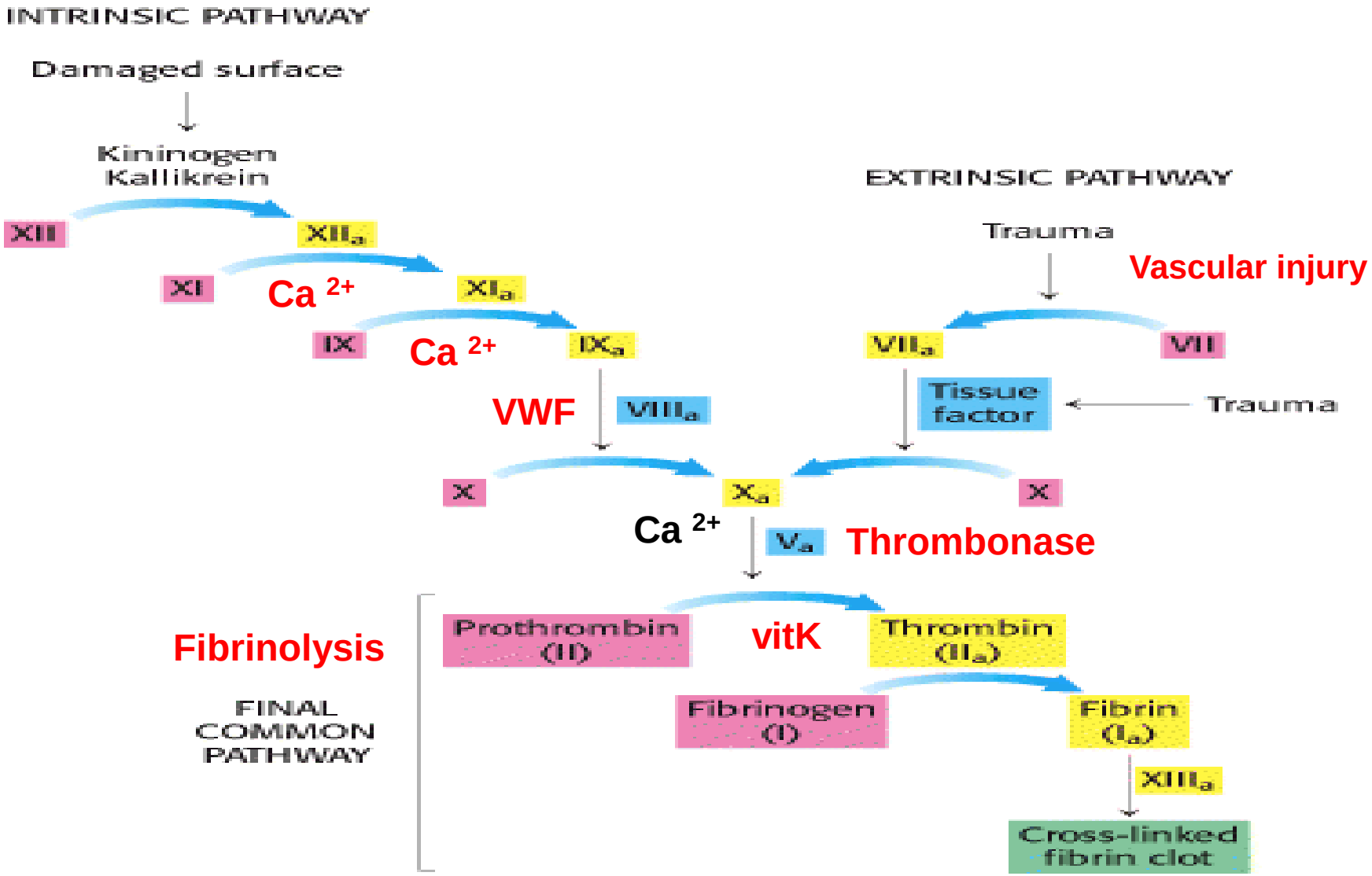
Molecular basis of some blood coagulation disorders

Blood coagulation cascade

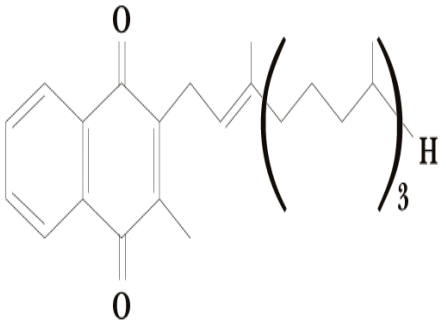


Factor Numerical	Description Name
I	Fibrinogen
II	Prothrombin
III	Tissue factor, tissue thromboplastin
IV	Calcium
V	Labile factor, proaccelerin, AC-globin
VI	Accelerin, eliminated by the International Committee on Blood Clotting Factors
VII	Proconvertin, stable factor
VIII	Antihemophilic A factor (AHF) Antihemophilic globulin (AHG)
IX	Plasma thromboplastin component (PTC), Christmas factor Antihemophilic B factor (AHB)
X	Stuart-Prower factor, autoprothrombin III
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor, glass factor, contact factor
XIII	Fibrin stabilizing factor (FSF), fibrinase
Prekallikrein	Fletcher factor
High molecular weight kininogen	Fitzgerald factor, Williams factor, Flau Jeac factor, Contact activation cofactor
(Designation) a	A factor that has been activated and is now functional

Vitamin K

Vitamin K_1 is abundant in vegetable oils and green leafy vegetables e.g. Spinach, peas and cabbage.

Vitamin K_2 is synthesized by intestinal flora and is found in animal tissues. Putrefied fish meal is a rich source.

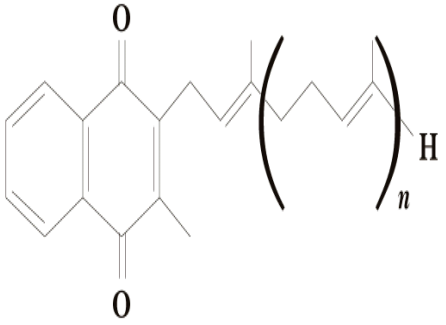


Vitamin K_1
(phylloquinone)

Sources of vitamin K include tomatoes, cheese, egg yolk, and liver.

Breast milk is NOT a good source of vitamin K.

Vitamin K is required for post translational modifications of several proteins required in the coagulation cascade.



Vitamin K_2
(menaquinone series)

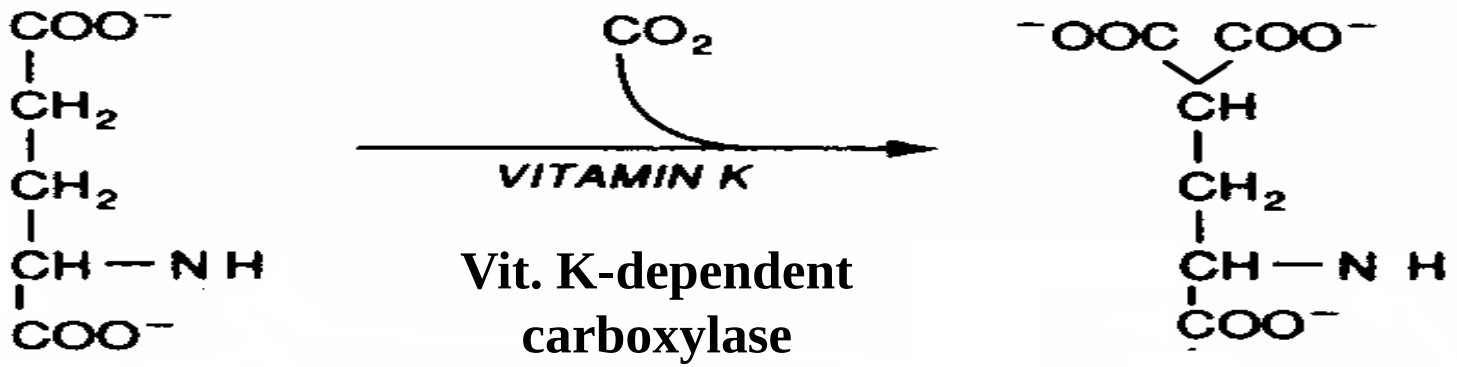
It converts blood clotting factors (II, VII, IX and X) to the active state. They are synthesized in liver in an inactive precursor form.

Mechanism of vitamin K-dependent activation for prothrombin

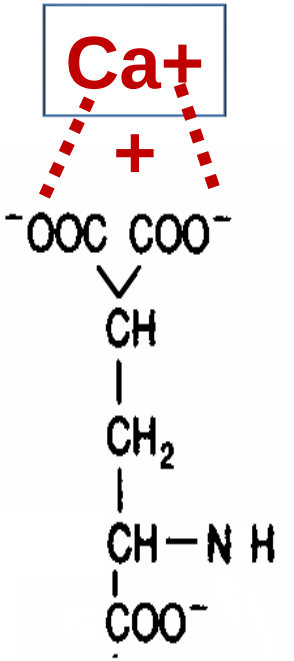
1. Prothrombin is synthesized in liver in an inactive precursor form called pre-prothrombin.
2. Pre-prothrombin (prothrombin precursor) conversion to prothrombin requires vitamin K-dependent carboxylation (of specific glutamic acid residues to γ-carboxyglutamic)

Pre-prothrombin → Prothrombin

(Glutamate) (γ caboxyglutamate)



3. The γ- carboxygulatmic acid residues are good chelators which allow prothrombin (active) to bind (chelate) calcium.
4. Prothrombin-Ca++-complex binds to phospholipids of cell membrane where proteolytic conversion to thrombin can occur.

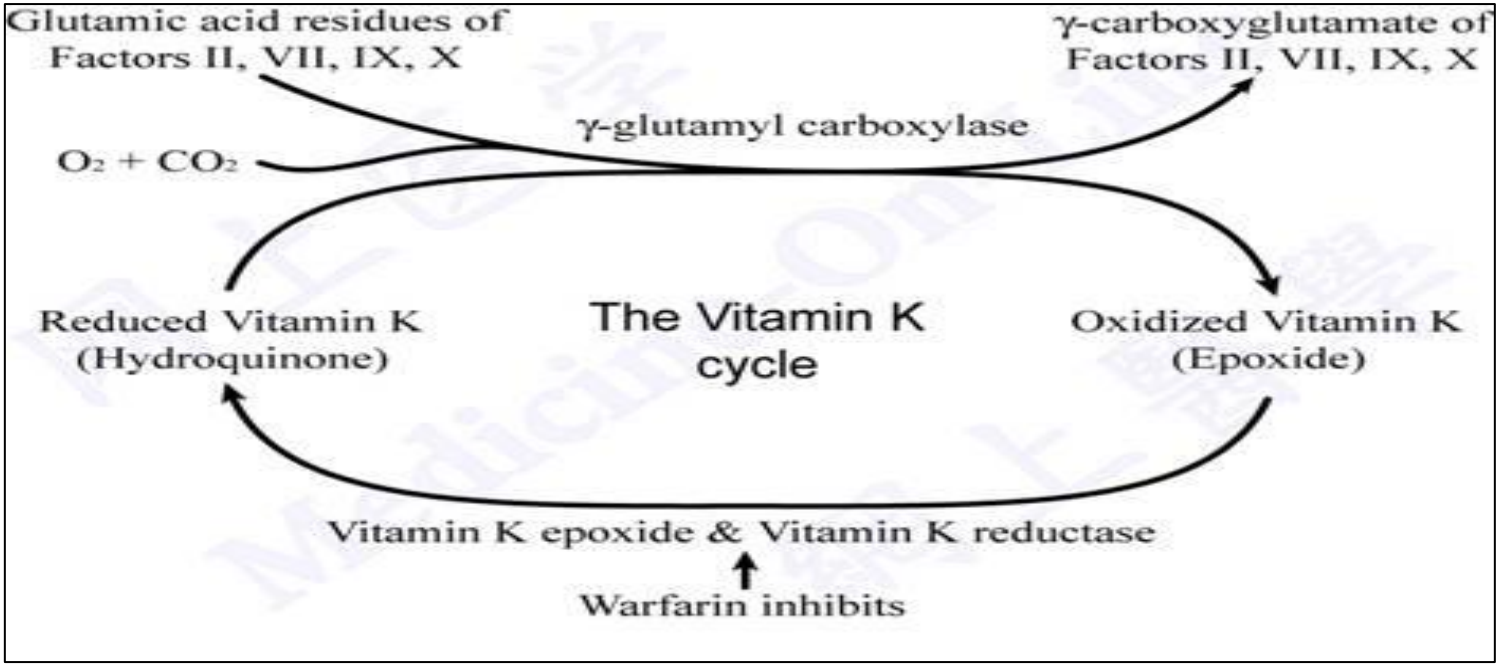


Function:

- Vitamin K is an essential cofactor for the carboxylase enzyme in specific protein molecules such as:

1- Blood clotting factors (II,VII, IX, X).
2- Bone calcium-binding proteins as osteocalcin.
3- The product of Growth arrest specific gene Gas6 which is involved in differentiation & development of nervous system.

Vitamin K cycle



Role of liver in blood clotting:

1. Site of clotting factors synthesis.
2. Site of bile salts synthesis (to help vit. K absorption).

Liver failure: results in severe bleeding problems.

Anti-coagulants

- Dicumarol & warfarin are antagonists of vitamin K (anti-coagulants).
- Are used to reduce blood coagulation in patients at risk of thrombosis. Thus, vitamin K is the antidote to an overdose of warfarin.

Deficiency

Causes:

Primary deficiency: rare

Secondary deficiency:

- Fat malabsorption.
- In newborn who lack bacterial colonization.
- long-term or high-dose administration of antibiotics (they kill the bacteria in large intestine).
- Anticoagulant Therapy.
- In patients suffering from Liver diseases (obstructive jaundice).

Vitamin K deficiency

Manifested by:

-Bleeding tendency (GIT, ecchymoses) from minor wounds.

- Nose & gum bleeding.
- Heavy menstrual bleeding.
- Increased risk for osteoporosis.

Diagnosed by:

- Prolonged blood coagulation time: prolonged prothrombin time (↑↑ PT). [blood takes 10-13.5 sec to clot].

Prevention: single shot of vit. K at birth in newborn.

The clotting process must be precisely regulated

- Hemorrhage and thrombosis must be regulated by mechanisms that normally limit clot formation to the site of injury.
- Activated factors are short-lived because they are diluted by blood flow, removed by the liver, and degraded by proteases

Regulation-Two Mechanisms

1- Va and VIIIa factors are digested by protein C, a protease that is switched on by the action of thrombin which has dual function:

a- It catalyzes the formation of fibrin

b- it initiates the deactivation of the clotting cascade.

2- Specific Inhibitors of clotting factors are crucial in terminating blood clotting as:

a- Tissue factor pathway inhibitor (TFPI), inhibits the complex of TF- VIIa - Xa .

b- Anti-thrombin-III, another inhibitor which is inactivates thrombin, its inhibitory action is enhanced by negatively charged heparin.

Diagnostic Tests

A- Activated partial thromboplastin time (aPTT): measures effectiveness of clotting factors (in seconds) (intrinsic pathway)
It is only elevated in:
1- Factor XI, IX, or VIII deficiency
2- Factor XI, IX, or VIII specific factor inhibitor
3- Heparin contamination
4- Antiphospholipid antibodies

B- Prothrombin time (PT) (extrinsic pathway)
It is only elevated in:
1- Factor VII deficiency
2- Congenital (very rare)
3- Acquired (Vit K deficiency, liver disease)
4- Factor VII inhibitor
5- Rarely in patients with modest decreases of factor V or X

C- Measurement of the amount of each factor in the plasma and aPTT test performed as routine diagnostic tests for bleeding disorders

D- ELISA detects the presence of antibodies to clotting factor proteins.

Molecular basis of some blood clotting disorders

1- Von Willebrand disease: most common inherited bleeding disorder

- The genetic mutations result in inherited deficiency of Von Willebrand
- It is associated with an increase in aPTT, thus prolonged bleeding time despite normal platelet count
- Because vWF binds factor VIII and stabilizes it, a deficiency of vWF gives rise to a secondary decrease in factor VIII levels.

Von Willebrand disease types

- Gene is located on chromosome 12
- Type-1 and type-3, both have reduced quantity of circulating vWF
- Type-1, an autosomal dominant disorder, accounts for 70% of all cases and the level of vWF in the blood range from 20%-50% of normal.
- Type-3 is autosomal recessive due to deletions or frameshift mutations with total deficiency, accounts for 5-10% of the cases.

- Type-2 is associated with qualitative defects in vWF, autosomal dominant due to missense mutations resulting in nonfunctional vWF levels.
- Accounts for 20% of all cases.
- Type 2 is broken down into four subtypes: type 2A, type 2B, type 2M and type 2N, depending on the presence and behavior of multimers of vWF.
- Acquired vWD: This type of vWD in adults results after a diagnosis of an autoimmune disease, such as SLE, or from heart disease or some types of cancer.
- Also, it can also occur after taking certain medications.

2- Classic Hemophilia

- Hemophilia A: most common blood clotting defect-permanent tendency for hemorrhage due to missing factor VIII of the intrinsic pathway or marked reduction of its activity. It is X-linked recessive disorder due to an inversion mutation in intron 1 (5%) or 22 (45%). Nonsense/stop mutations prevent factor production. Missense mutations may affect factor production, activity or half-life. Over 600 missense mutations identified
- Hemophilia B: factor IX deficiency (X-linked recessive disorder). Most cases associated with point mutations. Deletions in about 3% of cases. Promoter mutations in about 2%
- Hemophilia C: factor XI deficiency (autosomal recessive disorder).
- Parahemophilia: autosomal recessive disorder due to deficiency of factor V.
- Their clinical features are similar to that of hemophilia A
- The blood level of factor VIII in severe hemophilia A patient is less than 5% of normal.

- Patients have normal platelet count and bleeding time, but prolonged aPTT
- Patients are generally treated by blood transfusion of concentrated plasma fraction containing factor VIII, with its associated dangers:
    a- Hepatitis or HIV/AIDS
    b- Possibility of patients making auto-antibodies
- Recently, treatment has been made much safer as a result of cloning and expression of the gene for factor VIII (protein).
- Through the DNA recombinant technology, the pure protein can be isolated and administered to patients with none of those dangers.

3- <u>Thrombosis</u>

- Four primary influences that contribute to the pathogenesis:
    a- Endothelial injury (dominant)
    b- Abnormal blood flow
    c- Hypercoagulability (less)
    d- Alteration of the coagulation pathways
- May be primary (genetic) or secondary (acquired)

- Defects of the protein C pathway and increased levels of coagulation factors [due to a mutation in protein C (changed amino acid serine into proline at position 270)].
- Protein C is involved in deactivation of blood clotting factors (Va and VIIIa)
- Factor V and prothrombin mutations are common genetic risk factors for venous thrombosis.
-The factor V mutation produces a change in amino acid arginine 506 into glutamine rendering factor V resistant to cleavage by protein C.
- Most affected individuals develop venous thrombosis and are young adults or teenagers heterozygous for the deficiency with levels of functional protein C of 40 - 65%.

4- Thrombocytopenia

- Reduction in platelet number (less than 20,000-50,000).
- Could be non-immunogenic - mechanical injury
- Immunogeneic -development of autoantibodies against the platelets self antigens (membrane glycoproteins complexes Ib-IIIa and Ib-IX).

- Drug-induced thrombocytopenia as quinine, sulfonamide and other antibiotics.
- Heparin therapy, misdiagnosis can have severe consequences.

5- <u>Disseminated intravascular coagulation (DIC)</u>

- Disorders ranging from obstetric complications to advanced malignancy and bacterial sepsis
- Organ involved release thromboblastic substances, factor X, endotoxins and cytokines
- All increase tissue factor expression.
- Inhibit protein C activity by suppressing thrombomodulin expression on endothelium
- Sudden widespread of fibrin thrombi in the microcirculation
- Cause diffuse circulatory insufficiency, in the brain, lungs, heart and kidneys

6- Thrombotic thrombocytopenia purpura (TTP)

- Widespread formation of hyaline thrombi comprised of platelet aggregates in the microcirculation
- Patients are deficient in ADAMTS 13 (Willebrand factor-cleaving protease) gene which encodes an vWF metalloproteinase enzyme
- Deficiency may be inherited or acquired
- Enzyme normally degrades high molecular weight multimers of vWF
- Absence of this enzyme due to mutations causes multimers of vWF accumulates in the plasma leading to aggregation of platelets in the microcirculation
- ADAMTS 13 is also called disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13