Haemostasis

It is the stoppage of bleeding from an injured blood vessel.

Also it may be defined as the process in circulation where the blood is maintained fluid in vessels and without major loss in case of injury.

Another definition,

haemostasis is the process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue.

It is a highly balanced interaction between blood vessels, platelets, and soluble clotting factors that are involved in the formation and dissolution of blood clots.

- Haemostasis, composed of 4 major events that occur in a set order following the loss of vascular integrity:

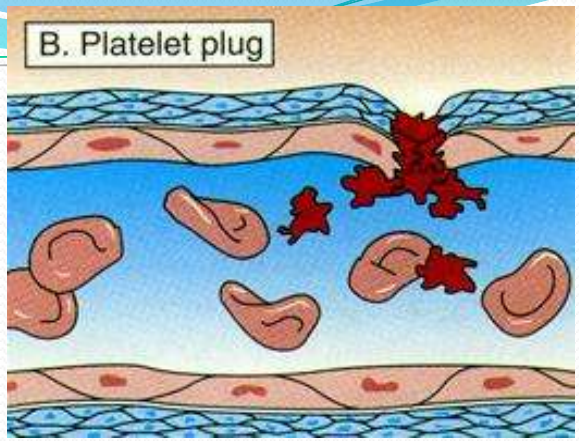
1. Local vasoconstriction occurs in the area of injury, it is caused by:

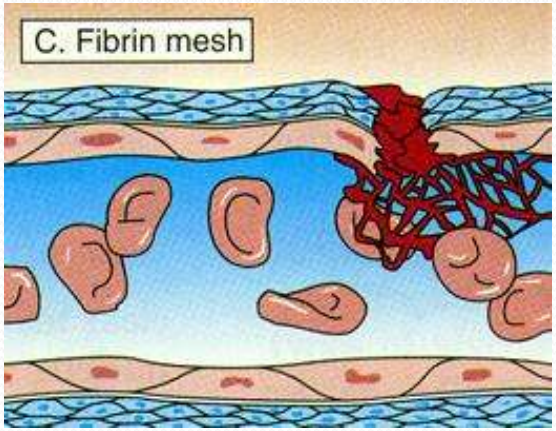
a. Local nervous reflex initiated by pain impulses in the traumatized area..

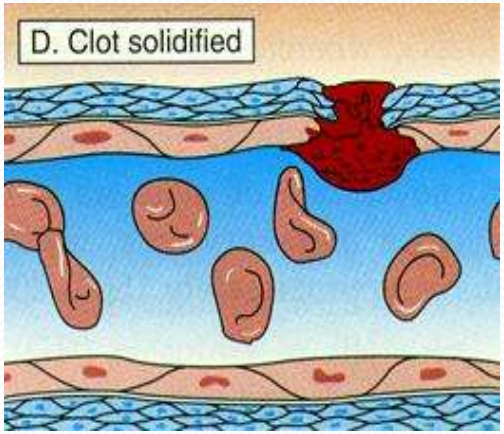
- b. Local vasoconstrictors released in the area from endothelial cells (Endothelin), from platelets(Thromboxane A2, Serotonin and ADP)

- c. Myogenic mechanism, contraction of the smooth muscles of injured blood vessel as a direct response to trauma.

2. Formation of platelet plug(**white thrombus**):It has been discussed before, it is enough to stop small bleeders.

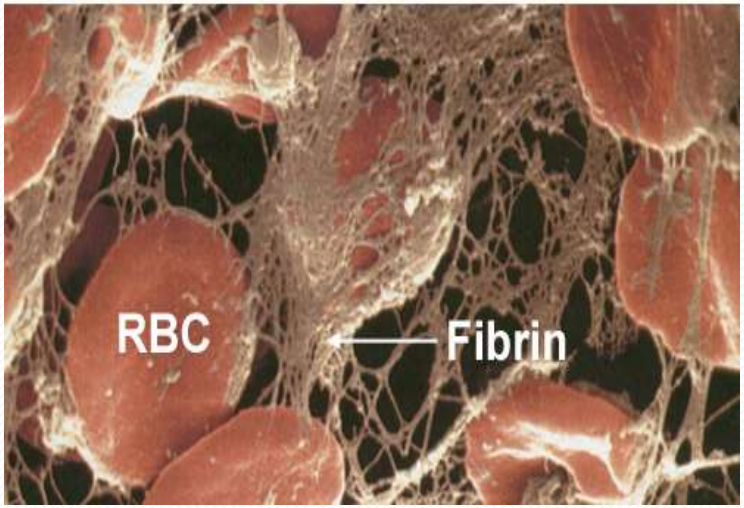






3. Formation of blood clot(blood coagulation):It is needed to close large injuries in the blood vessels (**red thrombus**).

Blood clot is formed of meshwork of fibrin threads entrapping blood cells and platelets.



4. Fibrinolysis is the final stage of restoring haemostasis - it prevents uncontrolled, widespread clot formation and breaks down the fibrin within blood clots. The two most important anticoagulants involved in fibrinolysis are antithrombin III (ATIII) and Protein C.

The end products of fibrinolysis are fibrin degradation products (FDPs).

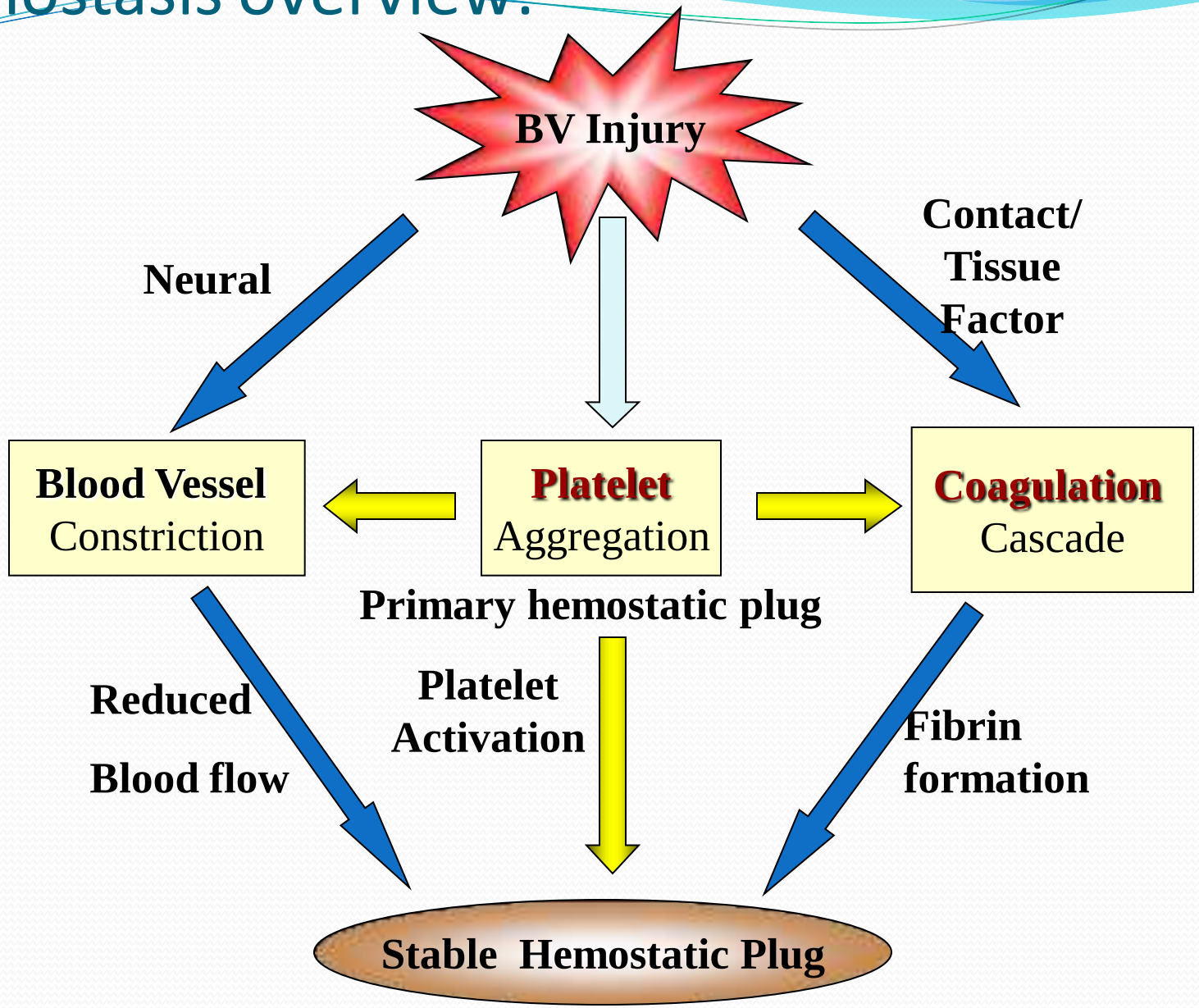
- Formation of blood clot needs the following clotting factors :

- *Formation of blood clot needs the following clotting factors:

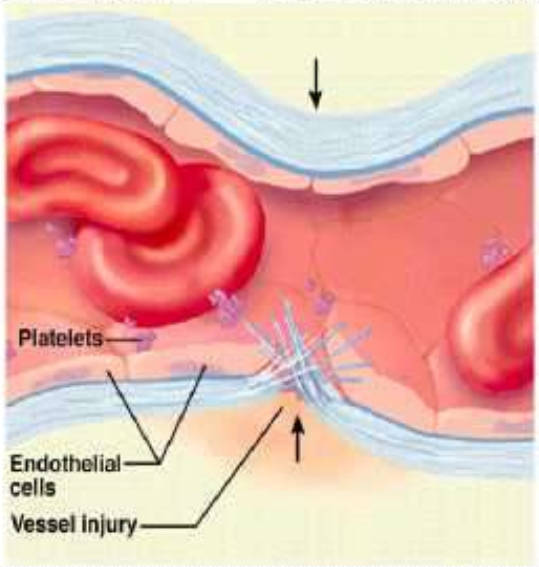
Factor	Name	Pathway
I	Fibrinogen	Common
II	Prothrombin	Common
III	Tissue factor	Extrinsic
IV	Ionized free calcium	All
V	Proaccelerin	Common
VII	Proconvertin	Extrinsic
VIII	Antihemophilic factor	Intrinsic
IX	Christmas factor	Intrinsic
X	Stuart factor	Intrinsic
XI	Plasma thromboplastin Antecedent	Intrinsic
XII	Hageman factor	Intrinsic
XIII	Fibrin stabilizing factor	Common
HM/WK	Fitzgerald factor	Intrinsic
PK	Fletcher factor	Intrinsic
PF3	PF3, Phospholipid surface	Intrinsic, common

I	Fibrinogen	VIII	Antihaemophilia factor A
II	Prothrombin	IX	Antihaemophilia B Christmas F plasma thromboplastin F
III	Tissue thromboplastin (tissue factor)	X	Stuart power factor
IV	Ca^{++}	XI	Antihaemophilia factor C
V	Proaccelerin	XII	Contact factor (Hageman factor)
VII	Proconvertin	XIII	Fibrin stabilizing factor

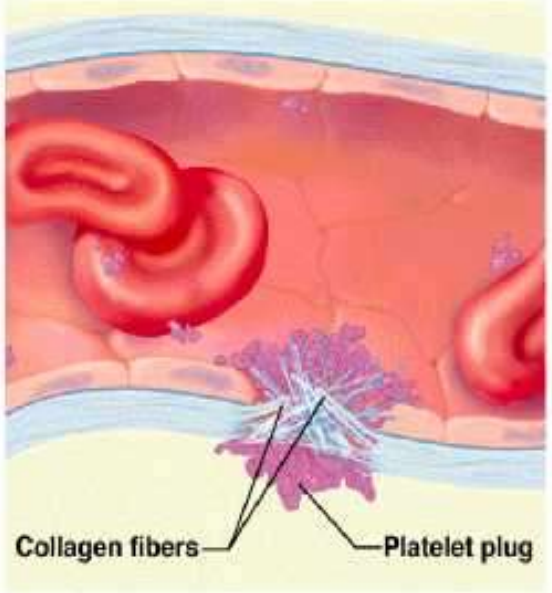
Haemostasis overview:



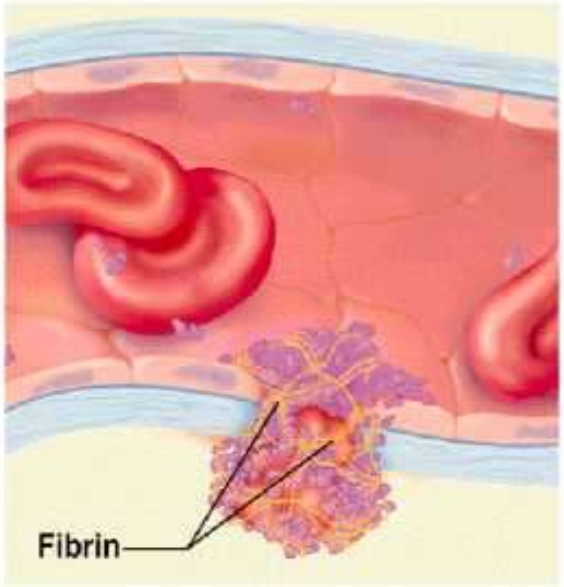
Hemostasis — Vasoconstriction



Hemostasis — Platelet Plug



Hemostasis — Blood Clot



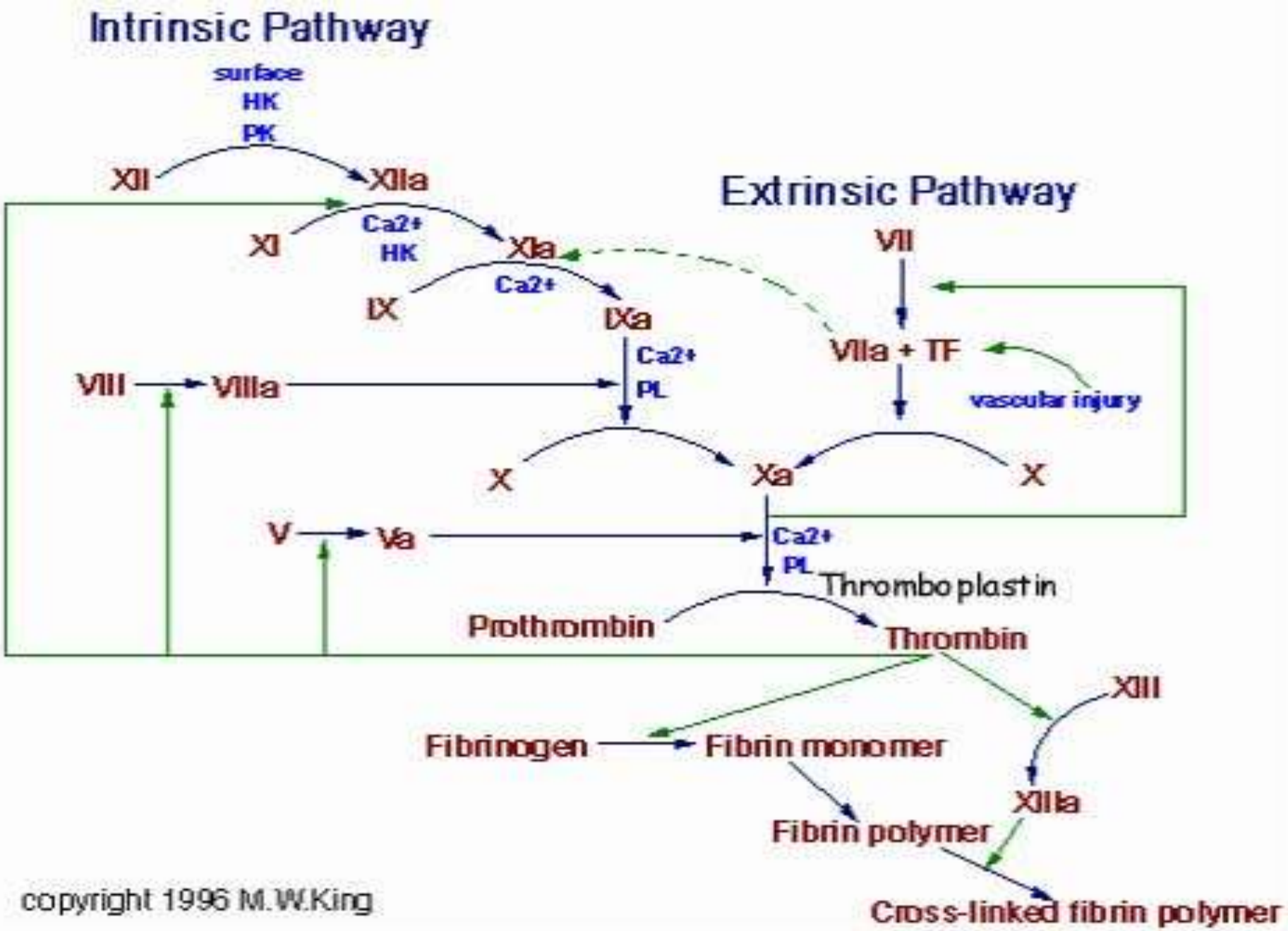
PF3, Prekallikrein (**PK, Fletcher factor**) and High molecular weight kininogen (**HMWK, Fitzgerald factor**), are considered as clotting factors.

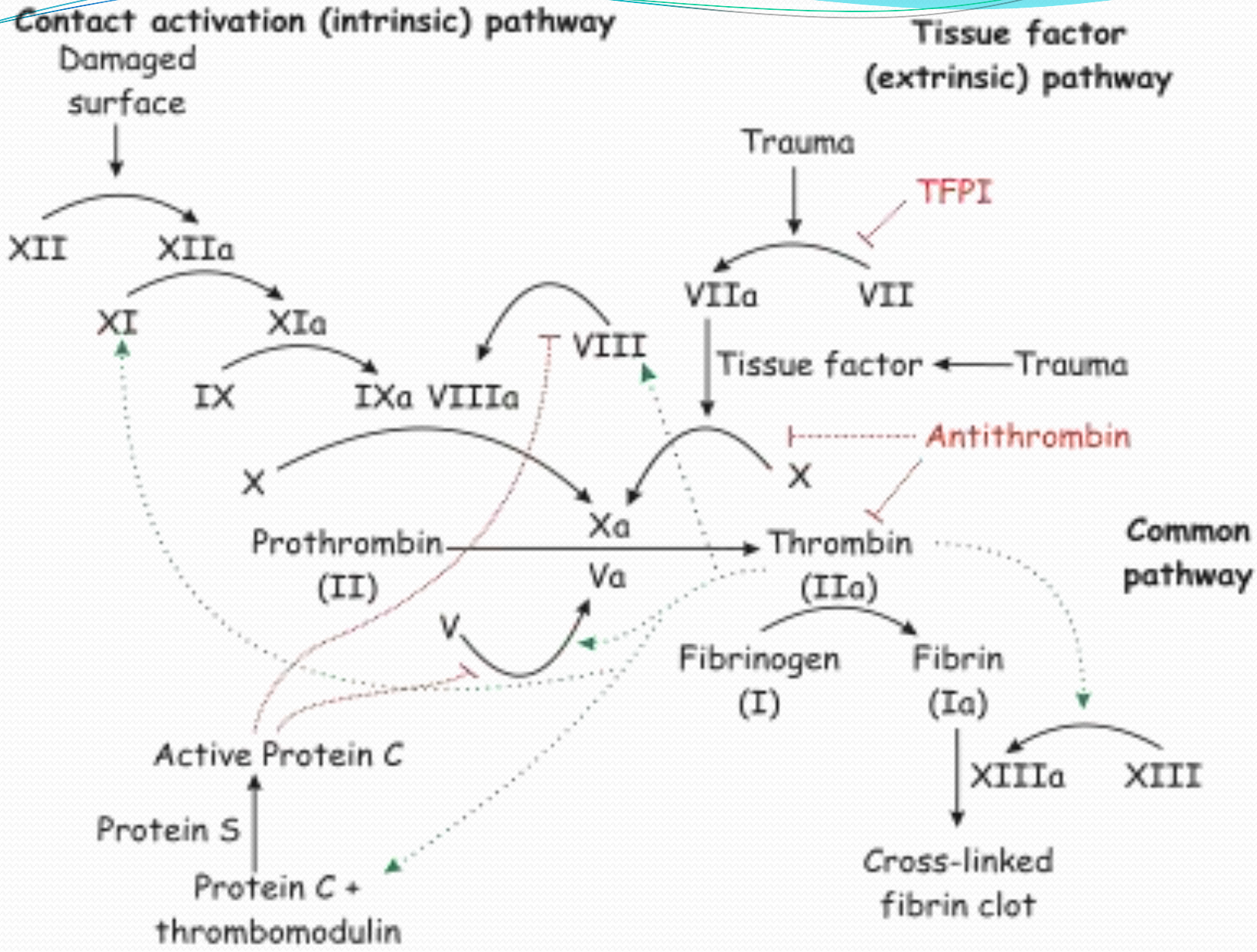
*The clot is formed by these factors through three pathways:

a. Intrinsic pathway (contact activation pathway):All clotting factors needed are present in the blood(Factor XII, XI, VIII, HMWK, pre-Kallikrien).

b. Extrinsic pathway (tissue factor pathway) : Need factors which are not present in the blood(Factor III,VII).

c. Common pathway: It includes factor(X , V, II, and I).

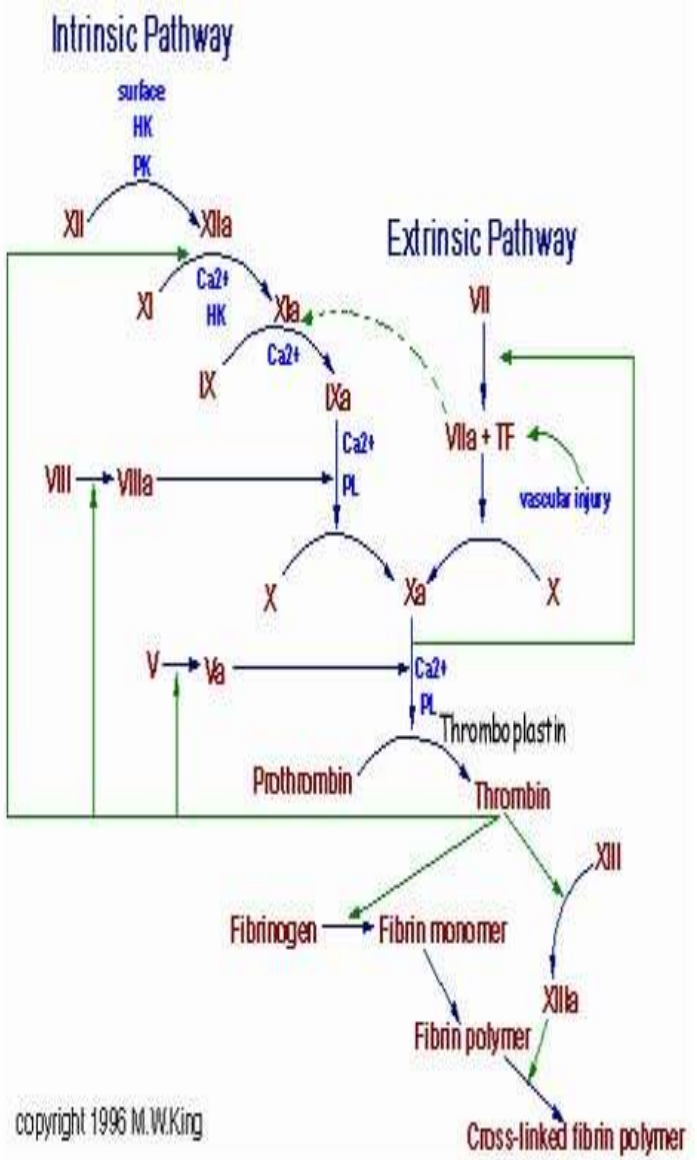

Intrinsic Pathway
surface
HK
PK
XII
XIIa
XI
Ca2+
HK
XIa
IX
Ca2+
IXa
VIII
VIIIa
Ca2+
PL
X
Xa
V
Va
Ca2+
PL
Prothrombin
Thrombo plastin
Thrombin
Extrinsic Pathway
VII
VIIa + TF
vascular injury
X
Fibrinogen
Fibrin monomer
XIII
XIIIa
Fibrin polymer
Cross-linked fibrin polymer
copyright 1996 M.W.King


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

The clotting cascades:

a. The intrinsic cascade

Initiated when contact is made between blood and exposed negatively charged surfaces as subendothelial collagen. Contact activation involves FXII,XI,PK(Fletcher F) and HMWK(Fitzgerald F).



- These molecules are activated when
  1.plasma interacts with negatively charged substance:
- a. In vivo (collagen, endotoxin or platelet phospholipid).

  b. In vitro(glass, kaolin).

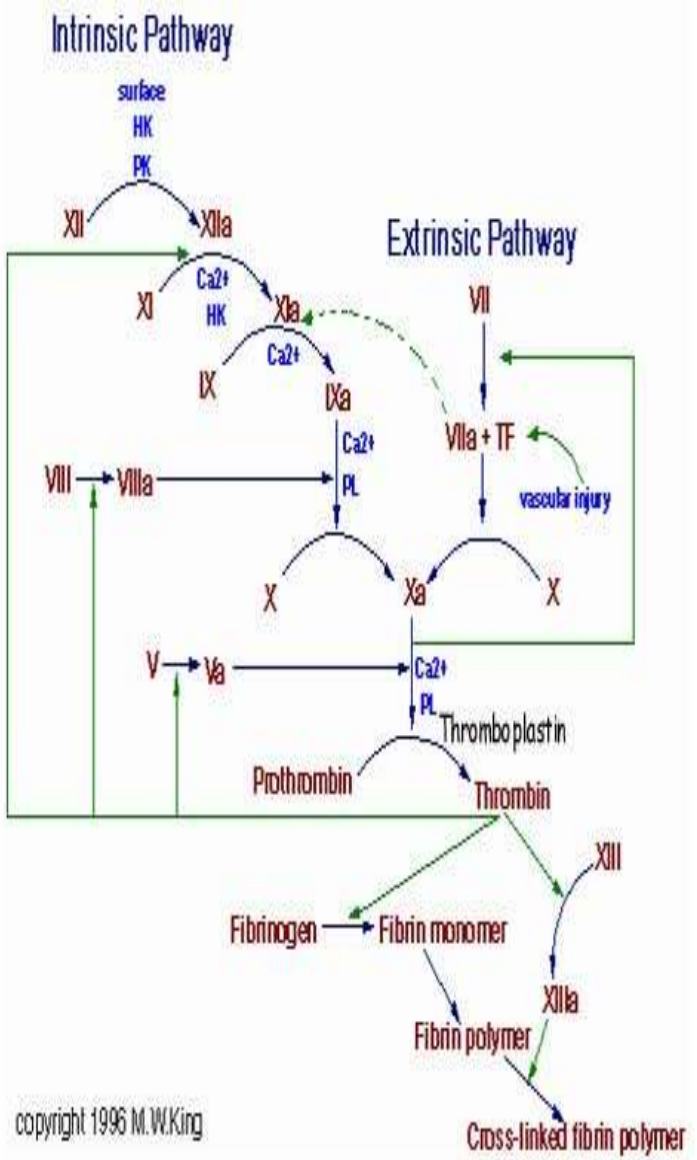
Then the product of contact activation of these factors is activation of factor XI, which subsequently activate F IX. In conjunction with thrombin- activated factor VIII, activated FIX subsequently activate F X in the presence of PF3 and Ca ions.

b. The extrinsic coagulation pathway Is initiated upon a. Vascular injury which leads to

b. Exposure of tissue factor(TF) (also identified as factor III, tissue thromboplastin); it is a sub endothelial cell-surface co protein ,

c. It will contacts and complexes with F VII and activates it (active Proaccelerin F) in the presence of Ca and PF3.

d. This complex can activate FIX of intrinsic pathway and FX of common pathway. Factor VII may also be activated by active factor XII, X and IX, once coagulation started.



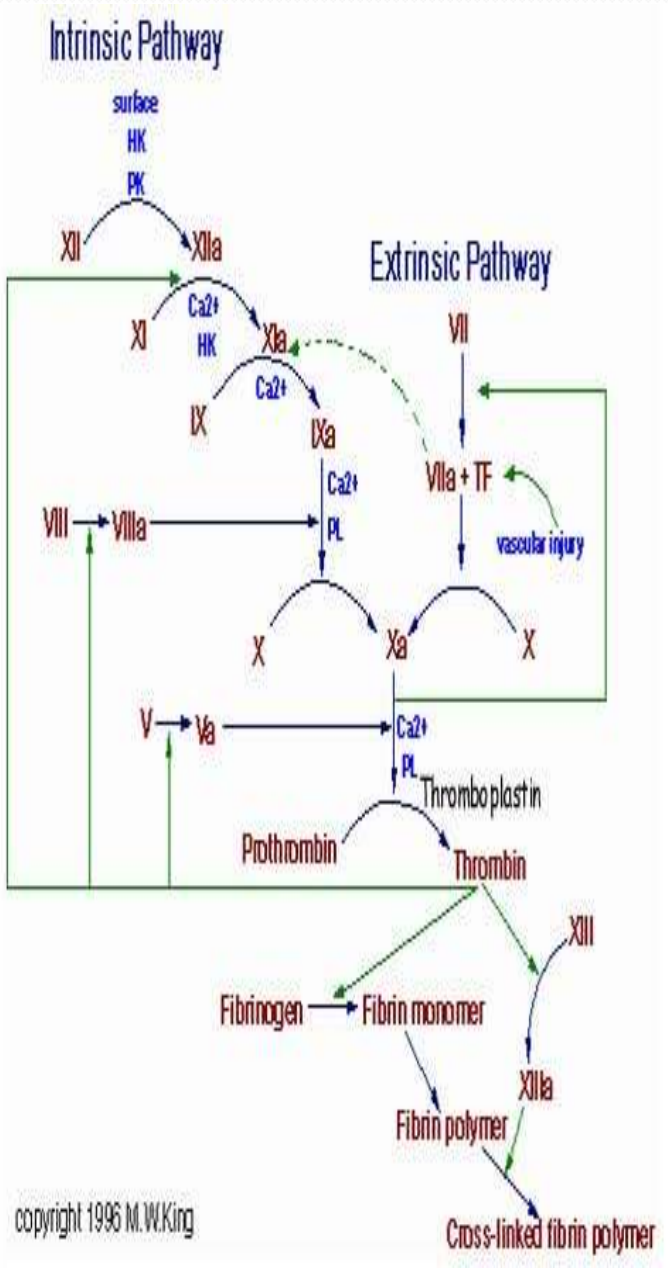
C. Common pathway:

1. The two pathways converge at the activation of factor X to Xa.

2. Factor Xa has a role in the further activation of factor VII to VIIa.

3. Active factor Xa hydrolyzes and activates Prothrombin(inactive FII) to thrombin.

4. Thrombin converts fibrinogen(inactive FI) to fibrin and to activate factor XIII to XIIIa, that cross-links fibrin polymers solidifying the clot. Also thrombin activates factors XI, VIII and V furthering the cascade.



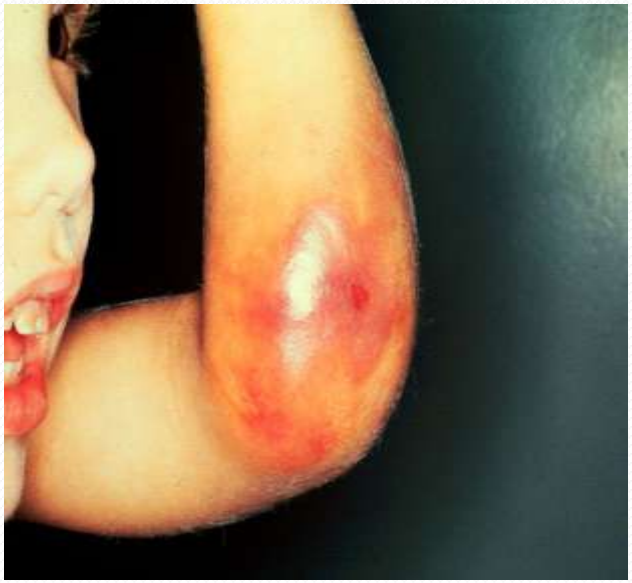
HMWK = high molecular weight kininogen (Fitzgerald F).

PK = prekallikrein(Fletcher F).

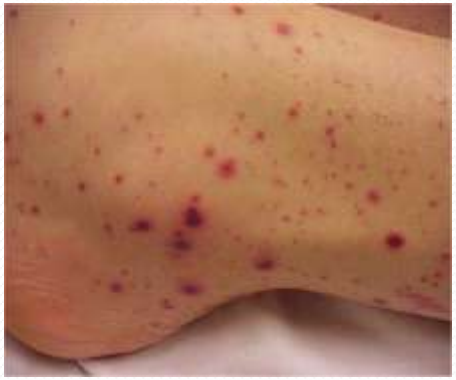
PL = phospholipid.

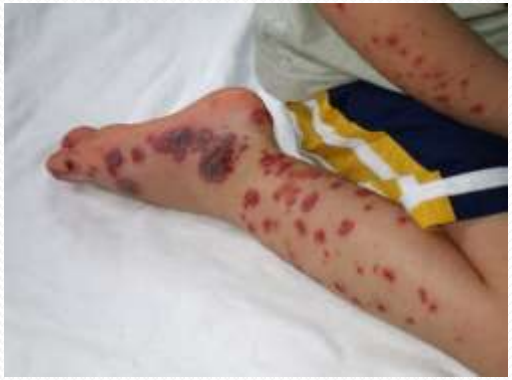
- Thrombin can then also activate factors XI, VIII and V furthering the cascade.

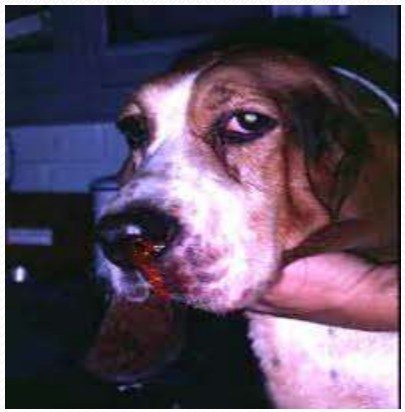
- Clinical presentation for disorders of secondary hemostasis: Resulting from coagulation factor deficiency and coagulation factor inhibitors. Large spreading hematomas, hemarthrosis, bleeding into body cavities, delayed, severe bleeding with routine surgery.



- Clinical presentation for primary hemostatic disorders: Resulting from blood vessel defects qualitative and quantitative platelet abnormalities. Superficial hemorrhage- petechiae, purpura, ecchymosis, spontaneous epistaxis, ocular hemorrhages, immediate oozing from surgical incision







Diagnostic approach:

- Case history.
- Physical examination.
- Laboratory diagnostic tests.

Both extrinsic and intrinsic factors are complementary , Ca, should be in the ionized form to be effective in promoting coagulation, except for the first two steps in the intrinsic pathway, Ca ions are essential for promotion of all reactions.

Evaluation of the coagulation system:

Tests are applied on blood collected from the patient and a normal animal of the same species, sodium citrate is used as an anticoagulant(9:1), blood should be kept cool and used within 30 minutes of collection.

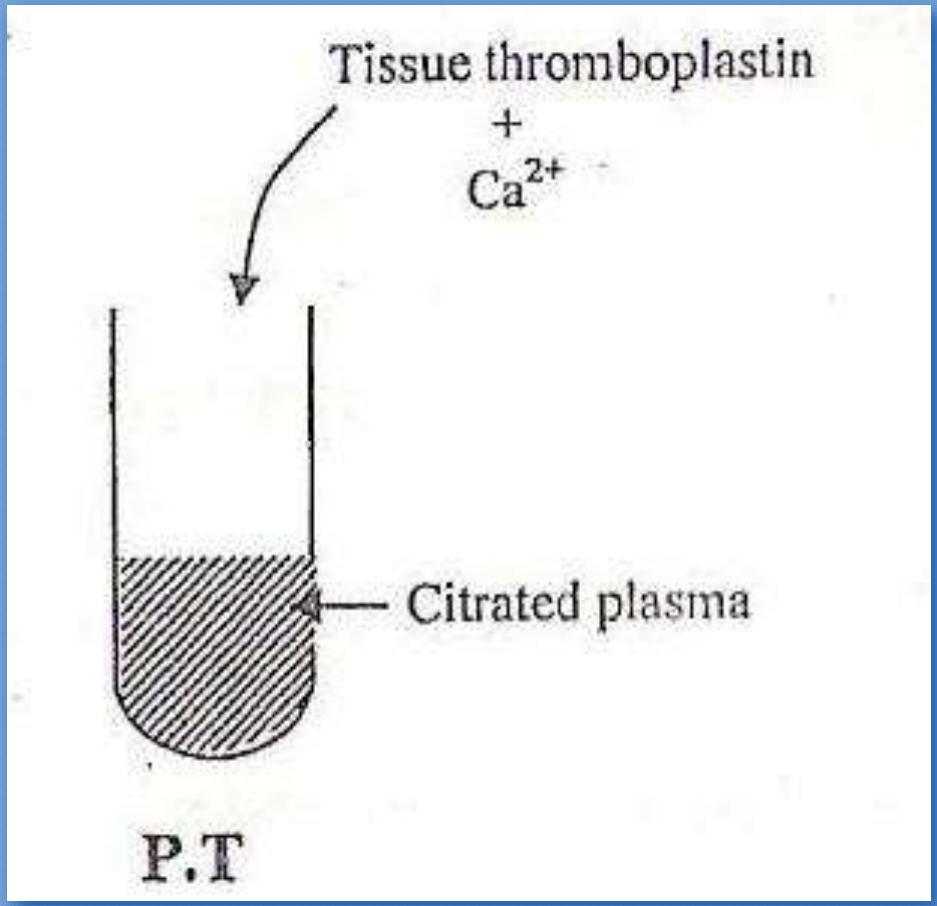
1. CBC.. Is always the first test to perform when a bleeding patient is presented to detect platelet number, platelet morphology presence or absence of anemia.

- **2. Activated clotting time (ACT): It measures intrinsic and common pathway similar to PPT but PPT is more sensitive.**
- The activated clotting time (ACT) allows rapid evaluation of secondary haemostasis.
- The ACT is the time taken for 2ml of fresh whole blood to clot in a tube with a contact activator (diatomaceous earth, silicon),
- The reaction must occur at body temperature to give a reliable indication of haemostatic ability: this can be achieved by the use of a warm water bath, or in an emergency by holding the tubes under an arm.
- The normal ACT is 90-120 seconds and <75 seconds in dogs and cats respectively.

Interpretation: ACT will therefore be prolonged when factors I, II, V, VIII, IX, X, XI or XII are deficient or abnormal, such as in DIC, liver disease, vitamin K antagonist toxicosis (ingestion of rodenticides in small animals or mouldy sweet clover in large animals represents the most common cause of coagulopathy in vet medicine) or haemophilia A or B.

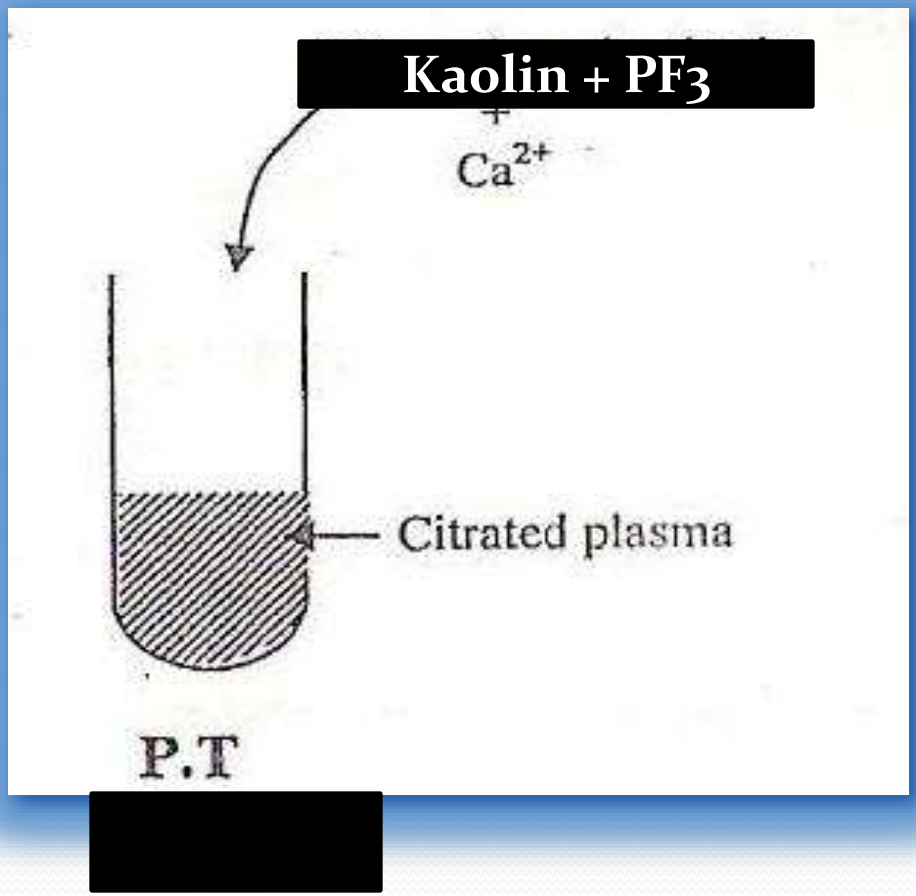
3. Prothrombin time (PT) : It is a laboratory test used to evaluate the extrinsic and common pathway. **Normal value** in man is from 10-14 seconds. In animals the patient´s PT value is compared with the value obtained from the blood of a normal control animal carried at the same time.

Method: Add to the citrated plasma of the patient tissue thromboplastin(III) and Ca(IV), calculate the time in seconds that pass till a clot is formed =PT.


Tissue thromboplastin
+
Ca^{2+}
Citrated plasma
P.T

4. Activated partial thromboplastin time(APTT): It is the time needed to form a clot after activation of contact factors without thromboplastin. **Normal value** depend on laboratory method used, **in man it is**(30-40 seconds),in animals it differ according to the lab. method applied , result is compared with the value of a control animal carried at the same time.

Method: To a citrated patient،s plasma add kaolin(a surface activator) and Ca^{++} and PF3. Calculate the time that pass till a clot is formed.



Prolongation of PTT with normal PT; localizes the problem to FXII, PK, HMWK,FIX, FXI or FVIII. Prolongation of PT and PTT indicate common pathway disorders (fibrinogen, prothrombin, FV, or FX.

3. Evaluation of plasma fibrinogen:

a. *The heat precipitation test* is a practical easy test but it is insensitive, centrifuged blood in two capillary tubes for PCV are used, one of them is broken above the buffy coat , drops of plasma are delivered to the prism of a refractometer, total plasma protein is measured (first reading) in g/dl, the second tube is heated in 54-56 C for three minutes,

plasma became cloudy by the coagulated fibrinogen, it is the only plasma protein that coagulate at this temperature, the heated capillary tube is re-centrifuged, the coagulated fibrinogen will be precipitated above the buffy coat, break the capillary tube and measure the remained protein(serum protein)(second reading),1st. reading –2nd. reading=Fibrinogen.

b. *Thrombin time(TT):* It measure the time (seconds) required for fibrin clot to occur in citrated plasma after the addition of Ca++ and thrombin(aII). The TT is largely dependent on the functional fibrinogen concentration. Hypofibrinogenaemia (less than 100mg/dl) will prolong the TT .

The rule of vit. K in blood coagulation: It is essential in a carboxylation reaction that results in the formation of active coagulation factors II, VII, IX and X. Dicumarol, a product of moldy sweet clover , inhibits the vit. K dependent carboxylation reaction. Consequently, dicumarol toxicity can result in haemorrhage in cattle and other species consuming moldy sweet clover. This led to the discovery of related compounds that are now used as rodenticides and therapeutic anticoagulants. This reaction takes place in the liver.

Blood Clotting Disorders: Hereditary factor deficiencies are uncommon in animals and specific factor assays must be performed to confirm these deficiencies.

Disorders of extrinsic pathway:

a. Hereditary deficiency of factor IIV (proconvertin) in dogs, it is a mild disease associated with easy bruising.

b. Acquired Vit. K deficiency , initially affect FVII because of its short half life, after that inactive FII,VII,IX and X may accumulate due to lack of vit. K-dependent carboxylation.

.

Disorders of intrinsic pathway:

a. Hereditary deficiency of PK (dogs and horses), affected animals may experience clinical bleeding.

b. Hereditary FXII deficiency(cats and dogs), resulting in PTT prolongation.

c. Hereditary FXI deficiency(dog and Holstein cattle), they have protracted bleeding after surgery.

e. Hereditary, sex-linked,

FIX deficiency(haemophilia B) in cats and dogs, affected young males have only 5-10% of normal FIX activity and experience bleeding with mild trauma. Carrier females are usually identified by decreased activity of FIX(40-60%), but do not experience spontaneous bleeding.

f. Hereditary, sex-linked, FVIII deficiency(haemophilia A, cats, horses, sheep, cattle and dogs), affected animals show bleeding disorders of various severity according to the species .

g. Acquired deficiency of clotting factors:

- *As in disseminated intravascular coagulation(DIC) which is associated consumption of the clotting factors and platelets that exceeds the rate of synthesis
- * Acquired deficiency of vit. k- dependent clotting factors: May occur in rodenticides toxicosis in small animals(Coumarine) and mouldy sweet clover poisoning in large animals, in addition to hepatic failure.