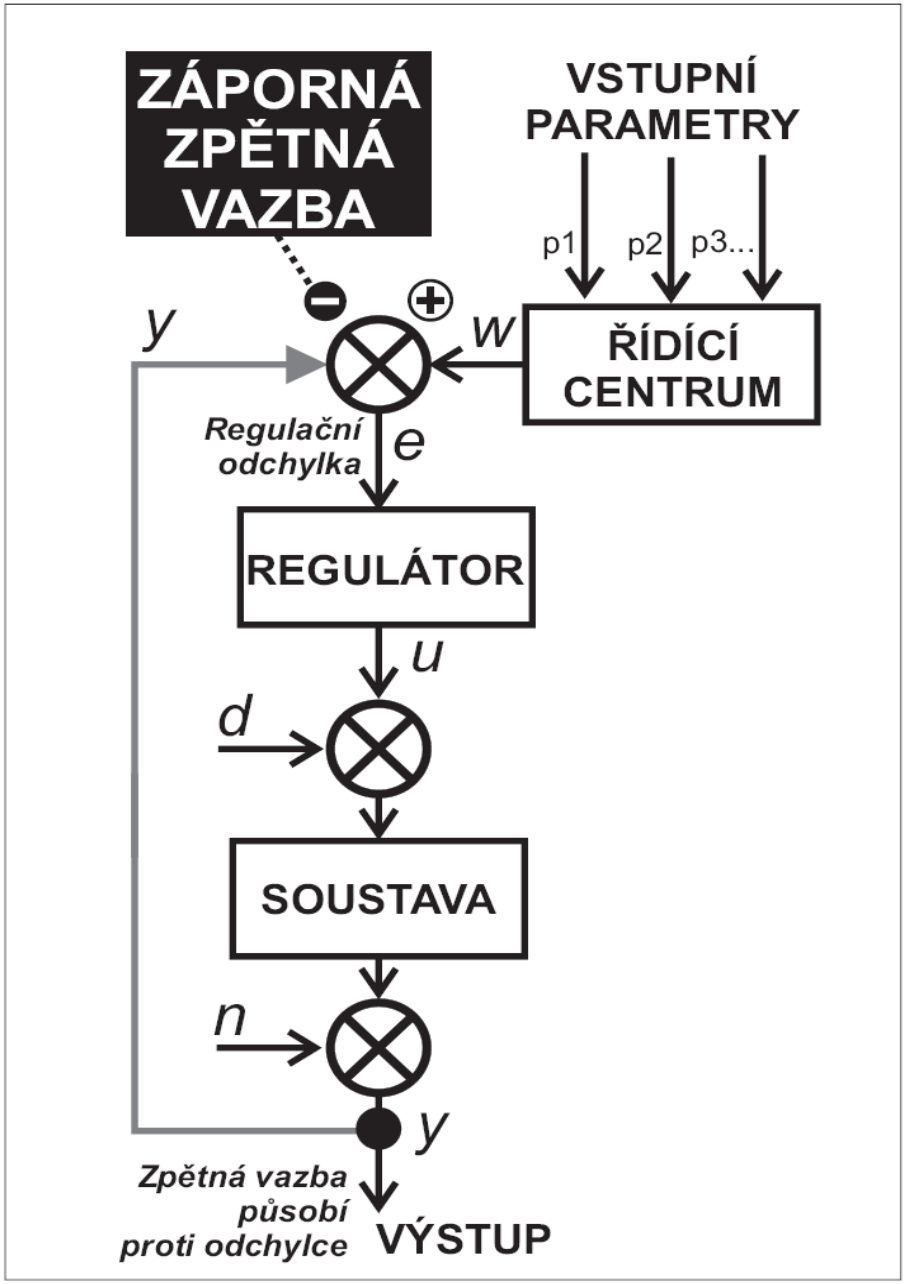

Vessel wall
Endothelium
Platelets
Plasma coagulation system

General description of control systems

Control system: Negative feed-back

y…controlled variable, I/O
w…pre-set value
e…error signal
u…actuating variable
d,n…disturbance variables

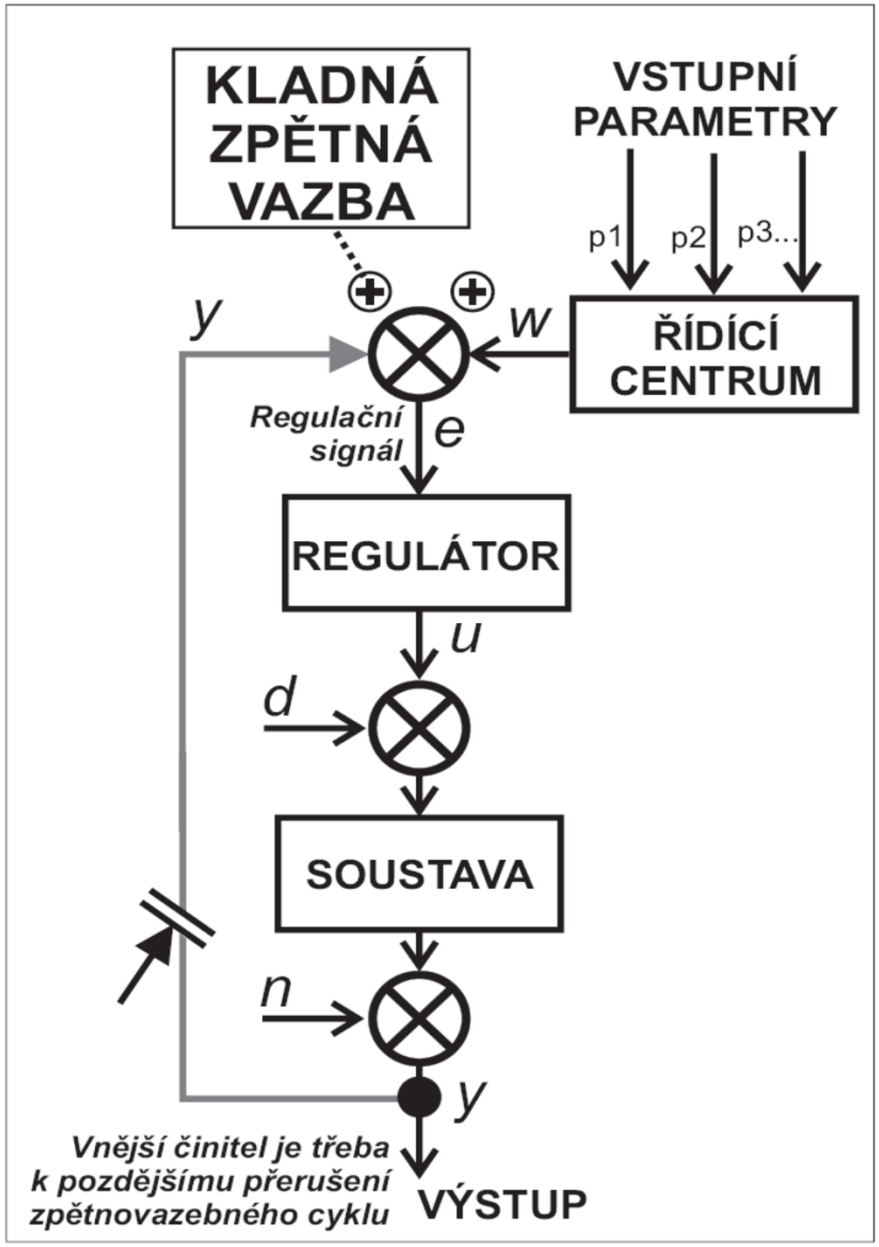
In **negative** feed-back, error signal *e* used for control is obtained by **subtraction** of the controlled variable (-*y*) from the pre-set value (+*w*), *e* = *w* - *y*.



Control system: Positive feed-back

y…controlled variable, i/o
w…pre-set value
e…error signal
u…actuating variable
d,n…disturbance variables

In **positive** feed-back, error signal *e* used for control results from **addition** of the controlled variable (+*y*) to the pre-set value (+*w*),
e = *w* + *y*.
Outer factor is needed to disconnect feedback cycle at the point from output back



Examples – negative and positive feed-back

Negative feed-back – easy, almost everything is controlled this way:
blood pressure, temperature, glycemia, …
in general – homeostasis…

positive feedback – fewer examples, more difficult:
1) in physiology/ pato-physiology:
Fever onset, ovulation, production of sex hormones in large,
„avalanche-like“ trigger reactions:
hemocoagulation, division of lymfocytes
during the immune reaction (e.g the pneumonia crisis)

2) Pathology (pathologic values of variables, vicious circles, failures).
Building up of a new, pathologic equilibrium, example: adaptation to the lower PO2
failure of blood pressure control -> shock, hypo-perfusion, hypoxia…

Endothelium

Antithrombotic Properties

Anti-platelet activities:

- **Endothelium covers highly thrombogenic basal membrane**
- **Uninjured endothelium does not bind platelets**
- **PGI2 (prostaglandin) and NO (nitric oxide) from endothelium inhibit platelet binding**
- **ADPase counters the platelet aggregating effects of ADP**

Endothelium

Antithrombotic Properties

Anticoagulant activities:

- **Heparin-like molecules ... activate anti-thrombin III (inactivates active proteases)**
- **Thrombomodulin ... changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa**
- **tPA (tissue plasminogen activator) ... activates fibrinolysis via plasminogen to plasmin**

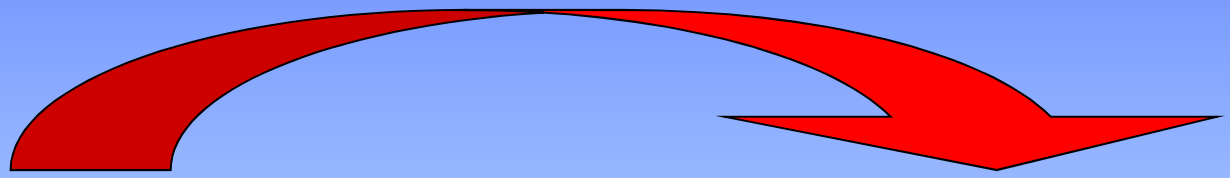
Endothelium

Prothrombotic Properties

- Synthesis of von Willebrand factor
- Release of tissue factor
- Production of PAI (plasminogen activator inhibitors)
- Membrane phospholipids bind and facilitate activation of clotting factors via Ca2+ bridges

Endothelium

Vessel injury

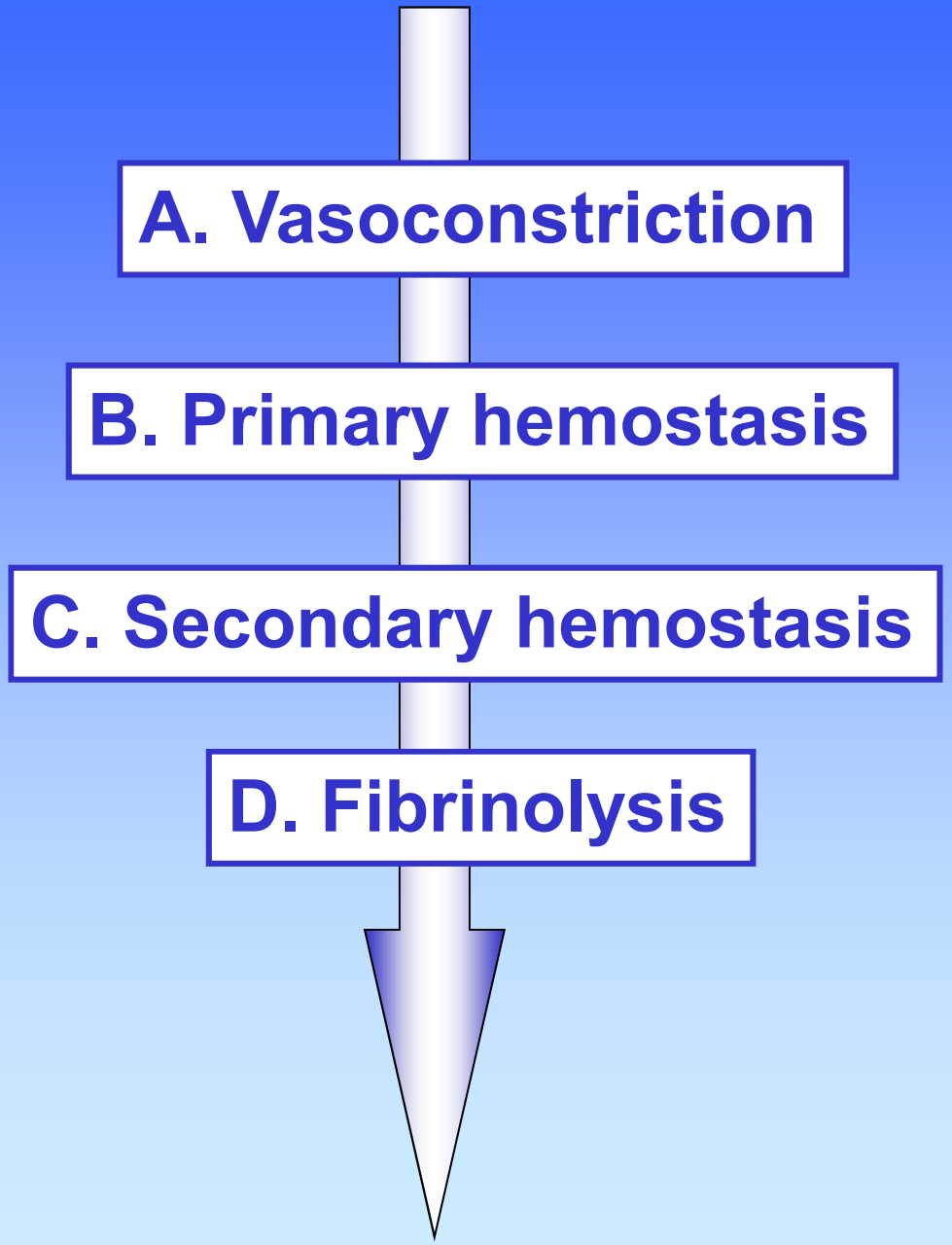


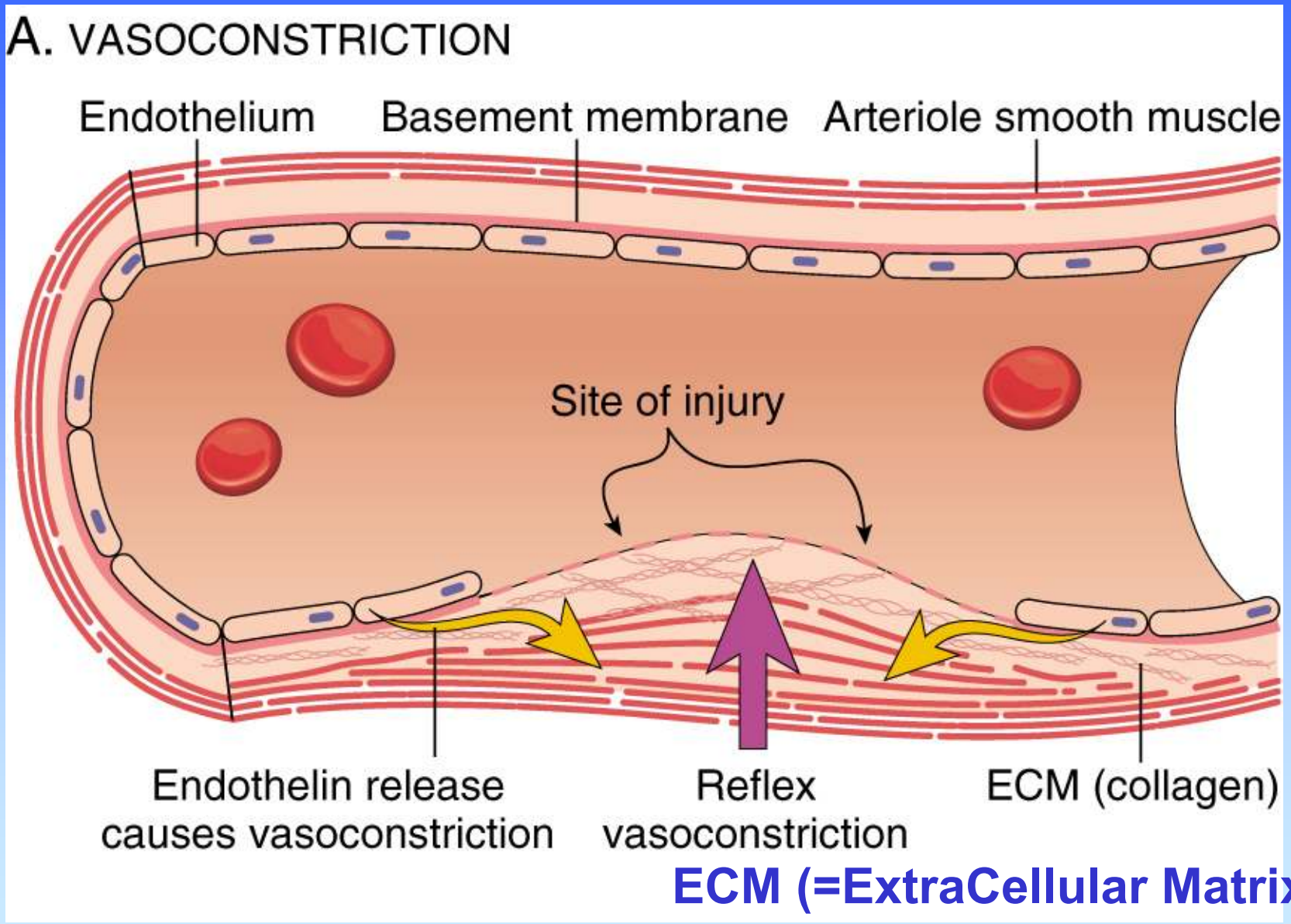
Antithrombogenic

(Favors fluid blood)

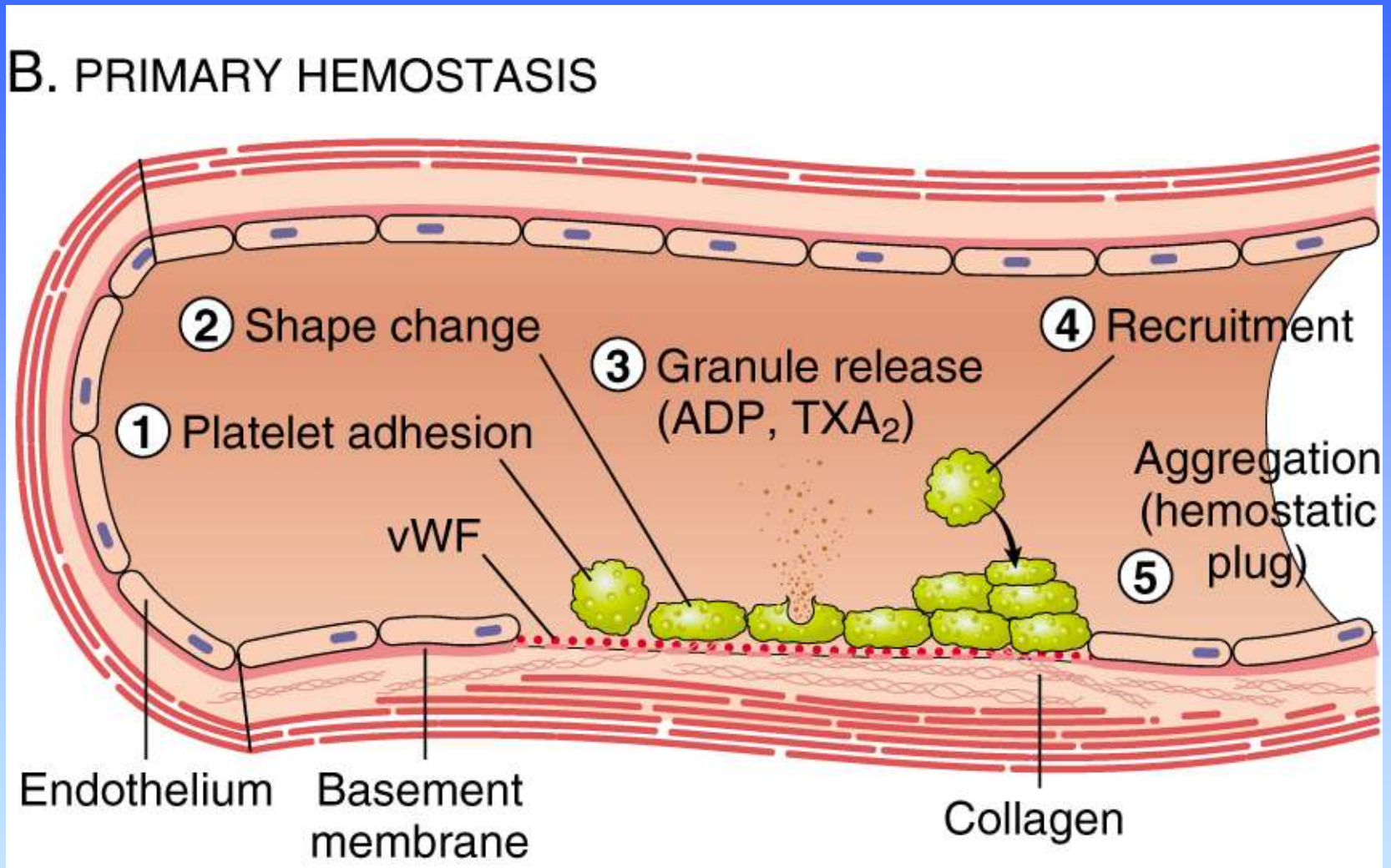
Thrombogenic

(Favors clotting)

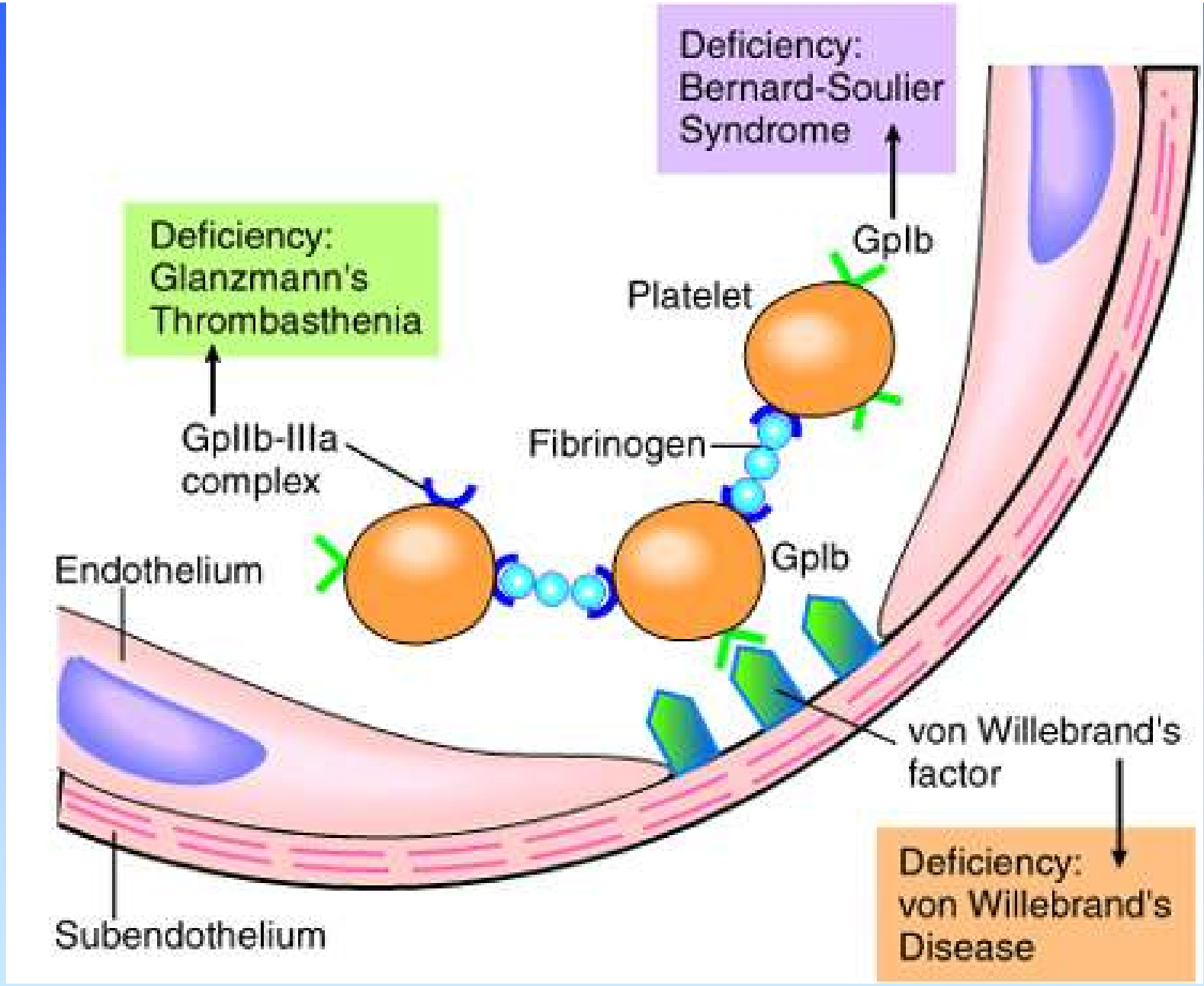

A. Vasoconstriction
B. Primary hemostasis
C. Secondary hemostasis
D. Fibrinolysis



ECM (=ExtraCellular Matrix)

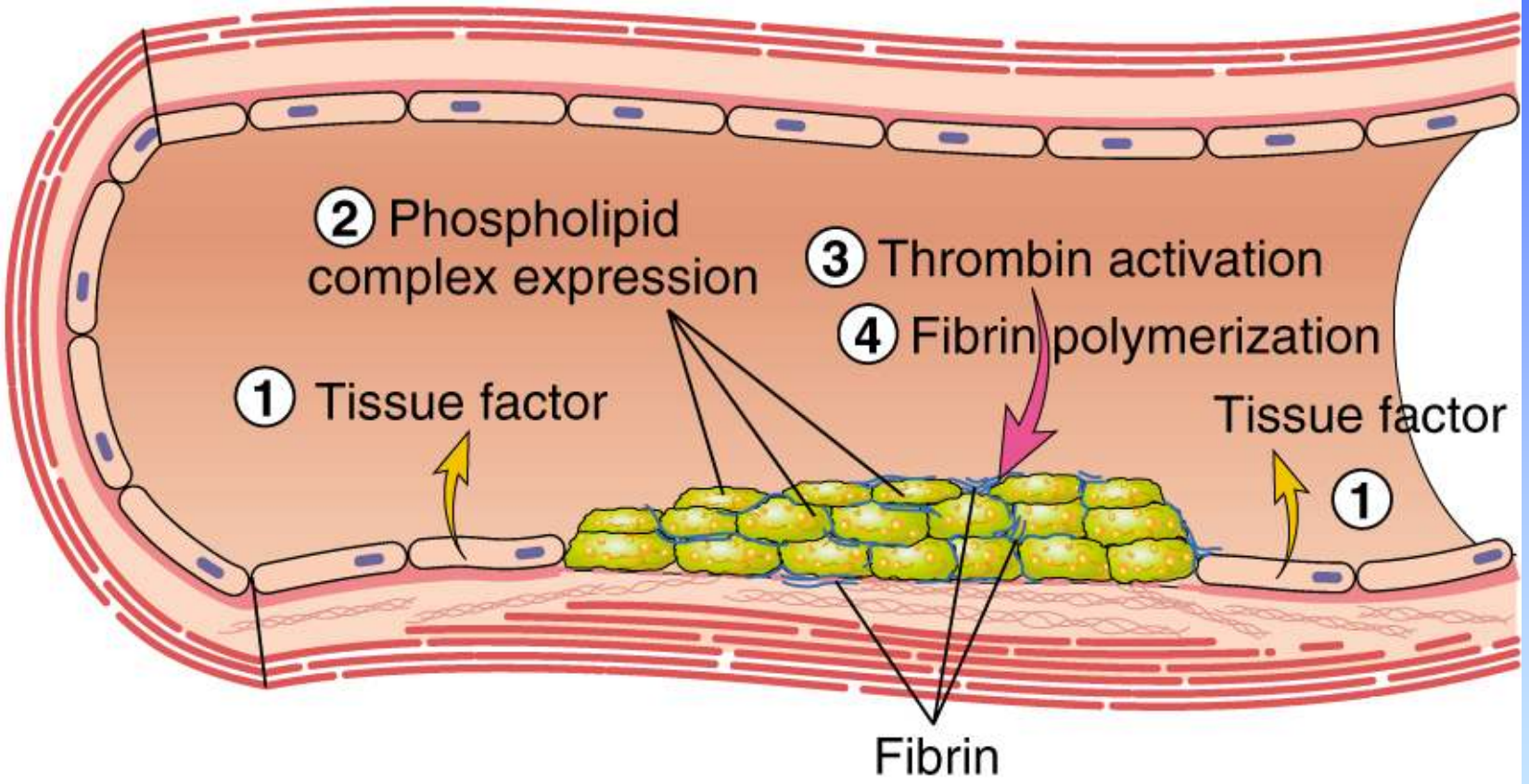


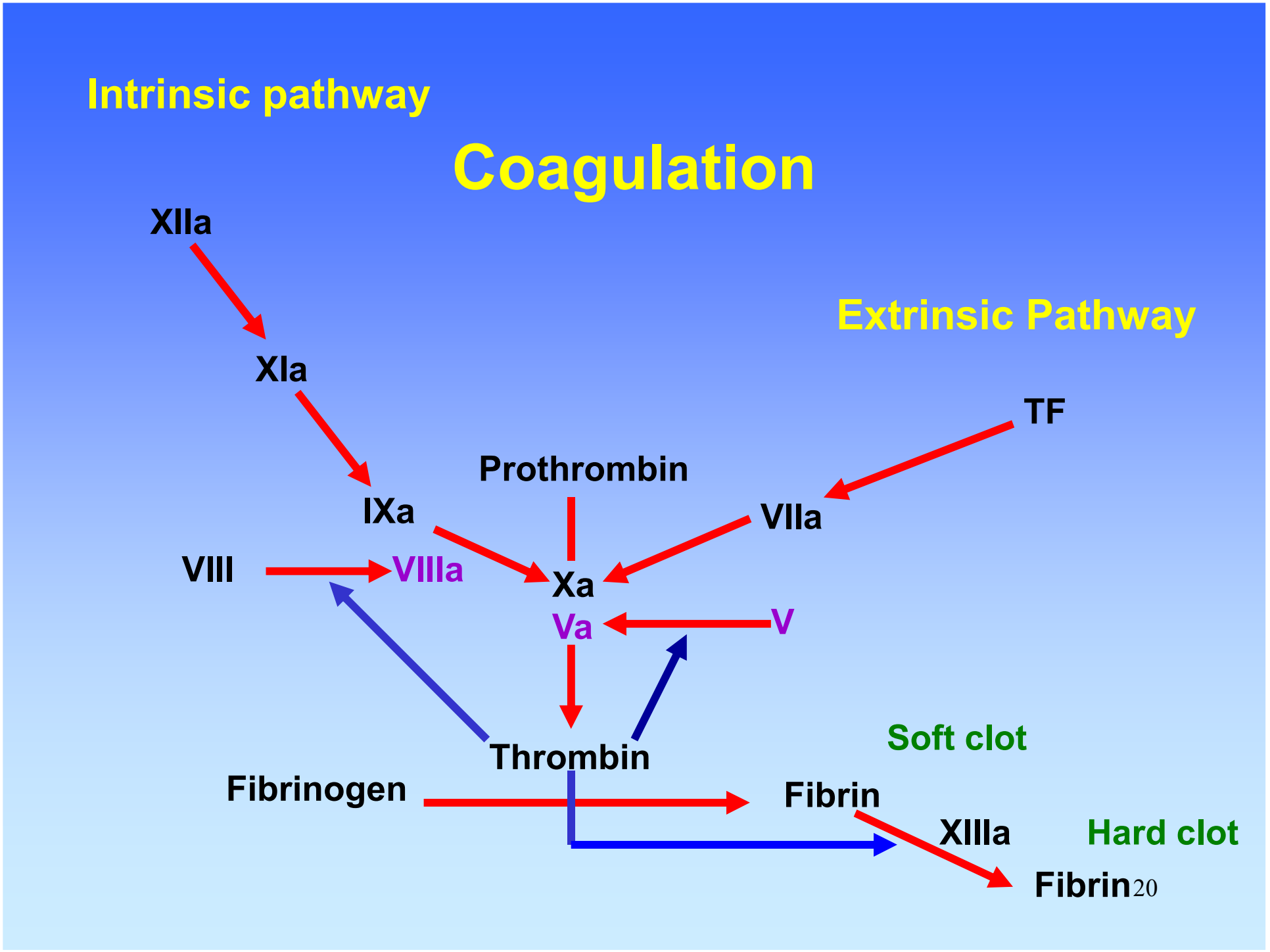
TXA2 (thromboxane A2, lipid)

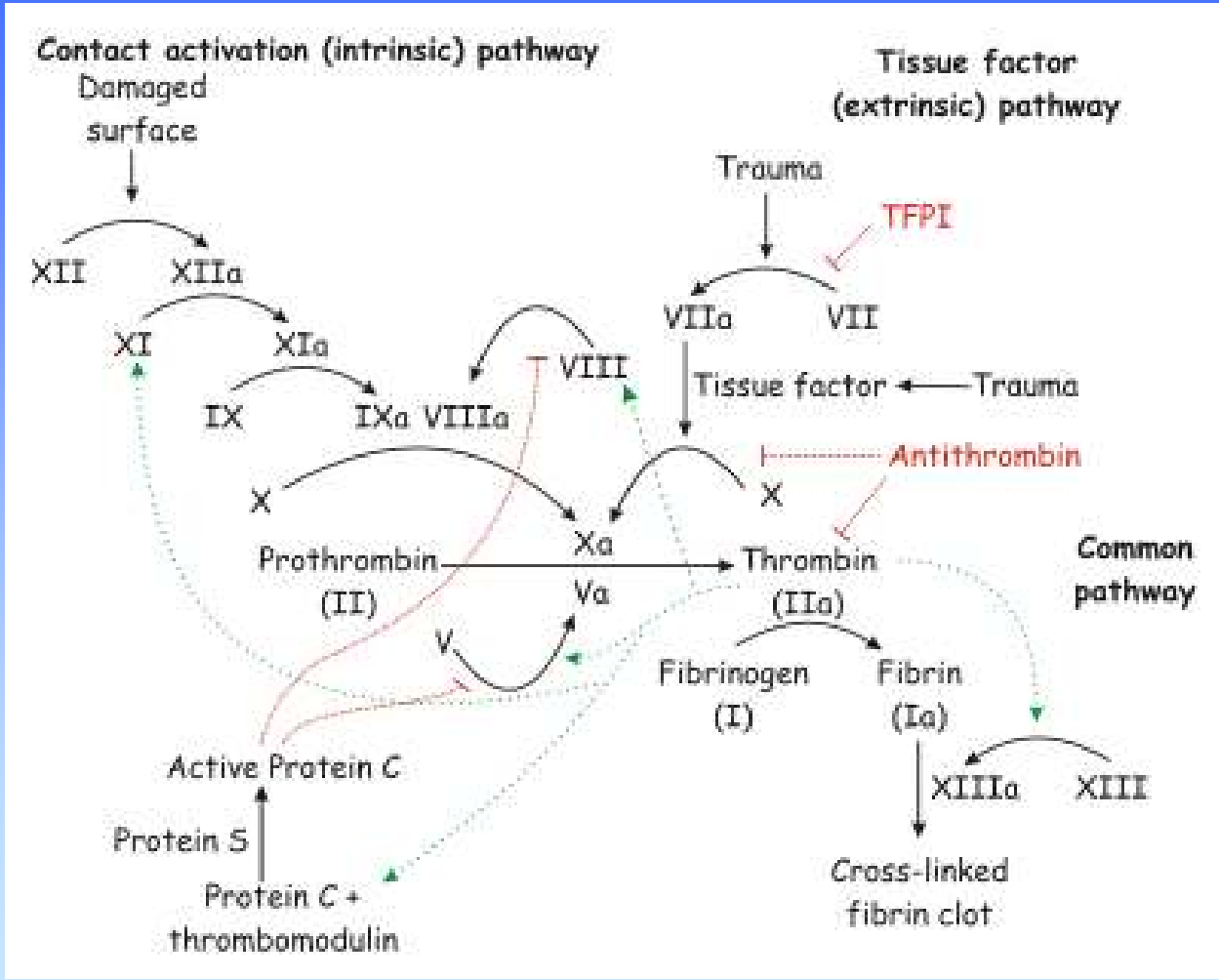


Gp – G-protein coupled receptors

C. SECONDARY HEMOSTASIS




Intrinsic pathway
Coagulation
XIIa
XIa
IXa
VIII
VIIIa
Prothrombin
Xa
Va
V
Extrinsic Pathway
TF
VIIa
Thrombin
Fibrinogen
Fibrin
Soft clot
XIIIa
Hard clot
Fibrin


Contact activation (intrinsic) pathway
Damaged surface
XII
XIIa
XI
XIa
IX
IXa VIIIa
VIII
X
Xa
Va
Prothrombin (II)
Thrombin (IIa)
V
Active Protein C
Protein S
Protein C + thrombomodulin
Tissue factor (extrinsic) pathway
Trauma
TFPI
VIIa
VII
Tissue factor
Trauma
Antithrombin
X
Common pathway
Fibrinogen (I)
Fibrin (Ia)
XIIIa
XIII
Cross-linked fibrin clot

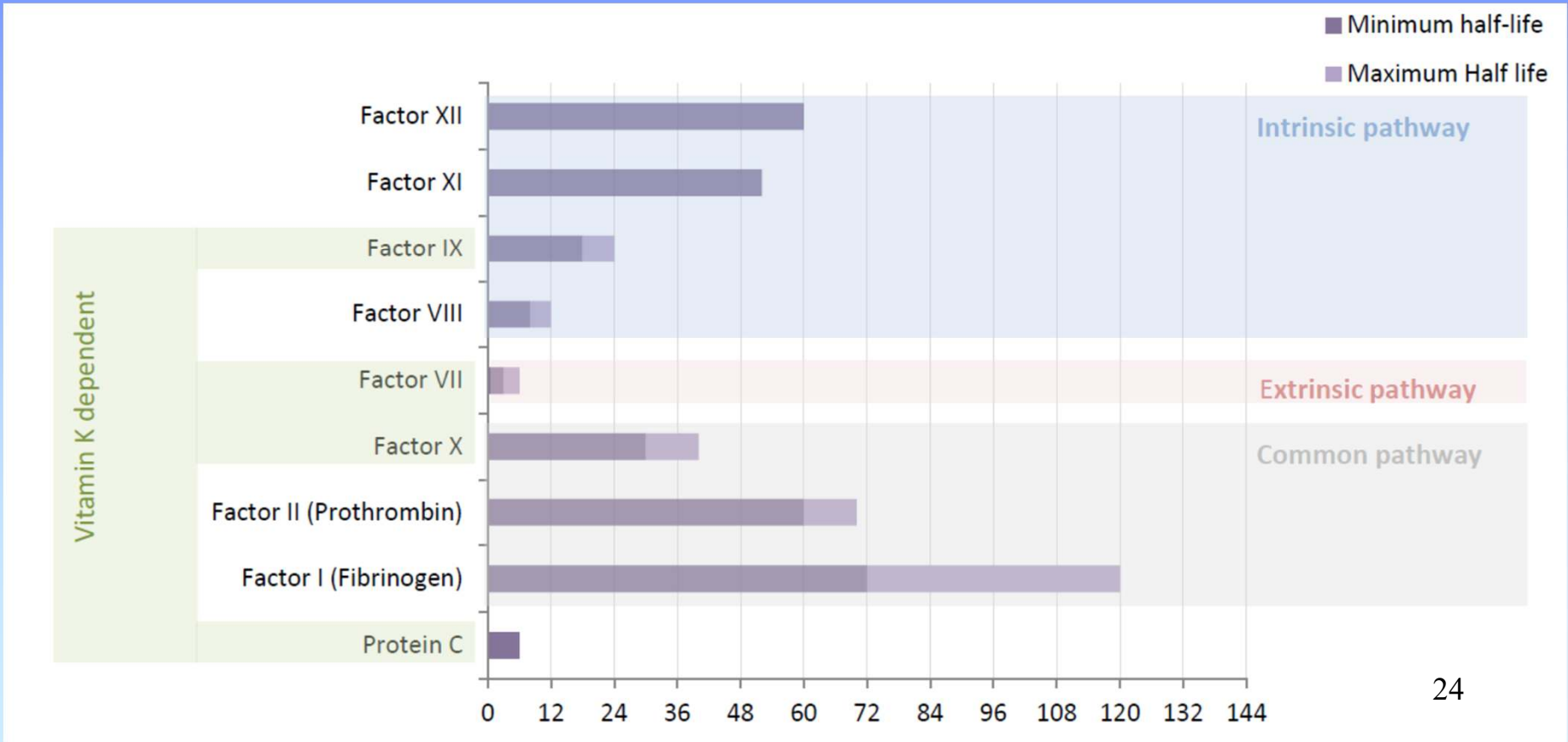
Coagulation

- Enzymatic cascade (amplification)
- Several serine proteases
- Produced by liver (most)
- Require vitamin K (several, 2, 7, 9, 10, C, S)
- Requires Ca^{2+} (the same, 2, 7, 9, 10, C, S)
- 3 protein cofactors (not enzymes)
- Reversible (via production of plasmin)

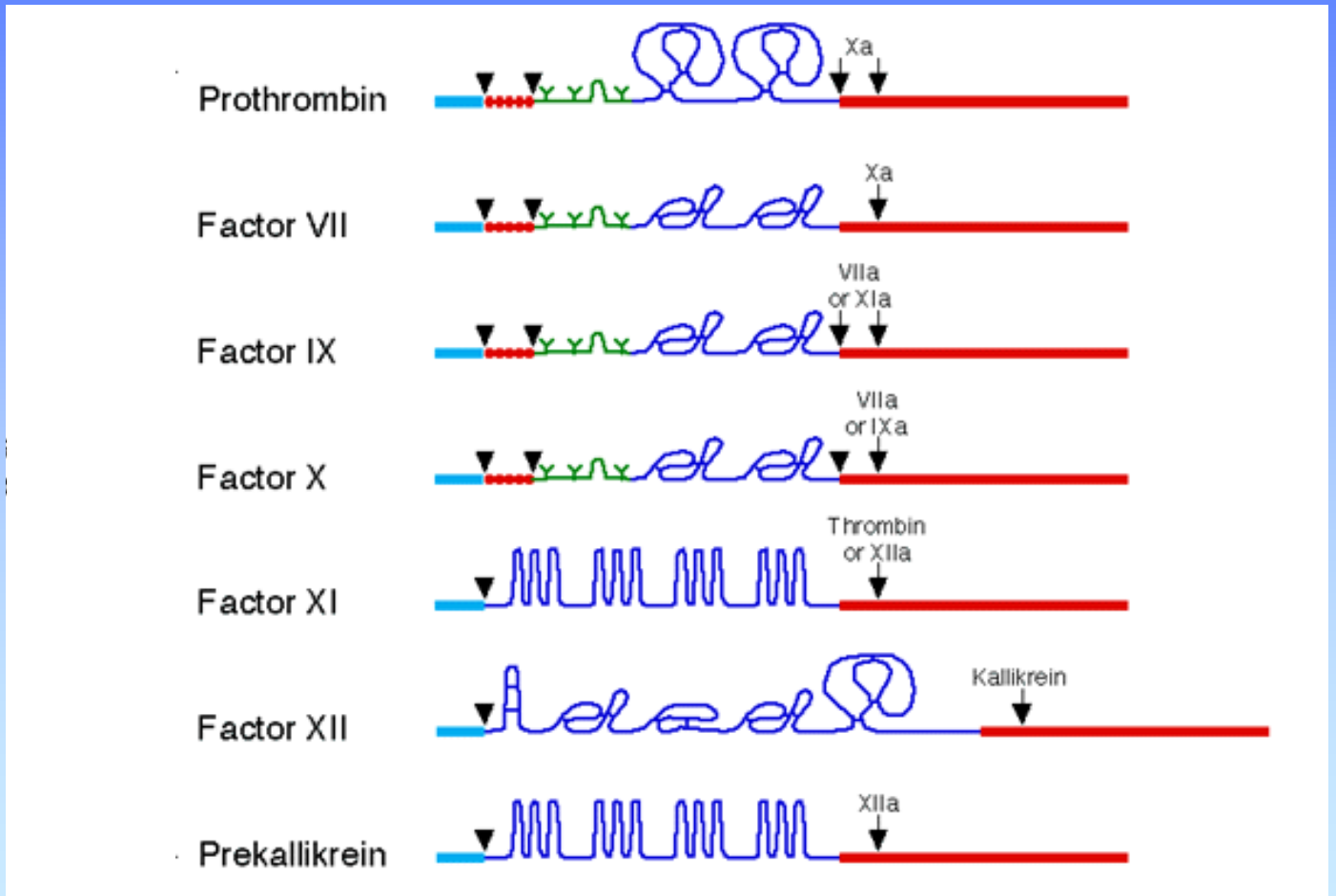
Coagulation

Factor	Name	Molecular Weight	Plasma concentration (µg/ml)	Required for hemostasis (% of normal)	Vit K dependency	Natural source
I	Fibrinogen	330,000	3000	30	No	Liver
II	Prothrombin	72,000	100	40	Yes	Liver
III	Tissue factor		--		No	Tissue
IV	Calcium ion		--	--	No	Plasma
V	Proaccelerin	300,000	10	10-15	No	Liver
VII	Proconvertin	50,000	0,5	5-10	Yes	Liver
VIII	Antihemophilic	300,000	0,1	10-40	No	RES
IX	Thromboplastin	56,000	5	10-40	Yes	Liver
X	F. Stuart	56,000	10	10-15	Yes	Liver
XI	Prethromboplastin	160,000	5	20-30	No	Liver
XII	F. Hageman	76,000	30	0	No	Liver
XIII	Fibrin stabilizing	320,000	30	1-5	No	Liver
vWF	Von Willebrand	140,000			No	Endothelium
Prot C					Yes	Liver
PKLK	Prekallikrein	82,000	40	0		
HMWK	HMW Kallikrein	108,000	100	0		

Half lives of coagulation factors

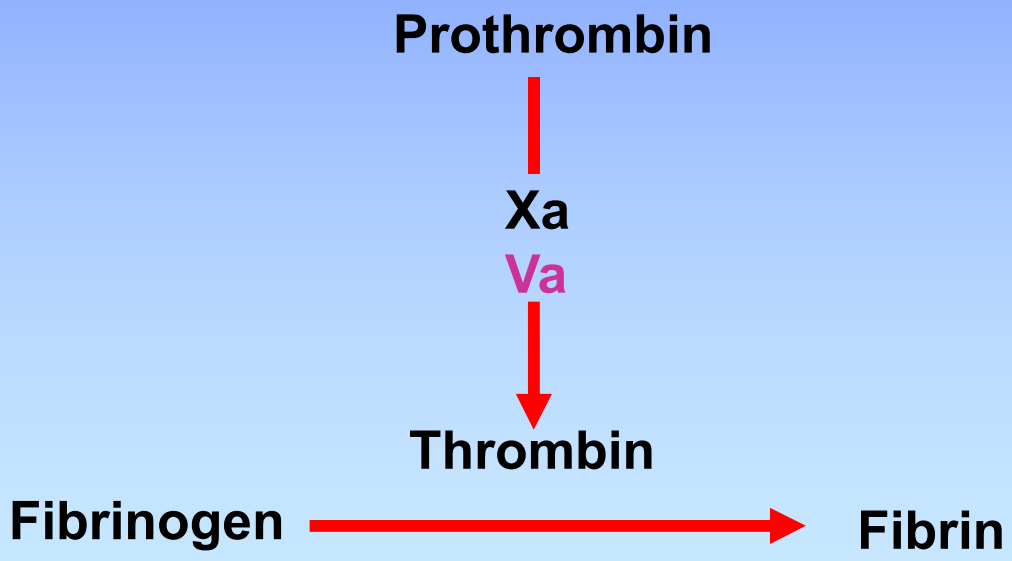


Coagulation

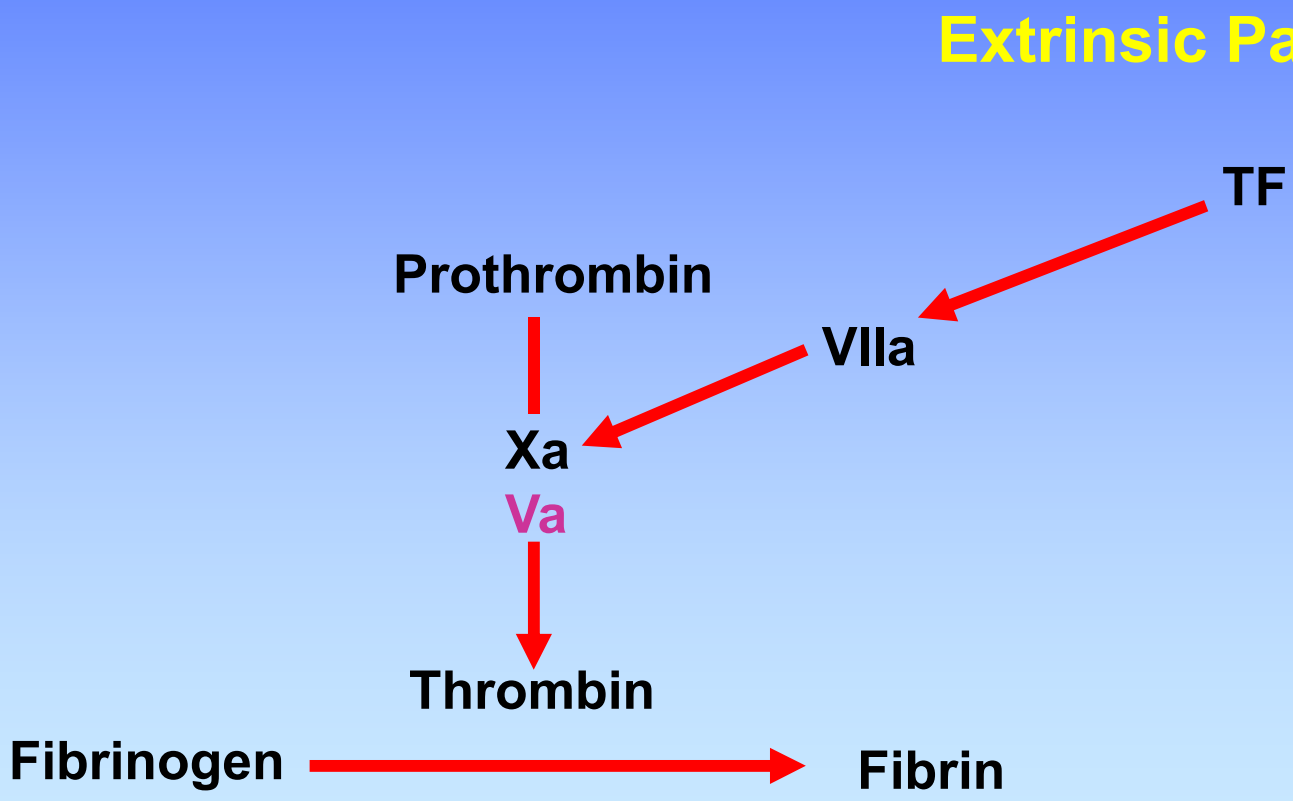


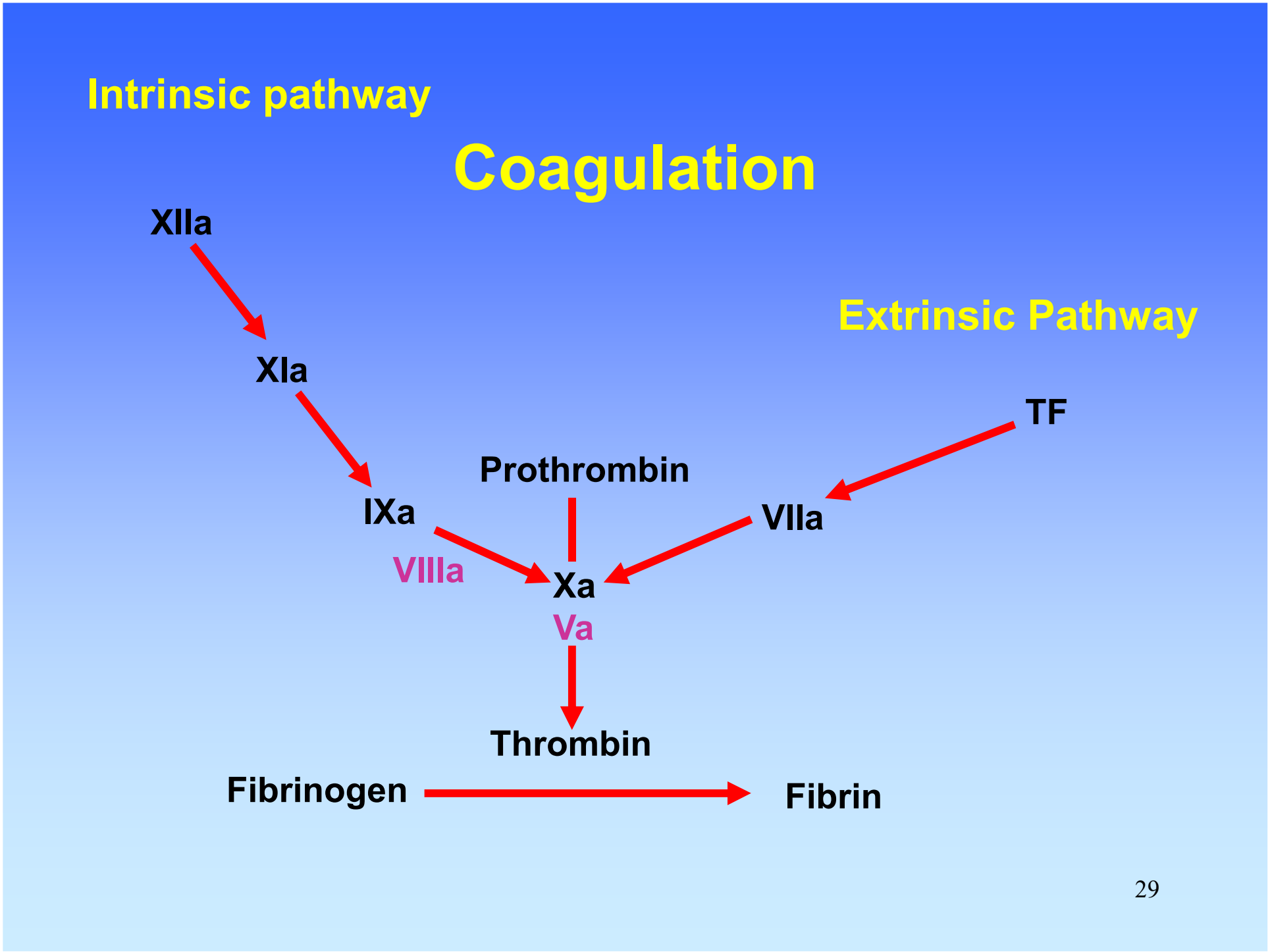
Coagulation

Coagulation



Coagulation

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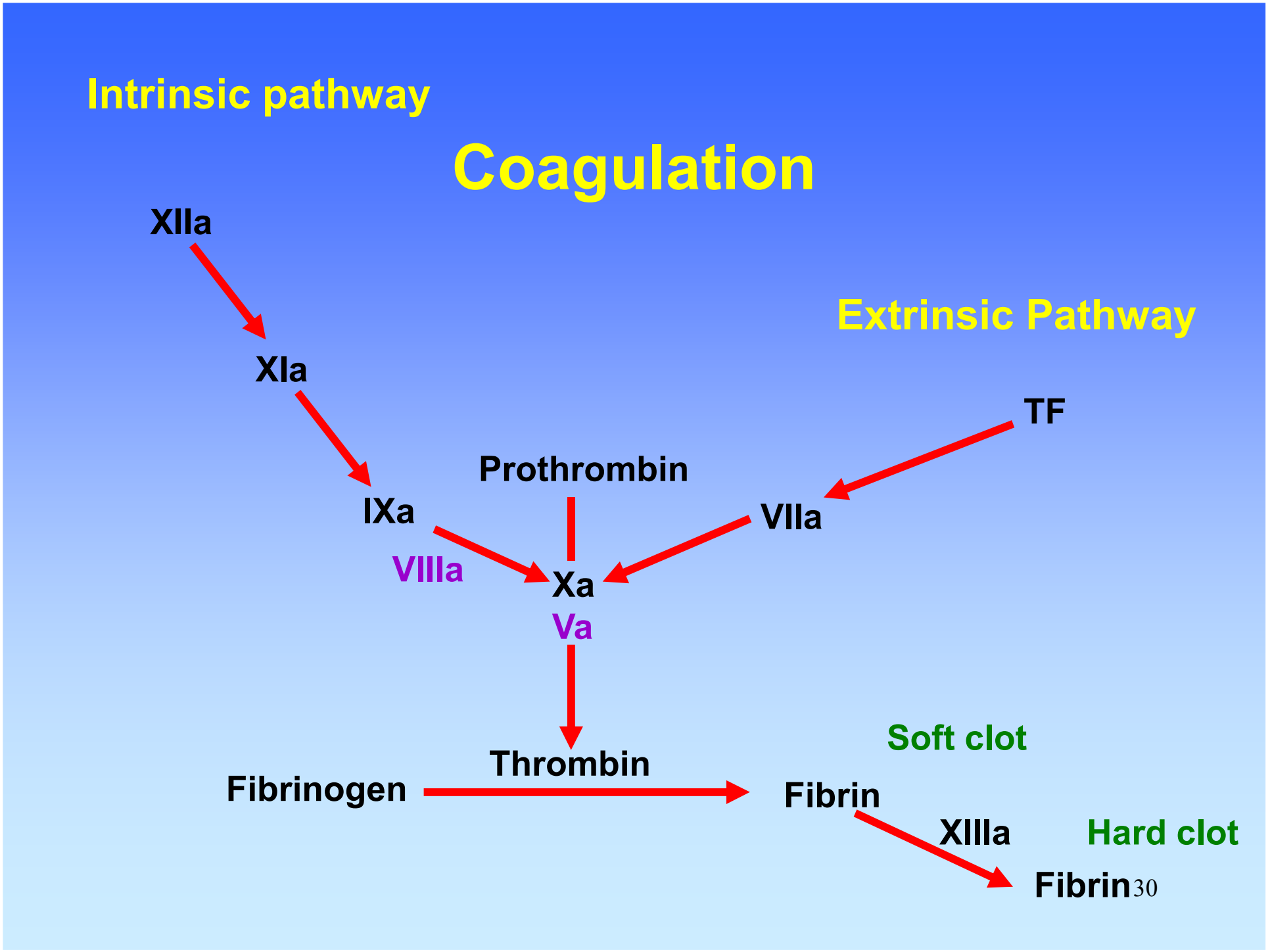
Fibrinogen
Fibrin
Extrinsic Pathway
TF
VIIa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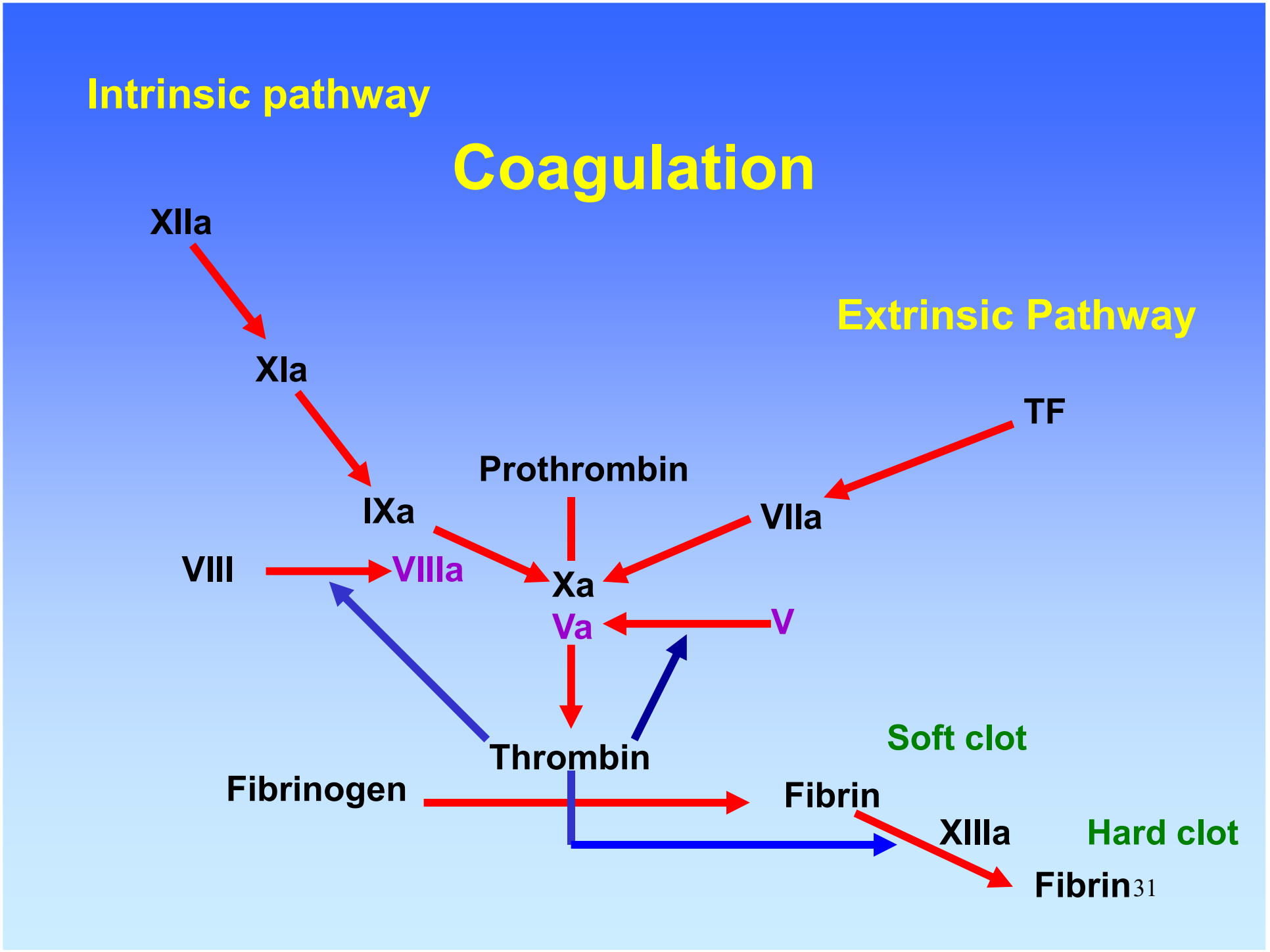
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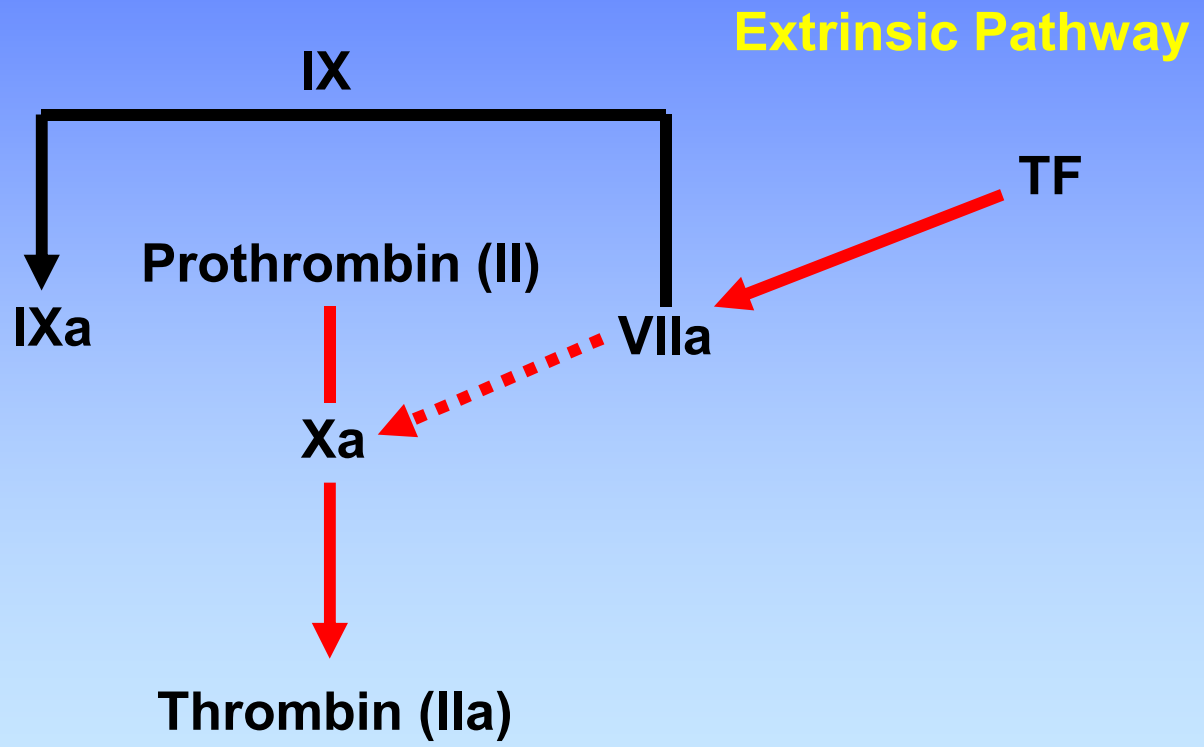
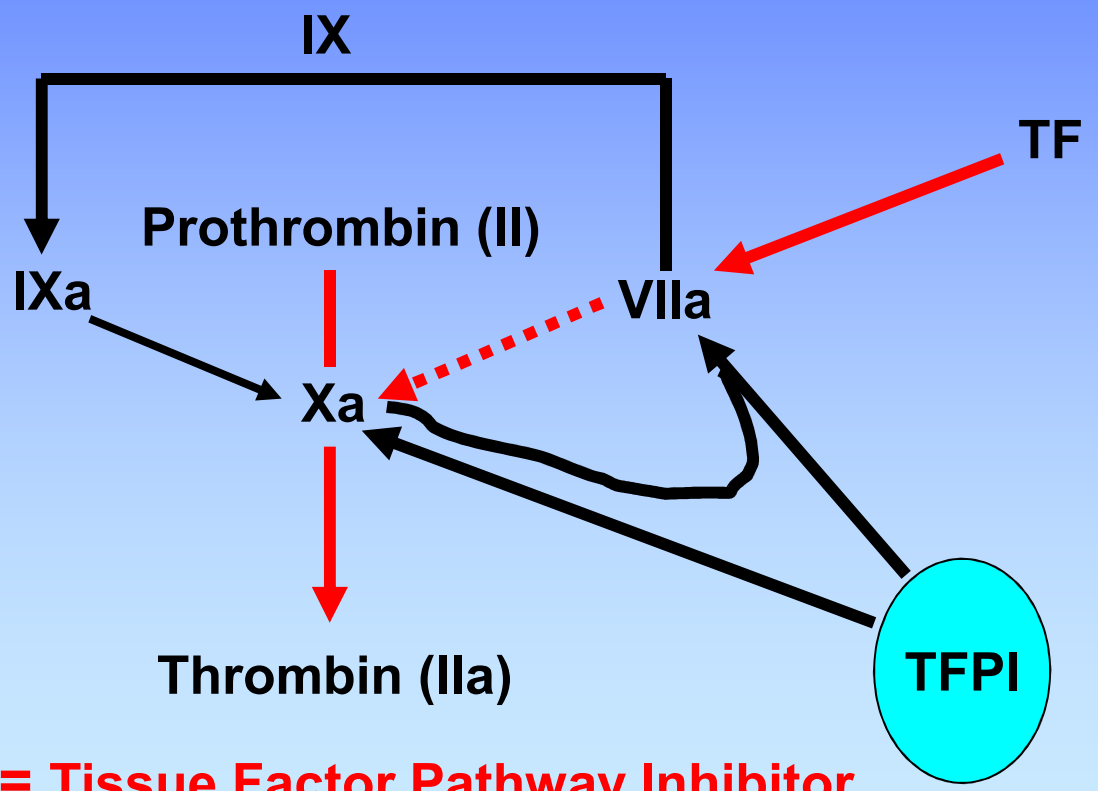
Fibrin

Revised tissue factor pathway



New: Production of IXa
Interaction of intrinsic and extrinsic pathways

Revised tissue factor pathway



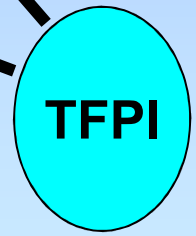
New: TFPI = Tissue Factor Pathway Inhibitor
... inhibition of Xa and VIIa

Revised tissue factor pathway

TFPI is protease inhibitor
34 and 41 kD forms in plasma (C-term truncation)

Activities:
- direct inhibition of Xa
- inhibition VIIa-TF complex in a [Xa]-dependent manner
- binding to LDL, HDL and Lp (a)

~10% present in platelets (endothelium also)



New: TFPI = Tissue Factor Pathway Inhibitor
... inhibition of Xa and VIIa

Revised tissue factor pathway

Net results:

Production of IXa

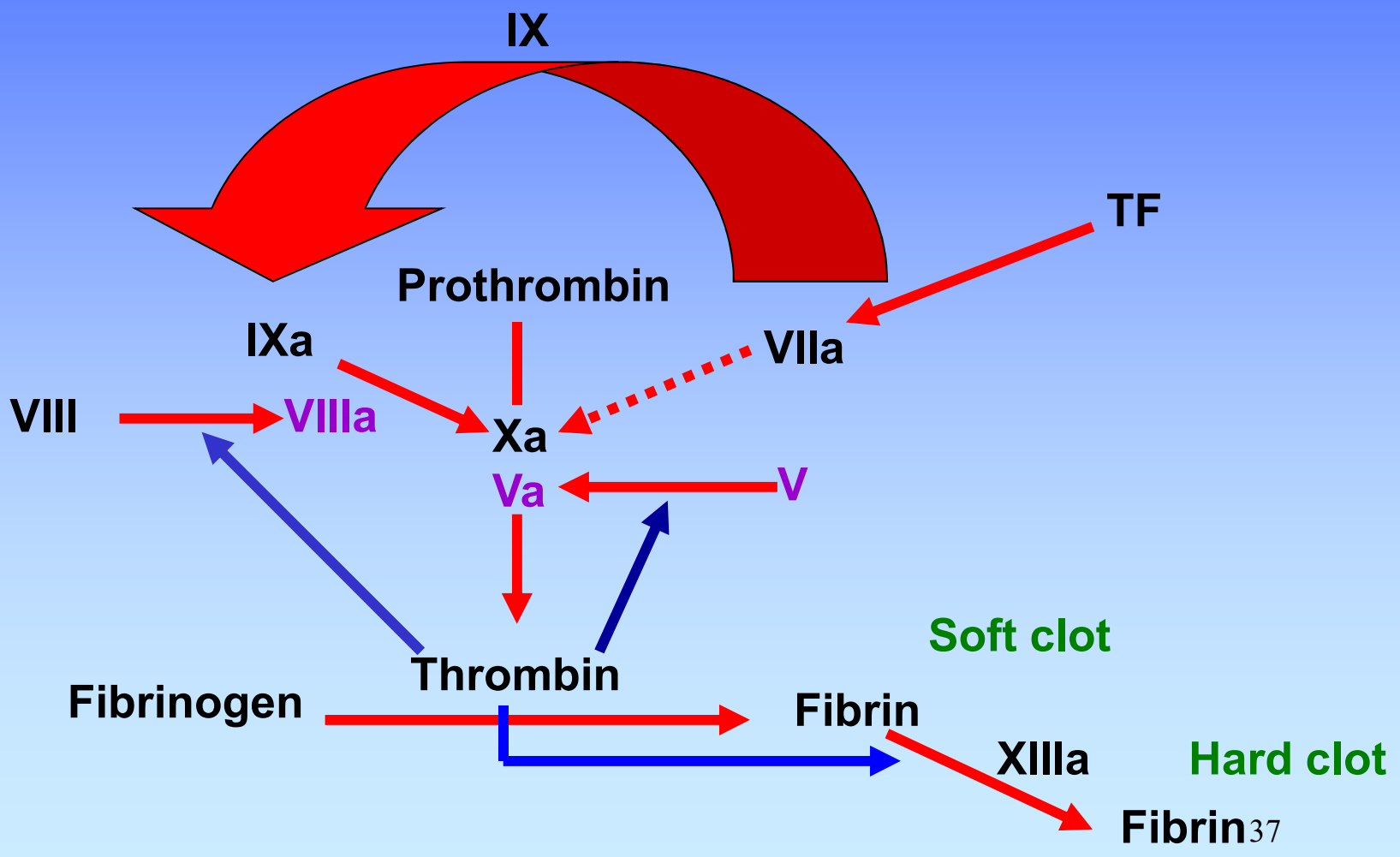
Production of small amounts of thrombin (IIa)

No or only little fibrin formed!

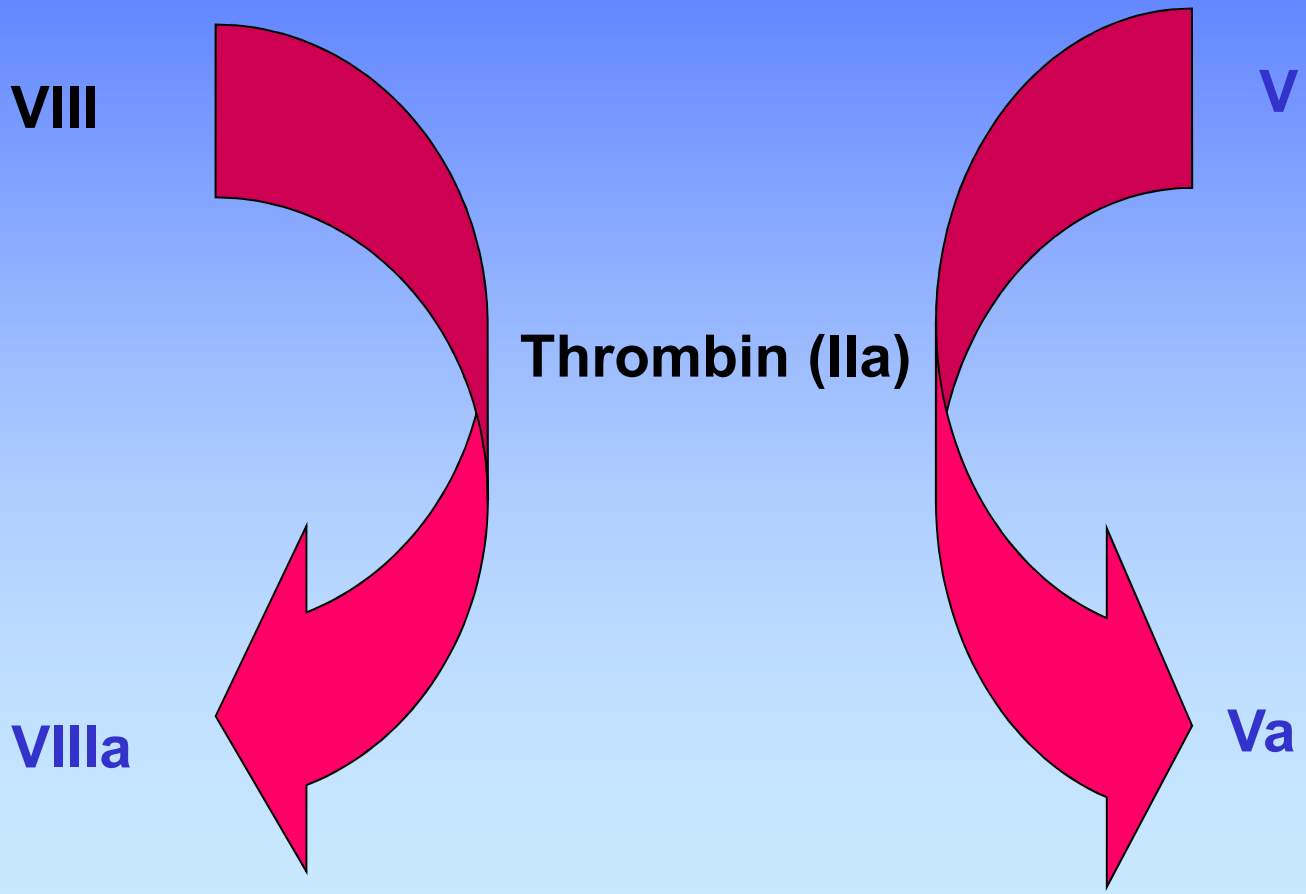
Revised tissue factor pathway

- VIIa forms via binding of VII to TF
- VIIa activates some X→Xa
- Xa converts a small amount of II to IIa; this thrombin is used to produce small amts of VIIIa and Va
- As the concentration of TF-VIIa-Xa-IIa increases, TFPI inactivates this complex stopping further production of thrombin.
- IXa, with VIIIa (produced as above), produces Xa; this Xa with Va produces new thrombin; this thrombin produces more VIIIa and Va and then we get lots of thrombin and fibrin.

Revised tissue factor pathway



Revised tissue factor pathway



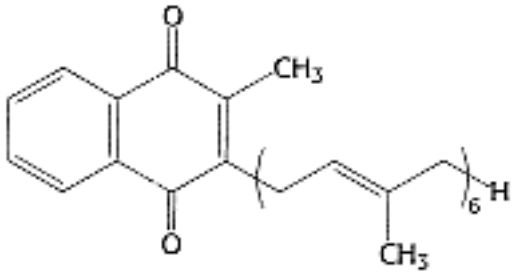
Role of vitamin K

Factors II, VII, IX, X, proteins C and S require a post-translational modification (PTM) before their activation

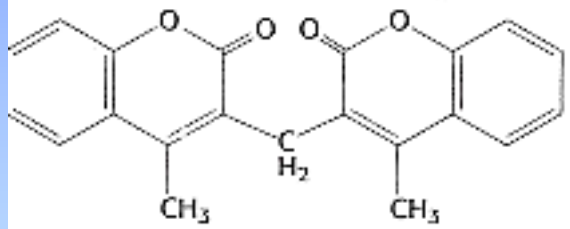
This PTM requires vitamin K

This PTM involves the addition of a COO^- to certain Glu residues in the clotting factors

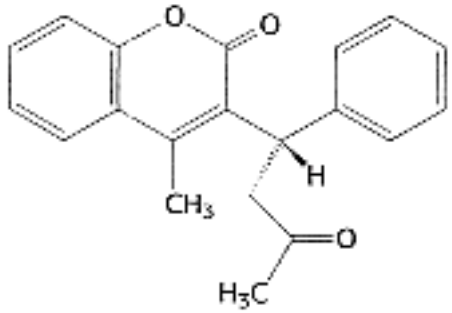
resulting in the formation of several gamma-carboxy glutamates



Vitamin K

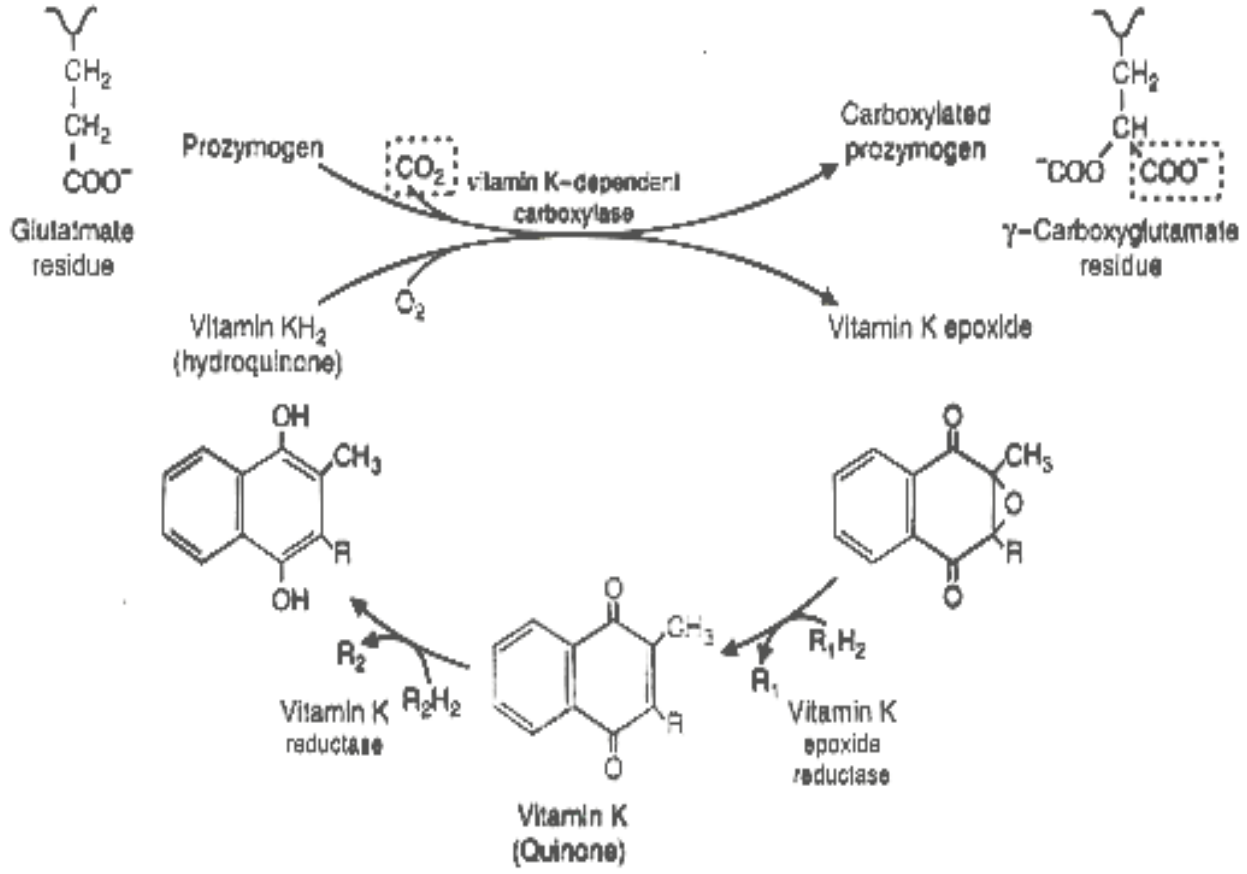


Dicoumarol



Warfarin

Role of vitamin K

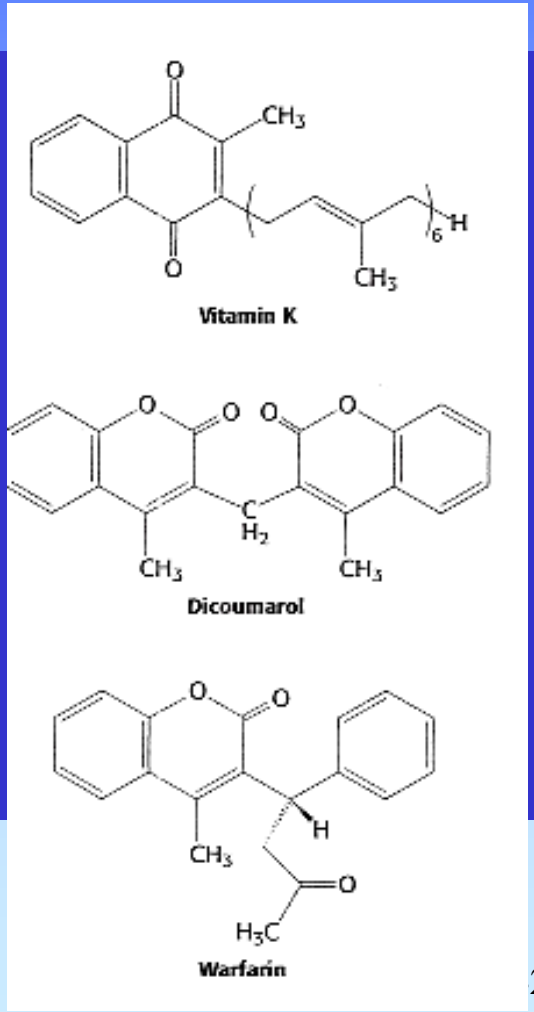


Physiologic inhibitors of coagulation

- **Antithrombin III**
  - SERPIN
- **Activated Protein C + protein S**
  - Inactivates Va and VIIIa (via proteolysis)
  - mutation: Factor V Leiden (APC resistance)
- **Thrombomodulin**
  - Binds to thrombin
  - Decreases ability to produce fibrin
  - Increases ability to activate Protein C

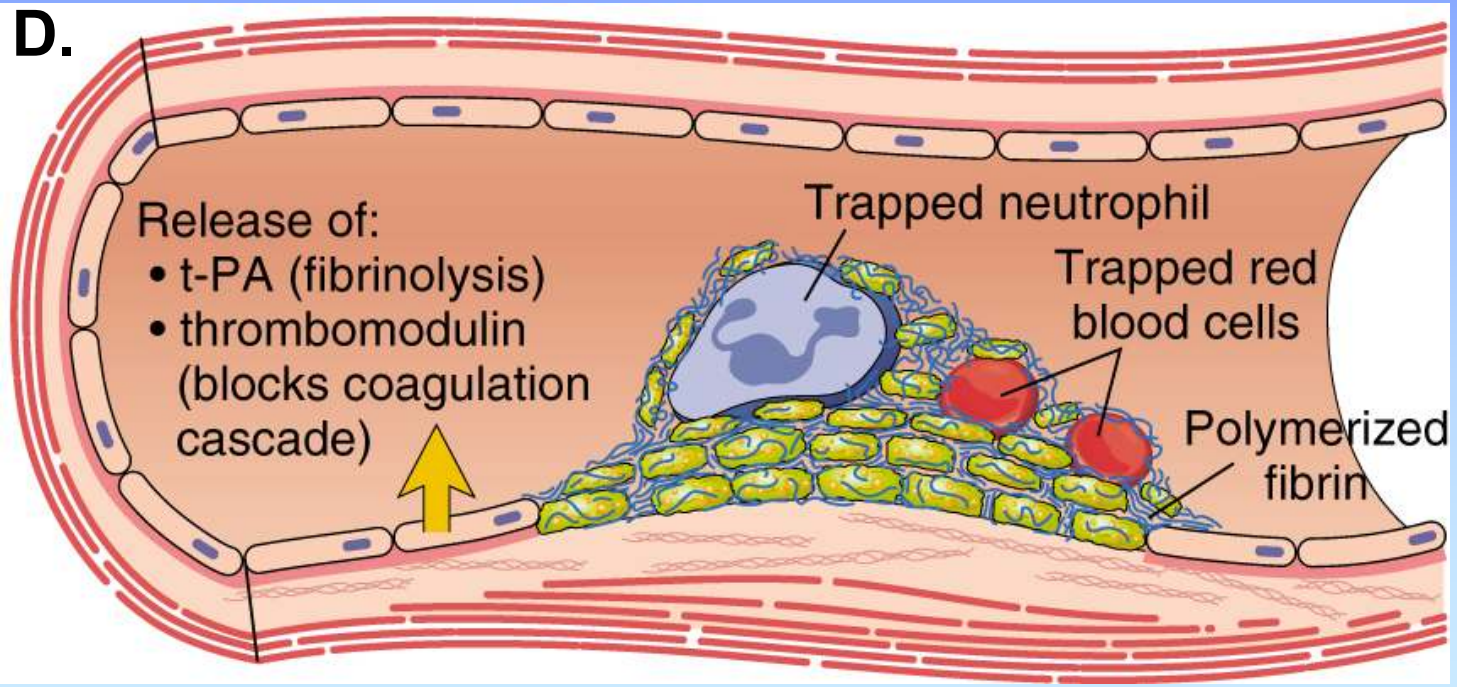
Non-physiologic inhibitors of coagulation

- **Vitamin K antagonists**
  (in vivo only)
- **Ca++ chelators**
  (in vitro only)
  - EDTA
  - Citrate
  - Oxalate
- **Heparin**
  (in vivo and in vitro)



Fibrinolysis

... Clot removal



Fibrinolysis

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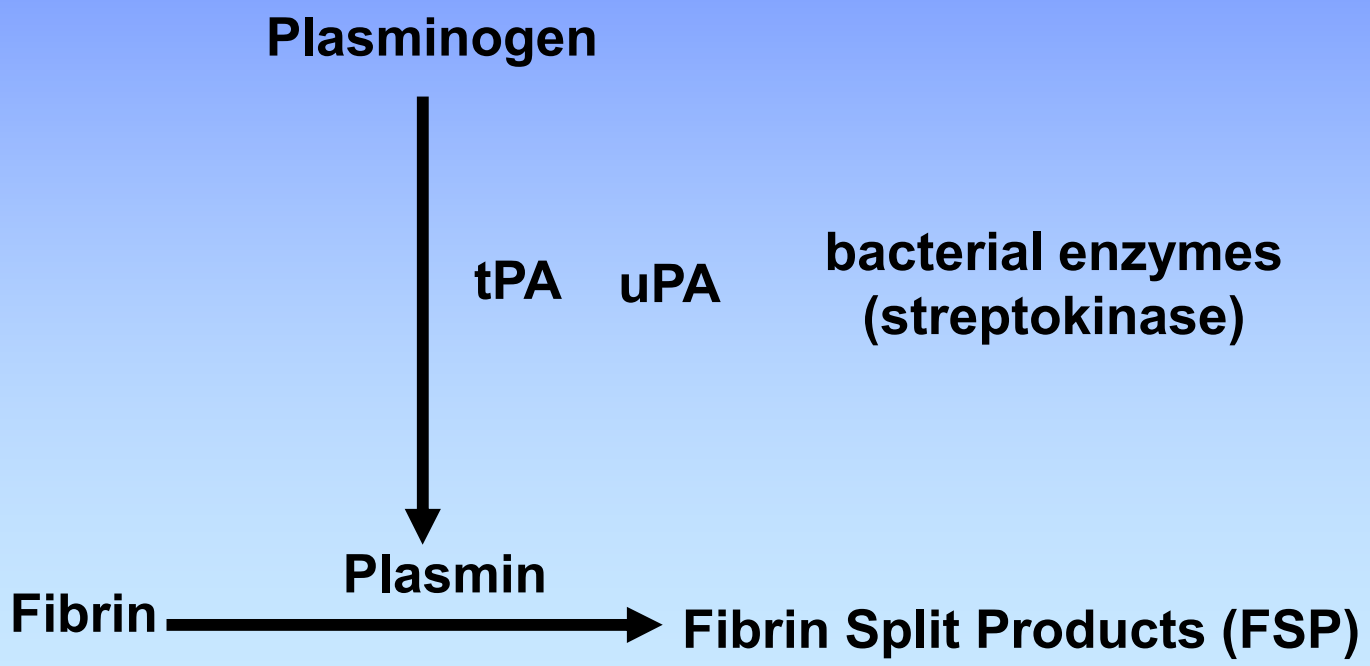

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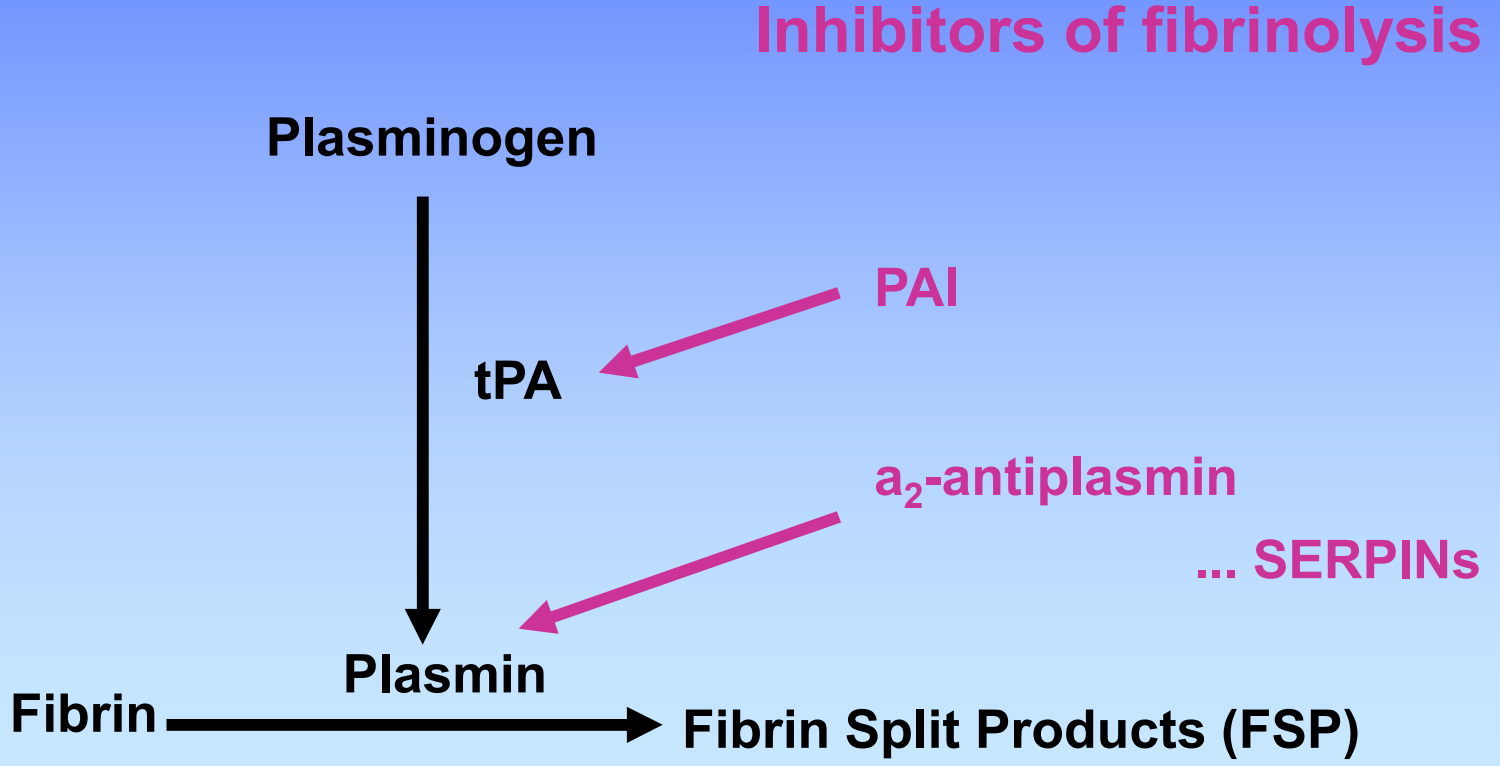
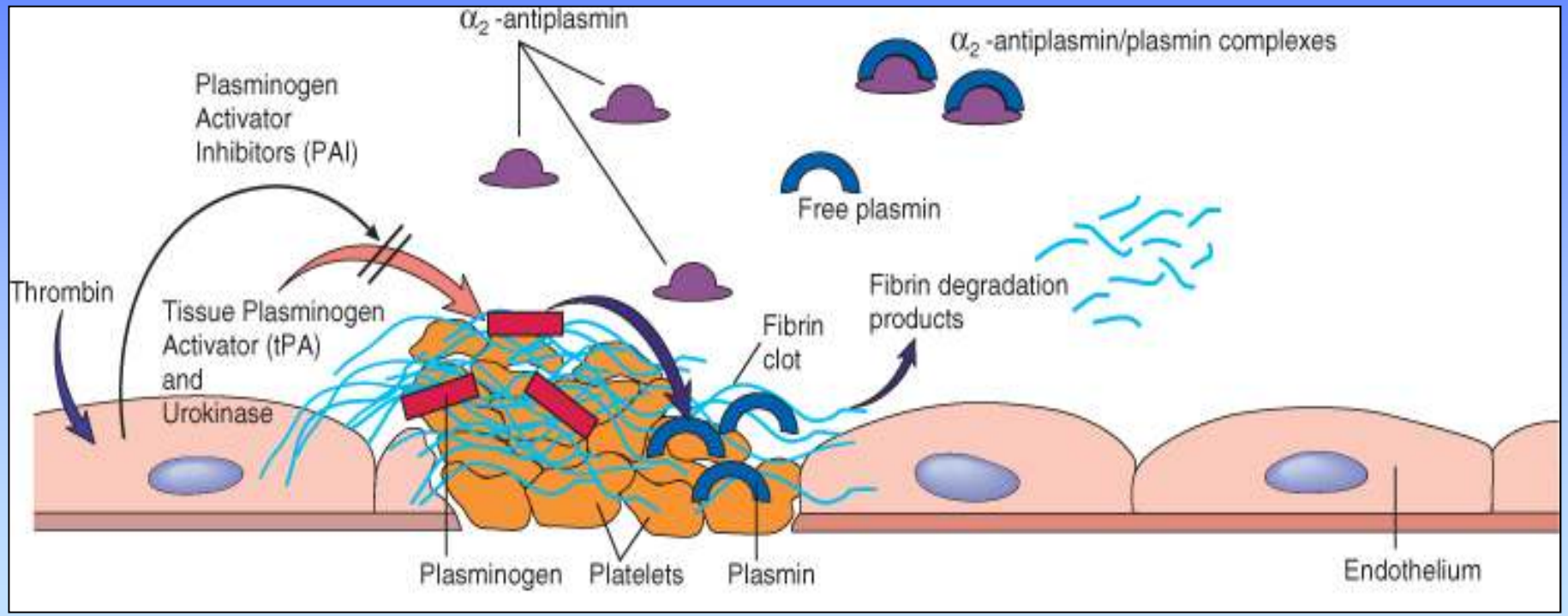
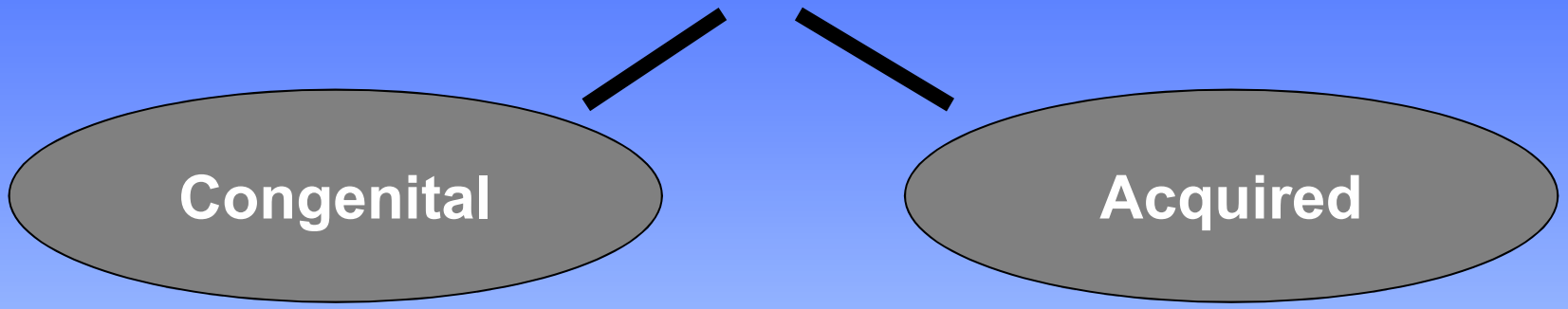


Fibrinolysis

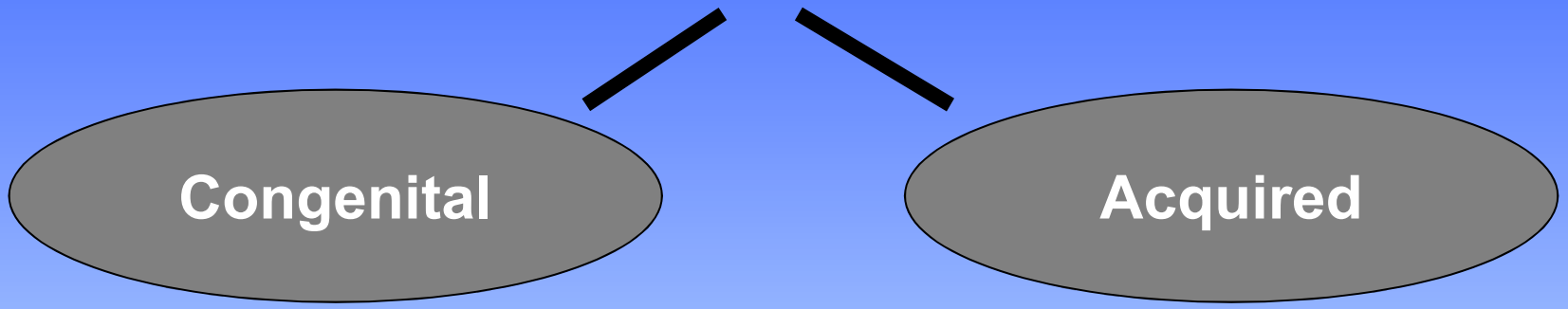


II.
Pathology

Coagulopathies

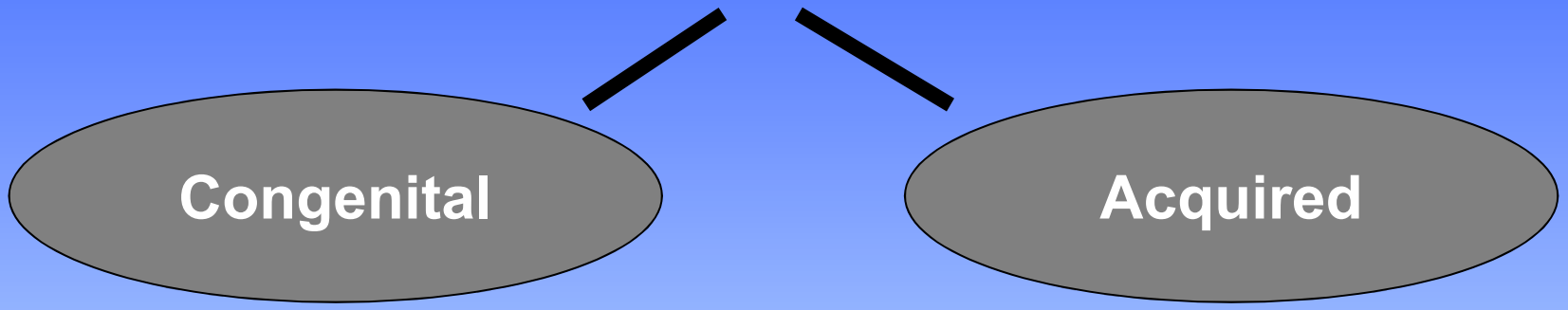


Coagulopathies



Hemophilia A ... f VIII
Hemophilia B ... f IX
Hemophilia C ... f XI
Dys- / A- fibrinogenemia
F V defic. (parahemophilia)
F XIII defic.
APC resistance

Coagulopathies



Congenital

- Hemophilia A ... f VIII
- Hemophilia B ... f IX
- Hemophilia C ... f XI
- Dys- / A- fibrinogenemia
- F V defic. (parahemophilia)
- F XIII defic.
- APC resistance

Acquired

- Liver proteosynthesis
- Vitamin K defic.
  - obstructive icterus
  - intestin. resorption
- Anticoagulant therapy
  - Dicumarol
  - Heparin

Vasculopathies

Congenital

Mb. Rendu-Osler-Weber

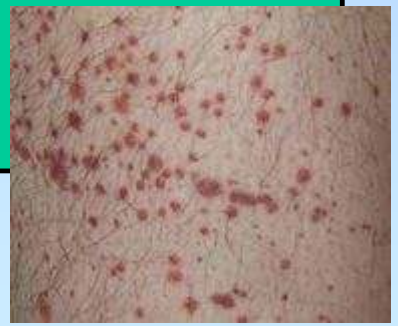
= hereditary hemorrhagic teleangiectasia
AD, TGFbeta1 rec.

Ehlers-Danlos Sy.

= defects in collagen synthesis

Acquired

Purpura Henoch-Schönlein
Scurvy (Scorbut)
Steroid purpura
Purpura simplex and senilis



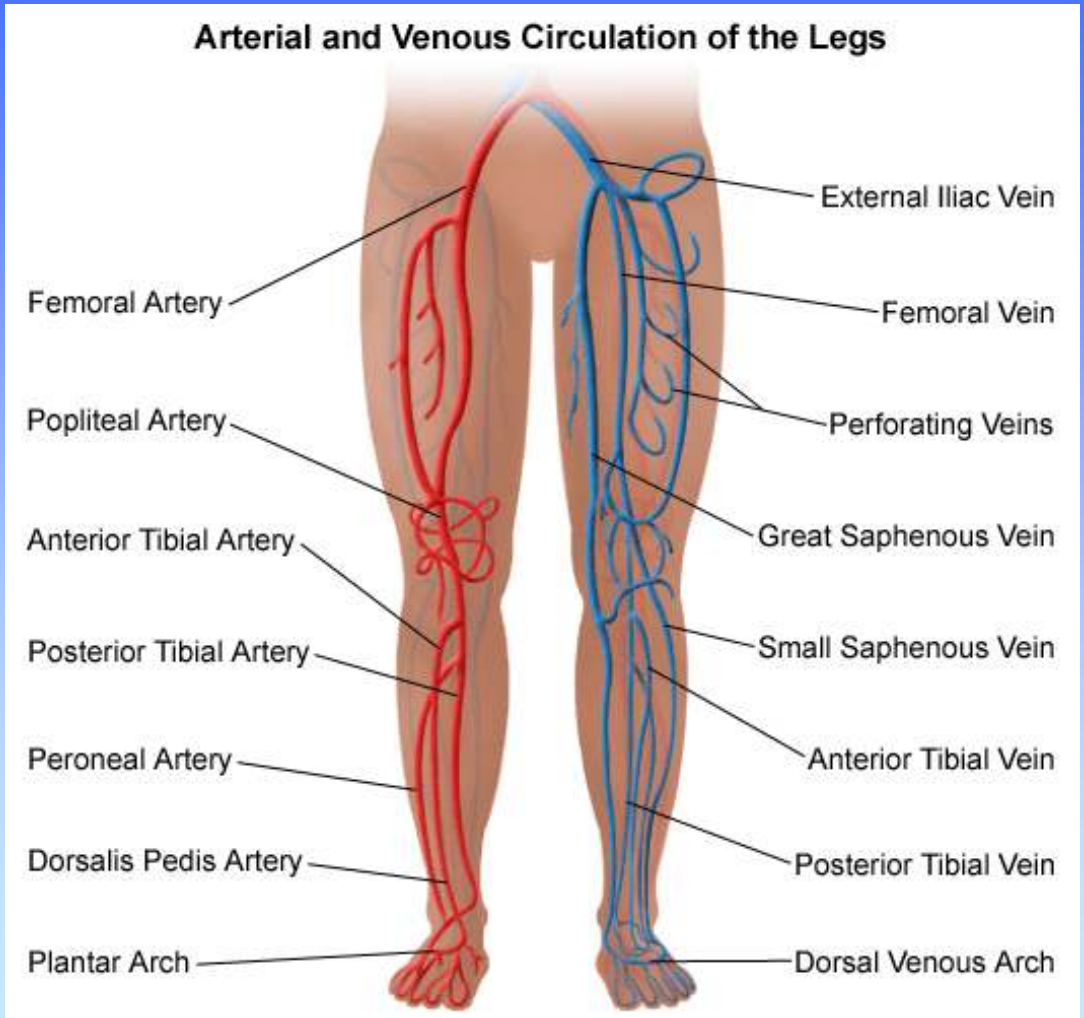
Vasculopathies / purpuras

- congenital
  - e.g. Ehlers-Danlos syndrom (defect of collagen)
- Acquired
  - **scurvy** (vitamin C deficiency)
  - **glucocorticoid excess**
  - Purpura senilis
  - Henoch-Schoenlein purpura (children after an upper respiratory infection xx DD DIC in meningococcal infection!)

Risc factors and examples of VTE (venous thrombo-embolism)

Risc factors:
-vessel oppression (e.g. phlebo-thrombosis of left lower extremity is circa 3 times more common than phlebo-thrombosis of right lower extremity ….Why is that so?)
-dehydration
-hyperviscosity
-stasis syndrom (e.g. right heart insufficiency, long airplane flight)
-immobility
-obesity
-activation of secondary hemostasis, e.g. Inflammation, infection, trauma, malignancies
-inborn hypercoagulable states

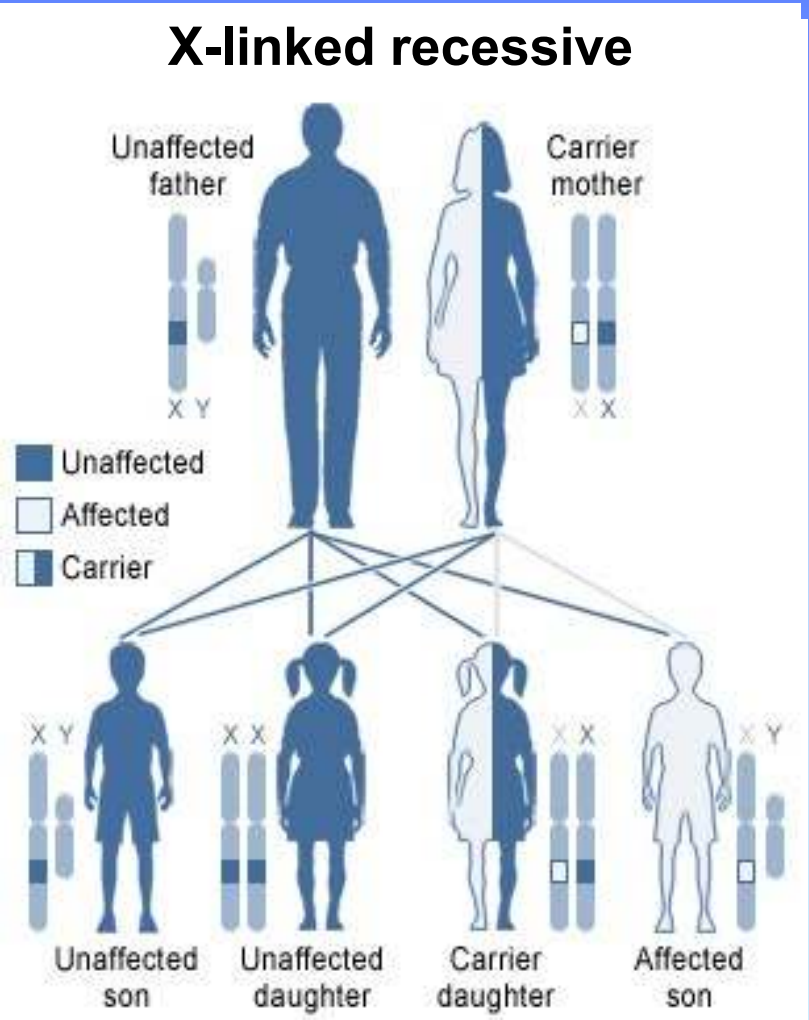
Examples:
-**phlebothrombosis** of deep veins of lower extremities
-**thrombophlebitis** of superficial veins of lower extremities
-**lung thrombembolism**
-**thrombosis of large visceral veins** (e.g. thrombosis of vena portae, hepatic vein thrombosis= **Budd-Chiari syndrome**)
-**Trousseau symptom** (migratory thrombophlebitis in malignancies)
-thrombotic complications in **chronic hemolytic anemias** (sickle cell anemia, thalassemias) and **clonal disorders of hematopoiesis** (MPN, PNH)


Arterial and Venous Circulation of the Legs
Femoral Artery
Popliteal Artery
Anterior Tibial Artery
Posterior Tibial Artery
Peroneal Artery
Dorsalis Pedis Artery
Plantar Arch
External Iliac Vein
Femoral Vein
Perforating Veins
Great Saphenous Vein
Small Saphenous Vein
Anterior Tibial Vein
Posterior Tibial Vein
Dorsal Venous Arch

Genetic examination

Hemophilia A

1 : 10 000



Queen Victoria of Britain

Carrier of Hemophilia

She had 9 children and spread this new mutation into many noble houses of Europe

X-linked

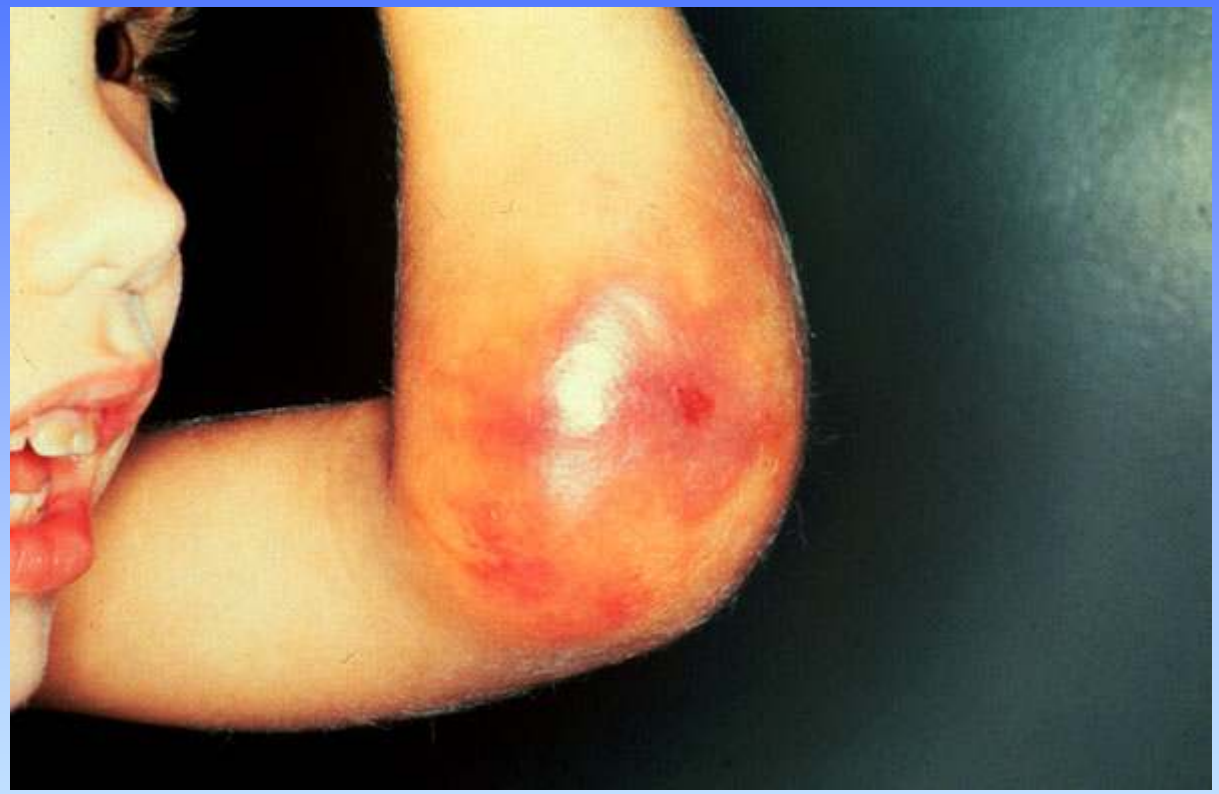
Great-grandson

Tsarevich Alexei

Sufferer of Hemophilia

Tsar Nicolai II of Russia

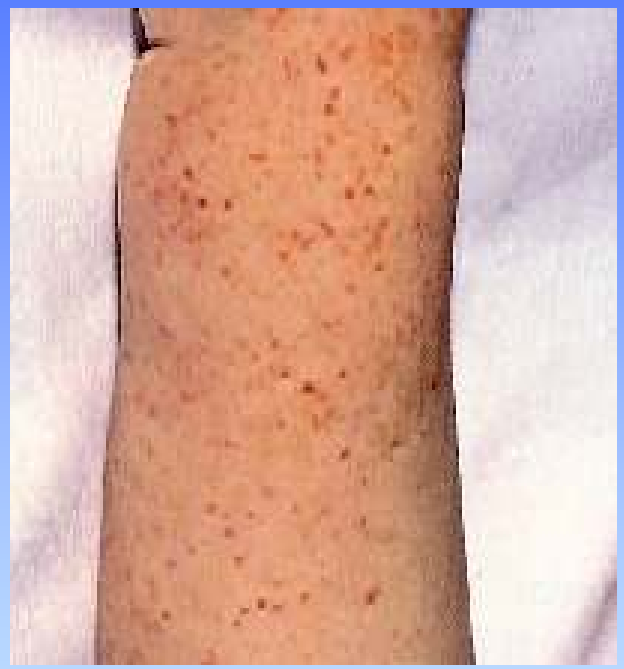
Clinical signs

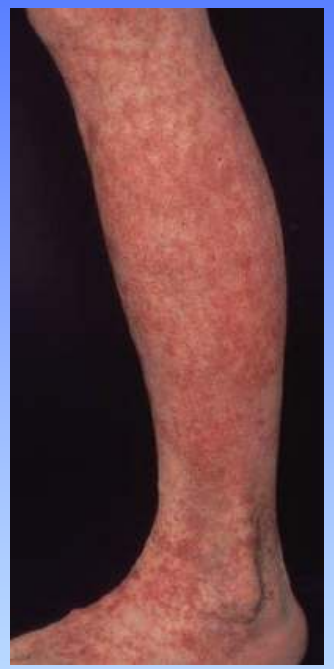


Hemophilia

Large hemorrhage after a small injury
Arthral hemorrhage
Secondary arthropathy

Clinical signs

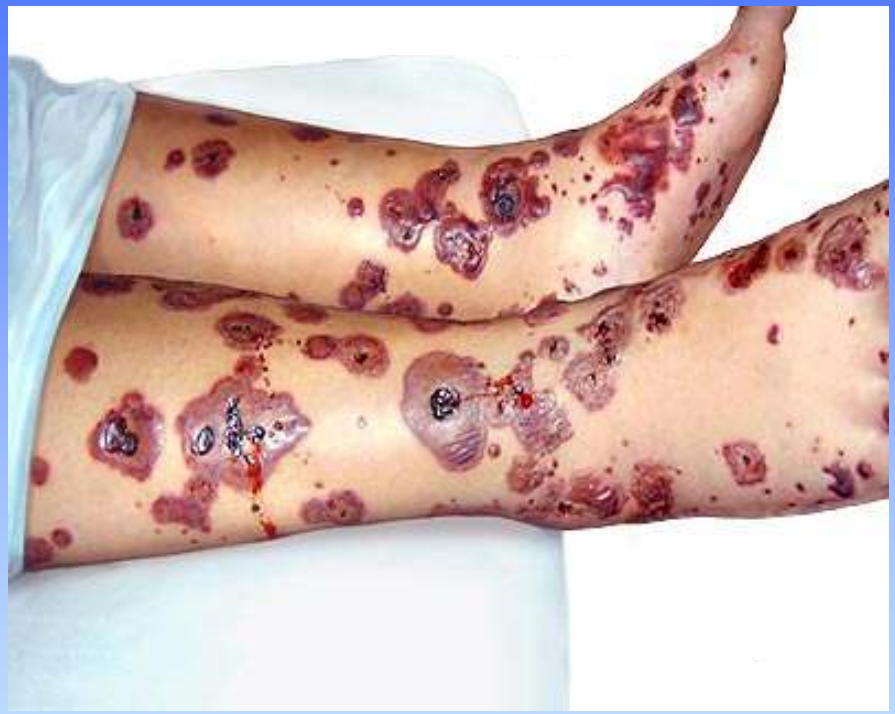




Thrombocytopenia

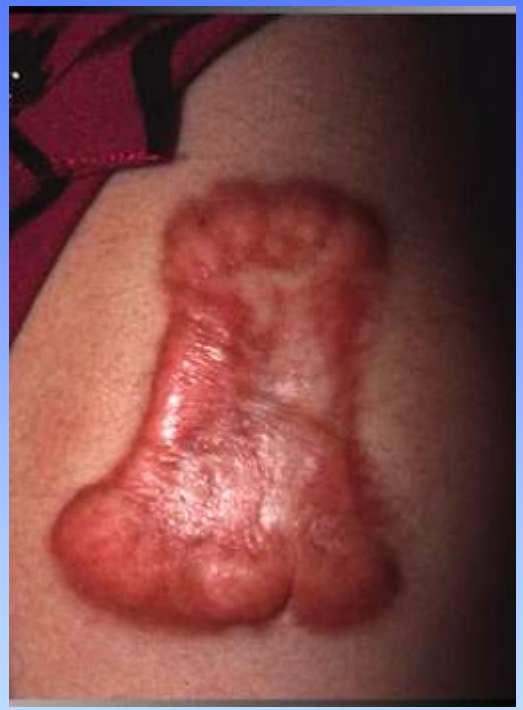
Petechiae, pigmentation

Clinical signs



Henoch-Schonlein

Clinical signs



F XIII deficiency

Late bleeding

Keloid scarring

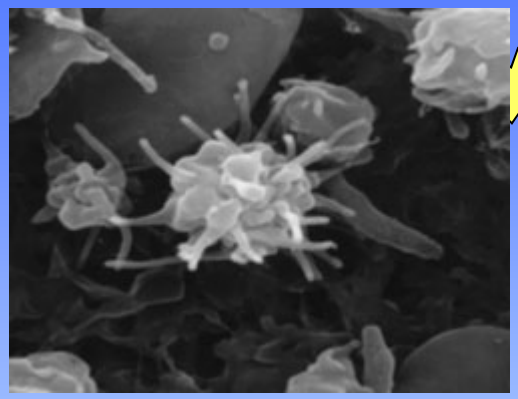
Thrombocytopenia

- 1) **Production decreased↓**
- 2) **Consumption increased ↑**
  - A) with increased activity of thrombin
  - B) imuno-thrombocytopenia
  - C) other
- 3) **Combination of both mechanisms**

Platelet count

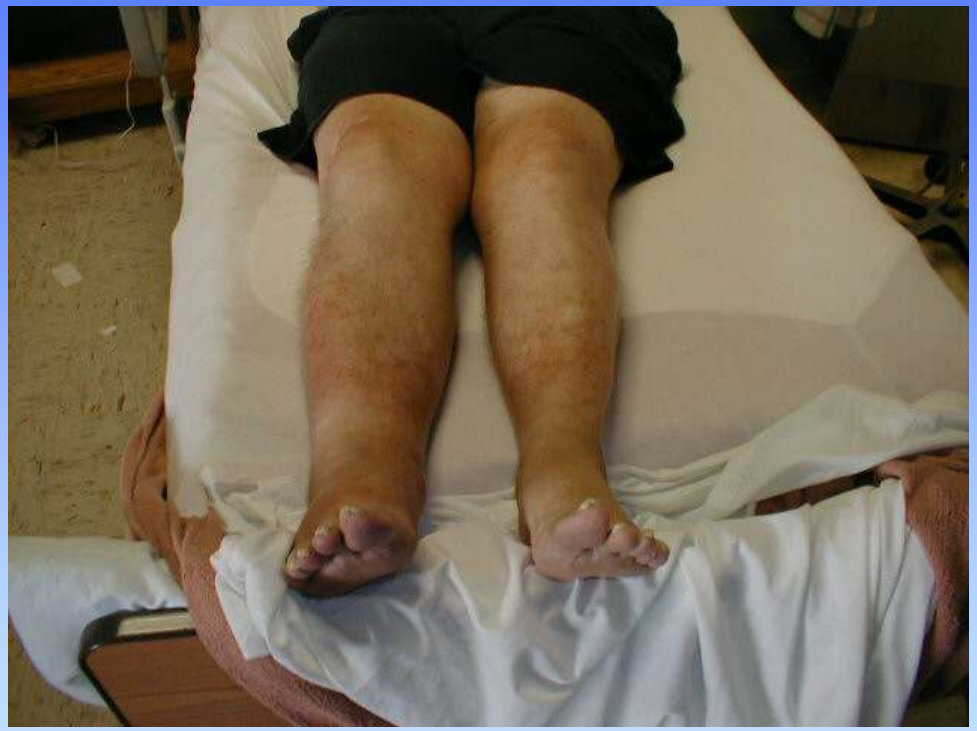
- 200 – 400 x $10^3/\mu L$ ($10^9/L$)

= 200 000 – 400 000 /μL



The risk of spontaneous bleeding is low if the number of platelets is > 30 000 /μL and blood vessels and coagulation system are intact

Clinical signs



Deep venous thrombosis

Pulmonary embolism

III.
Diagnostics and monitoring

Standard tests in Faculty General Hospital

Quick time, INR	0,8 - 1,2
Act.Part.Thromb.Time	27-35 s
Thrombin time	12 - 14 s
Fibrinogen	2 - 4 g/l
Antithrombin III	> 70%
Ethanol test	neg.
D-dimers (FDP)	neg.

Prothrombin Time (Quick test)

Principle: Stimulation of extrinsic (main) coag. system
Citrate plasma ... add TF (in excesive amount) + $CaCl_2$ ... fibrin fibre

Normal: PT = 12 - 15 s
INR = $(PT_P)^{ISI}$ / PTN
ISI = international index of sensitivity of used thromboplastin (commonly > 1)

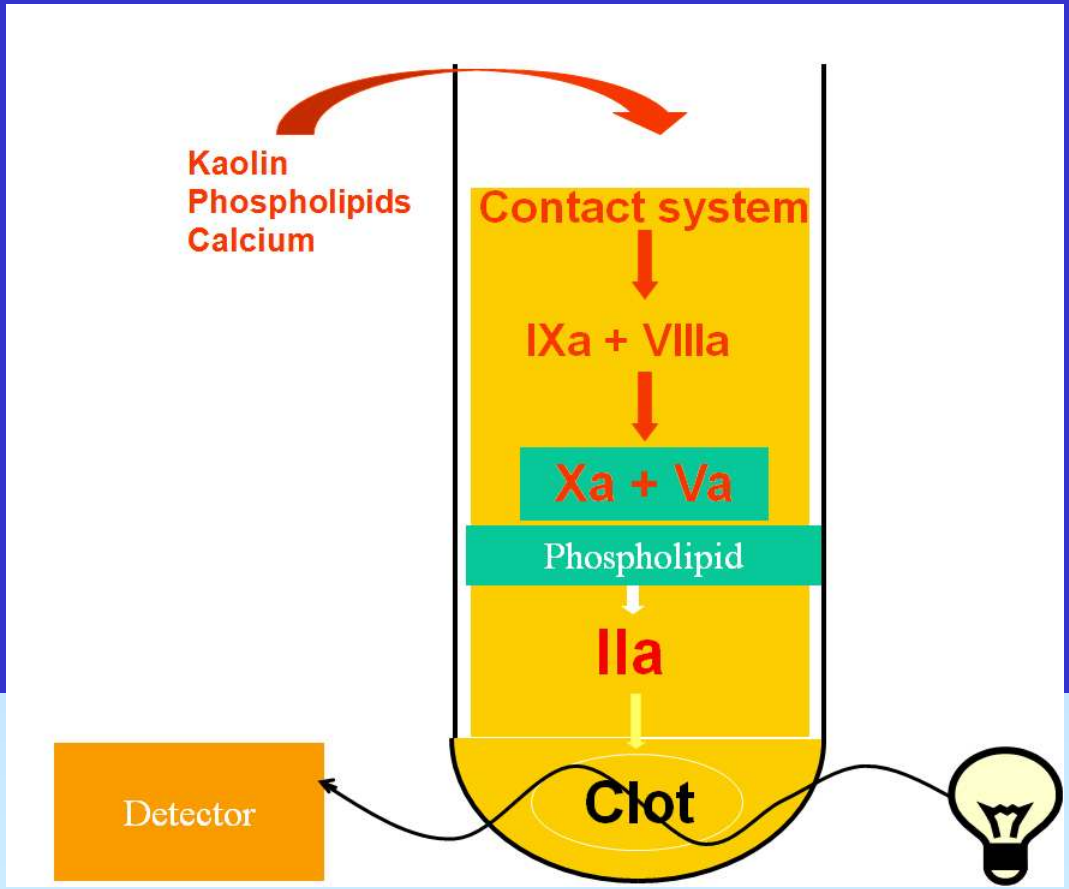
Prolongation: defic. vit. K dep. FII, VII, X, ↓↓Fbg
Usage: screening, monitoring of oral anticoagulants, liver proteosynthesis

Normal range	INR 0,8 - 1,2
Therapeutic range	INR = 2,5 - 4,5
Surgery	INR < 1,6

APTT, Activated partial thromboplastin time

Principle: Stimulation of intrinsic (contact) way of coag. system

Citrate plasma ... add contact activator (e. g. kaolin) + $CaCl_2$ ... fibrin fibre



APTT, Activated partial thromboplastin time

Principle: Stimulation of intrinsic (contact) way of coag. system
Citrate plasma ... add contact activator (e. g. kaolin) + $CaCl_2$ ... fibrin fibre

Normal: APTT = 27 - 35 s

Prolongation: defic. of VII, V, X, XII, VIII, XI, IX (hemophilia A,B,C), ↓↓Fbg, ↑↑FDP
Shortening: prothrombotic status
Usage: screening, diagnostics of coagul. deficits, monitoring of heparin therapy

Therapeutic range 1,2 - 2,5 x

Lee-White test

Cloting time of whole blood

Whole blood without anticoagulants ($CaCl_2$) ...
polystyrene or glass tube, 37°C ...
spontaneous stimulation of intrinsic

Normal: 4 - 10 min.

Usage: Basic, rough orientation in acute status

Thrombin Time

Whole blood without anticoagulants ($CaCl_2$) ... add thrombin in standard amount, 37°C ... fibrin fibre

Normal: 12 - 14 s

Prolongation:
- ↓↓ Fbg (acute stage of DIC)
- antithrombins
- fibrinolysis

Usage: DIC
- monitoring of fibrinolytic therapy

Fibrinogen, Fbg

Normal plasma levels = 2 - 4 g /l
Functional of immunological detection

High: Inflammation
DM
Smoking
Low: Low synthesis (congenital or low liver function)
Consumption (DIC)

Hypofibrinogenemia
Dysfibrinogenemia

FDP

Total degradation products of fibrin(-ogen)

ELISA or aglutination semiquantitative methods

High: Recent coagulation activity
(thrombo/ embolism, bleeding, surgery, DIC ...)

High sensitivity, low specificity

Paracoagulation tests (Ethanol, Protamin)

Principle: Ethanol catalyzes conversion of fibrin monomers + PDP → fibrin polymers

Low sensitivity and specificity

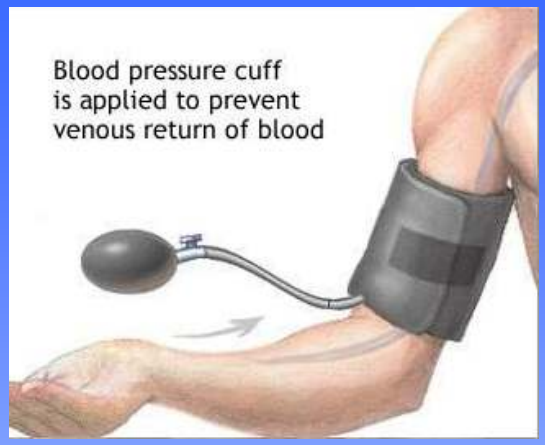
Usage: 1st stage of DIC

Duke test

Duke, 1910
Estimation of bleeding time
Time of spontaneous cutoff of bleeding after standard puncture to auricle of ear

Limits: 2 - 5 min., or 4 - 8 min. (depends on methods)

Prolongation - Disturbance of primary hemostasis:
Plt < 20 000 or Plt dysfunction, vW disease



Rumpel - Leede test

Capillary resistance

Number of petechia on forearm (area 4 x 4 cm) after a standard pressure (ruff 10,5 kPa for 10 min.) or after underpressure (Brown, 1949)

Limits: > 5 petechia ... higher capillary fragility (e.g. hereditary purpura Weber-Rendu-Osler)

Presumable results

Diagnosis	Plt	Duke	APTT	Quick	TT
Thrombocytopenia	↓	↑	N	N	N
Hemophilia A	N	N	↑	N	N
Hemophilia B	N	N	↑	N	N
Hemophilia C	N	N	↑	N	N
vWd	N	↑	N / ↑	N	N

Presumable results

Diagnosis	Plt	Duke	APTT	Quick	TT
F V defic.	N	N	↑	↑	N
F II defic.	N	N	↑	N	N
F VII defic.	N	N	N	↑	N
Warfarin / vit. K def.	N	N	↑	↑	N
Heparin i. v.	N	N / ↑	↑	N / ↑	↑
Heparin s. c.	N	N	N	N	N

Presumable results

Diagnosis	Plt	Ethan	APTT	Quick	TT
DIC 1st stage	↓	+	↑	↑	N
DIC 2nd stage	↓↓	-	↑↑↑	↑↑↑	↑↑

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