Ho Medical
ANAESTHESIA

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Physiology of Blood & Immune System

Physiol-09B12 Describe the production and function of red blood cells

1. Red blood cells are produced from haemopoetic pluripotent stem cells (HPSC) in the bone marrow. They are essential for the oxygen carrying capacity of the blood and as a buffer.

2. RBC production:
    a. Formation from pluripotent stem cells in the bone marrow which can differentiate into all blood line cells. The differentiation is stimulated by:
        i. Growth inducer factors – IL_3,
        ii. Stimulation inducer factors – erythrocyte CSF

$$\mathbf{HPSC} \Rightarrow \mathbf{BFU_E} \Rightarrow \mathbf{CFU_E} \Rightarrow \mathbf{erythroblast}$$

    b. Maturation from the erythroblast → RBC occurs in several stages in which the following changes occur:
        i. Mitosis
        ii. ↓cytoplasm
        iii. ↑ Hb content
        iv. Condensation nuclear content
        v. Extrusion of nucleus from pyknotic erythroblast to form a reticulocyte (circulates for 1-2 days)
        vi. RNA translated to protein from reticulocyte → erythrocyte

$$\mathbf{proerythroblast} \Rightarrow \mathbf{basophilic} \Rightarrow \mathbf{polychromatic\ E} \Rightarrow \mathbf{pyknotic\ E} \Rightarrow \mathbf{reticulocyte} \Rightarrow \mathbf{erythrocyte}$$

    c. Erythropoietin (EPO) is the hormone which controls RBC production. It is produced in the kidney (90%) and liver (10%) and promotes *differentiation* and *proliferation* of proerythroblasts → erythrocytes. It acts via tyrosine kinase linked receptor.

3. RBC Structure:
    a. Biconcave disc 2 x 7.5µm
    b. Membrane: bipolar lipid layer with 50% protein, 40% fat, 10% carbohydrate
    c. Haemoglobin: a metalloprotein weighing 65000 Da containing 4 polypeptide Haeme-globin chains.
        i. Haeme – synthesised in mitochondria by a series of reactions summarised:

$$\mathbf{glycine} + \mathbf{succinyl\ CoA} \Rightarrow \cdots \Rightarrow \mathbf{haeme}$$
$$\mathbf{Fe^{2+}} \nearrow$$

        ii. Globin – a polypeptide chain formed in ribosomes
        iii. Haeme + globin → globin chain
        iv. Types:
            1. Adult (HbA) – 2α + 2β
            2. Fetal (HbF) - 2α + 2γ

4. RBC function:
    a. O_2 carriage: each Fe^{2+} Haeme binds loosely to one O_2 molecule and each haemoglobin can carry 8 O-atoms. The binding of O_2 stabilises the αβ Haeme structure → relaxed state. The release of O_2 allows 2,3-DPG to enter the molecule and bind between the 2 β-chains.
    b. CO_2 carriage: each terminal amine group of the globin can form a carbamino compound with CO_2.

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$$\mathbf{HbNH_2 + CO_2 \Leftrightarrow HbNHCOO^- + H^+}$$

This allows CO_2 carriage which is more efficient when Hb is in the deoxygenated state (Haldane effect).

c. Buffering: Hb is a weak acid (pKa 6.8) and is the primary non-bicarbonate buffer of blood for acids. The imidazole groups of the histidine residues can bind with H^+. Its buffering capacity is raised because each Hb has 38 histidine residues.

$$\mathbf{H_2CO_3 \Leftrightarrow H^+ + HCO_3^-}$$
$$\mathbf{Hb + H^+ \Leftrightarrow Hb.H}$$

De-oxyHb is a better buffer than oxyHb. Thus deoxygenated blood facilitates CO_2 buffering. Acid environments cause displacement of O_2 from Hb to facilitate H^+ binding, releasing O_2, accounting for the Bohr Effect.

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Physiol-00A8 Briefly describe the breakdown of haemoglobin after red cell lysis.

1. The average lifespan for red cells is 120 days in the circulation. Given that the normal Hb content is 750g (150g/L x 5L), this equates to approximately 6g breakdown haemoglobin per day. There are several mechanism of red cell lysis:
    a. Intravascular lysis – haemoglobin escapes in the plasma and dissociates into α-β dimmers which are bound by haptoglobin for transport to the liver. Breakdown of Hb occurs in the liver.
    b. Extravascular lysis – aged RBCs are unable to maintain cell integrity and broken down in the spleen by the reticuloendothelial system. Haemoglobin is phagocytosed and degraded in the reticuloendothelial cells.

2. Structure of Haemoglobin: a metalloprotein weighing 65000 Da containing 4 polypeptide Haeme-globin chains.
    i. Haeme – synthesised in mitochondria by a series of reactions summarised:

$$\mathbf{glycine} + \mathbf{succinyl\ CoA} \Rightarrow \cdots \Rightarrow \mathbf{haeme}$$
$$\mathbf{Fe^{2+}} \nearrow$$

    ii. Globin – a polypeptide chain formed in ribosomes
    iii. Haeme + globin → globin chain

    Thus, haemoglobin consists for 3 parts: Haeme, globin and Fe^{2+}, each of which are broken down in different ways.

3. Breakdown of Components:
    a. Globin:
        i. degraded by macrophages → amino acids
        ii. amino acids reused for protein synthesis
    b. Iron:
        i. Released from Haeme oxygenase into circulation
        ii. Transported by transferrin → each molecule binds 2xFe^{2+}
        iii. Stored: Fe^{2+}→Fe^{3+} + apoferritin → ferritin within hepatocytes, GI mucosal cells and the reticuloendothelial system.
        iv. Reused as body's iron stores → bone marrow → Haeme synthesis
    c. Haeme:

$$\mathbf{haeme} \Rightarrow \mathbf{CO} + \mathbf{Fe^{2+}} + \mathbf{biliverdin}\ (\text{haeme oxygenase})$$

        i. Biliverdin → bilirubin (macrophage biliverdin reductase)
        ii. Bilirubin binds albumin and carried in plasma to the liver
        iii. Transferred via portal circulation to the liver, where bilirubin unbinds and enters the hepatocytes via an *active transport pump*.
        iv. Glucoronidation → bilirubin-glucoronide (conjugated).
        v. Excreted into bile canaliculi and to the gut as part of **bile**
        vi. In the small intestine, broken down into Urobilinogen (bacterial enzymes)
            1. 90% reabsorption into portal circulation for recycling (enterohepatic recycling)
            2. 5% converted stercobilinogen → faeces excretion
            3. 5% transferred from hepatocytes → systemic circulation → renal Urobilinogen excretion.

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Physiol-10A12/02B12/95B10 Briefly explain the changes that occur in stored whole blood

1. Whole blood contains RBCs, WBCs, platelets, plasma, proteins, coagulation factors, electrolytes and dissolved gases.
    a. 1 unit = 400-480mLs

2. Method of storage: aimed to maximise safe storage time (the time which allows > 70% survival of RBCs 24 hours post-infusion).
    a. Additives: CPDA1 (citrate-phosphate-dextrose-adenine) 63mLs
        i. Citrate - binds Ca^{2+} → anticoagulant
        ii. Phosphate – buffer, and provides phosphate for ATP synthesis
        iii. Dextrose – substrate for Embden-Meyerhof glycolysis ATP production
        iv. Adenine – substrate for ATP synthesis.

        Alternatively ACD (acid-citrate-dextrose) used
    b. Temperature: optimal 4°C
        i. ↓ rate metabolism, bactericidal, retains 2,3 DPG
        ii. Prevent freezing risk

3. Changes of constituents: known as the 'storage lesion' of blood

Constituent	**Change**
RBC	Cellular integrity diminishes with time (failure of Na/K ATPase) → spherical shape with ↑ rigidity. 79% survival 28 days. Post transfusion some spherocytes may recover normal shape
Free Hb	Haemolysis of RBC releases ↑free Hb (30% at 28 days)
2,3 DPG	Important for maintaining right shift O_2-Hb curve Produced via side-reaction of glycolysis (Rappoport Luebering shunt) Gradual depletion → left shift curve → P_{50} = 15mmHg Levels 5% at 28 days, Regenerates post-transfusion Depletion most significant in ACD storage (due to acidity)
WBC	Granulocytes lose phagocytic and bactericidal properties in 4-6 hours. Antigenic properties maintained Microaggregates → ↓macrophage activity, altered antigen presenting capacity
Platelets	Non-functional within 48 hours of storage Change shape discoid → spherical Massive transfusion causes coagulopathy due to dilutional thrombocytopenia
Coagulation factors	Factor V: ↓50% at 21 days factor VIII: exponential ↓75% 24 hours, 30% 21 days Normal haemostasis requires 35% factor VIII and 5-20% factor V.
Biochemical	↓ATP due to glycolysis and exhaustion of substrates ↑K: progressive impairment Na/K ATPase → ↑extracellular K 30mmol/L ↓ Na: due to ↓Na/K pump activity ↓ Ca: due to citrate ↓ glucose ↓pH: 6.7 after 28 days ↑ lactate
Bacteria	3/1000 contaminated by bacterial Pseudomonas, yersinia enterocilitica can grow at 4°C

Physiol-07B13 What are the physiological reasons making it safe to give O negative blood to patients

1. Blood transfusion involves the safe and compatible administration of RBCs from a donor to a recipient. The aim of which is to ↑O_2 carrying capacity of the blood by ↑amount of haemoglobin in RBCs.

2. Compatibility:
    a. Human blood contains many antigenic systems. The two most important are the ABO and Rh system.
    b. Incompatibility occurs when the transfusion recipient has antibodies to antigens in the donor blood. The immune reaction causes agglutination (cell clumping), cell haemolysis and a catastrophic transfusion shock syndrome.
    c. 30 other systems of blood antigens are known but do not induce a clinically significant incompatibility response → accounts for the common mild symptoms temp seen on transfusion.

3. ABO system:
    a. A set of antigenic carbohydrate chains expressed on the surface of RBCs and many other cells of the body (salivary glands, kidney, liver and lungs). The baseline antigen is H which codes for a transferase enzyme which places a fucose residue on a membrane glycophingolipid. The A and B antigens are derived from additions of carbohydrate chains onto the H antigen.

Group	**% Pop**	**Description**	**Antibodies**	**Donor compatilibiltiy**
O	43	H antigen – no A or B antigens.	A and B	Universal
A	45	N-acetylgalactosamine residue added to fucose end	B	Only to A or AB
B	9	Galactose residue added to fucose end	A	Only to B or AB
AB	3	Both A and B antigens present	None	Only to AB

    b. Individuals develop IgM antibodies to the antigens that are not displayed during the first decade of life (probably due to exposure to antigen in food or bacteria). Because type O blood has no antigens, it is safe to give in patient who have antibodies to A and/or B – hence it is part of the universal donor group.

4. Rhesus system:
    a. A set of antigens specifically displayed on RBC surface which consists of 3 alleles on chromosome 1, described by Dd/Ce/Ee. For the purposes of compatibility, only the D antigen has significant antigenicity such that individuals are classified into Rh positive (D antigen) or Rh negative (absence of D antigen).

Group	**% Pop**	**Description**	**Antibodies**	**Donor compatilibiltiy**
Rh +ve	85	D antigen (D)	Nil	Universal
Rh -ve	15	Absence of D antigen (d)	Anti-D upon exposure to D +ve	Only to A or AB

    b. Unlike the ABO system, Rh –ve people do NOT develop antibodies to D-antigen early in life. Upon exposure of Rh –ve person to Rh +ve, the individual develops IgM and then IgG antibodies. This most commonly occurs when Rh –ve mother exchanges blood with Rh +ve child. The antibodies to D will cause a haemolytic reaction if the 2nd child is Rh +ve. Hence, anti-D antibodies are given.
5. O negative blood can be safely transfused because it does NOT contain any of the significant A,B or D antigens that react with patient antibodies.

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Physiol-05B11/00B4 Outline the principles of compatibility testing of allogeneic (homologous) blood for transfusion

1. Blood transfusion involves the safe and compatible administration of RBCs from a donor to a recipient. Compatibility testing is carried out prior to transfusion to ensure clinical compatibility between the donor blood antigens, and the recipient plasma antibodies. This involves 3 major steps:
    a. Blood groups
    b. Antibody screening
    c. Cross-matching

2. Incompatibility: in the absence of compatibility testing, the following transfusion reactions can occur due to donor-recipient incompatibility –
    a. Haemolytic transfusion reactions
    b. Anaphylactic reactions
    c. Transfusion related acute lung injury
    d. Graft vs. host disease

3. Blood grouping: there are 2 clinically significant blood antigen systems which require compatibility grouping. Involves testing recipients **RBC antigens.**
    a. ABO system – defined by H, A and B polysaccharide antigens on the surface of cell membranes (fucose residues on membrane glycophingolipid). Individuals develop IgM antibodies to antigens they do not posses in the 1st decade of life.

Group	**% Pop**	**Description**	**Antibodies**	**Donor compatilibiltiy**
O	43	H antigen – no A or B antigens.	A and B	Universal
A	45	N-acetylgalactosamine residue added to fucose end	B	Only to A or AB
B	9	Galactose residue added to fucose end	A	Only to B or AB
AB	3	Both A and B antigens present	None	Only to AB
Rh +ve	85	D antigen (D)	Nil	Universal
Rh -ve	15	Absence of D antigen (d)	Anti-D upon exposure to D +ve	Only to A or AB

    b. Rh system – defined by D/d, C/e, E/e alleles on chromosome 1 in which the group is defined by the presence of absence of the D antigen. Rh –ve individuals only develop Anti-D antibodies upon exposure to Rh +ve blood. This of most significance in an Rh –ve mother with an Rh +ve child.
    c. Blood grouping – involves exposure of the recipients RBCs to serum containing known IgM anti-A, anti-B and anti-AB antibodies; and known IgG anti-D antibodies → observing for agglutination (cell clumping) *at room temperature in saline.* Agglutination suggests presence of the antigen and corresponding blood group.

4. Antibody screening: involves testing recipients **serum antibodies**
    a. Principle: there are over 30 other antigen blood systems which generally do not cause clinical incompatibility. Screening of recipient serum tests for presence of minor antibodies (e.g. Kelly, Duffy) which could lead to cell agglutination.

   b. Process: group matched RBCs with known minor antigens (Kelly, Duffy) are added to serum → agglutination suggests presence of minor antibodies → further Coomb's testing required.

5. Cross match: involves mixing a sample of the potential donor blood to the recipient serum and observing for agglutination.
   a. Saline Test: donor cells are suspended in saline and tested at room temperature with known IgM antibodies → reconfirms ABO type.
   b. Indirect Coomb's test:
      i. Principle: detection of IgG antibodies in the recipient serum which would cause RBC agglutination. These antibodies may be missed on initial screening. Used *only* on recipient blood that has positive antibody screen. Not used routinely.
      ii. Procedure:
         1. Incubation: of recipient serum with reagent RBCs → binding if IgG to their antigens on RBCs
         2. Washing: removal of serum (and all free IgG)
         3. Coomb's reagent: 'antiglobulin serum' which contains rabbit antibodies directly against human IgG → binds to IgG on RBCs → agglutination → positive Coomb's test.
   c. Major cross match: in-vitro testing recipient serum vs. donor RBCs
   d. Minor cross match: in-vitro testing recipient RBCs vs. donor serum (not used as antibody screening picks up irregular donor antibodies)

6. Summary: routine compatibility testing involves:
   a. Proper sample collection technique
   b. Patient identification
   c. Specimen handling and labelling
   d. Biochemical compatibility testing (As above)

Testing	**% safety**
ABO compatible	99.4
ABO + Rh compatible	99.9
ABO + Rh + antibody screen	99.94
ABO + Rh + antibody screen + Coomb's test	99.95

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MAKE-UP: Outline the complications of massive blood transfusions

1. Massive blood transfusion is defined as the transfusion of a volume of stored blood greater than the recipient's blood volume, in less than 24 hours.

2. Complications:

Complication	Description	Treatment
Volume-related		
Citrate toxicity	Citrate chelates Ca^{2+} → No Ca in stored blood Hypocalcaemia → tremor, ↓HR, widened QT, ↓contractility → cardiogenic shock	$CaCl_2$
Hyperkalaemia	Over time, ↓Na/K ATPase pump → ↑ extracellular K (up to 30mmol/L 28 days). Na/K ATPase pump usually active post-transfusion → usually not a problem. ↑ risk already hyperkalaemic patient	Usual treatment: Insulin / dextrose Ca-gluconate Resonium Dialysis
Acidosis	pH blood 2 weeks → 6.5 (lactate from glycolysis) Exacerbate existing acidosis Post-transfusion citrate → HCO_3^- Lactate → pyruvate (liver)	Bicarbonate
Hypothermia	Blood stored 4°C Each unit → ↓ patient temp 1°C Hypothermia → exacerbates L shift OHDC	Warm patient Warm room
2,3 DPG deficiency	2,3DPG depleted 5% at 28 days → L shift OHDC → ↓ delivery O_2 to tissues Rapidly replenished post-transfusion. CPDA preservative minimises this (buffers acid)	
Dilutional coagulopathy	↓ factor V, VIII → dilution patient coagulation factors. Platelets dysfunctional after 48 hours storage	Platelet and FFR transfusion
Volume overload	Exacerbated in patients with heart failure	Lasix
Idiosyncratic		
ABO incompatibility	Massive transfusion reaction → shock Intravascular haemolysis via IgG/IgM antibody-antigen complexes Urticaria, flushing, shock, haemolysis, circulatory collapse.	Stop transfusion
Graft vs. Host	Recipient blood contains antibodies against host antigens → mild immune response. Accumulation of donor lymphocytes in skin, GIT, liver.	Slow transfusion
Sepsis	Contamination, usually pseudomonas	Stop transfusion, antibiotics
TRALI	Acute onset non-cardiogenic pulmonary oedema Associated with FFP → ?immune mediated Antibodies against MHC in blood/FFP → sensitisation from previous transfusion	

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Physiol-07A10/99A2/95A7 Explain the mechanisms that prevent blood clotting in intact blood vessels (do not draw the clotting cascade)

1. Haemostasis is the physiological mechanism where blood is prevented from being lost from damaged vessels whilst allowing it to remain fluid in circulation. This is controlled by a delicate balance of pro-coagulant (1° and 2° haemostasis) and anti-coagulant systems.
    a. Procoagulation system is a bioamplification system involving activation of clotting substances which triggers a cascade of precursor enzymes. This can result in uncontrolled clotting unless the anticoagulant system is simultaneously activated.
    b. The anticoagulant system acts in mechanisms classified according to Virchow's triad:
        i. Endothelial surface factors
        ii. Blood flow factors
        iii. Blood constituent factors.

Property	**Mechanism**
Endothelial surface - thromboresistant	
No Collagen exposure	No binding site for vWF → no platelet adhesion No exposure of tissue thromboplastin → no activation of factor VII
Glycocalyx coating	Smooths endothelial surface → laminar flow Repels platelet and coagulation factors
PGI_2	Inhibits platelet aggregation (↑cAMP) Smooth muscle relaxation → vasodilatation → ↓vascular resistance → ↑ flow
NO (EDRF)	Inhibits platelet aggregation (↑cGMP) Smooth muscle relaxation → vasodilatation → ↓vascular resistance → ↑ flow
Thrombomodulin	Complexes with thrombin → activates protein C → inactivates factor Va, VIIIa + stimulated fibrinolysis
Heparan sulphate	Natural heparin → enhances ATIII activity → Inhibits IIa, Xa
TPA	Converts plasminogen → plasmin (activated by APC)
Blood flow	
Laminar flow	Minimises platelet contact time with endothelial surface (Axial streaming)
Dilution	Coagulant factors are washed away and diluted → removed via RES
Shear stress	Detaches weakly adhered platelets
Blood constituents: factors which inactivate the intrinsic, extrinsic and common coagulation pathways	
Coagulation factors	Circulate as inactive factors and generally require vessel wall damage to start coagulation cascade
Anti-thrombin III	Circulating protease inhibitors → inactivates factors IIa, IXa, Xa, XIa, XIIa Activity increased x 1000 by heparin Accounts for 70% plasma anti-coagulant properties
Extrinsic pathway inhibitor	Inhibits factor VIIa
α_2 macroglobulin	Inhibits IIa (thrombin)
α_2 antiplasmin, antitrypsin	Inhibits circulating serine proteases
Protein C + S	Thrombin-thrombomodulin → activates protein C Protein C + S adhere to platelet → activation of complex Complex inactivates factors Va and VIIIa Complex inactivates inhibitors of TPA → ↑TPA → ↑plasmin → fibrinolysis

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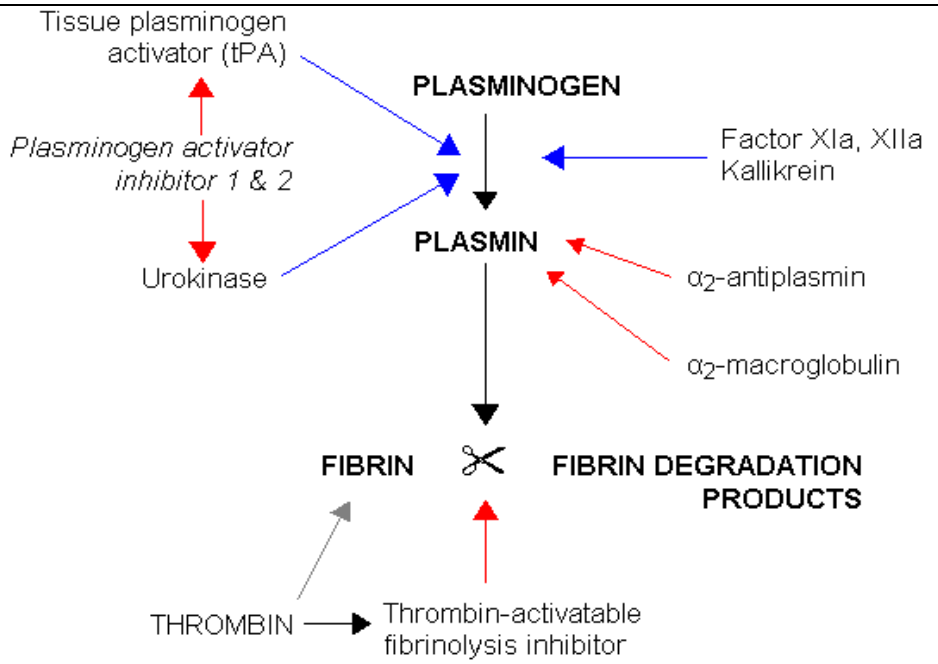
Fibrinolytic system	 Protein C (activated) → inactivates inhibitors of TPA → ↑TPA TPA: converts plasminogen → plasmin Plasmin: cleaves fibrin and fibrinogen → fibrin degradation productions (FDP) → lysis of fibrin plug.

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Physiol-06A11/97A6 Briefly outline the role of platelets in haemostasis

1. Platelets are small granulated cellular fragments which play an important role in the stages of haemostasis – the mechanism by which blood loss is minimised from damaged vessels whilst maintained fluidity of circulating blood.
    a. Derived from megakaryocytes
    b. Lifespan 7-10 days
    c. Normal level 150-300 x 10^6/mL

    The platelets play an important role in the 3 stages of haemostasis:
    d. Vasoconstriction
    e. Platelet plug (1° haemostasis)
    f. Blood clot (2° haemostasis)

2. Role of platelets:
    a. Vasoconstriction:
        i. Platelets release TXA_2 → potent vasoconstriction
    b. Platelet adhesion: the damage to a vessel wall exposes subendothelial substances which trigger haemostasis. Adhesion does not require metabolic activity.
        i. vWF binds to subendothelial collagen
        ii. Platelets adhere to vWF via linkage with glycoprotein 1b-IX (Gp1b-IX)
        iii. Facilitated adhesion via GpIa/IIa
    c. Platelet activation – release of factors
        i. Activation of platelets occurs by:
            1. Binding to vWF
            2. Factors: epinephrine, 5HT, ADP, thrombin
            3. Mediated via ↑PLC → ↑DAG, IP_3 → ↑PKC → ↑Ca^{2+} (required for activation)
        ii. Activation involves:
            1. Release of platelet dense granules → ADP, adrenaline, 5HT, Ca^{2+} → reinforce activation (positive feedback)
            2. Release of α-granules → fibrinogen, vWF, factor V m thrombospondin→ aggregation
            3. Change shape discoid → spherical with pseudopods
            4. Platelet contraction
    d. Platelet aggregation: this forms the primary platelet plug.
        i. Factors expose GpIIb/IIIa complex → platelet-platelet binding via fibrinogen
        ii. Formation of TXA_2 → vasoconstriction, platelet aggregation (via ↓cAMP)

$$\textbf{membrane phospholipid} \Rightarrow \textbf{arachidonic acid} \Rightarrow \mathbf{TXA_2}$$

        iii. ADP → positive feedback platelet aggregation (↑IIb/IIIa) and further ADP release
        iv. vWF, fibrinogen → released from α-granules stimulate adhesion
        v. thrombospondin: stimulates platelet aggregate

3. Procoagulant activity
    a. Platelet activation exposes platelet factor 3 (via ↑Ca^{2+}) which promotes coagulation pathways for secondary haemostasis:
        i. Intrinsic pathway reaction IXa, VIII, X → Xa
        ii. Common pathway reaction Xa, V, II → IIa (thrombin)
    b. Platelet activation releases α-granules:

   i. vWF: required for binding factor VIII preventing its degradation
   ii. factor V (common pathway)
c. Platelet contraction required for contraction of clot following fibrin haemostasis.

4. Anticoagulant activity: important to prevent disseminated coagulation
   a. PGI_2 acts on platelets → inhibit aggregation and activation
   b. Protein C, S → binds platelets → inactivate factors Va, VIIIa, inhibitors of TPA → ↑fibrinolysis.

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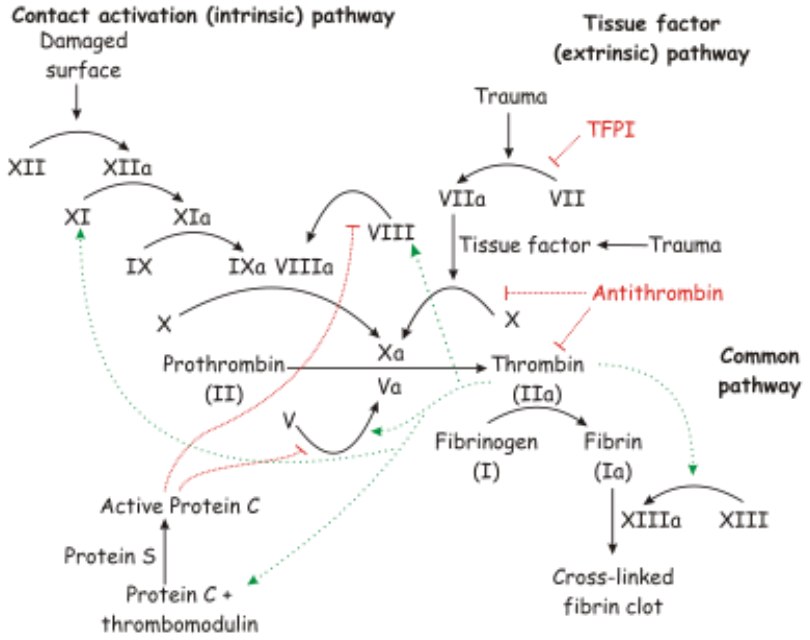
Physiol-01B7 Explain the main difference between the intrinsic and extrinsic pathways of coagulation

1. The coagulation pathway is a bioamplification system in which damage to a vessel wall triggers a cascade of activation of circulating precursor enzymes which convert soluble fibrinogen into fibrin. Fibrin is required to contract and stabilise the platelet plug (secondary haemostasis).

2. Coagulation pathways: there are 2 historical pathways which both end in a final common pathway – the activation of factor X → formation of fibrin.



3. Comparison table:

Property	**Intrinsic**	**Extrinsic**	**Common**
Importance	Less important in normal coagulation	More important in normal coagulation	Necessary for haemostasis
Activation	Contact with negatively charged surfaces in-vivo (collagen, subendothelium) and in-vitro (glass). Activates factor XII → XIIa	Tissue factor release, Ca^{2+} (via ADP, TXA) → activation factor VII → VIIa *Alternative pathway*: VIIa can activate IX → IXa. Thus, deficiencies in factors XII, XI do not cause bleeding disorders.	In-vivo: extrinsic pathway In-vitro: intrinsic (glass) pathway Inflammation: intrinsic
Factors	XII, XI, IX	VII	X, II
Co-Factors	PF3, Ca^{2+} , VIII	Ca^{2+}	V, PF3, Ca^{2+}
Speed	1-6 min (slow)	15 sec (fast)	
Investigations	APTT: contact activation by glass in the presence of PF3, Ca^{2+}	PTT/INR: activation time of thromboplastin (TF) in the presence of PF3, Ca^{2+}	Final clot formation: Thrombin (IIa) converts fibrinogen → fibrin monomer + fibrinopeptides. Monomers aggregate → soluble fibrin Thrombin converts XIII → XIIIa → forms insoluble fibrin.
Drug inhibition	Heparin: facilitates activity ATIII → inhibits Xa, IIa.	Warfarin: ↓ production of vitamin K factors II, VII, IX, X.	
Diseases	Haemophilia A – ↓ factor VIII Haemophilia B – ↓ factor IX Haemophilia C – ↓ factor XI		
Sepsis	↑ factor VIII production Intrinsic clotting becomes more important		

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Physiol-10A10 Describe how white blood cells defend the body against infection.

1. The white blood cells are produced from a common haemopoetic pluripotential stem cell and are important in protection against infection. They can be divided into:
    a. Phagocytes – neutrophils, basophils, eosinophils, monocytes
    b. Lymphocytes – lymphocytes, plasma cells

    These cells are important in both innate and acquired immunity, which work together in defence against infection.

2. Innate immunity: the natural immune mechanisms present at birth, lasting for life.

Cell	Role in defence	Time Course
Phagocytes – the process involves: Chemotaxis – margination along endothelium via adhesion molecules stimulated by cytokines from infection site (LTB_4, IL-8, C3b) and attracted by chemotactic factors (C5a). Phagocytosis – bacteria are coated with opsonins (C3b, CRP) and bound by neutrophil receptors → engulfment of organism by pseudopodia. Digestion – through Lysosomal enzymes → superoxide, H_2O_2, hydroxyl radicals.		
Neutrophils 62%	Dense nucleus containing azurophilic granules. Killing of bacteria and fungi.	Defence of invading organisms: 1st line: macrophages migrate (minutes) 2nd line: PMN migrate (one hour) 3rd line: monocytes released from blood and mature to macrophages 4th line: ↑ production and macrophages and monocytes.
Eosinophils 2.3%	Granules contain basic proteins toxic to parasites. Killing of parasites.	
Basophils 0.4%	Dark cytoplasmic granules containing heparin, histamine, serotonin Become mast cells in tissues Activation by C3a, C4a, C5a → degranulation → bronchial obstruction, itch, conjunctivitis, mucosal oedema	
Monocytes 5%	Become macrophages in tissues → differentiate as part of RES: - Kupffer cells in liver - Glial cells in CNS - Alveolar macrophages in lungs - Mesangial cells in kidney 2 roles: 1. Phagocytosis (innate) 2. Antigen presentation (acquired) – contain MHC II receptors for presentation of antigens to helper T cells.	
NK cells	Large granular lymphocytes Killing of tumour and viral infected cells without prior sensitisation → Cell mediated immunity. Two functions: - recognition of antigens on target cells - antibody-dependent toxicity (have Fc receptor)	

3. Acquired immunity: the 2nd line of defence in which the immune system acquires memory upon initial organism exposure, resulting in a more powerful specific defence response upon repeat exposure.

Lymphocytes: produced in bone marrow and thymus. 30% of white cell population. Divided into T and B cells which control cell-mediated and humoral immunity.	
T lymphocytes: arise from the thymus and circulate to rest in lymph glands of the body. Carry T-cell receptors (TCR) which recognise circulating antigens. 80% of lymphoid pool	
Cytotoxic T	Posses surface glycoprotein CD 8 which binds to MHC I molecules on all body cells. When stimulated → insertion of protein pores (perforins), injection of Cytotoxic substances

	→ target cell dies.
Helper T 10%	Posses surface glycoprotein CD4 which binds to MHC II molecules on antigen-presenting and B-lymphocyte cells. Non-phagocytic, Cytotoxic. When stimulated → release cytokines which regulate and stimulate all other aspects of the immune system: - macrophage phagocytic activity - Cytotoxic T-cell proliferation - B-lymphocyte proliferation and differentiation - positive feedback activation of other helper T-cells - differentiation into memory T cells Critically important in the immune response as other T and B cell responses are poor without helper T-cell stimulation.
Suppressor T	Regulatory lymphocyte which suppresses the immune response of other cells. Important in maintaining tolerance to self-antigens
Memory T	Differentiated from helper T-cells after stimulation by MHC II antigen. Persist in the body Upon subsequent exposure → rapid proliferation into Cytotoxic T-cells and release of cytokines to amplify an immune response.
B-lymphocytes: arise from the liver (foetus) and bone marrow (adults) and rest in lymph node germinal centres and MALT. Control humoral immunity. Require stimulation by helper T-cells to fully differentiate.	
Plasma cells	Exposure to antigen → differentiate specific plasma cells → produce antibodies (IgM/G/E/A/D) which bind to antigen. Antibody-antigen complex: - activation complement (classical pathway) → destruction - opsonisation for destruction by macrophages, neutrophils - chemically neutralize toxins
Memory B	Persist after initial infection is cleared, and reside in lymph nodes Activation by repeat binding or T-cells enables quicker antibody response

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Physiol-09B15/97B5 Describe the complement system.

1. The complement system is a proteolytic system presents in the plasma, and an important part of innate immunity. It consists of > 25 plasma proteins which are involved in a cascade of reactions culminating in amplified cell lysis and the acute inflammatory response. It consists of 2 pathways (classical, alternate) which end in a final common pathway.

2. Classical pathway:
    a. Consists of 11 enzymes
    b. Activation: antibody-antigen complexes (IgM, IgG), CRP (antibody independent)
    c. Pathway: activation C1 (C1q→C1r → C1s) → reaction with C2, C4 → C4b-C2a (C3 convertase) → cleaves C3 → C3a and C3b

3. Alternate pathway: does not require antibody activation
    a. Activation: Continuously active, accelerated by aggregated IgA, infection
    b. Pathway: C3 undergoes slow, spontaneous conversion → hydrolysed C3 → reaction with factor B and D → C3 convertase → cleaves C3 → C3a and C3b

4. Common pathway:
    a. Activation: classical and alternative
    b. Pathway:
        i. C3b (C5 convertase) splits C5 → C5a and C5b
        ii. C5b → combines with C6-9 → forms membrane attack complex (MAC)
        iii. MAC inserts itself onto cell membrane forming pores → cell lysis
        iv. C3a, C5a are anaphylatoxins → binds to granulocytes → activation

5. Function:

Function	**Mediators**
Cell lysis of bacterial cell	MAC
Solubilise antibody-antigen complex for easier breakdown	MAC
Release inflammatory mediators	C3a, C5a
Local vasodilation	C3a, C5a
Granulocyte chemotaxis and aggregation	C3a, C5a + C1-4
Opsonisation	C3b

6. Regulation:
    a. Some of the 25 proteins are regulators of the cascade to prevent uncontrolled inflammatory response.
    b. C1 esterase deficiency → hereditary angioedema
        i. Autosomal dominant
        ii. Danazol induces remaining C1 esterase synthesis from normal gene

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Physiol-05B12/98B6/90 Briefly discuss the physiological roles of plasma proteins.

1. Plasma is the fluid medium of the intravascular compartment which transports substances between tissues. The plasma protein are a group of globular molecules derived from amino acids which have numerous roles in the circulation. Most is produced in the liver except the immunoglobulins (macrophages, plasma cells), and they exist in equilibrium with tissue proteins.

2. Types of plasma proteins:

Type	**Amount (g/L)**	**Roles**
Albumin	45	Oncotic pressure – 80% Acid Base – 15% buffering of carbamino compounds Transport – bilirubin, Ca, hormones, free fatty acids, drugs Metabolism – intracellular breakdown into amino acids for catabolism
Globulins: α_1 α_2 β γ	25 10-15	Classified according to electrophoretic mobility α_1 antitrypsin – protease inhibitor, inhibits trypsin, chymotrypsin, plasma Lipoproteins – chylomicrons, VLDL, LDL, HDL → lipid transport α_1 acid glycoprotein – binding of basic drugs, acute phase reactant α_2 macroglobulin – protease inhibitor Prothrombin – coagulation Haptoglobin – scavenges free Hb (intravascular haemolysis) Caeroloplasmin – binds copper, acute phase reactant Transferrin – binds $2Fe^{3+}$ Immunoglobulins derived from B lymphocytes – IgG 76^, IgA 16%, IgM 7%, IgE 1%
Fibrinogen	3	Final step coagulation cascade → forms fibrin clot (thrombin, Ca^{2+}, factor XIIIa)
Others: Cytokines Complement CRP Coagulation		 IL, IFN, chemokines, lymphokines, TNF – mediators of immune response 25 proteins produced by liver, RES → Innate immunity amplification system Inflammatory mediator Biological amplification system → trigger coagulation cascade in vessel wall damage

3. Roles of plasma proteins: PROTEIM

Roles	**Description**
Proteolytic systems	Complement Coagulation Fibrinolytic Kinin systems
Role acid-base	15% protein buffering due to ionisation of imidazole groups of albumin Forms carbamino compounds with CO_2
Oncotic pressure	$\mathbf{NFP = k[(P_c - P_i) - \sigma(\pi_c - \pi_i)]}$ Capillaries are relatively impermeable to plasma proteins and exert an oncotic pressure of 25mmHg, holding fluid inside the capillaries. ↓ plasma protein → ↓π_c → interstitial oedema
Transport	Hormones ($T_{3/4}$) Drugs – competition for protein binding Metal cations Bilirubin, free fatty acids
Enzyme activity	Pseudocholinesterase
Immune response	Antibody production, cytokines
Metabolic	Amino acid breakdown for catabolic energy production

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