OVERVIEW OF BODY FLUIDS AND FLUID COMPARTMENTS

The major component of the human body is water, which accounts for 63% of an adult male. An increased body fat content is associated with ageing, obesity and being female. Consequently, the percentage of water in women falls to 52%. Dissolved within this water are carbon dioxide (CO\(_2\)), nutrients, proteins and charged particles (ions).

Fluid in the body is distributed into different compartments (Fig. 2.1):
- Intracellular fluid (ICF): the fluid inside the cells.
- Extracellular fluid (ECF): all fluids outside cells, comprising:
  - 75% interstitial fluid (ISF): the ECF that bathes the cells and lies outside the vascular system
  - 25% plasma: the non-cellular part of the blood (within the vascular system).

ISF and plasma are in a state of continual exchange via pores in the highly permeable capillary membrane. The two fluids therefore have a similar composition, with the exception of large proteins, which are trapped within the capillaries in the vascular system.

Transcellular fluid is another small compartment of body fluid. Although it can be viewed as a specialized type of ECF, their compositions vary greatly.

Osmolarity and osmolality

Adding solute dilutes the concentration of pure water, and so increasing solute concentration decreases water concentration (Fig. 2.2).

The tendency of water (solvent) to diffuse from a region of higher concentration to an area of lower water concentration until equilibrium is reached underlies the principle of osmosis. As explained in Chapter 1, osmosis is the diffusion of solvent through a selectively (semi-) permeable membrane from a less concentrated solution to a solution with a higher concentration of solute. Note that the membrane allows the passage of solvent but not solute. In biological systems, this solvent is water (Fig. 2.3).

The pressure at which water is drawn from the weak solution (on the left side of Fig. 2.3A) into the more concentrated solution (on the right side of Fig. 2.3A) is known as the osmotic pressure; the higher the solute concentration, the higher the osmotic pressure. Water will be drawn into the right side until there is osmotic equilibrium (Fig. 2.3B). If a pressure is applied on the more concentrated side (Fig. 2.3A), net movement of water can be prevented.
In an ideal solution, osmotic pressure is similar to the pressure of a gas, with respect to temperature and volume:

\[ P = \frac{nRT}{V} \]

Where \( P \) = pressure of a gas, \( n \) = number of particles, \( R \) = the gas constant, \( T \) = absolute temperature and \( V \) = the volume of the gas.

Therefore, at a given temperature, osmotic pressure will be proportional to the number of particles per unit volume. Solute particles are seen as the osmotically active particles, the total concentration of which, regardless of exact composition, is referred to as osmolality, which is expressed in osmoles (Osm).

In Figure 2.3, osmotic pressure exerted by the solution forces water to move from left to right, as hydrostatic pressure forces movement in the opposite direction.

In an ideal solution, osmotic pressure is similar to the pressure of a gas, with respect to temperature and volume:

**Fig. 2.1** Body composition and fluid components.
1 Osm = 1 mole ($6.02 \times 10^{23}$) solute particles.
1 mOsm = 1/1000 mole of solute particles.

The nature of the solute will dictate the osmolarity. Dissolving 1 mole of a non-ionizing compound, such as glucose, in water will give a solution of 1 Osm. However, dissolving 1 mole of sodium chloride (NaCl), which dissociates into Na$^+$ and Cl$^-$, will give a solution of 2 Osm.

Although the above is true for an ideal solution, it is not the case for bodily solutions, in which the number of osmotically active particles is less than the dissociated particles as a result of ion combinations. This effect is more pronounced with increasing concentration: the more NaCl dissolved per unit volume, the less it behaves like an ideal solution.

**Osmolarity**
- Osmolarity (mOsm/L) is defined as the number of osmoles per unit volume.

This can be illustrated by putting $x$ Osm of solute into a beaker then adding water to make up 1 L of solution. Clearly, the water added would be less than 1 L.

**Osmolality**
- Osmolality (mOsm/kg) is defined as the number of osmoles per unit weight.

Normally, osmolality is about the same as osmolarity. The normal value for plasma is 280–295 mOsm/kg. At these normal plasma values, there is no net water movement. Lower or higher values will cause cell swelling (with danger of lysis/bursting) or shrinking, respectively.

The selective permeability properties of the cell membrane play an important role in cell function by controlling the entry into cells of small molecules and ions. Diffusion across biological membranes can be divided broadly into passive and active forms of transport (see also Chapter 1).

**Passive transport**

The movement of small molecules and ions is dictated by their electrochemical concentration gradient. They move from high to low concentrations, or to neutralize a charge imbalance between two zones.

Size, electrical charge, shape and weight affect the rate of diffusion. If a substance is lipid soluble, diffusion across the cell membrane (which is formed...
from a lipid bilayer; see Chapter 1) will occur more readily. However, with polar substances, diffusion rates through water-filled ion channels are greater. If the solutions either side of a membrane comprise only diffusible ions, diffusion occurs until equilibrium is reached and the ion distribution on each side is the same. At this point, this is the value of diffusible anions × diffusible cations.

Non-ionic diffusion
Although weak acids and bases cross cell membranes with difficulty in their ionic and dissociated forms, some have increased solubility in their undissociated form. Diffusion of such undissociated substances is called non-ionic diffusion; it occurs in the kidneys and gastrointestinal tract.

The Gibbs–Donnan effect
Non-diffusible ions trapped on one side of a membrane affect the passage of other ions. Negatively charged proteins (anions) will attract positive ions (cations) but repel other anions. This effect is described by the Gibbs–Donnan equation. The concentration of diffusible ions on side A of the membrane is the same as that on side B:

\[
\frac{\text{cation A}}{\text{cation B}} = \frac{\text{anion B}}{\text{anion A}}
\]

and:

\[
\text{cation A} \times \text{anion A} = \text{cation B} \times \text{anion B}
\]

Active transport
Carrier proteins can transport substances against their electrical and chemical gradients by using energy from ATP hydrolysis. A typical example of active transport is the Na⁺/K⁺-ATPase, which moves Na⁺ out of and K⁺ into a cell.

FLUID MOVEMENT BETWEEN BODY COMPARTMENTS
The body’s fluid compartments are normally in osmotic equilibrium, although they contain different amounts of various ions (Fig. 2.4):

- ICF: K⁺ contributes ≈50% of osmolality.
- ISF and plasma: Na⁺ and Cl⁻ are responsible for ≈80% of osmolality.

Plasma and interstitial fluid exchange
In the capillaries, the osmolality of plasma is approximately 1 mOsm/L greater than the osmolality of ICF and of ISF. Much of this is due to plasma proteins. This osmotic pressure draws fluid into the capillaries and is counterbalanced by the capillary hydrostatic pressure, which is 20 mmHg greater than that of the ISF.

The exchange of water and ions occurs across the thin capillary wall, which is composed of endothelial cells. Substances can pass via:

- Vesicular transport (not discussed further but requires energy expenditure).
- Junctions between endothelial cells.
- Fenestrations (when present).

As well as this vesicular transport, simple diffusion and filtration are responsible for transport.

Simple diffusion
This is responsible for 90% of exchange – relating mainly to net efflux of O₂ and glucose from plasma and influx of CO₂ into plasma.
Filtration
This is responsible for 10% of exchange. The rate of filtration relies on Starling forces, which are derived from two aspects of the ISF and capillary fluid:

1. Oncotic pressure ($\pi$), which resists filtration.
2. Hydrostatic pressure ($P$), which favors filtration.

### Oncotic pressure
Oncotic pressure is the osmotic pressure produced by the proteins that are confined to a compartment/space by their relatively large size. Its value remains constant throughout the capillary. The effect of these proteins is to draw fluid into the compartment/space in which they are confined. In the plasma, oncotic pressure is 17 mmHg but, owing to imbalance of ions as the result of the Donnan effect, there are more Na$^+$ ions within the capillary and therefore a higher osmotic pressure of 25 mmHg.

### Hydrostatic pressure
The following determine vessel hydrostatic pressure:
- Arteriolar blood pressure.
- Venous blood pressure.
- Arteriolar resistance, on which depends the extent to which blood pressure is transferred along the capillary.

Hydrostatic pressure is maximal at the arteriolar end of the capillary (32 mmHg), where it exceeds oncotic pressure (which is 25 mmHg) and thus favors filtration. At the venous end, fluid re-entry into the capillary is favored; hydrostatic pressure (12 mmHg) is lower than oncotic pressure (25 mmHg) (Fig. 2.5).

### Exchange between interstitial fluid and the lymphatic vessels
The overall efflux of fluid from the capillaries would be expected to cause an increase in ISF hydrostatic pressure. However, this fluid, along with plasma proteins lost from the vascular space, enters a network of lymphatic channels, which is present in all organs and tissues. The fluid is returned to the circulatory system when the lymphatic system empties into the venous system via the thoracic duct in the neck. Normal lymph flow is 2–4 L per day.
This calculation relies on the principle of conservation of mass. However, in real life, excretion and metabolism of the indicator substance takes place. Hence, to measure concentration \( x \) hours after administering the injection:

\[
\text{Volume of distribution} = \frac{\text{Amount of substance injected} - \text{Amount metabolized after } x \text{ hours}}{\text{Concentration of substance after } x \text{ hours}}
\]

Dye can be injected to assist with measurement. The dye must be confined to the compartment being measured; it also needs to be distributed equally, as well as having no influence on water or other solute distribution. Two methods of measurement are used, depending on the rate of excretion from that compartment:

1. Single injection method: a test substance with a slow rate of excretion is used.
2. Constant infusion method: a test substance with rapid excretion is used.

**Slow injection method**

After introduction of the dye, the concentration is measured at intervals. The logarithm of concentration is plotted against time with the concentration of the substance (denoted by using square brackets: \([\text{substance}]\)) determined by extrapolating the straight portion of the graph back to time = 0 (Fig. 2.7):

\[
\text{Compartment volume} = \frac{\text{Amount of substance injected}}{[\text{substance}] \text{ at time } 0}
\]

**Constant infusion method**

After an initial loading dose, the substance is infused at rate equal to the rate at which it is being excreted, so that plasma concentrations remain constant (they are checked at intervals). The infusion is then stopped and urine collected until all the substance is excreted. The amount present in the body when the infusion is stopped = amount excreted (Fig. 2.8):

\[
\text{Compartment volume} = \frac{\text{Amount of substance excreted}}{\text{Plasma } [\text{substance}]}
\]

**Plasma volume**

This is measured by the dilution principle using a high-molecular-weight substance that persists in
the vascular compartment. Examples include radioiodinated human serum albumin or the diazo compound Evans blue dye (T-1824).

**Blood volume**

This can be derived from the haematocrit (fraction of total blood volume) and the plasma volume:

\[
\text{Total blood volume (TBV)} = \frac{\text{Plasma volume}}{1 - \text{haematocrit}}
\]

So, if plasma volume = 3.5 L and haematocrit = 0.3, then TBV = 5 L.

**Red cell volume**

Red cell volume (RCV) can be measured in a variety of ways, depending on what information is known:

1. \( \text{RCV} = \text{TBV} - \text{plasma volume} \)
2. \( \text{RCV} = \frac{\text{Haematocrit} \times \text{plasma volume}}{1 - \text{haematocrit}} \)
3. Direct dilutional method: radioactive chromium \((^{51}\text{Cr})\) is used to label or tag red blood cells. The fraction of red blood cells tagged is measured.

**Extracellular fluid volume**

The substances that are used to measure ECF by the dilutional principle also disperse in both plasma and ISF but are cell-membrane impermeable and so do not enter cells. Examples are:

- Thiosulphate.
- Inulin (can be excluded from bone and cartilage).
- Mannitol.

Small amounts of radiochloride or radiosodium diffuse intracellularly. The lymphatic system cannot be separated from the ECF and is therefore measured with it. Normal ECF volume is 15 L (20% body weight).

**Interstitial fluid volume**

Direct measurements of ISF cannot be made and it is therefore calculated as:

\[
\text{ISF} = \text{ECF} - \text{Plasma volume}
\]

Although absolute ECF volume is less in infants and children, the ECF:ICF ratio is larger. Hence, dehydration is frequently more severe and develops more rapidly in children than in adults.

**Total body water**

Radioactive water – tritium \((^3\text{H}_2\text{O})\) or deuterium \((^2\text{H}_2\text{O})\) – is used by the dilutional principle. Normal total body water values are:

- 63% in males = 45 L of 70 kg body weight.
- 52% in females = 36 L of 70 kg body weight owing to greater proportion of fat; fat cells have a lower water content than muscle.
Transcellular fluid

Transcellular fluid (TCF) is a small compartment usually considered as part of the ECF representing ~5% of ECF (~1L) and includes a number of small volumes such as cerebrospinal fluid, intraocular, pleural, peritoneal and synovial fluids. To include digestive secretions (which can be excessive and are, strictly speaking, outside the body) as transcellular may be debated.

BLOOD PHYSIOLOGY

Functions and components of the blood

Blood is the only liquid connective tissue. It comprises 8% of total body weight (5 L in a normal adult). The functions of blood relate to its composition:

- Transport: of gases, nutrients, waste products and hormones.
- Immunological: defence against bacteria, viruses and foreign bodies by leukocytes.
- Homeostatic: temperature, pH, haemostasis and fluid exchange.

There are two main components in blood (Fig. 2.9):

1. Plasma (55%): a watery substance containing dissolved solutes and proteins in suspension.
2. Cells and cellular fragments (45%):
   - erythrocytes (99%), also known as red blood cells (RBCs)
   - platelets (<1%)
   - leukocytes (<1%) or white blood cells (WBCs).

Plasma

Plasma comprises:

- Water: forms a medium for the suspension and transport of proteins, solutes and gases and so influences partial pressures and gas exchange. Water is important in temperature regulation because it releases heat. It also removes waste and breakdown products.
- Solute: electrolytes in particular create osmotic pressure. Ions, e.g. $\text{HCO}_3^-$, are important in buffering pH change.
- Proteins: are important buffers and exert oncotic pressure:
  - Albumin: particularly important for vascular oncotic pressure and fluid exchange. It also transports fatty acids, lipid-soluble hormones and some drugs
  - Globulins: $\alpha$ and $\beta$ globulins transport hormones and iron, respectively. $\gamma$ Globulins
(antibodies) defend against viruses and bacteria.

- Other components: including fibrinogen, which is important in the process of blood clotting.

**Cells**
- RBCs: small, flexible, anucleate cells containing haemoglobin (~8 μm diameter). The biconcave shape suits their function in gas exchange and their transport.
- Platelets: small cell fragments derived from megakaryocytes in the bone marrow. Initiate haemostasis and thrombus formation at injury sites.
- WBCs: numbers increase during infection, surgery or strenuous exercise:
  - neutrophils (60%): engulf and phagocytose bacteria; also involved in inflammation. Numbers increase with bacterial infection, inflammation, burns and stress
  - lymphocytes (20%): there are type B and type T cells, the immunological roles of which include generation of the specific immune response, including antigen–antibody reactions. Numbers increase in viral infections and some leukaemias
  - monocytes (5%): phagocytose after transforming into macrophages. Numbers increase in viral or fungal, infections, tuberculosis and some chronic diseases
  - eosinophils (3%): destroy worm parasites. In allergic reactions, they combat histamine. Numbers increase in parasitic infections, allergic reactions and autoimmune diseases
  - basophils (<1%): amplify the inflammatory response via the release of heparin and vasoactive substances. Numbers increase in allergic reactions, cancers and leukaemias

**Gas transport in the blood**
After gas exchange at the lungs, gas transport completes the O₂ and CO₂ trade between cells and the external environment.

**Oxygen transport**
The factors affecting O₂ delivery to tissues are:
- The speed of delivery or blood flow: this depends on vascular constriction/dilatation.
- The amount of O₂ that can be carried by the blood: this depends on the:
  - amount of dissolved O₂
  - proportion of haemoglobin in the blood
  - affinity of haemoglobin for O₂

Hence, O₂ is transported in the blood in two forms:
- Dissolved in plasma: 2%.
- In chemical combination with haemoglobin in RBCs: 98%.

O₂ dissolves in plasma up to about 3 mL/L and, in accordance with Henry’s law, is proportional to its partial pressure. This small amount reflects the low solubility of O₂ in plasma. Clearly, this is not sufficient to meet even the body’s resting metabolic needs of 250 mL O₂/min, as a normal cardiac output of 5 L/min would supply only 15 mL/min of O₂ in this form. Hence, a carrier molecule is needed to transport the required O₂. This molecule is haemoglobin.

**Henry’s law**
The weight of a gas absorbed by a liquid with which it does not combine chemically is proportional to the partial pressure of the gas to which the liquid is exposed.

**Haemoglobin**
The molecular structure of this conjugate protein molecule is largely responsible for its O₂-carrying properties (Figs 2.10 and 2.11). Each of the four subunits consists of a polypeptide chain attached to a haem group, which can reversibly bind with one O₂ molecule. Hence, up to four molecules of O₂ can combine with each molecule of haemoglobin (Hb); a combination that exhibits cooperativity among the four binding sites. Haemoglobin has a greater affinity for binding the second and third O₂ molecules than the first and fourth, and these differences in affinity give rise to the characteristic ‘S’ shape of the O₂ dissociation curve (see later).

- Men have 150 g Hb/L of blood (15 g/dL).
- Females have 130 g Hb/L of blood (13 g/dL).

In adults, normal Hb is known as HbA, and consists of two α and two β chains. Any change to
the sequence of the polypeptide chains results in structural differences, with consequent alteration to the properties of the Hb (see later).

**Haemoglobin binding**

The rapid reversible combination of O\(_2\) with Hb to form oxyhaemoglobin (HbO\(_2\)) is written as:

\[
\text{Hb} + \text{O}_2 \leftrightarrow \text{HbO}_2
\]

(DeoxyHb) (OxyHb)
(Purplish) (Bright red)

Depending on the number of binding sites occupied by O\(_2\), Hb can be considered as either saturated (carrying all four molecules of O\(_2\)) or partially saturated (carrying less than four molecules of O\(_2\)).

The O\(_2\)-carrying capacity of Hb is 1.34 mL O\(_2\)/g Hb. This figure takes into account the small amounts of methaemoglobin (ferric iron core) in the O\(_2\)-carrying state compared to the normal ferrous Fe\(^{2+}\) state, and also allows for Hb that has combined with carbon monoxide (CO): in neither state can Hb transport O\(_2\). Therefore, in an adult man with 15 g of Hb/dL blood, the O\(_2\)-carrying capacity will be:

\[
15 \times 1.34 = 20.1 \text{ mL O}_2/\text{dL blood}
\]

The actual O\(_2\)-carrying capacity will depend on the amount of Hb in the blood, with the pressure of O\(_2\) (PO\(_2\)) driving the O\(_2\) into the blood.

O\(_2\) saturation of Hb refers to the proportion of Hb bound to O\(_2\). When the amount of reduced (deoxgenated) Hb is >5 mg, the lips, tongue and oral mucosa in particular take on a bluish pallor, known as central cyanosis. This reflects an O\(_2\) loading problem through either reduced gaseous exchange or pulmonary blood flow. Peripheral cyanosis—blueness of the nail beds and other extremities—results from secondary local causes such as sluggish blood flow, vasoconstriction, cold temperatures; that is it can occur independently of, but always in the presence of, central cyanosis.

**The oxygen dissociation curve**

Blood PO\(_2\) not only determines the amount of dissolved O\(_2\) in plasma, but also how much O\(_2\) binds to Hb, i.e. the O\(_2\) saturation. This is represented by the O\(_2\) dissociation curve (Fig. 2.12), which relates the PO\(_2\) to the O\(_2\)-carrying capacity of Hb under the following conditions:

- pH 7.4.
- Temperature 37°C.
- Normal PCO\(_2\) (40 mmHg).

The graph is ‘S’-shaped because Hb undergoes four sequential reactions to enable each subunit to bind with O\(_2\). These reactions become progressively easier, except for the binding of the fourth oxygen molecule. The S shape of the curve is explained as follows:

- When PO\(_2\) is low: the curve is steep and small changes in PO\(_2\) lead to large changes in Hb saturation.
saturation. This corresponds to gas exchange between the systemic capillaries and tissues. The result is that, for a small drop in PO₂, the tissues can take large amounts of O₂.

- When PO₂ > 70 mmHg: the curve is almost flat. This confers the advantage that when alveolar PO₂ drops, Hb can still become fully saturated with O₂. Importantly, this means that someone with moderate lung disease or who is hypoventilating can still easily load blood with O₂.

Various influences alter the behaviour of O₂ dissociation by shifting the position of the O₂ dissociation curve to the right or the left (Fig. 2.13).

**Right shift** This equates to reduced O₂ affinity of Hb, which results in:
- Easier unloading of O₂ at tissues at a given PO₂.
- Lower O₂ saturation.

A shift to the right occurs under the following conditions:
- pH < 7.4.
- Temperature > 37 °C.
- ↑ PCO₂: known as the Bohr effect. This is attributed to its action on [H⁺] and is discussed later in the chapter.
- ↑ 2,3 diphosphoglycerate (2,3-DPG), which binds to the β chains of Hb.

**Left shift** This equates to increased affinity of Hb for O₂ resulting in:
- Easier O₂ binding and uptake at any PO₂.
- Higher O₂ saturation.

A shift to the left occurs when conditions causing a right shift are reversed, such as:
- pH > 7.4.
- Cooler temperatures: note that O₂ is more soluble in plasma at lower temperatures.

**Effects of 2,3-diphosphoglycerate** 2,3-DPG is a normal metabolic by-product of RBC metabolism...
and large increases in levels of 2,3-DPG are seen in chronic hypoxia (chronic lung disease or at high altitudes). Its function is to facilitate the unloading of \( O_2 \) for use by peripheral tissues by allowing greater \( O_2 \) release at any \( PO_2 \). It does this by binding to the \( \beta \) chains of Hb, thereby reducing its affinity for \( O_2 \).

2,3-DPG is lost from stored blood, so that 1-week-old blood has very low levels. This means that unless 2,3-DPG levels are corrected, the blood will have very little tissue unloading.

Other forms of haemoglobin

**Myoglobin** This haem protein is found within muscle. Like the Hb subunit, myoglobin consists of one haem group with a polypeptide chain. It has the following features:

- Higher affinity for \( O_2 \) than normal adult Hb (HbA): the \( O_2 \) dissociation curve is relatively to the left. Therefore, myoglobin takes up \( O_2 \) from Hb in the capillaries.
- Acts to transport and temporarily store \( O_2 \) in skeletal muscle and has limited availability during anaerobic conditions, lasting only a few seconds.
- Binds \( O_2 \) at \( PO_2 \) values greater than those in venous blood \( PO_2 \). Hence it would be unsuitable as a blood carrier for \( O_2 \) (because it would not surrender \( O_2 \) to the tissues).

**Fetal haemoglobin** The fetal circulation has a lower \( PO_2 \) (30 mmHg after deoxygenation) than the maternal uterine arterial blood from which \( O_2 \) diffuses. Fetal blood contains mainly fetal Hb (HbF), the structure of which differs from HbA in that \( \gamma \) chains replace the \( \beta \) chains of HbA. HbF is crucial in directing \( O_2 \) transfer from maternal circulation to the fetus:

- HbF has a higher affinity for \( O_2 \) and its dissociation curve is positioned to the left of that of HbA. Therefore, HbF is 20–50% more saturated with \( O_2 \) than HbA at low \( PO_2 \) levels.
- The concentration of Hb in fetal blood (the [HbF]) > the concentration in maternal blood ([HbA]). This results in greater carriage of \( O_2 \) in fetal circulation.
- \( CO_2 \) unloading from fetal to maternal circulation: the acidic affects of increased \( CO_2 \) levels promote HbA dissociation of \( O_2 \), shifting its curve to the right. As \( CO_2 \) diffuses from fetal blood into maternal, this Bohr effect works in both fetal and maternal directions: double Bohr shift. \( O_2 \) release from maternal to fetal circulation and increasing fetal blood \( O_2 \) capacity are thereby promoted.

**Sickle haemoglobin** The Hb variant HbS is found in sickle-cell disease. It consists of a single amino acid substitution at position 6 in the \( \beta \)-globin chain of HbA. This has the following consequences:

- The \( O_2 \) dissociation curve is shifted to the right.
- HbS is a poorly soluble deoxygenated form that polymerizes, rendering the RBC fragile and crescent- or sickle-shaped: this further predisposes to thrombus formation, and hence vaso-occlusive crises caused by blockage of blood vessels can arise.

Sickle-cell disease is an autosomal recessive disorder with heterozygotes symptomatic only when hypoxic. However, homozygotes experience manifestations of infarction and ischaemia from vaso-occlusive crises (such as leg ulceration and bone pain, among others).

**Thalassaemias**

This is a group of disorders where there is an imbalance in the 1:1 ratio of \( \alpha \)- and \( \beta \)-globin chains, secondary to defective synthesis in either of these chains. As a consequence, the globin chains precipitate, causing:

- Inhibition of erythropoiesis in RBC precursors.
- Haemolysis of mature RBCs.

Each chain is coded by four genes, with symptoms varying from a mild microcytic anaemia with two gene deletions, to the death of either the fetus or the newborn if all four genes coding a chain are deleted. Disease is described as minor, intermediate or major, depending on the number of normal genes.

**Carbon monoxide poisoning and carboxyhaemoglobin**

Carboxyhaemoglobin (carboxyHb) is formed when carbon monoxide (CO) binds to Hb. CO interferes with \( O_2 \) transport in a number of ways:

- CO competes with \( O_2 \) for Hb-binding sites and renders the iron atom to which it is bound unable to bind any \( O_2 \) molecules.
- The affinity of CO for Hb is significantly higher (250 times greater) than that of \( O_2 \). It also takes a long time to clear.
• Exposure to very low levels of CO can seriously compromise the O₂-carrying capacity of blood: inspiration of 0.1% CO halves the O₂-carrying capacity because 50% of Hb would be in the form of carboxyHb.
• CO shifts the O₂ dissociation curve to the left, even further to the left than the curve for myoglobin (Fig. 2.13), decreasing both O₂ loading at the lungs and O₂ unloading at the tissues.
• CO poisoning is detected by blood carboxyHb levels and cherry-red colouring of the patient (from its pigment colour, not cyanosis). There would also be few if any symptoms.

Carbon dioxide transport

CO₂ in the blood is found in the following forms:
• Dissolved in plasma.
• As bicarbonate ions (HCO₃⁻) in plasma.
• As carbaminoglycine compounds (chemical combination with proteins) in whole blood.

The relative proportions of the above are subject to arteriovenous differences.

Dissolved CO₂

Between 5 and 10% of CO₂ is carried dissolved in the plasma. As with dissolved O₂, the actual amount dissolved depends on the partial pressure of CO₂. Henry’s law applies and CO₂ is approximately 20 times more soluble than O₂:

\[
\text{CO}_2 \text{ solubility at } 37°C = 0.6 \times 10^{-3} \text{ mL/mmHg CO}_2/1 \text{ mL of plasma}
\]

Therefore, in 100 mL mixed venous blood \(\text{PCO}_2 = 45 \text{ mmHg}\), the amount of dissolved \(\text{CO}_2\) is:

\[
0.6 \times 10^{-3} \times 100 \times 45 = 2.7 \text{ mL CO}_2/\text{L blood}
\]

Bicarbonate ions

The majority of CO₂ is transported as HCO₃⁻: 90% of CO₂ in arterial blood and 60% of CO₂ in venous blood is in this form:

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-
\]

\(\text{H}_2\text{CO}_3\) is carbonic acid, which is a weak acid. The formation of \(\text{HCO}_3^-\) ions from \(\text{CO}_2\) consists of two reactions:

1. \(\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3\): the formation of carbonic acid. This reaction requires the enzyme carbonic anhydrase, which is present in RBCs and which speeds up the reaction so that it is thousands of times faster than in the plasma.

2. \(\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-\): the dissociation of carbonic acid into \(\text{H}^+\) and \(\text{HCO}_3^-\) is normally rapid. However, it is limited by accumulation of the products, which need to be removed if the reaction is to proceed. This can be achieved by:
   - Hb buffering of \(\text{H}^+\): the reduced form of Hb is less acidic than the non-reduced form and so accepts \(\text{H}^+\) more readily:
     - \(\text{H}^+ + \text{Hb}^- \rightarrow \text{HHb}\)
     - \(\text{H}^+ + \text{HbO}_2^- \rightarrow \text{HHb} + \text{O}_2\)
   - The chloride shift: \(\text{HCO}_3^-\) movement from RBC to plasma. A specific anion exchange (AE) is responsible for the movement of \(\text{HCO}_3^-\) out of and \(\text{Cl}^-\) into the RBC. This \(\text{Cl}^-\) movement is known as the chloride shift (Hamburger phenomenon) and is important in maintaining electrical neutrality.

Carbamino compounds

In the tissues, CO₂ reacts readily with the terminal amine (\(\text{NH}_2\)) group on blood proteins (i.e. Hb) to form carbamino compounds – including carbaminoglycineHb – that contribute up to 30% of CO₂ transport in venous blood. This reaction does not require an enzyme and occurs more readily with reduced (less acidic) Hb. At the lungs, CO₂ is easily released by carbamino compounds.

The Haldane effect and the Bohr effect

The carriage of CO₂ in the blood is increased by deoxygenation. This is a result of reduced Hb being a weaker acid than its oxygenated form. Hence reduced Hb has the following actions:

- Promotes \(\text{HCO}_3^-\) formation and carriage by more readily accepting \(\text{H}^+\) in RBCs.
- Reacts more readily with CO₂ to produce carbaminoHb.

At the tissues, both Bohr and Haldane effects facilitate peripheral gas exchange (Fig. 2.14):

- ↑ PCO₂ and ↓pH promote \(\text{O}_2\) unloading from HbO₂ (the Bohr effect), shifting the \(\text{O}_2\) dissociation curve to the right.
- ↓PO₂ encourages CO₂ transport by the mechanisms described above (the Haldane effect).

At the lungs, again these effects are important (Fig. 2.15):
Physiology of the blood and body fluids

Fig. 2.14 Red blood cell carbon dioxide uptake and oxygen release at the peripheral tissues.

- ↑PO₂ and ↓PCO₂ facilitate CO₂ release from carbamino compounds by forming oxyHb.
- Release of H⁺, which was buffered by Hb in its reduced form, is crucial for the reverse reaction that forms HCO₃⁻ and H⁺ and produces CO₂, which is then exchanged at the lungs and exhaled.

Importantly, pH remains stable at both sites of gas exchange:

- At the tissues, reduced Hb gains acid.
- At the lungs loss of acid from reduced Hb results in acidic oxygenated Hb formation.

Carbon dioxide dissociation curve
PCO₂ dictates the total blood concentration of CO₂ in all its different forms of transport (Fig. 2.16). Importantly, the CO₂ dissociation curve displays an absence of flat portions and therefore does not demonstrate any saturation. The variability of PCO₂ is therefore greater than that of PO₂.

Haemostasis
Haemostasis refers to the control of bleeding. Following injury to a blood vessel, responses occur in two phases:

1. Rapid: reactions of blood vessel and platelets:
   - slowing of blood flow
   - formation of a platelet mass at the site of injury
   - diffusion of tissue factors from the extravascular compartment, initiating the extrinsic coagulation pathway.
2. Slow: intrinsic coagulation pathway: formation of insoluble fibrin threads that stabilize the platelet mass.

Three mechanisms are involved with haemostasis:

1. Vasoconstriction.
2. Formation of a platelet plug.
3. Coagulation (formation of a blood clot) with eventual clot retraction.

Vasoconstriction
Immediately after an injury to a blood vessel, the smooth muscle in the vascular wall contracts, decreasing diameter and blood loss. This occurs in response to:

- Nervous reflexes: as a result of pain and traumatized vessels.
- Local myogenic spasm.
- Platelet thromboxane A₂: this is more important in smaller than in larger vessels.

Formation of a platelet plug
Platelet adhesion, activation and aggregation occur (Fig. 2.17).

Fig. 2.15 Red blood cell carbon dioxide unloading at the lungs.
Adhesion
Damage to the vascular endothelium exposes collagen and other connective tissue to which platelets adhere.

Activation
During activation, platelets undergo:
- Shape change: they swell then extend many projections that facilitate greater contact with other platelets.
- Granule release: contractile proteins contract, causing release of the following from granules:
  - ADP and thromboxane A₂: which activate and make platelets sticky
  - serotonin and thromboxane A₂: which cause vasoconstriction, so reducing blood loss.
- Expression of new receptors: IIb and III.
Activated platelets recruit nearby platelets so that the effects are amplified.

Aggregation
The sticky, activated platelets attract one another and adhere, forming a loose clump. This platelet plug is effective at blocking small holes and coagulation will not be necessary. However, injury to a blood vessel often requires coagulation.

Coagulation
Coagulation comprises a sequence of enzyme-catalysed conversions of inactive factors to more active forms, culminating in the conversion of fluid blood into a solid gel or clot.

Blood clot formation begins a matter of seconds after severe trauma to the vascular wall, and in minutes after injury to other areas of the body.
Two pathways are involved (Fig. 2.18):

1. The intrinsic pathway:
   - is triggered by trauma to blood and exposure to collagen
   - involves many enzyme-catalysed steps
   - is slow (minutes).
2. The extrinsic pathway:
   - requires factors external to blood vessels, e.g. tissue factor (tissue thromboplastin)
   - few enzymes and steps are involved
   - is limited by the amount of tissue factor
   - is fast (seconds).

Both pathways produce prothrombinase (prothrombin activator), the formation of which appears to be the rate-limiting step in haemostasis. After this stage, pathways follow a common set of reactions, which is known as the final common pathway (see Fig. 2.18):

1. Prothrombinase and ionized Ca\(^{2+}\) cause prothrombin $\rightarrow$ thrombin.
2a. Thrombin and ionized Ca\(^{2+}\) cause fibrinogen (soluble) $\rightarrow$ fibrin fibres (insoluble).
2b. Thrombin activates fibrin-stabilizing factor (XIII), which cross-links the fibrin fibres.

The cross-linked fibrin strands mesh plasma, the platelet plug, plasmin and RBCs to form a blood clot.

In clinical practice:
- The intrinsic pathway is monitored by partial or accelerated thromboplastin time (APTT).
- The extrinsic pathway is monitored using the prothrombin time (PT).

**Role of thrombin**

Thrombin formed in the first stage on the final common pathway exerts positive feedback effects on the coagulation cascade (Fig. 2.19):

- Acceleration of the formation of prothrombinase.
- Platelet activation.

### Actions of thrombin

- Polymerization of fibrinogen.
- Activation of factor XIII, which cross-links fibrin strands.
- Positive feedback effects on the coagulation cascade.

### Role of platelets

- Platelet phospholipids are required for the assembly of prothrombinase: these interact with activated factors X and V, and with Ca\(^{2+}\), to produce prothrombinase.
- IIb and IIIa receptors bind to fibrin causing platelet aggregation with the fibrin glue.

#### Thrombus formation

A thrombus is a clot that forms within an intact blood vessel. This results from inappropriate activation of haemostasis with one of the following consequences:

- The thrombus dissolves spontaneously.
- The thrombus remains intact, with the risk of embolization.

#### Venous and arterial thrombi differ:

- Arterial thrombi:
  - contain large platelet and small fibrin components
  - formation is associated with atheroma formation and turbulent blood flow
  - have a rough endothelial surface that attracts the platelets.
- Venous thrombi:
  - contain large fibrin and small fibrin components
  - formation is associated with slow blood flow, which causes a large increase in procoagulant factor concentration.

### Clot retraction

Within a few minutes of its formation, the clot begins to contract due to platelets applying tension to the fibrin fibres that are attached to damaged blood vessels, the ends of which are therefore brought closer together. In addition, fluid is squeezed out of the clot. Platelets are suited to clot retraction because they:

- Release factor XIII (fibrin-stabilizing factor): causing more cross-linking of fibrin and permitting further compression of the clot.
Activate the self-contractile proteins thrombosthenin, actin and myosin: these allow the platelet to pull harder on the fibrin fibres.

**Normal prevention of coagulation**

The normal vascular system employs a number of mechanisms to keep haemostasis in check:

**Prevention of activation of a haemostatic plug**

- Smooth endothelial cells discourage any activation of the intrinsic pathway.

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**Fig. 2.18 Coagulation pathways.**

**Fig. 2.19 Thrombin positive feedback loop.**
The glycocalyx layer on the endothelium repels both platelets and clotting factors.

**Inhibition of the coagulation cascade**
- Endothelial-bound thrombomodulin: binds thrombin and activates protein C, which is a plasma protein.
- Protein C: inactivates factors V and VIII.
- Antithrombin III (α-globulin and most important circulating anticoagulant): combines with thrombin, inactivating it for up to 20 minutes and blocking its effects on fibrinogen.
- Heparin molecule: is not active by itself but potentiates antithrombin III 100–1000-fold, with the added effect of removing activated factors XII, XI, X and IX.

**Fibrinolysis**
Plasmin is particularly important for removing inappropriately formed small blood clots; it acts to digest fibrin fibres and inactivate clotting substances: fibrinogen, prothrombin, factors V, VIII and XII. Plasmin is formed from the inactive plasma enzyme plasminogen by:
- Tissue plasminogen activator (t-PA): released by damaged endothelial cells at the periphery. It activates plasminogen in the presence of fibrin.
- Clotting factors: thrombin and activated factor XIII can activate plasminogen.