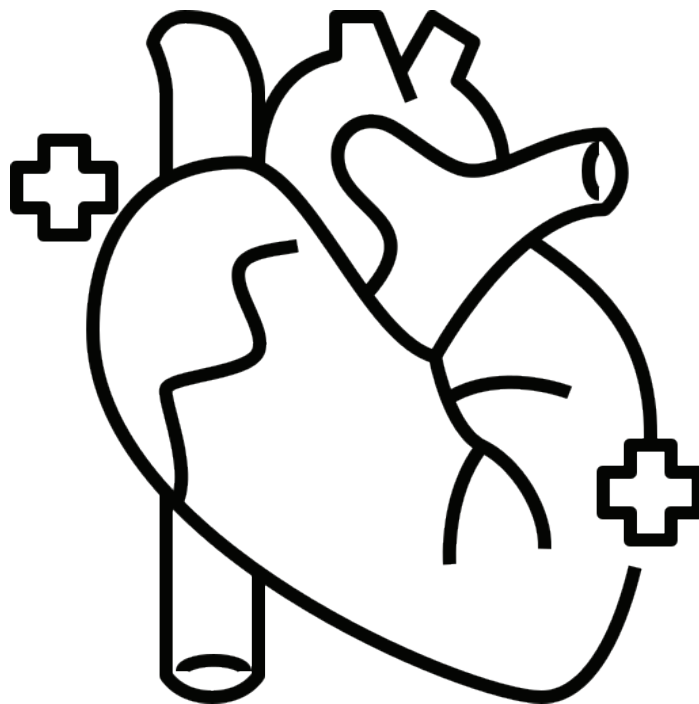


SYSTEM PHYSIOLOGY

ANIMAL



Dr. KAILASH CHOUDHARY | Dr. RAM PRAKASH SARAN

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PREFACE

Physiology is where anatomy is made to work — a body of organs and tissues becomes, chapter by chapter, a system that keeps itself alive, moves, senses, reproduces, and defends itself against a changing environment. This book was written for that experience of understanding: not a list of facts to be memorised before an exam, but a working account of how each organ system does what it does, in humans and, wherever the comparison sharpens the point, in the other animals whose solutions are older, stranger, or simply different.

It exists for a practical reason as well. Animal Physiology carries substantial weight in the Life Sciences examinations, and it is a core paper across the M.Sc and B.Sc life-science curricula, yet the standard reference texts are written for a semester, not for an examination hall six months away. Students preparing for these exams are usually working from scattered class notes, textbooks of uneven depth, and years of previous-year questions with no single place that ties the physiology to the questions actually asked of it. This book was built to be that place.

The fourteen chapters move outward from the blood to the mechanisms that connect one organ to another. Blood and Hemostasis opens with the tissue that reaches every other, its formed elements, and the two processes an examiner returns to most often — coagulation and blood-group compatibility. The Cardiovascular, Respiratory, Excretory and Digestive System chapters that follow take up the classical transport and exchange systems, each built the same way: the comparative picture across animal groups first, then the human system in the depth an examination demands, down to the kind of quantitative problem — a filtration rate, an oxygen-dissociation calculation, an acid–base derangement — that a solved numerical question requires. The Nervous, Motor and Sensory System chapters take the same approach to signalling and movement, from the ionic basis of the action potential to the phototransduction cascade and the sensory worlds — echolocation, electroreception, polarised-light vision — that a comparative question is most likely to test.

The middle third of the book turns to the systems that regulate the body as a whole: the Endocrine System, the Reproductive System, and Thermoregulation, each again worked through invertebrate and vertebrate biology before it settles on the human case. A separate chapter is given to Stress and Adaptation, tracing the sympatho-adrenal and hypothalamic–pituitary–adrenal axes through to allostatic load and the extremes of metabolic depression — torpor, hibernation, diapause — that examiners have increasingly drawn on in recent papers.

The last two chapters - Holobionts and the Gut Microbiome treats the animal as host to a metaorganism, with its own methods of study, its own gut–brain axis, and its own bearing on disease — a subject that barely featured in physiology courses a decade ago and now appears regularly in current question papers. Interorgan Communication closes the book by drawing the threads of the preceding thirteen chapters together: the signals by which the liver, the fat, the muscle and the brain announce their state to one another, and what goes wrong, systemically, when that signalling fails.

Every chapter ends with solved problems or practice questions worked through in full, because a physiological principle is tested, in these examinations, by being applied — to a graph, a clinical value, the action of a hormone — and not by being recited. Learning objectives at the start of each chapter and KEY POINT boxes throughout are meant to make each chapter as useful for revision as for a first reading, so that the same book serves a student meeting a system for the first time and a candidate returning to it the week before the exam.

We have tried, throughout, to write the physiology accurately first and the exam-readiness second, in the conviction that the second follows from the first and not the other way round. Where the two pulled in different directions we kept the physiology, trusting that an examiner rewards understanding over memorised phrasing. We hope this book earns a place on the desk of every student working toward CSIR-NET, GATE, or a life-sciences degree, and we welcome the corrections and suggestions of the teachers and students who use it.

Dr. Kailash Choudhary

Dr. Ram Prakash Saran

ACKNOWLEDGEMENTS

No book of this length writes itself, and this one least of all — fourteen systems, from the blood to the signals that pass between organs, each drafted, tested in a classroom, and rewritten until it held. We are glad, once the writing is done, to set down in one place who helped it hold.

Our first debt, as it was with the book that came before this one, is to the students of the Institute for Advanced Studies (IFAS). It was their questions about a coagulation cascade that would not balance, or a reflex arc that would not quite make sense on first reading, that decided which explanations in these pages earned their place and which were sent back to be rewritten. A textbook owes its clarity to the readers who refused to accept less of it, and this one is no exception.

We are grateful again to Er. Radheshyam Choudhary and Mr. DadaSaheb Sondge, who gave this manuscript the same careful, chapter-by-chapter reading they gave the last, and whose suggestions have left their mark on every system this book describes. Mr. Pranshu Dwivedi and Mr. Vishal Bhujbal worked through it section by section and figure by figure, and it is their eye, once more, that deserves the thanks wherever an explanation lands cleanly and a diagram says exactly what it needs to.

Mr. Lalji Kanoujia and Dr. Rakesh Kumawat took on, for this book as for the last, the considerable task of sorting and compiling its central concepts and setting each one against its proper chapter and section — the quiet structural labour that turns fourteen independent chapters into a single coherent reference. Mr. Lokesh and Mr. Mohit proofread the manuscript and verified its index with the same close attention that has become, across both books, their signature; a reference work is trusted in the small things, and they attended to all of them.

The book's finished appearance belongs, once again, to the IFAS Publication Team, and in particular to Mr. Vikendra Metha, whose cover design and diagram work give this volume — as they gave the one before it — a surface as considered as its contents.

To everyone whose hand touched these pages without their name appearing here, our sincere and continuing thanks. A book of this kind is the sum of many small, uncredited acts of care, and we remain conscious of every one of them.

With sincere thanks,
Dr. Kailash Choudhary
Dr. Ram Prakash Saran

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Blood and Hemostasis

Blood is the only tissue that reaches every other. It is also the only one that can be sampled from a vein and read like a report on the whole body, which is why almost every clinical investigation begins with it. This chapter follows blood from what it is made of, through how its cells are made and destroyed, to the two processes that matter most in an examination — the arrest of bleeding, and the antigens that decide whether one person's blood can safely enter another's.

LEARNING OBJECTIVES

- Describe the composition and physical properties of blood, and distinguish plasma from serum.
- Interpret a serum protein electrophoretogram and name the major plasma proteins and their functions.
- Identify the formed elements and quote their normal circulating values.
- Trace haematopoiesis from the stem cell, and explain how erythropoiesis is regulated and what nutrients it needs.
- Describe the structure, developmental forms and catabolism of haemoglobin.
- Outline innate and adaptive immunity and the leukocytes that carry them out.
- Explain primary and secondary hemostasis, the coagulation cascade, its natural brakes and its drugs.
- Explain the biosynthesis of the ABO and Rh antigens and apply the rules of transfusion compatibility.

1.1 Blood

Blood is a highly specialised *fluid connective tissue*: cellular elements suspended in a liquid extracellular matrix. The cells — collectively the *formed elements* — are the red blood cells (erythrocytes), the white blood cells (leukocytes) and the cell fragments called platelets (thrombocytes). The matrix, *plasma*, is what makes blood unique among connective tissues, for it is fluid rather than solid. In a large, complex body blood is the principal transport medium, delivering oxygen and nutrients to trillions of cells and carrying wastes away, while the heart drives it through a closed network of vessels.

1.1.1 Physical characteristics

Blood is opaque because of the cells, proteins and dissolved substances it carries; the haem pigment of haemoglobin gives it its colour. In health it is confined to the circulation and accounts for about **7–8%** of body weight in an adult — roughly 5 L in a 70 kg person. Blood volume is about 4.5–5.5 L in women and 5.0–6.0 L in men, the higher volume in men reflecting the stimulatory effect of testosterone on haematopoiesis. Volume is defended by the kidney's handling of salt and water, by the renin–angiotensin–aldosterone system, by antidiuretic hormone (vasopressin) and by the natriuretic peptides, so that circulating volume and pressure stay within narrow limits.

The *specific gravity* of whole blood is about 1.050–1.060, higher than that of water (≈ 0.99 g/mL), and depends mainly on cell number and plasma protein content. Individual cells differ in density — erythrocytes are the densest, granulocytes next, and lymphocytes, monocytes and platelets progressively lighter — which is the basis of separating them on a density gradient. Blood is also **3.5–5.5 times more viscous than water**, and its viscosity rises steeply as the haematocrit and the concentration of plasma macromolecules increase; in polycythaemia and in the paraproteinaemias the rise in viscosity makes flow to the extremities and to internal organs sluggish.

KEY POINT Blood cells are separated in the laboratory by two complementary principles: by *density*, using density-gradient centrifugation, and by *surface markers*, using fluorescence-activated cell sorting (FACS). Density separates by physical property and gives a bulk fraction; FACS separates by identity and can purify a defined population cell by cell.

1.1.2 Plasma and serum

Plasma is the clear, pale-yellow liquid left when the cells are removed by centrifugation of anticoagulated blood. If instead the blood is allowed to clot, the fluid expressed from the clot is *serum*. Serum has essentially the composition of plasma except that it lacks fibrinogen and the other factors consumed during coagulation (chiefly factors I, II, V and VIII). In short: **serum = plasma – fibrinogen and the consumed clotting factors.**

Water is the major component of plasma (about 92%); the remainder is dissolved solute — proteins, lipids, carbohydrates, amino acids, vitamins, minerals, hormones, metabolic wastes, cofactors, dissolved gases and electrolytes (Table 1.1). These solutes are central to homeostasis, not least in holding plasma pH and osmolality steady. Of the plasma buffers the **bicarbonate ($\text{HCO}_3^-/\text{H}_2\text{CO}_3$) system is the most important quantitatively**, because its acid component is volatile and can be blown off by the lung while its base component is regulated by the kidney; haemoglobin and the plasma proteins provide additional buffering capacity.

Most plasma proteins — albumin, fibrinogen and 50–80% of the globulins — are made by the *liver*; the γ -globulins (immunoglobulins) are the exception, being produced by plasma cells of lymphoid tissue. In liver cirrhosis the fall in plasma-protein synthesis lowers the *colloid osmotic (oncotic) pressure* of blood, so fluid leaves the capillaries and tissue oedema results.

Individual proteins serve specialised roles (Table 1.2): albumin maintains oncotic pressure and carries free fatty acids; transferrin carries iron; ceruloplasmin carries copper; haptoglobin and hemopexin scavenge free haemoglobin and free haem; transthyretin carries thyroid hormone and retinol.

Two examples matter clinically — **α_1 -antitrypsin** (α_1 -antiprotease), whose genetic deficiency causes emphysema because neutrophil elastase goes unopposed in the lung, and **α_1 -acid glycoprotein (AGP)**, an acute-phase reactant that rises in inflammation and binds many basic drugs.

Table 1.1 Composition of plasma.

Class	Substance	Normal range
Cations	Sodium (Na^+); Potassium (K^+); Calcium (Ca^{2+}); Magnesium (Mg^{2+})	136–145; 3.5–5.0; 4.2–5.2; 1.5–2.0 mEq/L
	Iron (Fe^{3+}); Copper (Cu^{2+})	50–170; 70–155 $\mu\text{g/dL}$
Anions	Chloride (Cl^-); Bicarbonate (HCO_3^-); Lactate; Phosphate	95–105; 22–26; 0.67–1.8; 3.0–4.5 mEq/L
Protein	Total protein (albumin 3.5–5.0 g/dL)	6–8 g/dL
Sugar	Glucose (fasting)	70–110 mg/dL
Other	Creatinine; blood urea; cholesterol	0.6–1.2; 7–18; 150–200 mg/dL

Table 1.2 The major plasma proteins and what they do.

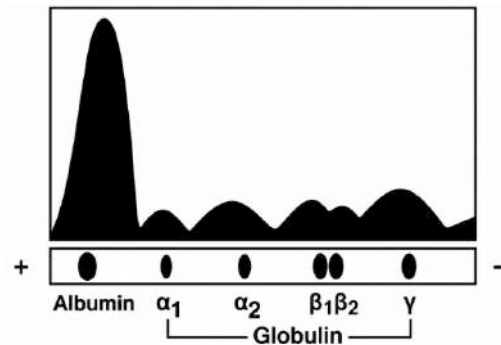
Protein	Function
Albumin	Maintains colloid osmotic pressure; carries free fatty acids, bilirubin, calcium and many drugs; contributes to buffering
γ -Globulins (immunoglobulins)	Humoral immunity; the only major plasma proteins not made by the liver
Transthyretin (prealbumin)	Transport of thyroid hormone and, with retinol-binding protein, of retinol
Transferrin	Iron transport in plasma
Ceruloplasmin	Copper transport; ferroxidase activity
Haptoglobin	Binds free haemoglobin released by intravascular haemolysis
Hemopexin	Binds free haem
C-reactive protein	Acute-phase reactant; marker of inflammation
α_1 -Antitrypsin (α_1 -antiprotease)	Protease inhibitor; deficiency causes emphysema and liver disease
α_1 -Acid glycoprotein (AGP)	Acute-phase reactant; binds basic drugs
von Willebrand factor	Platelet adhesion and carriage of factor VIII — made by endothelium and megakaryocytes, <i>not</i> by the liver

1.1.3 Serum protein electrophoresis

Serum protein electrophoresis (SPEP) is a simple and inexpensive method that separates proteins by net charge, size and shape. The two major fractions are albumin and the globulins (Figure 1.1). In a healthy person **albumin is the largest peak and migrates furthest toward the positive electrode (anode)** because it carries the greatest net negative charge. The globulins fall into five zones — α_1 , α_2 , β_1 , β_2 and γ — with the γ fraction nearest the cathode.

The pattern is diagnostically useful. In *acute inflammation* the acute-phase proteins of the α_1 and α_2 zones (α_1 -antitrypsin, AGP, haptoglobin, ceruloplasmin) rise while albumin falls, because albumin behaves as a negative acute-phase protein. A broad, *polyclonal* rise in the γ zone accompanies chronic inflammation and chronic infection; a narrow, *monoclonal* γ spike points instead to a plasma-cell disorder such as multiple myeloma.

Figure 1.1 Electrophoretic separation of serum proteins. Albumin lies nearest the anode; the globulins resolve into α_1 , α_2 , β_1 , β_2 and γ zones, the γ zone lying nearest the cathode.



1.1.4 Functions of blood

The cells and plasma act singly and together to perform four broad functions.

- **Transport** of respiratory gases, nutrients, wastes, hormones and heat between regions of the body.
- **Immunity** — defence against pathogens and abnormal cells.
- **Hemostasis** — the arrest of blood loss after vascular injury.
- **Homeostasis** — stabilisation of the internal environment (pH, ions, osmolality, temperature) through the three activities above.

1.2 The Formed Elements and Haematopoiesis

Blood contains three classes of formed element. Erythrocytes are by far the most numerous, at about **4–6 million per microlitre (μL)**; leukocytes number **4,500–11,000/ μL** and platelets **150,000–400,000/ μL** . The leukocytes are further divided by morphology and function (Figure 1.2), and normal circulating values are listed in Table 1.3.

Figure 1.2 The principal leukocytes. The granulocytes (neutrophil, eosinophil, basophil) have lobed nuclei and stainable granules; the mononuclear cells are the monocyte — which becomes the tissue macrophage — and the lymphocytes, of which the natural killer cell is one class.

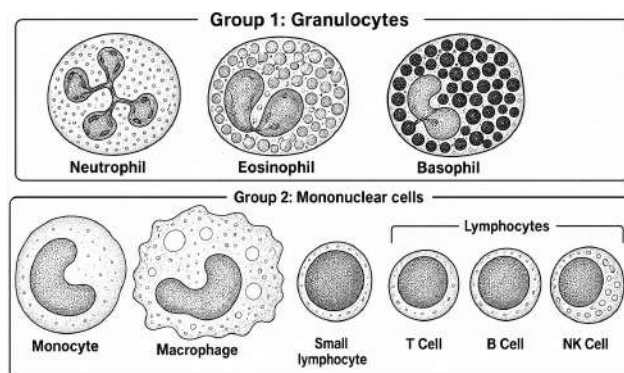


Table 1.3 Normal circulating levels of the formed elements in an adult.

Cell type	Absolute count per μL	Share of leukocytes
Erythrocytes (men / women)	$4.3\text{--}5.9 \times 10^6$ / $3.5\text{--}5.5 \times 10^6$	—
Leukocytes (total)	4,500–11,000	100%
Neutrophils	2,000–7,000	40–75%
Lymphocytes	1,000–3,000	20–45%
Monocytes	200–1,000	2–10%
Eosinophils	20–500	1–6%
Basophils	20–100	<1–2%
Platelets	150,000–400,000	—

Polymorphonuclear neutrophils are the most numerous leukocytes and the vigorous phagocytes of invading micro-organisms. The other granulocytes — eosinophils and basophils — are far fewer and act against parasites and in allergic and hypersensitivity reactions. The mononuclear leukocytes are the monocytes, which leave the vessels and mature into long-lived tissue macrophages, and the lymphocytes, which carry adaptive immunity. Because the leukocyte profile shifts characteristically in disease — neutrophilia in bacterial infection, eosinophilia on allergen or helminth exposure, lymphopenia in advanced HIV disease — the **differential leukocyte count** is one of the most informative simple investigations in medicine.

1.2.1 Erythrocytes

Each erythrocyte is a *biconcave disc* about 7 μm across and up to 2.5 μm thick, and **lacks a nucleus** — an adaptation that maximises the surface-to-volume ratio and so the efficiency of gas exchange, and that frees the whole cell interior for haemoglobin. Its shape is held by an elaborate membrane cytoskeleton, a meshwork of spectrin, ankyrin and band 4.1 anchored to the membrane, which gives the deformability needed to squeeze through capillaries narrower than the cell itself. The cytoplasm is rich in haemoglobin, and also contains antioxidant enzymes and the glycolytic machinery that supplies the ATP for the membrane pumps that keep intracellular K^+ high and Na^+ and Ca^{2+} low.

Each normal red cell contains about **29 pg of haemoglobin** — the mean corpuscular haemoglobin, MCH, with a reference range of 27–33 pg. The *haematocrit*, the fraction of blood volume occupied by red cells, is **highest in venous blood**, because there the cells have taken up CO_2 and water and swollen slightly. Red cells behave as osmometers: in hypotonic saline they swell and lyse. Haemolysis *begins* at about 0.45–0.50% NaCl and is *complete* at about 0.35%, whereas the cells remain intact in isotonic ($\approx 0.9\%$) saline. This graded behaviour is the basis of the **osmotic fragility test**, in which fragility is increased in hereditary spherocytosis and decreased in thalassaemia and iron deficiency.

1.2.2 Megakaryocytes and platelets

Large, multilobed *promegakaryocytes* of the bone marrow mature into **megakaryocytes**, giant polyploid cells that extend proplatelet processes into the marrow sinusoids and shed fragments of cytoplasm as platelets. A single megakaryocyte yields several thousand platelets. Their production is driven by **thrombopoietin**, made constitutively by the liver and cleared by binding to platelets, so that the free hormone concentration rises automatically whenever the platelet mass falls. Platelets are **anucleate**, about one-quarter to one-third the diameter of an erythrocyte (2–4 μm), and circulate for about **8–10 days**. Their granules store physiologically active molecules that are secreted when the platelet is activated.

1.2.3 Leukopoiesis

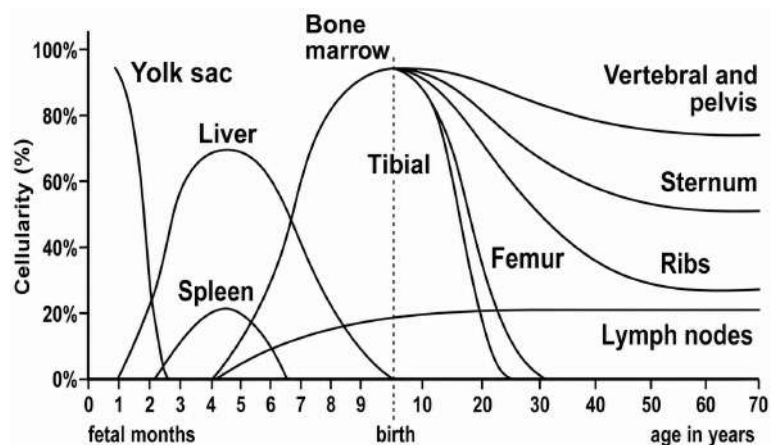
Leukocytes are produced in the marrow and can also divide within lymphoid tissue. Many are amoeboid, moving by pseudopodia, and leave the circulation by squeezing between endothelial cells — *diapedesis*. Granulopoiesis follows the myeloid line: a myeloblast becomes a promyelocyte, then a myelocyte whose cytoplasm fills with granules, then a metamyelocyte, a band form and finally the mature segmented cell. Myeloblasts are not normally seen in peripheral blood, though myelocytes may appear there in myeloproliferative disease and in severe infection. Three myelocyte types — eosinophilic, basophilic and neutrophilic, named for the dyes their granules take up — give rise respectively to eosinophils, basophils and neutrophils. Nuclei of mature granulocytes are divided into two to five lobes joined by thin chromatin strands, which is why they are called *polymorphonuclear*. Neutrophils survive only about 6–12 hours in the circulation and one to two days in tissue, so they must be replaced continuously.

1.2.4 Sites of blood-cell production

All blood cells derive from **haematopoietic stem cells (HSCs)**, undifferentiated cells able to generate the progenitors of every blood lineage. Their defining property is *self-renewal*: when an HSC divides, one daughter remains a stem cell while the other becomes committed. HSCs are **multipotent** and give rise not only to erythrocytes, granulocytes, lymphocytes and platelets but also to osteoclasts and tissue mast cells; they are not themselves derived from any more committed cell. The site of production shifts through development (Figure 1.3).

- **Early embryo** — the yolk sac.
- **Mid-trimester fetus** — the liver, then the spleen and lymphoid tissue.
- **After birth** — the red bone marrow becomes the sole significant site.

Figure 1.3 Sites of blood formation through life. Haematopoiesis passes from the yolk sac to the fetal liver and spleen and then to the marrow; within the marrow it retreats from the long bones and is confined in the adult to the axial skeleton.



2.6.3 Parasympathetic regulation

Vagal endings release acetylcholine onto the SA node, where it binds **M₂ muscarinic receptors** — named because they also respond to muscarine, the mushroom toxin. The M₂ receptor couples to an inhibitory G protein (G_i), lowering cAMP and closing the funny and T-type channels. Phase 4 flattens and the rate falls, toward 20–40 a minute with strong stimulation (Figure 2.31).

Because vagal fibres supply mainly the atria and very little ventricular muscle, vagal activity slows the heart far more than it weakens it — though very strong stimulation can reduce contractile strength as well.

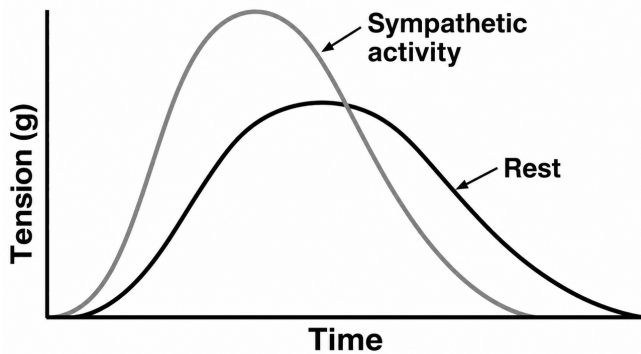


Figure 2.30 Cardiac muscle tension at rest and during sympathetic stimulation.

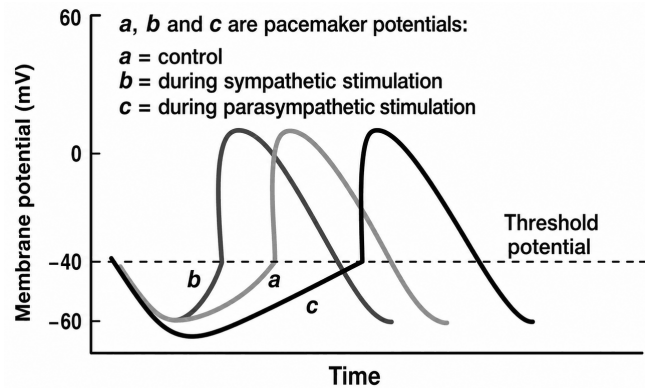


Figure 2.31 Frequency of pacemaker action potentials during sympathetic and parasympathetic regulation.

2.6.4 The HCN channel is where the two systems meet

Everything above comes down to one channel. The more HCN channels are open, the sooner threshold is reached and the sooner the next beat fires — and cAMP is what opens them (Figure 2.32).

Norepinephrine and circulating adrenaline bind **G_s-coupled β₁ receptors**, activate adenylate cyclase, raise cAMP, open more HCN channels and speed the heart. Acetylcholine binds **G_i-coupled M₂ receptors**, inhibits adenylate cyclase, lowers cAMP, closes HCN channels and slows it. (The α₂-adrenergic receptor is also G_i-coupled and lowers cAMP.) The two divisions are therefore genuinely antagonistic, acting on the same target from opposite directions (Table 2.3). Figure 2.33 puts the whole scheme on one nodal cell.

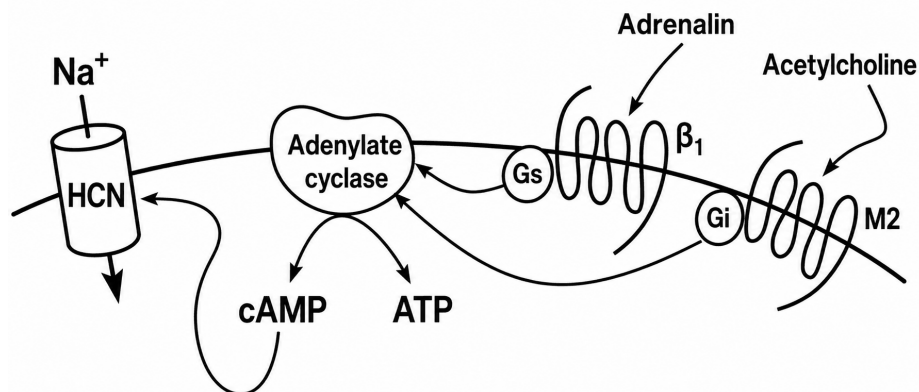
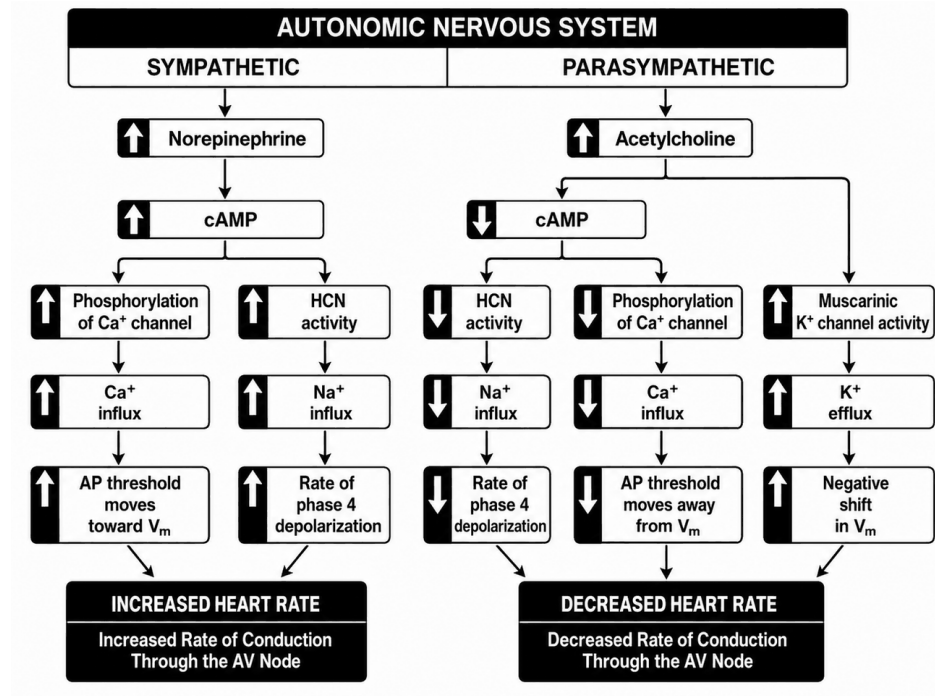


Figure 2.32 HCN-channel opening and closing are controlled by intracellular cAMP, which rises with β₁ (G_s) stimulation and falls with M₂ (G_i) stimulation.

Table 2.3 Effects of the autonomic nerves on the heart.

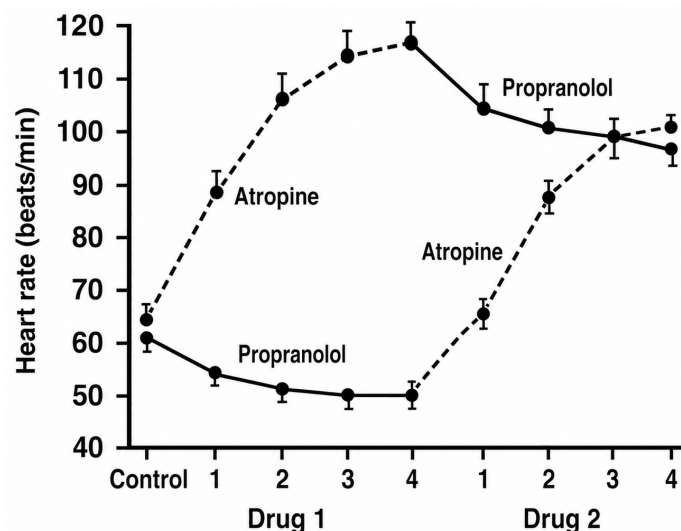
Region	Sympathetic	Parasympathetic
SA node	Faster rate	Slower rate
AV node	Faster conduction	Slower conduction
Atrial muscle	More forceful contraction	Less forceful contraction
Ventricular muscle	More forceful contraction	Little effect — few vagal fibres

Figure 2.33 Autonomic regulation of the nodal cells (HCN = funny channel; V_m = membrane potential).



Vagal tone normally predominates at rest, and a simple experiment proves it. Blocking the vagus with **atropine** raises the heart rate a great deal; blocking sympathetic effects with **propranolol** lowers it only slightly (Figure 2.34). Block both together and a young adult's heart settles at about 100 a minute — the **intrinsic heart rate**.

Figure 2.34 Effect of equal doses of atropine and propranolol on the heart rate of a healthy young adult.



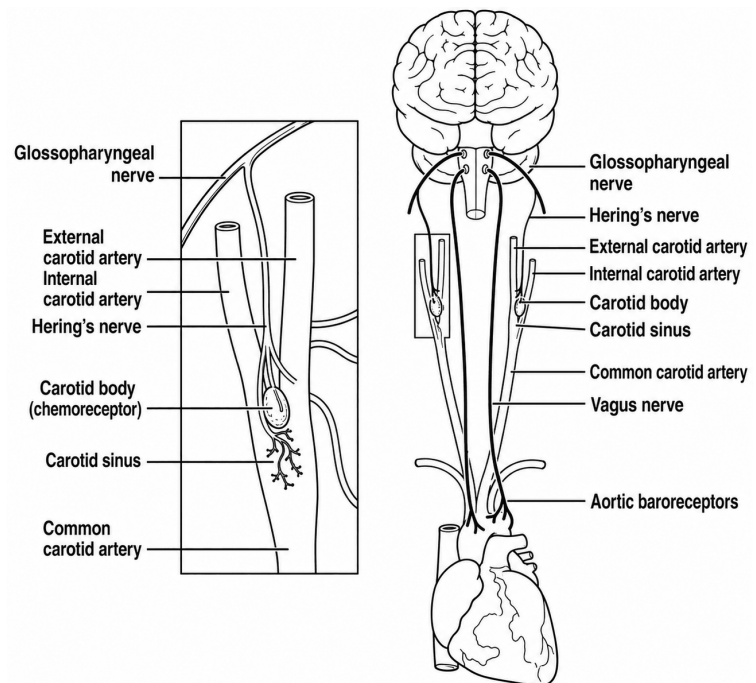
2.6.5 The baroreceptor reflex

Baroreceptors are stretch sensors in the walls of the aortic arch and the carotid sinuses. They give the body its fastest defence of arterial pressure: a sudden change in pressure triggers an opposite change in heart rate and vessel tone within a beat or two.

The receptors are spray-like nerve endings, densest in the **carotid sinus** just above the carotid bifurcation and in the **aortic arch**. Carotid-sinus signals travel in the nerve of Hering — whose cell bodies lie in the **petrosal ganglion** — into the glossopharyngeal nerve and on to the nucleus tractus solitarius of the medulla. Aortic-arch signals travel in the **vagus**, with cell bodies in the **nodose ganglion**, to the same nucleus (Figure 2.35).

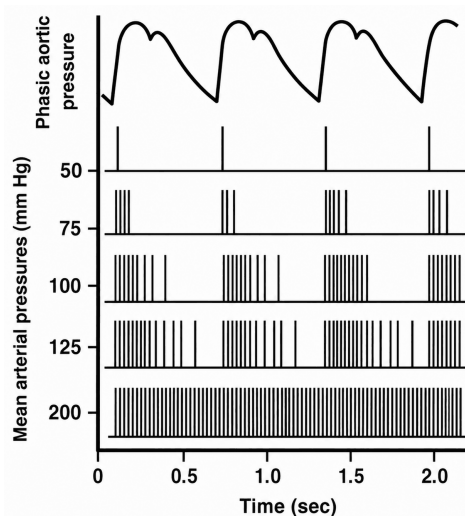
Baroreceptor firing rises when pressure rises and falls when pressure falls. More firing inhibits the medullary vasoconstrictor centre, so the vessels dilate, and excites the vagal centre, so the heart slows — both of which bring pressure back down.

Figure 2.35 Physiological anatomy of the baroreceptors.



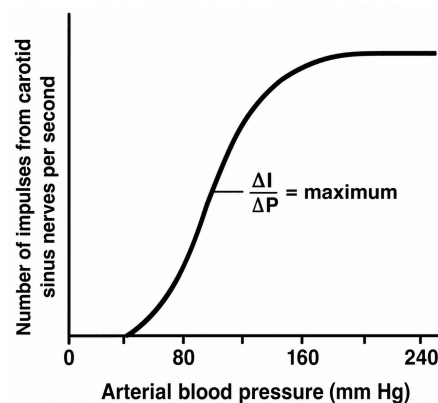
The carotid receptors are the more sensitive of the two, so a given change there produces a larger systemic response. They also respond far better to a *pulsating* pressure than to a steady one (Figure 2.36): at a normal mean pressure a single fibre fires a burst in early systole and falls almost silent later in the cycle.

Figure 2.36 Firing of a single carotid-sinus afferent fibre in relation to phasic aortic pressure at different mean arterial pressures.



Plotted against pressure, the carotid receptors are silent below about 60 mmHg, then increase their firing steeply and almost linearly between about 80 and 150 mmHg, reaching a maximum near 180 mmHg (Figure 2.37). They are most sensitive right around the normal operating pressure, which is exactly where a control system should be most sensitive. The aortic receptors behave the same way but operate about 30 mmHg higher.

Figure 2.37 Activation of baroreceptors at different levels of arterial pressure.



The pay-off is large. Myelinated mammalian fibres conduct at up to about **120 m/s**, against as little as **0.25 m/s** in small unmyelinated fibres — fast enough to support quick reflexes and complex processing in a large body.

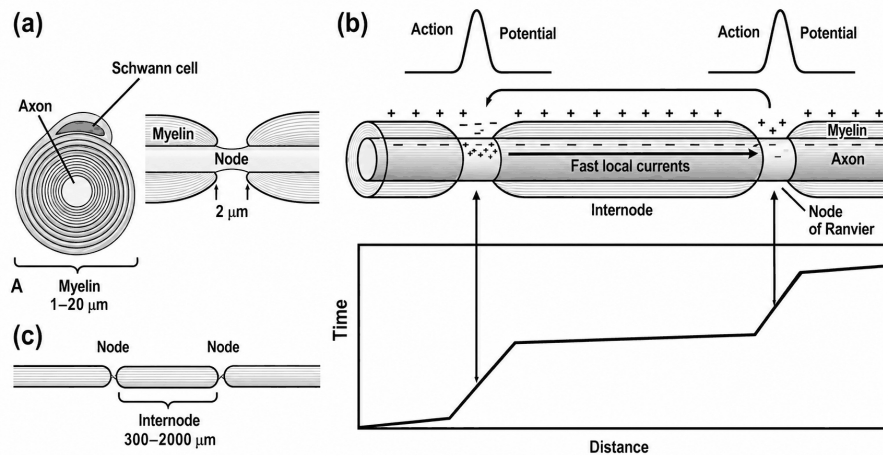


Figure 4.25 A Schwann cell wrapping an axon to form myelin, and the node of Ranvier between two internodes.

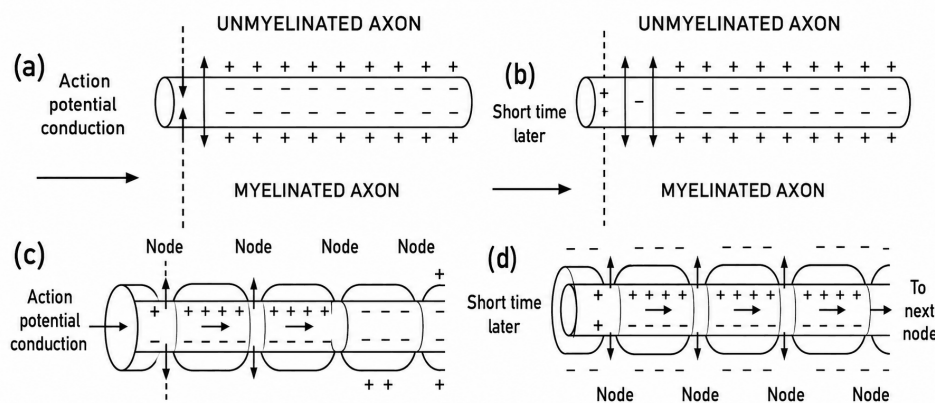


Figure 4.26 Continuous conduction in an unmyelinated axon (a, b) compared with saltatory conduction from node to node in a myelinated axon (c, d).

DO YOU KNOW? Demyelinating disease attacks the insulation, not the wire. In multiple sclerosis the myelin of CNS axons is lost, the length constant collapses, and impulses slow or fail altogether — which is why symptoms are so varied and so scattered.

4.5 Synapses and Synaptic Transmission

Neurons influence one another at **synapses** — specialised junctions where one cell passes a message to the next. The sending cell is **presynaptic**, the receiving cell **postsynaptic**. There are two kinds of junction: electrical and chemical (Figure 4.27).

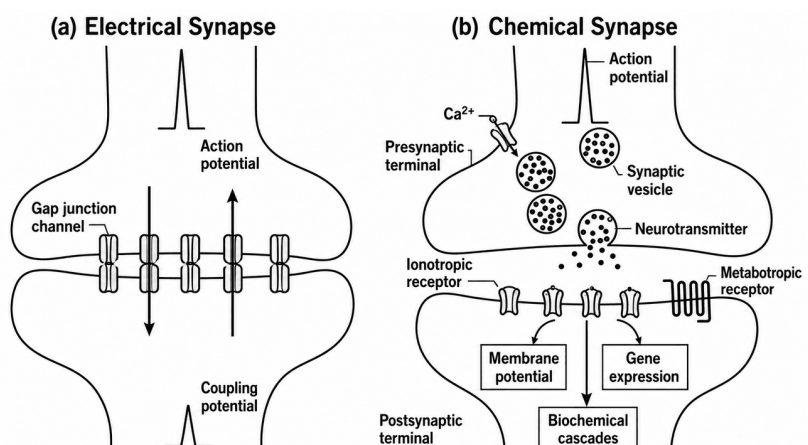


Figure 4.27 (a) An electrical synapse, coupled by gap junctions, and (b) a chemical synapse, separated by a cleft.

Thermo-Regulation

Every enzyme in an animal's body has an opinion about temperature, and none of them agrees with the weather. An animal can either accept whatever temperature the world imposes and build a biochemistry that copes, or it can burn fuel to hold one temperature of its own choosing and pay for it in food. Almost everything in this chapter follows from that single choice.

LEARNING OBJECTIVES

- Describe the four physical routes by which heat is exchanged, and write the heat-balance equation.
- Explain the Q_{10} , calculate it, and interpret a thermal performance curve.
- Distinguish acclimation from acclimatization, and describe metabolic compensation.
- Explain freeze avoidance and freeze tolerance, and the role of antifreeze proteins.
- Distinguish ectotherms from endotherms and poikilotherms from homeotherms
- Describe thermoregulation in invertebrates, fishes, amphibians and reptiles.
- Describe insulation, evaporative cooling, vasomotor control and countercurrent heat exchange
- Explain shivering and non-shivering thermogenesis, and the role of brown adipose tissue.
- Compare torpor, hibernation and aestivation.
- Describe the hypothalamic control of human body temperature and the thermoneutral zone.
- Explain acclimatization to heat, fever, hyperthermia and hypothermia.

Temperature is the most pervasive physical influence on an animal, because it sets the rate of every chemical reaction in the body. Warmth speeds reactions up — but only to a point. Above the temperature at which an enzyme works best, the rate collapses as the protein unfolds; and cold stiffens membranes until transport pumps and channels stop working.

Most cellular life is therefore confined to roughly **0–45 °C**, and each species occupies a much narrower band inside that range, matched to its habitat. A trout enzyme may work best at 10 °C and the human equivalent at 37 °C; neither would serve the other animal.

Thermoregulation — literally 'heat control' — is the set of processes by which an animal governs its internal temperature. In the animals that do it well, it draws on the nervous, endocrine, muscular, respiratory and circulatory systems at once, and it is one of the best examples in physiology of a genuine negative-feedback controller with a sensor, an integrator, effectors and a set point.

8.1 Heat Gains and Heat Losses

An animal produces heat continuously as a by-product of metabolism, and at the same time exchanges heat with its surroundings. Body temperature is simply the running balance of the two.

$$S = M \pm R \pm C \pm K - E$$

S = heat stored · M = metabolic heat · R = radiation · C = convection · K = conduction · E = evaporation

When S is zero the animal is in **thermal balance** and its temperature is steady. Note the signs: metabolism can only *add* heat and evaporation can only *remove* it, but radiation, convection and conduction can go either way depending on whether the surroundings are warmer or cooler than the body.

Heat always flows from warmer to cooler, and it does so by four physical routes whose relative importance depends entirely on the conditions (Figure 8.1, Table 8.1).

Figure 8.1 The physical routes of heat exchange between an animal and its surroundings, with the approximate share of each in a resting human at ordinary room temperature.

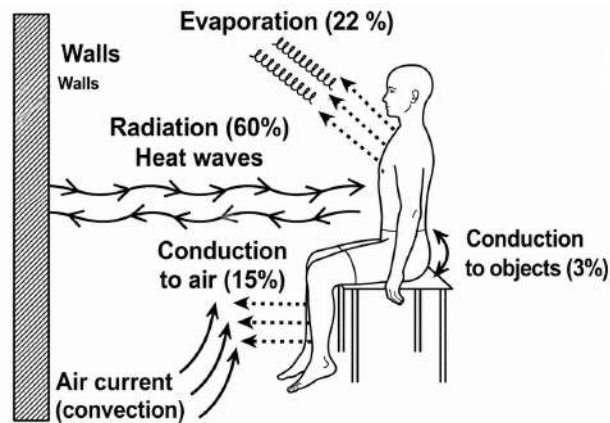


Table 8.1 The four routes of heat exchange

Route	Mechanism	Share in a resting human
Radiation	Infrared waves travelling between any two objects at different temperatures — no contact and no medium needed	≈ 60%
Conduction to air, carried off by convection	Heat passes into the layer of air touching the skin; that air, being warmer and lighter, rises and is replaced by cooler air	≈ 15%
Evaporation	Heat carried away in the latent heat of vaporisation as sweat or respiratory water evaporates	≈ 22%
Conduction to objects	Direct contact with a cooler or warmer solid — a chair, the ground, water	≈ 3%

Radiation is the largest single route in a resting human, and the only one that needs no medium at all — it is how the sun warms a basking lizard across 150 million kilometres of vacuum. **Conduction to solid objects** is the smallest, simply because so little of the body is usually in contact with anything.

Conduction and convection work together and are often counted as one route. Heat is conducted into the thin **boundary layer** of still air touching the skin, and convection then carries that layer away. Anything that thins or disturbs the boundary layer — wind, swimming, a fan — raises heat loss sharply, which is the physical basis of wind chill.

Evaporation is different in kind from the other three. Every gram of water evaporated removes about 0.58 kcal, and it does so **regardless of the direction of the temperature gradient**. Once the surroundings are hotter than the body, radiation, conduction and convection all deliver heat to the animal, and evaporation becomes the only route of loss left.

KEY POINT Evaporation's one weakness is that sweat cools only by **evaporating**. In humid air it drips off unevaporated and takes almost no heat with it. This is why a hot dry day is survivable and a hot humid day is dangerous, and why the wet-bulb temperature, not the air temperature, sets the true limit of human heat tolerance.

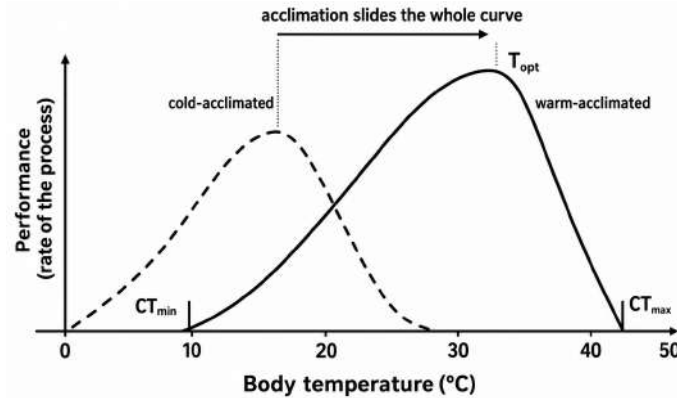
Insulation modifies all of this. Fur, feathers, blubber and clothing work not by being warm themselves but by **trapping still air or fat** and so thickening the barrier through which heat must be conducted. Insulation is measured in **clo** units — one clo is roughly the insulation of an ordinary business suit, enough to keep a resting person comfortable at 21 °C.

8.2 The Effect of Temperature on Animals

8.2.1 Thermal limits and the performance curve

Life occupies a narrow thermal range. Below 0 °C ice crystals pierce membranes and dehydrate the cytoplasm; above about 45 °C proteins begin to denature and membranes become too fluid to hold gradients. Between those limits, every process an animal performs has its own **thermal performance curve** (Figure 8.2).

Figure 8.2 A thermal performance curve. Performance rises to a maximum at T_{opt} and falls away on either side, ending at the critical thermal minimum and maximum. Acclimation slides the whole curve along the temperature axis.



Three landmarks define such a curve. T_{opt} is the temperature of best performance; CT_{min} and CT_{max} , the critical thermal minimum and maximum, are the temperatures at which the animal loses coordinated movement altogether. The span between them is the **thermal tolerance range**.

The curve is strikingly **asymmetric**. Performance builds gradually as temperature rises toward the optimum, but collapses over a very few degrees above it. That asymmetry is why overheating kills so much faster than chilling, and why a species living close to its T_{opt} has almost no thermal safety margin.

A **eurythermal** species tolerates a wide range of temperatures and has a broad, low curve; a **stenothermal** species tolerates only a narrow range and has a tall, narrow one. Antarctic fishes are the extreme stenotherms, dying at temperatures a temperate fish would find merely cool.

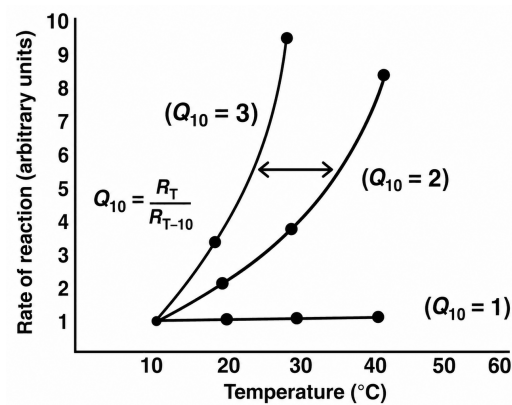
8.2.2 Temperature sensitivity: the Q_{10}

Even well inside the survivable range, a change in temperature disturbs function, because different reactions respond to it differently. The sensitivity of any rate process is expressed as the **temperature coefficient Q_{10}** — the factor by which the rate changes for a 10 °C rise in temperature.

$$Q_{10} = (R_2 / R_1)^{(10 / (T_2 - T_1))}$$

A process that is completely temperature-insensitive has a Q_{10} of **1**. Simple physical diffusion has a Q_{10} near **1.2–1.3**. Most enzyme-catalysed biological processes have Q_{10} values of **2–3**, meaning the rate doubles or triples for every 10 °C rise (Figure 8.3).

Figure 8.3 The effect of Q_{10} on the rate of a reaction. A Q_{10} of 1 gives a flat line; a Q_{10} of 2 doubles and a Q_{10} of 3 triples the rate for each 10 °C rise.



A worked example. A frog's heart beats 20 times a minute at 5 °C and 50 times a minute at 20 °C. Then $Q_{10} = (50/20)^{(10/15)} = 2.5^{0.667} \approx 1.85$ — a typical biological value. Notice that the exponent handles any temperature interval, so the two measurements do not have to be exactly 10 °C apart.

The trouble is that the many reactions making up a physiological process do **not** share a single Q_{10} . A change in tissue temperature therefore does not simply speed everything up together; it throws the reactions out of proportion with one another. That imbalance, more than the change in absolute rate, is what an animal must resist or compensate for.

8.2.3 Acclimation and acclimatization

Two words are used for the adjustments an animal makes to a sustained change in temperature, and the exam distinction between them is worth fixing. **Acclimation** is the response to a change in a *single* variable under controlled laboratory conditions. **Acclimatization** is the response to the natural, seasonal change in the *whole* environment.

Consider a fish whose body temperature simply follows the pond. Its metabolic rate is measured in summer at 24 °C; with a Q_{10} of 2, the rate at the winter temperature of 4 °C should be about a quarter as high. In fact the measured winter rate is far higher than that — almost as high as in summer (Figure 8.4).

As in humans the cycle is controlled by the hypothalamus (GnRH), the anterior pituitary (FSH and LH), the ovaries (progesterone, oestradiol and the inhibins) and the uterus (PGF2 α). Seasonal breeders take their day-length cue through the pineal melatonin signal described in Chapter 10.

11.10.1 Spontaneous and Reflex Ovulation

An important distinction among species is what triggers ovulation. In **spontaneous ovulators** — humans, rats, cattle — ovulation follows the hormonally generated LH surge whether or not mating occurs. In **reflex (induced) ovulators** the LH surge, and therefore ovulation, is triggered by the act of mating itself. Recorded examples include the rabbit, vole, cat, mink, ferret and camel.

11.10.2 The Rat Estrous Cycle

The rat is polyestrous and ovulates every four to five days throughout the year, which is what makes it the standard model for reproductive physiology. The four-day cycle divides into **proestrus**, **estrus**, **metestrus** and **diestrus**, and — usefully — the stage can be read directly from the cell types in a vaginal smear (Figure 11.26, Table 11.6).

Hormonally, prolactin, LH and FSH stay low and then rise in the afternoon of proestrus. Oestradiol begins to rise at metestrus, peaks at proestrus, and returns to baseline at estrus. Progesterone rises through metestrus and diestrus, falls, and then rises to a second peak late in proestrus alongside the gonadotropin surge. Ovulation occurs from the end of proestrus into early estrus.

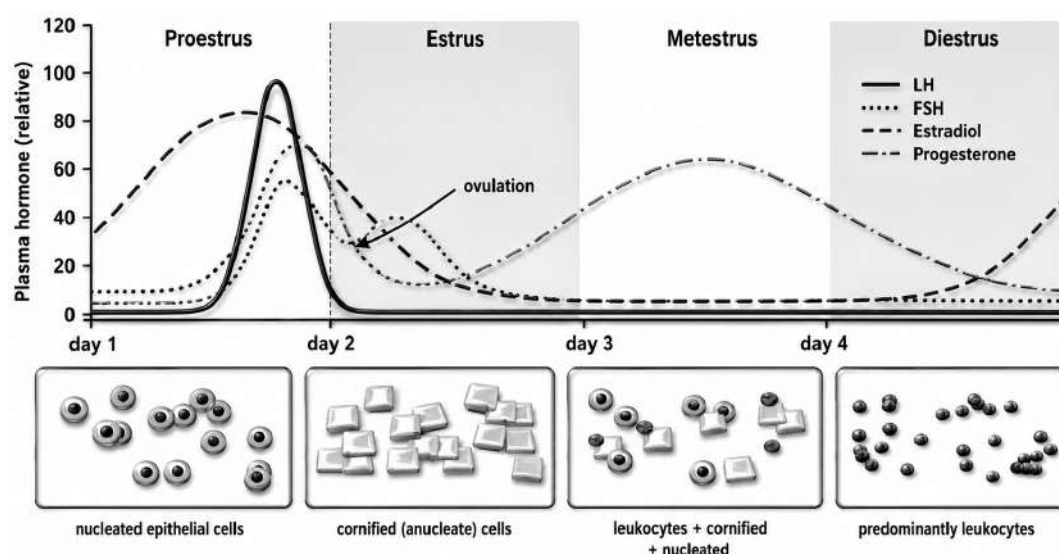


Figure 11.26 The four-day estrous cycle of the rat: plasma hormones above, and the vaginal smear cytology that identifies each stage below.

Table 11.6 Stages of the Rat Estrous Cycle

Stage	Predominant smear cells	Hormonal and ovarian note
Proestrus	Nucleated epithelial cells	Oestradiol peak; LH and FSH rise in the afternoon
Estrus	Cornified (anucleate) cells	Sexual receptivity; ovulation occurs
Metestrus	Leukocytes with cornified and nucleated cells	Oestradiol begins to rise; corpus luteum forms
Diestrus	Predominantly leukocytes	Progesterone dominant; quiescent phase

Table 11.7 The Menstrual and Estrous Cycles Compared

Feature	Menstrual cycle	Estrous cycle
Occurs in	Primates	Most other mammals
Fate of the endometrium	Shed as visible bleeding	Reabsorbed
Sexual receptivity	Throughout the cycle	Restricted to estrus ("heat")
Cycle length	About 28 days	Four days (rat) to a year (dog)
Follicular phase	Named for the ovary	Proestrus and estrus
Luteal phase	Named for the ovary	Metestrus and diestrus

KEY POINT Two facts about the estrous cycle repay firm memorisation. First, the stage is read from the vaginal smear — diestrus is dominated by leukocytes, estrus by cornified anucleate cells, proestrus by nucleated epithelial cells. Second, ovulation type differs by species: cats, rabbits, mink, ferrets and camels are reflex (mating-induced) ovulators, whereas rats, cattle and humans ovulate spontaneously.

11.11 Practice Questions

1. In the developing fetus, the substance secreted by the Sertoli cells that causes regression of the paramesonephric (Müllerian) ducts is:

- (1) Testosterone
(2) Dihydrotestosterone
(3) Müllerian-inhibiting substance (MIS/AMH)
(4) Inhibin B

Answer (3). MIS/AMH, a TGF- β -family homodimer from fetal Sertoli cells, drives Müllerian regression. Testosterone, acting through DHT in the target tissues, instead maintains the Wolffian ducts.

2. In the two-cell, two-gonadotropin model, the enzyme that converts theca-derived androgens to oestradiol-17 β in the granulosa cell is:

- (1) CYP17 (17,20-lyase)
(2) CYP19 (aromatase)
(3) 5 α -reductase
(4) 3 β -HSD

Answer (2). FSH induces granulosa-cell CYP19, which removes the C19 methyl group to turn C19 androgens into C18 oestrogens. The theca supplies the androgen because the granulosa cell cannot make it.

3. A vaginal smear from a laboratory rat is dominated by leukocytes. The stage is:

- (1) Diestrus
(2) Proestrus
(3) Estrus
(4) Metestrus

Answer (1). Diestrus is the leukocyte-dominated stage. Proestrus shows nucleated epithelial cells, estrus shows cornified anucleate cells, and metestrus shows a mixture of all three.

4. Which group of animals are reflex (induced) ovulators, in which the LH surge is triggered by mating?

- (1) Rat and human
(2) Cattle and sheep
(3) Cat, rabbit and mink
(4) Horse and pig

Answer (3). Cats, rabbits, mink, ferrets and camels ovulate in response to mating. Rats, cattle and humans are spontaneous ovulators.

5. Meiotic arrest of the oocyte before the LH surge is maintained by:

- (1) Low intra-oocyte cAMP
(2) High intra-oocyte cAMP kept up by GPR3
(3) Active PDE3A degrading cAMP
(4) Loss of gap junctions with the cumulus

Answer (2). GPR3 is constitutively active and keeps cAMP high, while cumulus-derived cGMP entering through gap junctions inhibits PDE3A. The LH surge cuts the cGMP supply, PDE3A becomes active, cAMP falls and meiosis resumes.

6. The mid-cycle LH surge is triggered when circulating oestrogen:

- (1) Falls below a threshold
(2) Rises briefly to any level
(3) Is replaced by progesterone
(4) Exceeds about 200 pg/mL for roughly fifty hours

Answer (4). The switch from negative to positive feedback requires both a high oestrogen concentration and a sustained duration. A brief rise, or a high level for only a few hours, will not do it.

7. Removing the ovaries at eight weeks of gestation ends the pregnancy, but at sixteen weeks it does not. The reason is:

- (1) The fetus begins to make its own progesterone
(2) The luteo-placental shift transfers progesterone production to the placenta
(3) hCG production ceases after ten weeks
(4) The endometrium no longer requires progesterone

Answer (2). Between about weeks 7 and 10 the placenta takes over progesterone production from the corpus luteum, after which the ovary is no longer needed to maintain the pregnancy.

8. A sustained rise in basal body temperature through the second half of the cycle is caused by:

- (1) Progesterone raising the hypothalamic set-point
(2) Oestradiol raising the metabolic rate
(3) The LH surge itself
(4) Inhibin A acting on the hypothalamus

Answer (1). Progesterone raises the thermoregulatory set-point by about 0.3 to 0.5 °C. Because the rise follows ovulation, basal body temperature is a retrospective marker rather than a predictive one.

13.5 The Microbiota and the Immune System

The mucosal immune system faces a problem with no obvious solution: it must remain tolerant of an enormous resident population while staying able to respond to a pathogen crossing the same surface. It solves the problem partly by being taught how, by the residents themselves.

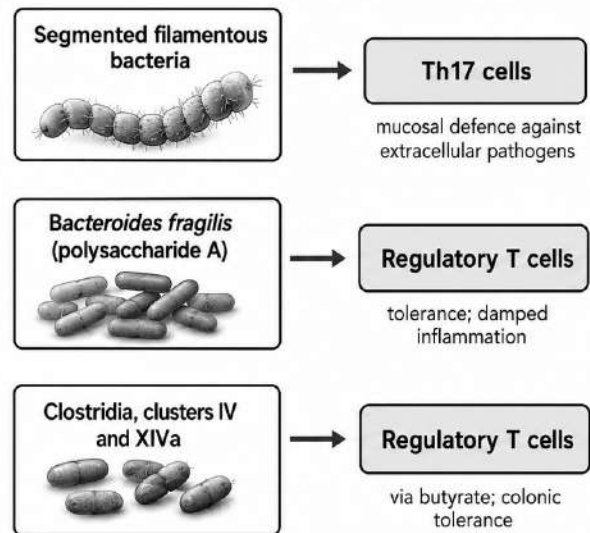
A germ-free animal is not merely a clean animal; it is an immunologically undeveloped one (Figure 13.12a). Its Peyer's patches are few and small, its isolated lymphoid follicles immature, its IgA plasma cells very scarce, and its lamina propria depleted of CD4⁺ cells. Th17 cells are nearly absent and colonic regulatory T cells are reduced. Its mucus layer is thin and single-layered, and its caecum is grossly enlarged.

More striking still, particular taxa instruct particular cell populations (Figure 13.12b). **Segmented filamentous bacteria** induce Th17 cells. The polysaccharide A of *Bacteroides fragilis* induces regulatory T cells. Clostridia of clusters IV and XIVa induce colonic regulatory T cells, largely through the butyrate they produce.

(a) Germ-free against conventional

Feature	Germ-free	Conventional
Peyer's patches	few, small	numerous, large
Isolated lymphoid follicles	immature	mature
IgA plasma cells	very few	abundant
Lamina propria CD4 ⁺ cells	reduced	normal
Th17 cells	almost absent	present
Regulatory T cells (colon)	reduced	normal
Mucus layer	thin	thick, two-layered
Caecum	greatly enlarged	normal

(b) Particular taxa instruct particular cells



A germ-free animal is not merely a clean animal — it is an immunologically undeveloped one.

Figure 13.12 (a) Germ-free and conventional animals compared. (b) Particular bacterial taxa instruct particular lymphocyte populations.

KEY POINT The relationship is instructional, not merely permissive. Colonise a germ-free mouse with segmented filamentous bacteria alone and Th17 cells appear; colonise it with *Bacteroides fragilis* alone and regulatory T cells appear. The immune system does not simply tolerate the microbiota — it is built by it.

13.5.1 How the Peace Is Kept

Instruction is only half the story. The host must also hold an enormous population at arm's length without declaring war on it, and it does so with five defences arranged in depth (Figure 13.13).

- 1. Segregation.** The two mucus layers of §13.2.2 keep the bulk of the community physically off the epithelium.
- 2. Chemical exclusion.** Paneth cells at the base of the crypt secrete α -defensins, lysozyme and the C-type lectin **RegIIIy** into the inner layer, which is what keeps it sterile.
- 3. Antibody coating.** Plasma cells in the lamina propria make dimeric IgA, which the **polymeric immunoglobulin receptor** carries across the epithelium. Secretory IgA binds bacteria and limits their access without triggering complement or inflammation — it is a restraining antibody, not a destructive one.
- 4. Controlled sampling.** **M cells** overlying Peyer's patches, and dendritic cells that extend processes between epithelial cells, take antigen from the lumen deliberately, on the host's own terms.
- 5. Containment.** Anything that does get through is stopped in the mesenteric lymph nodes, so the response stays local and the systemic immune system never meets the resident community at all.

How the gut stays tolerant without being defenceless

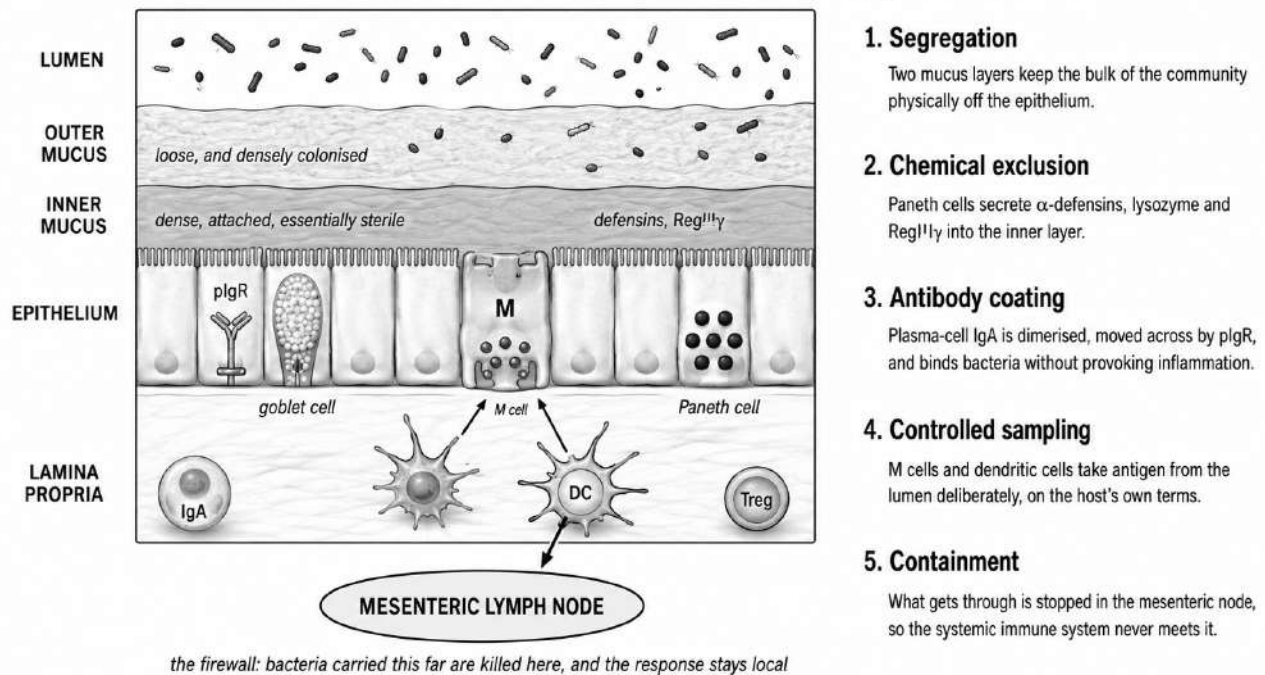


Figure 13.13 Five defences in depth: how the gut stays tolerant of its residents without being defenceless against a pathogen.

The whole arrangement is driven by the residents themselves. Microbial molecular patterns — lipopolysaccharide, flagellin, peptidoglycan — are read by the host's pattern-recognition receptors, and at the low, steady levels a healthy gut presents they maintain the barrier rather than inflame it. The same signal at the same receptor means "keep the wall in good repair" in the gut and "an infection has begun" in the bloodstream.

13.5.2 The Hygiene Hypothesis

The **hygiene hypothesis**, and its later reformulation as the "old friends" hypothesis, proposes that reduced early-life exposure to micro-organisms leaves the immune system poorly calibrated, and contributes to the rise in allergic and autoimmune disease in industrialised populations. The epidemiological association is strong; the mechanism is plausible and incompletely established.

13.6 Methods: How the Microbiome Is Read

Almost everything in this chapter depends on techniques that did not exist thirty years ago, and the limits of the techniques set the limits of what can honestly be claimed.

Culture alone is inadequate: the great majority of gut anaerobes were for a long time unculturable, and although culturomics — high-throughput culture under many conditions — has recovered a surprising number of them, the field is built on sequencing (Figure 13.14, Table 13.4).

- **16S rRNA amplicon sequencing** amplifies one conserved gene, present in every bacterium and archaeon, whose hypervariable regions differ between taxa. It is cheap and works on tiny samples, but resolves only to genus, misses fungi and viruses entirely, and is distorted by primer bias and by variation in 16S copy number between species. It answers *who is there*.
- **Shotgun metagenomics** sequences all the DNA in the sample. It resolves species and often strains, includes fungi, viruses and plasmids, and reports gene content — so it answers *who is there and what they can do*. It is far more expensive and requires host DNA to be removed.
- **Metatranscriptomics, metaproteomics and metabolomics** go further still, reporting what is being expressed or produced rather than what is merely encoded.

KEY POINT Both sequencing methods are **compositional**: they report proportions, not absolute numbers. If one taxon collapses, every other appears to rise even though none has changed in absolute terms. A great many published "increases" are artefacts of this. Quantitative methods — spiking in a known standard, or flow cytometry of the sample — are the correction.