CHAPTER 1

Biochemistry, Cell and Molecular Biology

Molecular biology is essentially the practice of biochemistry without a licence.
Erwin Chargaff

Single Best Answers for Medical Students: Basic Science, First Edition. Stuart Kyle.

1.1 In the Krebs cycle, which of the following is formed during the conversion of succinate to fumarate?
A Reduced NAD
B Reduced FAD
C Carbon dioxide
D ATP
E GTP

1.2 The influx of which of the following ions is responsible for neurotransmitter release from pre-synaptic neurones?
A Sodium
B Calcium
C Potassium
D Chloride
E Magnesium

1.3 Which of the following is **not** correct regarding apoptosis?
A The process is ATP-dependent
B Cell shrinkage occurs
C Cell contents are packaged into apoptotic bodies
D Can be physiological and pathological
E Karyolysis is a typical feature

1.4 Antimitochondrial antibodies are the main serological marker for which disease?
A Autoimmune hepatitis
B Primary biliary cirrhosis
C Sjögren's syndrome
D Systemic lupus erythematosus
E Goodpasture's syndrome

1.5 Which of the following sequence of events is correct with regard to haematopoiesis?
A Haemocytoblast → Myeloid progenitor → Plasma cell
B Haemocytoblast → Lymphoid progenitor → Macrophage
C Myeloid progenitor → Erythrocyte → Basophil
D Lymphoid progenitor → Megakaryocyte → Thrombocytes
E Monoblast → Monocyte → Macrophage

1.6 Which of the following shifts the oxyhaemoglobin dissociation curve to the left?
A Increase in temperature
B Increase in pH
C Increase in carbon dioxide concentration
D Increase in 2,3-DPG
E Increase in altitude

1.7 In control of metabolic processes, which of the following is **not** a second messenger system?
A Binding of glucagon to hepatocytes
B Diffusion of nitric oxide which stimulates cGMP synthesis
C cAMP activating protein kinases
D Binding of calcium ions to calmodulin
E G protein activating phospholipase C

1.8 Phenylketonuria is a genetic disorder in which the enzyme phenylalanine hydroxylase cannot convert phenylalanine into which amino acid?
A Tryptophan
B Methionine
C Histidine
D Tyrosine
E Proline

1.9 Which of the following is **not** an enzyme involved in glycolysis?
A Aldolase
B Hexokinase
C Enolase
D Isomerase
E Oxidase

1.10 Which of the following is a Gram-positive bacterium?
A *Haemophilus influenzae*
B *Neisseria meningitidis*
C *Salmonella typhi*
D *Clostridium difficile*
E *Helicobacter pylori*

1.11 In molecular biology, which of the following techniques can be used to detect methylated sites in a DNA sequence?
A Northern blot
B Western blot
C Eastern blot
D Southern blot
E Southwestern blot

1.12 Which of the following hormones is secreted by most carcinoid tumours?
A Dopamine
B Adrenaline
C Noradrenaline
D Aldosterone
E Serotonin

1.13 Which of the following amino acids is present in all types of collagen?
A Arginine
B Aspartate
C Alanine
D Glutamate
E Glycine

1.14 Which of the following is **not** a component of the extracellular matrix?
A Chondroitin sulphate
B Versican
C Fibronectin
D Laminin
E Glycophorin

1.15 In the polymerase chain reaction, which of the following statements is correct?
A Magnesium is an essential cofactor for DNA polymerase
B Elongation is performed at a temperature of 60 °C
C Extension occurs in the 3′ to 5′ direction on each strand
D The annealing temperature is typically 10 °C below the primer T_m
E DNA polymerase synthesises a new DNA strand using deoxynucleoside diphosphates

1.16 Which of the following is a diagnostic test for pheochromocytoma?
A Human chorionic gonadotrophin
B Alpha fetoprotein
C Vanillylmandelic acid
D 5-hydroxyindoleacetic acid
E Cortisol

1.17 Which of the following disorders is caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase?
A Ehlers-Danlos
B Tay-Sachs
C Lesch-Nyhan
D Budd-Chiari
E Peutz-Jeghers

1.18 Which of the following is **not** a human herpes virus?
A Varicella zoster
B Cytomegalovirus
C Epstein-Barr
D Coxsackievirus
E Kaposi's sarcoma-associated virus

1.19 Which of the following statements regarding coagulation is correct?
A Coeliac disease can cause vitamin K deficiency
B Disseminated intravascular coagulation is associated with thrombocytosis
C Warfarin affects the synthesis of factors 2, 7, 8 and 10
D The prothrombin time is prolonged in von Willebrand's disease
E The APTT is normal in haemophilia A

1.20 Which of the following statements regarding glucagon is correct?
A Released by beta cells
B Inhibits gluconeogenesis
C Secretion is stimulated by hyperglycaemia
D Secretion is inhibited by increased free fatty acids
E Composed of a 29-amino acid dimer

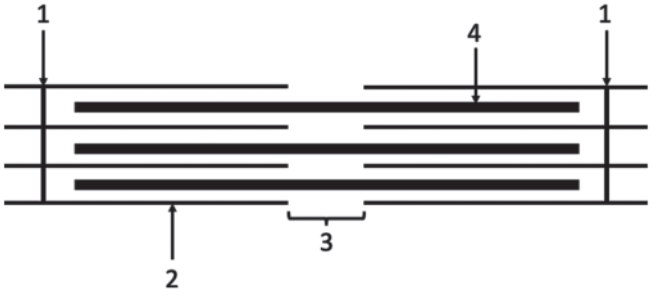
1.21 Which of the following statements regarding muscle contraction is correct?
A An action potential causes calcium ions to diffuse into sarcoplasmic reticula
B Calcium ions bind to tropomyosin
C During the power stroke, ADP and P_i dissociate from myosin
D Actin and myosin bind together to form disulphide bridges
E At the neuromuscular junction, noradrenaline binds to the sarcolemma

1.22 Which of the following carcinogens is associated with bladder carcinoma?
A Asbestos
B Aflatoxin
C 2-naphthylamine
D Chromium
E Benzopyrene

1.23 Which of the following viruses and their associated diseases are correctly matched?
A Herpes simplex type 1 – shingles
B Mumps – pancreatitis
C Coxsackie A – yellow fever
D Epstein-Barr – cervical cancer
E Varicella zoster – lymphoma

1.24 Which of the following regarding the sarcomere is correct?
A Thick filaments are composed mainly of the protein myosin
B H-zones and I-bands represent overlap between myosin and actin
C A-bands contain thin filaments only
D Within H-zones are Z-lines which represent the middle of the sarcomere
E Upon muscle contraction, Z-lines do not change their length

1.25 Which of the following statements regarding the ultrastructure of a single sarcomere (shown below) found in skeletal muscle is correct?



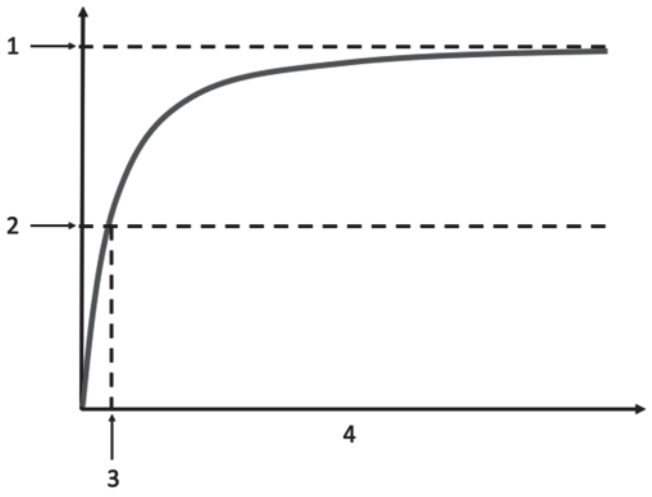
A Sarcomere length is the distance between each M line which corresponds to lines labelled 1
B Structure labelled 2 contains actin-binding sites
C Region labelled 3, the A band, shortens with muscle contraction
D Calmodulin and creatine kinase are found in region labelled 3
E Calcium ions bind to structure labelled 4

1.26 Which of the following regarding stages of mitosis is **not** correct?
A During prophase, microtubules of the cytoskeleton disaggregate
B During metaphase, duplicated chromosomes attach at the kinetochore
C During anaphase, chromosomes are positioned at opposite poles
D During telophase, chromosomes condense and cleavage furrows form
E During cytokinesis, two daughter cells are produced

1.27 Which of the following cells does omeprazole act on the surface of?
A Delta
B Gamma
C Chief
D Parietal
E Foveolar

1.28 Phosphatidylinositol is a phospholipid found on the cytosolic side of some eukaryotic cell membranes. Which of the following regarding phosphatidylinositol is **not** correct?
A It is amphiphilic
B It contains a glycerol backbone, two fatty acids and a modified phosphate polar head
C At physiological pH, it is zwitterionic
D Common fatty acids within its structure include arachidonic and stearic acid
E Inositol rings are commonly phosphorylated by kinases

1.29 Which of the following regarding Michaelis-Menten kinetics (shown below) is correct?



	1	2	3	4
A	V_{max}	$\frac{1}{2}V_{max}$	K_m	[E]
B	K_m	V_{max}	$\frac{1}{2}V_{max}$	V
C	$\frac{1}{2}V_{max}$	[S]	K_m	V
D	V_{max}	$\frac{1}{2}V_{max}$	K_m	[S]
E	K_m	$\frac{1}{2}V_{max}$	V_{max}	[S]

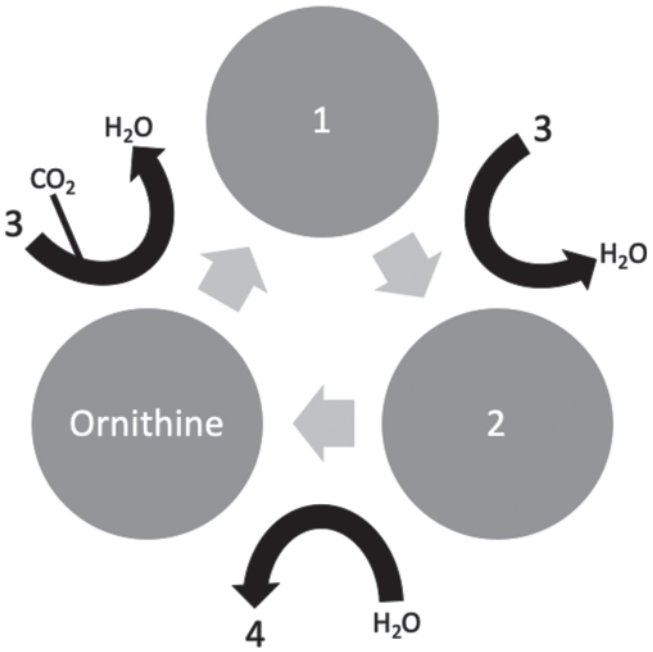
1.30 Which of the following diseases and causative organisms are matched correctly?

	Tuberculosis	Malaria	Measles	Tinea cruris	Schistosomiasis
A	Adenovirus	Fungus	Bacterium	Parasite	Fungus
B	Bacterium	Protist	Paramyxovirus	Fungus	Parasite
C	Rhinovirus	Bacterium	Bacterium	Parasite	Fungus
D	Bacterium	Protist	Adenovirus	Fungus	Parasite
E	Bacterium	Parasite	Paramyxovirus	Fungus	Parvovirus

1.31 Which of the following regarding adult haemoglobin is **not** correct?
A It is composed of two alpha and two beta chains
B It contains a porphyrin ring with a central Fe^{3+} which binds oxygen
C It has a quaternary structure
D Hydrophobic and hydrophilic groups face inwards and outwards, respectively
E It has four oxygen binding sites

1.32 Which of the following enzymes helps anneal DNA fragments through the formation of phosphodiester bonds?
A Ligase
B Polymerase
C Helicase
D Endonuclease
E Kinase

1.33 Which of the following is correct regarding this biochemical pathway?

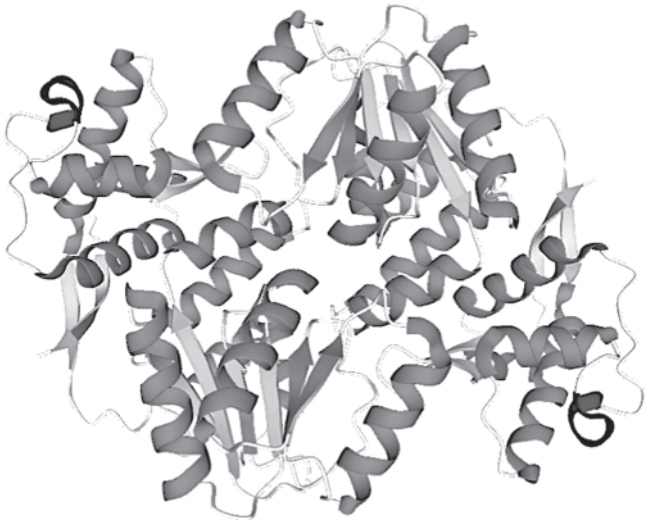


	1	2	3	4
A	Citrulline	Asparagine	Urea	Ammonia
B	Asparagine	Citrulline	Ammonia	Urea
C	Citrulline	Arginine	Ammonia	Urea
D	Methionine	Arginine	Urea	Ammonia
E	Arginine	Citrulline	Ammonia	Urea

1.34 Which of the following regarding organelles is correct?
A The endoplasmic reticulum transports lysosomes
B Mitochondria are approximately 300 nm in diameter
C Nuclei contain 80S ribosomes
D Peroxisomes contain the enzyme reductase
E Golgi bodies contain cristae

1.35 Which of the following techniques is used to determine 3D protein structure?
A Mass spectrometry
B X-ray crystallography
C Ion-exchange chromatography
D Gel electrophoresis
E Protein sequencing

1.36 Which of the following statements regarding this protein structure is **not** correct?



A It is homodimeric
B α-helices and β-pleated sheets are evident
C No random coils are evident
D Level of protein organisation is quaternary structure
E Prosthetic groups are not present

1.37 Which of the following amino acids would **not** form ionic bonds with lysine at physiological pH?
A Aspartate
B Glutamate
C Arginine
D Histidine
E Proline

1.38 Which of the following regarding the *trp* operon is correct?
A It encodes three structural genes
B Tryptophan acts as an activator
C Transcriptional attenuation occurs when tryptophan concentration is high
D It was first characterised in *Saccharomyces cerevisiae*
E The trp repressor is a pentamer, structurally similar to CRP

1.39 Which of the following proteins usually coat endocytic vesicles?
A Clathrin
B Actin
C Laminin
D Desmin
E Tubulin

1.40 Which of the following metabolic processes occurs in the mitochondria?
A Glycolysis
B Cholesterol synthesis
C Pentose phosphate pathway
D Fatty acid β-oxidation
E Fatty acid synthesis

1.41 Which of the following enzymes catalyses substrate-level phosphorylation?
A Pyruvate kinase
B Galactokinase
C Hexokinase
D Phosphofructokinase
E Glycerol kinase

1.42 Which of the following cell junctions allow intercellular communication via connexins?
A Gap junctions
B Tight junctions
C Adherens junctions
D Desmosomes
E Hemidesmosomes

1.43 Which of the following is the correct conversion of amino acid to neurotransmitter?
A Tyrosine → Glutamate
B Tryptophan → Acetylcholine
C Tyrosine → Gamma-aminobutyric acid (GABA)
D Tryptophan → Dopamine
E Tyrosine → Dopamine

1.44 Which of the following is directly formed from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) using HMG-CoA reductase?
A Squalene
B Ubiquinone
C Fumarate
D Mevalonate
E Palmitate

1.45 Which of the following diseases is **not** caused by a spirochete?
A Syphilis
B Lyme disease
C Leptospirosis
D Relapsing fever
E Leishmaniasis

1.46 Which of the following regarding calcium homeostasis is **not** correct?
A Thyroid gland releases calcitonin in response to hypercalcaemia
B Parathyroid glands release PTH in response to hypocalcaemia
C Increased PTH release causes direct osteoclast activation
D Increased PTH release causes hydroxylation of 25-dihydroxycholecalciferol in the small intestine
E Phosphate reabsorption is inhibited in the kidney and serum calcium increases

1.47 Which of the following regarding vitamin B_{12} metabolism is correct?
A Vitamin B_{12} is required for the conversion of methionine into homocysteine
B Vitamin B_{12} has high affinity for haptocorrin at high pH
C Methionine synthase is a folic acid- and B_{12}-dependent enzyme
D Paneth cells secrete intrinsic factor which binds vitamin B_{12}
E Intrinsic factor-vitamin B_{12} complex is absorbed by active transport

1.48 Which of the following regarding oxidative phosphorylation is correct?
A 30 ATP molecules are produced from a single glucose molecule
B There are three protein complexes found in the inner mitochondrial membrane
C Water is the final electron acceptor in the electron transport chain
D Carbon monoxide inhibits ATP synthase
E 5–6 ATP molecules are produced from 2 NADH in oxidative decarboxylation

1.49 Which of the following sequences of secretory pathways for proteins is correct?
A Smooth ER → cis-Golgi network → Golgi cisternae → Secretory vesicle → Cell surface membrane
B Rough ER → cis-Golgi network → Golgi cisternae → Secretory vesicle → Cell surface membrane
C Rough ER → trans-Golgi network → Golgi cisternae → Secretory vesicle → Cell surface membrane
D cis-Golgi network → Rough ER → Golgi cisternae → Secretory vesicle → Cell surface membrane
E Golgi cisternae → trans-Golgi network → Smooth ER → Secretory vesicle → Cell surface membrane

1.50 Which of the following is the correct DNA base triplet that corresponds to the tRNA anticodon AUG?
A UAC
B ATG
C TAC
D AUG
E TTC

Answers

1.1 *B – Reduced FAD*

The Krebs Cycle (or citric acid cycle/tricarboxylic acid cycle) is a series of chemical reactions in aerobic organisms that releases energy in the form of ATP. In eukaryotes, it occurs in the matrix of the mitochondria, and in prokaryotes, it occurs in the cytosol. The cycle generates reduced NAD which is used in oxidative phosphorylation (electron transport chain). In a single turn, the cycle yields three reduced NAD molecules, one reduced FAD molecule and one ATP/GTP/ITP molecule (ATP in plants, and GTP/ITP in animals). The cycle goes around twice for each molecule of glucose that enters respiration due to there being two pyruvate molecules and hence two acetyl Coenzyme A molecules for every glucose molecule.

Succinate is oxidised to the four-carbon molecule, fumerate. Two hydrogen atoms and accompanying electron are transferred to FAD producing reduced FAD. The reduced FAD can then transfer its electrons directly to the electron transport chain; hence B is the correct response. Reduced NAD and carbon dioxide are both formed during the conversions of isocitrate to α-ketoglutarate, and α-ketoglutarate to succinyl CoA. Reduced NAD is finally formed during the conversion of malate to oxaloacetate. ATP or GTP is only formed during the conversion of succinyl CoA to succinate.

1.2 *B – Calcium*

Intracellular ions include potassium and magnesium, whilst extracellular ions include calcium, sodium and chloride. Calcium ions are important in nerve transmission, blood clotting and muscle contraction. In neurones, an action potential arrives at the presynaptic neurone, depolarising the membrane and opening the voltage-gated calcium channels. Upon influx of calcium ions, intracellular calcium sensing proteins called synaptotagmins bind with calcium, which leads to synaptic transmission via the exocytotic release of neurotransmitters into the synaptic cleft by fusion of synaptic vesicles to the pre-synaptic membrane; hence B is the correct response.

In excitatory ion channel synapses, sodium ion channels are found on post-synaptic membranes. When neurotransmitters such as acetylcholine and glutamate bind to these ion channels, they open and sodium ions enter causing depolarisation, making an action potential more likely. In inhibitory ion channel synapses, neuroreceptors are found on chloride channels. Binding of the neurotransmitter such as GABA, opens the chloride ion channels and chloride ions flow in causing hyperpolarisation which makes an action potential less likely. Therefore, impulses arriving in one neurone at these synapses can inhibit an impulse in the next neurone. The Na^+/K^+ ATPase pump builds up an electrochemical gradient across neuronal membranes by helping to pump 3 sodium ions out of the cell and 2 potassium ions into the cell hence resulting in excess extracellular sodium ions and intracellular potassium ions. This pump helps maintain the resting membrane potential of the neurones at ca. −70 mV.

Magnesium ions are important cofactors in a variety of chemical processes in the body. They activate DNA/RNA polymerases, help in DNA synthesis and help regulate other ion and mineral concentrations inside and outside of cells. Magnesium ions help regulate calcium ions in cells by helping to pump them out of the cells, particularly in neurones. It should be remembered that in the clinical context, primary disturbances of magnesium are uncommon and deranged magnesium levels usually result from disturbances of fluid or other electrolytes.

1.3 *E – Karyolysis is a typical feature*

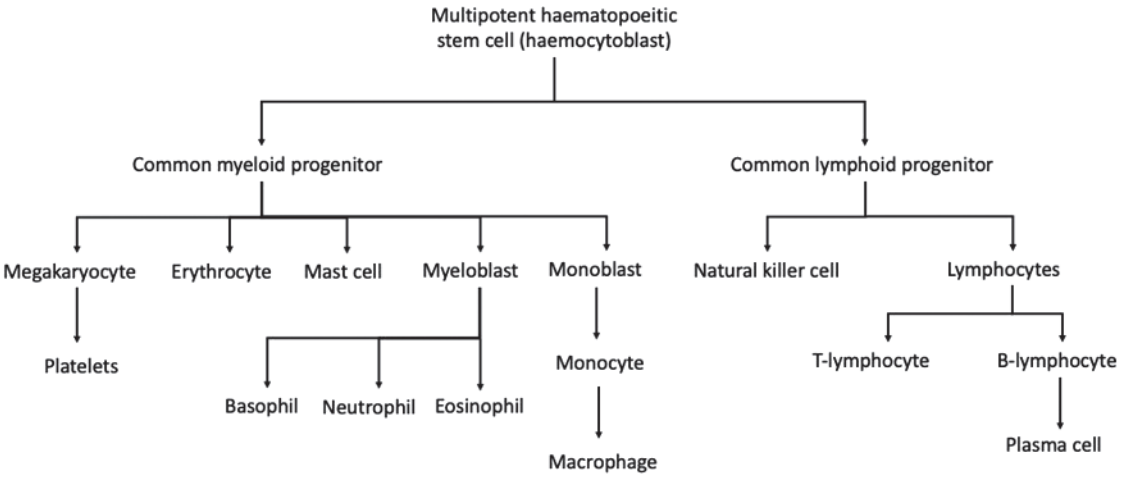
Apoptosis is programmed cell death. The process requires ATP. It has characteristic morphological features which involve single or clusters of cells, such as cell shrinkage, formation of membrane bound vesicles and a cytoplasm with organelles retained in apoptotic bodies, pyknosis (irreversible condensation of chromatin in the nucleus), karyorrhexis (destructive fragmentation of the nucleus) and blebbing of the plasma membrane. It does not involve karyolysis; hence E is the correct response. Karyolysis is the complete dissolution of chromatin of a dying cell as a result of enzymatic degradation of endonucleases. It is a common feature in necrosis. Apoptosis can be physiological or pathological. Necrosis is always pathological.

1.4 *B – Primary biliary cirrhosis*

Antimitochondrial antibodies are the main serological marker for primary biliary cirrhosis; hence B is the correct response. The presence of antinuclear antibody (ANA), anti-smooth muscle antibody (SMA), anti-liver kidney microsomal antibodies (LKM) and anti-soluble liver antigen (SLA) is more suggestive of autoimmune hepatitis. Anti-Ro and anti-La antibodies are suggestive of Sjögrens syndrome, in addition to ANA. Anti-dsDNA is highly suggestive of SLE. Anti-glomerular basement membrane (GBM) is suggestive of Goodpasture's syndrome.

1.5 *E – Monoblast → Monocyte → Macrophage*

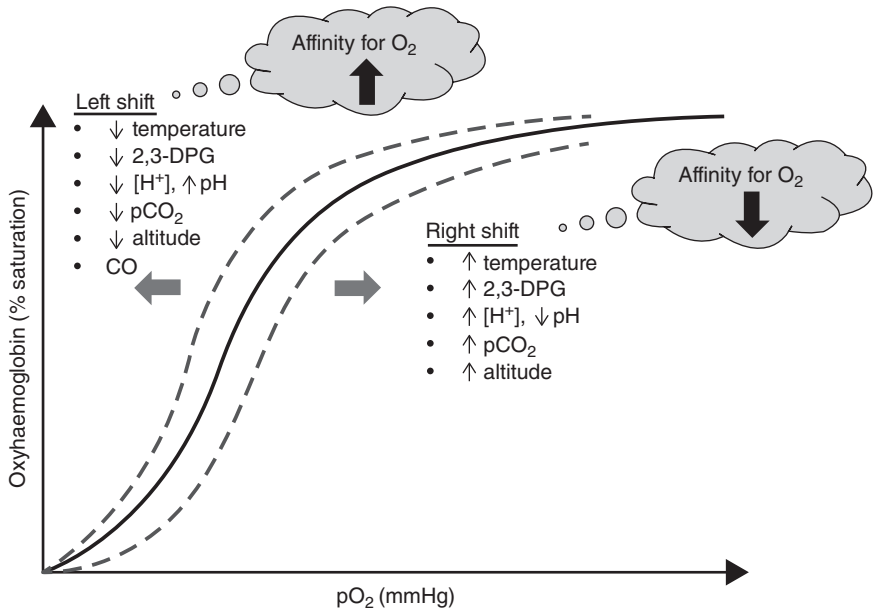
Haematopoiesis is the continuous process by which blood cell lineages are produced from haemopoietic stem cells. The figure below highlights the important cell lineages and the mature cells formed from haematopoietic stem cells; hence E is the correct response.



1.6 *B – Increase in pH*

The sigmoid shape of the oxyhaemoglobin dissociation curve results from interaction between oxygen and haemoglobin. The curve can be displaced such that the affinity for oxygen is altered. Factors that change the curve, shown in the figure below, include changes in temperature, pH (or hydrogen ion concentration), concentration of carbon dioxide and 2,3-diphosphoglycerate (2,3-DPG). Increasing pH shifts the curve to the left; hence B is the correct response. 2,3-DPG is a by-product made by erythrocytes during glycolysis. It reduces the affinity of deoxyhaemoglobin for oxygen and facilitates unloading in the tissues. Levels increase under hypoxaemia such as at high altitude and in congenital heart disease, in addition to conditions such as hyperthyroidism and pyruvate kinase deficiency. Interestingly, organisms living at higher altitudes, where partial pressure of oxygen is lower, have adapted and evolved to increase stores of myoglobin, which has a much higher affinity for oxygen than haemoglobin at a lower partial pressure.

The Bohr effect, through changes in carbon dioxide and hydrogen ion concentration in the blood, enables enhanced association/loading of oxygen in the lungs and dissociation/unloading of oxygen in the tissues. Carbon monoxide poisoning and hypophosphataemia can also shift the curve to the left, as can Hb variants such as foetal haemoglobin, methaemoglobin and carboxyhaemoglobin. Pregnancy, chronic anaemia and sickle cell anaemia shifts the curve to the right.



1.7 *A – Binding of glucagon to hepatocytes*

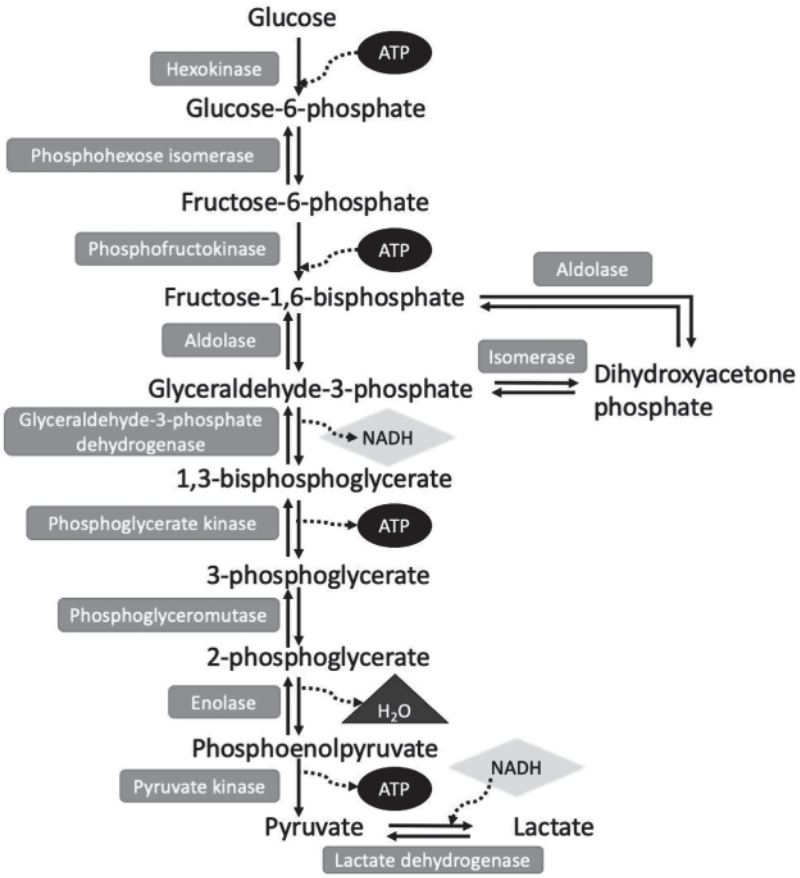
First messengers are any extracellular factors that elicit an intracellular response. They can range from environmental factors and small molecules, to larger proteins. Common examples include neurotransmitters, peptide hormones, cytokines, growth factors and drugs. First messengers tend not to physically cross the phospholipid bilayer; instead they need to be transduced into second messengers. Glucagon binding to hepatocytes is an example of a first messenger; hence A is the correct response. All other options are examples of second messengers. Second messengers trigger intracellular signalling cascades and may act as enzyme activators, inhibitors or cofactors. Examples include (1) activation of guanylyl cyclase (GC) by calcium or nitric oxide, which catalyses synthesis of cGMP from GTP. Subsequently, cGMP activates protein kinase G; (2) cAMP forms phosphodiesterases which are regulated through phosphorylation by protein kinase A and C. Adenylate cyclase is responsible for synthesising cAMP; (3) calcium ions bind to calmodulin which can activate nitric oxide synthase, and lead to vasodilation via nitric oxide; and (4) phospholipase C is activated through coupling to a G protein.

1.8 *D – Tyrosine*

Phenylketonuria is an autosomal recessive metabolic disorder (inborn error of metabolism) whereby there is a deficiency in the enzyme, phenylalanine hydroxylase which is required for the conversion of phenylalanine into tyrosine. Phenylalanine accumulates and is converted to phenylpyruvate which is excreted in the urine. Tyrosone is essential for the production of dopamine; hence D is the correct response.

1.9 *E – Oxidase*

Glycolysis occurs in the cytoplasm and is the process of breaking down glucose (or fructose and galactose) into two three-carbon compounds. It takes place in 10 steps. It is used by all cells in the body for the generation of energy. Glycolysis produces pyruvate and lactate in aerobic and anaerobic conditions, respectively. Pyruvate then enters the Krebs cycles for further energy generation. Glycolysis generates 2 molecules of ATP when 1 molecule of glucose is converted into 2 molecules of pyruvate. NAD^+ is an obligatory substrate in glycolysis, and if it is not generated, glycolysis will cease. In aerobic conditions, NADH is oxidised in the mitochondria to regenerate NAD^+. Anaerobically, NADH and NAD^+, and pyruvate and lactate are interconverted using lactate dehydrogenase. As shown in the following figure, the enzyme not used in glycolysis is oxidase; hence E is the correct response.



1.10 *D – Clostridium difficile*

The following table highlights common Gram-positive and -negative bacteria; hence D is the correct response, as all other options are examples of Gram-negative bacteria.

		Gram-positive	Gram-negative
Cocci	Aerobe	*Enterococcus* *Staphylococcus* *Streptococcus*	*Neisseria*
	Facultative anaerobe		
	Anaerobe	*Peptostreptococcus*	
Bacilli	Aerobe	*Bacillus* *Cornebacterium* *Lactobacillis* *Listeria* *Mycobacterium* *Nocardia*	*Pseudomonas*
	Facultative anaerobe		*Bordetella* *Brucella* *Escherichia* *Haemophilus* *Klebsiella* *Pasteurella* *Proteus* *Salmonella* *Shigella* *Vibrio* *Yersinia*
	Anaerobe	*Actinomyces* *Clostridium*	*Bacteroides* *Fusobacterium* *Prevotella*
	Microaerophile		*Campylobacter* *Helicobacter* (curved rod)
Spirochaetes	Aerobe		*Leptospira*
	Anaerobe		*Borrelia* *Treponema*

1.11 *D – Southern blot*

A blot is a method of transferring DNA, RNA or proteins onto a blotting membrane, often using gel electrophoresis. Transferred biological molecules are visualised using various staining methods, autoradiography or specific labelling techniques. All options in this question are techniques employed in molecular biology. Southern blot is a technique used to detect alterations in DNA, such as point mutations, translocations and DNA methylation; hence D is the correct response. Northern blots are used for analysing molecular size and abundance of mRNA, and individual gene expression. Western blot or protein immunoblotting is used to identify and locate specific proteins. Eastern blot is used to analyse post-translational modifications such as the addition of carbohydrates, lipids or phosphates to proteins. Southwestern blot, which combines aspects of Southern and Western blotting, is used to study DNA-protein interactions.

1.12 *E – Serotonin*

Carcinoid tumours are slow-growing neuroendocrine in origin, derived from enterochromaffin or Kulchitsky cells. Microscopically, a conglomerate of neuroendocrine cell types can be seen, containing many membrane-bound neurosecretory granules. These granules are composed of a variety of hormones, the most common being serotonin; hence E is the correct response. Other substances include histamine, prostaglandins, dopamine, kallikrein, substance P and corticotrophin. In addition to carcinoid-like symptoms such as flushing, wheezing and tachycardia, serology may be required to diagnose carcinoid. 5-hydroxyindoleacetic acid (5-HIAA) is a primary metabolite of serotonin which can be measured in a 24-hour urine collection. Serum chromograffin A can also be measured.

1.13 *E – Glycine*

Collagen is the main structural protein in the extracellular matrix. It is composed of amino acids that form a triple helix, consisting of two identical α1 chains and one α2 chain. The most common sequence of collagen follows the pattern Gly-X-Pro or Gly-X-Hyp, where X is any other amino acid, Pro is the amino acid proline and Hyp is hydroxyproline; hence E is the correct response. Glycine is essential in maintaining and stabilising the triple helix. It is required at every third position, as upon assembly, glycine is placed

inside the helix where no other larger side groups can be inserted. This means the proline and hydroxyproline rings are placed exteriorly in the helix. Proline and hydroxyproline provide the triple helix with thermal stability.

1.14 *E – Glycophorin*

Glycophorin is a sialoglycoprotein which spans the membrane of erythrocytes. It does not form part of the extracellular matrix; hence E is the correct response. The extracellular matrix is a highly dynamic support network which surrounds all cells and tissues. It is composed of macromolecules, regulatory factors and cells. Extracellular matrix molecules form part of a tightly regulated system for the maintenance of tissue homeostasis, repair and development. The major constituents in the extracellular matrix include collagens, elastins, and non-collagenous glycoproteins and proteoglycans. Adhesive extracellular matrix glycoproteins allow cells to adhere to the matrix, such glycoproteins include fibronectin, laminins, vitronectin, tenascins, thrombospondins, entactins and many others. Proteoglycans consist of a central protein core with glycosaminoglycan side chains, and include versican, aggrecan, decorin, biglycan, fibromodulin and others. Glycosaminoglycans (GAGs) are linear polysaccharides composed of many disaccharide repeating units including chondroitin sulphate, keratan sulphate and heparan sulphate.

1.15 *A – Magnesium is an essential cofactor for DNA polymerase*

Magnesium ions function as a cofactor for DNA polymerase activity by enabling incorporation of dNTPs during polymerisation. They help catalyse phosphodiester bonds between primers and phosphates of dNTPs; hence A is the correct response. Elongation, or the extension step, is carried out at ~72 °C as this is the optimum temperature for DNA polymerase to bind to primers and catalyse replication using dNTPs. This allows for new DNA strands to be synthesised. Extension occurs in the 5′ to 3′ direction on each strand. The annealing temperature, as a general rule of thumb, is 3–5 °C below the lowest primer T_m. DNA polymerase synthesises a new DNA strand using dNTPs (deoxynucleoside triphosphates).

1.16 *C – Vanillylmandelic acid*

Pheochromocytomas are rare, catecholamine-producing neuroendocrine tumours predominantly arising from chromaffin cells of the adrenal medulla. Adrenal tumours produce both norepinephrine and epinephrine, whereas extra-adrenal tumours exclusively produce norepinephrine. Twenty-four-hour urinary metanephrine metabolites can be measured or an end-stage metabolite of catecholamines – vanillylmandelic acid (VMA); hence C is the correct response. Elevated levels of VMA are useful in diagnosing pheochromocytoma. Human chorionic gonadotrophin (HCG) is produced by a growing embryo and the placenta. Measuring HCG levels can be helpful in identifying normal and pregnancies. Detection can also be useful in evaluating various trophoblastic diseases, such as hydatidiform moles and gestational trophoblastic neoplasia (invasive moles, choriocarcinomas). Elevated levels of alpha fetoprotein are useful markers of hepatocellular carcinoma. It can also be raised in patients with non-seminomatous germ cell tumours. 5-hydroxyindoleacetic acid (5-HIAA) is the primary metabolite of 5-hydroxytryptamine (serotonin). Measurement of 24-hour urinary excretion of 5-HIAA is useful in patients with primary midgut carcinoid tumours. These tumours originate from enterochromaffin cells of the intestine. Elevated levels of cortisol are typical of Cushing's syndrome (corticosteroid excess), exercise, stress, depression, obesity and alcohol. Cortisol hypofunction can result due to Addison's disease, hypothyroidism, reduced ACTH production, long-term use of corticosteroids and congenital adrenal hyperplasia.

1.17 *C – Lesch-Nyhan*

Lesch-Nyhan syndrome is a rare, X-linked disorder of purine metabolism, caused by deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). HPRT catalyses the conversion of hypoxanthine and guanine into their respective nucleotides, inosinic acid (IMP) and guanylic acid (GMP), respectively. The syndrome is also characterised by overproduction and accumulation of uric acid, which is most likely due to intracellular accumulation of phosphoribosyl pyrophosphate (PRPP) leading to hyperuricaemia; hence C is the correct response. Ehlers-Danlos is an inherited connective tissue disorder typically caused by mutations in collagen genes (*COL5A1* and *COL5A2*). Tay-Sachs disease is an autosomal recessive disorder resulting from deficiency in the lysosomal enzyme hexosaminidase A leading to accumulation of GM2 ganglioside within neuronal cells. Budd-Chiari syndrome can occur as a result of obstructive hepatic venous outflow which leads to hepatic venous congestion. The main cause being hepatic vein thrombosis with or without occlusion of the inferior vena cava. Other causes include hypercoagulable states, neoplasia, myeloproliferative disorders, pregnancy and contraceptive pills. Peutz-Jeghers syndrome is an autosomal dominant disorder characterised by the presence of multiple gastrointestinal hamartomatous polyps and skin hyperpigmentation. It is usually caused by germline mutation in the serine-threonine kinase tumour suppressor gene.

1.18 *D – Coxsackievirus*

Coxsackieviruses belong to the single-stranded positive-sense ssRNA viruses of the enterovirus family. Types A and B are pathogenic, commonly associated with pyrexial illnesses and are the causative organisms for aseptic meningitis, myocarditis, hand, foot and mouth disease and herpangina (common childhood illness characterised by ulcers in the mouth and throat); hence D is the correct response. All other options are human herpes viruses.

1.19 *A – Coeliac disease can cause vitamin K deficiency*

Coeliac disease is a gluten-sensitive enteropathy which can lead to malabsorption. It is a chronic, autoimmune, T-cell-mediated inflammatory disorder. Vitamin K deficiency may be due to malabsorption; hence A is the correct response. Other causes include inadequate stores and the use of oral anticoagulants. Disseminated intravascular coagulation is associated with thrombocytopenia (not thrombocytosis) due to platelets being consumed. Warfarin, a vitamin K epoxide reductase antagonist, inhibits the synthesis of vitamin K-dependent coagulation factors, including factors 2, 7, 9 and 10 (not 8!), as well as proteins C and S. Prothrombin time is normal in both haemophilia A and von Willebrand's disease. APTT is increased in both haemophilia A and von Willebrand's disease.

1.20 *D – Secretion is inhibited by increased free fatty acids*

Glucagon inhibits fatty acid synthesis (and glycolysis); hence D is the correct response. It is released by alpha cells of the pancreas; beta cells release insulin. Glucagon increases gluconeogenesis (rather than inhibiting it) and also glycogenolysis. It also increases ketone body production from fatty acids and stimulates lipolysis in adipose tissue. Glucagon increases blood glucose during hypoglycaemia, whereas insulin lowers blood glucose during hyperglycaemia. Glucagon is a 29-amino acid, single-chain polypeptide derived from a pre-proglucagon 180-amino acid precursor. It is not a dimer.

1.21 *C – During the power stroke, ADP and P_i dissociate from myosin*

The power stroke is related to the mechanical force generated by release of ADP and P_i, and to the conformational change in myosin which then exerts this force on actin. Myosin pulls the actin filament; the sarcomere shortens and the muscle contracts; hence C is the correct response. An action potential causes calcium ions to diffuse out of the sarcoplasmic reticulum (not in), which are released upon signalling from the transverse tubules (T-tubules). Calcium ions bind to troponin (not tropomyosin) which expose the actin-binding sites. Actin and myosin form cross-bridges (not disulphide bridges) and undergo cyclic binding interactions where they pull against one another. At the neuromuscular junction, acetylcholine (not noradrenaline) binds to the sarcolemma. Acetylcholine depolarises the sarcolemma and the action potential can then propagate across it and down the T-tubules.

1.22 *C – 2-naphthylamine*

Occupational exposure to 2-naphthylamine, an aromatic amine has been shown to increase the risk of bladder cancer, particularly those who work in the dye industry. It is also found in cigarette smoke; hence C is the correct response. High exposure to asbestos can cause mesothelioma. Aflatoxin is a mycotoxic carcinogen produced by certain strains of *Aspergillus* and has been implicated in the aetiology of hepatocarcinoma. Chromium compounds have been shown to increase the risk of lung carcinomas. Benzopyrene is a polycyclic aromatic hydrocarbon commonly found in cigarette smoke (tar). It can cause squamous cell, large and small cell and adenocarcinomas of the lungs.

1.23 *B – Mumps – pancreatitis*

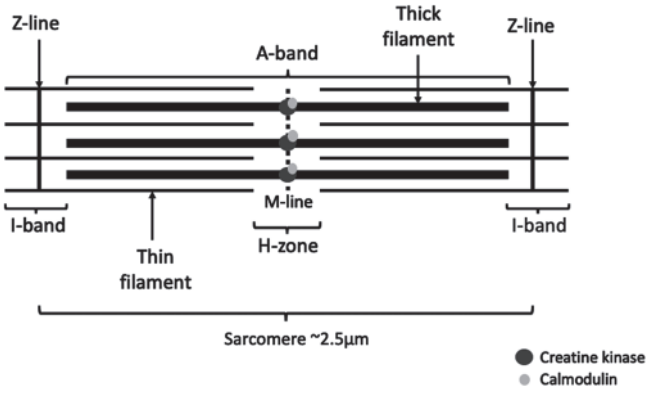
Mumps is caused by a paramyxovirus and spread by droplets. Associated conditions or complications include CNS involvement, epididymo-orchitis, oophoritis, myocarditis, mastitis, hepatitis and pancreatitis; hence B is the correct response. Herpes simplex type 1 causes mucocutaneous lesions, predominantly of the head and neck such as herpes labialis, keratoconjunctivitis, stomatitis and encephalitis. Shingles is caused by herpes (varicella) zoster. Coxsackie A viruses are spread by the faecal-oral route and are responsible for a broad spectrum of diseases such as hand, foot and mouth, herpangina, meningitis, encephalitis, myocarditis and myositis. Yellow fever is caused by a flavivirus and mainly confined to Africa and South America. Epstein-Barr (EBV) is a gamma herpes virus and causes infectious mononucleosis, Burkitt's lymphoma, oral hairy leucoplakia in AIDS patients, nasopharyngeal carcinoma and post-transplant lymphoma. Cervical cancer is caused by sexually acquired infection with human papillomavirus (HPV), predominantly types 16 and 18. Varicella zoster is an alpha herpes virus, like HSV types 1 and 2, but causes chickenpox in children and shingles in adults. Viruses that can cause lymphoma include EBV, human T-lymphotropic virus type 1, human herpesvirus type 8 and hepatitis C.

1.24 *A – Thick filaments are composed mainly of the protein myosin*

A sarcomere is the smallest contractile unit of a muscle fibre, composed of two myofilaments: thick filaments are composed mainly of myosin, and thin filaments are composed mainly of actin (and titin); hence A is the correct response. The H-zone and I-band are the regions that only contain the thick and thin filaments, respectively. The A-band contains the thick filaments with some overlap of the thin filaments. The Z-lines (or discs) borders the sarcomere and contain a central M-line, which anchors the thick filaments. In a contracted muscle fibre, the distance between the Z-lines, the I-band and the H-zone decreases, but the A-band remains unchanged. In a fully contracted muscle, the H-zone is no longer visible.

1.25 *D – Calmodulin and creatine kinase are found in region labelled 3*

Region labelled 3 is the H-zone which contains a central M-line (middle of the sarcomere). It serves to arrange the thick filaments into the A-bands maintaining their alignment and controlling stress distribution along the sarcomere during muscle contraction. The M-line is a dense complex of proteins which contains important structural linkers such as myomesin, calmodulin and creatine kinase; hence D is the correct response. Sarcomere length is the distance between each Z line (not M). Structure labelled 2 is the thin filament, primarily composed of actin so actin binding sites are found on structure labelled 4, myosin. Region labelled 3 is the H-zone, not the A-band. The A-band remains unchanged in muscle contraction. Calcium ions bind to troponin (a component of the thin filament, label 2) and not myosin (label 4). The following figure illustrates the important features of a sarcomere.



1.26 *D – During telophase, chromosomes condense and cleavage furrows form*

During telophase, chromosomes decondense as they arrive at opposite poles of the cell; hence D is the correct response. In addition, spindles start to break down, the nuclear envelope reforms and nucleoli reappear. In animal cells, cleavage furrows form during cytokinesis as the cytoplasm splits into two and the cell divides. All other options are correct.

1.27 *D – Parietal*

Omeprazole is a selective, irreversible proton pump inhibitor. It inhibits the H^+/K^+ ATPase pump found in parietal cells of the stomach; hence D is the correct response. Parietal or oxyntic cells are found in the lamina propria, specifically within the neck of fundic gland. They make hydrochloric acid and gastric intrinsic factor which are released into the lumen of the stomach.

Delta or D cells are somatostatin-producing cells that can be found in the stomach, pancreas and intestine. In the stomach, somatostatin acts on parietal cells and helps reduce acid secretions. In the pancreas, somatostatin has both paracrine and endocrine function. The paracrine effects are to inhibit the release of glucagon and insulin by α and β cells of the Islets of Langerhans, respectively. The endocrine effects are on reducing smooth muscle contraction in the gallbladder and alimentary canal. Another type of delta cell, D_1 produces vasoactive intestinal peptide which induces glycogenolysis and has important roles in regulating intestinal motility and the tone of smooth muscle.

Gamma, also called F or pancreatic polypeptide (PP) cells produce pancreatic polypeptide in the Islets of Langerhans of the pancreas. This hormone has roles in reducing appetite and inhibits exocrine secretions of the pancreas and the release of bile from the gallbladder. It helps stimulate the release of enzymes by chief cells and reduces the release of HCl by the parietal cells of the stomach.

Chief cells can be found in the stomach, parathyroid gland and in the carotid body. Chief or zymogenic cells make the enzymes lipase, pepsinogen and rennin and release them into the lumen of the stomach. They are found in the base of the fundic gland. They also make the hormone, leptin which inhibits the sensation of hunger.

Foveolar cells or surface mucous cells line the gastric mucosa of the stomach and secrete mucous which helps lubricate the gastric lining. Mucous neck cells are found in the neck of the fundic gland. They help reduce friction whilst food is being churned.

1.28 *C – At physiological pH, it is zwitterionic*

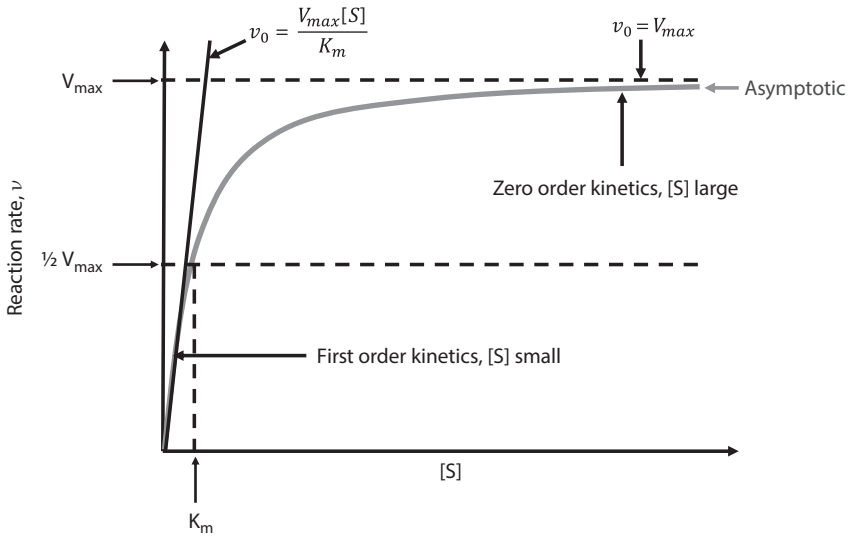
Phosphatidylinositol is a glycerophospholipid with an inositol head group, two fatty acid tails and a glycerol backbone. It is a precursor to inositol phosphates which are important in cell signalling. At physiological pH, the phosphate group that is substituted with the inositol polar head group renders an overall negative charge on the molecule; hence C is the correct response. It is not zwitterionic at physiological pH as there is no positive charge to balance the negatively charged phosphate. All other options are correct.

1.29 *D* – | V_{max} | $\frac{1}{2}V_{max}$ | K_m | $[S]$ |

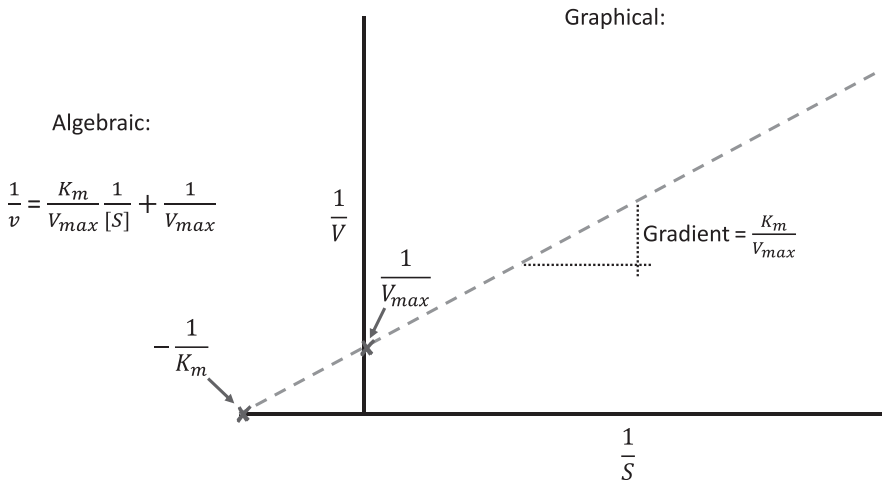
Michaelis-Menten kinetics, involving a single substrate and product, is a simple model that accounts for enzyme dynamics. The Michaelis-Menten equation relates the maximum velocity at maximum substrate concentrations when all enzyme active sites are saturated with substrate, V_{max}, the substrate concentration at which the reaction velocity is $\frac{1}{2}V_{max}$, K_m (the Michaelis constant – a measure of the affinity an enzyme has for its substrate), and the substrate concentration, [S], such that:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Graphically, this can be depicted as follows; hence D is the correct response:



Additionally, algebraic rearrangement of the Michaelis-Menten equation yields a linear, double-reciprocal plot which is far easier to interpret than an entire rectangular hyperbola, and which facilitates estimation of K_m. Plots such as the Lineweaver-Burk are commonly employed by enzymologists as shown below:



Throughout the 20th century, significant advances in enzyme kinetics were made by Haldane who introduced the concept of introducing more than one substrate into reactions, and Fersht who introduced the term *enzyme specificity*.

1.30 *B –* Bacterium | Protist | Paramyxovirus | Fungus | Parasite

Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis*, malaria is caused by the parasitic protist (or protozoan) *Plasmodium*, measles is caused by the paramyxovirus, tinea cruris ('jock itch'), a fungal infection of the superficial skin of the groin is caused by a dermatophyte, and schistosomiasis (also called bilharzia) is a parasitic worm (or fluke) infection; hence B is the correct response.

1.31 *B – It contains a porphyrin ring with a central Fe^{3+} which binds oxygen*

Adult haemoglobin contains a porphyrin ring in which a central Fe^{2+} (not Fe^{3+}) co-ordinately bonds and binds oxygen; hence B is the correct response. It exists as a tetramer containing two alpha and two beta chains (subunits). Since it has more than one polypeptide chain, it has a quaternary structure. Hydrophilic groups face outwards where water is found at the surface of the protein, while hydrophobic groups face inwards and bury among hydrophobic amino acids of the protein. Haemoglobin has four separate haem groups that can each bind a molecule of oxygen and this leads to a significant increase in the affinity for oxygen at the other haem groups.

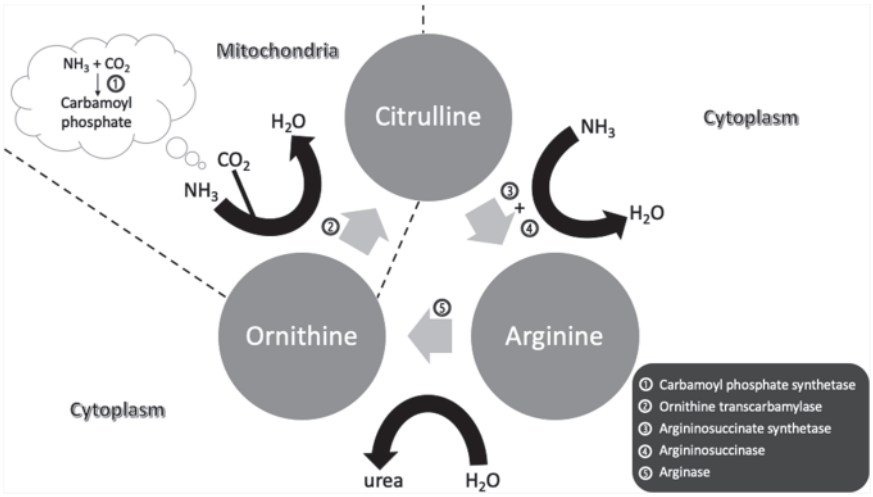
1.32 *A – Ligase*

DNA ligase (polydeoxyribonucleotide synthase) catalyses the formation of a phosphodiester bond by annealing DNA single strands; hence A is the correct response. Polymerases catalyse the polymerisation of nucleoside triphosphates into DNA or RNA in the form of dNTPs and rNTPs, respectively. A phosphodiester bond is formed between the 3′ end of the growing chain and the 5′ phosphate of the incoming nucleotide. In addition, hydrogen bonding occurs between complementary nucleotides. DNA polymerases require a primer for initiation, RNA polymerases do not. Helicase utilises energy from ATP hydrolysis to separate (unwinds) complementary strands of DNA or RNA duplexes. Endonucleases (restriction enzymes) cleave phosphodiester bonds within a polynucleotide chain, commonly doing so at specific nucleotide sequences. Kinases phosphorylate specific amino acids using the phosphate in ATP.

1.33 *C –* Citrulline | Arginine | Ammonia | Urea

The urea, or ornithine cycle, depicted with extra detail below, is a cyclic pathway which converts toxic ammonia into relatively inert urea. It occurs both in the cytoplasm and in mitochondria, and is the sole source of endogenous arginine, ornithine and citrulline production. Ammonia and urea are products of oxidative deamination and nitrogen metabolism, respectively; hence C is the correct response. The reaction exclusively occurs in periportal hepatocytes of the liver and urea is then transported to and excreted by the kidneys. Clinically, the major sequelae of urea cycle dysfunction are neurological, and accumulation of ammonia has been considered to play an important role in hepatic encephalopathy. The urea cycle links with the Krebs cycle, and the overall reaction can be summarised as:

$$NH_4^+ + HCO_3^- + 3ATP^{4-} + 2H_2O + \text{aspartate} \rightarrow \text{urea} + 2ADP^{3-} + 2P_i^{2-} + PP_i^{4-} + AMP^{2-} + 5H^+ + \text{fumarate}$$



1.34 *C – Nuclei contain 80S ribosomes*

Eukaryotic cells, i.e., those that have a nucleus contain 80S ribosomes (S meaning Svedberg units, a sedimentation coefficient) composed of 40S and 60S subunits (note these are not actual masses, otherwise the maths would be completely incorrect!); hence C is the correct response. Prokaryotes contain 70S ribosomes, composed of 30S and 50S subunits. The endoplasmic reticulum synthesises membrane proteins and soluble proteins and correctly folds them. These are then transported to the Golgi where proteins are processed, packaged, modified and subsequently released in secretory vesicles. Mitochondria range in size between

1 and 10 µm (300 nm = 0.3 µm). Peroxisomes, found in all eukaryotic cells, are involved in ß-oxidation of fatty acids and hydrogen peroxide metabolism. They contain high concentrations of oxidative enzymes such as urate oxidase and catalase (whereas reductases catalyse reduction reactions). Golgi bodies (apparatus/complex) contain around 3–6 flattened sacs called cisternae (cis, medial and trans-cisternae); the inner mitochondrial membrane contains folds called cristae.

1.35 *B – X-ray crystallography*

Globular proteins have a well-defined 3D structure, and when crystallised, this structure can be determined by X-ray crystallography. This is done by beaming x-rays through a crystal which interact with electrons, and therefore allow atomic positions to be calculated; hence B is the correct response. In addition, 2D- and 3D nuclear magnetic resonance (NMR) spectroscopy can be used to determine protein structure in solution. Mass spectrometry is used to accurately measure molecular mass of proteins, in addition to protein sequencing and identification. Proteins can be separated and purified based on their size using gel filtration chromatography, and charge using ion-exchange chromatography and gel electrophoresis. Gel electrophoresis can also be crudely used to determine protein size. Protein sequencing is an automated yet laborious process using a technique called Edman degradation. The order of the amino acid sequence can be deduced by sequencing peptides produced by enzyme digestion, and overlapping sequences are then compared.

1.36 *D – Level of protein organisation is quaternary structure*

This is the protein structure for a DNA helicase, and therefore it has tertiary structure (not quaternary); hence D is the correct response. It has two polypeptide chains that are identical; therefore it is homodimeric (not heterodimeric). The structure contains both α-helices and β-pleated sheets. There are no random coils evident. There are no prosthetic groups present within the structure.

1.37 *E – Proline*

Ionic bonding is the electrostatic attraction between oppositely charged species. There are 20 amino acids used as protein building blocks and they differ from each other only at the R-group. R-groups render amino acids as non-polar or hydrophobic, polar or hydrophilic, acidic or basic. Basic and acidic amino acids are capable of ionic interactions. Proline is a non-polar, hydrophobic amino acid, so would not ionically bond with lysine; hence E is the correct response. Aspartate and glutamate, and arginine and histidine are acidic and basic amino acids, respectively. The 20 alpha amino acids and the R-group character which they exhibit are shown below:

Amino acid	Abbreviation		R-group character	
Glycine	G	Gly	Hydrophobic Non-polar Neutral	Aliphatic
Alanine	A	Ala		
Valine	V	Val		
Leucine	L	Leu		
Isoleucine	I	Ile		
Methionine	M	Met		
Phenylalanine	F	Phe		Aromatic
Tyrosine	Y	Tyr		
Tryptophan	W	Trp		
Serine	S	Ser	Hydrophilic Polar Neutral	
Threonine	T	Thr		
Cysteine	C	Cys		
Proline	P	Pro		
Asparagine	N	Asn		
Glutamine	Q	Gln		
Lysine	K	Lys	Hydrophilic Polar Cationic, basic	
Arginine	R	Arg		
Histidine	H	His		
Aspartate	D	Asp	Hydrophilic Polar Anionic, acidic	
Glutamate	E	Glu		

1.38 *C – Transcriptional attenuation occurs when tryptophan concentration is high*

The *trp* operon encodes genes involved in tryptophan biosynthesis in prokaryotes. First characterised in *E. coli*, it contains five structural genes (*trp A–E*) which encode seven protein domains, a promotor (where RNA polymerase binds) and an operator (where the repressor binds). Transcription of the *trp* operon is tightly regulated and determined by the concentration of tryptophan. At high concentration, transcription is turned off and it is turned on when tryptophan is absent (or in short supply). Attenuation is the negative feedback mechanism by which premature termination of *trp* RNA synthesis occurs. It is a mechanism for reducing operon expression when levels of tryptophan are high; hence C is the correct response. The *trp* operon is controlled by both a repressor, which interacts with the operator when tryptophan is bound (reducing transcription), and the attenuator, a terminator (leader) sequence adjacent to the *trpE* gene (which prevents completion of transcription, rather than blocking initiation). Tryptophan acts as a co-repressor (not activator) which behaves as both a sensor and a switch – it can sense when tryptophan levels are high, and then switches the operon off (an efficient process preventing unnecessary enzyme production). The trp repressor is a dimer (not pentamer), structurally similar to the CRP protein and lac repressor. Importantly, control by the *trp* repressor (70-fold) and attenuation (10-fold) serve to allow approximately 700-fold overall regulatory control of transcription of the *trp* operon as a result of tryptophan levels.

1.39 *A – Clathrin*

Clathrin is a three-legged scaffold protein (known as a triskelion) which forms a lattice-like coat on membranes. The connection of the clathrin lattice to the membrane is mediated by clathrin adaptor proteins. Clathrin coats transport vesicles during membrane trafficking which allows clathrin-dependent endocytosis to import extracellular molecules; hence A is the correct response. Actin is a microfilament-forming protein which plays an integral role in muscle contraction and cell movement, in addition to helping to maintain and control cell shape and architecture. Laminin is a heterotrimeric glycoprotein of the basement membrane and non-collagenous component of the extracellular matrix (ECM). Alongside collagen type IV, laminin provides mechanical support, serving as a scaffold for other ECM components. Desmin is a cytoplasmic intermediate filament protein found in cardiac, skeletal and smooth muscle. Alongside vimentin, desmin plays an essential role in maintaining muscle cytoarchitecture. Tubulin, a dimeric protein essential to the eukaryotic cytoskeleton, polymerises and assembles into microtubules needed for the cell cycle.

1.40 *D – Fatty acid β-oxidation*

Fatty acid β-oxidation is the process by which energy is released through breakdown of fatty acids into acetyl-CoA in the mitochondrial matrix. Acetyl-CoA is then oxidised in the Krebs cycle, releasing CoA needed to maintain β-oxidation; hence D is the correct response. Glycolysis is the process of generating energy through the breakdown of glucose into pyruvate (aerobically) and lactate (anaerobically) in the cytosol. Pyruvate then enters the Krebs cycle. Cholesterol synthesis takes place in the cytosol and endoplasmic reticulum (ER). The pentose phosphate pathway (shunt) is an alternative to glycolysis that generates NADPH (reducing power for lipid and cholesterol synthesis) and pentoses (precursor for nucleic acid synthesis). The process occurs in the cytosol. Fatty acid synthesis is initiated by carboxylation of acetyl-CoA yielding malonyl-CoA required for elongating fatty acid chains. The process also utilises NADPH. Similar to β-oxidation, the process occurs in the cytosol and ER.

1.41 *A – Pyruvate kinase*

Substrate level phosphorylation is the process of forming ATP from ADP and phosphorylated intermediates in coupling reactions of glycolysis and the Krebs cycle. Pyruvate kinase catalyses the transfer of phosphate from phosphoenolpyruvate to ADP yielding pyruvate and ATP (last step of glycolysis); hence A is the correct response. Step 7 of glycolysis uses phosphoglycerate kinase to convert 1,3-bisphosphoglycerate to 3-phosphoglycerate, which is another example of substrate-level phosphorylation. In contrast, oxidative phosphorylation generates ATP from oxidised NADH and $FADH_2$ using electrochemical gradients. Galactokinase catalyses the conversion of α-D-galactose into galactose-1-phosphate by means of the Leloir pathway (galactose metabolism). This results in conversion to glucose-1-phosphate which can then enter glycolysis. Hexokinase is the initial enzyme used in glycolysis for the conversion of glucose to glucose-6-phosphate by ATP. Phosphofructokinase catalyses the phosphorylation of fructose-6-phosphate to fructose-1,6-diphosphate by ATP in glycolysis. Glycerol kinase catalyses the conversion of glycerol to glycerol-3-phosphate, predominantly in the liver. This is then oxidised into dihydroxyacetone phosphate which can then enter glycolysis or gluconeogenesis.

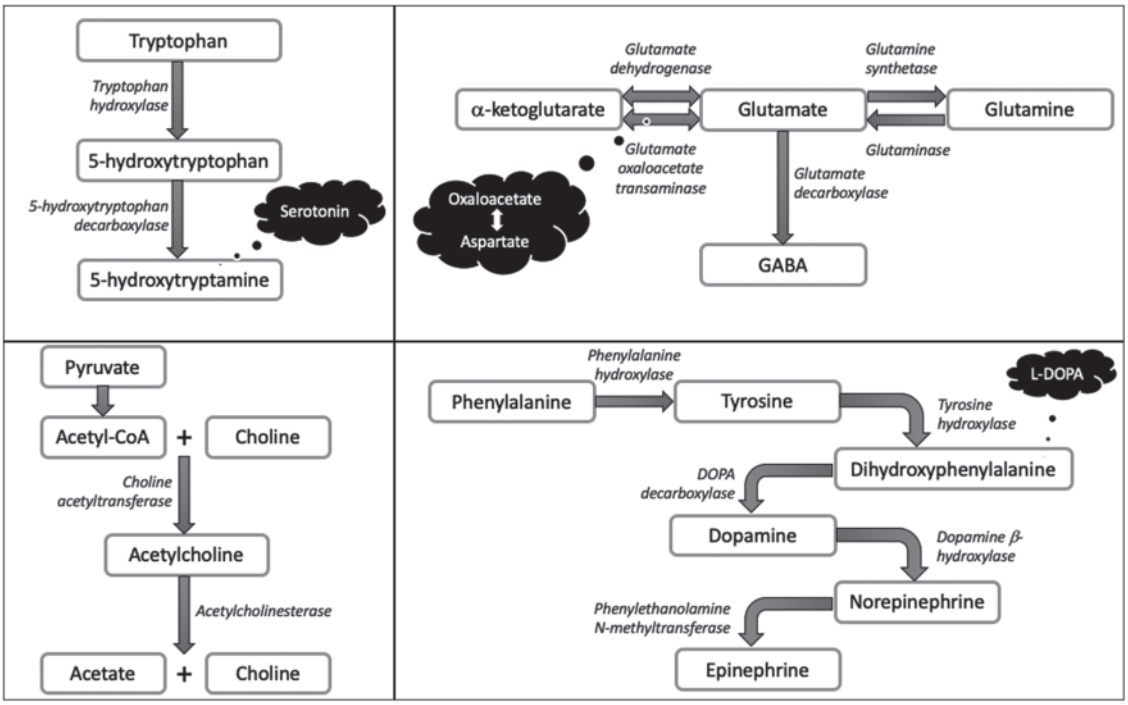
1.42 *A – Gap junctions*

Gap junctions are large channels that connect the cytosol of two adjacent cells and promote intercellular communication. They are dodecameric structures composed of connexin proteins which allow passage of smaller signalling molecules such as ions, second messengers and metabolites, but not larger molecules such as proteins; hence A is the correct response. Tight junctions (zonula occludens), which include transmembrane proteins such as occludin, claudin, and cingulin, act as a continuous intercellular barrier and restrict diffusion of solutes in epithelial and endothelial cells. They regulate the passage of proteins and

liquids across the cell membrane. Adherens junctions mediate cell–cell adhesions via integral membrane proteins such as cadherins and nectins. These junctions are important regulators in cell remodelling and proliferation, and tissue dynamics, architecture and morphogenesis. Desmosomes are membrane units that mediate cell–cell adhesion between adjacent cell membranes by providing structural and mechanical stability to cells subjected to physical stresses (heart, skin). They facilitate strong adhesion between adjacent epithelial cells. They are formed by two cadherin glycoproteins, desmoglein and desmocollin, which associate with other proteins such as plakoglobin, plakophilin and desmoplakin. On the other hand, hemidesmosomes connect basal epithelial cells and the underlying basement membrane or substratum via intermediate filaments. Instead of desmogleins and desmocollins in the ECM, hemidesmosomes utilise integrins to facilitate adhesion.

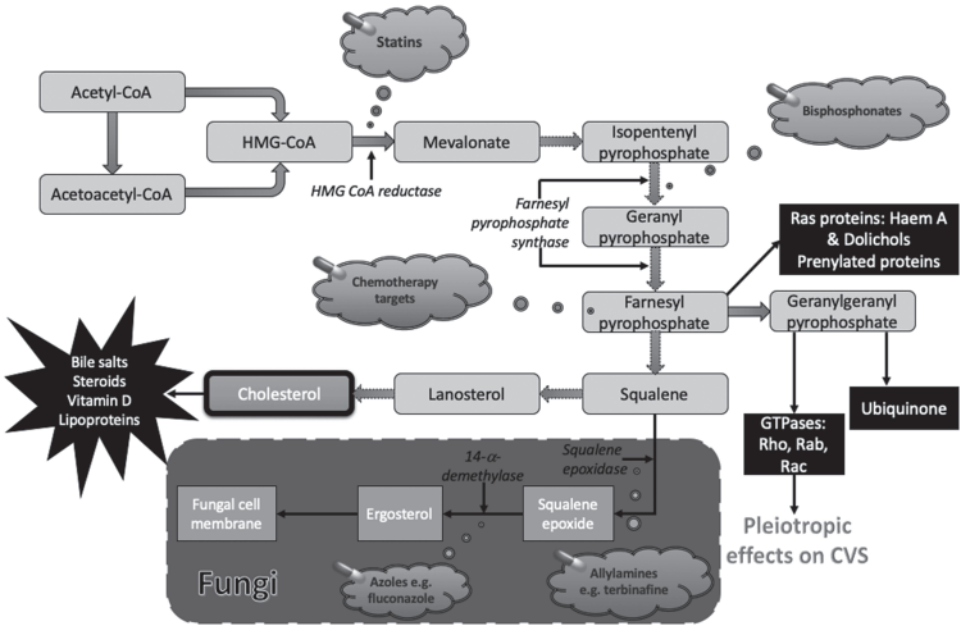
1.43 *E – Tyrosine → Dopamine*

The synthesis of various neurotransmitters from precursors is shown below. Phenylalanine is converted into tyrosine, which via L-DOPA, is converted into dopamine; hence E is the correct response.



1.44 *D – Mevalonate*

HMG-CoA reductase is the enzyme that reduces HMG-CoA to mevalonate using NADPH, in cholesterol biosynthesis; hence D is the correct response. The process begins with acetyl-CoA from the mitochondria being transported to the cytosol, and with acetoacetyl-CoA, is then converted to HMG-CoA. Numerous phosphorylation and condensation reactions in the endoplasmic reticulum yield squalene which is then cyclised to lanosterol. The conversion to cholesterol then proceeds via 19 reaction steps! As shown below, there are many therapeutic targets in the process. Statins are HMG-CoA reductase inhibitors which thwart conversion of HMG-CoA to mevalonate. Bisphosphonates are pyrophosphate analogues and stimulate osteoclast apoptosis and inhibit cholesterol synthesis. They decrease prenylation of proteins by blocking farnesyl pyrophosphate synthase (FPPS) which are required for cell function and survival. There has also been growing interest in the anticancer effects of FPPS inhibition, as lack of farnesyl pyrophosphate halts protein prenylation which is required for functioning oncogenic GTPases. Interestingly as an aside, in fungi (and with minimal effects on human cholesterol biosynthesis), squalene is converted to squalene epoxide using squalene epoxidase. In dermatophyte infections, allylamines, such as terbinafine, inhibit squalene epoxidase and therefore ergosterol biosynthesis, causing a fungicidal effect (in *Candida* spp. they are fungistatic). Azoles, such as fluconazole, miconazole and clotrimazole inhibit ergosterol synthesis by inhibiting lanosterol 14-α-demethylase, which is known to play an important role in membrane permeability. In fungi, azoles prevent demethylation of lanosterol into ergosterol using fungal cytochrome P450, and the cell membrane becomes permeable.



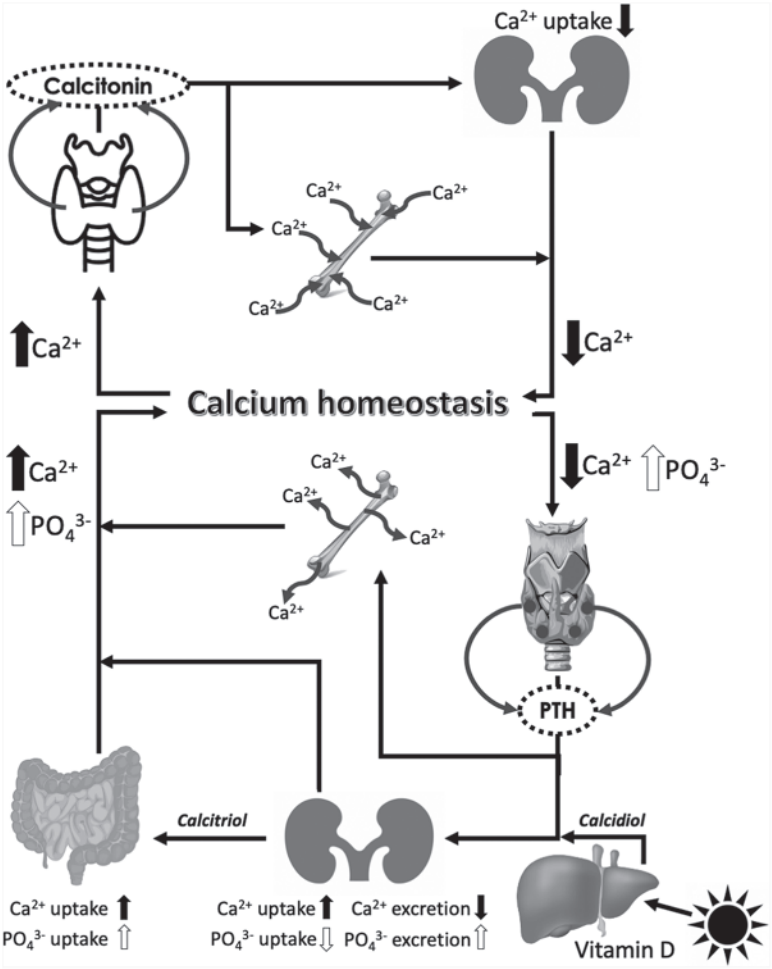
1.45 *E – Leishmaniasis*

Leishmaniasis is caused by a protozoan parasite transmitted by Phlebotomine (in the Old World) or Lutzomyia (in the New World) sand flies. It is endemic to tropics and sub-tropics and clinical manifestations include cutaneous (skin ulcers), mucocutaneous (mucosal ulcerations) and visceral (fever, weight loss, lymphadenopathy, splenomegaly, pancytopenia); hence E is the correct response. All other diseases listed are caused by spirochetes (Gram-negative, spiral bacteria): syphilis is caused by *Treponema pallidum*, Lyme disease is caused by *Borrelia burgdorferi*, leptospirosis is caused by bacteria of the genus Leptospira (primarily sp. *interrogans*) and relapsing fever is caused by various Borrelia species which can be transmitted via lice or ticks.

1.46 *D – Increased PTH release causes hydroxylation of 25-dihydroxyvitamin D in the small intestine*

PTH is released from the parathyroid gland in response to hypocalcaemia. PTH stimulates the production of active vitamin D (calcitriol, or 1,25-dihydroxycholecalciferol) in the tubular cells of the kidneys. Vitamin D_3 from sunlight or diet is converted into inactive vitamin D (calcidiol, or 25-hydroxycholecalciferol) in the liver. This is then hydroxylated in the kidneys (not 25-dihydroxycholecalciferol in the small intestine) by 1α-hydroxylase into active vitamin D. This increases absorption of calcium and phosphate in the intestines; hence D is the correct response. PTH also stimulates bone resorption of osteoclasts and release of calcium and phosphate from bone. Calcitonin is secreted by the parafollicular cells of the thyroid. Hypercalcaemia increases calcitonin secretion and it is known to directly inhibit bone resorption in osteoclasts. All other options are correct. Calcium homeostasis is summarised below.

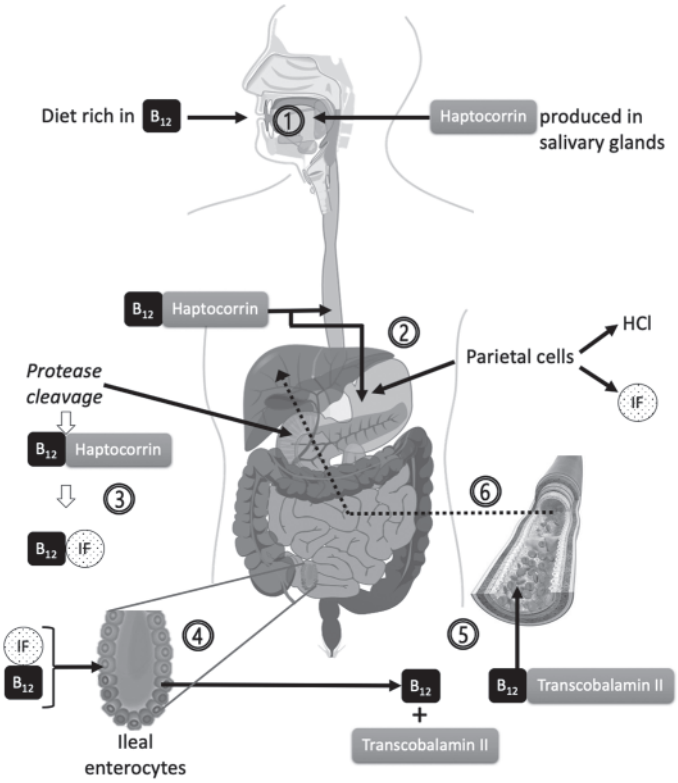
Interestingly as an aside, the growth factor, FGF23, plays an important role in calcium and phosphate homeostasis. FGF23 increases urinary excretion of phosphate in the kidney. It also inhibits 1α-hydroxylase and stimulates 24-hydroxylase (which increases 1,25-dihydroxycholecalciferol inactivation). Subsequently, 1,25-dihydroxycholecalciferol levels decrease, which leads to reduced calcium (and phosphate) reabsorption in the intestines. FGF23 also inhibits PTH secretion.



1.47 *C – Methionine synthase is a folic-acid and B_{12}-dependent enzyme*

The water-soluble vitamin B_{12} (cobalamin) is an important cofactor in many biochemical pathways. The physiologic mechanism of absorption is complex. Salient processes involved in absorption and metabolism are highlighted below. Vitamin B_{12} is key to the (re)methylation of homocysteine to methionine using methionine synthase in the cytosol. Methionine synthase also uses 5-methyltetrahydrofolate as a one-carbon donor; hence C is the correct response, as methionine synthase inexorably links folate and vitamin B_{12} metabolic pathways. In addition, vitamin B_{12} (adenosylcobalamin) is required for the conversion of methylmalonyl-CoA to succinyl-CoA using methylmalonyl-CoA mutase in the mitochondria (important in replenishing key intermediates in the Krebs cycle). As an aside and to a much lesser extent (as if the process was not complicated enough!), homocysteine can also be converted back into methionine via a folate-independent pathway using betaine-homocysteine methyltransferase in the liver and kidneys.

As illustrated below, vitamin B_{12} is bound to protein in the diet and upon digestion, released B_{12} binds to haptocorrin (previously known as R-binder or transcobalamin I) secreted by salivary glands. In the stomach (low pH), haptocorrin binds free B_{12} with much greater affinity than intrinsic factor (IF) (secreted by parietal cells of the stomach, not Paneth cells – secretory epithelial cells of the crypts of Lieberkühn which contribute to enteric innate immunity). In the small intestine, pancreatic proteases partially degrade the B_{12}-haptocorrin complex at neutral pH (not high pH), releasing B_{12} which then binds to IF. The B_{12}-IF complex binds to cubilin (an endocytic receptor for B_{12}-IF complexes) on enterocytes of the ileal mucosa and is internalised via endocytosis (not active transport). IF is then degraded. The B_{12}-transcobalamin II complex (active form of B_{12}) binds to and transports the newly internalised B_{12} in the circulation and to target cells where it is needed.



① B_{12} is released from protein-bound foods in the diet and haptocorrin (a B_{12} binder) is made in salivary glands

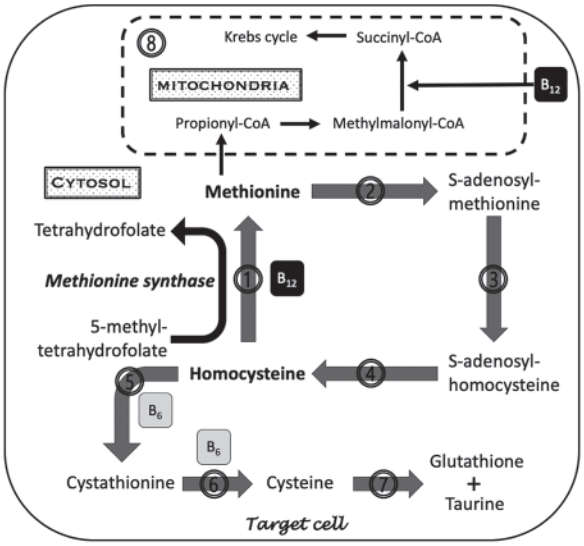
② B_{12} binds to haptocorrin. Gastric parietal cells produce HCl and intrinsic factor (IF)

③ Pancreatic proteases digest the B_{12}-haptocorrin complex, releasing B_{12} which forms a complex with IF

④ B_{12}-IF complex is recognised by the cubilin IF-receptor and is endocytosed into enterocytes of the distal ileum. IF is degraded, and B_{12} is released

⑤ Free B_{12} then binds to transcobalamin II and enters the portal circulation. It is then transported to tissues where the B_{12}-transcobalamin II complex is endocytosed, degraded in lysosomes and B_{12} is released into the cytosol

⑥ B_{12} is stored in the liver as adenosylcobalamin and released into the circulation when needed



① Methylation of homocysteine uses 5-methyl-tetrahydrofolate as a methyl donor and is catalysed by methionine synthase to methionine. B_{12} is an essential cofactor. Tetrahydrofolate is essential in purine and pyrimidine synthesis

② Methionine is catalysed by methionine adenosyltransferase into *S*-adenosyl-methionine, a methyl donor critical for gene regulation and protein methylation

③ *S*-adenosyl-methionine is converted to *S*-adenosyl-homocysteine by methyl-transferases as a result of transmethylation

④ *S*-adenosyl-homocysteine is then converted to adenosine and homocysteine by hydrolase

⑤ Via trans-sulphuration with serine in the presence of vitamin B_6, homocysteine can be converted to cystathionine using cystathionine β-synthase

⑥ Cystathionine is then converted to cysteine using cystathionase

⑦ Cysteine is involved in glutathione biosynthesis which has roles as an antioxidant, immune system enhancer and in detoxification
Cysteine can also be converted to taurine, which also functions as an antioxidant and is protective in cancer, heart disease and diabetes

⑧ Catabolised methionine, β-oxidation of odd-chain fatty acids and propionic acid from the gut flora produce propionyl–CoA. This is converted to methylmalonyl-CoA, and then succinyl-CoA using methylmalonyl-CoA mutase and B_{12} (adenosylcobalamin). This then enters the Krebs cycle

1.48 *E – 5–6 ATP molecules are produced from 2 NADH in oxidative decarboxylation*

In eukaryotic respiration, the total yield of ATP per glucose molecule has often been conflicting. Earlier studies showed a yield of 36–38 ATP per glucose molecule. However, more recent studies have shown this to be closer to 30–32 ATP per glucose molecule. The reason for this difference is based on electrochemical gradients in the mitochondria, as a result of electron carriers (NADH and $FADH_2$ from the Krebs). Four protons are needed to synthesise 1 ATP. In oxidative phosphorylation, 10 protons are pumped for every NADH, so therefore 1 NADH yields 2.5 ATP molecules; and 6 protons are pumped for every $FADH_2$, so therefore 1 $FADH_2$ yields 1.5 ATP molecules. This is in contrast to earlier studies where the ATP:NADH and ATP:$FADH_2$ were 3:1 and 2:1, respectively (yielding 36–38 ATP per glucose molecule). The maximum number of NADH and $FADH_2$ per glucose molecule is 10 and 2, respectively. Therefore, in oxidative phosphorylation, 28 ATP molecules are produced. Together with the 4 ATP molecules from substrate level phosphorylation, this yields a maximum of 32 ATP per glucose molecule. So why does the number range from 30 and 32? The electrons of the 2 NADH produced via glycolysis in the cytosol are transported into the mitochondria (as the mitochondrial membrane is impermeable to NADH) via two shuttle systems in the inner mitochondrial membrane: malate-aspartate and glycerol-phosphate. In the malate-aspartate shuttle, 2.5 ATP are produced when hydrogen from cytosolic NADH + H^+ is transferred to mitochondrial NAD^+. In the glycerol-phosphate shuttle, 1.5 ATP are produced when hydrogen from cytosolic NADH + H^+ is transferred to mitochondrial FAD. Hence the ATP yield depends on the electron carrier. The tables below show ATP yield depending on the ratio between ATP and electron carriers.

Assuming ATP : NADH = 2.5 : 1 and ATP : $FADH_2$ = 1.5 : 1					
Stage	Carbon flow	Molecules of reduced coenzymes produced	Net ATP molecules produced by substrate-level phosphorylation	Net ATP molecules produced by oxidative phosphorylation	Theoretical maximum yield of ATP molecules
Glycolysis	6C → 2 x 2C	2 NADH	2	3 ATP from 2 NADH■ or 5 ATP from 2NADH●	5 or 7
Link reaction	2 x 3C → 2 x 2C + $2CO_2$	2 NADH	0	5 ATP from 2 NADH	5
Krebs cycle	2 x 2C → $4CO_2$	6 NADH 2 $FADH_2$	2	15 ATP from 6 NADH 3 ATP from 2 $FADH_2$	20
TOTAL:	6C → $6CO_2$	10 NADH 2 $FADH_2$	4	26–28	**30–32**
■ : glycerol-phosphate shuttle ● : malate-aspartate shuttle					

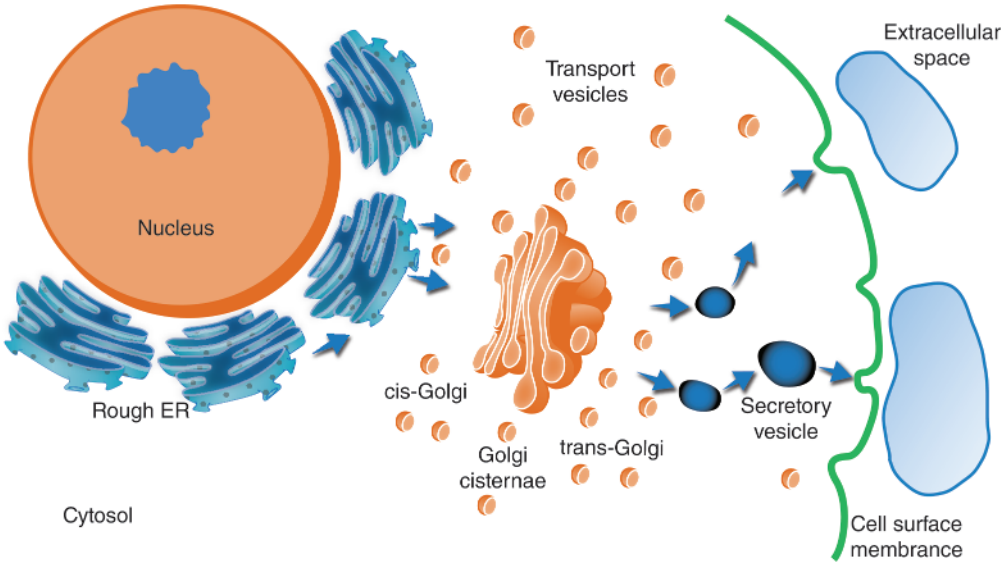
Assuming ATP : NADH = 3 : 1 and ATP : $FADH_2$ = 2 : 1					
Stage	Carbon flow	Molecules of reduced coenzymes produced	Net ATP molecules produced by substrate-level phosphorylation	Net ATP molecules produced by oxidative phosphorylation	Theoretical maximum yield of ATP molecules
Glycolysis	6C → 2 x 2C	2 NADH	2	4 ATP from 2 NADH■ or 6 ATP from 2NADH●	6 or 8
Link reaction	2 x 3C → 2 x 2C + $2CO_2$	2 NADH	0	6 ATP from 2 NADH	6
Krebs cycle	2 x 2C → $4CO_2$	6 NADH 2 $FADH_2$	2	18 ATP from 6 NADH 4 ATP from 2 $FADH_2$	24
TOTAL:	6C → $6CO_2$	10 NADH 2 $FADH_2$	4	32–34	**36–38**
■ : glycerol-phosphate shuttle ● : malate-aspartate shuttle					

Hence, as shown in the tables, oxidative decarboxylation (the link reaction) results in between 5 and 6 ATP molecules being produced from 2 NADH; E is therefore the correct response. Depending on the ATP:electron carrier, the net ATP molecules produced by oxidative phosphorylation is either 26–28, or 32–34 (not 30). There are four protein complexes in the inner membrane of the mitochondria (not three): *Complex I* – NADH ubiquinone oxidoreductase, *Complex II* – succinate ubiquinone reductase, *Complex III* – ubiquinol cytochrome c reductase, and *Complex IV* – cytochrome c oxidase. These function to translocate the 10 proteins from the mitochondrial matrix to the intermembrane space. In addition, ATP synthase is also present in the membrane, which pumps protons from the intermembrane space to the matrix, generating ATP. Complex IV receives electrons from cytochrome c and passes them to oxygen (the final electron acceptor, not water), which is reduced to water. Carbon monoxide (in addition to cyanide, sodium azide and hydrogen sulphide) inhibits cytochrome c oxidase (complex IV) in the respiratory chain, not ATP synthase. An example of an ATP synthase inhibitor (and oxidative phosphorylation) is the antibiotic, oligomycin.

1.49 *B – Rough ER → cis-Golgi network → Golgi cisternae → Secretory vesicle → Cell surface membrane*

Ribosomes synthesise proteins and become attached to the rough ER, where translation is finalised. Proteins can be inserted into the ER membrane, remain in the ER or get transported in transport vesicles. These fuse together to form *cis*-Golgi vesicles (nearest the ER) which progress into Golgi cisternae and *trans*-Golgi (farthest from the ER). During this stage, protein modifications occur. Some proteins remain in the *trans*-Golgi cisternae, while others are transported in secretory vesicles to the cell surface membrane, releasing contents by exocytosis; hence B is the correct response. The figure below summarises the secretory pathway of proteins.

Smooth ER are not involved in protein synthesis. Instead, they synthesise lipids (and cholesterol), phospholipids, steroids and carbohydrates. The lumen of the smooth ER is also an important storage site for intracellular calcium ions. The figure below summarises the secretory pathway of proteins.



1.50 *B – ATG*

Complementary base pairing is the formation of linear hydrogen bonds between adenine (A) and thymine (T), i.e., A=T, and cytosine (C) and guanine (G), i.e., C≡G, creating a stable DNA double helix. In addition, the distance between the base pairs is virtually identical at ca. 3.4 Å which enables the DNA double helix to form with the correct spatial geometry. It is the precision of base pairing that allows nucleic acids to act as templates for the synthesis of new complementary strands. The non-transcribed, coding or sense strand, read in the 5′→3′ direction, is identical to the pre-mRNA sequence (except uracil (U) in RNA replaces T in DNA). Whereas the transcribed, non-coding or antisense strand, read in the 3′→5′ direction, acts as the template and is complementary to the 5′→3′ strand. The resulting mRNA formed in transcription is transported to the ribosome where, together with tRNA, it directs protein synthesis in translation. Each codon on mRNA interacts with a specific tRNA anticodon which carries an amino acid. Hence, in this example (working in reverse), the anticodon on tRNA is AUG; therefore this is complementary to UAC of the mRNA codon. So, the sense DNA sequence would be ATG and the non-coding (antisense template) sequence would be TAC; hence B is the correct response, such that: