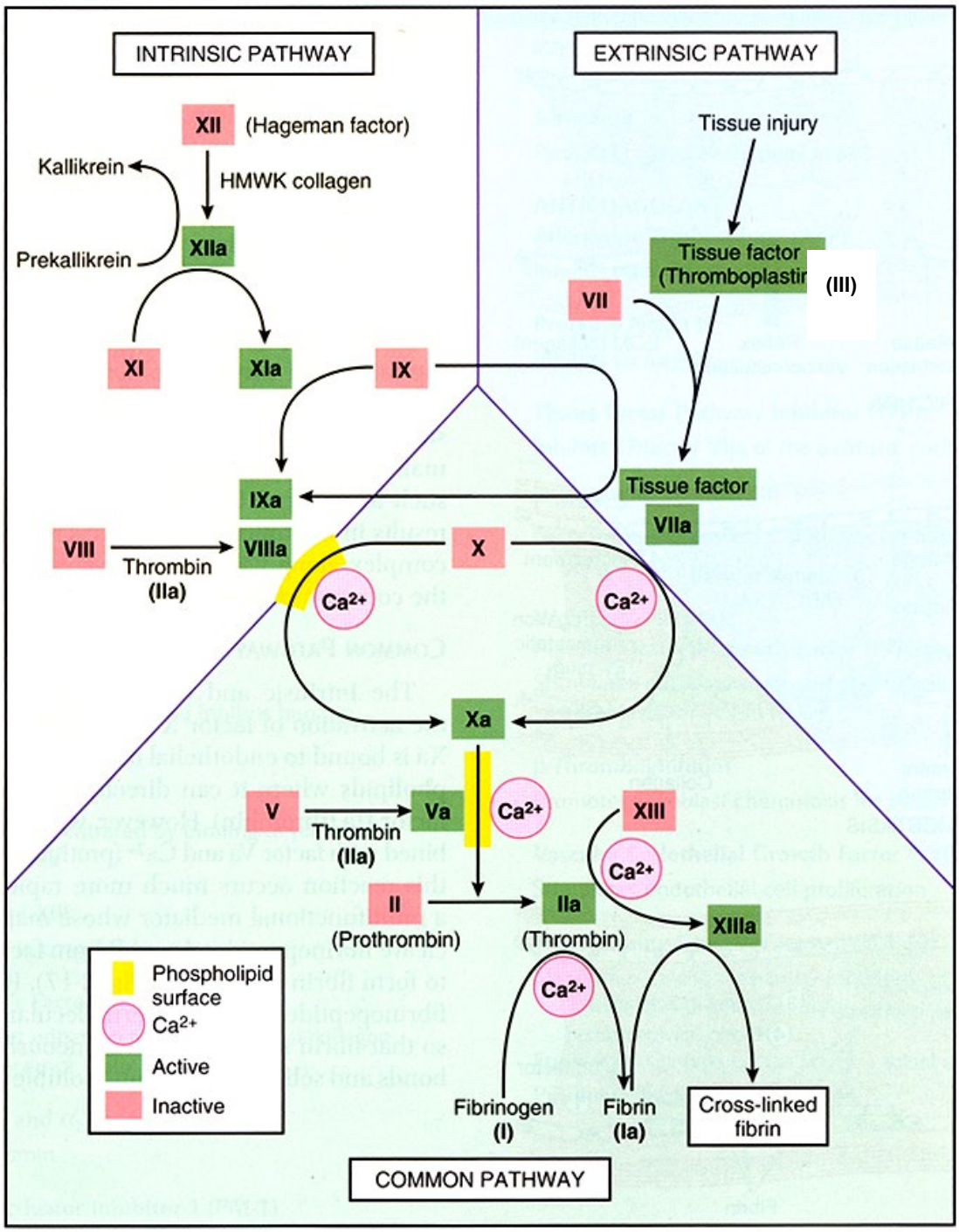
DISORDERS OF SECONDARY HAEMOSTASIS

- disorders of secondary haemostasis involve defects in the generation of fibrin or (rarely) the stability of fibrin formed via the coagulation cascade
- **Figure 1** provides a simple schematic representation of the coagulation cascade

Figure 1 – The Coagulation Cascade



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- **clinical signs suggestive of defective secondary haemostasis** include large volume bleeds, haematoma formation, and/or bleeding into body cavities or joints
- epistaxis and/or bleeding from other mucous membranes may occur but petechiae, purpura or ecchymoses in skin or mucous membranes are not usually seen
- haemorrhage may develop spontaneously or it may be delayed and protracted after a challenge to haemostasis (e.g. trauma, surgery)

- the **mechanisms responsible for defective secondary haemostasis** are:

  - **inherited deficiency of one or more coagulation factors**
  - **vitamin K antagonism** or **deficiency**
  - **severe acute** or **chronic hepatic parenchymal disease**
  - **excessive fibrinolysis** (or **fibrinogenolysis**)

- of these, **vitamin K antagonism is the most common mechanism** in domestic animals

INHERITED COAGULATION FACTOR DEFICIENCIES

- inherited coagulation factor deficiencies are most often identified in **purebred dogs** but also occur in crossbred dogs
- they occur rarely in cats and other domestic animal species

- **Table 1** lists the inherited coagulation disorders that have been identified in domestic animals

- deficiencies of factors III (tissue factor) and V and of high molecular weight kininogen (HMWK) are yet to be confirmed in domestic animals

- the severity of bleeding is usually inversely proportional to the activity of the clotting factor affected
- if the defect leads to severe bleeding, may see abortions, stillbirths and/or neonatal mortality ("fading puppy syndrome") (e.g. due to protracted post-natal bleeding from the umbilical cord) in affected families of animals

- some inherited coagulopathies may cause abnormal results in laboratory tests of haemostatic function but do **NOT** cause *in vivo* haemorrhage
- others cause only mild clinical haemorrhage
- these observations reflect redundancies and amplification loops in the coagulation cascade
- e.g. **factor XII deficiency** is subclinical
- e.g. **prekallikrein deficiency** may be subclinical or cause only mild haemorrhage
- e.g. **deficiency of factor VII** is usually either subclinical or causes only mild bleeding (e.g. increased bruising after trauma)

- **factor XI deficiency** (**haemophilia C**) usually causes only mild bleeding because factor X can be activated by the extrinsic system
- however, factor XI is necessary for sustained factor X activation; therefore, factor XI deficiency can cause massive bleeding following trauma (including surgical trauma) when sustained coagulation is required

Table 1 - Inherited Coagulopathies in Domestic Animals

Factor XIII	Toy poodle
Factor XII *(subclinical)*	Miniature poodle, standard poodle, German short-haired pointer, Shar pei Crossbred cats
Prekallikrein *(subclinical or mild haemorrhage)*	Poodle Belgian horse
Factor XI (haemophilia C) *(mild but severe haemorrhage if traumatised)*	English springer spaniel, Great Pyrenees, Kerry blue terrier Holstein cow
Factor IX (haemophilia B) *(variable but often severe haemorrhage)*	Saint Bernard, Shetland sheepdog, English sheepdog, French bulldog, Scottish terrier, Alaskan malamute, cocker spaniel, cairn terrier, black-and-tan coonhound Domestic shorthair cat, British shorthair cat, Siamese crossbred cats
Factor VIII (haemophilia A) *(variable but often severe haemorrhage)*	German shepherd (especially), beagle, chihuahua, English bulldog, greyhound, Irish setter, Labrador retriever, miniature poodle, Samoyed, schnauzer, Shetland sheepdog, Saint Bernard, Viszla, Weimaraner Domestic shorthair cat Arabian, Standardbred, Thoroughbred horse
Factor VII *(subclinical to mild haemorrhage)*	Beagle, Alaskan malamute, miniature schnauzer, boxer dog, bulldog, crossbred dogs
Factor X *(severe haemorrhage)*	American cocker spaniel, Jack Russell terrier, crossbred dogs
Factor II *(mild to severe haemorrhage)*	Boxer, cocker spaniel, miniature pinscher, otterhound
Factor I *(mild to severe haemorrhage)*	Saint Bernard, Bernese Mountain dog, Durrbach, Russian wolfhound (borzoi), Vizsla, collie, Lhasa Apso Saanen goat
Factors II, VII, IX and X (due to defective γ-glutamyl carboxylase) *(severe haemorrhage in neonates)*	Devon rex cat Labrador retriever Rambouillet sheep

- the **most severe haemorrhage** is associated with inherited deficiencies of **factor I, II** or **X** of the common pathway (all of these are rare), **factor VIII** or **IX** of the intrinsic pathway, or **combined factor deficiencies**

- **haemophilia A** (**inherited factor VIII deficiency**) is the **most common inherited coagulopathy in domestic animals and humans** due to the high rate of spontaneous mutation of the factor VIII gene

- it is inherited as an **X-linked recessive disorder** and manifests almost exclusively in hemizygous **males** (usually in the first six months of post-natal life)
- rarely, homozygous females that are the progeny of an affected male and a carrier female are clinically affected
- heterozygous female carriers are asymptomatic but may have abnormal results in laboratory tests of haemostatic capacity
- approximately 50% of the offspring of carrier females mated to unaffected males will inherit a defective X chromosome (i.e. 50% of male offspring will be affected and 50% of female offspring will be carriers of the trait)
- haemophilia A has been eradicated in German shepherds in Australia by genetic selection

- **haemophilia B** (**factor IX deficiency**) is less common than haemophilia A and is also inherited as an **X-linked recessive disorder**

- most cats with inherited coagulation factor deficiencies do **not** bleed spontaneously but instead bleed excessively after a challenge to haemostasis (e.g. protracted intra- or post-operative haemorrhage)
- however, **Devon rex kittens** with **inherited mutation of the gene encoding γ-glutamyl carboxylase** and hence **deficiency of the vitamin K-dependent factors (factors II, VII, IX and X)** often bleed severely in the neonatal period
- the latter condition is inherited as an **autosomal recessive trait** and has been reported in the UK, USA and Australia
- heterozygous Devon rex cats are asymptomatic but have abnormal results of laboratory haemostatic tests
- the condition in cats can be treated with oral/parenteral vitamin K_1

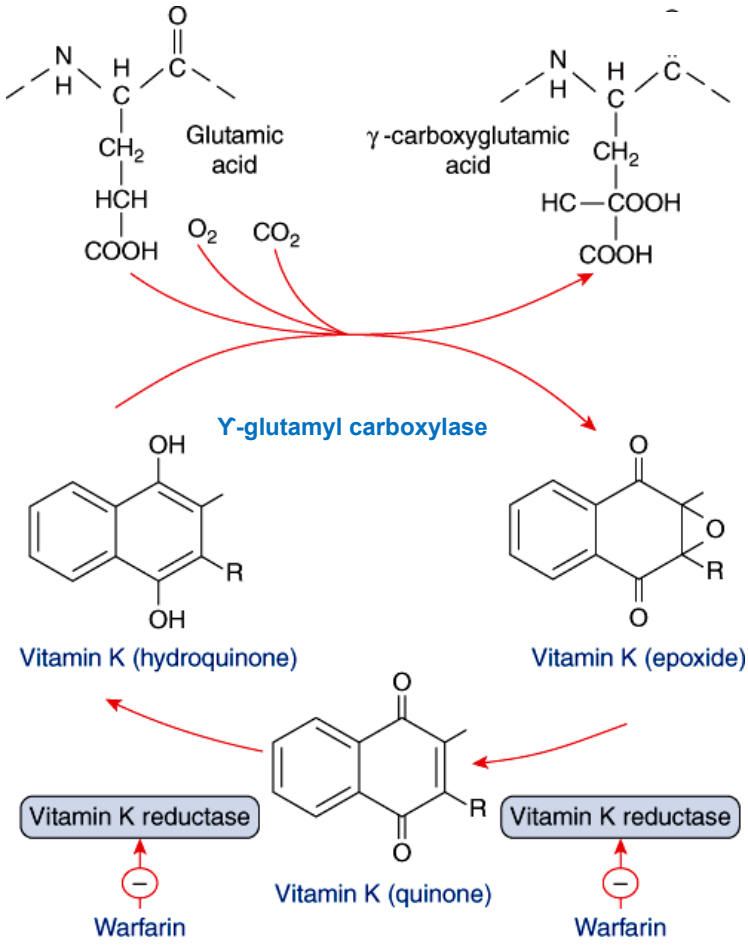
VITAMIN K ANTAGONISM OR DEFICIENCY

- **vitamin K** is required as a co-factor by the enzyme, **γ-glutamyl carboxylase**
- this hepatocellular enzyme is responsible for post-ribosomal carboxylation of glutamyl residues of the vitamin K-dependent coagulation factors (**factors II, VII, IX** and **X**) in order to render them functional in haemostasis

- during this carboxylation step, vitamin K is oxidised to its 2,3 epoxide form (**Figure 2**)
- it must then be converted back to its active reduced form (**hydroquinone**) via a two-step enzymatic process in order to participate again as a co-factor in carboxylation
- regeneration of reduced vitamin K from its oxidised form is catalysed by **reductase** enzymes
- these enzymes (particularly the epoxide reductase) can be antagonised by coumarins and related compounds

- if one or both reductase enzymes is/are antagonised or there is vitamin K deficiency, the liver produces proteins that are antigenically similar to factors II, VII, IX and X but, due to inadequate carboxylation, they have limited or no capacity to participate in the coagulation cascade
- these proteins are called **"proteins induced by vitamin K antagonism or absence"** (**PIVKA**)

Figure 2 – Role of Vitamin K in Carboxylation of Clotting Factors



Vitamin K Antagonism

- ingestion of the following can cause haemorrhage via **antagonism of vitamin K epoxide reductase**:

- **anticoagulant rodenticides** containing hydroxycoumarins or indandiones

- **mouldy sweet clover** (*Melilotus alba*) or **sweet vernal grass** (*Anthoxanthum odoratum)*
  - these plants naturally contain bishydroxycoumarin and the latter is converted to active dicoumarin when the plants spoil
  - **calves** are especially susceptible but adult cattle, pigs and rabbits may also be affected
  - sheep and horses are more resistant to poisoning

- **overdose of sulphaquinoxaline** (a coccidiostat) - reported in dogs
  - the drug potentiates the effect of anticoagulant rodenticides by suppressing intestinal microflora
    which are a potential source of vitamin K

- **overdose with therapeutic coumadins**
  - e.g. dogs ingesting warfarin medication prescribed to their human owners

Rodenticide Anticoagulant Poisoning

- **ingestion of anticoagulant rodenticides is a common cause of haemorrhage in cats and dogs** (and **rodents!**)

- first-generation anticoagulants (e.g. warfarin) generally cause poisoning after repeated ingestion
- the more potent second- and third-generation 4-hydroxycoumarin rodenticides (e.g. bromodialone, brodifacoum, difethalione) and indan-1,3-dione rodenticides (e.g. diphacinone) may induce life-threatening haemorrhage in cats and dogs after a single episode of ingestion and their longer half-life permits secondary intoxication of pets that consume poisoned rodents

- in mild cases of intoxication, vitamin K_1 administration may rapidly restore haemostatic capacity (within 12-48 hours of administration)
- in general, poisoning with first-generation antagonists requires vitamin K_1 therapy for one week
- 3-6 weeks of therapy may be needed for indandione or second- or third-generation coumarin compounds
- if the toxin identity is unknown, coagulation capacity is usually assessed after 7 days of therapy (24-48 hours after the last vitamin K_1 dose) to determine if ongoing therapy is needed and, if so, again after another two weeks of therapy

Vitamin K Deficiency

- **dietary vitamin K deficiency** is rare, with most reported cases being in **dogs**
- commercial animal diets usually contain excess vitamin K
- intestinal bacteria can also synthesise the vitamin and the liver stores several days' supply of vitamin K

- **prolonged anorexia** or **malnutrition** could cause or contribute to deficiency
- **oral antibiotic use** in cats and dogs can also eliminate intestinal bacteria that can synthesise vitamin K

- vitamin K is a **fat-soluble vitamin**
- rarely, animals with **chronic lipid maldigestion/malabsorption syndromes** may develop vitamin K deficiency, especially if there is concurrent oral antibiotic use
- e.g. **complete extrahepatic bile duct obstruction**
- e.g. **exocrine pancreatic insufficiency**
- e.g. **intestinal malabsorption**

HEPATIC PARENCHYMAL DISEASE

- **most of the coagulation factors are synthesised by hepatocytes** (some factors can be synthesised by vascular endothelial cells or macrophages; multiple cell types can synthesise factor III)

- significant decreases in coagulation factor activity can be experimentally induced in dogs by surgical removal of 70% of the liver
- **bleeding due to inadequate synthesis of coagulation factors** (potentially compounded by inadequate hepatocellular clearance of circulating anticoagulant factors) can be a feature of

severe acute hepatopathies or **chronic hepatopathies** in which the functional mass of hepatocytes has been reduced to ≤ 30%

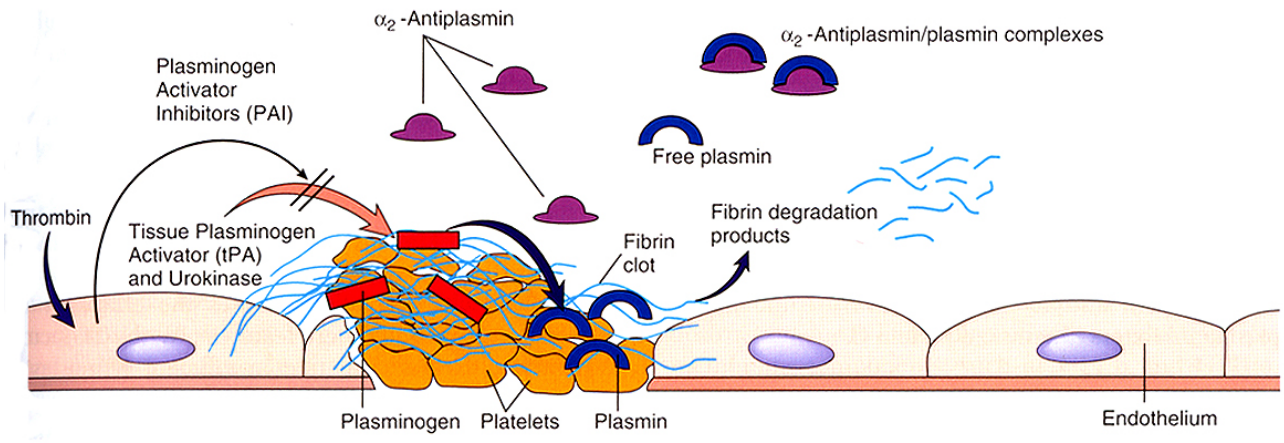
- surprisingly, most animals with hepatic disease of this severity do not bleed spontaneously
- instead, they are at risk of uncontrollable haemorrhage if haemostatic capacity is challenged (e.g. if a liver biopsy is undertaken!)
- therefore, **laboratory tests of haemostatic capacity are mandatory if subjecting an animal with known hepatic disease to liver biopsy or other invasive surgery**
- laboratory tests of haemostatic capacity can also be of use in assessing the severity of acute hepatic disease

- necrosis of hepatocytes can → damage endothelial cells lining the hepatic sinusoids → platelet consumption in haemostasis → **thrombocytopenia**
- chronic hepatic disease can also → **platelet dysfunction** (**thrombocytopathy**)
- thrombocytopenia and/or platelet dysfunction may contribute to the bleeding tendency in animals with hepatic disease, but deficiency of coagulation factors is more serious

- **hepatocytes also synthesise anticoagulants** (e.g. antithrombin, α_2-macroglobulin, α_1-protease inhibitor, protein C and protein S), **fibrinolytic agents** (e.g. plasminogen) and **fibrinolytic inhibitors** (e.g. α_2-antiplasmin) (**Figure 3**)
- the liver is also responsible for clearance from the circulation of many of the activated products of coagulation and fibrinolysis
- e.g. activated clotting factors are removed by hepatocytes whilst fibrin degradation products (FDP) generated by fibrinolysis are removed by hepatic Kupffer cells (and other tissue macrophages) (**Figure 3**)
- because of these various hepatic functions, animals with significant hepatic disease can be not only at risk of haemorrhage but also **at risk of thrombosis**

- severe hepatic necrosis can also trigger **disseminated intravascular coagulation** that commences with thrombosis in the microcirculation and may ultimately lead to haemorrhage (Lecture 29)

Figure 3 – Fibrinolysis



Reference: "Robbins Pathologic Basis of Disease" – R.S. Cotran, V. Kumar, T. Collins. 6th edition, W.B. Saunders Company, Philadelphia, 1999

EXCESSIVE FIBRINOLYSIS OR FIBRINOGENOLYSIS

- **fibrinolysis** (**enzymatic lysis of fibrin by plasmin**) (**Figure 3**) commences almost simultaneously with the onset of blood coagulation and is usually localised to the site of coagulation
- after haemorrhage has been controlled by formation of a secondary haemostatic (fibrin) plug, fibrinolysis contributes to restoration of vessel patency

- like coagulation, fibrinolysis involves a complex interaction amongst inactive proenzymes (e.g. plasminogen), activated enzymes (e.g. plasmin), activators (e.g. tissue-type plasminogen activators, t-PA), inhibitors (e.g. plasminogen activator inhibitors, PAI) and inactivators (e.g. α_2-antiplasmin)

- **excessive fibrinolysis** → premature breakdown of secondary haemostatic fibrin plugs → **haemorrhage**
- **fibrin degradation products** (**FDP**) generated by fibrinolysis also have anticoagulant properties (see Lecture 15) → risk of haemorrhage

- excessive fibrinolysis is **rarely recognised as a cause of bleeding in domestic animals other than in the context of disseminated intravascular coagulation** (Lecture 29)

- plasmin can also enzymatically degrade **fibrinogen** (**fibrinogenolysis**) → **fibrinogen degradation products** (also termed FDP)
- **excessive fibrinogenolysis** → inability to generate fibrin when needed → **haemorrhage**

- **excessive fibrinogenolysis is poorly documented in domestic animals** but can occur in the following circumstances:

  - snake envenomation - e.g. Eastern and Western diamondback rattlesnakes
  - administration of plasminogen activators - e.g. t-PA, streptokinase
  - excessive endothelial release of t-PA - e.g. shock, heat stroke, severe tissue trauma