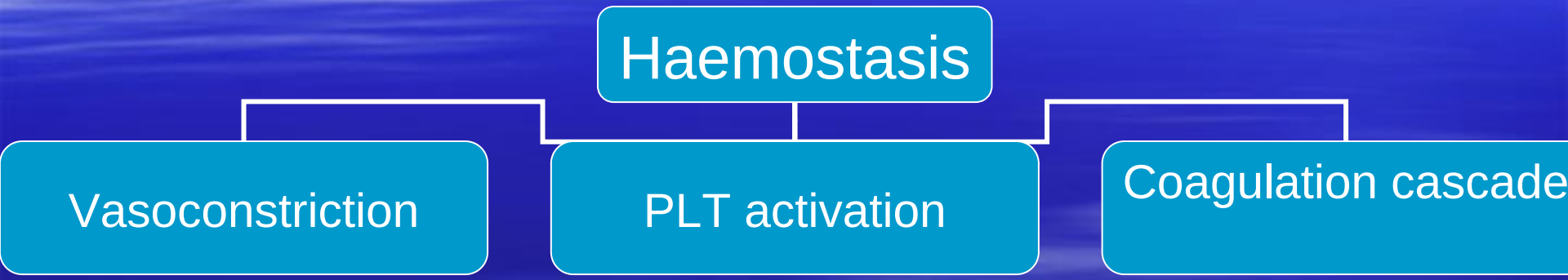
Haemostasis
Anti-Coagulation

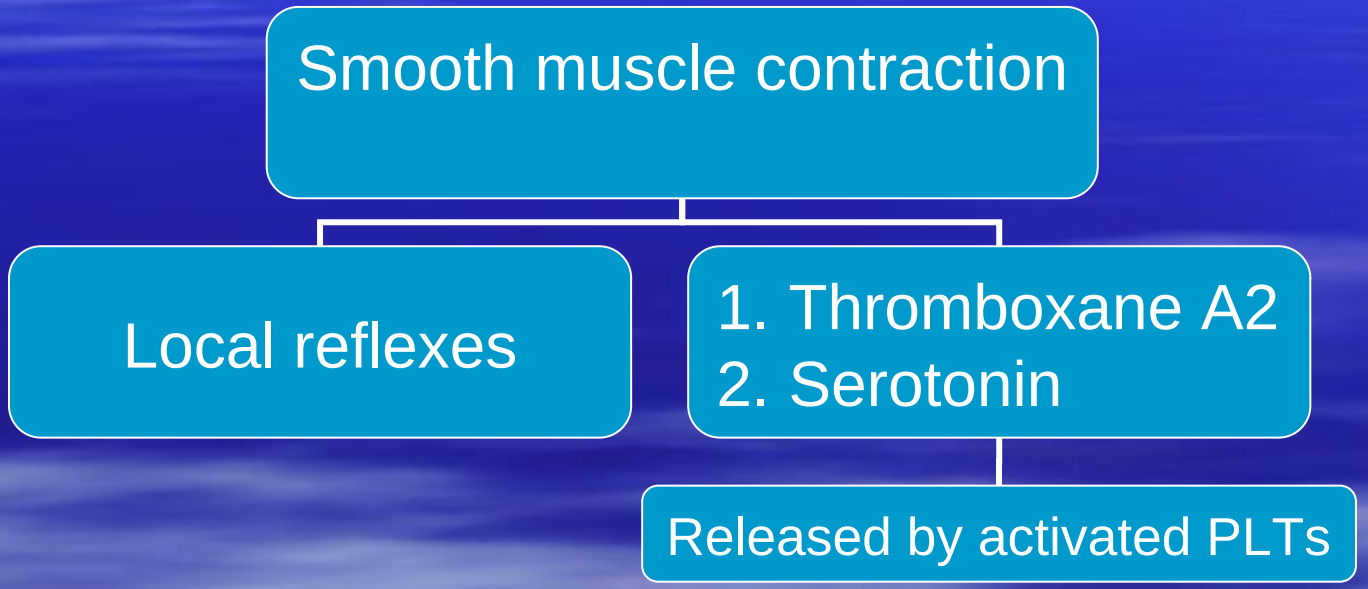
Sotiris Papaspyros

December 2006

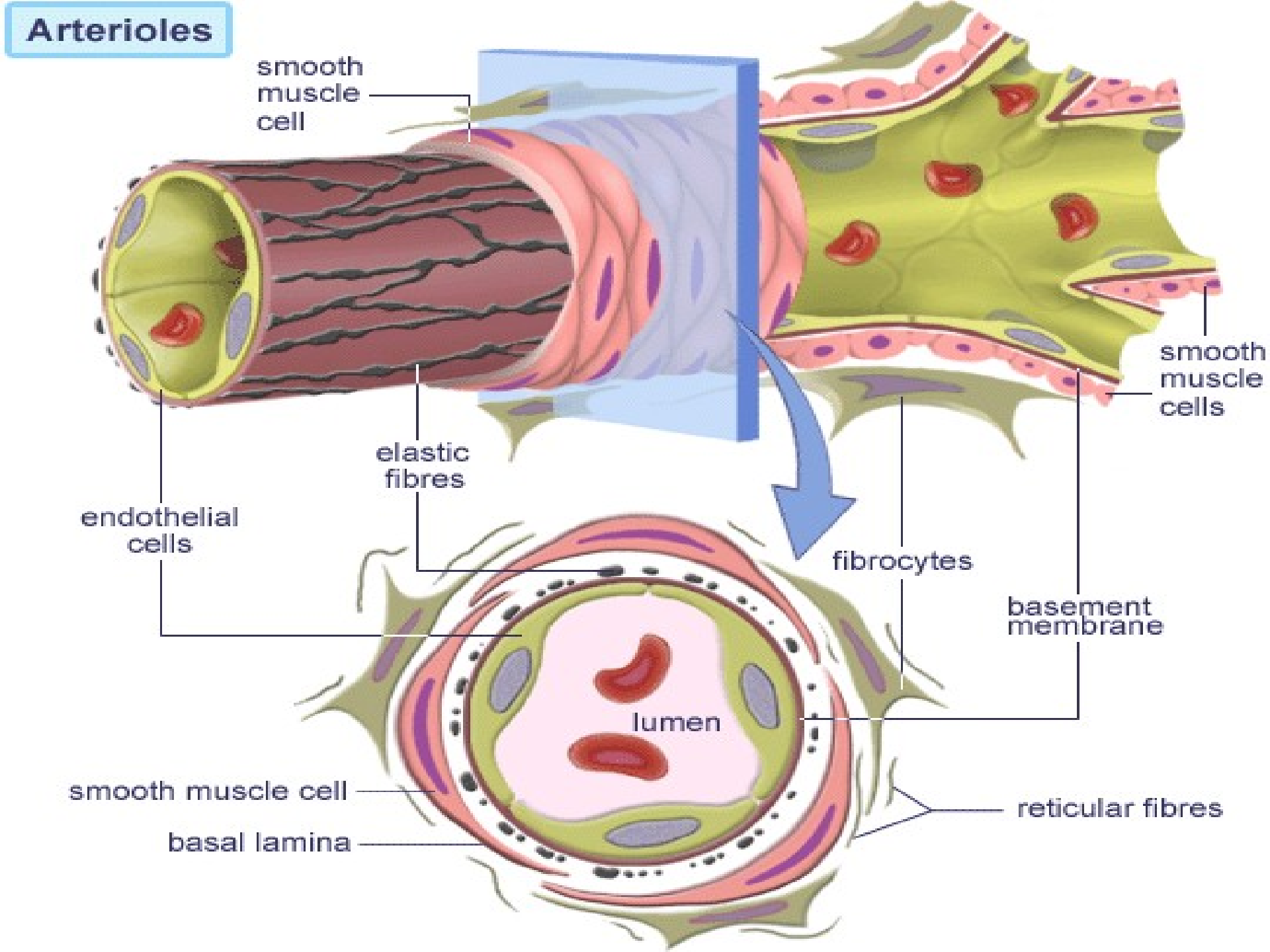
1. Haemostasis
   - Primary
     - PLTs
   - Secondary
     - Coagulation cascades
2. Assessment tests of haemostasis
3. Disorders of haemostasis
   - PLT and vessel wall disorders
   - Coagulation disorders
   - Disorders predisposing to thrombosis
4. Anticoagulant medication
   - Warfarin
   - Heparin
     - LMWH
   - Aspirin

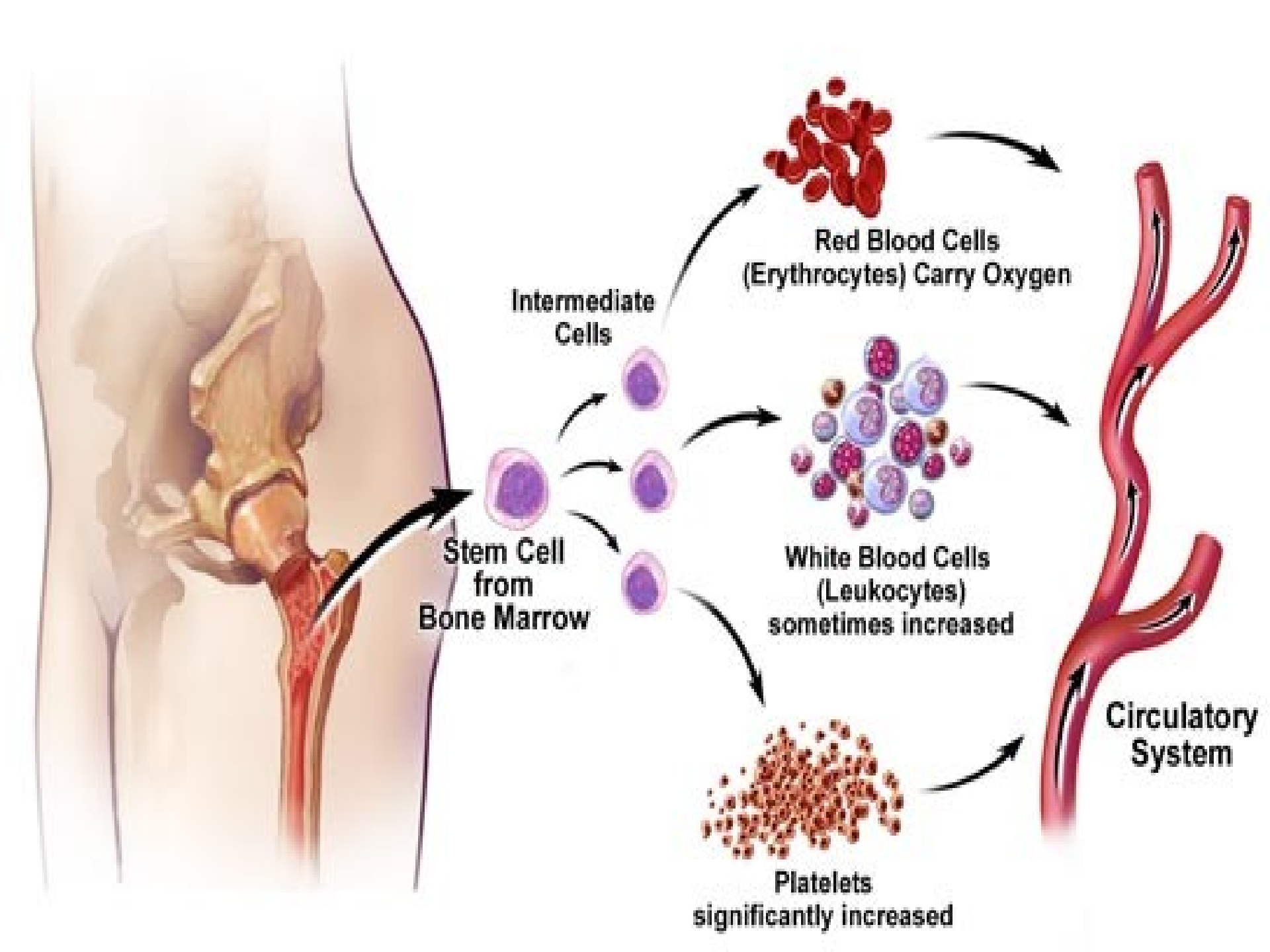

Haemostasis
Vasoconstriction
PLT activation
Coagulation cascade

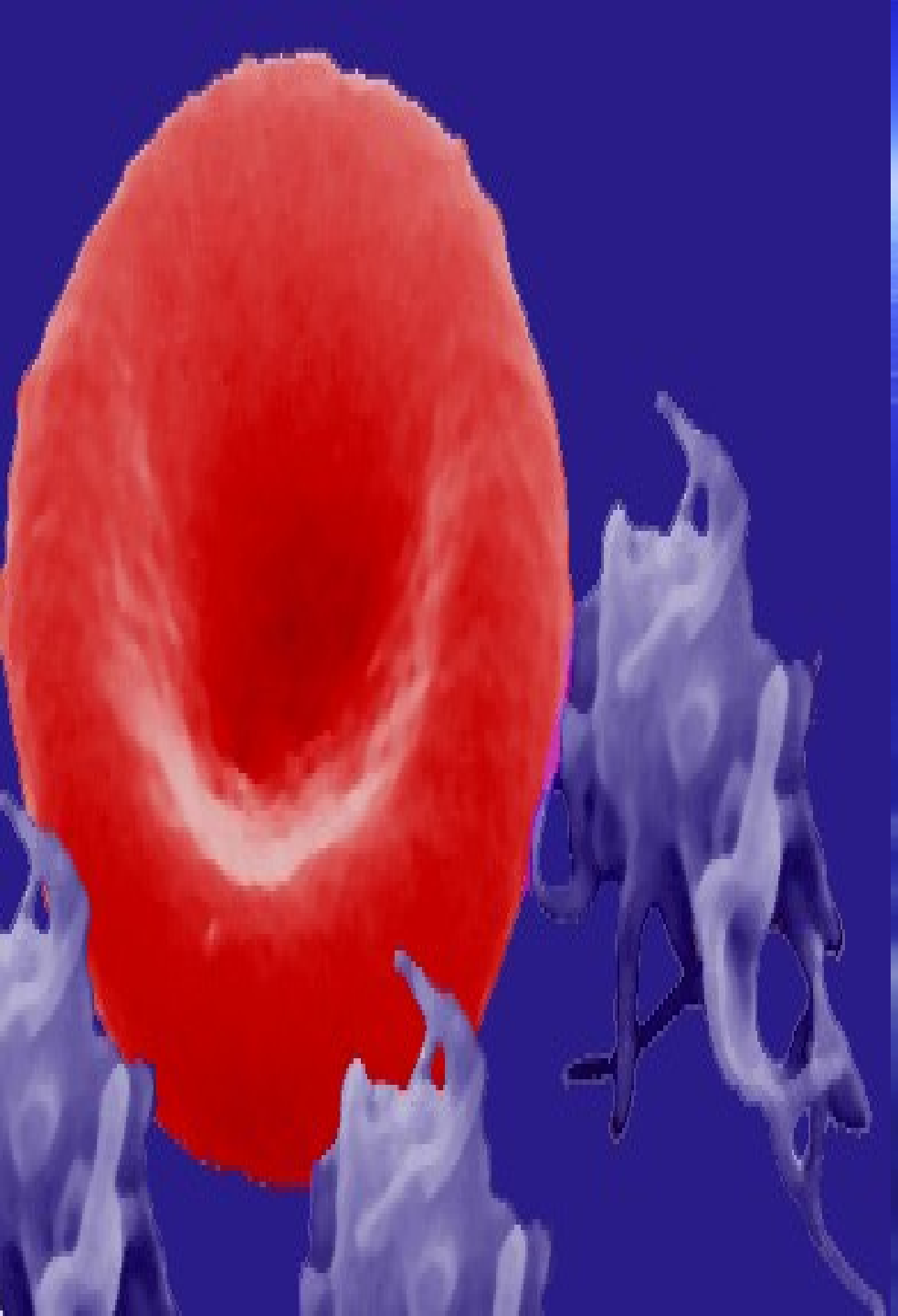
Vasoconstriction



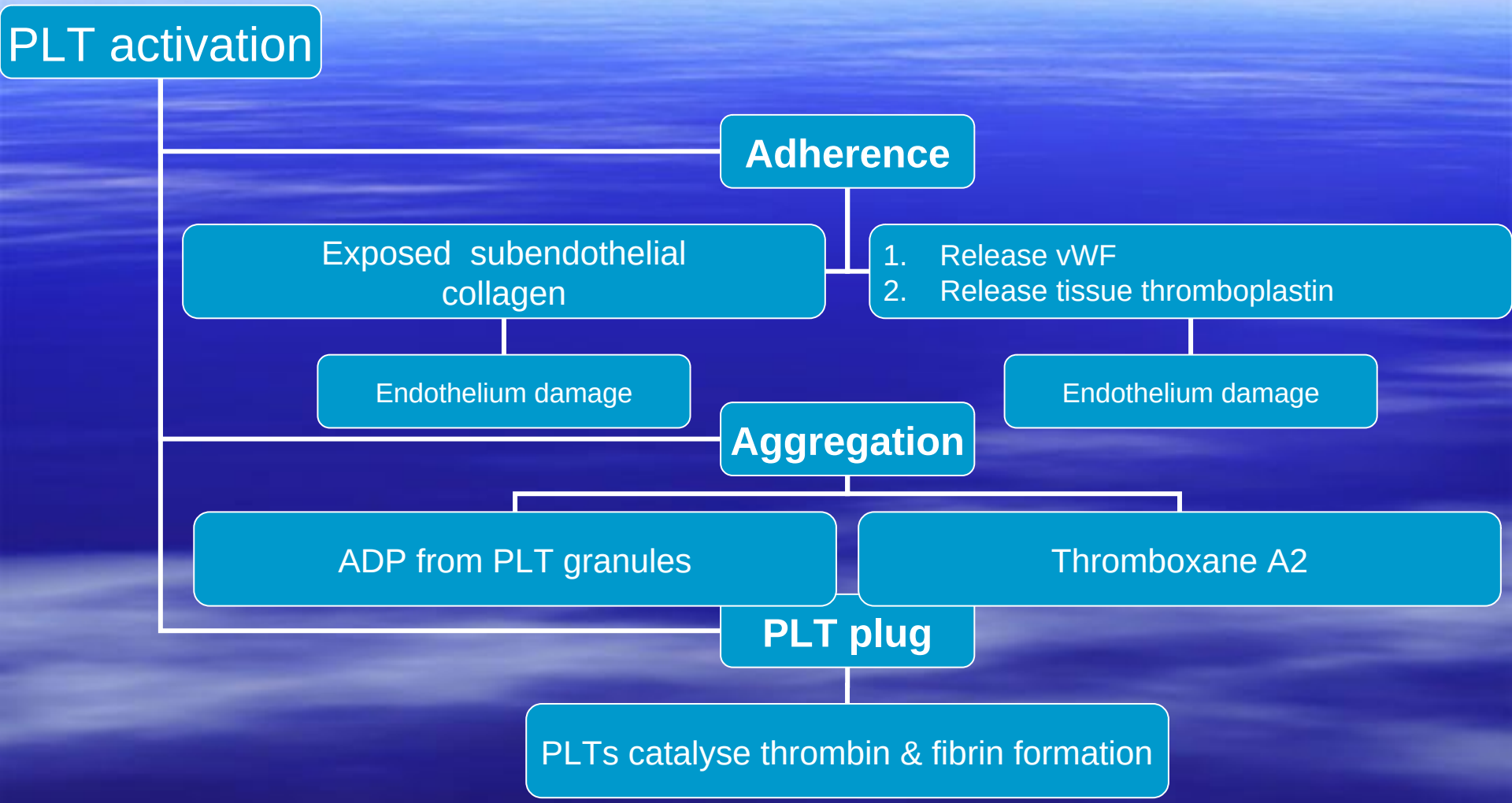
Arterioles


smooth muscle cell
smooth muscle cells
elastic fibres
endothelial cells
fibrocytes
basement membrane
lumen
smooth muscle cell
basal lamina
reticular fibres


Red Blood Cells
(Erythrocytes) Carry Oxygen
Intermediate
Cells
Stem Cell
from
Bone Marrow
White Blood Cells
(Leukocytes)
sometimes increased
Circulatory
System
Platelets
significantly increased



- Discoid non nucleated
- Contain granules
- Life 8-10 days
- Release the contents of their granules into the plasma, in turn activating other platelets and white blood cells. The platelets undergo a change in their shape which exposes a phospholipid surface for those coagulation factors that require it. Fibrinogen links adjacent platelets by forming links via the glycoprotein IIb/IIIa. In addition, thrombin activates platelets.


PLT activation
Adherence
Exposed subendothelial collagen
1. Release vWF
2. Release tissue thromboplastin
Endothelium damage
Endothelium damage
Aggregation
ADP from PLT granules
Thromboxane A2
PLT plug
PLTs catalyse thrombin & fibrin formation

1 Endothelium of vessel is damaged, exposing connective tissue; platelets adhere

2 Platelets form a plug

3 Seal is reinforced by a clot of fibrin

Collagen fibers

Platelet plug

Platelet releases chemicals that make nearby platelets sticky

Clotting factors from:

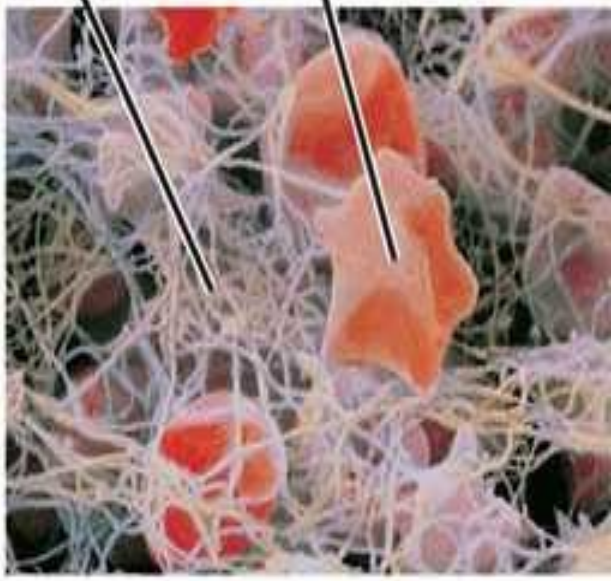
- Platelets
- Damaged cells
- Plasma (factors include calcium, vitamin K)

Prothrombin → Thrombin

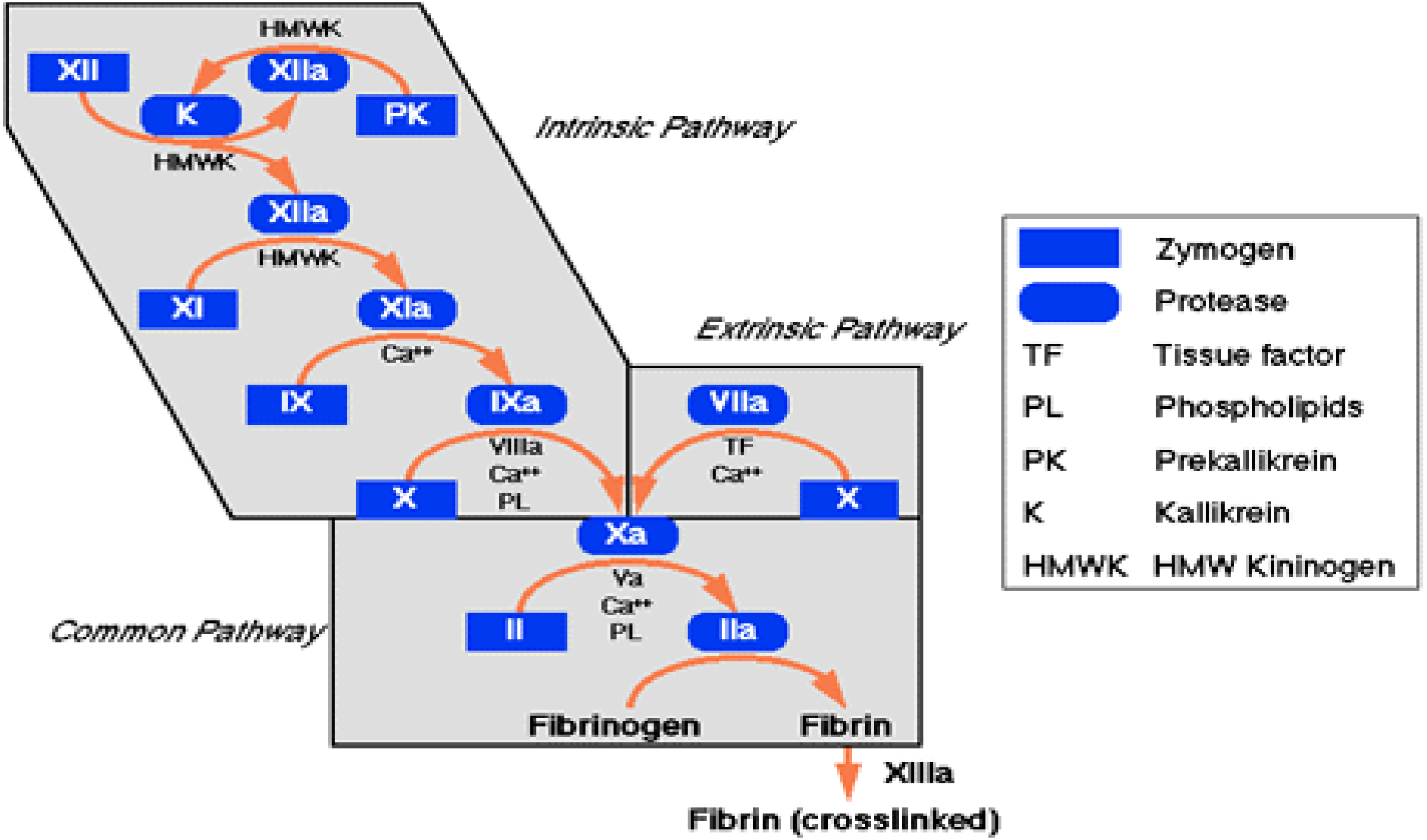
Fibrinogen → Fibrin

Fibrin clot

Red blood cell



5 μm



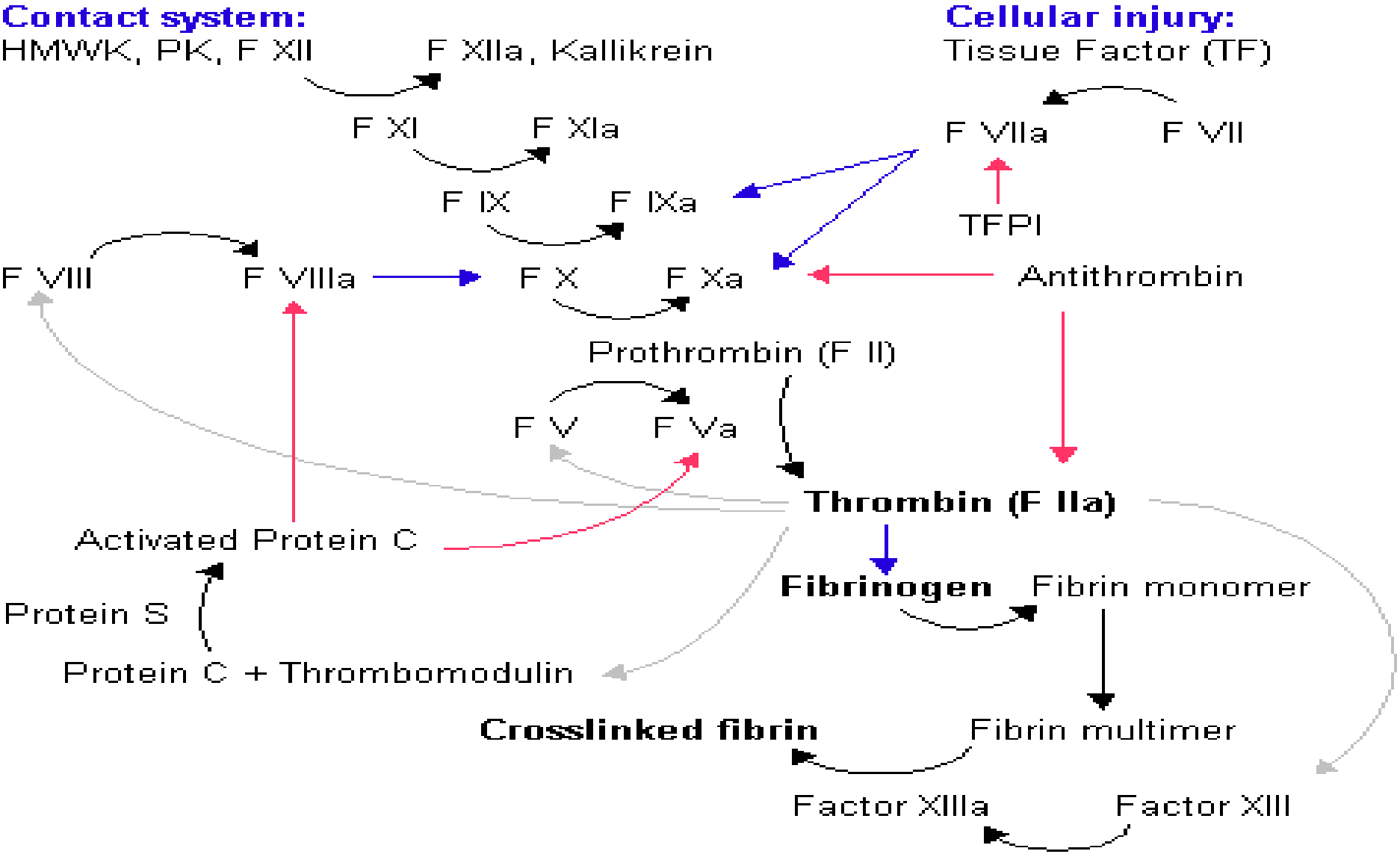
It is now known that the primary pathway for the initiation of blood coagulation is the *Tissue Factor* pathway.

The coagulation factors are serine enzymes except FVIII and FV which are glycoproteins.

The pathways are a series of reactions, in which a zymogen of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade.

Number and/or name	Function
I (fibrinogen)	Forms clot (fibrin)
II (prothrombin)	Its active form (IIa) activates I, V, VII, XIII, protein C, platelets
Tissue factor	Co-factor of VIIa (**formerly known as factor III**)
Calcium	Required for coagulation factors to bind to phospholipid (**formerly known as factor IV**)
V (proaccelerin, labile factor)	Co-factor of X with which it forms the prothrombinase complex
VI	*Unassigned* – old name of Factor Va
VII (stable factor)	Activates IX, X
VIII (antihemophilic factor)	**Co-factor of IX with which it forms the tenase complex**
IX (Christmas factor)	Activates X: forms tenase complex with factor VIII
X (Stuart-Prower factor)	Activates II: froms prothrombinase complex with factor V
XI (plasma thromboplastin antecedent)	Activates XII, IX and prekallikrein
XII (Hageman factor)	Activates prekallikrein and fibrinolysis
XIII (fibrin-stabilizing factor)	Crosslinks fibrin
von Willebrand factor	**Binds to VIII, mediates platelet adhesion**

prekallikrein	Activates XII and prekallikrein; cleaves HMWK
high molecular weight kininogen (HMWK)	Supports reciprocal activation of XII, XI, and prekallikrein
fibronectin	Mediates cell adhesion
antithrombin III	Inhibits IIa, Xa, and other proteases;
heparin cofactor II	Inhibits IIa, cofactor for heparin and dermatan sulfate ("minor antithrombin")
protein C	Inactivates Va and VIIIa
protein S	Cofactor for activated protein C (APC, inactive when bound to C4b-binding protein)
protein Z	Mediates thrombin adhesion to phospholipids and stimulates degradation of factor X by ZPI
plasminogen	Converts to plasmin, lyses fibrin and other proteins
alpha 2-antiplasmin	Inhibits plasmin
tissue plasminogen activator (tPA)	**Activates plasminogen**
urokinase	Activates plasminogen
plasminogen activators inhibitor-1 and 2 (PAI1, 2)	Inactivate tPA & urokinase (endothelial PAI)
cancer procoagulant	**Pathological** factor X activator linked to thrombosis in ca



Legend: **HMWK** = High molecular weight kininogen, **PK** = Prekallikrein, **TFPI** = Tissue factor pathway inhibitor. **Black arrow** = conversion/activation of factor. **Red arrows** = action of inhibitors. **Blue arrows** = reactions catalysed by activated factor. **Grey arrow** = various functions of thrombin.

- Tissue factor (Extrinsic)

1. The main role of the tissue factor pathway is to generate a "thrombin burst," thrombin being the single most important constituent of the coagulation cascade in terms of its feedback activation roles.
2. Thrombin then activates other components of the coagulation cascade, including protein C, FV and FVII (which activates FXI which in turn activates FIX), and activates and releases FVIII from being bound to vWF. FVIIIa is the co-factor of FIXa and together they form the "tenase" complex which activates FX and so the cycle continues.

- Contact (Intrinsic)

1. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK and prekallikrein do not have a bleeding disorder.

- Following activation by the contact factor or tissue factor pathways the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down regulated by the anticoagulant pathways.

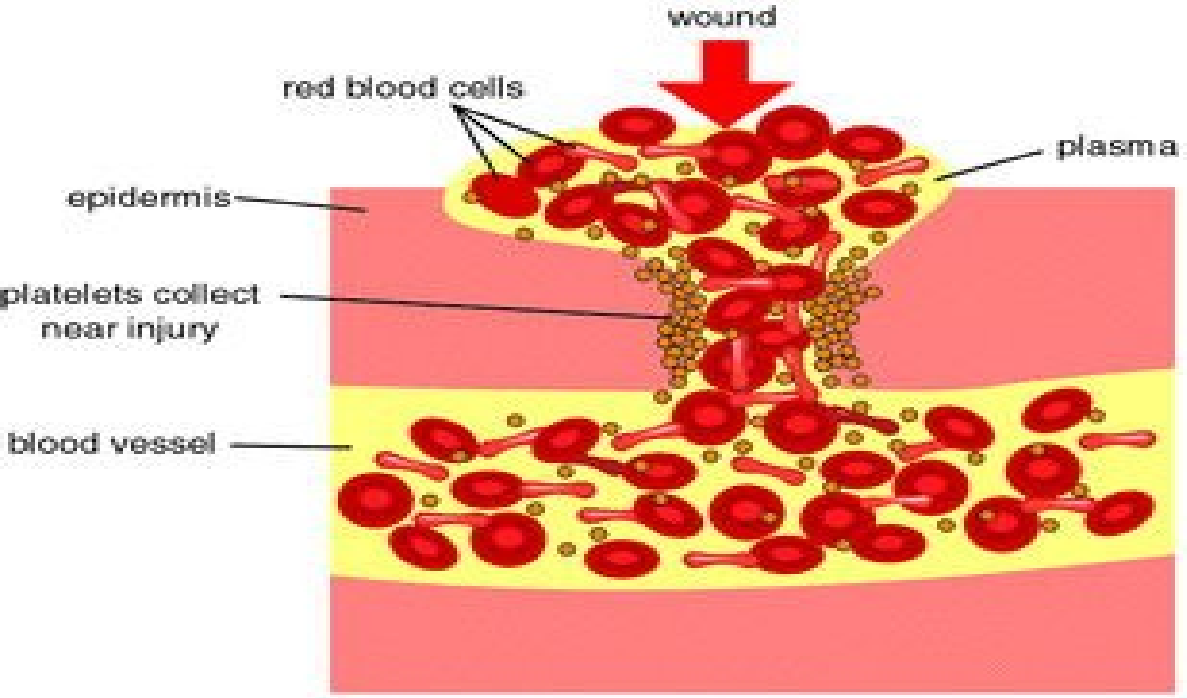
Co-factors and Inhibitors

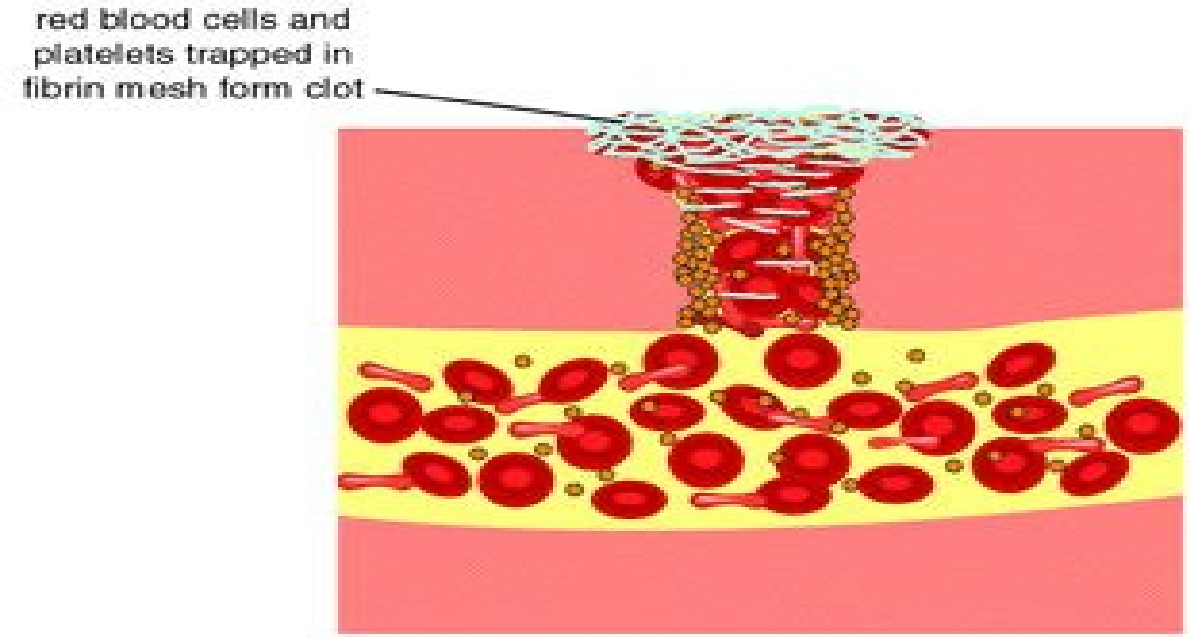
- Calcium and phospholipid (a platelet membrane constituent). Calcium mediates the binding of the complexes to the phospholipid surfaces expressed by platelets.
- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. Deficiency of vitamin K (e.g. in malabsorption), use of inhibiting anticoagulants (warfarin) or disease (cirrhosis) leads to the formation of PIVKA's (proteins formed in vitamin K absence) this causes partial or non gamma carboxylation.

Co-factors and Inhibitors

- Mechanisms that keep the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

1. Protein C degrades the co-factors FVa and FVIIIa. It is activated by thrombin and requires its co-enzyme Protein S to function. Quantitative or qualitative deficiency of either may lead to thrombophilia. Impaired action of Protein C , for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency.
2. Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases; thrombin and FXa, as well as FXIIa, and FXIa. It is constantly active, but its adhesion to these factors is increased by the administration of heparins. Quantitative or qualitative deficiency of antithrombin leads to thrombophilia.
3. Tissue factor pathway inhibitor (TFPI) inhibits F VIIa-related activation of F IX and F X after its original initiation.





injured tissues and platelets release the clotting factor **prothrombin activator** and **calcium ions**

prothrombin activator converts the blood protein prothrombin to **thrombin**

thrombin splits **fibrinogen** to form **fibrin**

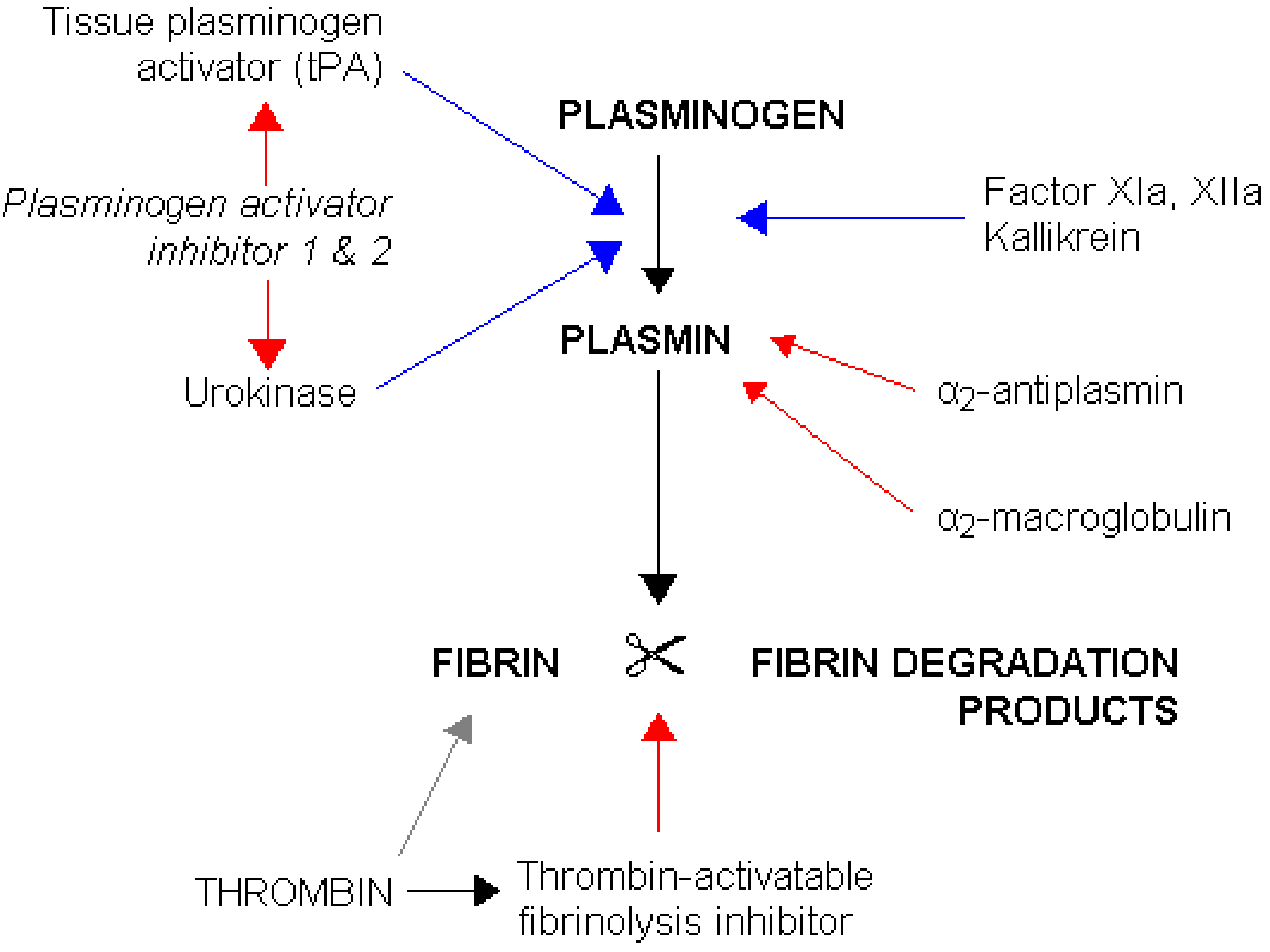
fibrin fibres form a mesh over wound, trapping red blood cells and platelets

bleeding stops

clot hardens and becomes smaller

new cells grow to repair wound site

enzyme **plasmin** is released to dissolve clot


Tissue plasminogen activator (tPA)
PLASMINOGEN
Plasminogen activator inhibitor 1 & 2
Factor XIa, XIIa
Kallikrein
PLASMIN
Urokinase
α2-antiplasmin
α2-macroglobulin
FIBRIN
FIBRIN DEGRADATION PRODUCTS
THROMBIN
Thrombin-activatable fibrinolysis inhibitor

Assessment of haemostasis
Common tests

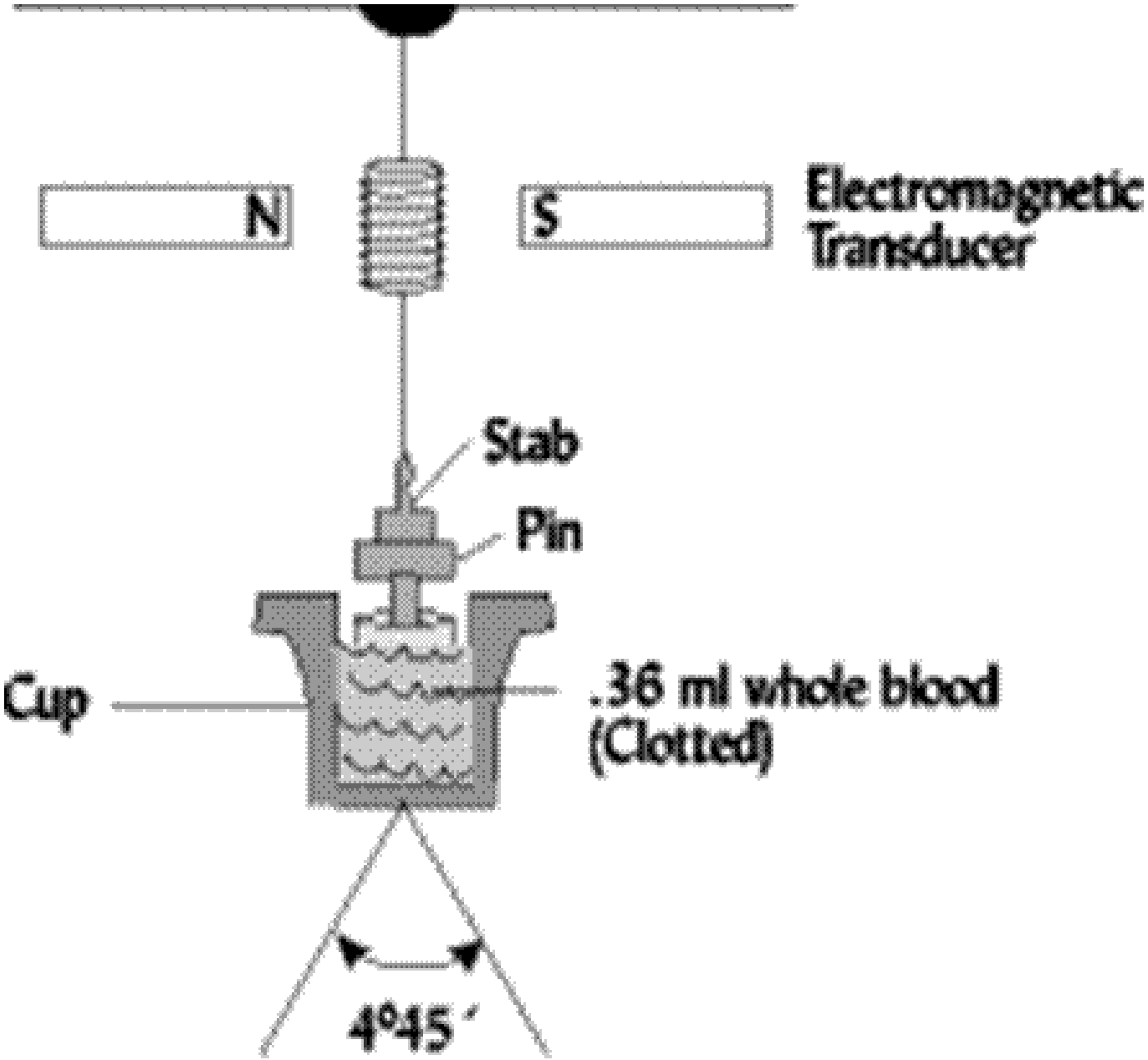
- PLT count
- Bleeding time
  - PLT function, vascular disorders and von Willebrand disease, and not by other coagulation factors so in haemophilia it is normal. Diseases that cause prolonged bleeding time include (DIC) and thrombocytopenia.
- INR (PT)
  - "tissue factor" (a specific cellular lipoprotein) pathway.
- APTT
  - Contact factor pathway
- TCT
  - quantitative and qualitative screening of fibrinogen
- D-dimer
  - to exclude thromboembolic disease where the probability is low.

Assessment of haemostasis
Other tests

1. mixing test
    1. to distinguish whether the underlying problem is a clotting factor deficiency on the one hand, or a factor inhibitor on the other hand.
2. antiphosholipid antibodies
    1. autoimmune production of antibodies against cell membrane constituents. ,
3. coagulation factor assays,
4. genetic tests (eg. factor V Leiden, prothrombin mutation G20210A),
    1. (the Leiden variant of factor V, cannot be inactivated by activated protein C) , dilute Russell viper venom test (dRVVT),
5. platelet function tests,
6. thromboelastography (TEG)
    1. depends on the activity of the plasmatic coagulation system, platelet function, fibrinolysis
7. thromboelastometry (ROTEG).

TEG

- **Thromboelastography** (**TEG®**) is a method of testing the efficiency of coagulation in the blood. It was first developed by Dr. Hellmut Hartert in 1948.
- **The original method**
- In **classical thromboelastography**, a small sample of blood (typically 0.36 ml) is placed into a cuvette (cup) which is rotated gently through 4º 45´ (cycle time 6/min) to imitate sluggish venous flow and activate coagulation. When a sensor shaft is inserted into the sample a clot forms between the cup and the sensor. The speed and strength of clot formation is measured in various ways (now usually by computer), and depends on the activity of the plasmatic coagulation system, platelet function, fibrinolysis and other factors which can be affected by illness, environment and medications.
- The patterns of changes in strength and elasticity in the clot provides information about how well the blood can perform hemostasis, and how well or poorly different factors are contributing clot formation.


N
S
Electromagnetic
Transducer
Stab
Pin
Cup
.36 ml whole blood
(Clotted)
4°45´

Disorders of haemostasis

PLTs and vessel wall

1. Thrombotic, immune thrombocytopenic purpura
2. Hemolytic-uremic syndrome (HUS)
3. Glanzmann's thrombasthenia
4. Bernard-Soulier syndrome (abnormal glycoprotein Ib-IX-V complex)
5. Storage pool disorders
6. Paroxysmal nocturnal hemoglobinuria
7. Gray platelet syndrome: deficient alpha granules.
8. Delta storage pool deficiency: deficient dense granules

Coagulation and thrombosis

1. Hemophilia A (Factor VIII deficiency)
2. Hemophilia B (Factor IX deficiency, "Christmas disease")
3. Hemophilia C (Factor XI deficiency, mild bleeding tendency)
4. Von Willebrand disease (the most common bleeding disorder)
5. DIC
6. Factor inhibitors

Predisposing to thrombosis

1. Heparin-induced thrombocytopenia and thrombosis ("white clot syndrome")
2. Antiphospholipid syndrome
3. Prothrombin mutation
4. Protein C deficiency
5. Protein S deficiency
6. Antithrombin deficiency
7. Factor V Leiden and Activated Protein C Resistence
8. Abnormally raised levels of Factor VIII and Factor XI

Pro-thrombotic states

Endothelium

1. Physical trauma (atherosclerosis)
2. Inflammation (vasculitis)
3. Hypertension
4. Bacterial endotoxins
5. Cigarette products

Blood flow

1. MI, AF
2. Aneurysms
3. Stasis, prolonged bed rest

Hypercoagulability

1. Factor V Leiden mutation (can't be deactivated)
2. Protein C,S deficiencies
3. Antithrombin III deficiency
4. Cancer (release procoagulant compounds)
5. Antiphospholipid antibodies

Bleeding tendencies

- **Vessel wall**

1. Infections(Neisseria,Ricketsia)
2. Collagen (Vit. C, Ehlers Danlos)

- PLTs

1. ↓production
2. ↓survival
   - immune,
   - non-immune,
   - **mechanical,**
   - dilutional
   - spleenic seq.
3. Dysfunction
   - congenital
   - acquired

- Factor abnormalities

1. Acquired
   - Vit K
   - Warfarin
   - Liver failure
2. Congenital
   - vWF
   - Haemophilias

Blood starts to coagulate throughout the whole body. This depletes the body of its platelets and coagulation factors, and there is a paradoxically increased risk of hemorrhage

DIC

Causes

1. Obstetric complications
2. Sepsis, particularly with gram-negative bacteria.
3. Tissue trauma such as burns, accidents, surgery or shock.
4. Malignant cancers, or widespread tissue damage (e.g. burns), or
5. hypersensitivity reactions all can produce the chemicals leading to a DIC.
6. Liver disease
7. Incompatible blood transfusion reactions
8. Envenomation by some venomous snakes

Diagnosis

1. full blood count (↓ PLTs)
2. ↑ FDPs or D-dimer tests (markers of fibrinolysis)
3. ↑bleeding time
4. ↓ fibrinogen levels.

Management

The underlying cause must be treated initially. Anticoagulants are not given, as by now all the coagulation factors and platelets have been used up. These must be replaced, by platelet transfusion and fresh frozen plasma, to restore normal levels. infusion with antithrombin may be necessary. recombinant activated protein C product.

Heparin-induced thrombocytopenia (HIT) with or without **thrombosis** (HITT) is thrombocytopenia due to the administration of heparin. While it is mainly associated with unfractioned heparin , it can also occur with expsoure to LMWH, but at significantly lower rates. Despite the low platelet count, it is a thrombotic disorder, with very high rates of thrombosis in the arteries with or without venous complications.

HIT

Pathophysiology

- Type I - Patients have a transient decrease in platelet count without any further symptoms. This recovers even if heparin is continued to be administered. Platelet counts rarely fall below 100. It occurs in 10-20% of all patients on heparin. It is not due to an immune reaction and antibodies are not found upon investigation.
- Type II - This form is due to an autoimmune reaction with antibodies formed against platelet factor 4 (PF4), neutrophil-activating peptide 2 (NAP-2) and interleukin 8 (IL8) which form complexes with heparin. The most common being to the heparin-PF4 complex. It appears that heparin binding to platelet factor 4 causes a confomational change in the protein, rendering it antigenic. Clot formation is mainly arterial and rich in platelets ("white clot syndrome"), in contrast with fibrin-rich clots (which are red due to trapped red blood cells). Most thrombotic events are in the lower limbs, skin lesions and necrosis may also occur at the site of the heparin infusion Type II HIT develops in about 3% of all patients on UFH and in 0.1% of patients on LMWH, and causes thrombosis in 30% to 40% of these patients.

Management

- Treatment is by **prompt withdrawal** of heparin and replacement with a suitable alternative anticoagulant.
- **Protamine sulfate**, the normal antidote for heparin, is not effective as the antibodies react with platelets independent of heparin.
- To block the thrombotic state, lepirudin (Refludan®), bivalirudin, argatroban, danaparoid or other direct thrombin inhibitors are used.
- Low molecular weight heparin is contraindicated in HIT.

Anticoagulants

- **Vitamin K antagonists**

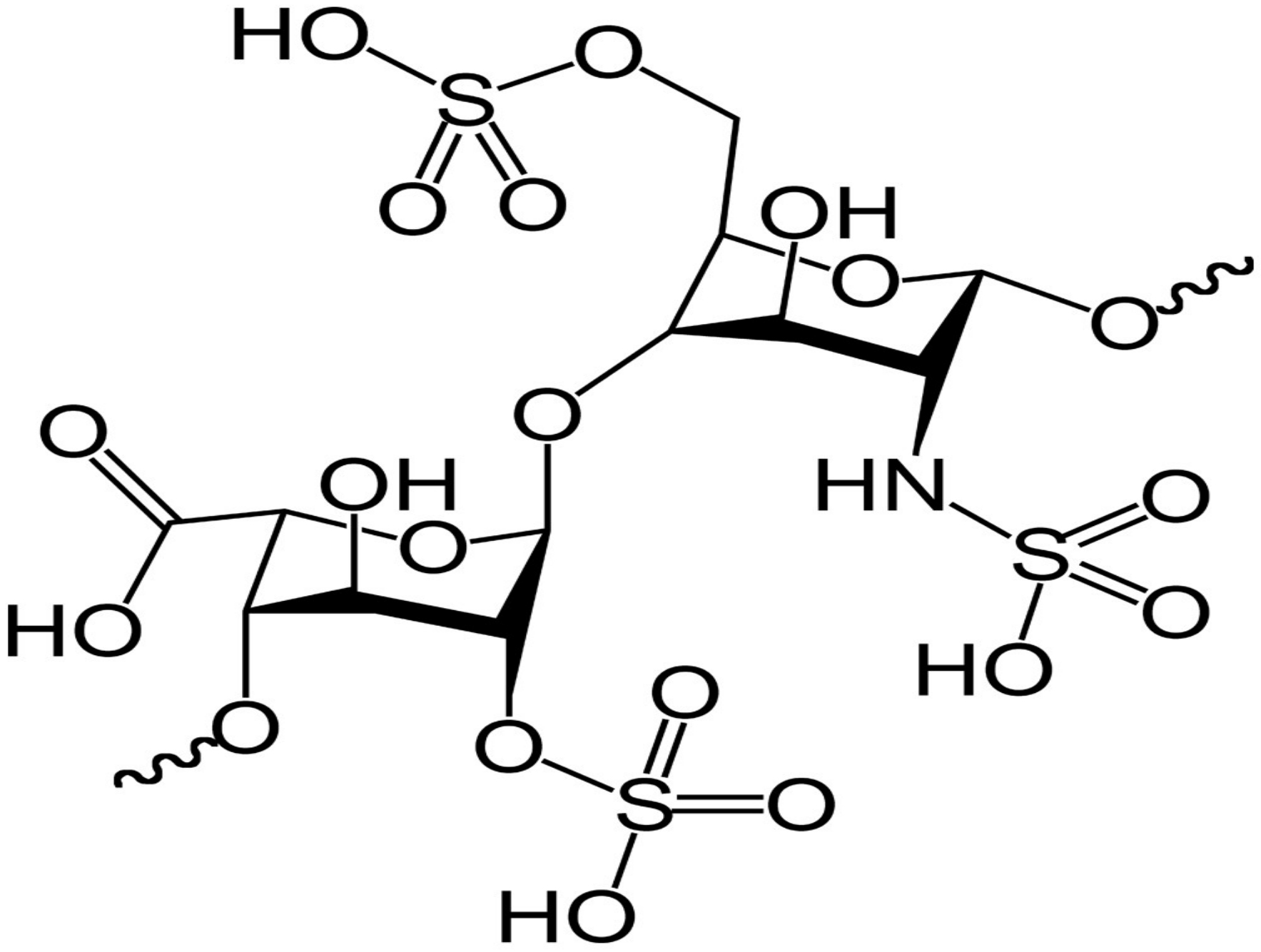
1. The oral anticoagulants are a class of pharmaceuticals that act by antagonizing the effects of vitamin K. It is important to note that they take at least 48 to 72 hours for the anticoagulant effect to develop fully. In cases when an immediate effect is required, heparin must be given concomitantly. Generally, these anticoagulants are used to treat patients with deep-vein thrombosis (DVT), pulmonary embolism, atrial fibrillation, and mechanical prosthetic heart valves.
2. These oral anticoagulants are used widely as poisons for mammalian pests, especially rodents. (For details, see rodenticide and warfarin.)
3. The most important oral anticoagulants are:
4. Warfarin (Coumadin). This is the main agent used in the U.S. and UK
5. Acenocoumarol and phenprocoumon This is used more commonly outside the U.S. and the UK
6. Phenindione

Anticoagulants

- **Heparin and derivative substances**
- Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used in vivo (by injection), and also in vitro to prevent blood or plasma clotting in medical devices. Vacutainer brand test tubes containing heparin are usually colored green.
- Low molecular weight heparin is a more highly processed product that is useful as it does not require monitoring of the APTT coagulation parameter (it has more predictable plasma levels) and has less side effects.
- Fondaparinux is a synthetic sugar composed of the five sugars (pentasaccharide) in heparin that bind to antithrombin. It is a smaller molecule than low molecular weight heparin.

Heparin

- **History**
- Heparin is one of the oldest drugs currently still in widespread clinical use. As it was discovered in 1916, its introduction predates the establishment of the United States Food and Drug Administration. It was originally isolated from liver cells, hence its name (*hepar* or "ηπαρ" is Greek for "liver"). Scientists were looking for an anticoagulant that could work safely in humans, and Jay McLean, a second-year medical student from Johns Hopkins University working under the guidance of William Henry Howell, found a compound extracted from liver that acted as an anticoagulant.


HO
S
O
O
O
OH
O
O
HN
O
S
O
HO
O
OH
O
HO
O
O
S
O
HO

Heparin

- **Mechanism of action**

1. Heparin is a naturally occurring anticoagulant produced by basophils and mast cells.
2. Heparin binds to the enzyme inhibitor antithrombin III (AT-III) causing a conformational change which results in its active site being exposed. The activated AT-III then inactivates thrombin and other proteases involved in blood clotting, most notably factor Xa.
3. The rate of inactivation of these proteases by AT-III increases 1000-fold due to the binding of heparin.

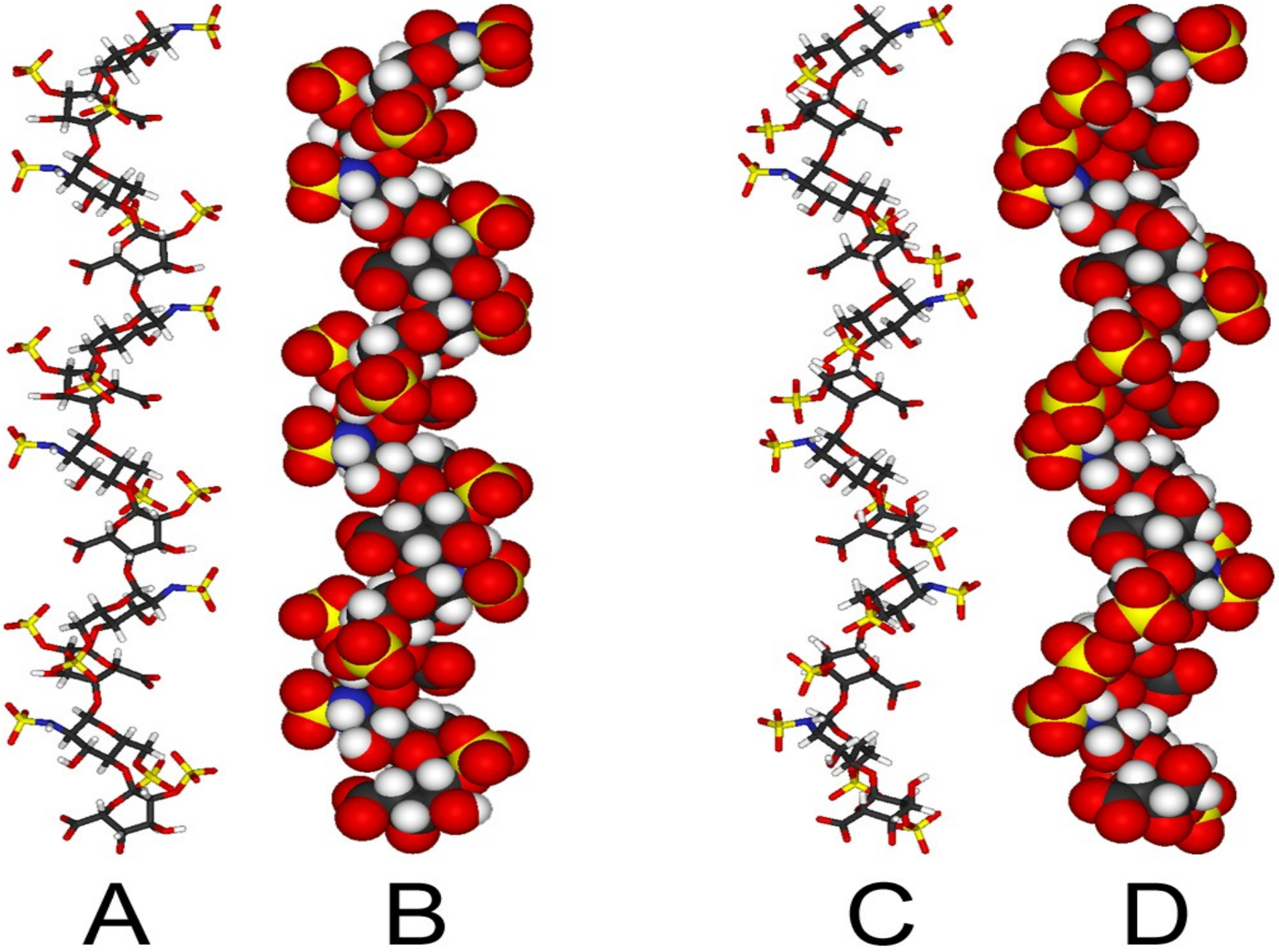
Heparin

- **Administration**

1. Heparin is given parenterally; it is digested when taken by mouth. It can be injected intravenously or subcutaneously (under the skin). Intramuscular injections (into muscle) are avoided because of the potential for forming hematomas.
2. Because of its short biologic half-life of approximately one hour, heparin must be given frequently or as a continuous infusion. However the use of low molecular weight heparin (LMWH) has allowed once daily dosing. Thus not requiring a continuous infusion of the drug.
3. If long-term anticoagulation is required, heparin is often only used to commence anticoagulation therapy until the oral anticoagulant warfarin takes effect.

Heparin

- **Adverse reactions**
- A serious side-effect of heparin is heparin-induced thrombocytopenia (HIT syndrome). HITS is caused by an immunological reaction that makes platelets aggregate within the blood vessels, thereby using up coagulation factors. Formation of platelet clots can lead to thrombosis, while the loss of coagulation factors and platelets may result in bleeding. HITS can (rarely) occur shortly after heparin is given, but also when a person has been on heparin for a long while. Immunologic tests are available for the diagnosis of HITS. There is also a benign form of thrombocytopenia associated with early heparin use which resolves without stopping heparin.
- Other side effects include alopecia and osteoporosis.
- **Treatment of overdose**
- In case of overdose, protamine sulfate can be given to counteract the action of heparin.


A
B
C
D

LMWH

- Differences with unfractionated heparin include:

1. Average molecular weight: heparin is about 20000 Da and LMWH is about 3000 Da
2. Once daily dosing, rather than a continuous infusion of unfractionated heparin
3. No need for monitoring of the APTT coagulation parameter
4. Possibly a smaller risk of bleeding
5. Smaller risk of osteoporosis in long-time use
6. Smaller risk of heparin-induced thrombocytopenia, a feared side-effect of heparin.

Anticoagulants

- **Direct thrombin inhibitors**
- Another type of anticoagulant is the direct thrombin inhibitors. Current members of this class include argatroban, lepirudin, and bivalirudin. An oral direct thrombin inhibitor, ximelagatran (Exanta®) may replace warfarin for some indications. It is awaiting Food and Drug Administration (FDA) approval.

Thank you