PERTURBATIONS OF FLOW 2

THROMBOSIS

- **thrombosis = inappropriate formation of a blood clot (thrombus) within the cardiovascular system**

- thrombosis is a dynamic ante mortem process in which activation of haemostatic mechanisms results in the formation of a solid intra-vascular mass (**thrombus**, singular; **thrombi**, plural) derived from blood constituents
- thrombosis results from the same complex interactions amongst vascular endothelial cells, exposed subendothelial collagen, and circulating platelets and coagulation factors that are responsible for physiological blood clot formation (**haemostasis)** following injury to blood vessels

- an ante mortem physiological blood clot forms as an **appropriate** response to vessel wall injury, is designed to staunch the flow of blood from the injury site, and is largely mural (involving the damaged vessel wall) to extravascular in location
- a thrombus is **always attached to an area of damaged vessel wall** and **protrudes to a variable extent into the vessel lumen** and hence **compromises blood flow**

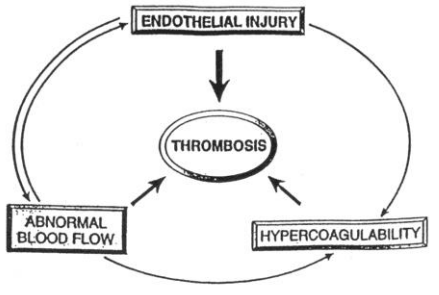
CAUSES OF THROMBOSIS

- three major mechanisms predispose to thrombosis:

  - **endothelial injury**
  - **abnormal haemodynamics** (**blood stasis** or **blood turbulence**)
  - **hypercoagulability of the blood**

- these factors are termed **Virchow's triad**, in honour of Dr Rudolf Virchow who first proposed the pathogenesis of thrombosis in 1845 (Figure 1)

Figure 1



Virchow's triad in thrombosis. Endothelial integrity is the single most important factor. Note that injury to endothelial cells can affect local blood flow and/or coagulability; abnormal blood flow (stasis or turbulence), in turn, can cause endothelial injury. The factors may act independently or may combine to cause thrombus formation.

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

Endothelial Injury

- **endothelial injury is the most important factor predisposing to thrombosis**
- **causes of endothelial injury** include: physical trauma, infectious agents (e.g. bacteria and endotheliotropic viruses), bacterial endotoxins or exotoxins, immune complex deposition, vasculitis, circulating toxins (e.g. uraemia), hypoxia/anoxia, blood turbulence etc

- endothelial cells modulate several aspects of normal haemostasis and possess both **procoagulant** and **anticoagulant properties**

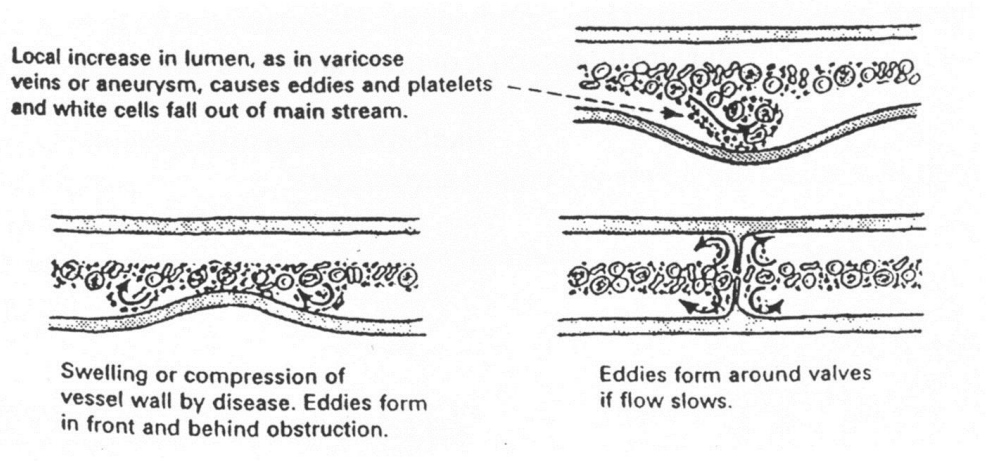
- **injured or activated endothelial cells adopt a procoagulant phenotype** that promotes local coagulation of blood and hence thrombosis
- e.g. injured endothelial cells release platelet activating factor and von Willebrand factor → local adhesion of platelets to exposed subendothelial collagen → subsequent platelet activation and aggregation
- e.g. injured or activated endothelial cells release or expose **tissue factor** (coagulation factor III) → activation of the extrinsic wing of the coagulation cascade → formation of fibrin
- e.g. injured endothelial cells can release plasminogen activator inhibitors → suppression of fibrinolysis (enzymatic lysis of fibrin by plasmin)

Abnormal Haemodynamics

- blood flow in arteries and veins is normally laminar (stratified)
- erythrocytes and leukocytes are in the centre of the stream, with platelets peripheral to them but separated from the vascular endothelium by a slower moving, narrow stream of plasma
- laminar flow of blood can be disrupted by either blood turbulence or blood stasis

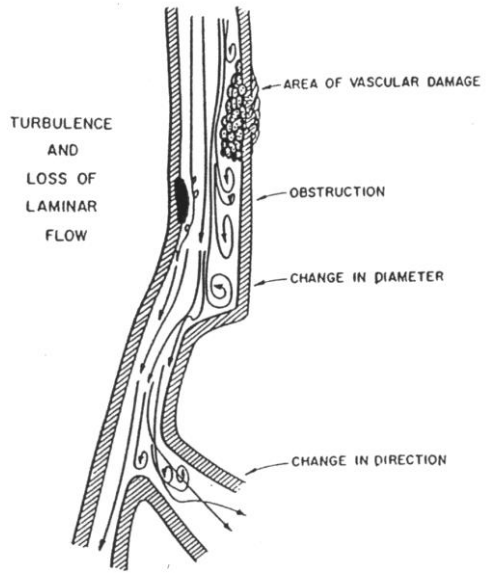
- there is a natural tendency for **blood turbulence** to develop in areas of hydraulic stress (e.g. sharp bends, changes in vessel lumen diameter, valves and branching points) (Figures 2 and 3)

Figure 2



Reference: "Pathology Illustrated" – A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh, 1995

Figure 3



Reference: "Mechanisms of Disease – A Textbook of Comparative General Pathology" – D.O. Slauson and B.J. Cooper, 2nd edition, Williams and Wilkins, Baltimore, 1990

- blood turbulence may also develop at sites of abnormal vessel wall dilation (arterial aneurysms and venous varicosities) or stenosis, with congenital heart malformations, and with acquired vessel wall thickenings such as arteriosclerosis and atherosclerosis

- blood turbulence → direct endothelial injury and formation of blood counter-currents and local pockets of blood stasis → increased likelihood of thrombosis
- blood turbulence also accelerates intravascular procoagulant cellular and enzymatic reactions that lead to blood coagulation

- conversely, **blood stasis** also promotes thrombosis, particularly within venous channels
- e.g. venous stasis in recumbent, immobile or anaesthetised patients and in pregnancy, shock and right-sided congestive heart failure
- decreased venous blood flow → increased blood viscosity → blood hypercoagulability
- decreased blood flow → hypoxic injury to endothelial cells → procoagulant state

- both blood stasis and turbulence may also prevent the dilution and removal of activated coagulation factors by fresh flowing blood, and retard the arrival of coagulation factor inhibitors

Blood Hypercoagulability

- an increased tendency of blood to clot intravascularly may be due to **increased procoagulant factors** or **decreased inhibitory factors** in the circulation

- e.g. oral contraceptives and pregnancy → increased hepatic synthesis of coagulation factors (e.g. factors I, VII, VIII and X) and decreased hepatic synthesis of antithrombin → blood hypercoagulability → increased risk of thrombosis
- e.g. disseminated malignancies, pancreatic necrosis, severe tissue trauma, severe burns and snake envenomation → release of tissue factor or tissue factor-like procoagulants into the

circulation → activation of the extrinsic wing of the coagulation cascade → increased risk of thrombosis
- e.g. severe protein-losing glomerulopathies → loss of antithrombin into urine + increased hepatic synthesis of clotting factors + increased platelet adhesion ("sticky platelets") due to hypoalbuminaemia → increased risk of thrombosis (as part of the nephrotic syndrome)
- e.g. cirrhosis → decreased hepatic synthesis of antithrombin → increased risk of thrombosis
- e.g. hyperglobulinaemia (e.g. multiple myeloma, feline infectious peritonitis), leukaemias (malignancies of haematopoietic cells with tumour cells in circulation) and polycythaemia/ erythrocytosis (increased erythrocyte count) → blood hyperviscosity → increased risk of thrombosis

THROMBUS FORMATION

- thrombosis involves activation of normal haemostatic mechanisms
- circulating **platelets adhere to exposed subendothelial collagen** (using collagen receptors +/- von Willebrand factor) and become **activated**, releasing molecules (e.g. ADP and thromboxane A_2) that recruit more platelets into the area
- the **platelets** then **aggregate** (bind to each other) via binding of soluble **fibrinogen**
- local activation of the coagulation cascade results in enzymatic conversion of the bound soluble fibrinogen molecules into an insoluble, cross-linked **fibrin polymer** that converts the aggregated platelets into a coagulum that is anchored at the point of vascular endothelial injury

- the resulting thrombus may protrude only partially into the vessel lumen (a **mural** or **non-occlusive thrombus**) or it may completely obstruct the lumen (an **occlusive thrombus**)

THROMBUS PROPAGATION AND THROMBOEMBOLISM

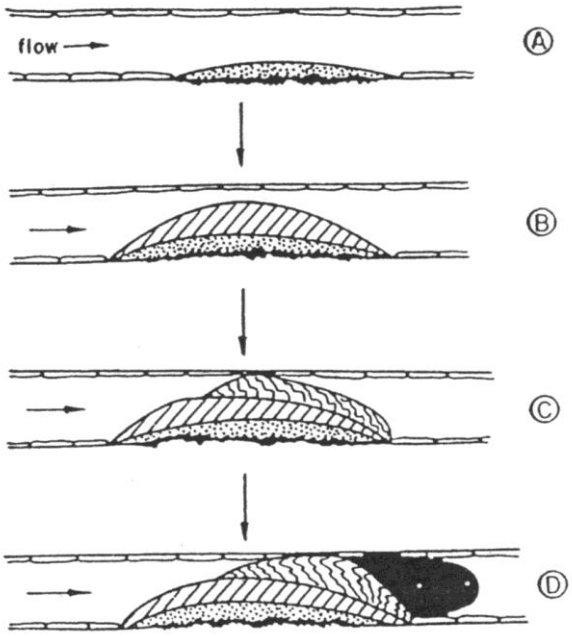
- continued flow of blood over a mural thrombus may permit its gradual enlargement (**propagation**) by allowing repeated layering of platelets and fibrin on its surface and entrapment of erythrocytes and leukocytes within it (Figure 4)
- release of mediators by entrapped platelets and leukocytes (especially neutrophils) may further damage the endothelium, as may also the local blood turbulence and stasis caused by the disruption of laminar blood flow → propagation of the thrombus

- **episodic blood coagulation** results in gross and microscopic lamination (**lines of Zahn**) of propagating thrombi, with alternating pale grey-white bands of platelets and fibrin and dark red bands containing entrapped erythrocytes (and leukocytes)
- lines of Zahn are significant only in that they **indicate that the thrombus was initially non-occlusive with continued blood flow over it**
- **laminations are most often seen in arterial or intra-cardiac thrombi** but may be observed in large venous thrombi (e.g. in the jugular veins)

- propagation results in elongation of mural thrombi, usually in the direction of blood flow but potentially also in the retrograde direction (Figure 4)
- over time, propagating mural thrombi may become **occlusive** (Figure 4)
- the protruding **head** and **surface** of a mural thrombus tends to receive new fibrin and platelet components because of its continued exposure to flowing blood

- the **tail** of the thrombus tends to accumulate whole clotted blood because of stagnant and turbulent blood flow; the tail of the thrombus therefore tends to be dark red

Figure 4



Thrombus Propagation
A thrombus grows by layering, particularly where blood flow is rapid. Endothelial damage leads to deposition of a layer of platelets (A), which is followed by the deposition of fibrin (B), and later by further fibrin deposition (C). A tail of clotted whole blood (D) may become attached in the area immediately downstream from the thrombus.

Reference: "Mechanisms of Disease – A Textbook of Comparative General Pathology" – D.O. Slauson and B.J. Cooper, 2nd edition, Williams and Wilkins, Baltimore, 1990

- not all of the thrombus may be attached to the vessel wall
- the downstream segment may be suspended in the vessel lumen as a floating tail; segments of the tail may break off as **thromboemboli (= fragments dislodged from an upstream thrombus that travel downstream in the blood to become trapped in distant vessels of smaller calibre)**

GROSS APPEARANCE OF THROMBI

- thrombi can develop anywhere in the cardiovascular system and are **always attached** to the underlying vessel wall or endocardium
- the gross shape, size, colour and consistency of thrombi are influenced by their location, their age, and whether or not they are infected (**septic thrombi**)
- the **rate of blood flow** (e.g. rapid flow in arterial vessels and within the heart versus sluggish flow in venous vessels) is one of the most important factors influencing the gross appearance of a thrombus

Intra-cardiac and Arterial Thrombi

- intra-cardiac and arterial thrombi usually develop at sites of **endothelial injury** or **blood turbulence**
- intra-cardiac thrombi **most often involve the surfaces of heart valves** (**valvular thrombi**) but can involve the inner walls of heart chambers (**mural thrombi**)
- large thrombotic masses attached to heart valves are commonly referred to as **vegetations** (because they grossly resemble vegetation or shrubbery) and are usually **septic** and contain bacterial colonies and leukocytes (see **vegetative valvular endocarditis** in lecture - Diseases of the Endocardium)

- intra-cardiac and arterial thrombi are usually firm and pale yellow (**pale** or **white thrombi**) and have a dull, dry, rough surface because they are chiefly composed of **platelets and fibrin**
- the high velocity and pressure of arterial and intra-cardiac blood flow sweep leukocytes and erythrocytes away, leaving only the tenacious platelets and fibrin

- because large areas of endothelium need to be damaged before thrombosis will occur in large arterial vessels or within the heart, arterial and intra-cardiac thrombi are usually **firmly anchored over a broad zone** to the underlying vessel wall, valve or heart chamber wall
- because of the fast and high pressure blood flow, such thrombi are also initially **mural** and **non-occlusive**

Venous Thrombi

- thrombosis of venous channels (**phlebothrombosis**) usually develops at sites of **blood stasis** (or, to a lesser extent, **turbulence**), especially near valves or within a venous plexus

- blood flow within the venous system is usually slow, especially in the larger distal veins of the limbs in which venous return to the right heart is opposed by gravity (venous blood flow in the distal extremities can virtually stop during periods of inactivity due to loss of the normal milking action of skeletal muscles)
- the slower blood flow allows thrombosis to develop **rapidly** within veins, with complete activation of the coagulation cascade and entrapment of red cells and leukocytes

- because of rapid development, venous thrombi are almost invariably **occlusive**
- they tend to form an almost perfect cast of the vessel and have a smooth, shiny surface
- venous thrombi are moister than arterial thrombi because they contain plasma fluid and are **dark red** (**red** or **stasis thrombi**) because of entrapped erythrocytes
- laminations are usually poorly defined or absent

- because they form in a slow flow area, venous thrombi do not usually generate strong points of attachment to the vessel wall; the **attachment points are usually fragile**
- venous thrombi also tend to be much **larger** than arterial thrombi, with **longer tails** (e.g. a thrombus originating in a branch of the femoral vein may grow into the femoral vein itself and thence into the iliac vein and even into the caudal vena cava) (Figure 5)
- for these reasons, **venous thrombi are more prone than arterial thrombi to generate thromboemboli**

- propagation is a major mechanism underlying thromboembolism because the unattached and growing tail of a venous thrombus is especially susceptible to fragmentation; as the advancing tail reaches a vessel bifurcation, rapidly flowing blood sweeps past the thrombus to cause

dislodgement of fragments

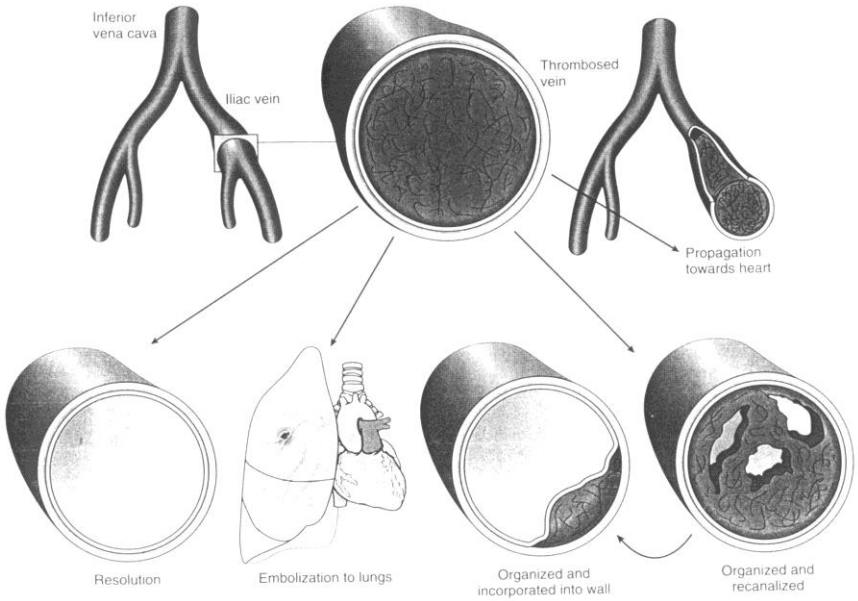
- ante mortem venous thrombi can be distinguished from **post mortem "red currant" blood clots** by detection of the (albeit weak) point of anchorage to the vessel wall, by detection of fine tangled strands of pale grey-yellow fibrin on their cut surface grossly, and especially by detection of evidence of congestion and oedema of tissues upstream

- the differences between arterial and venous thrombi are summarised in Table 1

Table 1 – Arterial/Intra-cardiac versus Venous Thrombi

Arterial and Intra-cardiac Thrombi	*Venous Thrombi*
- largely initiated by endothelial injury or blood turbulence	- largely initiated by blood stasis
- usually mural and initially non-occlusive	- usually occlusive
- slow propagation	- rapid propagation
- usually firm, dry and rough-surfaced	- usually fragile, smooth-surfaced and moist
- usually firmly anchored	- usually weakly anchored
- usually pale with prominent laminations	- usually dark red with faint fibrin strands but absent or indistinct laminations
- may give rise to thromboemboli	- usually larger than arterial thrombi, with longer tails and a greater propensity to generate thromboemboli

Figure 5



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

Septic Thrombi

- septic thrombi are infected with bacteria or, less often, fungi
- the infection may be present from the outset or develop secondarily in a pre-existent **bland** (**sterile**) thrombus
- septic thrombi tend to be softer and more friable than bland thrombi and are therefore more prone to generate thromboemboli
- infected thrombi may be heterogeneous in colour and texture and contain pockets of grossly obvious exudate (often cream-yellow due to large numbers of neutrophils)

RESOLUTION OF THROMBI

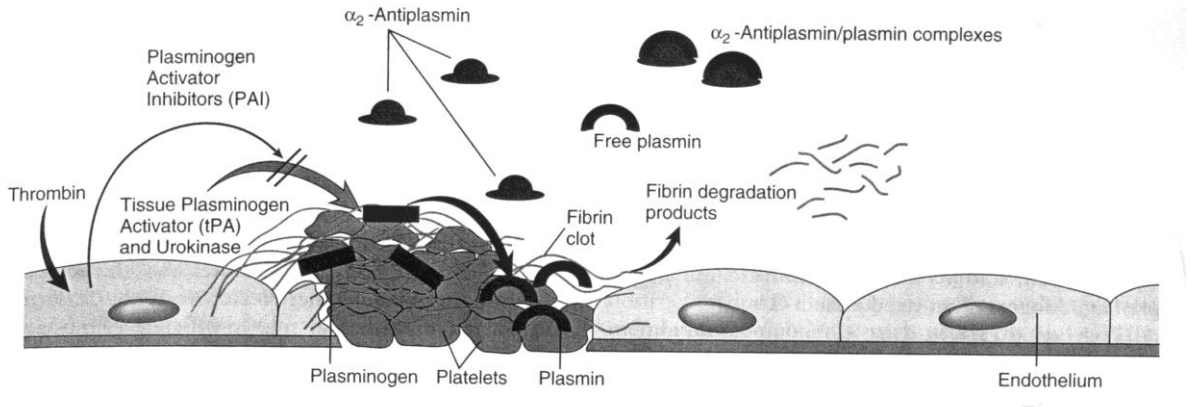
- if an animal survives the immediate effects of thrombosis, the thrombus may undergo **dissolution by fibrinolysis** and/or **organisation** (Figure 5)

Fibrinolysis

- **fibrinolysis = enzymatic breakdown of fibrin**

- fibrinolysis is effected by **plasmin** (Figure 6)
- **plasminogen** (the inactive precursor of plasmin) is produced by hepatocytes and circulates in health as a plasma protein
- plasminogen is activated to plasmin by proteolytic **plasminogen activators** (e.g. intrinsic activators such as kallikrein or activated coagulation factors XIIa or XIa; e.g. extrinsic activators such as **tissue-type plasminogen activator** (t-PA) derived from endothelial cells)

Figure 6



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- plasmin enzymatically digests fibrin into **fibrin degradation products** which have weak anticoagulant activity

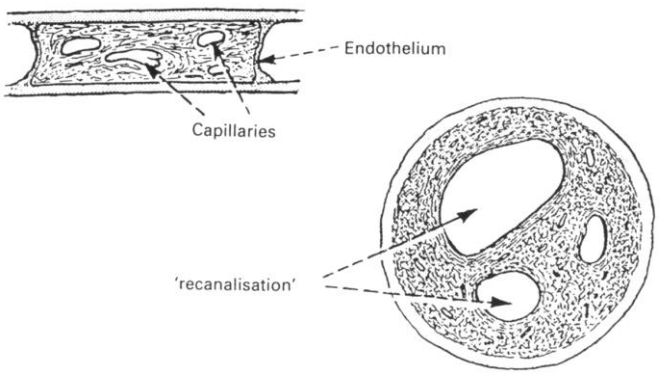
- fibrinolysis commences as soon as fibrin is generated
- activation of plasmin can allow rapid shrinkage and even total enzymatic lysis of **recently formed** thrombi

- in older thrombi, extensive fibrin polymerisation and cross-linking render the thrombus more resistant to proteolysis so that fibrinolysis is largely ineffective
- therefore, therapeutic infusions of fibrinolytic agents such as t-PA are only likely to be effective for a short time after thrombus formation

Organisation of Thrombi

- older thrombi not removed by fibrinolysis tend to become organised by phagocytosis by leukocytes (especially macrophages) and by ingrowth of endothelial cells and vascular smooth muscle cells and fibroblasts
- viable endothelial cells from the margins of the site of thrombus attachment begin to grow over the surface of the thrombus, ultimately insulating it from circulating blood and thereby reducing the risk of further propagation
- simultaneously, endothelial cells grow into the thrombus and form capillaries that provide nutrients for the infiltrating leukocytes that will phagocytose and enzymatically degrade the thrombus over time
- capillaries growing into an occlusive thrombus from either end may eventually anastomose and allow longitudinal **recanalisation** to restore some blood flow through the mass (see Figure 7)

Figure 7



Reference: "Pathology Illustrated" – A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh, 1995

- fibroblasts and vascular smooth muscle cells invade the thrombus and produce collagen which contracts as it matures
- over time, the organised thrombus is converted into a subendothelial fibrous scar (Figure 5)
- although the scar may cause focal narrowing of the vessel, adequate blood flow may be restored