1. Hemostasis 止血
2. Coagulation 凝固
3. Fibrinolysis 纖維蛋白溶解

Essential concepts-1

- **Hemostasis**
  - The balanced interaction of blood vessels, platelets, and soluble factors in the formation and dissolution of blood clots.
- **Platelets: Primary hemostasis- platelet plug**
  - Plugs endothelial wounds
  - Provide soluble factors that promote hemostasis

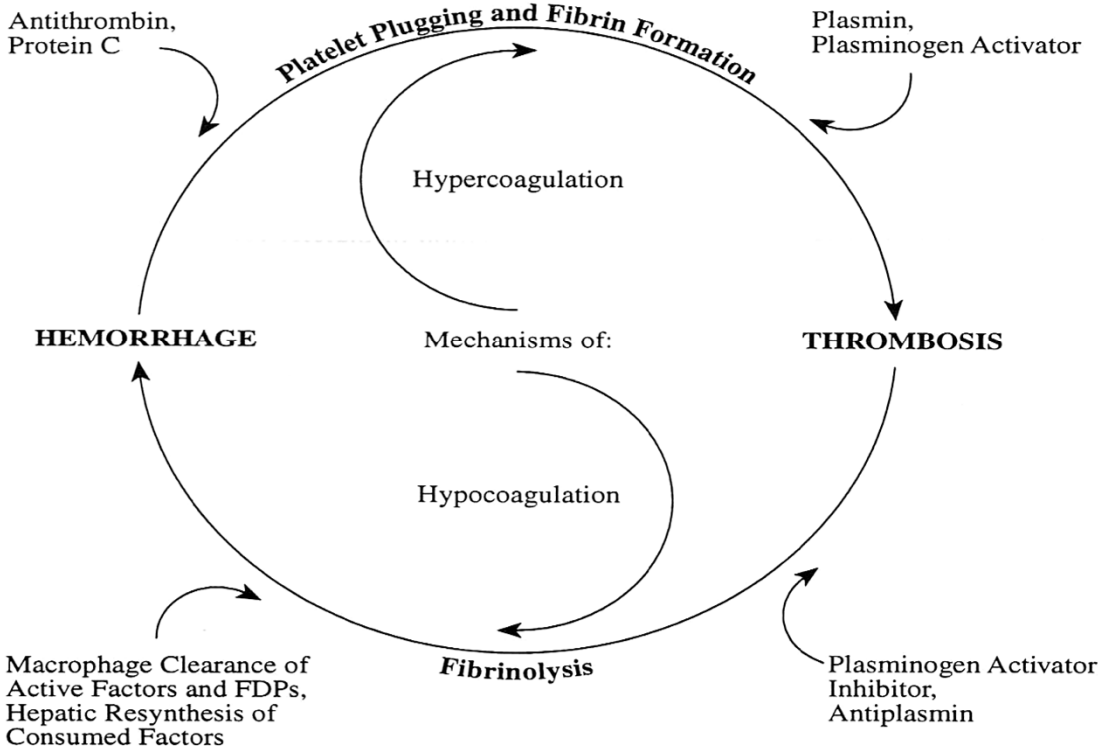
Essential concepts-2

- **Coagulation: Secondary hemostasis-fibrin formation**
  - –The process that results in the conversion of soluble fibrinogen to insoluble fibrin

Essential concepts-3

- **Fibrinolysis: Tertiary hemostasis-fibrin dissolution**
  - The process that involves activation of fibrinolytic factors , resulting in hydrolyzing fibrin

Disorders of hemostasis

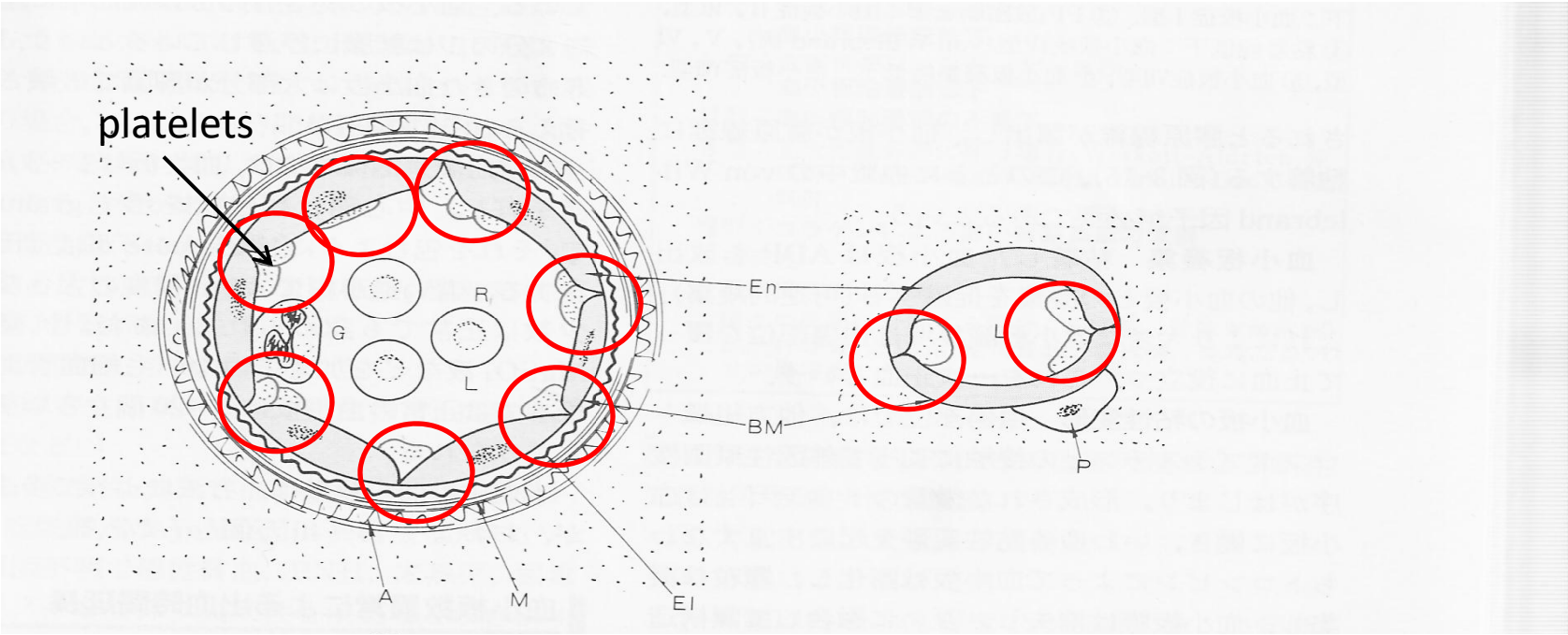


A kinetic scheme of hemostasis. Platelet plugging and fibrin formation are balanced by fibrinolysis. Activators and inhibitors regulate the balance. Imbalance leads to hypercoagulation (thrombosis) or hypocoagulation (hemorrhage)

Platelets

circulating in blood vessels

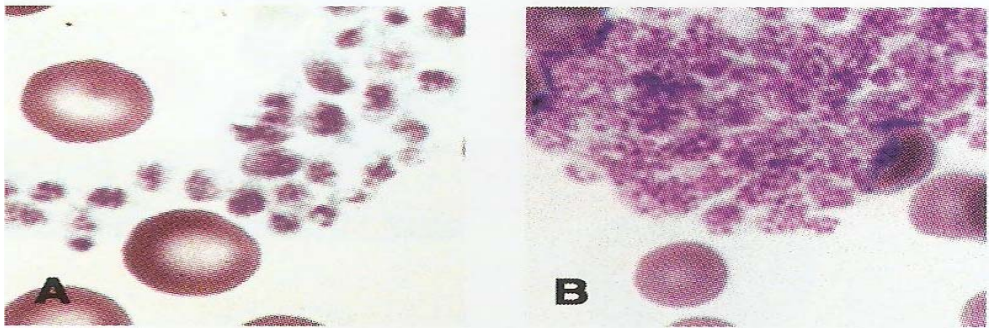
filling the space between endothelial cells and covering endothelium



Microvessel (left) and **capillary vessel** (right): embedded in connective tissues
A: tunica externa, BM: basement membrane, EL: lamina elastica interna**, En: endothelial cells**, G: granulocytes, L: lumen, M: musscle layer of tunica media , P: pericyte, R: red blood cell

Platelet morphology: mammals

- **A. Dog** B. **Cow**



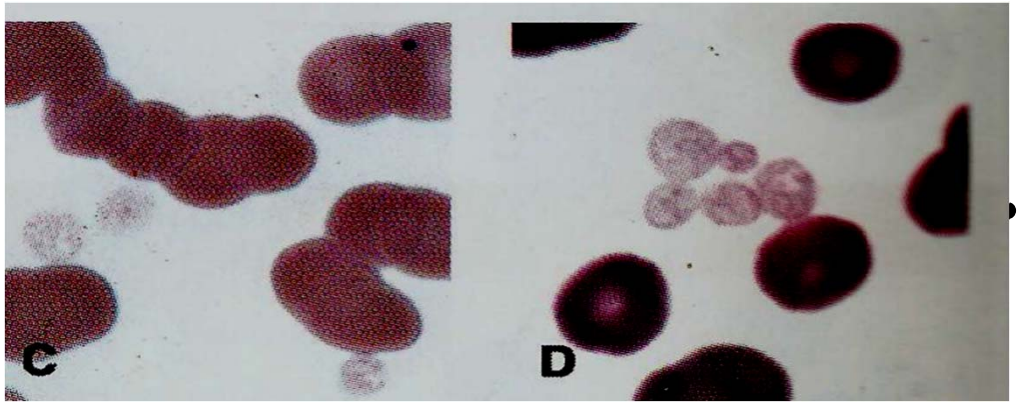
- **Anucleated cell fragments**
  - 200,000-500,000/μl
- **2-4 μm**, round to oval
- Cytoplasm
  - Light blue
  - Small reddish purple **granules**
    - **Conaining most of the active products of platelets**

- Dense granules: serotonin, ADP, ATP, Ca
- Alpha granules-PDGF, platelet factor IV, coagulation factos V and VIII, etc.

Platelet morphology: mammals

C. Horse: pale-staining-normal

- D. **Horse**



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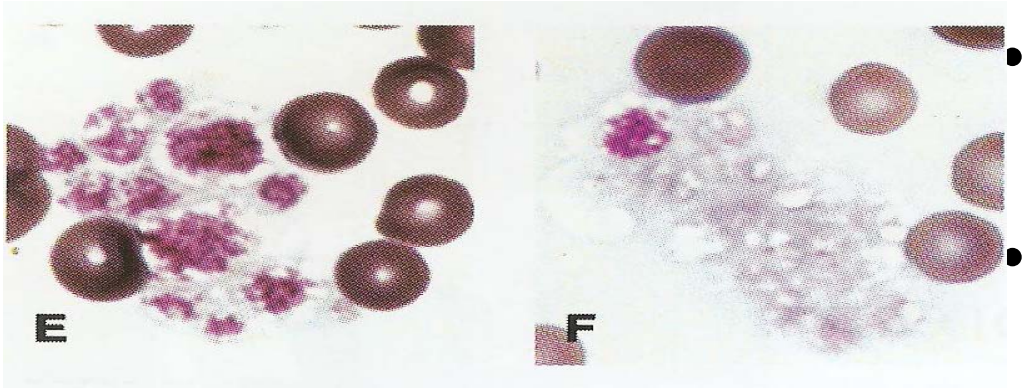
Platelet morphology: CAT



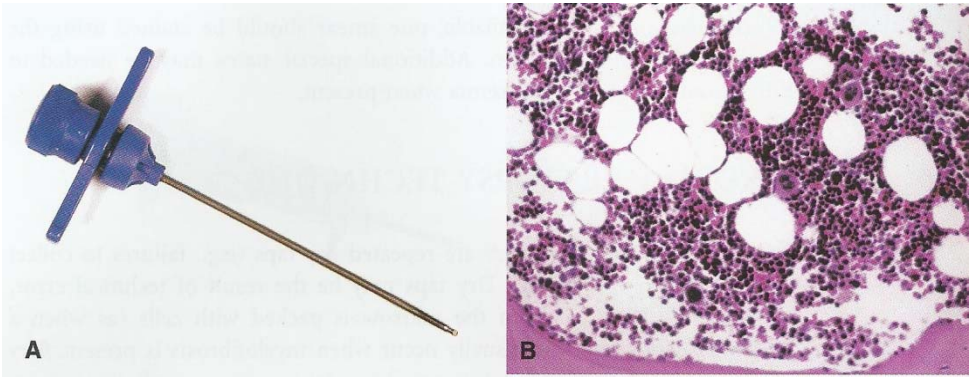
- Anucleated cell fragments
  - 200,000-500,000/μl
- 2-4 μm, round to oval
  - Cats: vary more in size

Cat platelets

-Sensitive to activation during blood collection

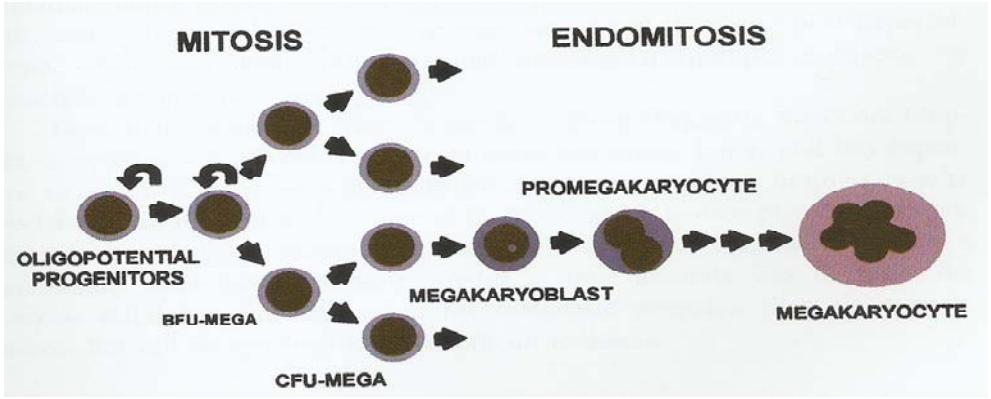
-Degranulated platelets aggregate

Platelet probone marrow



- **Cytoplasmic demarkation of megakaryocytes**
- **thrombopoietin**
  - Controlled by circulating platelet mass, not by number
  - Adsorbed to the platelet surface

Platelet probone marrow



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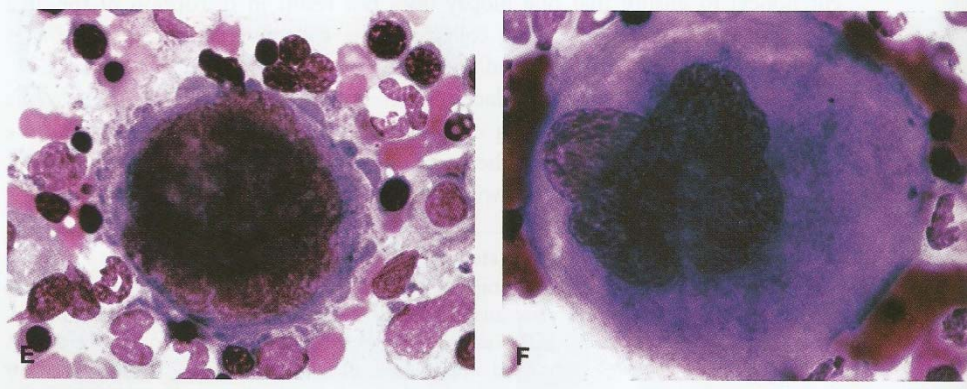


- **A splenic pool** of platelets
  - 20-30% of the circulating platelets
  - May affect platelet number
    - Splenic contraction – increases
    - Splenic congestion - decreases

Platelet function-1

1. Normal hemostasis

1. Maintenance of vascular integrity by sealing minor endothelial deficiencies.
2. Helping to arrest bleeding by the formation of platelet plugs following endothelial

Platelet function-2

1. **Normal hemostasis**

   3. Contributing membrane lipid procoagulant activity to facilitate coagulation

   4. Promoting vascular healing through platelet derived growth factor (PDGF)

Platelet function-3

1.Normal hemostasis

5. Wound repair through PDGF

6. Inflammation: cell to cell interaction and release soluble mediators

1. Release vasoactive substances, such as serotonin
2. Modulate neutrophil function

Platelet function-4

- TXA_2-induced irreversible aggregation
  - Several coagulation factors that are associated with platelets are released into the gel-like mass.
    - Fibrinogen, factor V, factor VIII

Platelet function-5

- Platelet membrane phospholipid is released
  - Accelerates cofactor of the intrinsic and common pathway of coagulation

Platelet function-6

- Other important platelet activators
  - Thrombin, endotoxin, epinephrine
- Various substances are released by aggregated platelets
  - Contractile protein, mitogens, serotonin,
  - heparin antagonist

Platelet function-primary hemostasis-1

- forming a **hemostatic plug**
  - –Sufficient to control bleeding from minute injuries of small vessels

Platelet function-primary hemostasis-2

- **Adherence between exposed subendothelial collagen and platelets**
  - Within seconds after injury
  - **von Willebrand's factor (VWF)**
  - **Gp1b/IX Receptor (collagen-platelet)**
  - Aggregation of platelets -**primary aggregation- reversible**

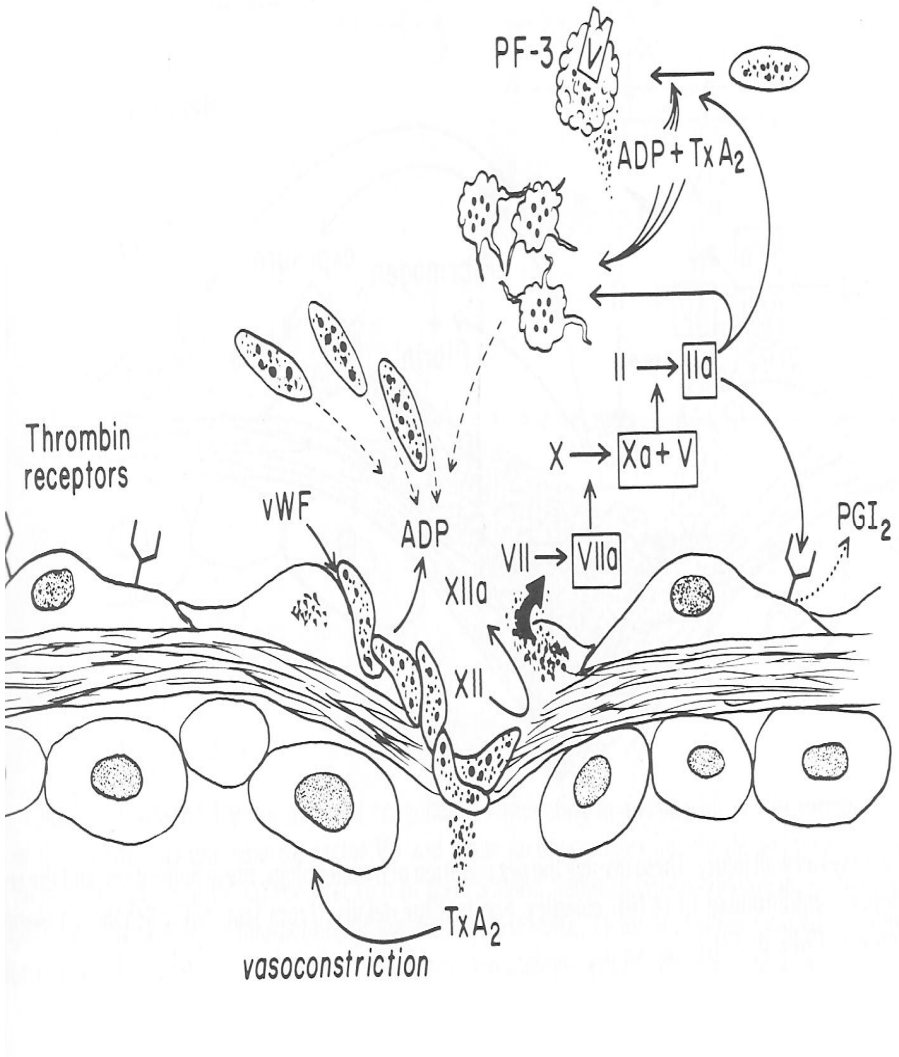
Platelet function-primary hemostasis-3

- The **aggregation of platelets resulting in platelet shape change**
  - **Adenosine phosphate (ADP) release from platelet granules**
    - **Further platelet adherence (platelet-platelet)**
    - **GPIIb/IIIa receptor**
    - Activate platelet phospholipase

Platelet function-primary hemostasis-4

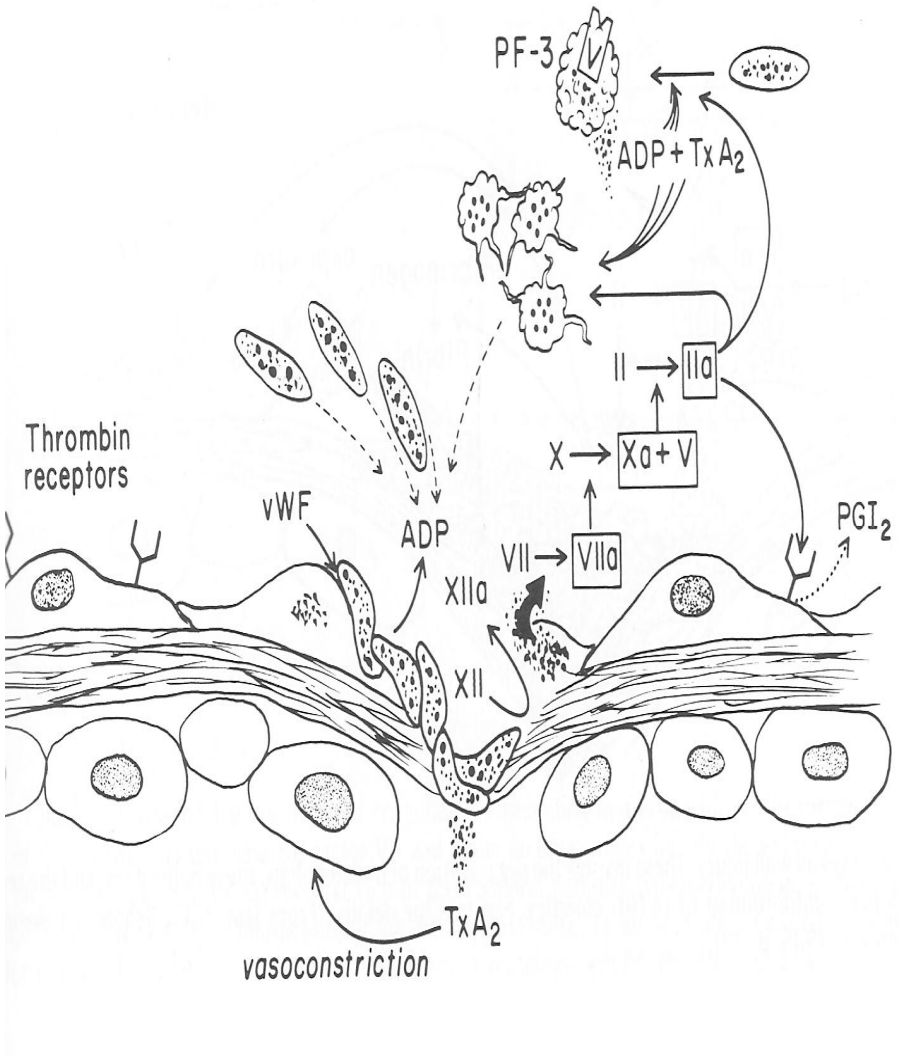
- The **aggregation of platelets resulting in platelet shape change**
  - **TXA_2**
    - **Marked, irreversible aggregation of platelet – secondary aggregation**
    - Viscous metamorphosis : the conversion of platelets to a gel-like mass
    - Locally intense vasoconstriction

Hemostatic events associated with vessel wall injury-1



- **Primary hemostasis**
- Exposed subendothelial collagen
- Activation of platelets
  - Adenosine diphosphate (ADP)
  - Thromboxane (TxA_2)

Hemostatic events associated with vessel wall injury-2



- Inhibition of platelet aggregation and release
  - PGI_2
- Initiation of clotting (secondary hemostasis)
  - Intrinsic system (XII→)
  - Extrinsic system (VII→)

Laboratory evaluation of platelet-1

A. Platelet count
B. Platelet evaluation from blood smear
C. Mean platelet volume and platelet distribution width
D. Examination of bone marrow smear for megakaryocytes
E. Bleeding time

Laboratory evaluation of platelet-2

F. VWF antigen assay

G. Antiplatelet antibody

H. Clot retraction

Laboratory evaluation of platelet-3

I. Other platelet function test

a. Platelet adhesion test: test for the ability of platelets to adhere to surfaces

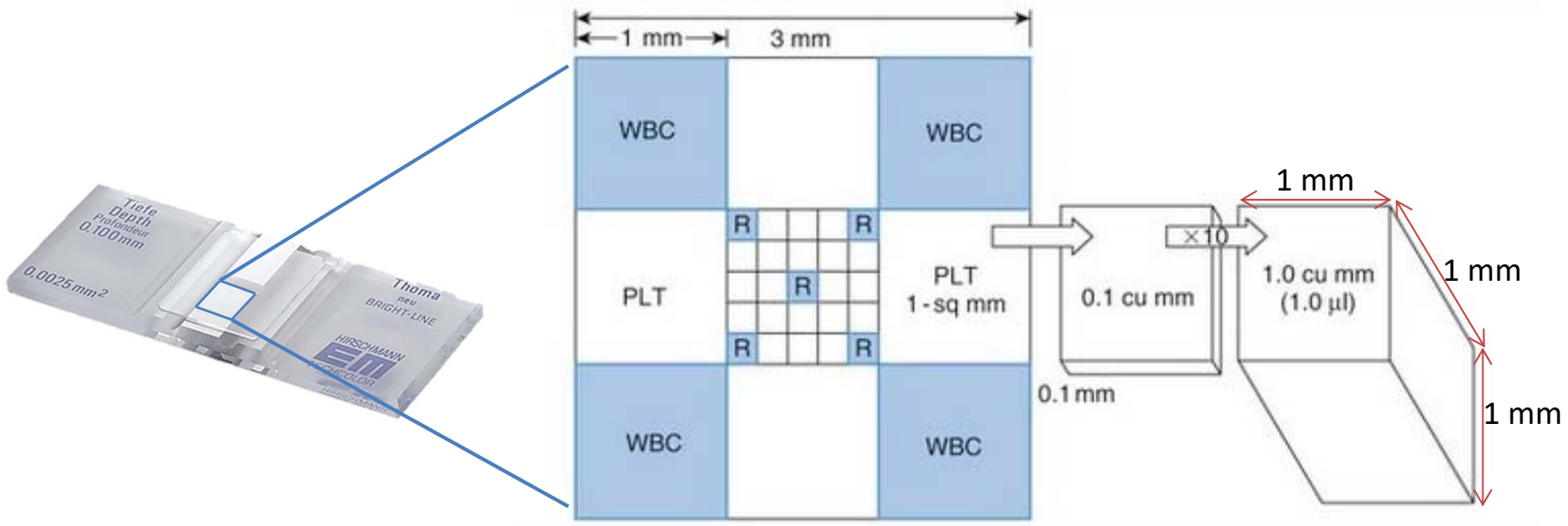
b. Platelet aggregation test: test for the ability of platelets to attach to one another

A. Platelet count-1

- Methods
  - Use EDTA for anti-coagulant
  - Manual counting : ammonium oxalate solution and hemocytometer
    - Poor precision
    - Experience required

A. Platelet count-2

- Hemocytometer for platelet count



Dilution ratio (common)	
Erythrocytes	1:200
WBC	1:20
Platelets	1:100

(Cells / mm^3) x 1/dilution ratio
= (Cells / µL) x 1/dilution ratio
= [(Cells x 10^9) / L] x 1/dilution ratio

A. Platelet count-3

- Methods
  - Electronic cell counters: whole blood
    - More precise
    - May be inaccurate in cats and cows
      - Platelet clumping
      - Small size of erythrocytes ; cats (platelet: large)
  - Manual or automatic counts should be verified by examination of a stained blood smear,

A. Platelet count-4

- Physiological factors influencing platelet number
  - splenic activity.
    - Splenic congestion and sequestration- low count
    - Splenic contraction that accompanies excitement (epinephrine response) –high count

A. Platelet count-5

- Thrombocytosis : counts above reference values
- Thrombocytopenia: counts below reference values
- Thrombocytopenia is clinically more important than thrombocytosis because thrombocytopenia is associated with excessive hemorrhage.

A. Platelet count-6

- Hypercoagulation from thrombocytosis
  - –Greater risk

- Hemorrhage from thrombocytopenia
  - –Will not occur until counts are <25,000/μl

A. Platelet count-7

PLT count (x $10^3/\mu L$)	At risk of hemorrhage	Subjective assessment of platelet number
<10	Yes (highest risk), usually spontaneous; induced by trauma or surgery	Marked decrease (very low)
<30	Yes, can be spontaneous, induced by trauma or surgery	Marked decrease (very low)
30-50	Yes, usually secondary to trauma or surgery; can be spontaneous if a concurrent platelet function defect	Moderate decrease (low)
50-100**	No, unless concurrent platelet function defect	Moderate decrease (low)
>100**	No, unless concurrent platelet function defect	Mild decrease (low)

** This is species dependent and applicable to dogs, cats, ruminants and camelids.

A. Platelet count-8

- Platelet counts in healthy greyhounds, Polish Ogar dogs, and Cavalier King Charles spaniels are generally lower than those measured in other dog breeds.

A. Platelet count-9

Breed	Platelets count	Reference interval of dogs of other breeds
Greyhounds	90-290 x 10^3 /µL	140-380 x 10^3 /µL
Polish Ogar dogs	167 x 10^3 /µL	344 x 10^3 /µL

- The lower limit of the reference interval for platelet counts in CKCS has been difficult to determine with certainty because there is a high incidence of an asymptomatic inherited thrombocytopenia with macrothrombocytes in this breed.

A. Platelet count-10

- Thrombocytosis is usually due to an increase in platelet production and release rather than an increased platelet lifespan.

- Usually, a platelet count higher than the reference interval for the species is a reactive thrombocytosis and not of direct pathologic importance.

A. Platelet count-11

- Causes of thrombocytosis
  - Drugs: corticosteroids and β-adrenergic drugs
  - Post-splenectomy: transient thrombocytosis
  - Reactive thrombocytosis: neoplasia, chronic inflammatory diseases, immune-mediated disease (IMHA), trauma and iron deficiency anemia
  - Neoplastic thrombocytosis(rare)

B. Platelet evaluation from blood smear-1

- Estimate decreased numbers
  - 8-10 platelets/ hpf : normal
  - <3-4 platelets/hpf: significant thrombocytopenia
  - 6-7 platelets/hpf: approximately 100,000 platelets/μl

B. Platelet evaluation from blood smear-2

- Estimate decreased numbers
  - <1 platelet/50 RBCs: thrombocytopenia*
  - # of platelets/# of WBC x WBC/μl= an estimate of the platelet #/μl

B. Platelet evaluation from blood smear-3

- Platelets are estimated by counting the average number of platelets seen per 100x oil immersion field in the monolayer.

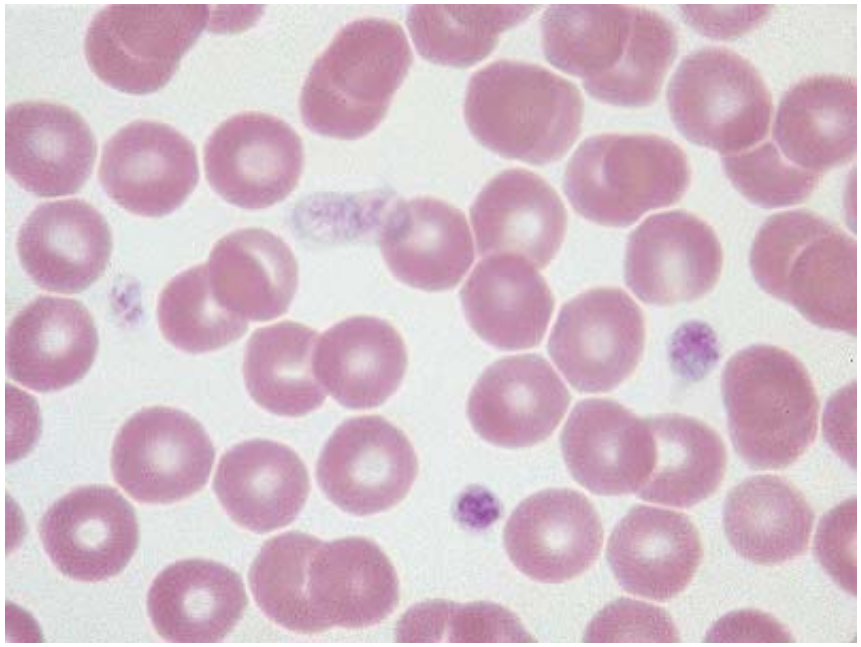
- In general, 10 oil immersion fields are counted and the results averaged.

Estimated platelet count/µL
= average count in 10 fields x 15,000 or 20,000

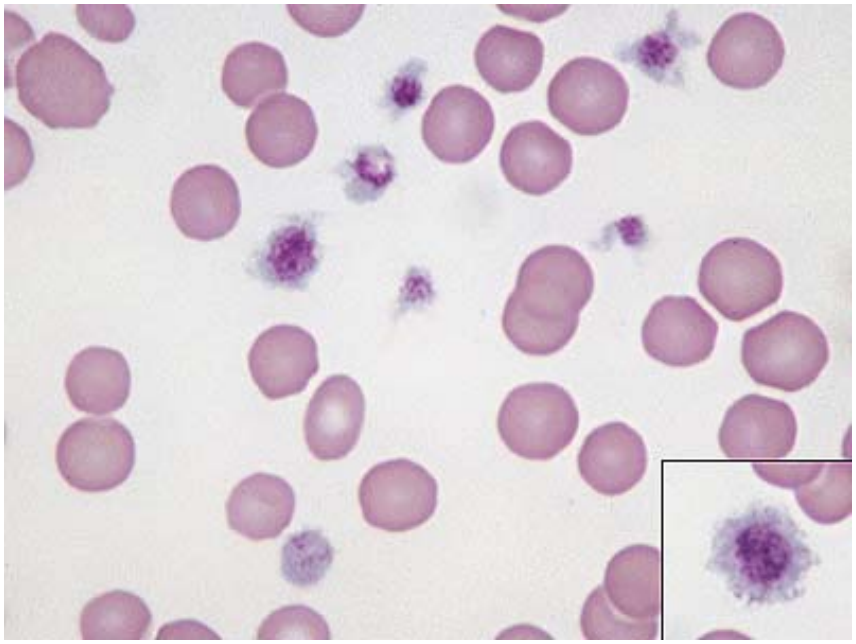
B. Platelet evaluation from blood smear-4

- Platelet morphology

Canine platelets



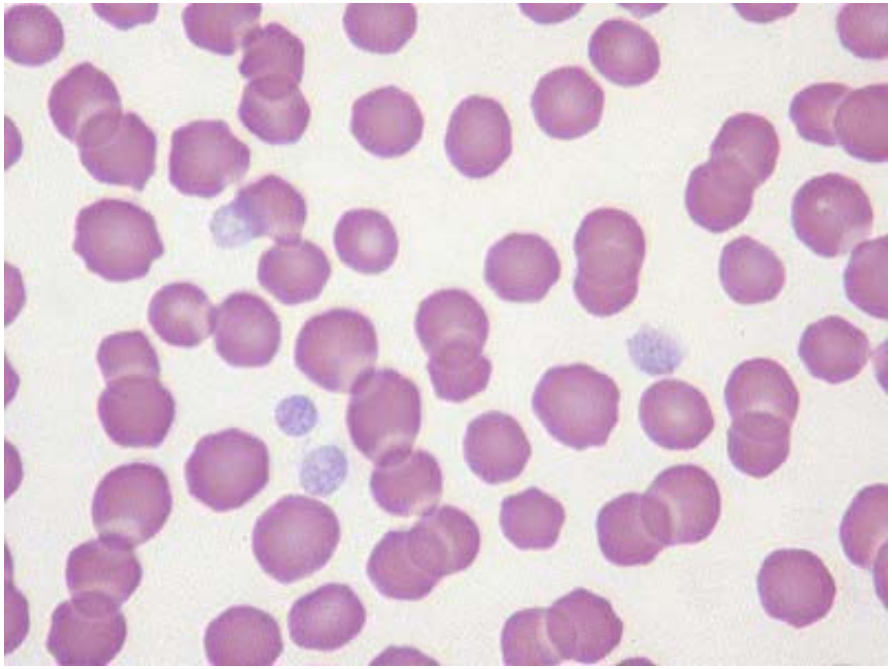
Feline platelets



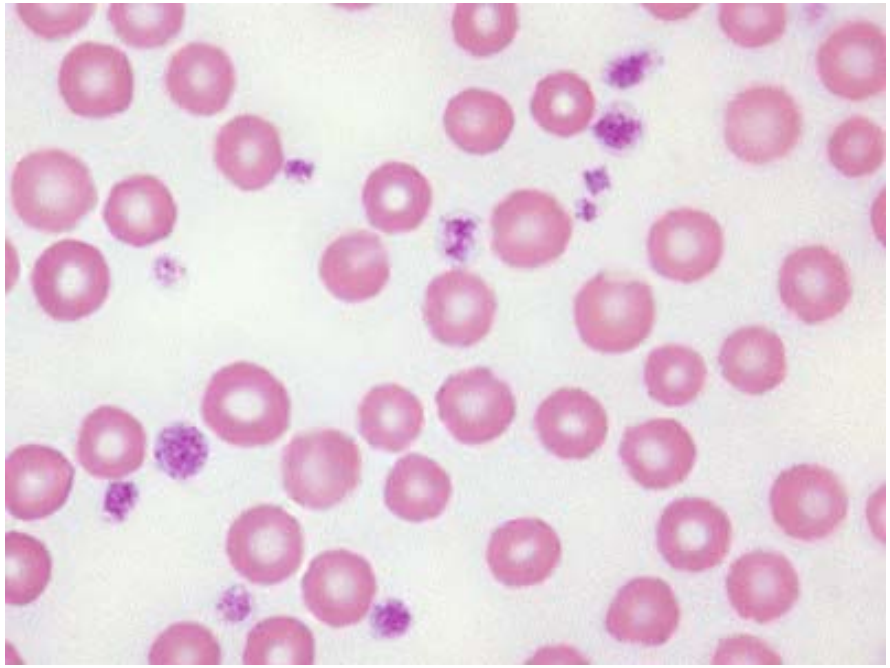
B. Platelet evaluation from blood smear-5

- Platelet morphology

Equine platelets



Bovine platelets



B. Platelet evaluation from blood smear-6

- Platelet morphology
  - –Large platelets: round or elongated
    - thrombocytopenia due to excessive platelet destruction
    - Infiltratife disease of bone marrow regardless of platelet count

B. Platelet evaluation from blood smear-7

- Platelet morphology
  - Platelet fragments or microplatelets
    - Iron-lack anemia (with thrombocytosis)
    - Bone marrow aplasia
    - Immune-mediated thrombocytopenia
  - Decreased granularity or vaculoes
    - may be seen in FeLV infection and DIC

C. Mean platelet volume (MPV)

- Determined by automatic counters
  - –Dogs: 6-9fl, Cats: 14-18fl, horses: <6fl
- Increased portion of large platelets released during responsive thrombopoiesis may yield an increased MPV.
- Platelet fragments may lower MPV if the portion in the circulation is sufficient

C. Mean platelet volume (MPV)

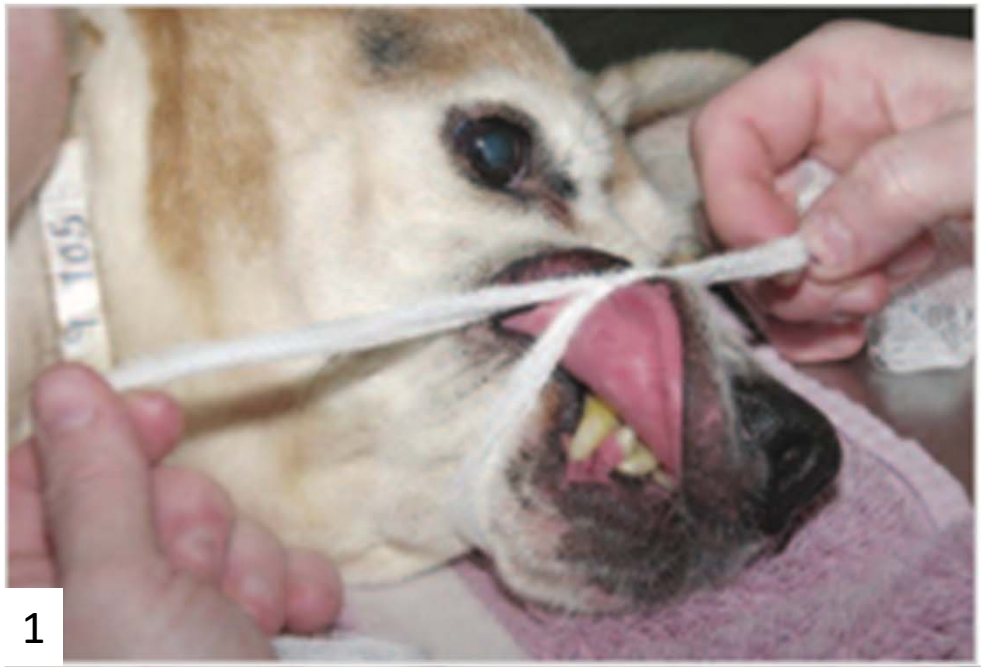
- MPV may be within normal range if high proportion of platelet fragments and large platelets co-exist.
- Canine MPV may be artifactually high in EDTA held more than 4 hours at 4 ℃.
- Platelet distribution width (PDW) is a measure of platelet anisocytosis.

D. Bone marrow smears for megakaryocytes

- Thrombocytopenia
- Normal or increased #
  - Excessive platelet consumption
  - Excessive platelet destruction
- Too few or no megakaryocytes
  - Decreased production

E. Bleeding time (BT)-1

- In vivo test
- **The functional ability** of **platelet to plug a minute wound**
- The duration of bleeding from a standardized incision of hairless skin or mucous membrane.



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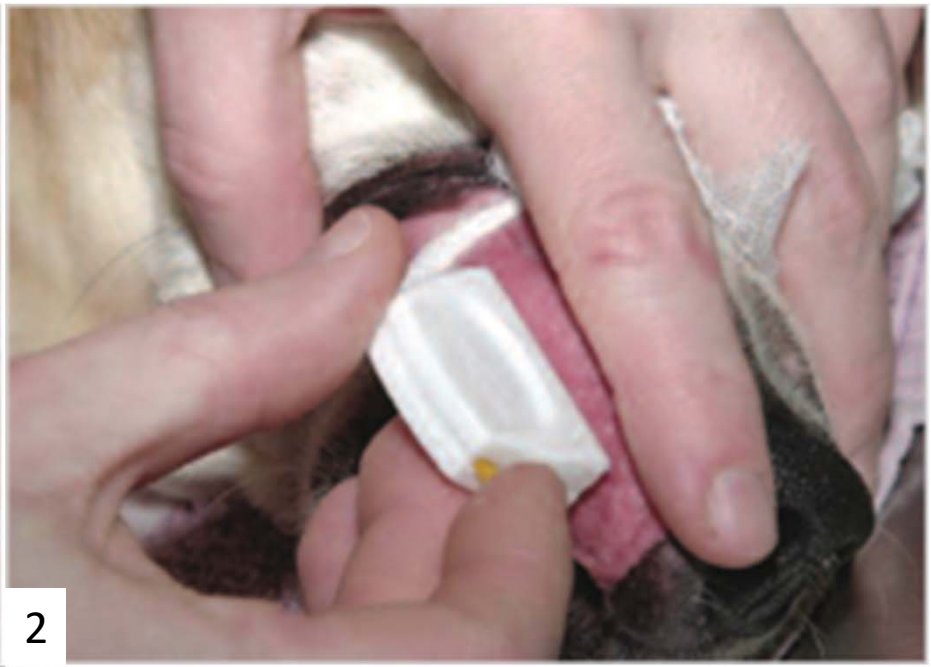

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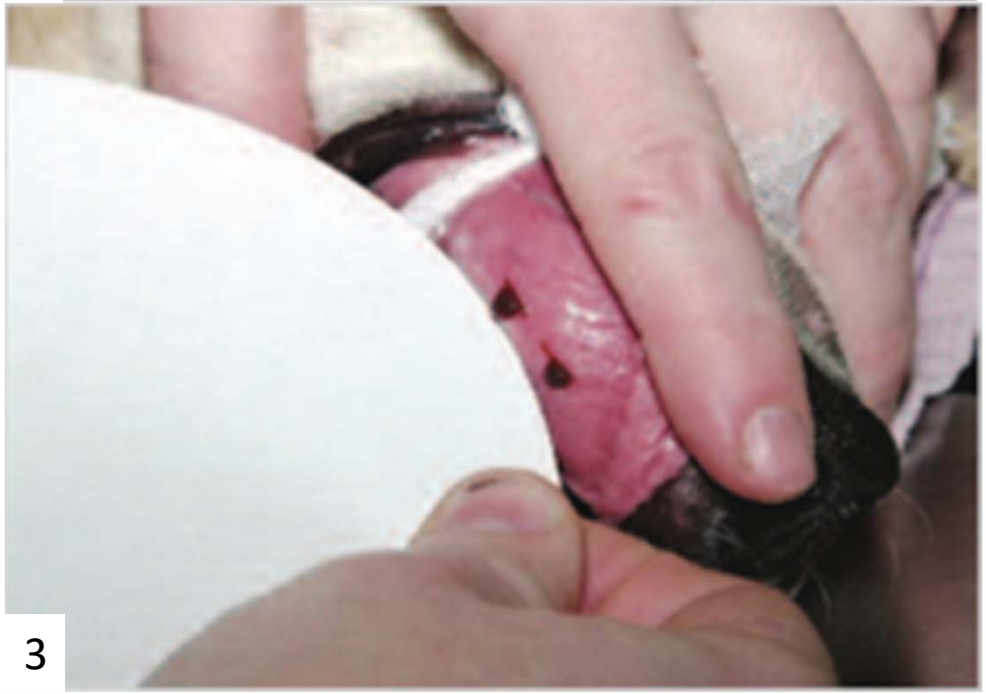

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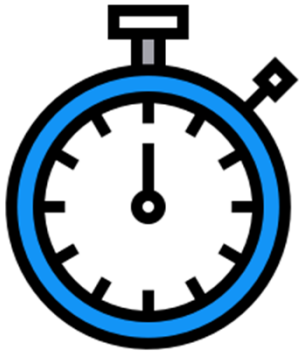


E. Bleeding time (BT)-2

- A simple clinical test to detect
  - Platelet function defect
    - vWD
    - Disseminated intravascular coagulation (DIC)
    - Uremia
  - Blood vascular disease
    - Vitamin C deficiency (scurvy) – in guinea pigs

E. Bleeding time (BT)-3

- The buccal mucosal bleeding time is done to evaluate platelet function in animals with normal or near-normal platelet counts.

Normal BMBT	
Healthy dogs	1.7 to 4.2 minutes (< 4 minutes)
Healthy cats	1 to 2.4 minutes (< 3 minutes)

- It provides no additional information in animals already known to have severe thrombocytopenia.

E. Bleeding time (BT)-4

- Only prolonged results are significant. Normal results may not eliminate the functional defect because of the low sensitivity of the test

- Coagulation factor deficiencies **do not** cause an abnormal bleeding time.

G. Von Willbrand's factor antigen assay-1

- **VWF**: adhesion factor, not coagulation factor
  - Produced in vascular endothelial cells or megakaryocytes
  - Polymers of 250.000 MW/unit
  - **2 functions**
    - Adhesion factor for platelet adherence to collagen
    - Carrier of coagulation factor VIII to stabilize

G. Von Willbrand's factor antigen assay-2

- Canine-specific vWF antigen assay is available
- Used to differentiate hemophilia A (factor VIII deficiency) from vWD (factor VIII deficiency with deficiency of VWF)

H. Antiplatelet antibody

- Immunofluorescent assay
- To test for immune-mediated thrombocytopenia

I. Clot retraction

- The amount of clear serum appearing around the clot in a non-anticoagulated blood sample is measured.
- Failure of serum separation
  - a platelet function defect and/or thrombocytopenia

J. Platelet adhesion and aggregation tests-1

- Sophisticated, but not adapted to practice laboratories
- Adhesion test: Glass-beads methods

J.Platelet adhesion and aggregation tests-2

- Aggregation test:

1. primary aggregation (reversible)
2. secondary aggregation (irreversible)

J. Platelet adhesion and aggregation tests-3

- To differentiate functional disorders
  - –vWD
  - –**Aspirin intoxication**
  - –Glanzmann disease;GPIIb/IIIa deficiency
  - –Bernard-Soulier syndrome: GPIb/IX deficiency
  - –Storage pool disease :

Disorders of platelets-1

- Functional disorders or thrombocytopenia
  - **Spontaneous bleeding from body surface**
  - **Petechiae or ecchymosis**
  - **Nose bleeding**

Diffuse petechiation of the skin over the abdomen, secondary to severe thrombocytopenia in a cat.

Disorders of platelets-2

- **Platelet dysfunction**
  - **Hereditary: vWD-the most common inherited bleeding disorder in animals**
  - **Acquired: Uremia, DIC, aspirin**

Mechanisms of thrombocytopenia-1

- Increased destruction or consumption
  - Immune-mediated thrombocytopenia
    - Autoimmunie, haptenic (promazine, infections),
    - Platelets adsorb immune complexes on their surfaces (infections, 7-21 days after modified live vaccine)

Mechanisms of thrombocytopenia-2

- Increased destruction or consumption
  - Infections
    - Direct infection
    - Consequence of hyperactive macrophage system
    - Ehrlichiosis, Anaplasma platys, and many bacterial and viral infections
  - DIC syndrome
  - Neoplasia

Mechanisms of thrombocytopenia-3

- Decreased production
  - Immune-mediated destruction of megakaryocyte precursors
  - Aplastic anemia
  - Ehrlichiosis (late stage) and other rickettsial diseases
  - Myelophthisis

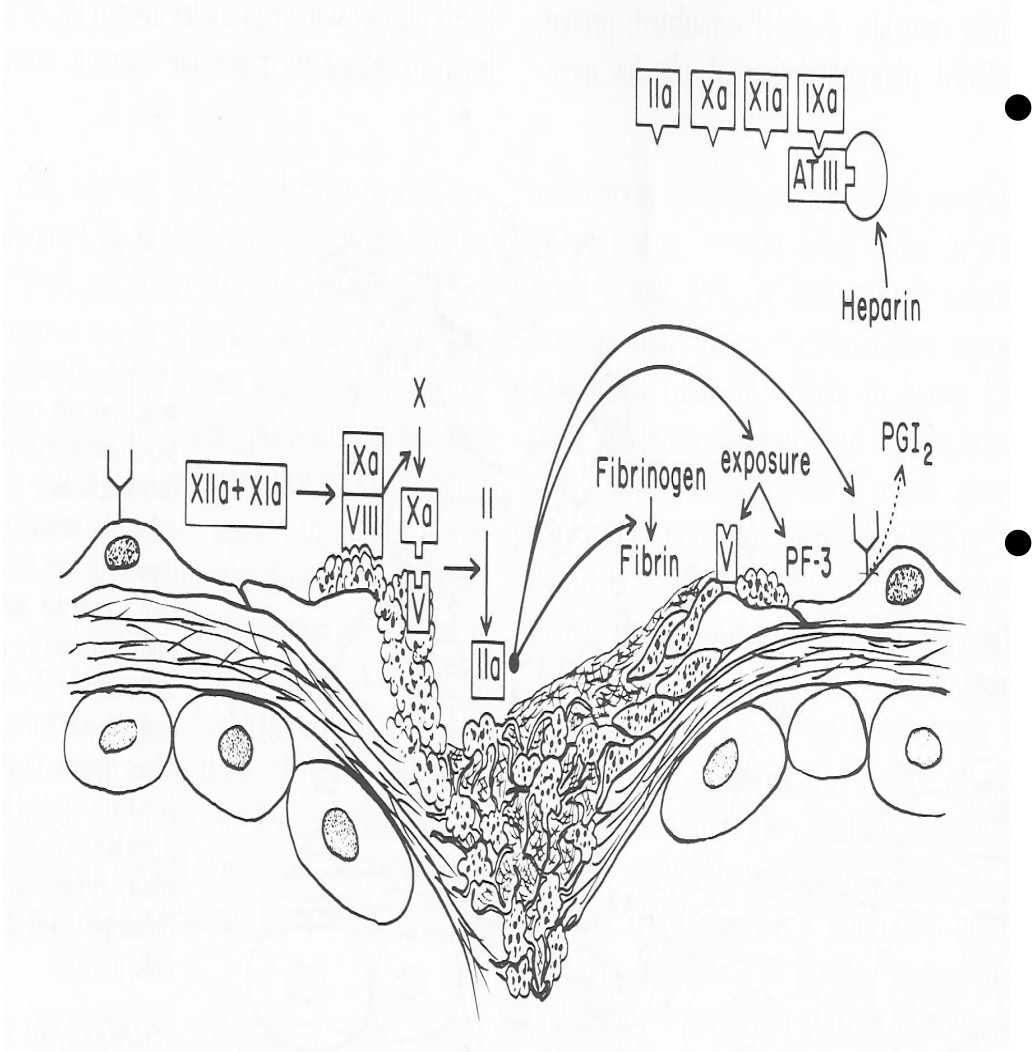
Causes of thrombocytosis

- Bleeding associated with
  - Trauma
  - Blood-sucking parasite
  - Neoplasm
- Iron-lack anemia: thrombopoietin
- FeLV-associated bone marrow disease
- Myeloproliferative disease

Hypercoagulation and DIC

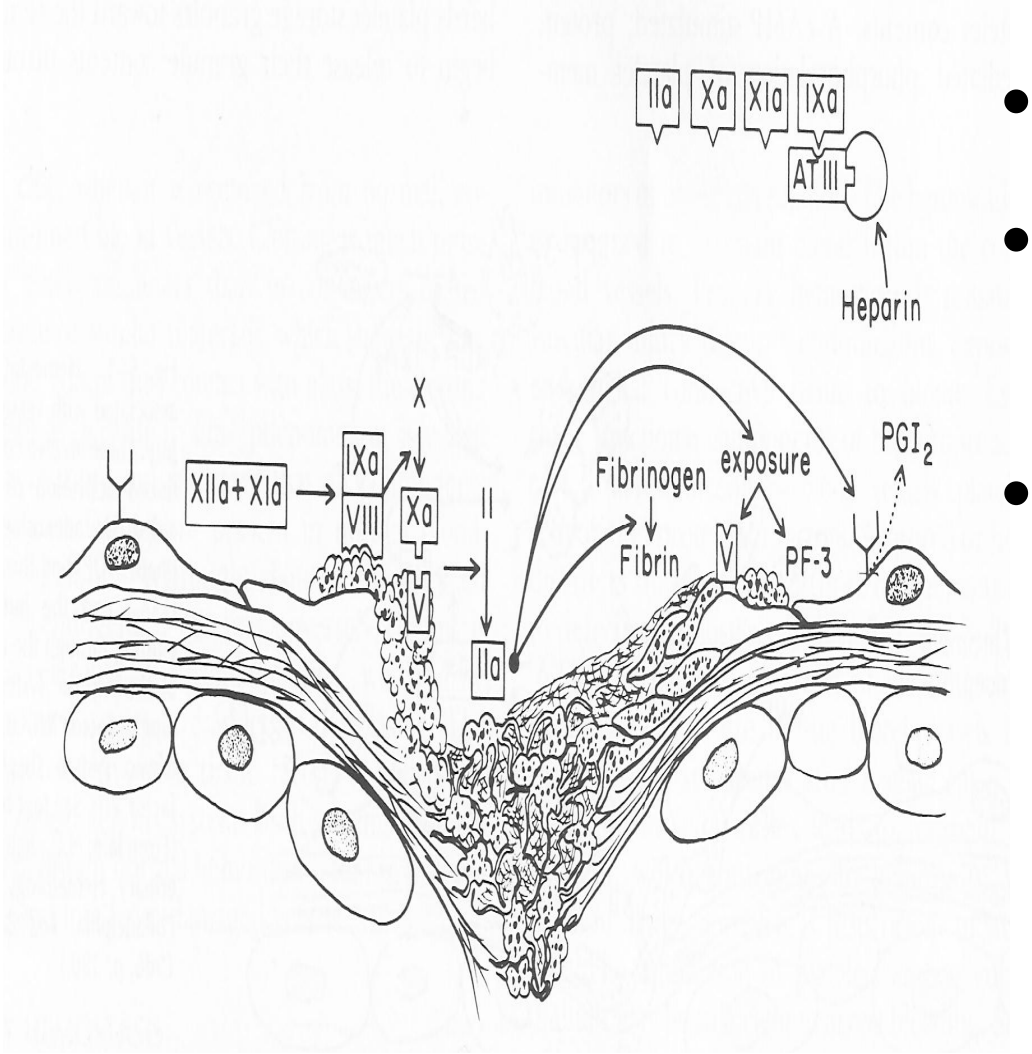
- May result from excessive platelet activation
- Blood stasis potentiates platelet activation
  - Severe dehydration
  - Shock
  - Bradycardia,
  - Hypotension,
  - Drugs
  - Polycythemia
  - Hyperviscosity syndromes

Hemostatic events associated with vessel wall injury-1



- Coagulation
  - –Secondary hemostasis
- Organization of platelet plugs.

Hemostatic events associated with vessel wall injury-2



- Fibrin deposition
- Stabilizing the platelet plug
- Regulatory actions
  - prostacyclin (PGI_2)
  - antithrombin III(ATIII)

Mechanisms of coagulation-1

A. Coagulation factors: produced in liver*
    1. Enzymatic factors: require activation of proenzyme to function

Mechanisms of coagulation-2

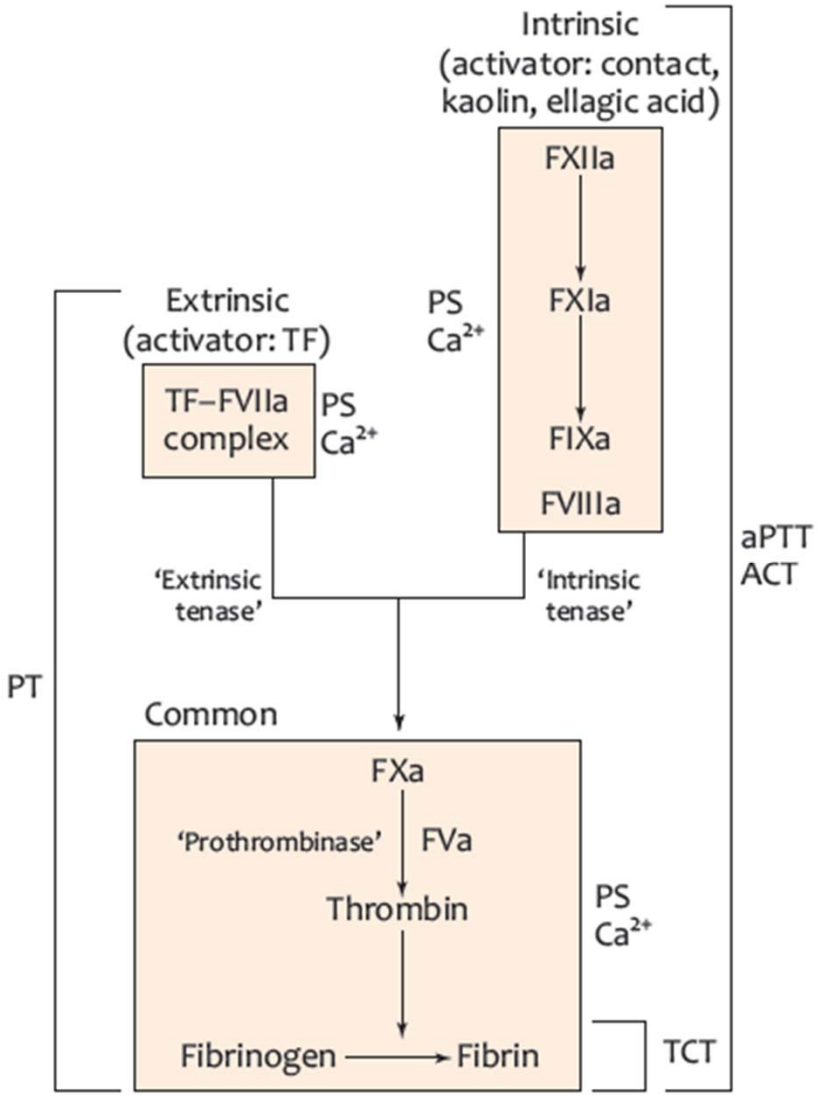
Non-enzymatic protein factors

B. Platelet phospholipid

C. Tissue thromboplastin

D. Calcium ion

Mechanisms of coagulation-3



A.Coagulation factors-1

1. Enzymatic factors: originate from liver, occur in plasma
   - Contact factors(XI and XII) and prekallikrein
   - Vitamin K-dependent factors: **II*, VII, IX**, and X
     - Vitamin K-dependency is conferred in the liver by **post-translational carboxylation of glutamic acid** residues **on certain parts of the coagulation factor molecules.**

A.Coagulation factors-2

– Vitamin K-dependent factors: **II*, VII, IX**, and X

- **The carboxylated regions of factors** II, VII, IX and X provide for **calcium binding** to platelet phospholipid, a coagulation cofactor, or tissue thromboplastin, a lipoprotein that triggers intrinsic system coagulation.

A.Coagulation factors-3

- Vitamin K-dependent factors: **II*, VII, IX**, and X
  - **In the absence of sufficient vitamin K** (e.g. coumarin poisonings), the coagulation factors are **secreted without sufficient carboxylation** and **have inadequate function**,. Coagulation inhibition factors, protein C and protein S
- Clot stabilizing factor, XIII

1-1. enzymatic factors cont.

- Metabolism
  - plasma half-life: hours to a few days
    - VII- 4-6 hours
  - After activation, enzymes are inhibited and removed by macrophages
  - Clotting does not consume these activated factors- present in serum

1-2. enzymatic factors cont.

- Natural coagulation inhibitors in vivo
  - Antithrombin III (complexed with heparin)
  - Protein C and Protein S ; Vitamin-K dependent
    - Thrombin that escapes clotting and inhibition moves downstream and binds with thrombomodulin on endothelial cells.

1-3. enzymatic factors cont.

- Natural coagulation inhibitors in vivo

  - Protein C and Protein S ; Vitamin-K dependent
    - Thrombin-activated protein C
      - Work with protein S (a co-enzyme) to function as an anticoagulant
        - »Hydrolyses Va,VIIa

2-1. Non-enzymatic protein factors

- Originate from liver, occur in plasma, associated with platelet membranes, localized in developing clots by platelet aggregation

2-2. Non-enzymatic protein factors

- V, VIII, and fibrinogen: acute phase proteins (globulin)
  - –Plasma concentrations increase during inflammatory or neoplastic diseases
  - –Plasma half-life: hours to days

2-3. Non-enzymatic protein factors

- V, VIII, and fibrinogen: acute phase proteins (globulin)
  - –VIII is also produced from the reticuloendothelial system
- Clotting consumes these factors- absent in serum
- Protein C controls factors V and VIII.

Nomenclature and some properties of coagulation factors

Factor	*Common Name*	*Molecular Weight*	*Plasma Level (μg/ml)*	*Half-life (hr)*	*Turnover Rate (μg/ml/day)*	*Biosynthesis*
I	Fibrinogen	340,000	2500	123	500	Liver, megakaryocytes
II	Prothrombin	70,000	100	100	40	Liver; vitamin K-dependent
III	Tissue thromboplastin	45,000	0	—	—	Virtually all tissues
IV	Calcium ions	—	—	—	—	
V	Proaccelerin	330,000	5–12	25	10	Liver, macrophages
VII	Proconvertin	63,000	1	5	2	Liver, vitamin K-dependent
VIII	Antihemophilic factor	1–2 million	7	10	25	VIII:C, probably liver vWF, endothelial cells, and megakaryocytes
IX	Christmas factor	62,000	4	20	2	Liver; vitamin K-dependent
X	Stuart-Prower factor	59,000	5	65	6	Liver; vitamin K-dependent
XI	Plasma thromboplastin antecedent	200,000	4	65	<2	Liver
XII	Hageman factor	80,000	29	60	<2	Liver
XIII	Fibrin-stabilizing factor	320,000	10	150	3	Liver; megakaryocytes
—	Prekallikrein	85,000	—	35	—	Liver
—	High-molecular-weight kininogen	120,000	—	156	—	Liver (?)

B. Platelet phospholipid

- Cofactor derived from platelet membranes
  - A structual micelle ,accelerates coagulation factor activation
- Calcium binding
  - Links the vitamin K-dependent coagulation factors to platelet phospholipid
  - Positions the enzymes for highly efficient action on respective substrates

C. Tissue thromboplastin-1

- A lipoprotein
- Injured cells of any kind release
  - Extrinsic pathway proceeds instantly
  - Blood sampling
    - Clean venipuncture – intrinsic pathway- 5-15 minutes until clot
    - Difficult venipuncture- blood samples contaminated by tissue thromboplastin- may clot in 12-15 seconds

C. Tissue thromboplastin-2

- The protein moiety: The factor specificity
  - –to react only with factor VII
  - –Caroxylation of factor VII
    - calcium linkage to tissue thromboplastin
    - Efficient action of these 2 factors

D. Calcium

- In hypocalcemia
  - –Sufficient calcium is always available for hemostasis
  - –Life-threatening neuromuscular disease may occur
- ANTICOAGULANTS
  - –Chelate Ca^{2+}
  - –EDTA, oxalates, citrate

2.Intrinsic pathway of coagulation-1

- Mechanisms
  - –XI, XII, prekallikrein, high molecular weight kininogen
  - –Activated when plasma interacts with negatively charged substances
    " contact activation"
    - In vivo: collagen, pyrophosphate from platelets, endotoxin
    - In vitro: glass, kaolin, celite

2.Intrinsic pathway of coagulation-2

- Mechanisms
  - –Activated factor XII: the product of contact activation
  - –Activates IX > activates IX> activates X , in conjuction with thrombin-activated VIII >>>common pathway

2.Intrinsic pathway of coagulation-3

- Deficiency of intrinsic factors
  - –Hemorrhage of varying severity
  - –Patterns of hemorrhage : due to abnormal secondary hemostasis

2.Intrinsic pathway of coagulation-4

- Deficiency of intrinsic factors
  - –deep-seated in tissues
    - intraarticular, subcutaneous, intramuscular
    - Gastrointestinal, urinary
    - Delayed onset of hemorrhage after vascular intervention
  - –*hemorrhage due to abnormal primary hemostasis: superficial bleeding

Disorders of intrinsic coagulation-1

- Hereditary deficiency
  - –Prekallikrein: dogs and horses, bleeding
  - –XII: cats, no bleeding, ± prolonged cloting time
  - –XI: dogs and Holstein cattle

Disorders of intrinsic coagulation-2

- Hereditary deficiency
  - **IX**: sex-linked, "**hemophilia B**", cats and dogs
  - **VIII**, sex-linked, "**hemophilia A**", cats and dogs
  - **Von Willebrand's disease**

Disorders of intrinsic coagulation-3

- **Acquired deficiency**
  - –VIII in DIC syndrome
    - The speed of consumption of the nonenzymnatic protein factors exceeds the rate of synthesis, resulting in hypocoagulation
      - –Fibrinogen, V, and VIII
    - Bleeding may not occur before nearly all factor activity is depleted

Acquired deficiency (cont.)

- **Vitamin K-dependent factors, II, VII*, IX, X****
  - Coumarin poisonings
  - Bile insufficiency
  - Liver disease
  - Cats are more sensitive
- Hypercoagulation and DIC
  - Exccesive contact activation occurs
    - Heat stroke, viremia, endotoxemia

3. Extrinsic pathway of coagulation

- Mechanisms
  - –VII and tissue thromboplastin
  - –The complex of activated VII and tissue thromboplastin
    - activates IX in the intrinsic cascade
    - activates X directly in the common pathway

Disorders of extrinsic coagulation-1

- Hereditary deficiency
  - –VII, dogs, mild bleeding and tendency of demodecosis

Disorders of extrinsic coagulation-2

- Acquired deficiency
  - Vitamin K-dependent factors, VII
  - In early stages or mild deficiency, only VII deficiency may be manifested

Disorders of extrinsic coagulation-3

- Acquired deficiency
  - VII has the shortest half-life among the vitamin K-dependent factors
  - VII will be replaced more rapidly by molecules lacking adequate vitamin K-dependent carboxylation.

Disorders of extrinsic coagulation-4

- **Hypercoagulation and DIC**
  - –Excessive tissue thromboplastin release
    - Intravascular hemolysis, massive necrosis, trauma, septicemia, endotoxemia

Common pathway of coagulation-1

- Mechanisms
  - –X, V, II
  - –X activated by either the intrinsic or extrinsic system> in the presence of V, converts II to **thrombin**

Common pathway of coagulation-2

- Mechanisms
  - **Thrombin: converts soluble fibrinogen to insoluble fibrin**
    - Activates VIII and V: acceleration of intrinsic and common pathway
    - Activates protein C
    - Platelet activation and aggregation

Disorders of common coagulation-1

- Hereditary deficiency
  - X, cocker spaniel
  - Prothrombin, dogs
- Acquired deficiency
  - V and VIII and fibrinogen in DIC
- Hypercoagulation and DIC
  - Due to direct activation of thrombin
  - Endotoxemia, certain venoms, mucin from adenocarcinoma

Disorders of common coagulation-2

- Antithrombin III deficiency: predisposed to hypercoagulation
  - –In canine nephrotic syndrome
  - –Colic horses

Disorders of common coagulation-3

- Protein C deficiency: predisposed to hypercoagulation
  - –Hereditary in horse
  - –Acquired in colic horse
  - –V and VIII remain to be active

5-1. Fibrinolysis

- Mechanisms
  - –Highly fibrin specific
  - –Plasminogen (proenzyme)
    - in plasma
    - platelet membranes

5-2. Fibrinolysis

- Mechanisms
  - Plasminogen activator
    - Derived from endothelium, platelets, and other cells
    - Balanced by plasminogen activator inhibitor (1:1)
    - Activates fibrin-bound plasminogen

5-3. Fibrinolysis

- Mechanisms

  – Plasmin hydrolizes fibrin

    - Fibrin degradation product

      – Cleared by macrophages

Disorders of fibrinolysis-1

- Poorly defined in veterinary medicine
- Normal reactivity of the components of fibrinolysis is a balanced process

Disorders of fibrinolysis-2

- Possible imbalance
  - –Decreased plasminogen: horse with ischemic colic
  - –Lack of plasminogen activators
  - –Lack of protein C

Disorders of fibrinolysis-3

- Possible imbalance
  - Lack of antiplasmin
  - Excess plasminogen activator inhibitor
  - Systemic plasminogen activation
    - Plasmin also hydrolyzes V and VIII
    - Potentiate DIC

6-1. **Laboratory evaluation of coagulation and fibrinolysis**

- Effective in hypocoagulative disorders (bleeding syndromes)

- MANY TESTS
  - **Whole blood clotting test**: ACT

6-2. Laboratory evaluation of coagulation and fibrinolysis

- MANY TESTS
  - Citrated plasma clotting test: APTT, PT, TCT
  - Fibrinogen concentration
  - Fibrin-fibrinogen degradation products (FDP)
- Differential diagnosis of major bleeding disorders in veterinary medicine

Activated clotting time (ACT)-1

- The time required for fibrin clot formation after contact activation in fresh whole blood
- Intrinsic and common pathway
- Sensitivity lower than APTT

Activated clotting time (ACT)-2

- Very reproducible with prewarmed tube and good venipuncture
- Commercial tubes containing a substance that potentiates contact activation
- More sensitive than Lee-White whole blood clotting time or capillary tube clotting time

Interpretation of ACT-1

- Increased ACT
  - –Indicates deficiency of coagulation factor(s) within the intrinsic or common pathway.
    - The factor deficiency must be <5% of normal to prolong ACT.

Interpretation of ACT-2

- Increased ACT
  - –Slight prolongation of ACT occurs in thrombocytopenia only if the platelt count is less than 10,000/μl.
    - A result of lack of platelet phospholipid.
- Hypofibrinogenemia is not detected by ACT

ACT and Clot Quality-1

- ACT tube can be used for subjective assessment of clot quality.
  - –After holding the tube at 37 ℃, the clot should be firm with clear serum appearing by 1-2 hours after collection.

ACT and Clot Quality-2

- ACT tube can be used for subjective assessment of clot quality.

  – A soft friable clot that liquefies in 30-60 minutes indicates excessive fibrinolysis or hypofibrinogenemia.

Pattern of clotting and serum

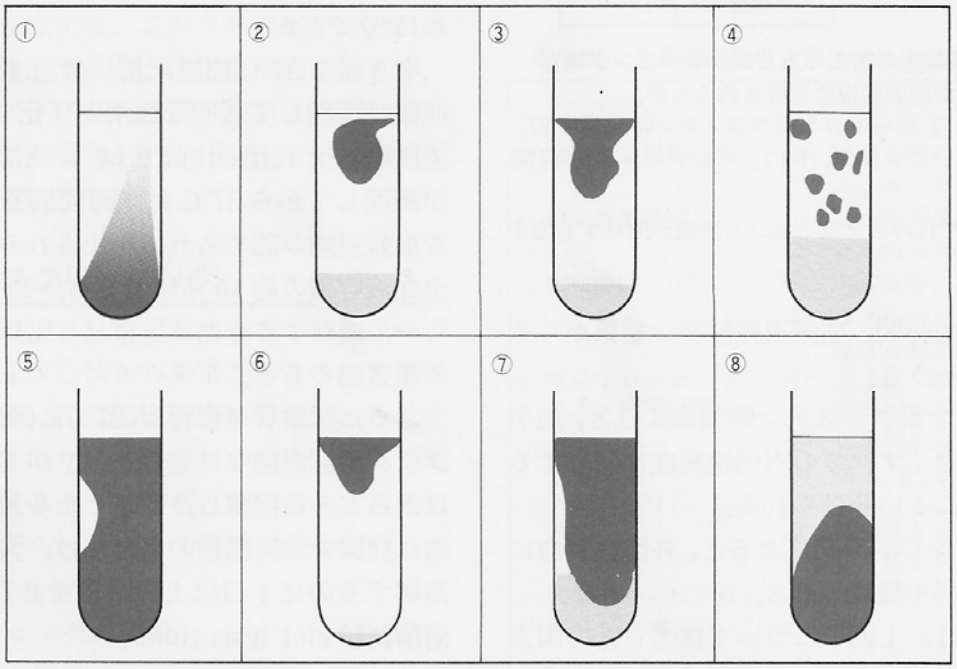


図 3-18　血液凝固試験における血餅および血清の病的所見（原図・河合）
① 血友病または赤沈値の著明な亢進，② 低フィブリノゲン血症，③ 第 XIII 因子欠乏症または異常フィブリノゲン血症，④ 線溶亢進，⑤ 血小板減少，⑥ 重症貧血，⑦ 多血症，⑧ 血清の異常：黄疸，溶血，淡黄色など

Activated partial thrombin test (APTT)-1

- Citrated plasma clotting tests
  - –APTT, PT, TCT, Russell viper venom test (Stypven time)
  - –Requires proper sample management

Activated partial thrombin test (APTT)-2

- Sample management
  - Use plastic syringes and commercial citrate tubes
  - Difficult venipuncture will contaminate the sample with tissue thromboplastin.

APTT

- Measures the time required for fibrin clot formation in citrated plasma after the addition of reagents
  - a contact activator, a phospholipid, and calcium

Increased APTT-1

- Increased APTT
  - Intrinsic or common coagulation factor deficiency
  - Hemophilia, XII deficiency, prekallikrein deficiency, vWD, DIC, vitamin K deficiency (coumarin poisonings, bile insufficiency, liver disease)

Increased APTT-2

- Increased APTT
  - Factors, including fibrinogen, must be <30% of normal
    - Hemophiliac carrier do not bleed because they have 40-60% of normal VIII or IX activity

Increased APTT-3

- Increased APTT
  - Factors, including fibrinogen, must be <30% of normal
    - vWD may have bleeding with a normal APTT because VIII activity is sufficient to result in a normal APTT but due to the platelet dysfunction (lack of vWF) bleeding occurs.

Increased APTT-4

- Increased APTT
  - Factors, including fibrinogen, must be <30% of normal
    - Frequently, mild deficiency of multiple factors (e.g. coumarin poisonings) results in clinical bleeding but APTT and PT are normal.

Increased APTT-5

- Increased APTT
  - –Thrombocytopenia has no effect on APTT because platelet phospholipid is provided in vitro

Other factors on APTT-1

- Therapeutic anticoagulation with heparin prolongs APTT. In dogs, 200IU/kg QID prolongs APTT 1.5-2.5 times baseline value.
  - Over time, the effect of heparin declines as heparin utilizes endogenous antithrombin III.

Other factors on APTT-2

- Increased fibrinogen and V and VIII in inflammation may shorten APTT.

- Specific factor analysis requires specialized laboratories working on animal bleeding disorders.
  - VIII (hemophilia A and vWD)
  - IX (hemophilia B)

Prothrombin time (PT)-1

- One-step prothrombin time (OSPT)
- The time required for fibrin clot formation in citrated plasma after adding tissue thromboplastin and calcium.

Prothrombin time (PT)-2

- Reagents that use human brain tissue thromboplastin give much longer PT on animal species.
  - Use reagents containing rabbit brain or synthetic tissue thromboplastin

Increased PT-1

- Indicates extrinsic or common coagulation factor deficiency
  - VII deficiency, DIC, vitamin K deficiency
  - Factors must be <30% of normal.

Increased PT-2

- Indicates extrinsic or common coagulation factor deficiency
  - In mild or early stages of coumarin poisonings, VII deficiency preceeds the other factor deficiency due to its shorter half life.
  - Fibrinogen must be <50% of normal.
- Thrombocytopenia has no effect on OSPT.

Thrombin clotting time (TCT)-1

- The time required for fibrin clot formation in recalcified, citrated plasma after the addition of thrombin.

Thrombin clotting time (TCT)-2

Increased TCT

- Hypofibrinogenemia : <100 mg/dl
- DIC, fibrinogen deficiency, dysfibrinogenemia, excessive FDP concentration (competes with patient fibrinogen for thrombin binding), dysproteinemia, heprinization

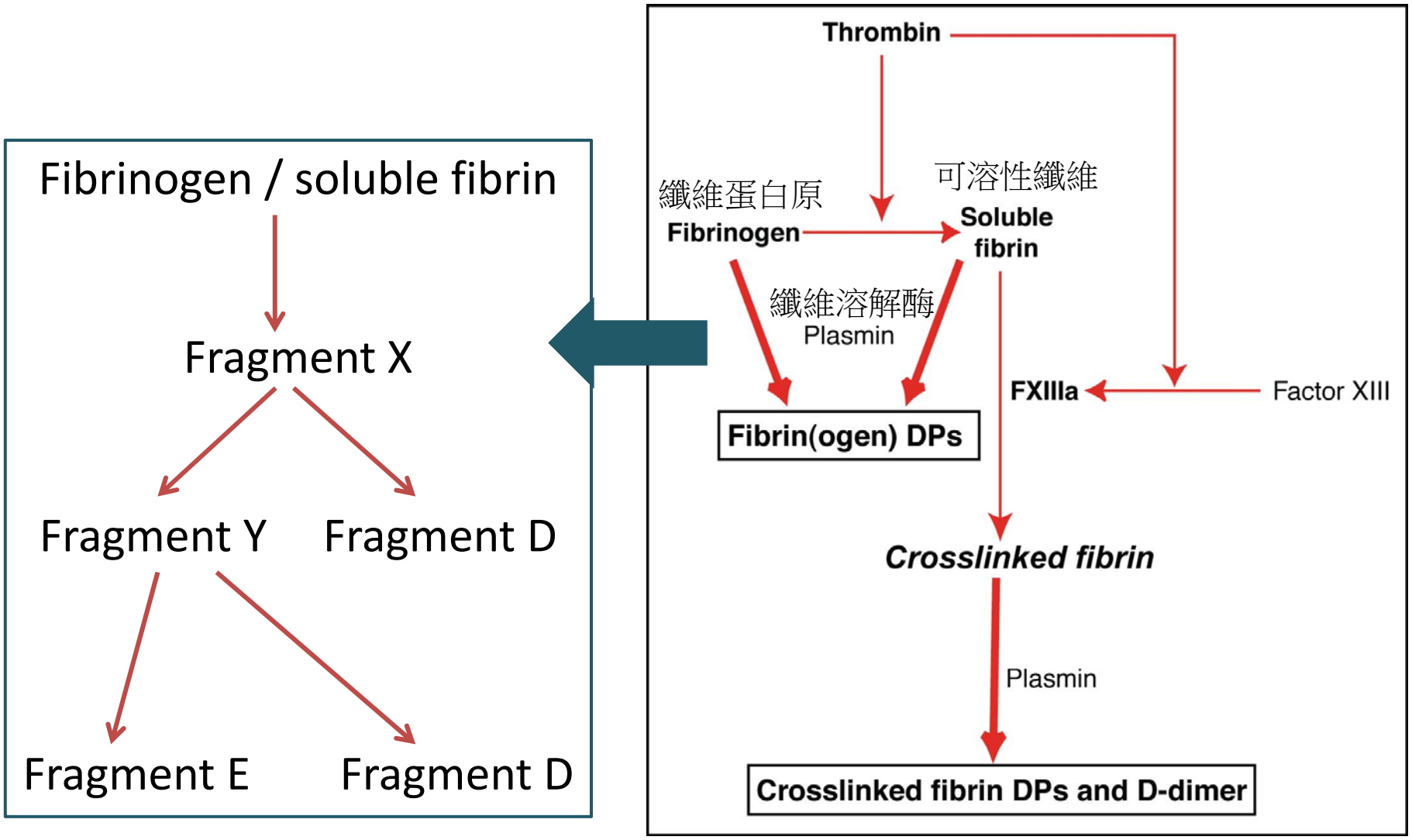
Russel viper venom test (Stypven time)

- Bypasses the extrinsic and intrinsic pathways
- Directly measures X, V, prothrombin, and fibrionogen (common pathway)
- Not commonly used
- Abnormal common coagulation can be assessed by the results of APTT and PT

fibrin-fibrinogen degradation product (FDP)-1

- Methods
  - –Agglutination of latex beads coated with antibody to human fibrinogen fragments, with commercial reagents
  - –The reactivity across animal species

fibrin-fibrinogen degradation product (FDP)-2



fibrin-fibrinogen degradation product (FDP)-3

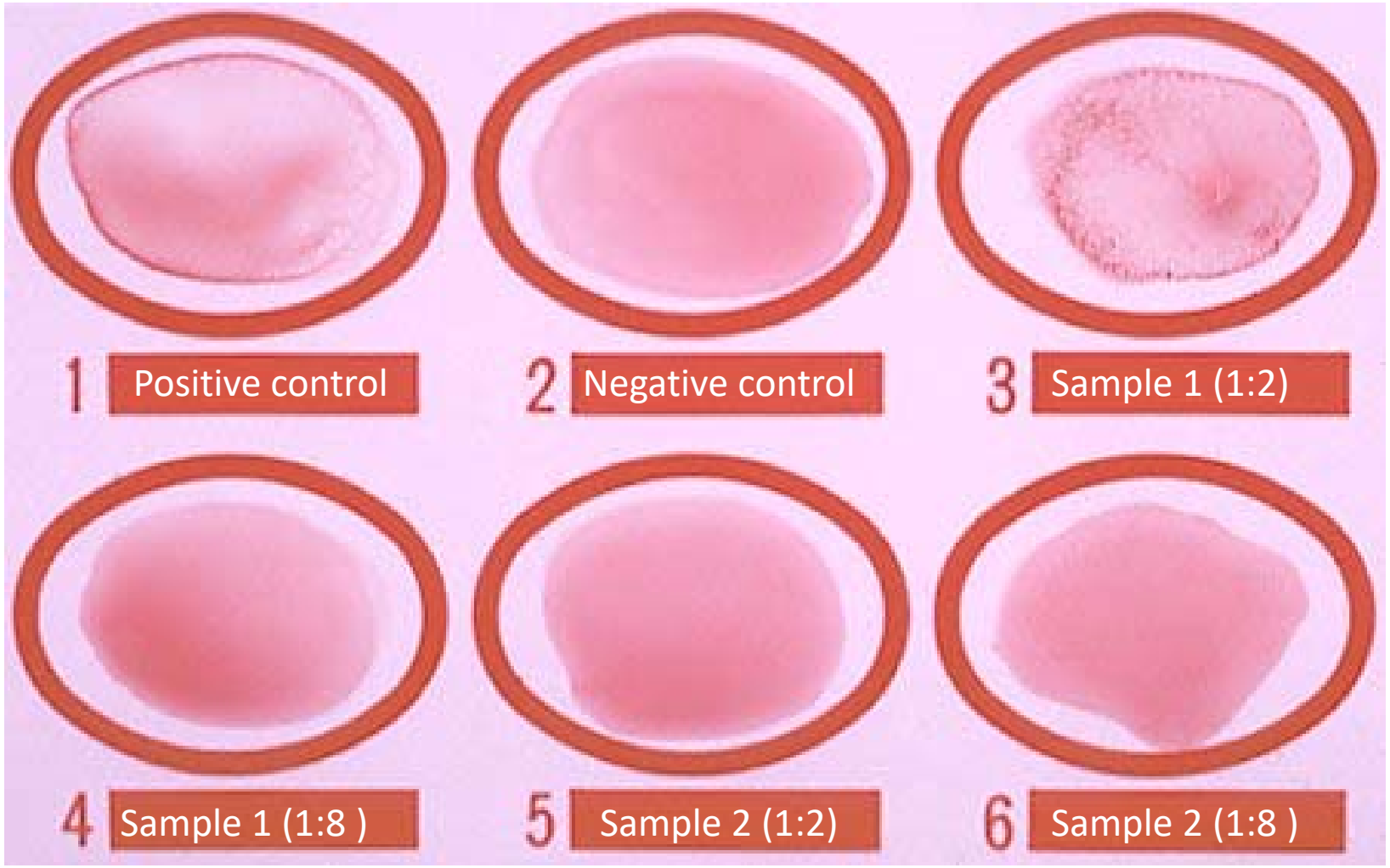
- Methods
  - Serum sample: preparation is required
    - Add thrombin to ensure all fibrinogen is consumed in the sample
    - Inhibit plasmin to ensure no in vitro fibrinolysis occurs
- Increased FDP
  - DIC

fibrin-fibrinogen degradation product (FDP)-4

- Newer latex-agglutination kits based on monoclonal antibodies
- Do not cross-react with intact fibrinogen.
- Results > 5 µl/mL are abnormal.

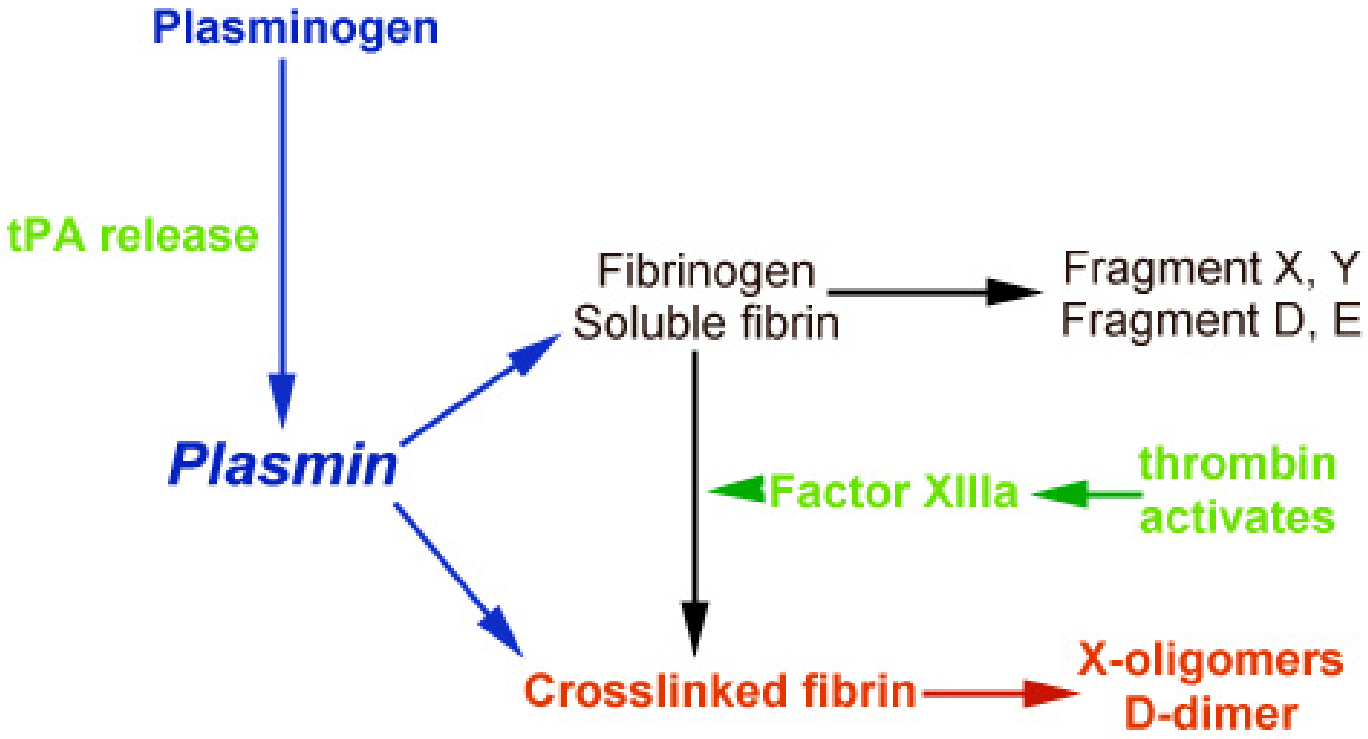
Plasma FDP concentration	1:2 dilution	1:8 dilution
< 5 µl/mL	Negative	Negative
5-20 µl/mL	Positive	Negative
> 20 µl/mL	Positive	Positive

Plasma FDP assay



D-dimer -1

- D-dimer is a specific plasmin-mediated breakdown product of crosslinked fibrin.



D-dimer -2

- D-dimer is more specific for fibrinolysis than FDPs, as its formation requires the action of thrombin (to activate factor XIII) to produce crosslinked fibrin and the cleavage of this fibrin by plasmin.

- Traditional FDP assays cannot distinguish between plasmin action on fibrinogen (fibrinogenolysis) and fibrin (fibrinolysis).

D-dimer -3

Normal values

- Normal dogs and cats:
  D-dimer values < 250 ng/mL

- Most healthy horses:
  D-dimer values < 500 ng/ml (but D-dimer can be as high as 1000 ng/mL in the horse).

D-dimer -3

Increased D-dimer

- D-dimer will be increased whenever there is activation of thrombin, to form crosslinked fibrin and fibrinolysis, i.e. thrombosis and fibrinolysis.

- The prototypical thromboembolic disease is *disseminated intravascular coagulation* (DIC) and D-dimer is often very high in this disorder

D-dimer -4

Species-specific interpretation

- **Dogs***:*
  - Plasma D-dimer values are often very high (> 1000 ng/mL) in dogs with documented thromboembolic disease, including DIC, or thrombosis (e.g. pulmonary thromboembolism)
  - High values have been reported in dogs secondary to neoplasia, inflammatory disease and hemorrhagic effusions.

D-dimer -5

Species-specific interpretation

- **Cats:**
  - D-dimer has been evaluated in cats with cardiac disease, who are predisposed to aortic thromboembolism.
  - High D-dimer levels in cats with conditions associated with DIC, e.g. feline infectious peritonitis virus infection.

D-dimer -6

Species-specific interpretation

- **Horses**:
  - D-dimer is increased in plasma in horses with severe colic and is a sensitive diagnostic test for the presence of underlying DIC.
  - In some studies, a high D-dimer was a negative prognostic indicator for outcome.

- Thank you for your attention!