E.S.R (erythrocyte sedimentation rate)

Sedimentation Rate (Sed Rate); Erythrocyte Sedimentation Rate (ESR): Sedimentation occurs when the erythrocytes clump or aggregate together in a column-like manner (rouleaux formation). These changes are related to alterations in the plasma proteins. Normally, erythrocytes settle slowly because normal RBCs do not form rouleaux. The ESR is the rate at which erythrocytes settle out of anticoagulated blood in 1 hour. This test is based on the fact that inflammatory and necrotic processes cause an alteration in blood proteins, resulting in aggregation of RBCs, which makes them heavier and more likely to fall rapidly when placed in a special vertical test tube. The faster the settling of cells, the higher the ESR. The sedimentation rate is not diagnostic of any particular disease but rather is an indication that a disease process is ongoing and must be investigated. It is also useful in monitoring the progression of inflammatory diseases.

- **There are two methods**

1- Wastergerns methods.
2- Wintrobes methods.

Wastergerns methods:

Equipment: wasterges tube 30cm long 2.5mm internal diameter.

- Volume: 1 ml
- Calibration: calibrated in millimeter from top to bottom zero-200mm.
- Dilution of blood: (1) of 3.13% trisodium citrate to (4) parts of venous blood. Mean (0.4 ml) of citrate to (1.6 ml) of venous blood.

- **Stages in E.S.R**

1- Rouleux formation (10 minites)
2- Sinking of rouleux (30-40 min)
3- Final stage of packing.

Normal range:
Men: 1-10 mm, women: 1-15 mm.

- **Factors affecting E.S.R**

1- Plasma factor
2- R.B.C. factor
3- technical factor

- **Plasma factor**

Increased levels of fibrinogen and globulin (alpha and beta globulin) speed up E.S.R.

2- Albumin retard E.S.R.

3- Cholesterol speed up E.S.R.

4- Lecithin retard E.S.R.

- **R.B.C. factor**

Anemia speeds up E.S.R. microcyte sediment slowly macrocyte sediment rapidly. Sickle cells and spherocyte retard E.S.R. because abnormal shapes of R.B.C. prevent rouleux formation. E.S.R. should be done within 2.5 hrs. After collection of blood.

- **Technical factors**

Dilution of blood 1:4, temperature (18-25c^{o}) higher temp. Increase E.S.R. Tube- vertical. Hemolysed blood should not be used.

Coagulation factors

Classification of Coagulation factors		
1-	**Factors I**	**Fibrinogen**
2-	**Factors II**	**Prothrombin**
3-	**Factors III**	**Thromboplastin**
4-	**Factors IV**	**Ca^{++}**
5-	**Factors V**	**proaccelerin**
6-	**Factors VI**	**obsolete**
7-	**Factors VII**	**Proconvertin**
8-	**Factors VIII**	**Anti hemophilic factor**
9-	**Factors IX**	**Plasma hemoplastin component**
10-	**Factors X**	**Stuart power factor**
11-	**Factors XI**	**Plasma thromboplastin anticodent**
12-	**Factors XII**	**Hagemen factor**

Notes:

- In the modern classification factor III and factors IV are not included.
- Factors I, II, V, VIII lost completely during clotting process.
- Factors V, VIII are labile factors.
- Factors II, VII, IX and X are vitamin K dependent factors.

- **Fibrinogen**

A protein of large MW (340.000 Da) synthesized by liver consist of three pairs of polypeptide chain ά, ß and **λ.**

Thrombin remove A and B polypeptide forming ά fibrin monomer lead to polymerized to visible clot.

- **Prothrombin**

A stabile protein (ά2 glubulin) contains 18 amino acids. Synthesized in the liver by help of vitamin K. MW (63.000 Da).

- **Thromboplastin**

Can be divided to:

1- Intrinsic thromboplastin (blood thromboplastin): complex substance generation by the interaction of factor XI, IX and VIII and platted factor III, XII, XI, IX, VIII and Ca^{++}.

2- Extrinsic thromboplastin (tissue thromboplastin): released from injured tissue, lung, brain and placenta are full source of tissue thromboplastin (lipoprotein).

- **Ca^{++} calcium ion**:

Needed for the coagulation mechanism at three points:

1- For the activation of thromboplastin.

2- For conversations of prothrombin to thrombin.

3- For the fibrin formation.

- Normally: 9-11.5mg/dl.

- **Proaccelerin:**

A globulin which is labile consumed fully during clotting. Not found in serum deficiency leads to Para hemophilia.

- **Proconvertin:**

Is beta globulin, stable factors not consumed during clotting of blood in test tube. So present in serum.

• **Anti hemophilic factor:**
Extremely labile factor not found in the serum. Deficiency leads to hemophilia. Synthesized by spleen and endothelial cells.

• **Plasma hemoplastin component (P.T.C.)**
Stable protein factor not consumed during clotting so present in serum. It is required for generation of intrinsic thromoboplastin.

• **Stuart power factor:**
Alpha globulin, not consumed during clotting of blood M.W 350 kDa. found in serum.

• **Plasma thromboplastin anticodent (P.T.A.):**
Beta globulin, partially consumed during clotting so found in both serum and plasma. Needed for the generation of intrinsic thromboplastin.

• **Hagemen factor:**
A stable globulin not consumed in clotting process, it initiates the intrinsic coagulation mechanism. Activated by exposed collagen, glass and other negatively charged compound.

• **Fibrin stabilizing factor (fibrinase):**
An enzyme which stabilized soluble fibrin to insoluble fibrin in (5) major area.

• **Hemostasis and coagulation mechanism**
Hemostasis: is the entire mechanism by which bleeding is controlled and finally stopped from blood vessel.
There are two pathway involved in clotting of blood:
1- Intrinsic coagulation mechanism.
2- Extrinsic coagulation mechanism.

• **Intrinsic coagulation mechanism**
The clotting of blood without the help of tissue thromboplastine, there are three phase:
1-Generation of intrinsic thromoplastin.
2- Convertatioin of prothrombine to thrombin.
3- Convertatioin of fibrinogen to fibrin.

• Extrinsic coagulation mechanism

The clotting of blood with the help of tissue thromboplastine**.**

• Cause of dearaged hemostasis mechanism

1- Deficiency of coagulation factors. (Congenital or acquired).
2- Presence of inhibitory substance.
3- Excessive fibrinolysis
4- Thrombocytopathy.
5- Combination of some of the above defect.

Bleeding Disorders

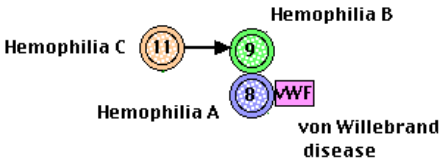
A deficiency of a clotting factor can lead to uncontrolled bleeding.

The deficiency may arise because

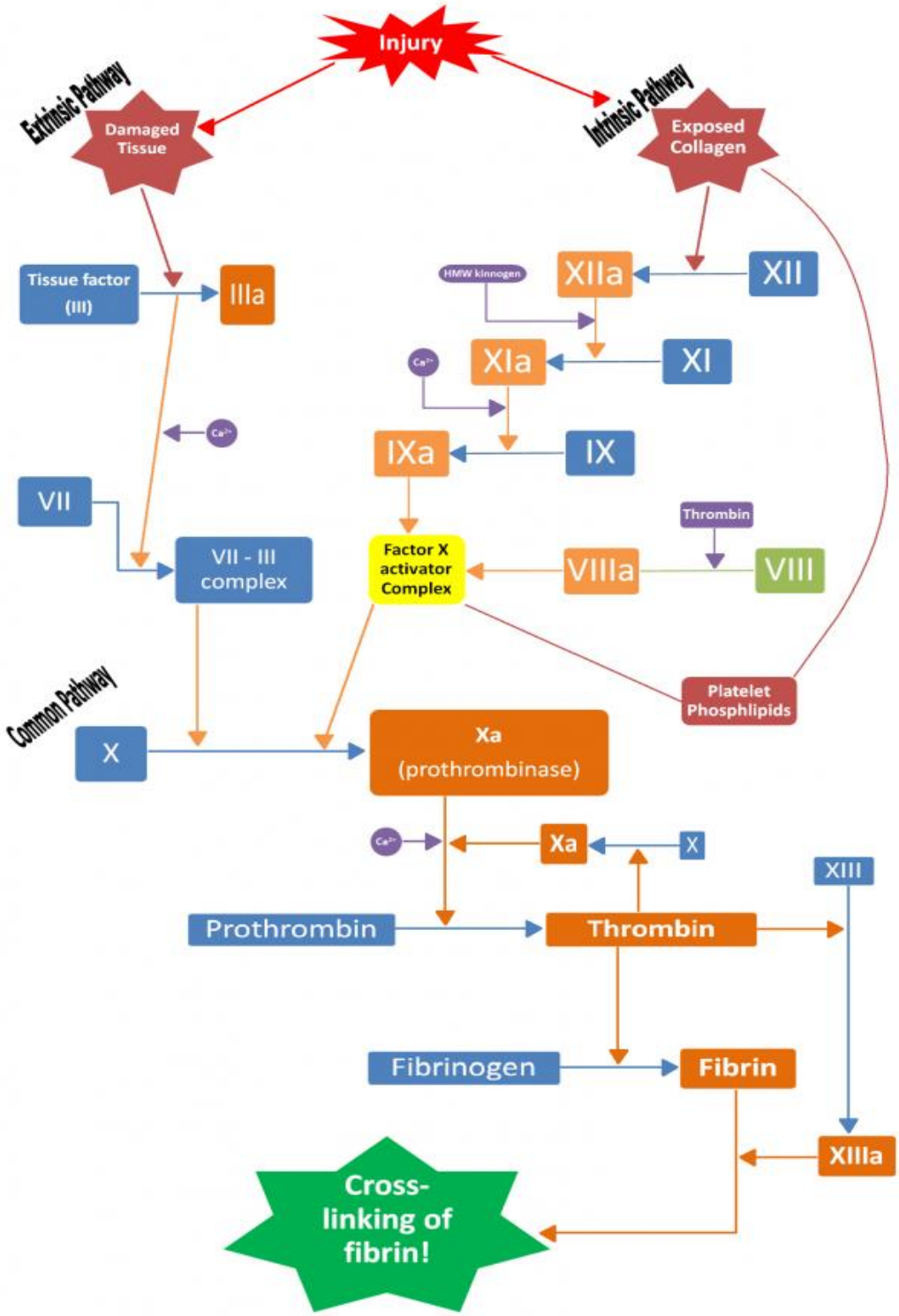
- not enough of the factor is produced or
- a mutant version of the factor fails to perform properly.

Examples:

- **von Willebrand disease** (the most common)
- **hemophilia A** for **factor 8** (VIII) deficiency
- **hemophilia B** for **factor 9** (IX) deficiency.
- **hemophilia C** for factor **11** (XI) deficiency



In some cases of von Willebrand disease, either a **deficient level** or a **mutant version** of the factor eliminates its protective effect on factor 8. The resulting low level of factor 8 mimics hemophilia A.


Injury
Extrinsic Pathway
Damaged Tissue
Intrinsic Pathway
Exposed Collagen
Tissue factor (III)
IIIa
HMW kinnogen
XIIa
XII
XIa
XI
IXa
IX
VII
VII - III complex
Factor X activator Complex
VIIIa
Thrombin
VIII
Platelet Phosphlipids
Common Pathway
X
Xa (prothrombinase)
Xa
X
XIII
Prothrombin
Thrombin
Fibrinogen
Fibrin
XIIIa
Cross-linking of fibrin!

Screening test done on a patient with bleeding disorder

1- Hess, s test
2- Bleeding time
3- Platelets count
4- Examination of blood film for platelet morphology.
5- Whole blood clotting time
6- Prothrombin time
7- Thrombin clotting time
8- Partial thromboplastin time with kaolin

1- Hess, s test: capillary resistance test, measure capillary resistance capillary fragility under condition of increased pressure.
Hess, s test is positive capillary disease (scurvy) and thrombocytopenia.

2- Bleeding time: time required for the spontaneous arrest of bleeding from skin puncture under standard conditions.
The two methods:

1- Ivy methods
Time: 2-7min
Bleeding time: is prolonged thrombocytopathy and after aspirin treatment.

2- Duke methods
Time: 2-5min
Bleeding time: is prolonged thrombocytopathy and after aspirin treatment.

- **Platelets count:**

The normal rate: 150.000-400.000 cell/ml.
Examination of blood film for platelets morphology. Normal size: 2-4 in diameter. Round, oval or rod shape. Pale purple giant cells suggest platelet abnormality.

- **Whole blood clotting time**. (Lee& white method).

Normal time: 4-9 min at 37c° is prolonged in the deficiency of factor involved in intrinsic coagulation mechanism most common cause deficiency in factor VII, IX and heparin therapy.

- **Prothrombin**:

Normal rate: 10-14 second, it is the clotting of citrated plasma after the addition of tissue thromboplastin calcium mixture. Measure the extrinsic coagulation mechanism.

- **Causes of prolonged Prothrombin time**

1- Oral anticoagulant therapy (comarine, warfarin)
2- Liver disease.
3- Nephritis.

- **Thrombin clotting time**:

The time required for the clotting of citrated plasma after the addition of thrombin. Prolonged in heparin therapy and normal rate: 9-11min.

- **Partial thromboplastin time with kaolin**

Three substance phospholipids kaolin and calcium are added to plasma. Normal rate: 35-45 second, prolonged due to deficiency of factors XII, IX, VIII and V this test is most useful in hemophilia.

- **Controlling Clotting**

While the ability to clot is essential to life, the process must be carefully regulated. Inappropriate clot formation, especially in the brain or lungs, can be life-threatening.

1-Antithrombin III

As its name suggests, this plasma protein (a serpin) inhibits the formation of thrombin.

It does so by binding to and thus inactivating:

- prothrombin
- factor 9
- factor 10

2-Heparin is a mixture of polysaccharides that bind to antithrombin III, inducing an allosteric change that greatly **enhances** its inhibition of thrombin synthesis. Some surgical patients, especially those receiving hip or heart valve replacements, and people at risk of ischemic stroke (clots in the brain), are given heparin. Heparin is found naturally in the body in small amounts and is produced by mast cells and basophils. Mast cells and basophils basically the same cells, but mast cells are found in connective tissue, and basophils are found in the blood stream.

3-Protein C

With its many clot-promoting activities, it is probably no accident that thrombin sits at the center of the control mechanism.

- Excess thrombin binds to cell-surface receptors called **thrombomodulin**.
- The resulting complex activates a plasma protein called **Protein C** and its cofactor **Protein S**.
- Together these inhibit further thrombin formation
  - directly — by inactivating Factor **5** and
  - indirectly — by inactivating Factor **8**.

Some inherited disorders that predispose to spontaneous clots, especially in the leg veins:

- inherited deficiency of **Protein C** or **Protein S**;
- inherited mutation in the **Factor 5** gene producing a protein that no longer responds to the inhibitory effect of Protein C.

Recombinant Protein C is now available to treat people threatened with inappropriate clotting, e.g., as a result of widespread infection (sepsis).

Vitamin K
Vitamin K is a cofactor needed for the synthesis (in the liver) of

- factors **2** (prothrombin), **7**, **9**, and **10**
- proteins **C** and **S**

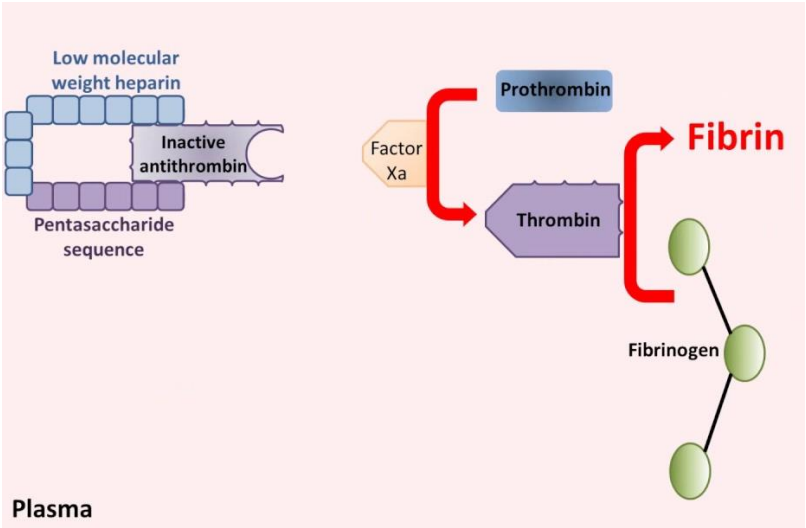
So a **deficiency** of Vitamin K predisposes to bleeding.

Conversely, blocking the action of vitamin K helps to prevent inappropriate clotting.

- **Drugs that affect coagulation**

Heparin: This combines with antithrombin-III, and force a conformational change in the compound, so that it acts at various stages of the clotting cascade to reduce clotting. It increases the effectiveness of antithrombin-III by over 1000x.

Mechanism of action: Heparin does not act directly on the extrinsic pathway. The low MW of heparin in bind to inactive form of antithrombin, leading to bind with Xa factor that is help to converted prothrombin to thrombin and then converted fibrinogen to fibrin. It is also inactivating many of the factors produced in clotting: 2, 8, 9, 10, 11, 12 are the ones affected (thrombin and 8-12).



2-Warfarin (Coumadin)

It is the most important oral anticoagulant. Other examples with a similar mechanism of action include pheninidione. Patients on warfarin (and other vit K antagonists) need to have individualised doses, and this means the treatment in both inconvenient and has a low margin of safety.

Mechanism

Inhibits enzymatic reduction of vitamin K to its active form hydroquinone. Bindingis competitive.

The effect takes several days to develop, as it is dependent on the half life of the already active factors, 2, 7, 9 and 10.

VII – has a half life of 6 hours

IX – has a half life of 24 hours

X – has a half life of 40 hours

II – has a half life of 60 hours

Pharmacokinetics

Absorbed rapidly and completely from the gut
Binds well to albumin
Peak time of action is about 48 hours after administration, but peak concentration in the blood is about an hour after administration
The effect on prothrombin time is initially seen after 12-16 hours, and lasts approximately 4-5 days.
Warfarin treatment is tricky because the therapeutic window (neither too much nor too little) is very narrow, and there is substantial variability between people in their response. So treatment requires regular monitoring of clotting time until the proper dosage is established.

Unwanted effects

1-Haemorrhage this is especially common to the bowel and brain. This can be counteracted by the administration of vitamin K, or giving fresh plasma containing clotting factors.

2-Teratogenicity (causes birth defects).

3-Necrosis of soft tissues this occurs mainly to tissues in the buttock and breast and is a result of thrombosis in venules. It generally occurs shortly after administration, and is a result of inhibition of synthesis of **protein C**.