The haemostatic system: do you really know enough about it?

Louise O'Dwyer MBA BSc(Hons) VTS(ECC)
DipAVN(Medical & Surgical) RVN
PetMedics Veterinary Hospital, Manchester M28 2LY, UK

Louise O'Dwyer MBA BSc(Hons) VTS(ECC)
DipAVN(Medical & Surgical) RVN

Louise gained her Diploma in Advanced Veterinary Nursing (Surgical) in 2004 followed by her Diploma in Advanced Veterinary Nursing (Medical) in 2007 and Veterinary Technician Specialist (Emergency and Critical Care) in 2011. She has contributed to more than 20 journal articles and books, and lectures regularly on all aspects of anaesthesia, emergency and critical care and infection control. Louise joined PetMedics Veterinary Hospital, the largest emergency clinic in the UK, in 2000, progressing to head nurse and now to the post of Clinical Director. Louise is the co-author of *Practical Emergency and Critical Care Veterinary Nursing* as well as *Wound Management in Small Animals: A Practical Guide for Veterinary Nurses and Technicians*, the BSAVA *Pocketbook for Nurses* and the forthcoming *A Veterinary Nurse's Guide to Infection Prevention and Control* (due 2015). She is currently working towards the VTS (Anaesthesia) – her final exam in Indianapolis is in September 2014 – as well as studying part-time towards a PhD in Antimicrobial Resistance in Companion Animals.

Louise holds a position on the European Veterinary Emergency and Critical Care Society's membership development committee and will take over as vice-chair in June 2014 at the EVECCS Congress in Prague; she is also on the British Small Animal Veterinary Association Congress committee. Louise was recently nominated for the prestigious Royal College of Veterinary Surgeons Golden Jubilee Award in Veterinary Nursing.

To cite this article use either
DOI: 10.1111/vnj.12159 or Veterinary Nursing Journal VOL 29 pp246–249

Introduction

Within the haemostatic system, complex processes are at play. These act to maintain the blood in a liquid state within the intact vascular system, while at the same time allowing clot formation in areas where damage, through both injury and natural 'wear and tear', has occurred to the vascular endothelium. Multiple mechanisms are involved to both promote and inhibit blood coagulation in order to maintain the balance. A further role of haemostasis is the limitation of any coagulation to the site of injury. This means that the haemostatic system requires additional functionality in order to break down clots and therefore restore normal blood flow to the state which existed prior to vascular injury.

Primary haemostasis

Primary haemostasis is triggered when damage to small blood vessels or capillaries occurs. The immediate response to this damage is the promotion of vasoconstriction, a process that in itself can diminish or decrease blood loss, due to slowing of blood in the vessels; it also allows more intimate contact of the blood cells and platelets with the vessel wall. Vasoconstriction (a reflexive response) is neurohormonal, and is localised to reduce the area for the clot to form.

When damage occurs to the blood vessel or capillary, its endothelial lining is exposed, which results in collagen (a supportive protein) also being exposed. This provides a surface for platelet adhesion. The adhesion process is mediated by von Willebrand Factor (vWF), a large glycoprotein produced in the endothelium, megakaryocytes and the subendothelial connective tissue. Its primary function is to bind to other proteins, particularly Factor VIII. vWF plays a dual role in haemostasis: it helps platelets to plug the injured blood vessel walls and is also a carrier for Factor VIII, which is vital in the clotting cascade. In the absence of vWF, blood coagulation and the formation of the platelet plug could be abnormal. This would affect both primary and secondary haemostasis. Primary haemostasis can be described as the formation of the platelet plug.

When platelets come into contact with the collagen fibres in the vascular wall or damaged endothelial cells, they begin to swell and take on irregular forms. These irregular forms have many radiating (star-shaped) projections, which protrude from their surfaces (pseudopods) (Mischke, 2012). The contractile proteins forcibly contract causing the release of granules that contain multiple active factors. They become sticky and adhere to the collagen fibres, forming a plug.

Platelets

Platelets are small, anucleate cells which are produced by megakaryocytes in the bone marrow and pulmonary vasculature. In the absence of vessel injury, platelets will remain in the circulation as discoid cells and have minimal interaction with other blood components or with the vessel wall. When stimulated, platelets function as the primary defence mechanism against bleeding.

The function of platelets is to maintain capillary integrity, and they do this by plugging the spaces between the endothelial cells of the capillary bed, initiating the process of coagulation, and retracting clots. They circulate in the bloodstream for around 5–9 days before being removed from the circulation by the spleen and liver (Garcia & South-Bodiford, 2012).

The pseudopods on platelet surfaces contain granules which have an excretory ability. Two of the proteins excreted during degranulation are histamine and serotonin. As platelets adhere to the damaged area of the vessel, degranulation occurs. The combined action of the platelets' ability to adhere to the site of injury and to each other, and the degranulation process, which produces ADP and thromboxane A2 in a normal patient, ensures the formation of a platelet plug (see below).

Normal platelet counts for canine and feline patients fall within the range of 200,000 to 500,000 per microlitre. Electronic counters will underestimate the platelet count if platelets are very large (which occurs in some cats and dogs, such as Cavalier King Charles Spaniels) (**Figures 1 and 2**), or if there are clumps, which is more commonly seen in cats (Waddell & Plumber, 2014). Cats also tend to have larger platelets that are more variable in size than other species.

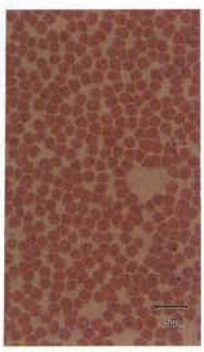

Figure 1. Normal platelet count and size on a blood smear

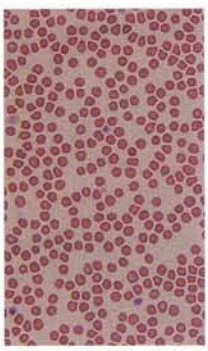

Figure 2. Large platelets seen on a blood smear taken from a Cavalier King Charles Spaniel

Adenosine diphosphate

Adenosine diphosphate (ADP) functions as a platelet agonist. An agonist is simply a chemical that binds to a cell receptor and is responsible for triggering a response from that cell. ADP can/may allow platelets to change shape as well as allow aggregation. It is also responsible for the generation of thromboxane A2, which is another platelet agonist.

Thromboxane A2

Thromboxane A2 is the product of activated platelets. It has prothrombotic properties and is responsible for increasing platelet aggregation and also stimulates the activation of new platelets. Thromboxane is named for its role in clot formation (thrombosis).

Secondary haemostasis

Secondary haemostasis begins with the exposure of blood to the negatively charged surfaces of damaged endothelium (intrinsic coagulation system) or to extravascular tissues (extrinsic coagulation system). The common end point of both the intrinsic and extrinsic coagulation systems is the conversion of prothrombin to thrombin, which is the critical step in the formation of a fibrin clot (Garcia & South-Bodiford, 2012).

Traditional model of blood coagulation

The coagulation cascade consists of a series of transformations of inactive serine proteases into their active forms, with thrombin as the end product. Thrombin then acts on the soluble fibrinogen and converts it to fibrin. This transformation is known as secondary haemostasis. All reactions require an enzyme (the coagulation factor), a substrate and a cofactor. In normal haemostasis, the substrate consists of the platelet surface (phospholipid layer) and calcium acts as the cofactor by holding the components together. In this way, clot formation is limited to the area around activated platelets. The coagulation cascade is traditionally divided into the intrinsic, extrinsic and common pathways (**Figure 3**). The extrinsic pathway is the main initiator of the clotting process.

Circulating within the blood are inactive clotting factors. These are produced mainly in the liver. Factors have been named and identified with Roman numerals. Once the factor has become activated, the suffix 'a' is then added to it. For example, Factor II (prothrombin) becomes Factor IIa (thrombin) when it has been activated by prothrombinase, calcium and thromboplastin.

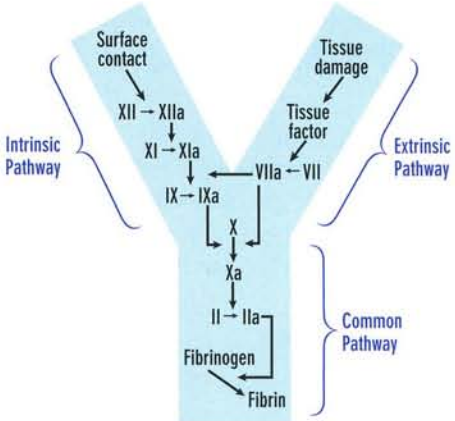

Figure 3. The coagulation cascade has traditionally been divided into intrinsic, extrinsic and common pathways (Good & Manning, 2003)

Secondary haemostasis is defined as the formation of fibrin through the coagulation cascade. This involves circulating coagulation factors (**Table 1**), which act as enzymes (which require activation) and cofactors (Factors V and VIII), calcium and platelets (Waddell & Plumber, 2014). Platelets provide a source of phospholipid (PF3) and a binding surface upon which the coagulation cascade proceeds.

The liver is responsible for synthesising the enzymes. The presence of Factor IV (calcium) is required for most coagulation factors to become effective. Vitamin K, which is synthesised in the intestinal tract by bacteria, is essential for functional synthesis of Factors II, VII, IX and X.

Formation of the clot is governed by the degree of trauma, typically beginning within 15–20 seconds in the case of severe vascular wall trauma, and within 1–2 minutes if the trauma has been minor. Approximately 3–6 minutes following the rupture of a vessel, the broken end of the vessel or the entire opening is filled with a clot (if the vessel opening is not too large). Within 20 minutes to an hour, retraction of the clot begins, further closing the vessel.

Table 1. Coagulation factors

Coagulation factor	Name
I	Fibrinogen
II	Prothrombin
III	Tissue factor
IV	Calcium
V	Proaccelerin
VI	*No factor VI*
VII	Proconvertin
VIII	Antihaemophilic
IX	Christmas factor
X	Stuart factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin-stabilising factor

The coagulation cascade

There are two separate pathways that activate the clotting cascade but these often take place at the same time. Both pathways eventually merge into the common pathway, resulting in the formation of stabilised fibrin.

The **extrinsic pathway** involves a protein called tissue factor (Factor III) that is found on various cells including those of the subendothelium. In damaged blood vessels, circulating Factor VII works with tissue factor (Factor III) and forms an active protease (VIIa) that eventually results in prothrombin being converted into the key active enzyme thrombin.

The **intrinsic pathway** is triggered when Factor XII (Hageman factor) comes into contact with collagen on the sub-endothelium of damaged blood vessels and is activated by the enzyme kallikrein to Factor XIIa. This ultimately also results in prothrombin being converted into the key active enzyme thrombin.

Coagulation factors

With the exception of calcium and thromboplastin, the coagulation factors (**Tables 1 and 2**) are proteins and can be divided into three families: the fibrinogen, prothrombin and contact families. The fibrinogen family includes fibrinogen and Factors V, VIII and XIII. The prothrombin family includes Factors II, VII, IX, X, protein C and protein S. The contact family of plasma coagulation proteins includes Factor XII, Factor XI (Fletcher factor or prekallikrein (PK)) and high molecular weight kininogen (HMWK). They are all involved in the mechanism that generates insoluble fibrin as a final product, via the coagulation cascade.

Disorders of secondary haemostasis frequently involve changes in the coagulation proteins which can involve a decreased level of a particular factor or a defect in the way the factor functions.

Extrinsic pathway

The primary role of the extrinsic pathway is to create an immediate response following vessel damage. After damage has occurred, Factor II (prothrombin) is released from the circulation and comes into contact with Factor III (tissue factor). Damaged tissue releases Factor III (thromboplastin), which, with the aid of calcium, will activate Factor VII. These form the activated complex of VII (VIIa) and this initiates the extrinsic mechanism.

Intrinsic pathway

The intrinsic pathway is the intrinsic factors that are found within the vascular space. Prekallikrein activates Factor XII. Prekallikrein is a pro-enzyme circulating in the plasma with high molecular weight kininogen (HMWK). Factor XIIa converts HMWK into bradykinin, an important mediator of vasodilatation, inflammation and fibrinolysis. Factor XII is a plasma protein that, when released from active

Table 2. Coagulation factors: how they are formed/manufactured and what they do (from Waddell & Plumber, 2014)

Coagulation factor	Formation and action
Fibrinogen (Factor I)	• a high molecular weight protein in the plasma • the liver produces most of the fibrinogen found in the circulating blood • converted into fibrin by the action of thrombin
Prothrombin (Factor II)	• formed by and stored in the liver; vitamin K is essential for the liver to be able to synthesise prothrombin • converted into its active form (thrombin – Factor IIa) by cleaving • prothrombin is split from a complex molecule into a simpler molecule
Thrombin (Factor IIa)	• an enzyme formed by prothrombin, calcium and thromboplastin in the plasma
Factor III (tissue factor or tissue thromboplastin)	• does not occur in the circulating blood • involved in the extrinsic pathway and activates Factor X
Factor IV (calcium)	• required for prothrombin activation (Factor IIa) and fibrin formation
Factor V (proaccelerin)	• present in plasma but not in serum • involved in both the intrinsic and extrinsic pathways • accelerates the conversion of prothrombin (Factor II) to the active thrombin (Factor IIa)
Factor VI	• no longer considered to be a separate factor as it is now realised to be an activated form of Factor V (Factor Va)
Factor VII (proconvertin)	• found to be present in both serum and plasma • participates in the extrinsic pathway • acts with Factor III (thromboplastin) in the presence of calcium to enable the conversion of Factor IX into IXa and Factor X into activated Factor Xa
Factor VIII (also known in human medicine as antihaemophilia factor (AHF))	• in the presence of calcium and phospholipids, is a cofactor for Factor IXa • part of the complex which converts Factor X into Xa
Factor IX (Christmas factor)	• involved in the intrinsic pathway • activates Factor X • deficiency results in haemophilia B
Factor X (Stuart factor)	• involved in both intrinsic and extrinsic pathways; it is this union that begins the common pathway • once activated, complexes with calcium, phospholipid and Factor Va to form prothrombinase, which splits and activates prothrombin to thrombin
Factor XI (Plasma thromboplastin antecedent)	• part of the intrinsic pathway • when activated by Factor XII, activates Factor IX thus accelerating thrombin formation
Factor XII (Hageman factor)	• initiates coagulation through the intrinsic pathway by activating Factor XI
Factor XIII (fibrin-stabilising factor)	• polymerises fibrin monomers thus enabling fibrin to form a firm blood clot • deficiency causes a clinical haemorrhagic diathesis

platelets, will activate Factor XI, thus initiating the intrinsic mechanism. Both Factor VIIa and Factor XIa will promote cascade reactions, eventually activating Factor X (Waddell & Plumber, 2014).

Active Factor X (Xa), along with Factor III, Factor V, calcium and platelet thromboplastic factor (PF3), will activate prothrombin activator. The intrinsic mechanism of prothrombin activator formation begins with trauma to the cellular components of blood, or exposure of blood to collagen in a traumatised vessel wall. This usually also results in damage to fragile platelets.

The formation of a clot by this mechanism usually takes 1–6 minutes, depending on the extent of damage. This cascade begins with the activation of Factor XII and the release of platelet Factor 3 (PF3) from damaged platelets. Activated Factor XII cleaves and activates Factor XI and prekallikrein (PK). Factor XII is also activated by activated prekallikrein (aPK) in an internal amplification loop. Calcium is required for the initial three steps. The prothrombin activator in the intrinsic pathway is very similar to the activator in the extrinsic pathway.

Fibrinolysis

The fibrinolytic component of the haemostatic mechanism is of paramount importance in the normal individual, but for many people it is the least understood aspect of haemostasis. Multiple mechanisms are available to limit the extent of clot formation. Negative feedback on thrombin partially limits the induction of clot formation, but mechanisms to promote clot breakdown (i.e. fibrinolysis) also exist.

The prothrombin activator converts prothrombin to thrombin. This is an enzymatic C cleavage, or splitting. Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated Factor X (Xa). The activity of Factor Xa is greatly enhanced by binding to Factor Va. Thrombin, acting as a serine protease, converts the soluble fibrinogen into insoluble strands of fibrin. Fibrin initially forms a loose mesh, but then Factor XIII causes the formation of covalent cross links, which convert fibrin to a dense aggregation of fibres. Platelets and red blood cells become caught in this fibre mesh, thus forming a blood clot.

Cell-based model of haemostasis

More recently, a cell-based model of haemostasis has been developed which will replace the classical model of the coagulation cascade. This model incorporates the role of cells and research has shown that haemostasis occurs on different cell surfaces in three overlapping steps: initiation, amplification and propagation. The first phase (initiation) occurs on a tissue factor (TF)-bearing cell. In the amplification phase, platelets and co-factors are activated in order to prepare for large-scale thrombin generation. Finally, propagation occurs on the surface of platelets and results in the propagation of large amounts of thrombin. For further details of the events that are involved in this mechanism, refer to Yagi (2013).

Conclusion

The haemostatic system is a complex one involving elements which control thrombosis, antithrombosis, fibrinolysis and antifibrinolysis. These processes interact with each other through complicated feedback systems to form and then break down clots where appropriate to maintain the intact vascular system. vnj

References

GARCIA, J., & SOUTH-BODIFORD, R. (2012). *Haematology in Small Animal Internal Medicine for Veterinary Technicians and Nurses*, pp. 161–192. Oxford. Wiley-Blackwell.

GOOD, L. I., & MANNING, A. M. (2003). *Thromboembolic Disease: Physiology of hemostasis and pathophysiology of thrombosis*. Compendium. Vol. **25**, No. 9, September 2003.

MISCHKE, R. (2012). Overview of haemostasis. In: DAY, M. J. & KOHN, B. Eds. *BSAVA Manual of Haematology and Transfusion Medicine*. 2nd Ed., pp. 182–188. Gloucester: British Small Animal Veterinary Association.

WADDELL, K., & PLUMBER, P. (2014). *When good coagulation goes bad – coagulopathies in the canine and feline*. VSPN online. February 2014.

YAGI, K. (2013). *Hemostatic Disorders*. IVECCS Proceedings 2013. San Diego. Veterinary Emergency and Critical Care Society.

NEWS REVIEW by Jean Turner

Elanco launches new ordering process for Recuvyra™

Elanco Companion Animal Health has launched a new ordering process for Recuvyra™, its transdermal fentanyl solution for the control of post-operative pain relief in dogs.

The new process will enable veterinary practices to purchase Recuvyra, which is a Schedule Two Controlled Drug, without having to complete the online training programme which has been mandatory since the launch of the product in April 2013. Training is, however, highly recommended for every veterinary professional who handles Recuvyra due to the innovative nature of the product delivery system. This can be via the short online training course available at www.recuvyratraining.eu or by reading the training leaflet provided with every vial of Recuvyra purchased.

Alice Laurens, Recuvyra product manager at Elanco Companion Animal Health, comments: 'Veterinary practices will still need to follow their wholesalers' procedures for ordering Schedule Two Controlled Drugs when purchasing Recuvyra, however any vet from the practice can now order and sign for delivery of the product, making it easier to purchase.'

Recuvyra 50 mg/ml transdermal solution for dogs is the first transdermal fentanyl solution to be licensed for the control of post-operative pain associated with major orthopaedic and soft tissue surgery.