ORIGIN & IMPORTANCE OF BLOOD GROUPS

- **ABO Group**: the most antigenic of the blood groups
  - H substance = glycoprotein + L-fucose
  - A antigen = H substance + N-acetyl galactosamine
  - B antigen = H substance + D-galactose

  - The naturally occurring Anti A or B antibodies are IgM immunoglobulins, and can be present without previous exposure to blood in “O” positive individuals.
  - some people can develop anti-A or anti-B antibodies, but these are IgG
  - ABO antigens are present in the cell membranes of many cells in the body, not just red cells.

- **Rhesus system**: RhD antigen is a protein of molecular weight 30,000; its serological activity depends upon the amino-acid sequence & specific phospholipids; is the second most antigenic blood group
  - There are 3 pairs of alleles, Dd, Cc & Ee; 85% rhesus positive have the D antigen & this is the most antigenic
  - immune red cell antibodies can develop (IgG), most important being anti-D (Rh antibody which is acquired & warm), others include P, Lewis & MN which are low antigenicity & react at low temperatures
  - there are no naturally occurring Rhesus antibodies, development requires previous exposure
  - Rhesus antigens are present only on the red blood cell

**Cross-Matching Blood**:
Compatibility testing requires
- blood grouping, i.e. determination of the recipient’s ABO & Rhesus status, achieved by adding a reagent known to contain a specific antibody, and suspend in saline, observing for agglutination (saline agglutination test). ABO compatible blood 99.4% relative safety; ABO and Rhesus 99.8%.
- screening for the presence of other antibodies in the recipient serum, to other red cell antigens; tested for by adding a panel of red cells of the same ABO & Rhesus group but which are known to express other antigens (e.g. Kell, Duffy, Lewin, Kidd) to the recipient’s serum, and observing for agglutination. A Coomb’s reagent may be used. ABO, Rhesus and negative antibody screen 99.94% relative safety. These antibodies only form after prior exposure to the antigens, and subsequent exposure will then result in adverse reactions.

- cross matching is the next test, mixing a sample of the potential donor’s red blood cells with the recipient’s serum, and observing for agglutination. This is the major cross match, and the minor cross matching involves the mixing of donor serum & recipient cells.

- An indirect Coomb’s test is the final to check for IgG antibodies which would not cause agglutination in the direct testing, but which may still cause a transfusion reaction. ABO, Rhesus and negative antibody screen plus Coombs test 99.95% relative safety.

Tests
a. saline agglutination test → RBC + antibodies at room temperature, which detects IgM antibodies (complete) such as anti-A, anti-B, anti-Lewis & anti-M
b. albumin & papain → remove positive charges of sialic acid on the cell surface
c. LISS → low ionic strength saline
d. Coombs test (antiglobulin) → detects incomplete IgG (rhesus) & IgM (Kidd & Duffy) antibodies
e. Indirect Coomb’s test - aims to detect the presence of any IgG antibodies directed against red blood cell membrane antigens which could cause reactions if transfused. These IgG antibodies do not cause agglutination by themselves in the other testing but can still cause problems if transfused, hence they are tested for using the indirect Coomb’s test.

The first step is incubation to bind any IgG antibodies to their antigens on the red cell membrane. Then the cells are washed to remove any unbound IgG as it could cause a false positive result. Coomb’s reagent is then added. The reagent is serum that contains rabbit IgG antibodies directed against human IgG antibodies. These antibodies bind to any IgG present on the red blood cells and binds multiple cells together, causing agglutination. Agglutination is a sign of a positive Coomb’s test, i.e. the presence of IgG.

The final step is to double check the above reaction, and involves the addition of Coomb’s control cells to a negative solution left from the previous action. The control cells are coated with IgG and if the Coomb’s reagent was active, will now cause agglutination, thus the Coomb’s test is truly negative. If the Coomb’s reagent was inactive, there will be no agglutination at this step, and need to repeat whole test again.

CONSTITUENTS & FUNCTION OF PLASMA
Constituents: 5% of body weight; 40-50mL/kg (3500mL in 70kg person)
90% of plasma is water, 8% leukocytes & platelets, 2% organic compounds (phospholipids, cholesterol, fat, glucose, urea, uric acid, lactic acid, creatinine, bilirubin, and bile salts) & electrolytes

Function of plasma –
- Supplies nutrients & removes waste products to & from tissues
- Dissipates heat generated by oxidative reactions
- Distributes hormones
- Transports white cells & antibiotics
- Transports platelets & coagulant proteins to stop bleeding

Difference between plasma & serum – serum has no coagulation factors
Serum is the fluid expressed from a blood clot as it contracts, and thus has no fibrinogen & very little other coagulation factors. Serum thus cannot clot, while plasma can.
**Plasma proteins**: comprise 50-80g/L; major classes are albumin (45g/L), globulins (25g/L – immunoglobulins 10-15g/L of this) & fibrinogen (3g/L)

**Function**
- plasma oncotic pressure, involved in the balance of Starling's forces (plasma proteins contribute to 19mmHg, and the further 4-6mmHg from electrolytes retained in the intravascular compartment due to Gibbs-Donnan equilibrium)
- carrier/transport function – albumin a low-specificity high-capacity carrier that is involved in the transport of bilirubin, calcium, free fatty acids, hormones (cortisol, thyroxine), copper & drugs (mainly acidic); alpha-1 acid glycoprotein (globulin) transports mainly basic drugs
- acid-base balance – proteins involved in buffering acids in the blood; carry carbon dioxide as carbamino compounds on the N-terminal amino group
- immune function – immunoglobulins & cytokines, complement
- fibrinolytic & coagulation systems
- energy substrates for metabolism in cells
- proteolytic systems

**PLATELETS & THEIR ROLE IN COAGULATION**
- **Platelet Production**

\[ 
\text{PLURIPOTENT STEM CELL} \quad \xrightarrow{\text{CFU}} \quad \text{megakaryoblast} \quad \xrightarrow{\text{megakaryocyte}} \quad \text{granular megakaryocyte} \quad \xrightarrow{\text{fragmentation}} \quad \text{PLATELETS} 
\]

- **Platelet Structure**
Peripheral –
a. GLYCOCALYX - (external coat) 150-200nm thick with glycoprotein receptors (a large carbohydrate tail forms the surface coat)

<table>
<thead>
<tr>
<th>Type</th>
<th>Complex</th>
<th>Function</th>
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<tbody>
<tr>
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<td>IIa</td>
<td>collagen receptor for adhesion</td>
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<tr>
<td>Ia</td>
<td>IX</td>
<td>VWF receptor for subendothelial adhesion</td>
</tr>
<tr>
<td>Ic</td>
<td>IIa</td>
<td>fibronectin receptor</td>
</tr>
<tr>
<td>Iib</td>
<td>Illa</td>
<td>receptor for fibrinogen, VWF, fibronectin &amp; vitronectin</td>
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<tr>
<td>IIIa</td>
<td>Iib</td>
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</tr>
<tr>
<td>IV</td>
<td></td>
<td>thrombospondin receptor</td>
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<tr>
<td>V</td>
<td></td>
<td>thrombin substrate for aggregation</td>
</tr>
<tr>
<td>IX</td>
<td>Iib</td>
<td>adhesion of platelets to subendothelial matrix</td>
</tr>
</tbody>
</table>

b. UNIT MEMBRANE PHOSPHOLIPIDS –
c. SUBMEMBRANE – translation of signals

Soc-Gel Zone –
→ Cytoplasmic matrix
→ Microtubular system (contractile role) – marginal bundle of 12 filaments at equator of platelet
  - polymerised tubulin which maintains asymmetry of cell
  - at aggregation, the tubular system is depolymerised to filaments
→ Microfilament (provides support via α polymers) – short actin filaments associated with GP Ib-IX, Ia, Ila, which stabilise the platelet membrane & shape
  - on activation, pseudopod extension due to activation of actin, α-actin, tropomyosin & actin-binding protein – causes a spiny platelet
  - central contraction then occurs, with granules squeezed into centre
→ Glycogen (storage function)

Membrane Canaliculi –
a. dense tubular system (calcium & prostaglandins) – narrow tubules randomly distributed in cytoplasmic matrix derived from endoplasmic reticulum
  - functions
    i. enzymes – peroxidase & G-6-phosphatase
    ii. prostaglandin synthesis – PG endoxide synthetase & phospholipase A
    iii. contain & regulate cytoplasmic level of calcium via Ca pump & Ca/Mg/ATPase (which controls the polymerisation of the microtubular system, causing a discoid shape & contractile action)
b. open (surface) canalicular system (increases surface area)
  → transport pathway for uptake of solutes & small particles (e.g. ferritin) by endocytosis
  → excretory route for release of various substances

Organelles –
→ α granules – round, membrane bound granules 0.15-0.4µm, forming 10% volume of platelets, containing:

<table>
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<th>function</th>
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<tbody>
<tr>
<td>Platelet Specific Proteins</td>
<td>endothelial antithrombosis; chemotaxis; heparinoid neutraliser, increases aggregation &amp; secretion</td>
</tr>
<tr>
<td>platelet factor 4</td>
<td></td>
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</table>
Platelet derived growth factor: mitogenesis & vessel repair  
Beta-thromboglobulin: chemotaxis  
**Coagulation Factors**  
VonWillebrand factor: adhesion & aggregation  
Fibrinogen: adhesion & aggregation  
Factor V: prothrombinase  
**Others**  
Thrombospondin: aggregation  
Fibronectin: fibroblast & platelet adhesion  
Plasminogen activator inhibitor: inhibit fibrinolysis

→ dense bodies – 0.17µm diameter

<table>
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<tr>
<td>ADP</td>
<td>aggregation &amp; vasoconstriction</td>
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<tr>
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</tr>
<tr>
<td>5HT</td>
<td>vasoconstriction &amp; aggregation</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>aggregation &amp; vasoconstriction</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Pyrophosphate</td>
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</table>

→ mitochondria  
→ lysosomes

- **Role of Platelets:**
  - **Physiological** –
    → Haemostasis  
    → Endothelial support (re-endothelial growth via platelet-derived growth factor)  
    → Detoxifies serotonin  
    → Phagocytosis  
  - **Pathological** –
    → Inflammatory reactions – increases permeability; chemotaxis; proteases  
    → Transplant rejection  
    → Thrombosis
Normal Haemostasis: three primary events

1. **Haemostatic response** – platelet plug formation & vascular response
   - vessel wall response – platelets form pseudopods & release ADP, serotonin & thromboxane A2 (which vasoconstricts)
   - vonWillebrand factor also enhances platelet adhesion

2. **Coagulation Mechanisms** – formation of tough fibrin clot, which stops bleeding & initiates tissue repair

3. **Fibrinolysis** – removal of fibrin which re-establishes blood flow after healing
Intrinsic Pathway: physiological role in coagulation in vivo is questionable, but is definitely important in interaction of blood with foreign surfaces (e.g. in cardiopulmonary bypass). Named “intrinsic” as theoretically components are already present in circulating blood.

Factor XII appears to bind to negatively charged surfaces, leading to its activation catalysed by high molecular weight kininogen & kallikrein. This may be brought about by the exposure of blood to the negatively charged collagen fibres & platelet factor 3 underlying endothelium in blood vessels.

The intrinsic pathway contributes to inflammatory responses, complement activation, kinin formation & fibrinolysis.

Activated factor XII catalyses the activation of factor XI, which catalyses activation of factor IX. Factor X (which is the start of the common pathway) is activated by IXa with factors VIIIa, V, calcium & platelet factor 3 as cofactors. Factor Xa then catalyses conversion of prothrombin (II) to thrombin (IIa) which catalyses fibrinogen (I) to fibrin (Ia). Fibrin is converted to a stable fibrin monomer by the cofactor XIIIa.

Activated protein C (with protein S as cofactor) inhibits activated factors V & VIII, decreasing the production of thrombin.

The intrinsic pathway can be tested for using APTT & ACT.

Deficiencies in factors XII, XI, prekallikrein & HMWK lead to increased APTT, but are not associated with clinical bleeding (hence the theory of relative unimportance in vivo). Factor IX deficiency however is associated with clinical bleeding as well as abnormal APTT.

Extrinsic Pathway: appears the more important system in vivo, and is named extrinsic as begins with traumatised tissue, which is extrinsic to the vessel.

Tissue factor (thromboplastin or factor III) is the cellular receptor & cofactor for factor VII. Its expression is highly cell specific, being on adventitial cells surrounding blood vessels, and epithelial cells of the renal glomerulus & epidermis. Tissue factor can also be induced on endothelial cells by inflammatory mediators such as endotoxin, interleukin-1 & TNF-α.

The extrinsic system is important in activating coagulation in the normal response to vascular injury, and in “inflammatory states” such as septic shock & malignancy.

Coagulation is initiated by the exposure of blood to tissue factor. This causes activation & binding of factor VII which catalyses the conversion of factor X to its activated form, using calcium ions as cofactors. This bypasses the need for the factor VIIIa/IXa complex of the intrinsic pathway. The main effect of this pathway is to generate thrombin, which then feeds back to the intrinsic pathway, activating & amplifying the system (activated factors IX & VIII).
Haemophiliacs who lack factor VIII still have normal extrinsic coagulation cascade, and as the brain has a substantial amount of tissue factor, the extrinsic cascade can prevent cerebral haemorrhages, however the lesser amount of this in their joints mean this is the major site of bleeding.
NORMAL MECHANISM OF PREVENTING THROMBOSIS

Haemostasis depends on a fine balance of procoagulant & anticoagulant factors. To prevent clot formation in the intact blood vessel, these factors err on the anticoagulant side →

Flow & Streaming of Blood: flow in vessels is normally laminar, and this decreases turbulence, and causes axial streaming of platelets, minimising their endothelial contact, thus minimising activation.

High blood flow dilutes activated intermediates of coagulation & disperses loose aggregates of platelets, thus limiting the generation of thrombin. The coagulation factors are normally present in the inactive form, but once activated, are removed by blood flow & broken down in liver & reticuloendothelial system

Endothelial Lining: non-thrombogenic & anticoagulant, yet when damaged is pro-coagulant.

Intact blood vessel has a smooth endothelial surface with a mucopolysaccharide glycocalyx layer which prevents contact activation of platelets & coagulation factors.

Endothelial cells produce prostacyclin & nitric oxide which inhibit platelet aggregation & cause vasodilatation (thus not inhibiting blood flow).

The luminal surface of endothelium contains heparin sulphate which binds antithrombin III; AT III is a major inhibitor of factors IXa, Xa, Xla, XIIa & IIa.

Endothelial cells also contain thrombomodulin which binds thrombin (thus slowing the coagulation cascade amplification) & the complex activates protein C, inactivating factors Va & VIIIa.

Anticoagulant Factors in Blood: clotting factors exist in an inactivated form and are washed away when activated, and inactivated by the liver & reticuloendothelial system (phagocytic cells in liver remove particular thromboplastins from blood, and hepatocytes can degrade activated coagulation factors)

Thrombomodulin prevents clotting by complexing with thrombin which has diffused away from the clot, and this complex then activates protein C, with protein S as a cofactor (which inactivates factors Va & VIIIa), and stimulates fibrinolysis.

Antithrombin III (which is accelerated by binding heparin sulphate) inactivates factors IXa, Xa, XIa, XIIa, IIa & activates protein C as well.

α2 macroglobulin & α1 antitrypsin inactivate thrombin.

If a clot is formed, the fibrinolytic process is activated → tPA & plasminogen are activated by any process which triggers coagulation cascade; protein C inactivates plasminogen activator inhibitor-1, which stimulates fibrinolysis. This system limits the further development of clot, and breaks down that which has already formed.

FIBRINOLYSIS

Fibrinolysis is clot lysis to restore blood flow:

1. Plasminogen circulates & locates clot, where it is incorporated & activated to plasmin
2. Plasmin cleaves fibrin & fibrinogen to FDP; it is inactivated by anti-plasmins so it does not circulate
3. Fibrin Degradation Products prevent cross-linking of fibrin & inhibit platelet function (half life 9 hours)
ASSESSING COAGULATION, PLATELET FUNCTION & FIBRINOLYSIS

- **Platelet Function**:
  - Platelet count
  - Platelet aggregation to adrenaline via aggregometry
  - Skin bleeding time (2-9 minutes)
    → Not a very dependable test –
      - highly operator dependent;
      - horizontal & vertical incisions different
      - increased time with skin fragility

- **Coagulation**:
  - *Intrinsic & Common Pathway* –
    1. ACT (normal 90-120 seconds)
      → fresh blood & kaolin/celite to activate factors XII & XI
      → utilised in cardiac surgery to assess adequacy of heparin
    2. APTT (normal 25-35 seconds)
      → citrated blood & rabbit brain phospholipid, and added kaolin (increases surface area)
      → measures all factors except VII
  - *Extrinsic & Common Pathway* –
    1. Prothrombin time (normal 10-12 seconds)
      → tissue thromboplastin & calcium
      → measures VII, X, V, II, I activity
  - *Common Pathway* –
    1. Thrombin time (normal 14-16 seconds)
      → measures conversion of fibrinogen to fibrin

- **Fibrinolysis**:
  1. Fibrinogen (normal 300mg/100mL)
     → significant if less than 100mg/100mL
  2. FDP (normal <10µg/mL)
     → DIC if >20µg/mL
PHYSIOLOGICAL CONSEQUENCES OF ACUTE & CHRONIC ANAEMIA

• Anaemia is when Hb falls below 2 standard deviations of the mean for a normal population (140 for males, 120 for females at sea level)
  - when fully oxygenated, each gram of haemoglobin carries 1.34mL of oxygen
  - 100mL of normal arterial oxygenated blood with Hb 150, carries 21mL oxygen (of which 4.5mL are delivered to the tissues)
  - if Hb is half normal (75) 100mL blood carries only about 10mL oxygen

• Compensatory Mechanisms: increased extraction of oxygen by the tissues & peripheral vasodilatation to increase tissue blood flow
  - Acute –
    → INCREASED 2,3-DPG – shift oxygen-haemoglobin dissociation curve to the right, increasing the amount of oxygen released from each gram of haemoglobin at normal capillary oxygen pressures
    → INTERNAL REDISTRIBUTION BLOOD FLOW – increases flow to tissues where oxygen supply must be maintained, causing shunting away from splanchnic bed (anorexia & indigestion), from genitals (abnormal menstruation & impotence) and skin (pallor; hypersensitivity to cold)
    → DECREASED BLOOD VISCOSITY – this combined with local vasodilatation decreases total peripheral resistance
    → INCREASED CARDIAC OUTPUT – to compensate for decreased TPR; increased stroke volume; cardiac output curve is shifted up and left, and venous return curve up
    → SODIUM & WATER RETENTION – due to drop in MAP, which stimulated renal retention of sodium & water, increasing plasma volume
  - Chronic –
    → ERYTHROPOIETIN PRODUCTION – 85% from endothelial cells of peritubular capillaries & 15% from live Kupffer cells; increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors (subsequently over 2-3 days, numbers of erythrocytes increase)

PRODUCTION OF BLOOD CONSTITUENTS

• Haemopoiesis: originate from common pluripotent haemopoietic stem cell (PHSC)
  - Development –
    a. intrauterine – 3-4 weeks, mesenchymal cells in the yolk sac; 6 weeks-7 months, liver & spleen; 6-7 months, bone marrow
    b. extra-uterine – first 5 years, bone marrow of long bones; 18-20 years, confined to the marrow of the vertebrae, pelvis, ribs, sternum, skull & proximal end of femur and humerus
  - precursors of PHSC are “colony forming units”
  - haemopoietic growth factors amplify cell division, differentiation & maturation (glycoprotein, colony stimulating factor, erythropoietin, interleukin & TNF)
Red Blood Cell:

- **Erythropoietin** – glycoprotein derived from kidney peritubular cells (90%) & liver centrilobular cells (10%) with a half life of 3-8 hours
  - Production is stimulated by decreased PaO2, O2 content, beta sympathetic activity, and local mediators (PGE1, PGE2)
  - Acts to increased mitosis of burst forming units, and increase DNA in erythroblasts
- Red blood cells are biconcave, with a large "surface area : volume" ratio; membrane consists of a bipolar lipid membrane with proteins
- Destroyed in the reticuloendothelial system, especially in the spleen

White Blood Cells:

- **Neutrophils** – consists of a dense nucleus with acid phosphatase, myeloperoxidase, esterase & lysozymes
  - 2 pools – circulating (half life 6-12 hours) & marginating (attached to vascular endothelium)
- **Eosinophils** – circulate for 3-8 hours, then enter tissues & epithelial lining
  - Contain histaminase
- **Monocytes** – develop from CFU_granulocyte/monocyte
→ Half life 20-40 hours
→ Found in reticuloendothelial system
→ Function – phagocytosis, & production of monokines (interleukin, prostagladin, TNF & IF)
→ Defence via chemotaxis, opsonisation & pinocytosis

- Basophils – dark cytoplasmic granules, containing heparin, histamine, serotonin
  → Become mast cells in tissues

- Lymphocytes –
  → T-LYMPHOCYTES –
    . helper (CD4 & HLA_D)
    . suppressor (CD2, 3, 8 & HLA_A&B)
    . cell-mediated immunity provided via lymphokines, i.e. IL2, IL3, TNF
  → B-LYMPHOCYTES – provide humoral immunity via natural killer cells & ADC
    (antibody dependent cytotoxic)

CONSTITUENTS OF BLOOD PRODUCTS & THEIR SOURCE, ROLES & RISKS

Blood Products :
1. Platelet concentrates – pooled for 4-6 units (6 x 10^6 platelets) in special polyelfin packs
   → constant agitation at 20-26°C
   → contains antigens HLA, ABO & Rh
2. Fresh frozen plasma – II, VII, IX, X rich
   → shelf life 1 year at -30°C
3. Cryoprecipitate – -70°C at separation
   → rich in factor VIII, fibrinogen, fibronectin
   → stored at -30°C
4. Freeze dried factor VIII & IX

Preservatives :
- CPD & CPD adenine – 63mL (citrate, phosphate, dextrose)
- Sodium citrate - 1.6g
- Dextrose – 1.46g
- Citric acid – 2.1 mg
- Sodium phosphoric acid – 1.6mg
- Adenine - 0.3mg

Risks of Blood Transfusion

Blood transfusion can cause autoimmune reactions via IgM antibodies to A and B antigens causing type II hypersensitivity reaction. Antibodies against donor leukocytes and cytokines can produce transient fever and rash.

Blood transfusion can also be associated with a risk of infection – the risk is 1:34,000. Asymptomatic donor bacteraemia can be a significant problem, and viral donor screening is not completely effective in detecting window period infections. There are microaggregate filters which can be used against viraemia and bacteraemia.

Hypothermia and circulatory problems can also result from blood transfusions. Transfusion-related acute lung injury is through to be mediated by complement-activating leucoagglutinating HLA-specific antibodies. Transfusions can also contribute to volume overload, hypocalcaemia, hyperkalaemia, coagulopathy and hypothermia.
Massive transfusion (replacement of more than two times the blood volume in less than 24 hours) is associated with more hazards. Can result in dilution of platelets and coagulation factors. The citrate anticoagulant used as a preserver can cause hypocalcaemia, especially in liver disease (poor citrate detoxification) Transient hyperkalaemia can result (usually short lived as quickly enters cells) and acidosis. This can be followed by metabolic alkalosis as citric acid is metabolised to pyruvate and bicarbonate. Profound hypothermia can result from massive blood transfusion, therefore best to use warmers.

**CHANGES DURING BLOOD STORAGE & PROBLEMS OF MASSIVE BLOOD TRANSFUSION**

- **Changes in Stored Blood**:
  5. RBC – become spherical; metabolic survival reduced
  6. WBC – lose phagocytic & bactericidal action within 4-6 hours; antigenic properties
  7. platelets – non-functional within 48 hours
  8. factor V – linear decrease to 50% at 21 days
  9. factor VIII – exponential decline in quantity → 75% at 24 hours; 30% at 21 days
  10. ATP – reduced by 75% at 28 days
  11. 2,3-DPG – decreased by 50% at 14 days; less than 5% left at 28 days
  12. K+ - progressive rise to 28-30 mmol at 30 days

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<td>12</td>
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- **Complications of Massive Blood Transfusion**:
  1. *Citrate toxicity* – if transfusion exceeds 1L in 10 minutes, or rapid exchange transfusion → manifests as prolonged QT interval & ST segment, and bradycardia
  2. *Hyperkalaemia* – not usually a problem as K+ diffuses into red blood cell, unless acidosis & hypotension exist
  3. *Acidosis* – initially occurs, but later can lead to alkalosis
  4. *Hypothermia* – VF can occur at 28-30°C → oxyhaemoglobin dissociation at low temperatures
  5. 2,3-DPG – less in transfused cells, and theoretically this will decrease peripheral oxygen unloading, however in studies this has been not shown to cause any tissue hypoxia; levels will improve by 6 hours, and normalise by 24-48 hours
  7. *Microaggregates*
  8. reactions – → immunological – rare
    a. immediate – due to ABO antibodies, caused by IgM & IgG; causes RBC haemolysis ++
    b. delayed – incomplete Abs to RBC (Kidd, Diffy, Rh, & Kell groups)
    c. white cells
    d. graft versus host reactions
e. plasma proteins
f. adult respiratory distress syndrome — cause, but related to massive transfusion
→ non-immune – septicaemia, hepatitis, CMV, HIV

**BREAKDOWN OF HAEMOGLOBIN**

**Formation of Haemoglobin:**

Begins in the proerythroblasts, and continues for some time in the reticulocyte stage. Heme is formed mainly from glycine & acetic acid in the mitochondria. Acetic acid is changed in the Kreb’s cycle to succinyl Co-A, and two of these molecules combine with two glycines to form a pyrrole compound. Four pyrrole compounds combine to form a protoporphyrin compound. The protoporphyrin combines with iron to form the heme molecule.

Each heme molecule combines with a long polypeptide chain called a globin, which is synthesised from amino acids by ribosomes. This heme & globin forms a haemoglobin chain, which is either alpha, beta, gamma or delta, and has a molecular weight of ~16,000. Four of these haemoglobin chains bind loosely together to form the haemoglobin molecule, with a molecular weight of 64,458.

There are variations in the subunit haemoglobin chains depending on the amino acid sequence of the polypeptide portion. Adult haemoglobin involves a combination of alpha & beta chains; foetal haemoglobin alpha & gamma chains.

**Breakdown of Haemoglobin:**
ABNORMAL HAEMOGLOBINS & THEIR CLINICAL SIGNIFICANCE

- In an adult, 96-98% circulating haemoglobin is haemoglobin A (2α & 2β chains)
  - Adults have small amounts of Haemoglobin A2 (α2δ2) & HbF (α2γ2)

- Haemoglobinopathies:
  - HbS, the α chains are normal, but the β chains have one glutamic acid residue replaced by a valine residue in the β polypeptide chain, due to mutation of adenine to thiamine in position 2 of the sixth codon of the β globulin
    → HbS is very insoluble at low oxygen tensions, and red cells shift from bioconcave discs to sickle-shaped and haemolyse

- Thalassaemias: defect in globulin synthesis but the actual polypeptide chains are normal
  - β major – severe anaemia in neonates & death unless transfused
  - β minor – mild hypochromic microcytic anaemia
  - α - 4 varieties
**KAM’S QUESTIONS**

1. OUTLINE THE DIFFERENT BLOOD GROUPS AND WHAT DETERMINES THEM.

   The term “blood groups” refers to the various groups of genetically determined antigens present in the membranes of red blood cells. The importance of the blood group depends upon its antigenicity. The most antigenic groups are the ABO system and the Rh system. These are the most likely to cause a transfusion reaction if incompatible red cells are transfused.

   There are three antigens in the ABO system, A, B and H.
   The H antigen consists of a fructose residue on the end of a membrane glycoprophospholipid. Individuals with just H antigen are grouped as “O”. They have circulating anti-B and anti-A antibodies, which they may have without prior exposure to blood.
   The A antigen consists of a N-acetyl galactosamine residue on the end of the H antigen.
   The B antigen consists of a galactose residue on the end of the H antigen.
   The ABO antigens are present not only on red blood cells, but on the cell membranes of many other cells in the body. Naturally occurring antibodies (anti-A & anti-B) can exist without prior exposure, usually IgM. IgG antibodies can develop on exposure to unmatched blood or tissue.

   The Rhesus system is the next most important group in terms of antigenicity. Each person will have up to 4 Rhesus genes due to two closely related loci (“D’ and “CcEe”) on chromosome 1. The Rhesus status of a person can either be described based on the Rh antigens present, or Rh positive or negative based on the D antigen status. The D antigen is the most antigenic of the common Rhesus antigens.
   There are no naturally occurring antibodies to the Rhesus antigens, and development of them requires prior exposure to the antigen.
   Rhesus antigens occur only on the red blood cell.

   Other blood groups include MNS, P, Lutheran, Lewis, Kell and Duffy, the genes controlling them being carried on pairs of chromosomes.

2. WHAT IS THE RH SYSTEM? HOW DO YOU ASSESS THE SYSTEM AND OUTLINE ANY PROBLEMS THAT MAY BE ASSOCIATED WITH RH INCOMPATIBILITY

   The Rhesus system is the second most important blood group in terms of antigenicity. There are two closely related loci on chromosome 1 that are identified as D and CcEe. They produce antigens D, d, C, c and E, e. The “d” antigen is really the absence of the “D” antigen, as no anti-d antibodies have been described.

   The Rhesus status of a person is either based on the antigens present (e.g. CDe) or as either “Rh positive” or “Rh negative” based on the D antigen status. The D antigen is the most antigenic of the Rhesus system.

   The Rhesus antigen exists only on red blood cells (as compared with ABO which are present on many cell membranes in the body). There are no naturally occurring Rhesus antibodies, therefore development of the antibodies requires exposure to the antigen.

   Rhesus antigen is tested for using the indirect Coombs test. The blood is incubated to bind IgG, washing to remove the unbound antibodies, then testing with the Coomb’s reagent which will cause agglutination of IgG antibodies to bound IgG if positive.
3. WHAT IS A CROSS MATCH PROCEDURE? ELABORATE ON THE TESTS USED. WHAT IS A COOMBS TEST?

Cross matching involves a trial match of the recipient's serum against the donor's washed cells. The total testing takes 30-60 minutes. The testing aims to avoid serious transfusion reactions by testing compatibility of donor to recipient blood.

Phase I of the test involves an ABO test, and check against P, MN and Lewis systems. This takes 1-5 minutes. The saline agglutination test is used for this phase, mixing red blood cells and antibodies at room temperature. It detects the IgM antibodies against ABO and anti-Lewis and anti-M.

Phase II of the crossmatch involves incubation with Rhesus antibodies over 20-30 minutes. Mixing with albumin and papain removes the positive charges of sialic acid on the cell surface. Low ionic strength saline can be used instead. The Coombs antiglobulin test can then be used to detect IgG and IgM antibodies.

Phase III involves indirect antiglobulin tests for antibodies in Kidd, Kell and Duffy systems.

The indirect Coomb’s test aims to detect the presence of any IgG antibodies directed against red blood cell membrane antigens which could cause reactions if transfused. These IgG antibodies do not cause agglutination by themselves in the other testing but can still cause problems if transfused, hence they are tested for using the indirect Coomb’s test.

The first step is incubation to bind any IgG antibodies to their antigens on the red cell membrane. Then the cells are washed to remove any unbound IgG as it could cause a false positive result. Coomb’s reagent is then added. The reagent is serum that contains rabbit IgG antibodies directed against human IgG antibodies. These antibodies bind to any IgG present on the red blood cells and binds multiple cells together, causing agglutination. Agglutination is a sign of a positive Coomb’s test, i.e. the presence of IgG.

The final step is to double check the above reaction, and involves the addition of Coomb’s control cells to a negative solution left from the previous action. The control cells are coated with IgG and if the Coomb’s reagent was active, will now cause agglutination, thus the Coomb’s test is truly negative. If the Coomb’s reagent was inactive, there will be no agglutination at this step, and need to repeat whole test again.

4. WHAT IS A DIRECT ANTI-GLOBULIN TEST AND AN INDIRECT ANTI-GLOBULIN TEST? WHAT IS THEIR CLINICAL SIGNIFICANCE?

Direct antiglobulin test is when immunoglobulin detected on Coombs reagent addition. First the donor serum is incubated with red cells to bind any IgG antibodies to their antigens on the red cell membrane. Then the cells are washed to remove any unbound IgG as it could cause a false positive result. Coomb’s reagent is then added. The reagent is serum that contains rabbit IgG antibodies directed against human IgG antibodies. These antibodies bind to any IgG present on the red blood cells and binds multiple cells together, causing agglutination. Agglutination is a sign of a positive Coomb’s test, i.e. the presence of IgG.

The indirect anti-globulin test is a double-check of the negative direct test. Coomb’s control cells are added to the negative solution left from the direct reaction. The control cells are coated with IgG and if the Coomb’s reagent was active, will now cause agglutination, thus the Coomb’s test is truly negative. If the Coomb’s reagent was inactive, there will be no agglutination at this step, and need to repeat whole test again.

5. LIST THE CHANGES THAT OCCUR WITH STORAGE OF BLOOD.
Usually blood donated in 500mL units mixed with 64mL preservative, e.g. citrate-phosphate-dextrose (citrate as anticoagulant, binding calcium; phosphate as a buffer and dextrose as metabolic substrate for red blood cells). If add adenine to the solution can increase storage time to 5 weeks as Adenine acts as a substrate for ATP synthesis. Stored at 4°C to inhibit bacteria and minimise glycolysis (\(\frac{1}{30}\) that at 37°C).

Red blood cells become more spherical, and their metabolic survival is reduced. This is due to decreased activity of the sodium/potassium/ATPase pump which allows the cells to lose potassium to the surrounding fluid and gain sodium from it. These causes loss of deformability which increases fragility. These changes are not irreversible, and after transfusion, the red blood cells can be destroyed rapidly by macrophages in the spleen and elsewhere. At 7 days there are 98% of original RBC’s left; by 14 days 85%, 21 days 80% and 28 days 75%. Their 2,3-DPG levels decrease significantly with storage beyond 1 week. At day 7 99% remains, which decreases to 50% by day 14, 15% by day 21, and 5% by day 28. ATP in stored blood gradually reduces from 96% at day 7 to 75% at day 28.

White blood cells lose their phagocytic and bactericidal action within 4-6 hours. Platelets become non-functional by 48 hours. Factor V linearly decreases to 5% by 21 days. Factor VIII decreases rapidly to 75% by 24 hours, then declines more slowly with 30% present at 21 days.

pH of stored blood also gradually decreases, being 7.2 on collection, 7.0 at day 7, 6.9 at day 14, 6.8 at day 21, and 6.7 at day 28.

Electrolytes also change in stored blood. Sodium content decreases from 168mmol/L on collection to 155mmol/L by day 28. Potassium increases significantly, with the normal 4 on collection increasing to 11.9mmol/L by day 7, 17mmol/L a week later, 21mmol/L by day 21, and 28mmol/L by day 28. Glucose content gradually decreases as well, with 350mg/dl on collection decreasing to about 200mg/dL by day 28.

6. **WHY ARE RED BLOOD CELLS BIOCONCAVE DISCS? WHAT ARE THE ADVANTAGES?**

Red blood cells are about 7.5\(\mu\)m diameter and 2\(\mu\)m thick, structured as a bioconcave disc. This structure is achieved by the protein network in the cell membrane. The bioconcavity increases the surface area to volume ratio. It contributes their flexibility.

If the red blood cells become spherical (hereditary, failure of membrane Na/K/ATPase pump, low osmotic pressure of surrounding fluids) they become more fragile and easily lysed.

7. **WRITE AN ACCOUNT ON THE HAZARDS OF BLOOD TRANSFUSION.**

Blood transfusion can cause autoimmune reactions via IgM antibodies to A and B antigens causing type II hypersensitivity reaction. Antibodies against donor leukocytes and cytokines can produce transient fever and rash.

Blood transfusion can also be associated with a risk of infection – the risk is 1:34,000. Asymptomatic donor bacteraemia can be a significant problem, and viral donor screening is not completely effective in detecting window period infections. There are microaggregate filters which can be used against viraemia and bacteraemia.
Hypothermia and circulatory problems can also result from blood transfusions. Transfusion-related acute lung injury is thought to be mediated by complement-activating leucoagglutinating HLA-specific antibodies. Transfusions can also contribute to volume overload, hypocalcaemia, hyperkalaemia, coagulopathy and hypothermia.

Massive transfusion (replacement of more than two times the blood volume in less than 24 hours) is associated with more hazards. Can result in dilution of platelets and coagulation factors. The citrate anticoagulant used as a preserver can cause hypocalcaemia, especially in liver disease (poor citrate detoxification). Transient hyperkalaemia can result (usually short lived as quickly enters cells) and acidosis. This can be followed by metabolic alkalosis as citric acid is metabolised to pyruvate and bicarbonate. Profound hypothermia can result from massive blood transfusion, therefore best to use warmers.

8. WHAT IS THE COMPOSITION OF FRESH FROZEN PLASMA, CRYOPRECIPITATE AND SUPERNATANT?

Fresh frozen plasma is separated from erythrocytes and platelets and stored at -18°C. It is thawed in a water bath when required and needs to be used within a few hours of thawing. Cross matching is not necessary. It contains all coagulation factors found normally in fresh plasma, but no platelets. FFP is preserved with citrate, thus rapid infusion can cause hypocalcaemia. Each 250mL unit raises most coagulation factor concentrations by 3-5%.

Cryoprecipitate is genetically engineered vonWillebrand factor, obtained from regular donor, with FFP freeze-thawed to concentrate fibrinogen, factor VIII, and vWF into 10% of its original volume. Standard doses are pooled from 12-20 donors. Carries the same risks of transfusion as plasma.

9. HOW WOULD YOU DETECT OR INVESTIGATE A BLOOD TRANSFUSION REACTION?

10. WHAT IS A PLATELET AND WHAT IS ITS FUNCTION?

Platelets are small granulated bodies 2-4µm diameter with a half life of about 4 days. They are derived from the pluripotent stem cell in the bone marrow, which form megakaryocytes and then platelets, production regulated by colony stimulating factors.

Platelets have a ring of microtubules around their periphery and an extensively invaginated membrane with an intricate canicular system in contact with the extracellular fluid. Membranes contain receptors for vonWillebrand factor, fibrinogen and collagen. Cytoplasm contains actin, myosin, glycogen, lysosomes and two types of granules. Dense granules contain the non-protein substances secreted in response to platelet activation (serotonin and ADP). Alpha-granules contain secreted proteins like platelet derived growth factor.

When a blood vessel is injured, the platelets adhere to the exposed collagen, laminin and vonWillebrand factor in the wall via integrins on the platelet surface. Platelets are then activated and release their granules, change their shape and bind to other platelets, forming a plug. Platelet activating factor is also released by neutrophils, monocytes and platelets, and acts on platelets via a receptor that activates phospholipase C and diacylglycerol causing further granule content release. DAG and cytoplasmic calcium activate phospholipase A to cause release of arachidonic acid from membrane phospholipids. The arachidonic acid is converted to thromboxane A2 which facilitates further calcium ion influx and phosphatidylinositol breakdown.
11. DISCUSS THE DETAILED SEQUENTIAL CHANGES IN STORED BLOOD

Red blood cells become more spherical, and their metabolic survival is reduced, due to decreased activity of the sodium/potassium/ATPase pump which allows the cells to lose potassium to the surrounding fluid and gain sodium from it. (this causes loss of deformability which increases fragility and these changes are not reversible thus red blood cells can be destroyed rapidly by macrophages in the spleen and elsewhere after transfusion). At 7 days there are 98% of original RBC’s left; by 14 days 85%, 21 days 80% and 28 days 75%. Their 2,3-DPG levels decrease significantly with storage beyond 1 week. At day 7 99% remains, which decreases to 50% by day 14, 15% by day 21, and 5% by day 28. ATP in stored blood gradually reduces from 96% at day 7 to 75% at day 28.

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12. OUTLINE THE FUNCTIONS OF PLASMA.

The fluid portion of the blood is referred to as plasma. Normal plasma volume is 5% body weight (3500mL in a 70kg man). Plasma contains an immense number of ions, inorganic molecules, an organic molecules that are in transit to various parts of the body. (serum has essentially same composition, but no fibrinogen, or clotting factors II, V and VIII). 8% is leukocytes and platelets, 90% water and 2% organic compounds (phospholipids, cholesterol, urea, glucose, lactic acid, creatinine, bilirubin) and electrolytes.

Contains plasma proteins (albumin, globulin and fibrinogen) which function in oncotic pressure, transport & carrier functions, acid-base balance, proteolytic systems, immune response, enzyme activity and metabolism.

Plasma functions as a medium for transporting red blood cells which carry oxygen and carbon dioxide to and from cells. It also supplies nutrients and removes waste products. Plasma dissipates heat which is generated by oxidative reactions. It also distributes hormones. Plasma transports white cells and drugs such as antibiotics. It also transports platelets and coagulation proteins that act to stop bleeding.

13. OUTLINE THE FUNCTIONS OF PLASMA PROTEINS.

Plasma proteins consist of albumin (40-45g/L), globulin α1, α2, β1, β2 & γ (25g/L) and fibrinogen (3g/L). Major functions of the plasma proteins are in maintaining oncotic pressure, and transport carriers (e.g. transporting thyroid, adrenocortical and gonadal hormones), function in acid base
balance as buffers and to transport carbon dioxide, in proteolytic systems (complement, kinin, coagulation, fibrinolytic) and in metabolism (provide amino acids to tissues for synthesis and catabolism).

Most plasma protein binding for basic drugs is to $\alpha_1$-acid glycoprotein, while acidic drugs bind to albumin.

14. WHAT IS THE HALF LIFE OF THE VARIOUS FACTORS IN BLOOD?

   Red blood cells 120 days  
   Platelets 4 days  
   Neutrophils 6 hours  
   Monocytes 72 hours  
   Albumin 20 days

15. OUTLINE THE COAGULATION CASCADE.

   The coagulation cascade has been traditionally considered to consist of intrinsic and extrinsic pathways that join to form a common pathway leading to the formation of a fibrin clot. It is controversial the relative importance of the two pathways, but it appears the extrinsic system is more important in normal coagulation.

   The intrinsic system is activated on contact with certain surfaces (e.g. subendothelial collagen). The initial reaction results in activation of factor XII which activates factor XI. XIa activates factor IX, then in a reaction that requires platelet factor 3, calcium and factor VIII, XIa converts factor X to activated factor X.

   The extrinsic system is activated by exposure of tissue thromboplastin in the subendothelium, activating factor VII which in turn activates factor X, and IX.

   The final common pathway involves activated factor X converting prothrombin to thrombin. This reaction also required factor V, calcium and platelet factor 3 (platelet thromboplastin). Thrombin converts fibrinogen to fibrin monomer and fibrinopeptides A & B. The monomers then aggregate non-covalently to produce soluble fibrin. Activation of factor XIII by thrombin causes covalent cross-linking which converts the fibrin polymer to insoluble fibrin.

   The clot then contracts, decreasing the size of the defect in the vessel wall. Platelets are required for this contraction.
16. OUTLINE THE FIBRINOLYTIC SYSTEM.

The fibrinolytic system is activated by initiation of coagulation, and limits the extent of the clotting.

Plasminogen activator is released from local endothelium in response to local thrombin production. This converts plasminogen bound to fibrin, to the active serine protease plasmin. Plasmin begins to break down the clot, forming fibrin degeneration products in the process. The Fibrin Degradation Products prevent cross-linking of fibrin & inhibit platelet function (half life 9 hours).

17. WHAT IS THE ROLE OF PROTEIN C AND PROTEIN S?

Protein C and Protein S are part of the thrombomodulin system. Activated protein C and its cofactor protein S inactivate factors Va and VIIIa, and inactivate an inhibitor of tissue plasminogen activator, increasing the formation of plasmin.

18. WHAT IS THE FUNCTION OF PLASMIN?

Plasmin or fibrinolysin is the active component of the plasminogen fibrinolytic system. It lyses fibrin and fibrinogen, producing fibrinogen breakdown products that inhibit thrombin.

Plasmin is formed from its inactive precursor, plasminogen, by the action of thrombin and tissue-type plasminogen activator. It is also activated by urokinase-type plasminogen activator. Plasminogen receptors are located on the surfaces of many different cells, and are plentiful on endothelial cells. When plasminogen binds to its receptors it becomes activated so intact blood vessel walls are provided with a mechanism that discourages clot formation.

19. OUTLINE THE SYNTHESIS AND BREAKDOWN OF HAEMOGLOBIN.

Haemoglobin is the oxygen-carrying pigment on red blood cells, a protein with molecular weight of 64,450. Haemoglobin is a globular molecule made up of 4 subunits. Each subunit contains a heme moiety conjugated to a polypeptide. In normal adult haemoglobin there are two alpha and two beta subunits.
Red blood cells have a circulating life of around 120 days. When enzymes function less efficiently, the cell becomes more fragile, and is destroyed by tissue macrophages in bone marrow, spleen and liver. They split the globulin portion off, releasing the amino acids, and convert the heme portion to biliverdin under the influence of heme oxidase. Most biliverdin is converted to bilirubin, attached to albumin and carried to the liver where it is conjugated and excreted in bile. The iron from the heme is carried by transferring and reused for haemoglobin synthesis, or placed in the iron stores.

20. WHAT IS LYMPH? OUTLINE THE FUNCTION OF LYMPHATIC FLUID, AND HOW IT IS RECYCLED.

Lymph is tissue fluid that enters the lymphatic vessels. The lymphatic system functions as an overflow mechanism, controlling protein concentration in interstitial fluid, volume of the interstitial fluid and pressure on the interstitium. According to Starling’s law, most fluid that filters out of the arterial end of the capillary is reabsorbed at the venous end. About one tenth however enters the lymphatic channels that are present in almost all body tissues (except cartilage, bone, CNS and epithelium). The lymphatics flow back to the thoracic duct and into the venous system via IVC. This system allows for the reabsorption of high molecular weight substances such as proteins that cannot be reabsorbed into the venous end of the capillary.

Lymph is derived from interstitial fluid, therefore has the same composition. Protein content varies according to tissue region drained – 2g/dL in most tissues, 6g/dL in liver, 3-4g/dL in intestines – thoracic lymph 3-5g/dL. Fat content is also variable. After a fatty meal, thoracic duct lymph can contain 1-2% fat. Lympha also contains clotting factors and lymphocytes.

Total lymph flow is 120mL/h, but is ultimately determined by interstitial fluid pressure and activity of the lymphatic pump.

If the lymphatics did not act to remove protein from interstitial fluid, the protein would remain and act as an oncotic force drawing fluid from capillaries causing tissue oedema. The lymphatic system also removes foreign particles such as bacteria, which filter through the lymph nodes.
MULTIPLE CHOICE QUESTIONS

1. Which of the following decrease platelet aggregation & cause vasodilatation
   A. PGE2
   B. PGF2α
   C. TBXA2
   D. PGD2
   E. PGI2

   PGE2 is a vasodilator which relaxes arteriolar smooth muscle, but does not affect platelets (∴ not A)
   PGF2α is a vasoconstrictor (∴ not B)
   TBXA2 is a vasoconstrictor & stimulates platelet aggregation (∴ not C)
   PGD2 is a vasodilator (∴ not D)
   PGI2 is a vasodilator & decreases platelet aggregation (∴ E correct)

2. Which is associated with inhibition of platelet aggregation
   A. prostaglandin I
   B. prostaglandin E
   C. Prostaglandin F

   Platelet aggregation is inhibited by PGI2 & PGE1 (∴ A & B correct)
   Platelet aggregation is stimulated by TXA2.

3. Which one of the following causes bronchodilatation
   A. PGE2
   B. PGF2α
   C. TBXA2
   D. LTB4
   E. LTD4

   Bronchodilatation is due to PGE2, PGI2 (prostacyclin) & PGE1 (∴ A correct)
   Bronchoconstriction is due to TXA2, PGF2α & leukotrienes (∴ not B, C, D, E)

4. In a patient receiving 24 units of blood in 24 hours, the complication most likely to be seen would be
   A. hypercalcaemia
   B. increased oxygen uptake in the lungs
   C. coagulopathy
   D. hypokalaemia

   Massive transfusion results in – dilution of platelets & coagulation factors (∴ C correct);
   hypocalcaemia due to citrate toxicity (∴ not A), hypomagnesaemia, hyperkalaemia (∴ not D),
   acidosis, hypothermia & 2,3-DPG deficiency, which results in left-shift of the oxygen-
   haemoglobin dissociation curve, facilitating uptake of oxygen in the lungs (∴ B also correct
   theoretically, but not borne out in clinical studies)

5. Problems of massive transfusion most often involve
   A. metabolic alkalosis
   B. hyperkalaemia
   C. coagulopathy due to hypocalcaemia
As above, not A, but B correct
There is little evidence of hypocalcaemia having a role in the coagulopathy that results from massive transfusion (Faunce p.144) ∴C also incorrect

6. The effect which is least likely to occur after transfusion of 25 units of whole blood is
   A. hypocalcaemia
   B. dilutional coagulopathy
   C. metabolic alkalosis
   D. increased affinity of haemoglobin for oxygen
   E. hyperkalaemia

As above, but for whole blood, coagulation factors are transfused as well.
C correct answer

7. Erythropoietin is a glycoprotein which
   A. stimulates white & red cell production
   B. is broken down in the kidney
   C. has a half life of days
   D. levels are reduced by high haematocrit
   E. erythropoietin increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors and mature erythrocytes (∴not A).
   The principle site of inactivation of erythropoietin is the liver (∴not B)
   It has a half life of 5 hours in the plasma, though an increase in circulating red blood cells is not seen for 2-3 days (∴not C)
   Erythropoietin synthesis is enhanced by hypoxia or loss of red cells, while when red cell volume increases, the erythropoietin release decreases (∴D correct)
   Production can also be stimulated by androgens & cobalt salts. Facilitated by beta-adrenergic stimulation & by adenosine.

8. Erythropoietin :
   A. red cell maturation in 24-72 hours
   B. inactivated by Kupffer cells
   C. Metabolised in the liver
   D. Half life is 5 minutes

As above, C the most correct (not sure of cell type in the liver for metabolism, and maturation is really 48-72 hours)
Half life in plasma 5 hours ⊿ not D

9. Antithrombin III inactivates which coagulation factor ?
   A. XIIa
   B. Xa
   C. IIa
   D. Ixa
   E. All of the above

Antithrombin III binds to serine proteases in the coagulation system, blocking their activity. Inhibits the active forms of factors IX, X, XI & XII ⊿ E correct
10. Vitamin K neutralises
   A. factor V
   B. heparin
   C. antithrombin III
   D. plasminogen

Vitamin K is a cofactor for the enzyme that catalyses the conversion of glutamic acid residues to gamma-carboxyglutamic acid residues. Proteins involved are factors II, VII, IX, X and protein C & S.
Activated protein C (with protein S as cofactor) inhibits activated factors V & VIII, decreasing the production of thrombin, but none of the above selection is one of these.
?? answer

11. Desmopressin
   A. increases factor VIII levels/activity
   B. has an anti-heparin effect
   C. has pressor activity

DDAVP increases the release of vonWillebrand factor, increasing activity of factor VIII :: A correct

12. Post-translational modification occurs with
   A. factor V
   B. vonWillebrand factor
   C. factor XII
   D. protein C

Post translational modification is the alteration of the polypeptide chain to form the final product protein, occurring by hydroxylation, carboxylation, glycosylation or phosphorylation of amino acid residues
Activated vitamin K is a cofactor in the gamma hydroxylation of coagulation factors, II, VII, IX, X & protein C :: D correct

13. Post-translational modification
   A. involves the removal of introns
   B. modification of amino acid residues in proteins
   C. self-splicing
   D. tRNA involved

As above, B correct

14. Haemoglobin breakdown
   A. iron excreted by the kidney
   B. heme is broken down to biliverdin
   C. heme is converted to bilirubin then transported to the liver bound to albumin

When old red blood cells are destroyed, in the tissue macrophage system, the globin portion is split off & heme is converted to biliverdin, producing CO in the process. Most biliverdin is then converted to bilirubin, and excreted in bile.
Bilirubin is bound to albumin for transport to the liver where it is conjugated. B is the most correct.

15. Platelet activation will not occur without
   A. calcium
   B. vessel wall damage
   C. vonWillebrand factor
   D. fibrinogen
   E. serotonin

   Activation of platelets is initiated by binding to collagen, platelet activating factor, vonWillebrand factor, ADP & thrombin.
   The initial release reaction is calcium-dependent. ∴ A correct

Ganong p.508; Faunce p.138

16. Platelet activation requires
   A. vessel wall damage
   B. calcium ions
   C. vonWillebrand factor
   D. cyclooxygenase
   E. prostaglandins

   ?? appears to require all

17. Which of the following statements about FFP is not true
   A. must be group specific
   B. contains all clotting factors except for platelets
   C. does not need to be crossmatched
   D. contains all clotting factors except deficient in factors V & VIII
   E. is not useful in treating coagulopathy

   FFP is separated from red cells & platelets. It need not be group specific (∴ A correct & not C) &
   contains all coagulation factors found in plasma (∴ not B)
   Factors V & VIII decrease rapidly in stored FFP, but are present in fresh donation (∴ not D)
   It is used in treating coagulopathy (∴ E correct)

18. Haemoglobin contains
   A. one protoporphorin ring & 4 ferrous ions
   B. four protoporphorin rings & one ferrous ion
   C. four protoporphorin rings & four ferrous ions
   D. one protoporphorin ring & one ferrous ion
   E. none of the above

   Haemoglobin is made up of 4 subunits, each containing a heme moiety which is a porphyrin derivative, conjugated to a polypeptide iron on each subunit (∴ C correct)

19. Thrombin inhibits
   A. factor Xa
   B. tPA
   C. protein C
   D. platelets
E. none of the above

Thrombin is a procoagulant which activates factors V & VIII. When it binds to thrombomodulin, it becomes an anticoagulant, activating protein C, and the APC then inactivates the factors V & VIII.
SHORT ANSWER QUESTIONS

1. EXPLAIN THE MAIN DIFFERENCE BETWEEN THE INTRINSIC & EXTRINSIC PATHWAYS OF COAGULATION (01B7)

The intrinsic & extrinsic pathways of coagulation involve a series of clotting proteins that form an amplification cascade. The distinction as “intrinsic” and “extrinsic” pathways is becoming less distinct than previously suggested, but essentially divides the coagulation cascade into those factors initiated by factors intrinsic to the vessel or extrinsic to the vessel. Both pathways end in the common pathway producing thrombin and fibrin, and involve positive feedback to amplify the system.

The intrinsic pathway theoretically involves those proteins already present in the circulating blood. It appears to be initiated by factor XII binding to negatively charged surfaces, such as collagen fibres underlying endothelium of blood vessels. The binding of factor XII causes the formation of activated factor XII (XIIa) catalysed by high molecular weight kininogen & kallikrein which catalyses the activation of factor XI, then factor IX & factor X. Activated factor X is the beginning of the “common pathway” which using activated factor VIII, factor V & platelet factor 3 as cofactors, activates factor II (prothrombin) to form thrombin, and fibrinogen to form fibrin. The fibrin is formed into cross-linked fibrin catalysed by activated factor XIII, forming a more stable clot.

The intrinsic pathway is tested for by activated partial thromboplastin time (APTT) involving the mixing of citrated blood with rabbit brain phospholipid & kaolin, and by ACT which involves mixing fresh blood with kaolin.

The extrinsic pathway is initiated by traumatised tissue (i.e. factors external to the vascular system) and involves the activation of factor VII by tissue factor (factor III or tissue thromboplastin). This tissue factor is expressed on adventitial cells surrounding blood vessels & epithelial cells of renal glomerulus & epidermis, and induced on endothelial cells by inflammatory mediators. The activated factor VII catalyses the activation of factor X in the presence of calcium ions which then activates prothrombin & fibrinogen as the common pathway outlined above. The extrinsic pathways is measured by prothrombin time or INR.

Whichever the pathway initially activated, thrombin and fibrin are the final common products, which act as positive feedback signals to factors VIII & IX of the intrinsic coagulation cascade, amplifying the system. The cross-linked fibrin clot formed reinforces the platelet plug that was formed locally after damaged endothelium initiated both platelet & coagulation cascade activation.

2. OUTLINE THE PRINCIPLES OF COMPATIBILITY TESTING OF ALLOGENIC (HOMOLOGOUS) BLOOD FOR TRANSFUSION (00B4)

The initial process of compatibility testing involves blood grouping. The red cell membrane may have A or B antigens & antibodies develop naturally against them. The naturally occurring anti-A & anti-B antibodies are IgM antibodies, and can be present without prior exposure. Some individuals also develop IgG antibodies, occurring after exposure. ABO-compatible blood has a 6% chance of causing a transfusion reaction. The saline-agglutination test is used to detect the IgM & IgG antibodies, and involves adding a reagent known to contain a specific antibody to the recipient’s serum, and suspend in saline, observing for agglutination.

Rhesus antigen is another that commonly causes the development of antibodies. These are IgG antibodies, the most important being anti-D, and develop only on exposure to the antigen. Other antibodies can also develop to P, Lewis & MN. Blood tested for Rhesus & ABO compatibility have
0.2% chance of causing a transfusion reaction. To test for Rhesus antibodies, the recipient’s serum is incubated against Rhesus antibodies in albumin or low ionic strength salt solution, for 20-30 minutes, at 37°C.

Over 50 other antigens have been identified, e.g. Kell, Lewin, Duffy & Kidd; and these can cause minor incompatibilities. Prior exposure to these will induce antibody formation & subsequent exposure causes an adverse reaction. Presence of these antibodies is tested for by a panel of labelled red blood cells of the same ABO & Rhesus grouping, tested against the recipient’s serum. Coomb’s reagent may be required. The first step in Coomb’s testing is incubation to bind any IgG antibodies to their antigens on the red cell membrane. Then the cells are washed to remove any unbound IgG as it could cause a false positive result. Coomb’s reagent is then added. The reagent is serum that contains rabbit IgG antibodies directed against human IgG antibodies. These antibodies bind to any IgG present on the red blood cells and binds multiple cells together, causing agglutination. Agglutination is a sign of a positive Coomb’s test, i.e. the presence of IgG. An indirect Coomb’s test is the final to check for IgG antibodies which did not cause agglutination in the direct testing, but which may still cause a transfusion reaction. The indirect Coomb’s test follows the Coomb’s test, i.e. addition of Coomb’s control cells to a negative solution left from the Coomb’s testing. The control cells are coated with IgG and if the Coomb’s reagent was active, will now cause agglutination, thus the Coomb’s test is truly negative. If the Coomb’s reagent was inactive, there will be no agglutination at this step, and need to repeat whole test again. ABO, Rhesus and negative antibody screen plus Coombs test 99.95% relative safety.

Cross matching is the next test, mixing a sample of the potential donor’s red blood cells with the recipient’s serum, and observing for agglutination. This is the major cross match, and the minor cross-matching involves the mixing of donor serum & recipient cells.

3. BRIEFLY DESCRIBE THE BREAKDOWN OF HAEMOGLOBIN AFTER RED CELL LYSIS (00A8)

Mature red blood cells usually circulate for 100-130 days. As enzymes within the red blood cells begin to decrease in function, the cells are destroyed by the tissue macrophages in the bone marrow, liver & spleen.

The macrophages split the globulin portion from the heme portion of haemoglobin. The globulin is broken down into amino acids & re-enter the body amino acid pool. The heme ring is opened by the macrophages to form iron & protoporphyrin under the influence of heme oxidase. The iron is carried by transferrin & is reused in haemoglobin synthesis, or placed in the body iron stores.

Protoporphyrin is further broken down into biliverdin & carbon monoxide. Biliverdin is converted into bilirubin and released by the macrophages to the plasma, transported to the liver bound to albumin. In the liver, bilirubin is absorbed through the hepatic cell membrane, and is conjugated with glucuronic acid, sulphate & other substances, and excreted by an active transport process into the bile canaliculi & excreted in bile.

Once in the intestine, about 50% of the bilirubin is converted to urooblinogen by bacterial action. Some of this is reabsorbed by intestinal mucosa – enterohepatic recirculation. Some is then lost to the body in faeces, after being further oxidised to form stercobilinogen. Of the recycled urooblinogen, most is returned to the liver & re-excreted in bile to the gut. About 5% is excreted in the urine, and oxidised to urooblin on exposure to air.
4. EXPLAIN THE MECHANISMS THAT PREVENT CLOTTING IN INTACT BLOOD VESSELS (99A2; 95A7)

Haemostasis depends on a fine balance between procoagulant & anticoagulant factors. In the intact blood vessel, these can be described by Virchow’s triad, i.e. factors in the wall of the vessel (endothelium), factors in the blood & blood flow itself.

Intact blood vessels have constant flow which prevents clot formation. Laminar flow decreases turbulence & causes axial streamlining of platelets, minimising platelet endothelial contact, reducing their activation. It also removes & dilutes activated coagulation factors & disperses loose aggregates of platelets, thus preventing the amplification of the system that would result in clot formation. These activated coagulation factors are inactivated by the reticuloendothelial system & the liver.

The intact blood vessel has a smooth endothelial surface & glycocalyx layer which prevents the contact activation of platelets & coagulation factors. Endothelial cells produce prostacyclin & nitric oxide which inhibit platelet aggregation & relax vascular smooth muscle, maintaining flow within the vessel (which inhibits clot formation as above). The endothelial cells express thrombomodulin which complexes with any thrombin formed (activated factor II), slowing the coagulation cascade if it is activated inappropriately (since thrombin acts as a positive feedback on the coagulation cascade normally amplifying the system). The thrombin-thrombomodulin complex also activates protein C, which (with Protein S as a cofactor) inactivates coagulation factors Va & VIIIa. The luminal surface of the endothelium also expresses heparin sulphate, which binds antithrombin III, and accelerates its inactivation of factors IXa, Xa & thrombin. Any disruption in the endothelium will cease the production of these factors, thus allow the formation of a clot in the vessel.

Anticoagulant factors in the blood also maintain a check on clot formation in the blood vessel. In the intact vessel, procoagulant & anticoagulant factors remain in balance, thus preventing the formation of unnecessary clot. Once this balance is disrupted, e.g. by massive coagulation cascade amplification as a result of vessel injury, a clot will be formed. Antithrombin III (which complexes with heparin sulphate present on endothelial cells to accelerate its effect) is activated by the formation of thrombin, and inactivates factors IXa, Xa, XIa, XIIa, and IIa (thrombin) and activates protein C. This catalyses the formation of plasmin from plasminogen. Activated protein C, using Protein S as a cofactor also inhibits the activated coagulation factors V & VIII. Alpha-2 macroglobulin & alpha-1 antitrypsin inhibit thrombin as well. Thrombolytic factors are also activated when clotting is activated, acting to dissolve the clot and limit clot extension within the vessel – plasminogen activators, plasmin & the above mechanisms.

5. BRIEFLY DISCUSS THE PHYSIOLOGICAL ROLE OF PLASMA PROTEINS (98B6; 92A; 90)

Plasma proteins comprise 50-80g/L, and consist mainly of albumin, globulins & fibrinogen. Albumin is the plasma protein present in the highest concentration (75% of total plasma proteins), and is almost the only one which is not a glycoprotein. It is synthesised in the liver, at a rate of 12-15g/day (25% of total hepatic protein synthesis) and has an intravascular half life of ~20 days.

One of the major functions the plasma proteins serve is maintaining oncotic pressure (plasma colloid osmotic pressure). The plasma proteins exert an oncotic pressure of ~19mmHg within the capillary, calculated by the vanHoft equation. The remaining 4-6mmHg is exerted by the electrolytes maintained within the capillary by Gibbs Donnan equilibrium caused by the plasma proteins. Oncotic pressure is involved in the Starling’s equilibrium of forces promoting & opposing fluid flux across the
capillary membrane. The capillary oncotic pressure serves to balance the high hydrostatic pressure required in the capillary to ensure blood flow. A reduction in oncotic pressure can lead to excess fluid filtration out of the capillary, and depending on the tissue’s lymphatic capacity, may lead to peripheral oedema.

Albumin & alpha-1 acid glycoprotein are important in binding & transporting poorly water soluble substances such as bilirubin, fatty acids, hormones (e.g. thyroid hormone & cortisol), copper and exogenous drugs. Decreased plasma protein concentrations will lead to increased free proportion of these substances, which is especially significant in those substances which have high protein binding.

Plasma proteins have a role in the proteolytic systems, in complement, kinin, coagulation & fibrinolytic systems. The globulins are involved in immune function, especially immunoglobulin (antibodies) & cytokines.

Plasma proteins have an important function in acid-base balance. The terminal amine groups of proteins are able to buffer excess acids. This is especially important in the transport of carbon dioxide from the capillaries back to the lungs for excretion from the body.

Plasma proteins can also be used as energy substrates by the tissues, broken down into amino acids & used in metabolic pathways.

6. BRIEFLY OUTLINE THE ROLE OF PLATELETS IN HAEMOSTASIS (97A6)

Haemostasis is the mechanism that prevents the loss of blood from a damaged vessel, and involves vasoconstriction, formation of a platelet plug, and then formation of a blood clot. Platelets are involved in all of these steps of haemostasis. Platelets are portions of cytoplasm extruded from megakaryocytes.

Damage to a blood vessel exposes subendothelial collagen & endothelial vonWillebrand factor. These bind to the glycoprotein (Gp) Ia/IIa & Gp Ib/IX receptors on the platelet glycocalyx. This causes the platelet to adhere to the site of injury & a coating of platelets (carpet) forms over the damaged area.

This binding triggers platelet activation & aggregation. Depolymerisation of the microtubules causes the platelets to flatten & actin polymerisation causes projection of pseudopodia. Damage to the endothelium also causes the release of phospholipids, including lipoprotein factor 3, that stimulates the coagulation cascade. Thrombin thus formed binds to a G-protein coupled receptor on the platelets, acting via protein kinase K to cause influx of calcium & release of calcium from the dense tubular network. The increased calcium concentration activates diacylglycerol, causing release of platelet granule contents – serotonin, ADP, adrenaline, fibrinogen, vonWillebrand factor & others. The increased calcium also induces calcium-calmodulin binding which activates phospholipase, producing thromboxane A2. Activation of the platelet exposes the GpIIb/IIIa receptor complex, which binds vonWillebrand factor released by the platelets, ultimately causing the aggregation of platelets together to form the platelet plug.

The release of vasoactive substances (serotonin, histamine, ADP) from the platelet granules causes local vasoconstriction, limiting blood loss & preventing the loss of activated platelet & coagulation factors from the site of vessel damage.

The ADP, fibrinogen & thrombin released cause positive feedback, amplifying the coagulation cascade & platelet binding, resulting the further platelet plug formation.
Eventually, stabilisation of the platelet plug occurs through platelet contraction & formation of fibrin mesh by the coagulation cascade.

7. **BRIEFLY EXPLAIN THE CHANGES THAT OCCUR IN STORED WHOLE BLOOD (02B12; 95B10)**

In stored blood –
- red blood cells become spherical & their metabolic survival time is reduced. A small amount of haemolysis occurs, with free haemoglobin increasing from 1.7 at collection to 30 by day 28. Red cell count decreases from 98% present at 7 days to 85% at 14 days, 80% at 21 days & 75% at 28 days.
- Cold storage of blood (at 4°C) reduces red cell metabolism, thus reduces the amount of 2,3-DPG (which is formed as a shunt off the glycolytic pathway) This causes a left-shift of the oxygen-haemoglobin dissociation curve, theoretically decreasing the amount of oxygen unloading in the peripheral tissues once the red cells are transfused. The amount of 2,3-DPG reduces from 100% on collection (10.6mmol/gHb) to 99% at day 7, 50% at day 14, 15% day 21, and 5% day 28
- ATP in the stored blood decreases to 75% at day 28
- White blood cells lose their phagocytic & bactericidal activity
- Platelets rapidly become non-functional within 48 hours when stored as whole blood
- Coagulation factors are not significantly altered up to 21 days, except factors V & VIII, which rapidly decline in concentration. Factor VIII levels are 50% by 24 hours, and 6% after 21 days. Factor V decreases to 50% at 14 days.
- Blood becomes more acidic the longer it is stored, pH falling from 7.2 at collection to 6.9 at day 14, and 6.7 at day 28
- Biochemical changes →
  - Potassium in the stored unit is 4mmol/L at collection, and increases to 12mmol/L at day 7, 17mmol/L day 14, 21mmol/L day 21, and 28mmol/L day 28. Once re-transfused, the potassium is taken up by the re-warmed & metabolically active red blood cells, and also is diluted through the extracellular compartment. Catecholamines also increase cellular uptake of potassium. Hyperkalaemia is usually only an issue with massive transfusion.
  - Sodium concentration in the stored unit of blood falls slightly as it is taken up by red blood cells (decreasing from 165mmol/L to 155mmol/L by day 28)
  - Glucose concentration falls from 6.4mmol/L at time of collection to 3.5mmol/L at 28 days

To store a unit of blood, it is mixed with a preservation solution & stored at 4°C. Aseptic techniques & sterile equipment also aim to minimised bacterial contamination. The preservative solution contains citrate (anticoagulant), phosphate (buffer), dextrose (for glycolysis) & adenine (for ATP synthesis). When stored in this solution at 4°C, blood has a 70% survival when stored for 35 days.