

WBC DIFFERENTIAL COUNT

- Objective:

determine the percentage of each type of WBC in well stained blood film.

Count 100 WBCs using Bright field microscope 40x or 100x.

N	L	E	M	B	TOTAL /10
1111 11	111				10
1111 1111					10
111	1111	1	1		10
1111	111	1	1		10
111	111	1	11	1	10
1111	1111	1	1		10
1111	1111	11			10
11	1111	1	111		10
11	1111	1	111		10
1111	1	1	111		10
45%	31%	9%	14%	1%	100

Haemostasis:

- ▣ A balance to keep the blood inside the vein in a liquid state if there is bleeding disorder.
- ▣ It is a mechanism that results from a balance between coagulation & anticoagulation & fibrinolysis.

Function of Haemostasis:

- ▣ To maintain blood in a fluid state within the vascular system.
- ▣ To arrest bleeding at the site of injury or blood loss.
- ▣ To ensure complete removal of the plug when healing is complete

Component of Homeostasis:

- ▣ Blood vessels
- ▣ Platelets
- ▣ Plasma coagulation factors
- ▣ Plasma coagulation inhibitors
- ▣ Fibrinolytic system

1-blood vessels

- ▣ Artery is bigger and thicker than a vein and it has ↑ elastic fiber capable of vasoconstriction help in arrest the blood loss.
- ▣ A good site for adhesion of platelets.

2-Platelets:

produced in the BM by fragmentation of cytoplasm derived from megakaryocyte
(CFU_{GEMM})

Platelets Functions:

- ▣ adhesion to injured vessels
- ▣ Secretion of fibrinogen & heparin
- ▣ Fusion in blood vessels so stimulate the coagulation activity
- ▣ platelets Aggregation on the wall of blood vessels to make temporary block in site of injury

platelets

- ▣ Normal life span :8-14 days
- ▣ Normal platelets count: $150-450 \times 10^9$ cell/L
 $150-450 \times 10^3$ cell/ μ L
- ▣ Low platelets count =thrombocytopenia
- ▣ High platelets count=thrombocytosis

3- Plasma coagulation factors

- ▣ Activation of clotting factors to form fibrin clot by intrinsic & extrinsic pathway.

4-Plasma coagulation inhibitors

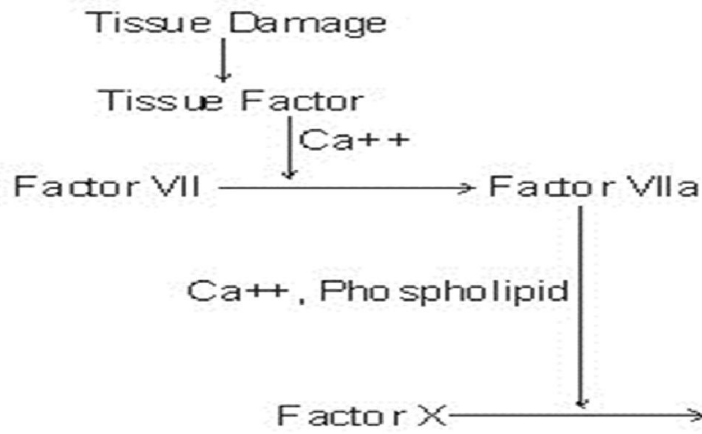
- ▣ Activation of coagulation inhibitors to localize & limit the formation of fibrin clot.

5-fibrinolysis

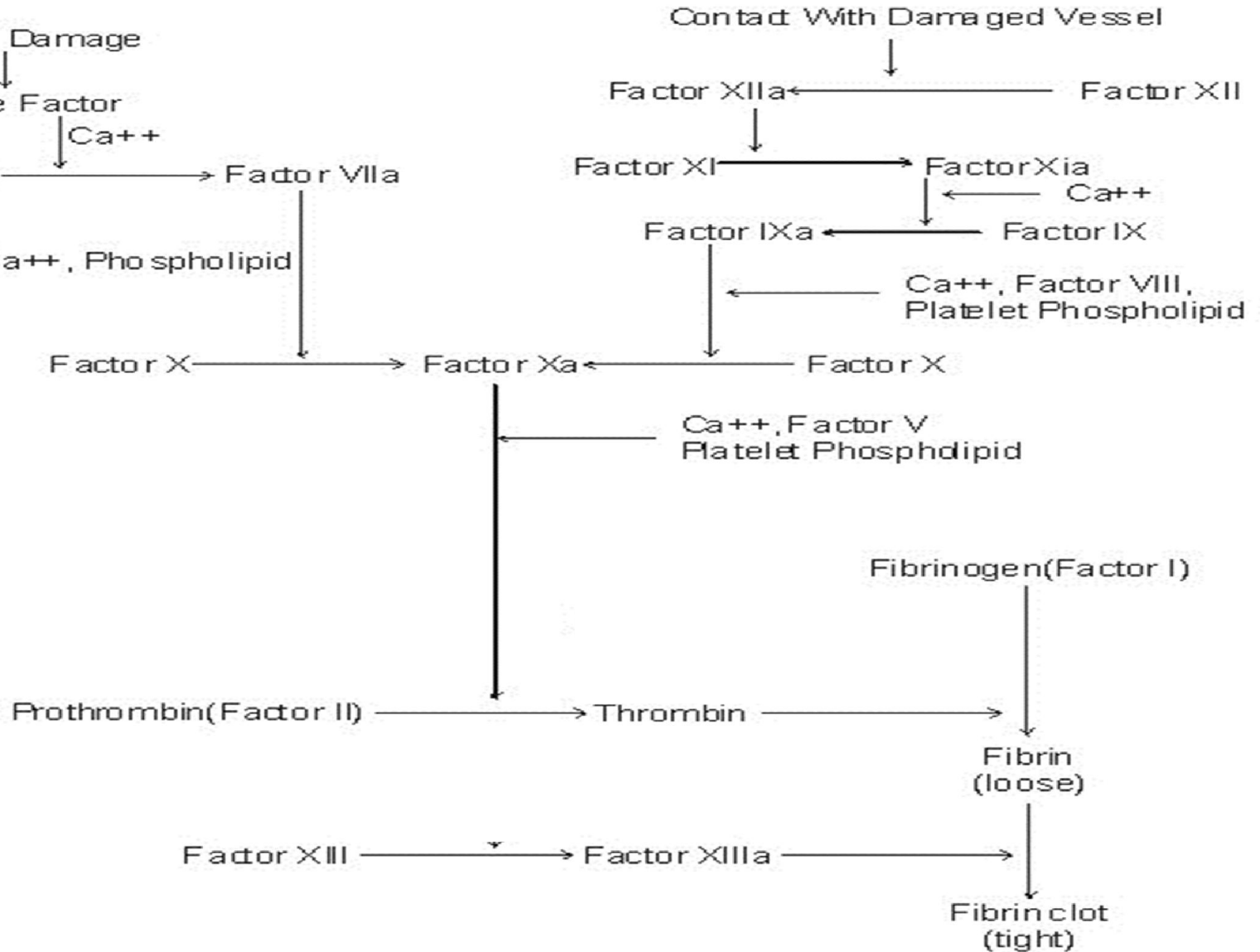
- ▣ To get rid from fibrin clot after healing of the wound (FDPs)

Coagulation Cascade

Extrinsic Pathway



Intrinsic Pathway



Causes of hemorrhagic disorder:

- ▣ **Deficiency of clotting factors**
- ▣ **Platelet disorders**
 - **Quantitative** : Thrombocytopenia
 - **Qualitative** -:Platelet function disorders
- **Defective capillaries**
- **Excessive fibrinolysis**

Screening Tests :

- ▣ Prothrombin time Pt (extrinsic)
- ▣ Activated partial thromboplastin time Aptt (intrinsic)
- ▣ Bleeding time BT (blood vessels contracting)
- ▣ Fibrinogen titer concentration
- ▣ Platelets count
- ▣ fibrinogen degradation products FDPs

BT bleeding time:

Principle:

A standard incision is made on the volar surface of the forearm and the time of the incision bleeds is measured

- ▣ **Normal range** :2 – 7 min
- ▣ **Prolong BT is indicative of** capillary defect or platelets dysfunction or thrombocytopenia

Platelets count

▣ In platelets count use diluent → 3.2% formal citrate = RBC diluent → transparency → gives color to RBCs and platelets and lyses the WBC

▣ **method:**

20 μL from EDTA blood

+

4mL from diluent(formal citrate)=4000 μL

Platelets count

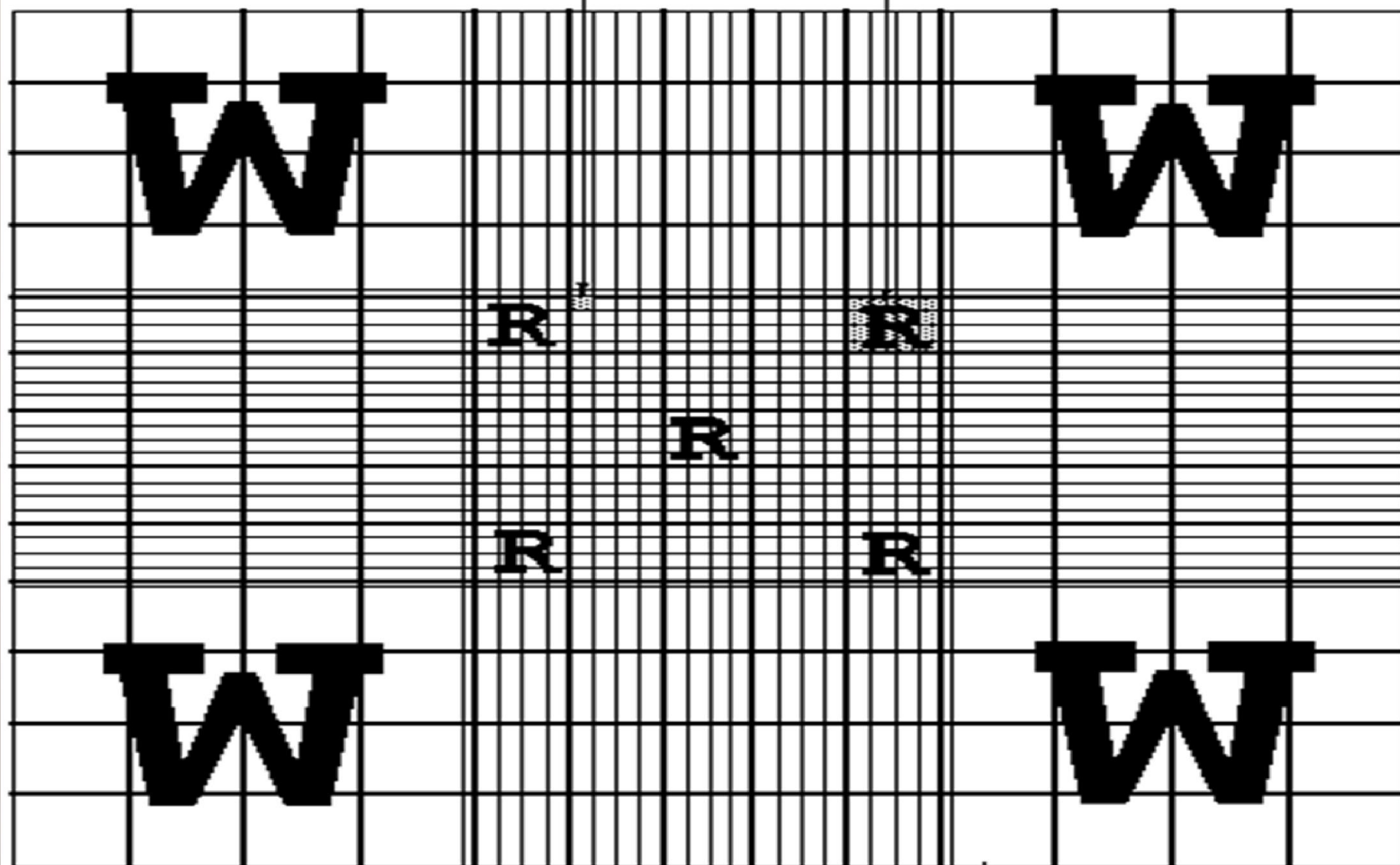
- ▣ Using Improved neubauer counting chamber Called (**haemocytometer**)

Put the haemocytometer in wet apetridish to allow the platelets to settle down for 10 min.

- ▣ count at 40 X objective

Small square = $1/400$ sq. mm.

$1/25$ sq. mm.



← 1 millimeter →

↑ Counting grid (central area)

▣ They appear small and shiny
while the RBC s are big

▣ Haemocytometer=Contain 9 squares
each square area size is 1mm X 1mm ,
each square contain a volume of 0.1ML
of diluted blood.

▣ we count the platelets in 5 squares

$$N = \frac{0.1}{5} = 0.02 \text{ ML}$$

Dilution factor= 200



Number of platelets in 1ML in diluted blood=

$$= \frac{N}{0.02} = \frac{N}{\frac{2}{100}} = \frac{NX100}{2} = NX50$$

Number of platelets in 1ML in whole blood =

$$NX50 \times 200$$

$$= NX10000$$