HAEMATOLOGY

(a) ***To explain the origin and importance of blood groups.***

(b) ***To outline the constituents and functions of plasma.***

(c) ***To describe platelets and their role in coagulation.***

(d) ***To describe the intrinsic and extrinsic coagulation pathways.***

(e) ***To describe the normal mechanisms of preventing thrombosis including endothelial factors and natural anticoagulants.***

(f) ***To describe fibrinolysis and its regulation.***

(g) ***To outline the methods for assessing coagulation, platelet function and fibrinolysis.***

(h) ***To explain the physiological consequences of acute and chronic anaemia.***

(i) ***To outline the production of blood constituents including red blood cells, haemoglobin, and plasma proteins.***

(j) ***To outline the constituents of blood products, their source, role and risks.***

(k) ***To describe the changes during blood storage and the problems of massive blood transfusion and their management.***

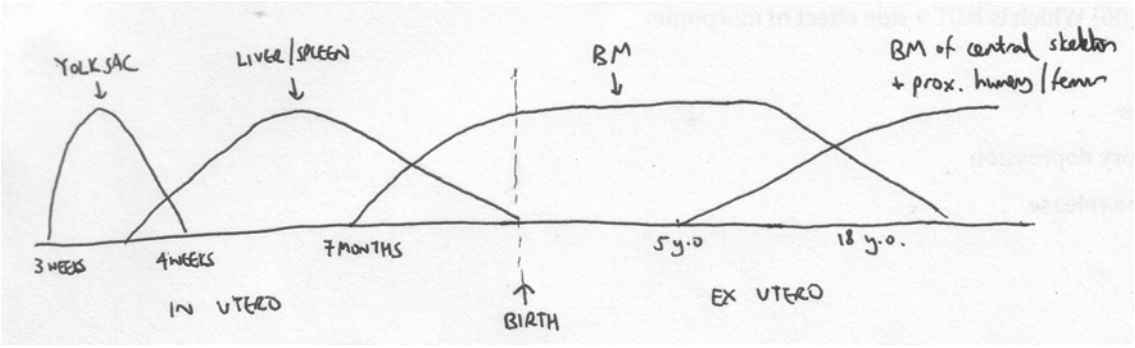
(l) ***To describe the breakdown of haemoglobin.***

(m) ***To describe abnormal haemoglobins and their clinical significance.***

(I) **Overview of Haemopoiesis**:

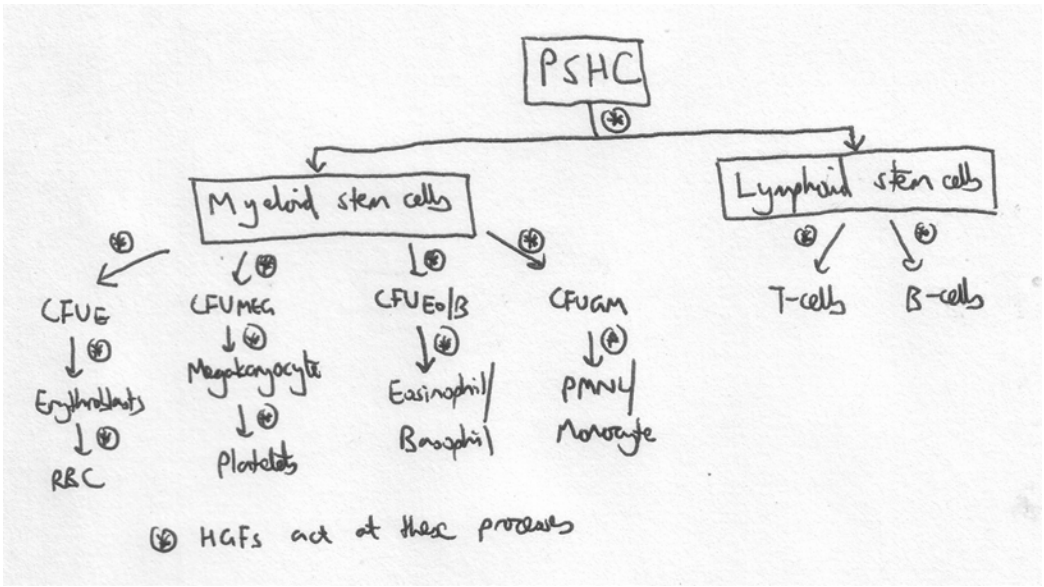
Sites of haemopoiesis:

- (i) Mesenchymal stem cells in yolk sac → 3-4 weeks of embryonic growth
- (ii) Liver (and spleen to lesser extent) → 6 weeks to 7 months of foetal life (and up to birth)
- (iii) Bone marrow → from 7 months of foetal life onwards
- (iv) Bone marrow of proximal ends of femur/humerus and central skeleton (vertebrae, pelvis, ribs, sternum and skull) → progressive fat replacement of long bone marrow occurs by age 18-20 y.o.



All blood cells originate from a common "pluripotential haemopoietic stem cell" (PHSC) found at the organ site of haemopoesis → produces two lineages, each associated with various stages of haemopoeitic precursor cells:

- (i) Myeloid stem cells → produce granulocytes (PMNL, basophils, eosinophils), erythrocytes (RBC), monocytes, megakaryocytes (platelets)
- (ii) Lymphoid stem cells → produce T- and B-lymphocytes



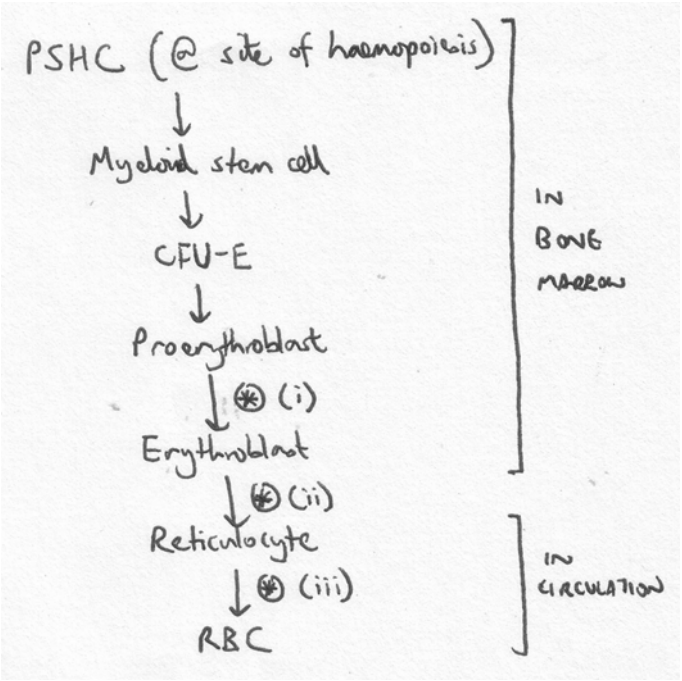
Haemopoeitic precursor cells are stimulated by "haemopoeitic growth factors" (Eg. IL-1, TNF, CSF, Etc.) → glycoprotein hormones synthesised by various cells (esp endothelial cells, stromal cells, T-cells, monocytes, macrophages; EXCEPT EPO which is synthesised by kidneys) that act on specific receptors on haemopoeitic precursor cells to regulate their production, differentiation and maturation

(II) **Red Blood Cells**:

Structure of RBC:

- Biconcave disc (7.5 um wide and 2 um thick) → large SA:volume ratio (allows ↑ gaseous diffusion in/out of cell) and easily deformable (allows it to traverse microvasculature)
- RBC membrane is a phospholipid bilayer that consists of various structural and contractile proteins, enzymes and surface Ag → contents are 50% protein, 40% fat, 10% CHO
- Along inner side of RBC membrane → lattice of proteins (esp spectrin, actin, ankyrin, band 4.1) that maintains biconcave shape of RBC
- Contains Hb within cytoplasm → has NO nucleus (nil DNA/RNA) or mitochondria

Formation of RBCs:



* Note:
  - (i) Proerythroblasts undergo series of cell division and maturation to form progressively smaller erythroblasts → contain more Hb and more condensed nuclear chromatin with each cycle
  - (ii) Pyknotic nucleus is extruded from late erythroblasts to form reticulocytes
  - (iii) Reticulocytes are released from BM into the circulation → they contain some RNA and continue to synthesise Hb → after 1-2 days, they eventually lose the RNA (and can no longer synthesise Hb) and become "mature" RBC)

Important to note – Final RBC maturation requires Vitamin B12 and Folic acid for DNA synthesis and nuclear maturation → otherwise large and fragile RBC are produced with ↓ t ½'s

Important to note – RBC formation is regulated by "Erythropoietin" (EPO):

- A glycopeptide hormone produced by interstitial cells of peritubular capillaries of the kidneys (90%) and also the liver (10%) → released as "pro-EPO" and forms an active 165 a.a. hormone once terminal Arg a.a. is removed in the circulation → t ½ 6-9 hrs
- Its is synthesised and released in response to renal tubular hypoxia → ↓ O_2 delivery due to (i) systemic hypoxaemia or (ii) anaemia
- It enhances erythropoiesis → it acts on immature erythroid cells in BM (by activating EPO surface receptor → tyrosine-kinase receptor that activates Tf's and RNA synthesis) to cause them to differentiate and proliferate into "mature" RBC → ↑ O_2 carrying capacity of blood

Haemoglobin (Hb):

Structure of Hb:

- Metalloprotein complex (MWT 65 kDa) with contains 4x subunits
- Each subunit contains:
  - (i) Haem group → iron (in ferrous or Fe^{2+} state) within protoporphyrin ring

- (ii) Globin → polypeptide chains

> Important to note – "Haem" is an iron-containing tetrapyrrole with 4 rings joined by methenyl bridges → found in several "Haemproteins" (which includes Hb, Mb, cytochrome, catalyses,)

Functions of Hb:

- (1) O_2 transport in blood → Hb can bind up to 4 O_2, with each subunit can bind O_2 reversibly ($Hb + O_2 \leftrightarrow HbO_2$)

> Important to note – Multimeric structure allows "cooperative binding" (↑ O_2 affinity with ↑ O_2 binding) → gives "sigmoidal" Hb O_2 dissoc'n curve:
> - Tense (T) – Deoxygenated Hb has ↓ O_2 affinity → b/c β-chains pulled apart which ↑ 2,3-DPG binding instead
> - Relaxed (R) – Oxygenated Hb has ↑ O_2 affinity → b/c β-chains pulled together which ↓ 2,3-DPG binding

- (2) CO_2 transport in blood → CO_2 bound to N-terminal of globin (as carbamino-Hb)
- (3) Buffering role in blood → Basic histadine moieties of globin buffer H^+
- (4) Iron storage → Iron in haem forms 65-70% of total iron stores

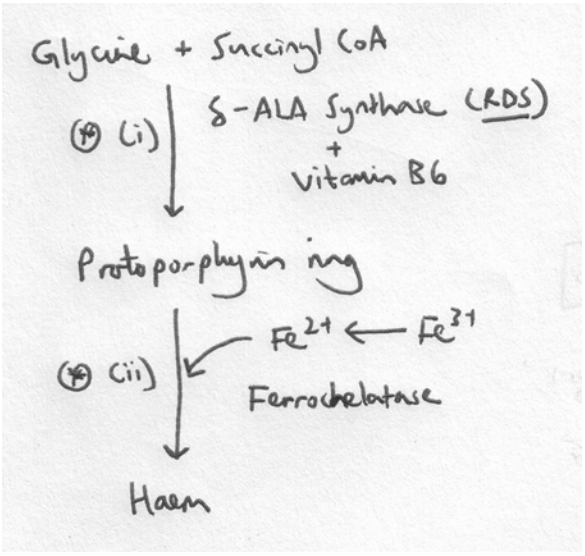
Variants of Hb → mainly characterised by the structure of the globin chains:

- HbA (adult Hb) is $\alpha_2\beta_2$ → 96-98% of adult Hb content
- HbA2 is $\alpha_2\delta_2$ → forms 1-3% of adult Hb content
- HbF (foetal Hb) is $\alpha_2\gamma_2$ → forms 1% of adult Hb content

> Others Hb variants to note:
> - Met-Hb → iron in haem is oxidised to Fe^{3+} (ferric) state
> - Hb-S → "Sickle-cell" variant due to abnormal a.a. substitution on β-chain

Hb production:

- Hb is formed by tetramerisation of 4x globin chains each combined with its a haem group:
  - (i) Haem synthesis → occurs in mitrochondria



* Note:

- (i) Protoporphyrin ring is formed by condensation of glycine + succinyl CoA using δ-aminolaevulinic acid synthetase (and pyridoxal phosphate (Vitamin B6) as a coenzyme) → rate-limiting step
- (ii) Protoporphyrin ring combines with Fe^{2+} to form haem (using Ferrochelatase)

> Aside – ALA synthase is controlled by –ve feedback (Ie. synthesis and activity of enzyme ↓ by haem) → Nb. its activity is ↑ by barbiturates in inducible (variegate) porphyria)

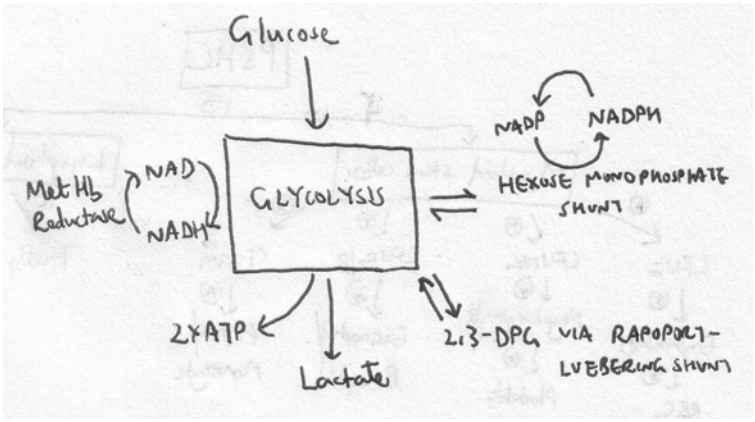
  - (ii) Globin synthesis → occurs in ribosomes

Hb destruction → See below in "RBC destruction"

<u>RBC metabolism</u>:

- (i) Energy is generated solely by anaerobic glycolysis (as it has NO mitochondria!) → produces 2x ATP + 1x lactate per glucose → ATP is used by Na^+/K^+ ATPase, which is implicated in maintaining RBC shape, volume and flexibility

- (ii) NADH is also generated by glycolytic pathway → used by "MetHb reductase" to reduce oxidised Hb (aka. MetHb) to Hb
- (iii) 2,3-DPG is interconverted from 1,3-DPG (glycolytic intermediate) via "Rapoport-Luebering shunt" → ↑ glycolysis = ↑ 2,3-DPG production, which ↓ Hb affinity for O_2 causing ↑ O_2 unloading to tissues
- (iv) NADPH is produced via the "Hexose monophosphate shunt" → maintains reduced glutathione store, which maintains an sulphydryl (-SH) groups intact in cells



<u>RBC destruction</u>:

- RBC remain in the circulation for 120 days → subsequently removed by phagocytosis in the "Reticulo-endothelial system" (mainly spleen, but also liver and BM) at a rate of 6 g Hb/day (or 0.8%/day)

> Note – With ageing, there is ↓ energy to maintain RBC cellular integrity (due to ↓ glycolytic capacity and ATP formation) → abnormal RBC passes through splenic sinuses and are removed from the circulation by macrophages via phagocytosis

- Within the RE system, it is broken down by macrophages as follows:
  - (1) Globin chains → broken to a.a. and re-enter a.a. pool
  - (2) Haem → metabolised by "Haem oxygenase" (microsomal enzyme) into:
    - (a) Biliverdin

> Biliverdin is then metabolised as follows:
> - (i) Reduced to bilirubin by "Biliverdin reductase" in the macrophage
> - (ii) Bilirubin (unconjugated or indirect) is extruded into plasma where it is bound to albumin (due to its low H_2O solubility)
> - (iii) It is taken up by hepatocytes via passive facilitated diffusion
> - (iv) Bilirubin is conjugated in the hepatocyte to UDP-glucuronic acid → produce bilirubin monoglucuronide, then bilirubin diglucuronide to ↑ its H_2O solubility
> - (v) Conjugated (or direct) bilirubin is actively secreted into bile
> - (vi) Intestinal bacteria converts bilirubin to stercobilinogen → some is reabsorbed into plasma (then excreted by kidney as urobilinogen) while rest is excreted in faeces as stercobilin

    - (b) Fe^{2+} → converted to Fe^{3+} and recycled to body's iron pool → then reutilised mainly for Hb synthesis
    - (c) C.O. (small amounts) → Nb. this is the ONLY source of endogenous C.O. production!
- Small amounts RBC (10%) breakdown prematurely in blood and release free Hb → binds to (i) Haptoglobin (α2-globulin), then (ii) Haemopexin (when haptoglobin saturated) → this prevents renal excretion of haem and loss of iron from the body!

<u>Red cell antigen and antibodies</u>:

- Red cell antigens → allow recognition of self from foreign cells by the immune system
    - "Blood groups" (ABO and Rh) are the most important Ag's b/c they are highly antigenic → individuals that lack a certain blood group produce Ab's against the Ag of that blood group, such that a transfusion reaction will occur if RBC of that blood group is given
    - There are > 400 other types of antigens (Eg. P, Lewis, MN, Kell) → less important role as they are weak and Ab's develop only after multiple exposures
- Red cell antibodies → there are two types:
    - (i) Naturally-occurring Ab are present in plasma of individuals who lack red cell Ag of a certain blood group → Ab are IgM only, optimally reactive at 4°C, and develop in the ABO system only (Eg. anti-A or anti-B Ab) after 3 months of age due to presence of Ag in bacteria/food that resemble A- and B-antigens
    - (ii) Ab can also develop upon exposure to RBC with Ag of a blood group that the individual lacks (Ie. via transfusion or transplacental passage during pregnancy) → Ab are IgG (and IgM initially), optimally reactive at 37 °C, and develop in the Rh system only (Eg. anti-D Ab)

> Important to note → IgG can pass transplacentally from mother to foetus → this has major significance with "erythroblastosis foetalis", where anti-D IgG passes from mother to have Rh-D +ve baby

(III) **Effects of Anaemia**:

"Anaemia" → a condition (acute or chronic) a/w deficiency of RBC or Hb in blood (Eg. due to haemolysis, chronic disease, Fe-deficiency, Vitamin B12/folate deficiency, haemorrhage, Etc.)

Consequence of ↓ Hb content of blood is ↓ O_2 content of blood (CaO_2), and thus ↓ O_2 delivery to tissues (DO_2) → risk of tissue hypoxia (Eg. organ ischaemia (esp heart and brain), ↑ lactic acidosis 2° to ↑ anaerobic metabolism, Etc.):

$$DO_2 = (C.O.) \times (CaO_2)$$

$$CaO_2 = ([Hb] \times 1.34 \times SaO_2) \times (0.003 \times PaO_2)$$

There are 4 compensatory mechanisms that maintain tissue oxygenation during anaemia:

- (1) ↑ C.O. to maintain tissue DO_2 despite ↓ CaO_2 → this occurs by 2 mechanisms:
  - (i) Hypoxic tissues induce arteriolar vasodilation (as part of "tissue autoregulation" response) to ↑ tissue blood flow and ↑ O_2 supply to meet its O_2 demand → this occurs collectively in all tissues, such that generalised vasodilation causes ↓ SVR and ↑ VR of blood back to heart, which leads to ↑ C.O.
  - (ii) ↓ BP 2° to ↓ SVR causes ↓ renal perfusion → elicits humoral response (RAAS and ADH) that ↑ plasma volume → ↑ C.O.

  > Important to note – ↑ C.O. is the only variable in "O_2 flux equation" that can be acutely increased (BUT it is a/w ↑ MRO_2, which will be compromised if [Hb] is very low (< 5 g/L))

- (2) ↑ tissue O_2 extraction for a given tissue DO_2 → due to:
  - (i) ↑ pO_2 gradient for diffusion of O_2 due to lower tissue pO_2
  - (ii) Right shift of OHDC due to ↑ RBC [2,3-DPG] → this ↑ P50 of Hb to 30 mmHg, which ↑ O_2 unloading in tissues
- (3) Redistribution of blood flow to tissues where adequate O_2 delivery is more critical (esp heart and brain)
- (4) ↑ erythropoiesis → hypoxia in renal tubular cells (due to ↓ renal tissue DO_2) stimulates release of EPO, which stimulates BM to produce new RBC and restore normal Hb within 4-8 weeks

> Important to note – Techniques to maintain tissue O_2 balance in a patient with anaemia:
> - ↑ tissue O_2 supply (or DO_2) by:
>   - (i) ↑ C.O. → volume load to significantly ↑ C.O. as per Frank-Starling mechanism (preferably with PRBC to replace Hb also, but OK with colloids/crystalloids)
>   - (ii) ↑ [Hb] → replace Hb with PRBC transfusion, and support ongoing haemopoiesis with haemotinic factors (Eg. Fe, vitamin B12, folate)
>   - (iii) ↑ SpO_2 and PaO_2 → supplemental O_2 (FiO_2 100%)
> - ↓ tissue O_2 demand by:
>   - (i) Sedation, paralysis and artificial ventilation → ↓ muscle MRO_2 a/w activity and respiration
>   - (ii) Maintain normal core body temperature → avoid hypothermia and shivering 2° to hypothermia, which ↑ MRO_2
>   - (iii) Minimal use of inotropes to maintain C.O. → avoid ↑ cardiac MRO_2

(IV) **Iron metabolism**:

Total iron body store:

- Adult male has 3500-3700 mg of iron in the body (↓ in females due to ↓ [Hb], ↓ weight and menstrual loss)
- Iron is ALWAYS protein-bound in the body (as free iron is chemically active and toxic due to its oxidative tendencies) → it is distributed in this form within 3 major pools:

Functional pool	- Bound to Hb → 65-70% or 2500 mg - Bound to Mb → 3-5% or 130 mg - Bound to other proteins (Eg. cytochromes) → 0.2% or 8 mg
Transport pool	Bound to transferrin → 0.1% or 4 mg
Storage pool	Bound to ferritin/haemosiderin (esp in RE system → liver, spleen, BM) → 25-30% or 1000 mg

Important to note → Protein-binding of iron within the body means that there is no physiological mechanism to regulate the excretion of iron from the body (Ie. no "free" iron to be removed) → thus, control of body's iron content relies solely on regulation of iron absorption in the GI tract

Functional uses of iron:

- Iron is incorporated in haem which is protein-bound → forms "haemproteins" (Eg. Hb, Mb, cytochromes, catalases, oxidases, Etc.)
- They are involved in (i) O_2 carriage/storage (Eg. Hb, Mb) or (ii) oxidative reactions (Ie. cellular respiration by cytochrome)

Regulation of iron balance:

Absorption of dietary iron:

- The average diet consists of 10-15 mg of iron of which ~ 10% is normally absorbed (but ↑ to 20-30% with pregnancy or iron-deficiency)
- Dietary iron is present in food in two forms:
    - (i) Bound to "haemproteins" → mainly Fe^{2+} form in Mb/Hb of red meats
    - (ii) Bound to organic ligands → mainly Fe^{3+} form complexed to proteins or as ferric-hydroxide
- Iron is absorbed by enterocytes in the duodenum and upper jejunum:

Important to note:

- Dietary Fe^{3+} (ferric) form is converted to Fe^{2+} (ferrous) form in the stomach → this is promoted by (i) ↑ gastric acidity and/or (ii) reducing agents (Eg. ascorbic acid)
- Fe^{2+} (ferrous) form is more easily released from organic ligands it is bound to and remains soluble in the alkaline environment of the small intestines (unlike Fe^{3+} (ferric) form) → thus, ↑ available for absorption
- Certain chelating agents affects the solubility of iron – (i) At ↓ pH's, certain substances (Eg. a.a.) can bind Fe^{3+} to form a "soluble chelate" to ↑ absorption, (ii) certain substances (Eg. phytates, tannates, phosphates) bind iron and form "insoluble chelates" to ↓ absorption

    - Along the apical surface of the enterocyte:
        - (i) Fe^{2+} is transported across by "Divalent metal transport 1" (DMT1)
        - (ii) Fe^{3+} is either converted to Fe^{2+} by "Ferrireductase" (then transported across by DMT1) or transported across directly by "Mobbilferrin-integrin pathway"

        - (iii) Haem is absorbed intact → broken down by haem oxygenase intracellularly to release Fe^{2+}
    - Within the cytoplasm of the enterocyte:
        - (i) Iron is transported across basolateral membrane by "Ferroportin" (coupled to "Ferrioxidase" which converts Fe^{2+} to Fe^{3+}) → binds to "Transferrin" in plasma in Fe^{3+} form
        - (ii) Excess absorbed iron binds to "Apoferritin" as Fe^{3+} to form "Ferritin" → forms iron store in enterocyte, which is shed into gut lumen when enterocyte dies in 3-4 days

Excretion of body iron:

- Most iron (0.5-1 g) is lost in the faeces (by desquamation of GIT enterocytes)
- Small amounts of iron are lost in urine, hair and sweat

> Important to note → iron loss in females is also the result of (i) menstruation or (ii) transplacental transfer during pregnancy

Control of body iron stores:

- To maintain homeostasis of body iron stores → absorption of dietary iron = excretion of body iron (Ie. ~ 1-1.5 g of iron is absorbed and excreted each day)
- Control of body iron stores occurs at the level of the small intestinal enterocytes → via "mucosal block" mechanism, which prevents excess iron from entering body:
    - If body iron stores are low → plasma transferrin levels are ↑ and transferrin saturation is ↓ → thus, more iron passes from ferritin stores in enterocyte into transferrin in blood
    - If body iron stores are adequate → plasma transferrin levels are ↓ and transferrin saturation are ↑ → thus, less iron is passed from ferritin stores in enterocyte into transferrin in blood. Instead, iron remains in ferritin stores in the enterocyte, which is later lost when the enterocyte is shed in 3-4 days time

> In summary:
> - Iron is readily absorbed across the apical surface of the enterocyte, but its absorption across the basolateral membrane into plasma is tightly controlled by (i) enterocyte ferritin levels and (ii) plasma transferrin levels and saturation
> - Any excess iron absorbed is stored as ferritin within the enterocyte and lost when the enterocyte is shed

<u>Transport of iron</u>:

- "Transferrin" serves to transport iron in plasma → each transferrin binds 2x Fe^{3+}
- It picks up most iron from macrophages within the RE system (Ie. recycles iron from destroyed ageing RBCs) → when it is saturated with iron, it delivers it to various cells in the body (Ie. delivers iron for use in mitochondrial cytochrome for [O] phosphorylation)

> Important to note – Transferrin binds to specific "Transferrin-receptors" → complex is then internalised by endocytosis, whereby the iron is released intracellularly and transferrin is returned intact into plasma

- Transferrin is normally 33% saturated with iron and has a t ½ of 8-10 days

<u>Storage of iron</u>:

- Reticulo-endothelial system (esp liver, spleen and BM) is a major store of "non-functional" iron → stores 25-30% (or 1000 mg) of total body iron

> Important to note – Hb is the largest store of iron in the body (65-70% or 2500 mg) of which the store is "functional"

- This storage pool of iron is bound either to:
    - (i) Ferritin (65%) – Water-soluble protein-iron complex that consists of outer protein shell (Apoferritin) and an inner core (Ferric hydroxyphosphate) → single ferritin can contain up to 4000x Fe^{3+}→ major storage form of iron in the body
    - (ii) Haemosiderin (35%) → insoluble cellular iron stores of partially degraded ferritin

(V) **Haemostasis**:

(A) Overview of haemostasis:

"*Haemostasis*" → property of blood that (i) allows it to remain fluid within an intact vessel AND (ii) allows the circulation to respond to vessel injury in a way that minimises blood loss

It involves interaction of – (i) blood vessels, (ii) platelets and (iii) plasma proteins (pro- and anti-coagulants)

In response to vessel injury, haemostatic processes act to arrest or minimise blood loss in 3 stages:

- (1) Vasoconstriction of damaged vessels
  - Both (i) neural reflexes and (ii) vasoactive mediators (esp TXA2 and ADP released from activated platelets) constrict damaged vessels → promote stasis of blood which retard blood loss and aids blood clot formation
- (2) Formation of platelet plug at damaged vessel
  - Exposure of subendothelium from vessel damage causes platelet adhesion, activation (incl release reaction) and aggregation (see below) → this forms temporary haemostatic plug only to retard blood loss
- (3) Formation of a blood clot to seal the defect
  - Exposure of subendothelium of damaged vessels (Eg. collagen, tissue factor) initiates the coagulation cascade (see below) to form a fibrin mesh that forms a more permanent haemostatic plug to arrests blood loss → this process is facilitated by procoagulant activity a/w platelet activation (see below)
  - This latter leads to the lay down of new collagen by fibroblasts to permanently seal vessel breach

Important to note → platelets participate in all three stages of haemostasis!!!!

(B) Haemostasis: Platelets

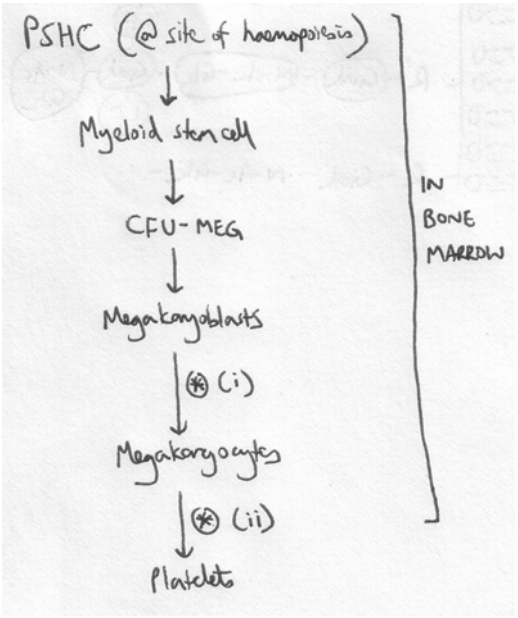
Overview of platelets:

- Small (2-4 um) disc-shaped cellular fragments of a megakaryocyte
- Normally 150,000 to 300,000/mm^3 in plasma (Nb. 50,000/mm^3 are required for surgery; spontaneous haemorrhage occurs when < 5000/mm^3)

Important to note → various anti-platelet drugs (Eg. aspirin) renders platelets non-functional, causing prolonged bleeding despite a quantitatively normal platelet count!

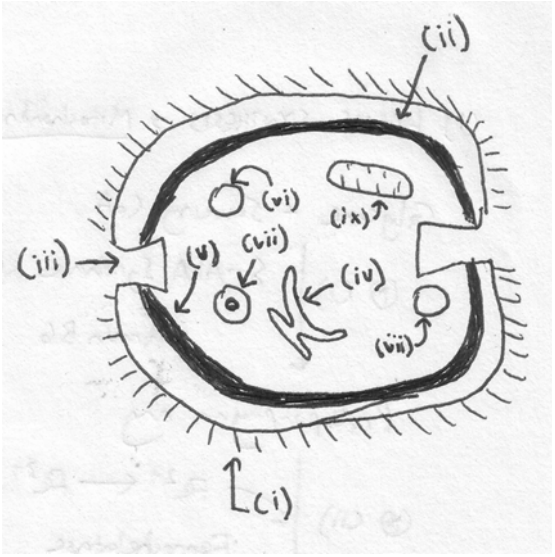
Turnover of platelets:

* Note: - (i) Megakaryblasts undergo "non-mitotic nuclear replication" → cell enlarges with increasing cytoplasmic volume with each cycle of replication - (ii) Cell membrane of megakaryocytes invaginates → causes mature platelets to "bud off" from the surface



- The synthesis of platelets is under the control of "Thrombopoietin" (protein hormone produced by the liver) → it binds to specific membrane receptors on megakaryocytes and induces terminal differentiation of megakaryocytes → ↑ "budding off" of platelets
- Platelets circulate in blood for 8-12 days → then removed by macrophages within the "reticulo-endothelial" system (esp in spleen and liver)

Platelet structure:



Platelets are fragments of megakaryocyte cytoplasm enclosed within a membrane that contain:
- (i) *Glycocalyx* → outer layer of membrane glycoprotein (Eg. Gp Ia, Ib, IIb, IIIa) surrounding the platelet membrane vital for platelet adhesion and aggregation
- (ii) *Platelet membrane* → contains (i) phospholipid bilayer → source of A.A., PAF and platelet factor 3 (vital for CF X and CF II activation), and (ii) proteins → surface Ag's such as HLA class I, ABO system, HPA 1-5
- (iii) *Open canalicular system* → invagination of platelet membrane with a large SA to absorb plasma CFs
- (iv) *Dense tubular system* → residual ER rich in Ca^{2+}, ATPase, AChE, Etc.
- (v) *Cytoskeleton* → peripheral submembranous zones of micro-tubules and –filaments that help maintain the discoid shape of platelets
- (vi) *a granules* → contain β-thromboglobulin, fibronectin, fibrinogen, PF4, PDGF, vWF, thrombospondin
- (vii) *Dense granules* → contain 5-HT, ADP, ATP, pyrophosphate
- (viii) *Lysosomes* (contain hydrolytic enzymes) and *microperoxisomes* (contain catalase)
- (ix) *Mitochondria* → ATP generated by glycolysis and TCA cycle (aerobic and anaerobic)

Important to note → platelets LACK nucleus → cannot synthesise protein!!!!

Platelet function:

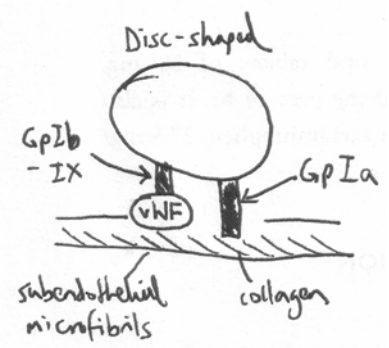
Platelets are involved in all 3 stages of haemostasis:
- (1) Local vasoconstriction of damaged vessel → due to release of vasoconstrictors (Eg. TXA2, 5-HT) during release reaction
- (2) Formation of platelet plug at damaged vessel → temporary haemostatic plug is formed by platelet adhesion to exposed subendothelium, then subsequent platelet activation (incl release reactions) and aggregation
- (3) Initiation of coagulation (by an activated platelet) → leads to formation of permanent haemostatic plug

Phases of platelet formation of haemostatic plug are listed below:

(1) Platelet adhesion:

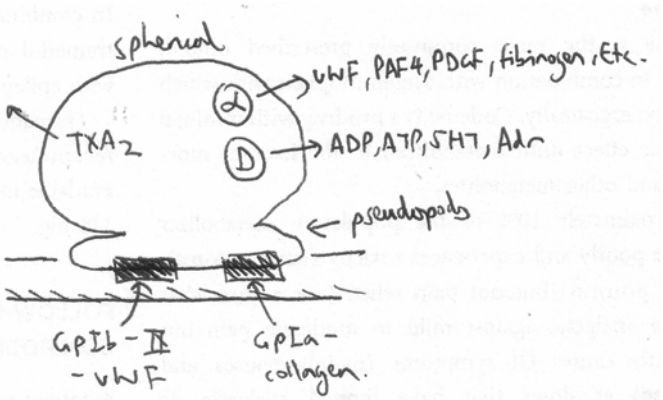
- Blood vessel damage exposes the subendothelial CT → causes platelet in circulating blood to adhere to it to initiate formation of the "platelet plug"
- Adhesion occurs via – (i) Platelet Gp Ia binding to subendothelial collagen, and (ii) Platelet Gp Ib-IX complex binding to subendothelial microfibrils via vWF

Note – Exposed subendothelial microfibrils allows circulating vWF to bind to it → this allows platelet Gp Ib-IX complex to indirectly adhere to the subendothelium through vWF



(2) Platelet activation:

- Following adhesion, platelets are activated by – (i) adhesion to proteins (esp collagen), (ii) soluble agonists (esp thrombin, Adr, ADP, 5-HT), and (iii) cell contact during platelet aggregation
- This stimulates various IC metabolic processes (esp activation of PLA2 and PLC2 → ↑ DAG/IP3 and PKC activation → ↑ Ca^{2+} → ↑ Ca^{2+}-dependent and calmodulin-dependent reactions)
- This leads to induction of – (i) Platelet release reactions (see below) and (ii) Platelet aggregation (Ie. exposes Gp IIb-IIIa complex on membrane) – and facilitates platelet adhesion to subendothelial CT (Ie. transforms platelet from compact disc into sphere with long pseudopodia that spreads along subendothelium



(3) Release reaction:

- Release reactions are mainly stimulated by (i) thrombin and (ii) collagen exposure
- Dense granules release its contents (ADP, ATP, Adr, 5-HT) within 30 secs → reinforce platelet activation
- α granules release its contents (fibrinogen, β-thromboglobulin, PAF-4, vWF, PDGF, thrombospondin) after 30 secs → reinforce platelet adhesion and aggregation
- TXA-2 is produced from platelet membrane (by PLA2) → causes local vasoconstriction, stimulates further release reaction, and induces platelet aggregation

(4) Platelet aggregation:

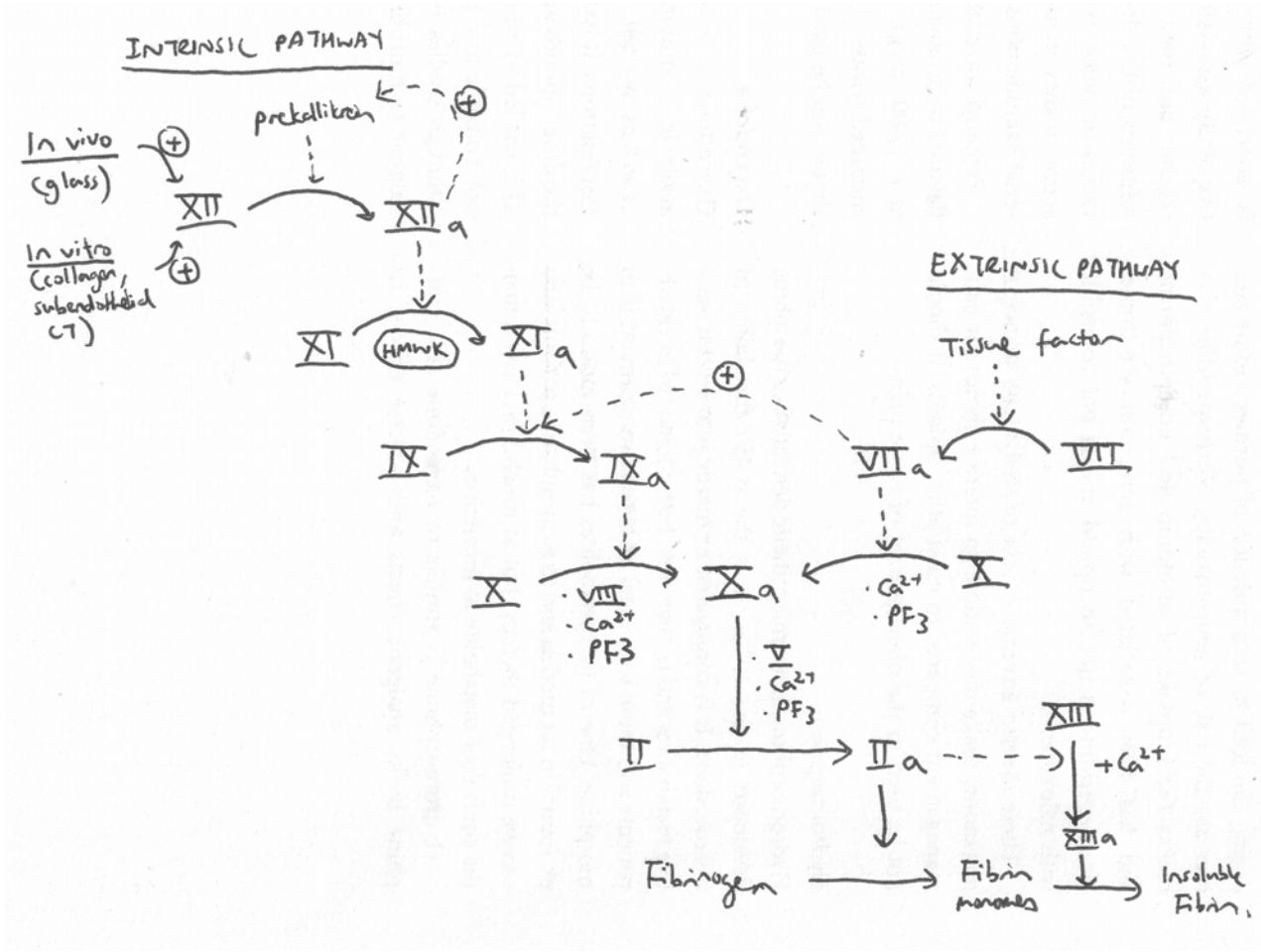
- Platelet aggregation is stimulated by mediators released during the release reaction – TXA2, ADP, fibrinogen, vWF and thrombospondin
- Platelet aggregation is formed by linkage of their GpIIb-IIIa complexes to vWF and fibrinogen → causes spreading of platelets on subendothelial matrix

(5) Procoagulant activity:
- Procoagulant activity of platelets is stimulated by (i) thrombin and (ii) collagen exposure
- This causes Ca^{2+} influx across plasma membrane → leads to re-orientation of phosphatidyl serine from inner layer to outer layer of platelet membrane (esp platelet factor 3 or PF3) → forms binding sites for two vital CF complexes (Tenase and Prothrombinase – see below) that allow the coagulation cascade to proceed

(C) Haemostasis: Coagulation Cascade

"Coagulation cascade" – A biological amplification system that consists of an intrinsic and extrinsic pathway that join to form a final common pathway → generates thrombin which converts fibrinogen into fibrin that enmeshes platelet plug at site of vascular injury → forms a firm and stable haemostatic plug

Coagulation cascade involves several "clotting factors" (CF) → either:
- (i) Precursors of serine proteases (enzymes) → activated by proteolysis (Eg. most CFs)
- (ii) Co-factors (Eg. CF V and VIII)



Important to note → Extrinsic pathway is MORE important to coagulation than intrinsic system → b/c (i) CF XII, CF XI or prekallikrein deficiencies are not associated with bleeding disorder, and (ii) CF VIIa (from intrinsic pathway) can activate CF IX!

(1) Intrinsic pathway:
- Activated by contact with –vely charged surfaces → (i) In vivo (Eg. exposure of collagen or subendothelial CT of damaged vessels), or (ii) In vitro (Eg. glass)

- Process:
  - (i) CF XII + -ve charged surface → generates CF XIIa
  - (ii) CF XIIa + HMWK (HMWT kininogen) + CF IX → generates CF IXa
  - (iii) CFXIa + CF IX → generates CF IXa
  - (iv) CF IXa + CF X + co-factors (CF VIII + Ca^{2+}) + PF3 (platelet phospholipid) → forms "Tenase" complex on platelet membrane → generates CF Xa

> Note:
> - (i) Contact phase involving prekallikrein, HMWK, CF XII and XI only occurs "in vitro" (Ie. deficiencies in these factors do NOT cause coagulopathy!)
> - (ii) CF VIIa (from extrinsic pathway) actually activates CF IX "in vivo"
> - (iii) CF XIIa activates prekallikrein → generates additional XIIa → +ve feedback of initial response

(2) Extrinsic pathway:
- Activated by contact with "Tissue factor" (or thromboplastin) → released from exposed subendothelium of damaged vessels
- Process:
  - (i) Tissue factor + CF VII → generates CF VIIa
  - (ii) CF VIIa + CF X + Ca^{2+} on platelet phospholipid membrane → generates CF Xa

> Note → Role of CF VIIa in vivo is to activate CF IX in intrinsic pathway (rather than CF X!)

(3) Final common pathway → process:
- (i) CF Xa + Prothrombin (CF II) + co-factors (CF V + Ca^{2+}) + PF3 (platelet phospholipid) → forms "Prothrombinase" complex on platelet membrane → generates thrombin (CF IIa)
- (ii) Thrombin hydrolyses soluble fibrinogen → fibrinopeptide A/B + fibrin monomers
- (iii) Fibrin monomers aggregate non-covalently via H-bonding → form "soluble fibrin"
- (iv) CF XIII is activated by thrombin + Ca^{2+} → generates CF XIIIa which covalently links fibrin polymers to form "insoluble fibrin"

> Note → Thrombin has +ve feedback on CF V, CF VII and CF XI

> Important to note – Hypocalcaemia and coagulation:
> - Ca^{2+} is essential for several steps of the coagulation cascade (Ie. formation of CF Xa, IIa, XIIIa) → BUT the degree of hypocalcaemia needed to adversely impact coagulation is below that associated with cardiac arrest/death
> - Thus, hypocalacaemia generally does NOT cause coagulopathy!

(D) Mechanisms that prevent blood clotting within a normal intact blood vessel:

Uncontrolled disseminated coagulation (and clot formation) interferes with blood delivery to tissues → as a result, there are several mechanisms that prevent this from happening:

(1) *"Thrombo-resistance" of an intact vessel endothelium*:
- (i) Intact vessel endothelium prevents exposure of blood to collagen (so vWF and platelets cannot bind) and tissue factor (so extrinsic pathway cannot be activated)
- (ii) Mucopolysaccharide (glycocalyx) coating of endothelium → repels platelets and CFs
- (iii) "Smooth" intact endothelial surface prevents contact activation of intrinsic system

- (iv) Endothelium produces "prostacyclin" (PGI2) → inhibits platelet aggregation and causes vasodilation (via vascular SM relaxation)
- (v) Endothelium produces "thrombomodulin" → activates protein C
- (vi) "Heparan sulphate" (a natural heparin) is present on endothelial cells → enhance activity of AT III by 750x
- (vii) Endothelium produces "tissue plasminogen activator" (in response to local thrombin) → enhances fibrinolysis

(2) *Continuous blood flow*:
- (i) Washes away and dilutes active pro-coagulant mediators
- (iii) Limits build-up of weakly bound platelet masses along endothelium

(3) *Circulation of coagulation factors in an inactive form* → circumstances causing conversion to active form do not usually occur in intact vessels

(4) *Inhibitory systems in plasma*:
- (a) Physiological inhibitors of coagulation:
    - (i) Antithrombin III (* most important * → 70% of coagulation inhibition)
        - Inhibit ALL serine proteases to varying degrees (esp thrombin and CF Xa) by combining with them via a peptide bond → form a stable complex
        - Note – This process is enhanced by heparin (and heparan)
    - (ii) Protein C and S
        - Both are vitamin K-dependent serine proteases
        - Protein C is activated by thrombomodulin (which is formed by thrombin binding to endothelial surface), which destroys co-facators CF VIIIa and CF Va → this process is enhanced by Protein S, which binds Protein C to the platelet surface
    - (iii) Tissue factor pathway inhibitor
        - Released by local platelet activation → inhibits CF Xa, CF VIIa and TF
- (b) Fibrinolysis → process where fibrin and fibrinogen are cleared by plasmin
    - Plasminogen (β-globulin) in plasma and tissue fluid is converted to Plasmin (a serine protease), which lyses fibrin and fibrinogen to fibrin-degradation products and D-dimers (cleavage products of cross-linked fibrin)
    - This conversion is initiated by:
        - (i) Extrinsic activation (from the tissues) → by endothelial tPA (enhanced by local thrombin production), which circulates and activates ONLY fibrin-bound plasminogen (Ie. localised to the clot)
        - (ii) Intrinsic activation (from the vessel wall) → byCF XIIa, kallikrein

(E) <u>Haemostasis: Von Willebrand Factor and CF VIII</u>

Von Willebrand Factor:
- HMWT multimeric plasma glycoprotein → behaves as an "acute phase protein" (↑ levels with inflammatory conditions)
- It is synthesised by – (i) Endothelial cells (stored in Weibel-Palade bodies) and (ii) Platelets (stored in α-granules)
- It has key functions:
    - (1) Platelet adhesion to subendothelium of damaged vessels → circulating vWF binds to exposed subendothelium filaments, and then binds to platelets via Gp Ib-IX
    - (2) Platelet aggregation → activated platelets generate surface Gp IIb-IIIa which allows platelets to bind via vWF
    - (3) Platelet activation → vWF released during the "release reaction" +vely feedbacks on platelet activation

CF VIII → a co-factor that consists of 2 components:
- (i) CF VIII C (coagulant) → synthesised in liver and inactivated by protein C (and S)
- (ii) CF VIII vWF (von Willebrand factor)) → see above

(F) Monitoring Haemostasis: APTT, PT, INR and PCT

(1) *Prothrombin time* (PT) → normally 10-14 secs
- Measures extrinsic system (CF VII) and factors common to both systems → sensitive to 75% of vitamin K-dependent CFs (II, VII and X only)
- Requires tissue thromboplastin (brain extract) and Ca^{2+} added to citrated plasma

(2) *International normalised ratio* (INR) → normally 1-1.5
- INR is the ratio of "sample PT" to "control international standard PT"
- INR is used to standardise PT values b/ different labs → this is b/c commercial PT reagents at different labs vary in responsiveness to warfarin-induced ↓ in CFs

(3) *Activated partial thromboplastin time* (APTT) → normally 30-35 secs
- Measures intrinsic system (CF VII, IX, XI, XII) and factors common to both systems
- Requires phospholipid, surface activator (kaolin) and Ca^{2+} added to citrated blood

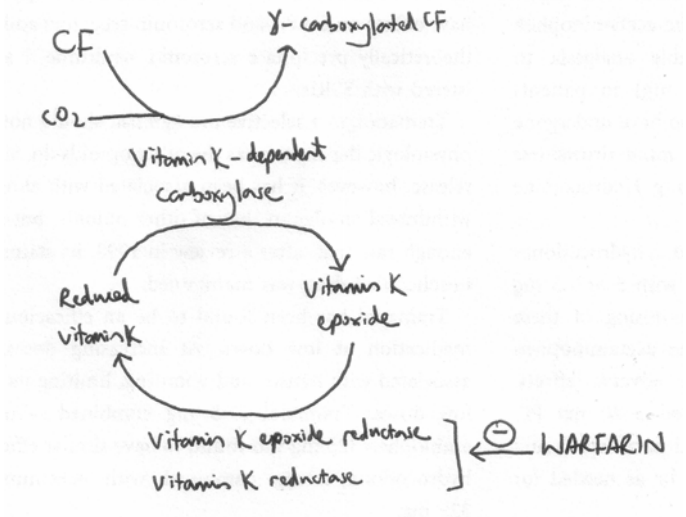
(4) *Activated coagulation time* (ACT) → normally 90-120 secs
- Used to monitor heparin and antagonism by protamine (Ie. for CPB)
- Requires mixing whole blood with an activation substance with a large SA (kaolin or celite) → induces clotting cascade → machine measures onset of clot formation

(VI) **Vitamin K**:

Vitamin K is a "fat-soluble vitamin" that is vital for coagulation

Its main role is post-translational modification (and activation) of vitamin K-dependent CFs (CF II, VII, IX, X, protein C and S) via the following processes:

- Vitamin K is used as a "co-factor" by "Vitamin K-dependent carboxylase" which catalyses γ-carboxylation of glutamatic acid residues in CFs → places a 2nd carboxyl group on the a.a. residue, giving it 2x –ve charges to chelates divalent Ca^{2+}
- Vitamin K is oxidised during this reaction (from "Reduced vitamin K" to "Vitamin K epoxide) → it needs to be reduced back to the active co-factor via 2 enzymes – (i) "Vitamin K epoxide reductase" and (ii) "Vitamin K reductase"



Aside – Warfarin inhibits "vitamin K epoxide reductase" and "vitamin K reductase" → prevents recycling of vitamin K and activation of vitamin K-dependent CFs

Vitamin K in the body is derived from diet (from plants (Eg. green leafy vegetables) and some animals (Eg. meats)) → absorption requires bile salts, which solubilises it for absorption in terminal ileum of small intestines

Important to note – Large amounts of vitamin K are produced in the intestines (esp colon) due to bacterial action, BUT colonic vitamin K is NOT absorbed → only vitamin K derived in the small intestines can be absorbed as bile salts here can solubilise it for absorption in the terminal ileum

Vitamin K is commonly used clinically to treat or reverse anticoagulation effects of warfarin (esp pre-operatively) → but it has issues:

- (i) Reversal of warfarin anticoagulation is NOT immediate (as it requires synthesis of new CFs) → if rapid reversal is need, FFP or activate CFVIIa should be given instead
- (ii) Vitamin K preparations contain cremophor EL as a solubilising agent → causes anaphylactoid reactions, esp with IV use
- (iii) High doses (IV 10 mg) prevents rapid re-establishment of warfarin anticoagulation post-operatively → thus, if post-operative anticoagulation is required, only IV 1-2 mg should be given

Newborn infants are susceptible to vitamin K deficiency → cause "Haemorrhagic disease of the newborn":

- They are deficient in vitamin K (and thus deficient in vitamin K-dependent CFs) b/c:
    - (i) It does not cross placenta in sufficient amounts → so hepatic stores of vitamin K are very low in a newborn
    - (ii) Bacterial production of vitamin K in the intestines does not occur in newborns (until end of 1st week) as the bowel is sterile and not colonised yet by bacteria

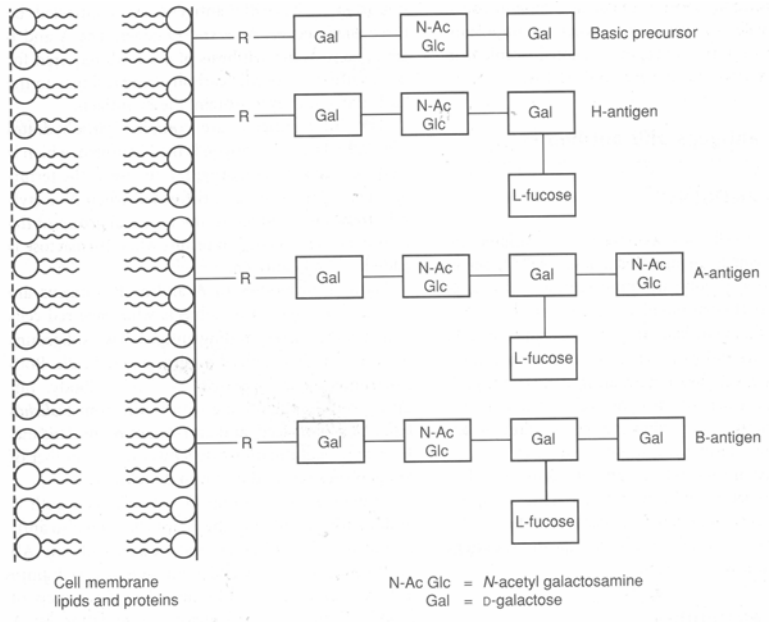
    - (iii) Dietary intake is initially poor and breast milk contains low amts of vitamin K
    - (iv) Some drugs given to mother may worsen vitamin K deficiency of the newborn (Eg. warfarin, phenytoin, antibiotics) → either inhibits activity, storage, or intestinal bacterial production of vitamin K
- It is easily prevented by routine vitamin K injections to all infants soon after birth

(VII) **Blood Groups**:

"Blood group" → classification system of blood based on the presence (or absence) of certain groups of genetically-determined antigens present on RBC membranes

Clinical importance of any certain blood group is based on its degree of antigenicity:
- The most antigenic (and thus most important) blood groups are the ABO and Rh system → these are most likely to cause reaction if group-incompatible blood is present
- Other blood groups (Eg. Kell, P, MN, Lewis) are less antigenic (and thus less important) → unlikely to cause reactions if group-incompatible blood is present

(1) ABO system:

Presence	ABO antigens are present in (i) RBC, (ii) most other body cells (Eg. WBC, platelets, kidney, liver, lungs, Etc.), and (ii) body fluids in a H_2O-soluble form (Eg. plasma, saliva, semen, urine, tears, bile, gastric juice → but NOT CSF) → in 80% of population who possess a "secretor gene"
ABO antigens	- ABO antigens are determined by 3 genes that are inherited in a simple Mendelian manner: o (i) H gene – Encodes α-L-fucosyltransferase, which attaches L-fucose to the end of a membrane glycosphingolipid → forms "H-antigen" o (ii) A gene – Encodes α-N-acetyl-D-galactosaminyl transferase, which attaches "N-acetyl-D-galactosamine" to terminal residue of H-antigen → forms "A-antigen" o (iii) B gene – Encodes α-D-galactosyltransferase, which attaches "D-galactose" to terminal residue of H-antigen → forms "B-antigen"  Important to note → antigenic specificity is determined by the CHO moiety attached to terminal group of H-antigen - These ABO antigens produces 4 main ABO blood groups: o Group A (45% of pop'n) → possess A-antigen only o Group B (9% of pop'n) → possess B-antigen only o Group AB (3% of pop'n) → possess A- and B-antigens o Group O (43% of pop'n) → possess H-antigen only
ABO antibodies	- Naturally-occurring Ab to A- and/or B-antigens (as anti-A and anti-B antibodies) are present in plasma of individuals who have RBC that lack the corresponding blood group antigen:

	o Group O individuals lack A- and B-Ag's → possess anti-A and anti-B Ab's o Group AB individuals have A- and B-Ag's → lack anti-A and anti-B Ab's o Group A individuals have A-Ag only → possess anti-B Ab's only o Group B individuals have B-Ag only → possess anti-A Ab's only - These are IgM only, optimally reactive at 4°C, and develop naturally after 3 months of age due to presence of Ag in bacteria/food that resemble A/B-Ag's

(2) Rhesus system:

Presence	Rh antigens are present ONLY on RBC (cf. ABO system)
Rh antigens	- Rh antigens are determined by 3 closely linked pair of alleles on chromosome 1 (Dd, Cc, Ee) Important to note → "d-Ag" alone does NOT exist as "d-allele" does not produce a protein product → it actually suggests an absence of "D-Ag" - Rh status is stated either: o (1) Based on Rh Ag present (Eg. EDe/ce) o (2) Based on presence of D-Ag (as D-Ag is MOST antigenic) → either (i) Rh+ve (85% of pop'n) or (ii) Rh-ve (15% of pop'n)
Rh antibodies	- Anti-Rh antibodies do NOT occur naturally (cf. ABO Ab's) → it requires exposure to Rh Ag (Ie. transfusion or transplacental exposure of Rh+ve blood) - There are Rh-Ab's to each specific Rh-Ag, EXCEPT the d-Ag which does not exist (Ie. anti-C, anti-c, anti-E, anti-e, anti-D exist only) - These Ab are IgG mainly (can be IgM on initial exposure) and optimally reactive at 37 °C Important to note – "Erythroblastosis foetalis" should not arise in a Rh-ve mother who is pregnant with a Rh+ve foetus, UNLESS anti-D Ab develop due to previous exposure to Rh+ve blood (Ie. previous pregnancy with Rh +ve foetus or Rh+ve blood transfusion) or lack of anti-D passive immunisation

(3) Other blood groups (Eg. P-antigen, Lewis, MN, Kell system) → these are weakly antigenic. Naturally-occurring Ab's are present in plasma but react at low temperatures only

Important to note – "Kell system" is the 3rd most important blood group:
- K antigen is present on RBC, leukocytes, and platelets → very immunogenic
- Isoimmunisation occurs in patients with multiple blood transfusions

(VIII) **Blood Transfusion**:

Blood Compatibility Testing:

Blood transfusion involves infusion of compatible blood (or its components) from donor to the recipient → so prior to transfusion, donor blood needs to be tested for compatibility with recipient blood to avoid serious transfusion reactions (esp haemolysis of transfused RBC) due to either blood group incompatibility or presence of reacting Ab's in the recipient

There are 3 procedures for compatibility testing:

(1) Blood group typing:
- (a) ABO group determination
  - (i) Test individual's red cells using anti-sera with IgM anti-A and anti-B Abs → +ve agglutination indicates blood type
  - (ii) Test individual's serum using known group A, B, and O red cells ("Reverse blood grouping") → +ve agglutination indicates blood type
- (b) Rh(D) group determination
  - Test individual's red cells against anti-serum containing sufficiently potent IgG against Rh(D)-positive cells → +ve agglutination indicates Rh +ve blood group

(2) Antibody screening:
- Screening for "minor" anti-RBC Ab's (Ie. Ab's against blood group Ag's other than A and B, such as RhD, Kell, Duffy, Etc.) is routinely done on pre-transfusion specimens from prospective recipients and prenatally on maternal specimens
- This is done by adding group matched RBC with known a selection of "minor" Ag's to the recipient's serum → +ve agglutination indicates recipient has "minor" Ab

(3) Cross-match:
- There are two types:
  - (a) "Major cross match" – Test in *vitro* serological compatibility between recipient's serum and donor's red cell
  - (b) "Minor cross match" – Test *in vitro* serological compatibility between donor's serum and recipient's red cells

> Important to note → minor cross matches are RARELY performed as donated blood is generally tested for and made free of irregular Ab's

- "Major cross-match" is done in two stages:
  - (a) Saline agglutination test
    - Used to reconfirm ABO blood grouping → it tests for presence of IgM (Eg. anti-A and anti-B Ab's) in recipient serum against donor RBC
    - Donor's RBCs are suspended in saline with recipient serum (which may contain Ab's against RBC) at room temperature → +ve agglutination means incompatible ABO blood grouping
  - (b) Indirect Coomb's test
    - Used to reconfirm presence of "minor" Ab's → it tests for presence of haemolytic IgG Ab's (Eg. anti-D Ab's, anti-Kell Ab's, anti-Duffy Ab's, Etc.) in recipient serum against donor RBC
    - There are two stages to the test:
      - (i) Donor RBCs are incubated at 37 °C with recipient serum (which may contain Ab's against RBC) → if the serum does contain Ab's, it will coat the RBC in vitro

- (ii) RBC washed with saline to remove the globulins and "anti-human globulin" (AHG) is then added → if there is agglutination, it means the recipient serum contains an Ab against donor RBC

> Aside – AHG is produced in animals (Eg. rabbit) after injection of human specific Ig → it is an IgG that is specific to human IgG, so when AHG is added to human RBC coated with human IgG, it causes them to them to cross link and agglutinate

Important to note:

- Administration of ABO-Rh compatible blood is already very safe → additional procedures in cross-match add very small extra safety margin

  ABO and Rh compatible blood → 99.8% relative safety
  ABO and Rh compatible blood AND –ve Ab screen → 99.94% relative safety
  ABO and Rh compatible blood and –ve Ab screen AND Coomb's test → 99.95% relative safety

- Most "minor" Ab's can be detected by a suitable selection panel of "minor" Ag's used during "Antibody screening" → thus "Indirect Coomb's test" is commonly omitted from routine cross-matching (UNLESS the antibody screen was +ve!)

<u>Types and Preparations of Blood Products</u>:

(1) Whole blood and red cell preparations:

Important to note → to preserve donor blood:

- (i) Donor blood is mixed with preservative solution at time of collection
  - CPD or CPDA → commonly used

    Citrate → chelates Ca^{2+} to anticoagulate blood
    Phosphate → buffers that provides PO_4^{3-} for metabolism
    Dextrose → substrate for glycolysis
    Adenine → substrate for ATP synthesis
  - SAG-M (Saline, Adenine, Glucose, Mannitol)
  - ACD (Acid, Citrate, Dextrose) → rarely used now

    Citric acid allows citrate and dextrose solutions to be autoclaved together → BUT acidic pH promotes ↑ rate of 2,3-DPG loss (unlike alkaline pH with CPD or CPDA which prevents 2,3-DPG loss)
- (ii) Storage at 4-6 °C → inhibit cellular metabolism (esp energy consumption) and bacterial growth in blood (Ie. present in blood or introduced during collection)
- (iii) Aseptic techniques/sterile equipment to minimise risk of bacterial contamination

- (a) Whole blood:
  - 400-480 mL blood is taken with 63 mL of preservatives → dilutes plasma by 20%
  - When CPDA1 preservative is used and blood is stored at 4-6 °C → shelf-life of 35 days
  - Properties of whole blood changes with – (i) Type of anticoagulant/preservative used and (ii) Duration of storage:

RBC and free Hb	- RBC becomes spherical and ↑ rigid (2° to metabolic changes) causing haemolysis → 75-80% survive after 21-28 days of storage - Free Hb ↑ from 1.7 to 29 mmol/L at 28 days (due to haemolysis)

WBC	Granulocytes lose phagocytic/bactericidal function after 4-6 hrs of collection
Platelets	Platelets are non-functional within 48 hrs at 4-6 °C
Clotting factors	- Labile CFs (CF V and VIII) deplete rapidly → CF V ↓ to 50% within 24 hrs; CF VIII ↓ to 50% after 14 days - Other CFs deplete only after 21 days of storage
2,3-DPG	↓ to 5% after 28 days due to ↓ metabolism → causes left shift in ODC (esp with ACD preservative cf. CPD or CPDA)
Biochemistry	- ↑ K^+ (30 mmol/L at 28 days) due to progressive loss from RBC into plasma a/w impaired RBC metabolism and Na^+/K^+ ATPase function - ↓ Na^+ (from 170 to 154 mmol/L at 28 days) due to impaired RBC metabolism and Na^+/K^+ ATPase function - ↓ Ca^{2+} due to citrate anticoagulant - ↓ pH (from 7.2 to 6.7 at 28 days) - ↓ ATP levels (to 75% at 28 days) due to ↓ metabolism - ↓ glucose (from 19.2 to 12.2 mmol/L at 28 days)

- (b) Packed red cells (Haematocrit > 0.75)
    - Obtained by removing 200-250 mL plasma after centrifugation or sedimentation of 1 unit of whole blood
    - Similar changes in blood seen (cf. whole blood) EXCEPT ALL CFs deplete more rapidly (and not just labile factors) b/c it contains little plasma → especially when it is transfused along with crystalloids to replace blood loss
- (c) Heparinised blood
    - 500 mL of blood with 30 mL heparin → short shelf-life of 24 hours
- (d) Frozen RBC
    - Treated with glycerol (cryoprecipitate) and stored in liquid N_2. RBC must be thawed and washed extensively with electrolyte solutions to remove glycerol prior to transfusion!

(2) Platelet concentrations:

- One unit contains 6 x 10^{10} platelets and is generated as either – (a) Pooled concentrates from 4-6 units of blood or (b) Single concentrate from single donor
- To keep platelets functional during storage, they need to be kept:
    - (i) At a temperature b/t 20-25 °C → prevent irreversible disc-to-sphere transformation (which occurs at 4 °C)
    - (ii) At pH 6.2-7.9 → platelets are packed in a "polyolefin plastic bag" which allows them to be aerated → this permits gas exchange of O_2 (promotes aerobic metabolism → ↓ lactate production) and CO_2 (↓ H^+ production) → prevent swings in pH that causes irreversible disc-to-sphere transformation
    - (iii) Constantly agitated → maintains pH range by assisting gas exchange across the storage ball wall (Ie. by minimising diffusion distance)

> Important to note → platelets ONLY have a shelf-life of 5 days due to risk of bacterial growth when stored at 20-25 °C

- Platelets express mainly HLA-1 Ag's, BUT contamination by leukocytes and RBC cause "alloimmunisation" → thus, ABO- and Rh-compatible platelets are normally used

> Note – Patients with repeated transfusions develop HLA Ab's (which ↓ efficacy and function of transfused platelets) → they require HLA-matched platelets!

- With each unit transfused, up to 33% of transfused platelets are sequestered in the spleen → as a result, plasma platelet levels ↑ of 5000-10,000/mm^3 per unit given

(3) Human plasma preparations:
- (a) Fresh frozen plasma
  - Prepared from fresh blood and frozen rapidly → stored at -30 °C with a shelf-life of 1 year → used to replace CFs
- (b) Cryoprecipitate
  - Prepared from freshly separated plasma by freezing at -70 °C then thawing at 4 °C → stored at -30 °C with shelf-life of 12 months → rich in CF VIII, fibrinogen and fibrinectin
- (c) Freeze-dried CF VIII concentrate
  - Concentrate of CF VIII (and some fibrinogen) is prepared from FFP and stored at 4°C (HIGH risk of hepatitis transmission!)
- (d) Freeze-dried CF IX concentrate
  - To treat Christmas disease or haemophilia B (CF IX deficiency). Contains CF II, VII and X also

<u>Complications of Blood Transfusion</u>:

Of patients receiving blood products, approximately 3% of them develop a reaction and very few (1:50,000) have a fatal reaction

(1) *Immunological reactions*:
- These reactions can be either immediate or delayed → severe reactions manifest as urticaria, flushing, chest pain, dyspnoea, rigors, tachycardia and shock → RBC destruction manifests as jaundice, haemoglobinuria, ARF and DIC
- Main reactions are due to:
  - (i) Massive intravascular haemolysis due to complement activation by IgM or IgG (Eg. ABO Ab's)
  - (ii) Extravascular haemolysis due to IgG coating of RBC (Eg. Rh Ab) → less severe
- Other types of immunological reactions:
  - (a) White cell reactions – Febrile reactions in 2% transfusions due to donor WBC reacting with alloantibodies induced by previous transfusions or pregnancy
  - (b) Graft-versus-host reactions (Very rare) – Deposition of donor lymphocytes in recipient's skin, liver or GIT causing rash, hepatitis or diarrhoea
  - (c) Post-transfusion purpura – Consumptive thrombocytopaenia occurs 1 week after transfusion of blood product (self-limiting within 4-6 weeks)
  - (d) Anaphylaxis to plasma proteins – Life-threatening situation that generally occurs in IgA-deficient patients whose sera contains anti-IgA

(2) *Non-immunological reactions*:
- (a) Septicaemia – 3 in 1000 units of blood stored at 4°C may be contaminated with bacteria (esp Pseudomonas strains) → higher risk in platelets as it is stored at 22°C
- (b) Disease transmission – Hepatitis (HAV, HBV and HCV), CMV, HIV, malaria, toxoplasmosis, syphilis
- (c) Air embolism
- (d) Circulatory overload
- (e) Iron overload (esp with repeated red cell transfusions due to Fe deposition in RE tissue)

<u>Massive Blood Transfusion</u>:

"Massive blood transfusion" → transfusion of volume of stored blood greater than recipient's blood volume in < 24 hours

There are several complications of massive blood transfusion:
- (1) Citrate toxicity causing hypocalcaemia

    - Occurs if transfusion rate is > 1 L per 10 minutes OR when exchange transfusion carried out within 2 hour
    - ↑ risk of toxicity with hypothermia or shock (due to ↓ HBF) → due to impaired hepatic metabolism of citrate
    - Hypocalaemia presents as involuntary muscle tremors, bradycardia with QT and ST prolongation, and ↓ C.O./BP 2° to myocardial depression → treat with IV CaCl
- (2) Hyperkalaemia
    - Stored blood has ↑ plasma K^+ (up to 30 mmol/L at 30 days) due to leakage from RBC
    - Hyperkalaemia after blood transfusion is not common b/c – (i) K^+ is taken back up into RBC (as normal RBC metabolic activity and Na^+/K^+ ATPase function resumes), (ii) K^+ is diluted in recipient plasma, and (iii) slow transfusion rates minimises sudden ↑ K^+ → UNLESS the patient is acidotic, already hyperkalaemic or is receiving massive transfusion
- (3) Acidosis
    - With storage, blood becomes acidotic (pH 6.5) such that massive transfusion can aggravate any pre-existing acidosis in the patient
- (4) Hypothermia
    - Rapid transfusion of unwarmed blood causes hypothermia, which can lead to – (a) Ventricular arrhythmias (esp VF and asystole), (b) Impaired O_2 delivery (due to Bohr effect), and (c) Aggravated citrate toxicity
- (5) 2,3-DPG deficiency:
    - 2,3-DPG levels ↓ with storage, thus ↓ O_2 delivery → BUT within 24 hours of transfusion, transfused RBC regenerates 2,3-DPG!
    - Use of CPDA preservative reduces this problem as depletion then occurs slowly
- (6) Dilutional coagulopathy
    - Occurs if blood volume is replaced > 2x in a 24 hr period
    - This is b/c stored blood has ↓ levels of CFs (esp CF V and VIII) and functional platelets (rather than citrate-related hypocalcaemia)
    - Coagulopathy due to dilutional thrombocytopaenia occurs first, before coagulopathy due to dilution of CF's
- (7) Microaggregates
    - Clumps of fibrin (MAJOR), platelets and leukocytes form in stored blood which enters the patient's circulation and becomes trapped in pulmonary vessels → causes release of lysosomes and precipitates ARDS

(IX) **Plasma**:

"Plasma" → part of ECF that forms the fluid medium of the intravascular compartment

> Important to note – Contents of plasma are the same as interstitial fluid (extravascular portion of ECF), EXCEPT plasma contains more protein!

Plasma contains several components, such as:

(1) Water/electrolytes:
- Plasma is 4% of TBW (40-50 mL/kg)
- 93% of plasma is water and electrolytes (esp Na^+ 140 mmol/L, Cl 100 mmol/L, K^+ 4 mmol/L, Ca^{2+} 1 mmol/L and Mg^{2+} 2 mmol/L)

(2) Plasma carbohydrates:
- Glucose is the main carbohydrate (4-8 mmol/L) in plasma
- There are small amounts of fructose, galactose and complex carbohydrates

(3) Plasma lipids:
- Most plasma FAs are esterified as TAGs → complexed with cholesterol and phospholipids in "Lipoproteins" (Eg. chylomicrons, VLDL, LDL and HDL)
- Small amounts of plasma FAs are unesterified → usually bound to albumin

(4) Plasma proteins:

Overview of plasma proteins:
- Plasma proteins are globular molecules → range from simple unconjugated proteins (Eg. albumin) to complex proteins (Eg. lipoproteins, glycoproteins, metalloproteins)
- Most are synthesised in liver (except few by plasma cells) → enter into and circulate in plasma (total plasma protein [ ] ~ 60-80 g/L) → plasma proteins exist in equilibrium with tissue proteins as an "exchangeable pool"
- There are 3 major classes:
    - (1) Albumin (40-45 g/L)
    - (2) Globulin (25 g/L) → subdivided into α1, α2, β and γ-globulins
    - (3) Fibrinogens (3 g/L)

Types of plasma proteins:
- (1) Albumin
    - Most abundant protein in plasma (40-45 g/L) → MWT 69 kDa
    - Synthesised in liver (13 g turnover/day) → released into plasma (usually unconjugated form with t ½ 20 days) → metabolised in liver, kidneys and gut
    - 50-60% albumin exists in extravascular space (only 40-50% in blood) → small amounts enter ISF from capillaries via pinocytosis (Nb. [albumin] in ISF >>> plasma!) → then (i) renters circulation via lymphatics or (ii) taken up by tissues for metabolism
    - Role → (i) Transport function (bilirubin, FFAs, Ca^{2+}, thyroid hormone, cortisol, acidic drugs), (ii) Main contributor of plasma oncotic pressure (60-80%), and (iii) Cellular metabolism of a.a. (as a source of energy)
- (2) Globulins → 4 subtypes:
    - (a) α1-globulins
        - α1-antitrypsin → serine protease inhibitor (inhibits trypsin, chymotrypsin, plasmin and other proteases in plasma)
        - α1-lipoproteins → role in TG/cholesterol transport in body (as chylomicrons, VLDL, LDL, HDL)

        - α1-antiglycoprotein → acute phase protein, binds basic drugs
    - (b) α2-globulins
        - α2-macroglobulin (80% of α2-globulins) → plasma protease inhibitor (trypsin, plasmin, chymotrypsin) mainly made by infectious organisms
        - Prothrombin → coagulation
        - Haptoglobin → binding free Hb
        - Caeruloplasmin → Cu^{2+} transport, acute phase protein
    - (c) β-globulins
        - Transferrin → Fe^{3+} transport
        - Haemopexin → haem binding
    - (d) γ-globulins (main plasma globulin → 10-15 g/L of total globulins)
        - Synthesised from plasma cells of BM, spleen, LN and gut (NOT liver!)
        - 4 types → (i) IgG (76%), (ii) IgA (16%), (iii) IgM (7%), (iv) IgE (min.)
        - Roles in innate/acquired immunity (Eg. activate complement, hypersensitivity reactions, protecting mucosal surfaces, binding Ag, Etc.)
- (3) Fibrinogen
    - Synthesised in liver → Role in coagulation

Functions of plasma proteins:
- (1) Transport/carrier function
    - Carriers of hormones, vitamins/ metals, drugs, metabolites and waste products
    - Eg. Albumin → bilirubin, FFAs, Ca^{2+}, thyroid hormone, cortisol, acidic drugs
    - Eg. Globulins → Lipoproteins (TG/cholesterol), Transferrin (Fe^{3+}), Caeruloplasmin (Cu^{2+}), TBG (T3/T4, Transcortin (cortisol), Etc.
- (2) Plasma oncotic (colloid osmotic) pressure
    - Plasma proteins (as colloids) exert 0.5% total osmotic pressure of 5545 mmHg → only 28 mmHg (most due to albumin)
    - Vital in transcapillary fluid dynamics between ISF and intravascular space → it prevent net loss of fluid from circulatory system into ISF
- (3) Haemostasis
    - (i) Coagulation → PT, fibrinogen, CFs (II, VII, IX, X), PC/PS, ATIII
    - (ii) Fibrinolysis → Plasminogen
- (4) Immune function
    - Innate/acquired immune response → acute phase proteins, complement system, antibodies, cytokines
- (5) Metabolic function
    - Source of a.a. to tissues for energy, anabolism (Eg. build enzymes), catabolism
- (6) Enzymes/hormonal function
    - (i) Plasma cholinesterase → drug metabolism
    - (ii) Anti-proteolytic enzymes (α1AT, α2-macroglobulin, Etc.)
    - (iii) Kinins → vasodilator hormone
- (7) Acid-base balance
    - (i) Buffer system (minor cf. other systems) → due to imidazole groups (pKa 6.8) in histidine residues
    - (ii) CO_2 transport (minor role cf. Hb) → as carbamino compounds