Biochemistry

Unit 5 · Bioenergetics and Carbohydrate Metabolism

How a cell captures energy from glucose and spends it: the free-energy rules behind ATP, the ten steps of glycolysis, the citric acid cycle, the electron transport chain and ATP synthase, and the pathways that make glucose, NADPH, ribose and polysaccharides. After this unit you can draw each pathway with its enzymes, explain how it is switched on and off, and calculate the ATP yield of glucose oxidation.

Taught at 8 of 10 | Usually Sem 3 | 8 chapters

About this unit

This unit follows glucose through the cell. It starts with the rules: what metabolism is, why enzymes are needed, and how free energy decides which reactions can run (chapters 5.1 and 5.2). Then it walks the main road of energy capture: glycolysis splits glucose into pyruvate (5.3), the citric acid cycle strips the carbons to CO_2 (5.4), and the electron transport chain turns the collected electrons into ATP (5.5). The last three chapters run the other way or branch off: gluconeogenesis makes glucose again (5.6), the pentose phosphate pathway makes NADPH and ribose and the glyoxylate cycle lets plants turn fat into sugar (5.7), and the final chapter builds starch, sucrose, cellulose and the bacterial cell wall (5.8).

Chapters

1. Metabolism overview; enzymes as biocatalysts ADVANCED
2. Bioenergetics, high-energy compounds and ATP
3. Glycolysis: steps, energetics and regulation
4. Citric acid (TCA) cycle
5. Biological oxidation: electron transport and oxidative phosphorylation
6. Gluconeogenesis
7. Pentose phosphate pathway and glyoxylate cycle
8. Biosynthesis of starch, sucrose, cellulose and peptidoglycan ADVANCED

How to use this book

- Read the lead paragraph and the figure of each chapter first; they carry the one idea you must keep.

- Cover the solution of each worked example and do it yourself on paper, with units, before you read the steps.
- Answer every "Check yourself" question in your head or on paper before you open it; the ones you get wrong are your revision list.

5.1 Metabolism overview; enzymes as biocatalysts

Metabolism is the full network of enzyme-catalysed reactions in a cell. Catabolism breaks large molecules down and captures part of their energy as ATP and reduced carriers; anabolism spends that ATP and reducing power to build. Every step is run by an enzyme, a biological catalyst that speeds a reaction millions of times without changing where its equilibrium lies.

Catabolism, anabolism and the three stages

A **metabolic pathway** is a series of enzyme steps in which the product of one step is the substrate of the next. Pathways can be linear (glycolysis), cyclic (the citric acid cycle, where the last product regenerates the first substrate) or branched. Hans Krebs described catabolism in three stages, shown in Figure 5.1.1.

1. **Stage 1:** polymers are broken into building blocks: polysaccharides to sugars, fats to fatty acids and glycerol, proteins to amino acids. Little energy is captured here.
2. **Stage 2:** the building blocks are converted to a few common intermediates, mainly acetyl-CoA. Some ATP and NADH are made (glycolysis belongs here).
3. **Stage 3:** the citric acid cycle oxidises acetyl-CoA to CO_2, and oxidative phosphorylation turns the electrons into most of the cell's ATP.

Anabolism runs the other way: from a few small precursors to many different large molecules. A pathway that serves both directions, like the citric acid cycle, is called **amphibolic**. Catabolic and anabolic routes between the same two compounds are never simple reverses of each other; at least one step differs, so the cell can switch one on and the other off (chapter 5.6 shows this for glucose).

Primary metabolites (sugars, amino acids, nucleotides, lipids) are needed for growth in every cell. **Secondary metabolites** (antibiotics, alkaloids, pigments) are made by particular organisms, often after growth stops, and are not needed for survival in the lab. Penicillin from *Penicillium* is the classic example.

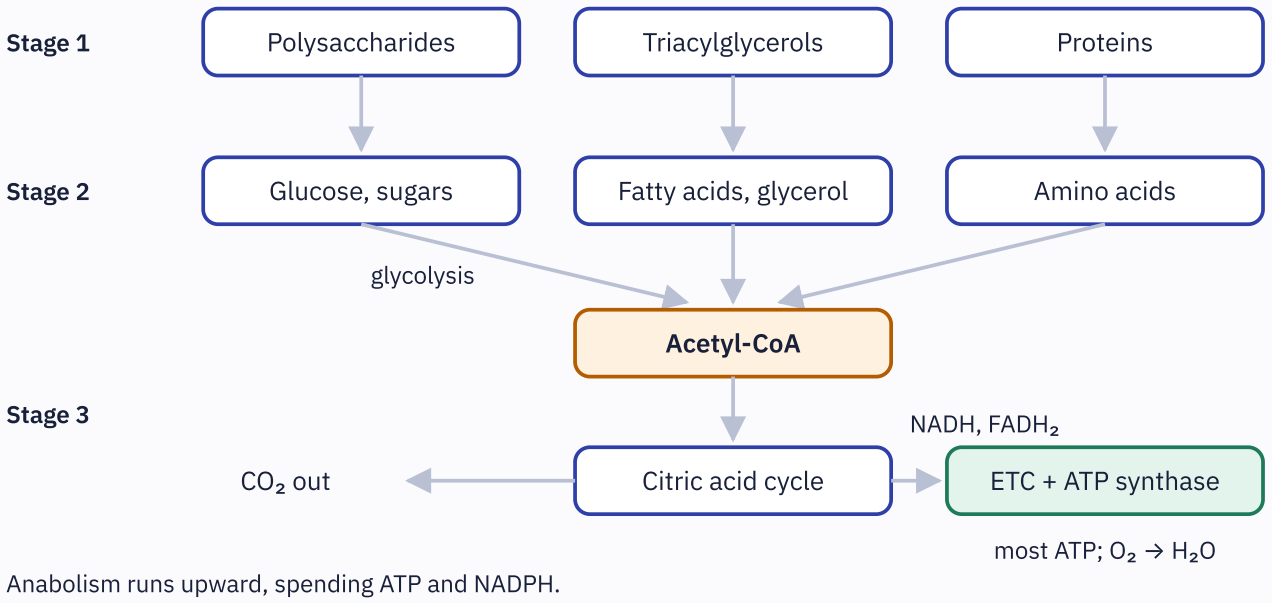


Figure 5.1.1 The three stages of catabolism. Many fuels funnel into one intermediate, acetyl-CoA, so a single citric acid cycle and one electron transport chain serve them all. Notice that most ATP is made in stage 3, not in the breakdown steps.

Enzymes as biocatalysts

An enzyme (almost always a protein; a few catalytic RNAs, ribozymes, also exist) binds its substrate in a pocket called the **active site** and stabilises the transition state. This lowers the **activation energy** E_a, the energy barrier the reactants must climb. It does not change ΔG, the free-energy difference between substrate and product, so it cannot make an unfavourable reaction favourable and it does not shift the equilibrium. It only gets the reaction to equilibrium faster.

Compared with chemical catalysts, enzymes work at body temperature and near neutral pH, are highly specific for one substrate or one bond, and can be regulated. Many need a non-protein helper: a metal ion or a **coenzyme** derived from a vitamin (NAD^+ from niacin, FAD from riboflavin, thiamine pyrophosphate from vitamin B_1; see Unit 4, Water-soluble vitamins). Enzymes are named by the EC system of the IUBMB. There were six classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases); a seventh, translocases (EC 7), was added in 2018 for enzymes that move ions or molecules across membranes.

How a pathway is controlled

The flow through a pathway is set by a few enzymes that catalyse steps far from equilibrium, usually the first **committed step**. The cell controls them in four ways: allosteric effectors (an end product inhibits an early enzyme, called feedback inhibition), covalent modification (phosphorylation by kinases, often under hormone control), the amount of enzyme (gene

expression, slow), and compartments (fatty acid oxidation in mitochondria, synthesis in the cytosol).

WORKED EXAMPLE

Question. An enzyme lowers the activation energy of a reaction by 30 kJ/mol at 37 °C. Assuming the pre-exponential factor of the Arrhenius equation is unchanged, by what factor does the rate increase?

1. The Arrhenius equation is $k = A \cdot e^{(-E_a/RT)}$, where k is the rate constant, A the pre-exponential factor, $R = 8.314$ J mol^{-1} K^{-1} and T the temperature in kelvin.
2. Ratio of rates: $k(\text{enzyme})/k(\text{none}) = e^{(\Delta E_a/RT)}$, where ΔE_a is the drop in activation energy.
3. $T = 37 + 273 = 310$ K, so $RT = 8.314 \times 310 = 2577$ J/mol.
4. $\Delta E_a/RT = 30\,000 / 2577 = 11.64$.
5. $e^{11.64} \approx 1.1 \times 10^5$.

Answer. About 110 000 times faster. A useful rule: at 37 °C every 5.9 kJ/mol taken off the barrier gives a tenfold speed-up ($RT \ln 10 = 5.9$ kJ/mol).

EXAM TRAPS

- "Enzymes shift the equilibrium" is wrong. They change the rate, never K'eq or ΔG.
- Anabolism and catabolism of the same compound are not the same steps run backwards; at least one step is different.
- Amphibolic means a pathway serves both catabolism and anabolism, not "found in both plants and animals".
- Secondary metabolites are not "less important"; they are simply not essential for the producer's growth.

CHECK YOURSELF

– REMEMBER **Name the seven EC classes of enzymes.** 2 marks

Oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, translocases.

– UNDERSTAND **Why does an enzyme not change the equilibrium constant of a reaction?** 2 marks

It lowers the barrier for the forward and reverse reactions equally; ΔG between substrate and product is unchanged, and K'eq depends only on ΔG°'.

– UNDERSTAND **Distinguish primary and secondary metabolites with one example each.** 2 marks

Primary: needed for growth in all cells (glucose, alanine). Secondary: organism-specific, not needed for growth (penicillin, nicotine).

– APPLY **By what factor does the rate rise if E_a falls by 17.8 kJ/mol at 310 K?** 3 marks

17.8/5.93 ≈ 3, so the rate rises about 10^3 = 1000 times.

– ANALYZE **Why does the cell regulate a pathway at its committed step rather than at a near-equilibrium step?** 4 marks

A near-equilibrium step runs both ways and just follows substrate and product levels; changing its enzyme changes little. The committed, far-from-equilibrium step sets the flux, and controlling it avoids building up intermediates that have no other use.

BEYOND THE SYLLABUS

Systems biologists now handle metabolism as a network: genome-scale models list every reaction an organism can run and predict growth by flux balance analysis. The same models guide metabolic engineering of *E. coli* and yeast to make drugs and biofuels. Metabolomics measures hundreds of metabolites at once by mass spectrometry. Search "flux balance analysis" and "KEGG pathway database" to see where this goes. None of this is for the exam.

5.2 Bioenergetics, high-energy compounds and ATP

Bioenergetics is thermodynamics applied to the cell. One quantity, the free-energy change ΔG, tells you whether a reaction can run on its own. Cells run uphill reactions by coupling them to the hydrolysis of ATP, the "energy currency" made in catabolism and spent in anabolism, movement and transport. A few compounds, called high-energy compounds, release even more free energy than ATP when hydrolysed, and that is how they can make ATP.

You need: the overview of catabolism and anabolism in chapter 5.1.

Free energy and the standard state

The Gibbs free-energy change of a reaction is ΔG = ΔH − TΔS, where ΔH is the enthalpy change (heat), T the absolute temperature and ΔS the entropy change. If ΔG is negative the reaction is **exergonic** and can proceed; if positive it is **endergonic** and needs an energy input; at ΔG = 0 the reaction is at equilibrium.

Biochemists use a standard state written with a prime, ΔG°': all solutes at 1 M, 25 °C (298 K), pH 7, and water treated as 1. Two equations connect it to real conditions:

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ΔG°' = −RT ln K'eq
ΔG   = ΔG°' + RT ln ( [products] / [reactants] )
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Here R = 8.314 J mol⁻¹ K⁻¹, K'eq is the equilibrium constant at the standard state, and the bracket term is the mass-action ratio Q of the actual concentrations. ΔG°' is a fixed property of the reaction; ΔG depends on the concentrations in the cell, and only ΔG decides the direction. A reaction with a positive ΔG°' can still run if the product is removed quickly, which keeps Q small.

Free-energy changes of consecutive reactions add up. This is the basis of **coupling**: an endergonic reaction runs when it shares an intermediate with a more exergonic one, so the sum is negative.

High-energy compounds

A high-energy compound has a bond whose hydrolysis has a ΔG°' more negative than about −25 kJ/mol. The "energy" is not stored in the bond itself; it is the free-energy difference between the compound and its hydrolysis products. The main groups are:

- **Phosphoanhydrides**: ATP, GTP, ADP, pyrophosphate (PPi).
- **Acyl phosphates**: 1,3-bisphosphoglycerate (1,3-BPG), from glycolysis.
- **Enol phosphates**: phosphoenolpyruvate (PEP), the highest in the cell.
- **Guanidinium phosphates**: phosphocreatine in vertebrate muscle, phosphoarginine in invertebrates.
- **Thioesters**: acetyl-CoA and succinyl-CoA (not phosphates, but ΔG°' of hydrolysis about −31 kJ/mol for acetyl-CoA).

The phosphoryl group transfer potential is −ΔG°' of hydrolysis. A compound higher in Figure 5.2.1 can pass its phosphoryl group to an acceptor lower down. ATP sits in the middle, which is

the whole point: PEP and 1,3-BPG can make ATP from ADP, and ATP can phosphorylate glucose or glycerol.

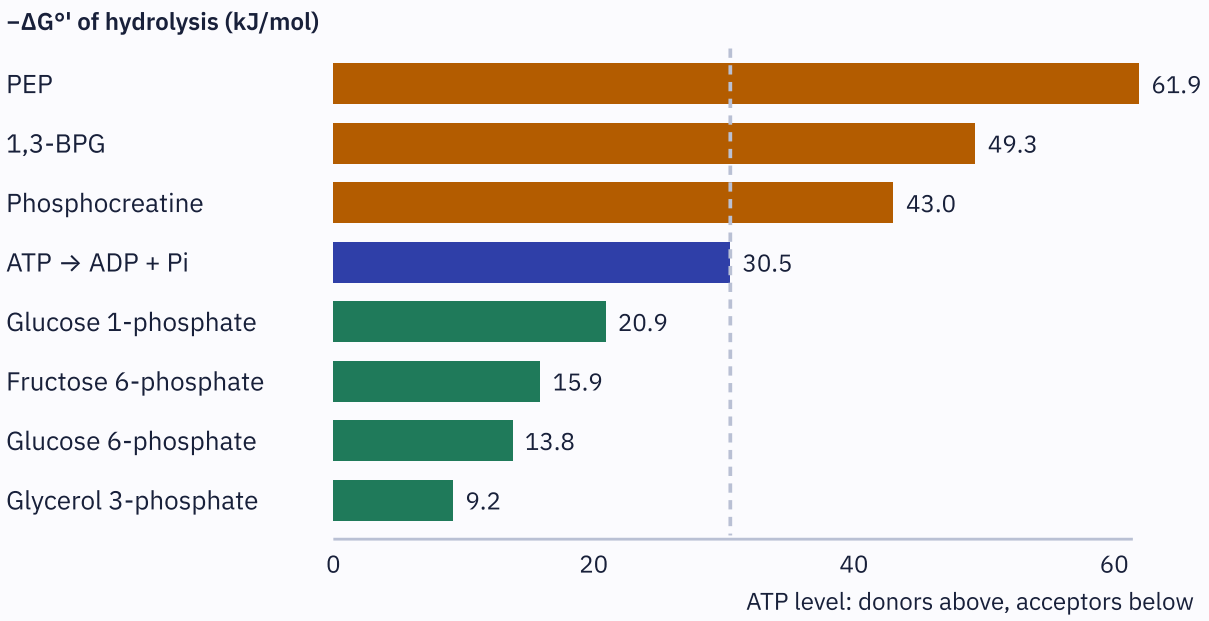


Figure 5.2.1 Phosphoryl group transfer potentials (standard values in common textbooks, e.g. Lehninger). Orange compounds can phosphorylate ADP to ATP; ATP (blue) can phosphorylate the green compounds. Notice that ATP is intermediate, which lets it act as the carrier between the two groups.

Why ATP hydrolysis releases so much energy

1. **Charge repulsion.** At pH 7 the triphosphate carries about four negative charges close together; hydrolysis separates them.
2. **Resonance.** The product Pi has more resonance forms than the bound phosphoryl group, so it is more stable.
3. **Ionisation.** ADP releases a proton at pH 7, and the low H^+ concentration pulls the reaction forward.
4. **Solvation.** ADP and Pi are better hydrated by water than ATP.

For PEP there is an extra push: the enol form of pyruvate released on hydrolysis turns at once into the far more stable keto form (tautomerisation). In cells ATP is bound to Mg^{2+}, and the Mg–ATP complex is the true substrate of most kinases.

The ATP cycle. ATP is made by two routes. **Substrate-level phosphorylation** transfers a phosphoryl group directly from a high-energy compound to ADP (1,3-BPG and PEP in glycolysis, succinyl-CoA in the TCA cycle). **Oxidative phosphorylation** uses the electron transport chain and ATP synthase (chapter 5.5); in plants, photophosphorylation does the same with light. The cell holds only a small pool of ATP and regenerates it continuously. Muscle buffers it with

phosphocreatine: creatine kinase makes ATP from phosphocreatine and ADP in the first seconds of a sprint. Adenylate kinase (2 ADP ⇌ ATP + AMP) squeezes ATP out of ADP when ATP runs low, and the rising AMP is a signal that switches catabolism on.

WORKED EXAMPLE 1: ΔG OF ATP HYDROLYSIS IN A CELL

Question. In human red blood cells [ATP] = 2.25 mM, [ADP] = 0.25 mM and [Pi] = 1.65 mM. Calculate ΔG for ATP hydrolysis at 37 °C. ΔG°' = −30.5 kJ/mol.

1. Convert to molar: ATP 2.25×10^{-3} M, ADP 0.25×10^{-3} M, Pi 1.65×10^{-3} M.
2. Q = [ADP][Pi]/[ATP] = $(0.25 \times 10^{-3})(1.65 \times 10^{-3})/(2.25 \times 10^{-3}) = 1.83 \times 10^{-4}$.
3. RT at 310 K = 8.314 × 310 = 2577 J/mol = 2.577 kJ/mol.
4. RT ln Q = $2.577 \times \ln(1.83 \times 10^{-4}) = 2.577 \times (-8.60) = -22.2$ kJ/mol.
5. ΔG = −30.5 + (−22.2) = −52.7 kJ/mol.

Answer. About −52.7 kJ/mol, much more negative than the standard value, because the cell keeps ATP far above its equilibrium level. This actual value is called the phosphorylation potential.

WORKED EXAMPLE 2: COUPLING

Question. Glucose + Pi → glucose 6-phosphate + H_2O has ΔG°' = +13.8 kJ/mol. Show how hexokinase makes the reaction favourable and find K'eq at 25 °C before and after coupling.

1. Write the two reactions: glucose + Pi → G6P (+13.8) and ATP + H_2O → ADP + Pi (−30.5).
2. Add them: glucose + ATP → G6P + ADP; ΔG°' = 13.8 − 30.5 = −16.7 kJ/mol.
3. RT at 298 K = 2.478 kJ/mol. Uncoupled K'eq = e^(−13.8/2.478) = 3.8×10^{-3}.
4. Coupled K'eq = e^(16.7/2.478) ≈ 8.5×10^{2}.

Answer. Coupling to ATP raises K'eq about 2×10^{5}-fold, from strongly backward to strongly forward. Note that hexokinase transfers the phosphoryl group directly; ATP is not first hydrolysed to free Pi.

EXAM TRAPS

- $\Delta G^{\circ\prime}$ and ΔG are not the same. A positive $\Delta G^{\circ\prime}$ does not mean the reaction never runs in the cell.
- "High-energy bond" does not mean a strong bond. It means a large negative ΔG of hydrolysis; the P–O bond itself is ordinary.
- Keep units straight: R in J mol^{-1} K^{-1} gives RT in J/mol; convert before adding to $\Delta G^{\circ\prime}$ in kJ/mol.
- Glucose 6-phosphate is a low-energy phosphate (−13.8 kJ/mol). Examiners like asking which listed compound is not high-energy.
- Substrate-level phosphorylation needs no oxygen and no membrane; oxidative phosphorylation needs both.

CHECK YOURSELF

– REMEMBER **State the biochemical standard state used for $\Delta G^{\circ\prime}$.** 2 marks

1 M solutes, 25 °C (298 K), pH 7 ($[H^+] = 10^{-7}$ M), water taken as 1; often 1 mM Mg^{2+}.

– REMEMBER **Give one example each of an acyl phosphate, an enol phosphate and a guanidinium phosphate.** 3 marks

1,3-bisphosphoglycerate; phosphoenolpyruvate; phosphocreatine.

– UNDERSTAND **Why can PEP make ATP but glucose 6-phosphate cannot?** 3 marks

PEP has a phosphoryl transfer potential of 61.9 kJ/mol, above ATP's 30.5, so transfer to ADP is exergonic. Glucose 6-phosphate (13.8) is below ATP, so the transfer would be endergonic.

– UNDERSTAND **List four reasons for the large negative $\Delta G^{\circ\prime}$ of ATP hydrolysis.** 4 marks

Relief of charge repulsion; greater resonance stabilisation of Pi; ionisation of ADP at pH 7; better solvation of products.

– APPLY **A reaction has K'eq = 10 at 25 °C. Find $\Delta G^{\circ\prime}$.** 2 marks

$\Delta G^{\circ\prime} = -RT \ln K'_{eq} = -2.478 \times 2.303 = -5.7$ kJ/mol.

– APPLY **Find $\Delta G^{\circ\prime}$ for phosphocreatine + ADP → creatine + ATP.** 2 marks

−43.0 + 30.5 = −12.5 kJ/mol; favourable, so creatine kinase makes ATP when ADP rises.

– ANALYZE **Why is it an advantage that ATP's transfer potential is intermediate rather than the highest in the cell?** 4 marks

An intermediate value lets ATP both accept phosphoryl groups from higher compounds (PEP, 1,3-BPG) and donate them to lower ones (glucose, glycerol), so one molecule can carry energy between catabolism and anabolism. If it were highest, nothing could make it by substrate-level transfer.

BEYOND THE SYLLABUS

Researchers now measure ATP and the ATP/ADP ratio inside living cells with genetically encoded fluorescent sensors, and follow phosphocreatine in human muscle by ^{31}P magnetic resonance spectroscopy. Thermodynamic analysis of whole pathways, which finds the steps that keep the pathway driving forward, is used to design engineered pathways. Search "max-min driving force" and "eQuilibrator" for tools that estimate ΔG'° from structures. This is not for the exam.

5.3 Glycolysis: steps, energetics and regulation

Glycolysis splits one glucose (6 carbons) into two pyruvate (3 carbons each) in ten cytosolic steps, making a net 2 ATP and 2 NADH. It needs no oxygen, runs in almost every cell on Earth, and is the only ATP source for red blood cells, which have no mitochondria. It is also called the Embden–Meyerhof–Parnas (EMP) pathway.

You need: ΔG°', coupling and high-energy compounds from chapter 5.2; glucose and fructose ring structures from Unit 1, Monosaccharides.

The ten steps

The first five steps are the **preparatory (investment) phase**: two ATP are spent to make a symmetrical six-carbon bisphosphate that splits into two triose phosphates. The last five are the **payoff phase**, run twice per glucose, which make four ATP and two NADH.

1. **Hexokinase**: glucose + ATP → glucose 6-phosphate (G6P) + ADP. Traps glucose in the cell, since the phosphorylated sugar cannot leave by the glucose transporter. Irreversible.
2. **Phosphohexose isomerase** (glucose-6-phosphate isomerase): G6P ⇌ fructose 6-phosphate (F6P), an aldose to ketose change.

3. **Phosphofructokinase-1 (PFK-1)**: F6P + ATP → fructose 1,6-bisphosphate (F1,6BP) + ADP. Irreversible and the committed step of glycolysis.
4. **Aldolase**: F1,6BP ⇌ dihydroxyacetone phosphate (DHAP) + glyceraldehyde 3-phosphate (GAP).
5. **Triose phosphate isomerase**: DHAP ⇌ GAP, so both halves continue as GAP.
6. **Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)**: GAP + Pi + NAD^+ → 1,3-bisphosphoglycerate (1,3-BPG) + NADH + H^+. The only oxidation step; the energy of oxidising an aldehyde is trapped in an acyl phosphate.
7. **Phosphoglycerate kinase**: 1,3-BPG + ADP → 3-phosphoglycerate (3PG) + ATP. First substrate-level phosphorylation.
8. **Phosphoglycerate mutase**: 3PG ⇌ 2-phosphoglycerate (2PG).
9. **Enolase**: 2PG → phosphoenolpyruvate (PEP) + H_2O. Removing water creates the high-energy enol phosphate.
10. **Pyruvate kinase**: PEP + ADP → pyruvate + ATP. Second substrate-level phosphorylation. Irreversible.

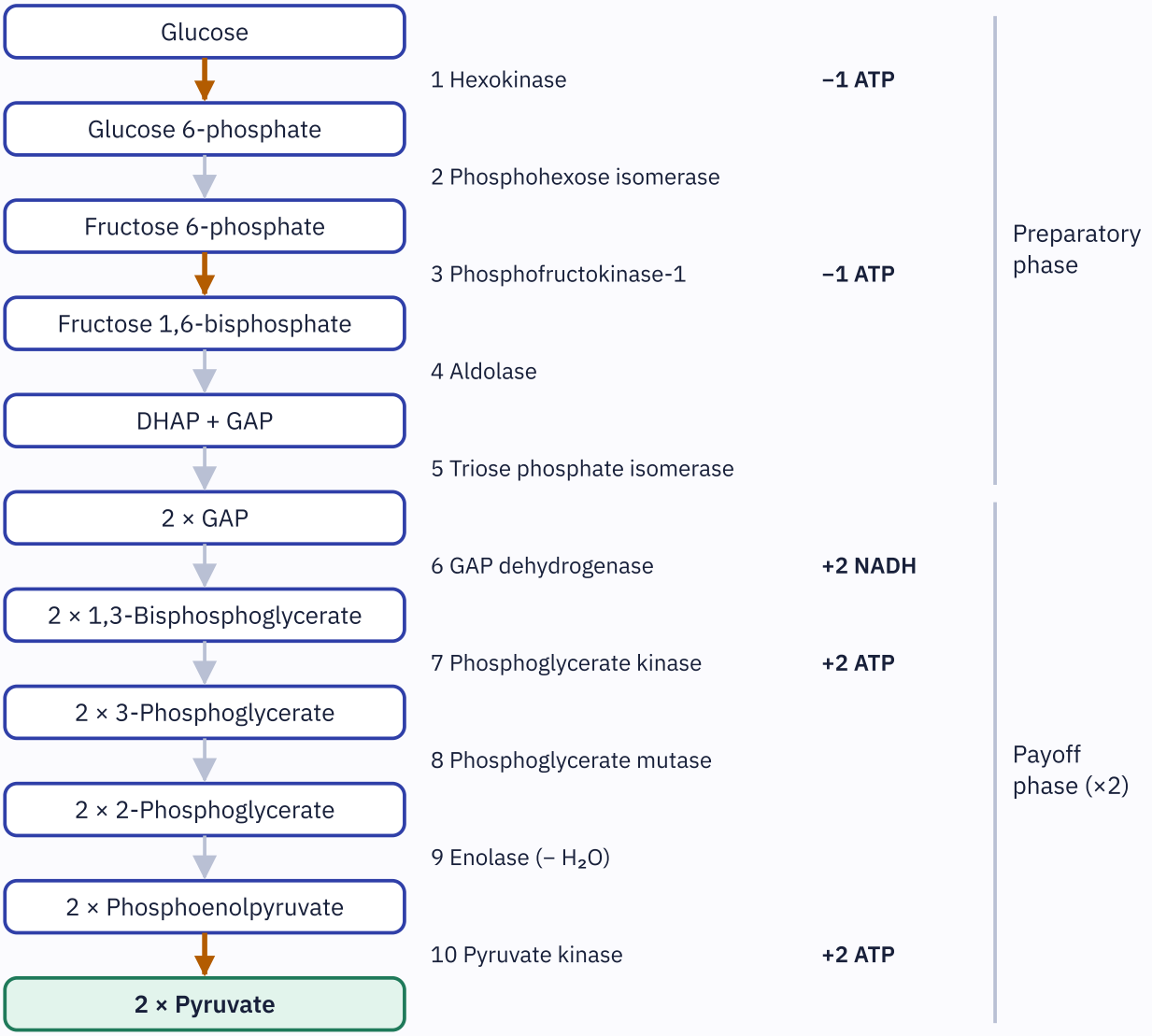


Figure 5.3.1 Glycolysis. Thick orange arrows mark the three irreversible steps (1, 3, 10), which are the control points and the steps gluconeogenesis must bypass. Notice that ATP is spent before the split and made after it, and that every step after the split happens twice per glucose.

Energetics

Add the steps and the net equation is:

$$\text{Glucose} + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ H^+ + 2\ ATP + 2\ H_2O$$

ATP balance: −2 (steps 1 and 3) + 4 (steps 7 and 10, two each) = net +2 ATP. Conversion of glucose to pyruvate has $\Delta G^{\circ\prime} \approx -146$ kJ/mol; about 61 kJ/mol of that is kept in the two ATP, so the overall $\Delta G^{\circ\prime}$ of glycolysis including ATP synthesis is about −85 kJ/mol. Several individual steps have positive $\Delta G^{\circ\prime}$ (aldolase +23.8 kJ/mol, the largest), but in the cell their ΔG is close to zero because the products are used at once. Only steps 1, 3 and 10 have large negative ΔG in the cell.

Fate of pyruvate and NADH. The cytosol has little NAD^+, so glycolysis stops unless NADH is re-oxidised. With oxygen, the electrons go to the mitochondria by a shuttle (chapter 5.5) and pyruvate enters the TCA cycle via pyruvate dehydrogenase (chapter 5.4). Without oxygen, muscle and red cells use **lactate dehydrogenase** (pyruvate + NADH → lactate + NAD^+); yeast uses pyruvate decarboxylase (thiamine pyrophosphate as coenzyme) then alcohol dehydrogenase to make ethanol and CO_2. Either way the net yield stays 2 ATP per glucose. Red blood cells also divert some 1,3-BPG to 2,3-bisphosphoglycerate (the Rapoport–Luebering shunt), which lowers haemoglobin's oxygen affinity; that detour skips step 7 and costs ATP.

Regulation

- **Hexokinase** is inhibited by its own product, G6P. Liver has **glucokinase** (hexokinase IV), which has a much lower affinity for glucose and is not inhibited by G6P, so the liver takes up glucose mainly when blood glucose is high after a meal.
- **PFK-1**, the main control point, is inhibited by ATP and citrate (signals that energy and building blocks are plentiful) and activated by AMP, ADP and **fructose 2,6-bisphosphate**, the strongest activator, whose level is set by insulin and glucagon (chapter 5.6).
- **Pyruvate kinase** is activated by F1,6BP (feed-forward) and inhibited by ATP, alanine and acetyl-CoA. In liver, glucagon causes its phosphorylation, which inactivates it.

Classic inhibitors: **iodoacetate** blocks GAPDH (step 6); **arsenate** replaces Pi at step 6 and forms 1-arseno-3-phosphoglycerate, which hydrolyses on its own, so step 7 makes no ATP; **fluoride** inhibits enolase (step 9), which is why blood for glucose estimation is collected in sodium fluoride tubes.

WORKED EXAMPLE

Question. (a) Calculate the net ATP made by anaerobic glycolysis of one glucose to lactate. (b) What is the net ATP yield if arsenate fully replaces phosphate at the GAPDH step? Show the count step by step.

1. ATP spent: hexokinase 1 + PFK-1 1 = 2.
2. ATP made: phosphoglycerate kinase 1 × 2 trioses = 2; pyruvate kinase 1 × 2 = 2; total 4.
3. NADH: 2 made at GAPDH, 2 used by lactate dehydrogenase; net 0.
4. (a) Net = 4 − 2 = **2 ATP per glucose**.
5. (b) With arsenate, the arseno compound breaks down to 3-phosphoglycerate without phosphoglycerate kinase acting, so step 7 gives 0 ATP. ATP made = 0 + 2 (pyruvate kinase) = 2; spent = 2.
6. Net = 2 − 2 = **0 ATP**, although pyruvate is still made and NADH still forms.

Answer. 2 ATP normally; 0 ATP with arsenate. Arsenate uncouples oxidation from phosphorylation at step 6.

EXAM TRAPS

- Gross ATP is 4, net is 2. Say which you mean.
- The only oxidation step is GAPDH (step 6); no step of glycolysis uses oxygen.
- PFK-1 is the rate-limiting, committed step, not hexokinase, because G6P also feeds glycogen synthesis and the pentose phosphate pathway.
- Fructose 2,6-bisphosphate is a regulator, not an intermediate of glycolysis. Do not confuse it with fructose 1,6-bisphosphate.
- Enolase removes water (a dehydration); it is not an oxidation.

CHECK YOURSELF

– REMEMBER **Name the three irreversible enzymes of glycolysis.** 3 marks

Hexokinase (or glucokinase), phosphofructokinase-1, pyruvate kinase.

– REMEMBER **Which steps of glycolysis are substrate-level phosphorylations?** 2 marks

Phosphoglycerate kinase (1,3-BPG → 3PG) and pyruvate kinase (PEP → pyruvate).

– UNDERSTAND **Why must NADH be re-oxidised for glycolysis to continue?** 3 marks

GAPDH needs NAD^+. The cytosolic NAD^+ pool is small, so without re-oxidation (by lactate dehydrogenase, alcohol dehydrogenase or a shuttle to the ETC) GAPDH stops and so does glycolysis.

– UNDERSTAND **How do glucokinase and hexokinase differ, and why does it matter for the liver?** 4 marks

Glucokinase has much lower glucose affinity and no product inhibition by G6P; it works mainly at high blood glucose, letting the liver store excess glucose without competing with other tissues when glucose is low.

– APPLY **How many net ATP are made when a glucose unit from glycogen (released as glucose 1-phosphate) goes to lactate?** 2 marks

3 ATP: glucose 1-phosphate becomes G6P without hexokinase, so only 1 ATP is spent (PFK-1) and 4 are made.

– APPLY **Glucose labelled with ^{14}C at C-1 is glycolysed. Which carbon of pyruvate is labelled?** 3 marks

C-1 of glucose goes to DHAP, which becomes GAP; it ends up as the methyl carbon, C-3, of pyruvate.

– ANALYZE **Explain why PFK-1 is inhibited by ATP even though ATP is also its substrate.** 4 marks

PFK-1 has a separate allosteric site with lower affinity for ATP. At high ATP the regulatory site fills and lowers the enzyme's affinity for F6P, so glycolysis slows when energy is plentiful; AMP and fructose 2,6-bisphosphate relieve the inhibition.

BEYOND THE SYLLABUS

Many cancer cells take up glucose fast and turn it to lactate even with oxygen present, the Warburg effect. PET scans exploit this with the tracer ^{18}F-fluorodeoxyglucose, which hexokinase traps as a phosphate in tumour cells. Pyruvate kinase M2, a tumour isoform, is a current drug target, and red-cell pyruvate kinase deficiency now has a licensed activator drug (mitapivat). Search "Warburg effect" and "PKM2". This is not for the exam.

5.4 Citric acid (TCA) cycle

The citric acid cycle, also called the tricarboxylic acid (TCA) cycle or Krebs cycle, oxidises the two carbons of acetyl-CoA to two CO_2 in eight steps in the mitochondrial matrix. It makes little ATP directly; its real product is reduced carriers (3 NADH and 1 $FADH_2$ per turn) whose electrons drive ATP synthesis in chapter 5.5. It is the common final pathway for carbohydrate, fat and protein, and its intermediates also feed biosynthesis.

You need: pyruvate from chapter 5.3; coenzymes thiamine, riboflavin, niacin, pantothenate and lipoic acid from Unit 4, Water-soluble vitamins.

The link reaction: pyruvate dehydrogenase complex

Pyruvate enters the mitochondrion and is converted to acetyl-CoA by the **pyruvate dehydrogenase (PDH) complex**, an oxidative decarboxylation:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \qquad \Delta G^{\circ\prime} \approx -33.4\ \text{kJ/mol}$$

The complex has three enzymes and five coenzymes. E1, pyruvate dehydrogenase, removes CO_2 using thiamine pyrophosphate (TPP). E2, dihydrolipoyl transacetylase, carries the acetyl group on a lipoamide arm and transfers it to coenzyme A. E3, dihydrolipoyl dehydrogenase, re-oxidises the lipoamide using FAD and passes the electrons to NAD^+. A memory aid: "TLC For Nancy" (TPP, Lipoate, CoA, FAD, NAD^+). The step is irreversible, which is why animals cannot turn acetyl-CoA, and so fatty acids, into net glucose (chapter 5.6).

PDH is inhibited by its products, acetyl-CoA and NADH, and by ATP. It is also switched off by phosphorylation: PDH kinase (activated by ATP, NADH and acetyl-CoA) phosphorylates E1, and PDH phosphatase (activated by Ca^{2+} in working muscle and by insulin) reactivates it.

The eight steps

1. **Citrate synthase**: acetyl-CoA (2C) + oxaloacetate (4C) + H_2O → citrate (6C) + CoA. Strongly exergonic ($\Delta G^{\circ\prime} \approx -32.2$ kJ/mol) because the thioester is hydrolysed.
2. **Aconitase**: citrate ⇌ isocitrate, through cis-aconitate. An iron–sulphur enzyme that moves the hydroxyl group so it can be oxidised.
3. **Isocitrate dehydrogenase**: isocitrate + NAD^+ → α-ketoglutarate (5C) + CO_2 + NADH. First CO_2 and first NADH; the main rate-limiting step.

4. **α-Ketoglutarate dehydrogenase complex**: α-ketoglutarate + CoA + NAD^+ → succinyl-CoA (4C) + CO_2 + NADH. Same mechanism and coenzymes as PDH.
5. **Succinyl-CoA synthetase** (succinate thiokinase): succinyl-CoA + GDP + Pi → succinate + GTP + CoA. The cycle's only substrate-level phosphorylation; some tissues and plants make ATP instead of GTP.
6. **Succinate dehydrogenase**: succinate + FAD → fumarate + $FADH_2$. The only enzyme of the cycle bound to the inner membrane; it is also Complex II of the electron transport chain.
7. **Fumarase** (fumarate hydratase): fumarate + H_2O → L-malate.
8. **Malate dehydrogenase**: L-malate + NAD^+ ⇌ oxaloacetate + NADH. $\Delta G^{\circ\prime} \approx +29.7$ kJ/mol; it runs forward only because citrate synthase keeps oxaloacetate very low.

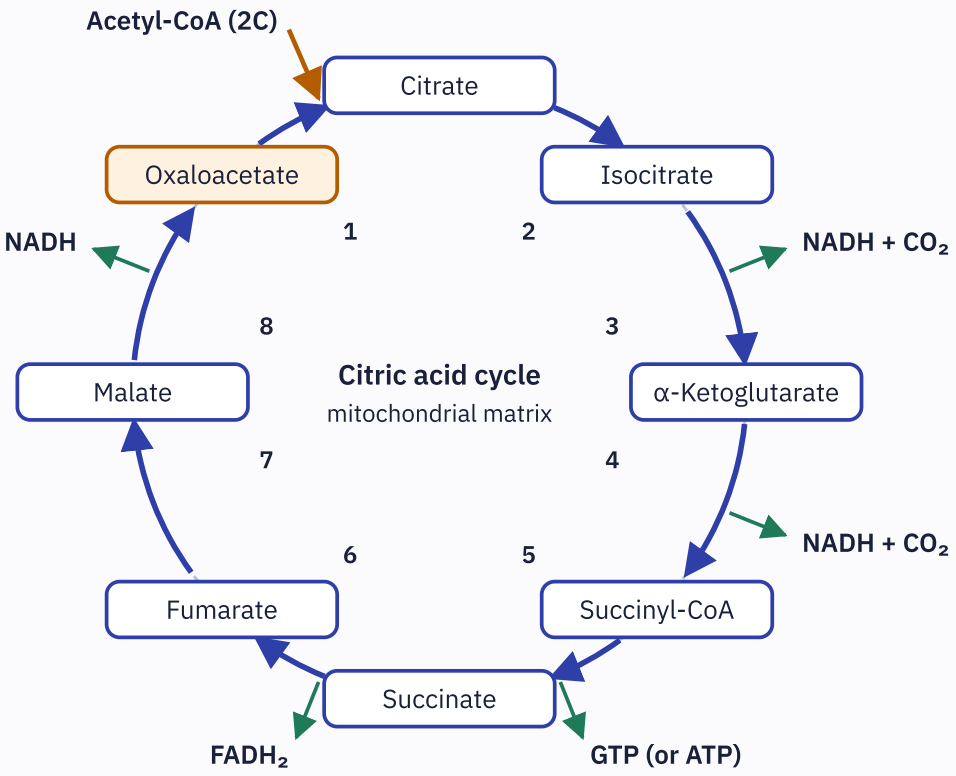


Figure 5.4.1 One turn of the citric acid cycle; numbers match the steps above. Acetyl-CoA (2 carbons) joins oxaloacetate (4 carbons), two carbons leave as CO_2 at steps 3 and 4, and oxaloacetate is regenerated. Notice the four oxidations (steps 3, 4, 6, 8) that load NADH and $FADH_2$.

Energetics and regulation

The net reaction for one turn is:

```
Acetyl-CoA + 3 NAD⁺ + FAD + GDP + Pi + 2 H₂O
      → 2 CO₂ + 3 NADH + 3 H⁺ + FADH₂ + GTP + CoA
```

With the modern P/O ratios (2.5 ATP per NADH, 1.5 per $FADH_2$; explained in chapter 5.5), one turn gives 3 × 2.5 + 1.5 + 1 = **10 ATP**. Older textbooks use 3 and 2, which gives 12. Oxygen is not used by any step, yet the cycle stops without it, because NAD^+ and FAD are regenerated only by the electron transport chain.

The cycle is controlled at three far-from-equilibrium steps. **Citrate synthase** is limited by oxaloacetate and acetyl-CoA supply and inhibited by NADH, succinyl-CoA and citrate. **Isocitrate dehydrogenase** is activated by ADP and Ca^{2+} and inhibited by ATP and NADH. **α-Ketoglutarate dehydrogenase** is inhibited by succinyl-CoA and NADH and activated by Ca^{2+}. A high NADH/NAD^+ or ATP/ADP ratio therefore slows the cycle, and muscle contraction (Ca^{2+}) speeds it up.

An amphibolic pathway

Cycle intermediates leave for biosynthesis: citrate for fatty acids, α-ketoglutarate and oxaloacetate for amino acids (glutamate and aspartate), succinyl-CoA for haem, and oxaloacetate for glucose (chapter 5.6). Reactions that refill the cycle are called **anaplerotic**. The main one in animals is **pyruvate carboxylase**, which needs biotin and is activated by acetyl-CoA: pyruvate + CO_2 + ATP → oxaloacetate + ADP + Pi. When acetyl-CoA piles up, it signals that more oxaloacetate is needed, and pyruvate carboxylase provides it.

Inhibitors examiners ask about: **fluoroacetate** (a rat poison) is converted to fluorocitrate, which blocks aconitase; **malonate** is a competitive inhibitor of succinate dehydrogenase, because it looks like succinate; **arsenite** binds the lipoamide of PDH and α-ketoglutarate dehydrogenase.

WORKED EXAMPLE

Question. Calculate the ATP yield from the complete aerobic oxidation of one molecule of pyruvate to CO_2 and H_2O. Give the answer with the modern P/O ratios and with the older ones.

1. PDH: 1 NADH.
2. One turn of the cycle: 3 NADH, 1 $FADH_2$, 1 GTP.
3. Totals: 4 NADH, 1 $FADH_2$, 1 GTP.
4. Modern: 4 × 2.5 + 1 × 1.5 + 1 = 10 + 1.5 + 1 = **12.5 ATP**.
5. Older (3 and 2): 4 × 3 + 1 × 2 + 1 = **15 ATP**.

Answer. 12.5 ATP per pyruvate (15 in older books). Write the P/O values you used, because examiners accept either set when it is stated.

EXAM TRAPS

- The PDH reaction is not part of the cycle; it is the link between glycolysis and the cycle. Count its NADH separately.
- The two CO_2 of one turn do not come from the acetyl group that just entered; they come from carbons that were in oxaloacetate. The acetyl carbons leave in later turns.
- Succinate dehydrogenase uses FAD, not NAD^+, because the $\Delta E^{\circ\prime}$ for oxidising succinate is too small for NAD^+.
- The cycle makes GTP (or ATP) only once per turn. Most ATP comes from the ETC.
- Malonate inhibits succinate dehydrogenase competitively; fluorocitrate inhibits aconitase. Do not swap them.

CHECK YOURSELF

– REMEMBER **Name the five coenzymes of the pyruvate dehydrogenase complex.** 3 marks

Thiamine pyrophosphate, lipoic acid (lipoamide), coenzyme A, FAD, NAD^+.

– REMEMBER **Which enzymes of the cycle release CO_2?** 2 marks

Isocitrate dehydrogenase and the α-ketoglutarate dehydrogenase complex.

– UNDERSTAND **Why is the citric acid cycle called amphibolic? Give two examples.** 4 marks

It oxidises acetyl-CoA (catabolism) and supplies precursors for synthesis (anabolism): α-ketoglutarate for glutamate, succinyl-CoA for haem, citrate for fatty acids, oxaloacetate for glucose and aspartate.

– UNDERSTAND **The cycle uses no oxygen. Why does it stop under anaerobic conditions?** 3 marks

Its dehydrogenases need NAD^+ and FAD, which are regenerated only when the ETC passes electrons to O_2. Without O_2, NADH builds up and the cycle halts.

– APPLY **How many ATP does one glucose yield through PDH and two turns of the cycle only (ignore glycolysis)?** 3 marks

2 × 12.5 = 25 ATP (modern) or 30 (older).

– APPLY **A tissue is treated with malonate. Which intermediate accumulates?** 2 marks

Succinate, because succinate dehydrogenase is blocked.

– ANALYZE **Why does a shortage of oxaloacetate slow acetyl-CoA oxidation even when acetyl-CoA is abundant, and how does the cell respond?** 4 marks

Oxaloacetate is a catalyst of the cycle: each acetyl-CoA needs one to form citrate. If it is drained (for gluconeogenesis, say), acetyl-CoA cannot enter. Acetyl-CoA activates pyruvate carboxylase, which replenishes oxaloacetate (anaplerosis); in liver the excess acetyl-CoA forms ketone bodies (Unit 6).

BEYOND THE SYLLABUS

Several cycle enzymes are now known as cancer genes: mutant isocitrate dehydrogenase makes 2-hydroxyglutarate, an "oncometabolite", and drugs against mutant IDH1 and IDH2 are in clinical use for some leukaemias. Succinate and fumarate also act as signals that change gene expression. Researchers trace carbon through the cycle with ^{13}C-labelled glucose and mass spectrometry. Search "oncometabolite" and "^{13}C metabolic flux analysis". This is not for the exam.

5.5 Biological oxidation: electron

transport and oxidative phosphorylation

NADH and $FADH_2$ from glycolysis, the link reaction and the TCA cycle hand their electrons to a chain of carriers in the inner mitochondrial membrane. As electrons fall from NADH to oxygen, the chain pumps protons out of the matrix; the protons flow back through ATP synthase, which makes ATP. This coupling of electron flow to ATP synthesis is oxidative phosphorylation, and it gives about 90 percent of the ATP from glucose.

You need: ΔG°' from chapter 5.2; the NADH and $FADH_2$ counts from chapter 5.3 and chapter 5.4.

Redox potential and free energy

Oxidation is loss of electrons; reduction is gain. Each redox pair (oxidised form/reduced form) has a **standard reduction potential** E°', measured in volts at pH 7 against the hydrogen electrode. A more negative E°' means a stronger tendency to give electrons away; electrons flow spontaneously from a pair of lower E°' to one of higher E°'.

REDOX PAIR	E°' (V)
NAD^+ / NADH	−0.320
Pyruvate / lactate	−0.185
Fumarate / succinate	+0.031
Ubiquinone / ubiquinol	+0.045
Cytochrome c (Fe^{3+} / Fe^{2+})	+0.254
½ O_2 / H_2O	+0.816

The free-energy change of electron transfer is $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$, where n is the number of electrons transferred, F is the Faraday constant (96.5 kJ V^{-1} mol^{-1}) and $\Delta E^{\circ\prime} = E^{\circ\prime}$(acceptor) − $E^{\circ\prime}$(donor). Actual potentials follow the Nernst equation, $E = E^{\circ\prime} + (RT/nF)\ \ln([\text{oxidised}]/[\text{reduced}])$.

The electron transport chain

The chain has four complexes and two mobile carriers, arranged in order of rising E°' (Figure 5.5.1).

1. **Complex I** (NADH:ubiquinone oxidoreductase, NADH dehydrogenase) takes 2 e^- from NADH through FMN and iron–sulphur centres and passes them to ubiquinone (Q). It pumps

4 H^+ out per pair of electrons.

2. **Complex II** (succinate dehydrogenase, the TCA enzyme) passes electrons from succinate through FAD and Fe–S centres to Q. It pumps no protons, which is why $FADH_2$ yields less ATP.
3. **Ubiquinone** (coenzyme Q) is a small lipid-soluble carrier that moves within the membrane and collects electrons from Complexes I and II (and from the glycerol 3-phosphate shuttle).
4. **Complex III** (cytochrome bc_1 complex) passes electrons from ubiquinol (QH_2) to cytochrome c through the Q cycle, releasing 4 H^+ into the intermembrane space per pair.
5. **Cytochrome c** is a small haem protein on the outer face of the inner membrane that carries one electron at a time to Complex IV.
6. **Complex IV** (cytochrome c oxidase; haems a and a_3, copper centres CuA and CuB) gives electrons to O_2: $4\ e^- + 4\ H^+ + O_2 \rightarrow 2\ H_2O$. It pumps 2 H^+ per pair of electrons.

Totals: **10 H^+** pumped per NADH (4 + 4 + 2) and **6 H^+** per $FADH_2$ entering through Complex II (0 + 4 + 2).

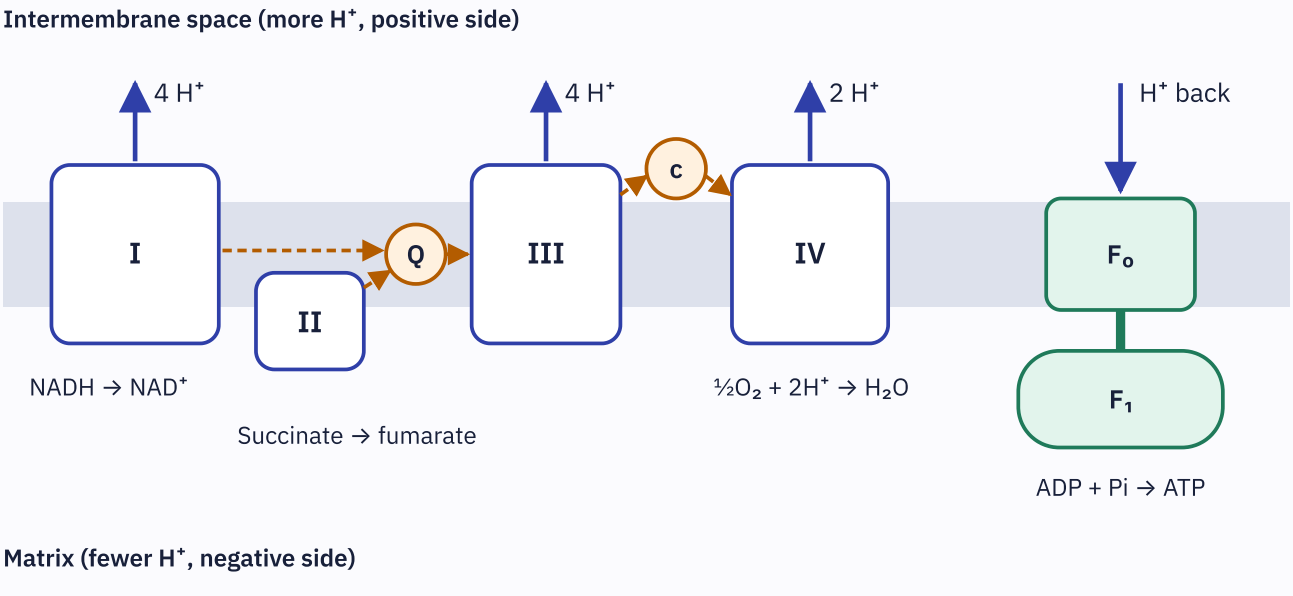


Figure 5.5.1 The respiratory chain and ATP synthase in the inner mitochondrial membrane. Dashed orange arrows trace electrons; blue arrows show protons pumped out by Complexes I, III and IV and returning through F_o to drive ATP synthesis in F_1. Notice that Complex II feeds Q but pumps no protons.

Chemiosmosis and ATP synthase

Peter Mitchell's **chemiosmotic theory** explains the coupling. Pumped protons create a **proton-motive force** with two parts: an electrical potential (the matrix side is negative) and a pH

gradient (the matrix is more alkaline). The inner membrane is impermeable to H^+, so the protons can return only through ATP synthase.

ATP synthase (Complex V, F_oF_1-ATPase) has two parts. F_o sits in the membrane and contains a ring of c subunits; protons passing through turn the ring. The ring turns the central γ stalk inside F_1, a knob in the matrix made of three α and three β subunits. In Paul Boyer's **binding change mechanism**, each β subunit cycles through three states: open (O, releases ATP and takes in ADP + Pi), loose (L, holds them) and tight (T, joins them into ATP). One full turn of γ makes 3 ATP. The rotation has been watched directly in single molecules.

P/O ratio. The P/O ratio is the ATP made per pair of electrons (per oxygen atom reduced). About 4 H^+ are needed per ATP: roughly 3 to turn the motor and 1 to bring Pi and ADP into the matrix (the ATP leaves by the ADP–ATP translocase). So NADH gives 10/4 = **2.5 ATP** and $FADH_2$ gives 6/4 = **1.5 ATP**. Older books use 3 and 2.

Cytosolic NADH cannot cross the inner membrane. In skeletal muscle and brain the **glycerol 3-phosphate shuttle** delivers its electrons to Q as $FADH_2$ (1.5 ATP). In liver, heart and kidney the **malate–aspartate shuttle** delivers them to mitochondrial NAD^+ (2.5 ATP).

Inhibitors and uncouplers. Rotenone and amytal block Complex I; antimycin A blocks Complex III; cyanide, azide and carbon monoxide block Complex IV; oligomycin blocks the F_o channel of ATP synthase; atractyloside blocks the ADP–ATP translocase. **Uncouplers** such as 2,4-dinitrophenol carry protons back across the membrane, so electrons flow and oxygen is used but no ATP is made; the energy is lost as heat. Brown fat does this on purpose with the protein thermogenin (UCP1) to keep newborns warm. Normally the rate of respiration is set by ADP supply, called **respiratory control**.

WORKED EXAMPLE 1: ENERGY RELEASED BY NADH OXIDATION

Question. Calculate $\Delta G^{\circ\prime}$ for the transfer of two electrons from NADH to O_2, and the efficiency of capture if 2.5 ATP are made ($\Delta G^{\circ\prime}$ of ATP synthesis = +30.5 kJ/mol).

1. $\Delta E^{\circ\prime} = E^{\circ\prime}(\text{acceptor}) - E^{\circ\prime}(\text{donor}) = 0.816 - (-0.320) = 1.136$ V.
2. $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime} = -2 \times 96.5$ kJ V^{-1} $mol^{-1} \times 1.136$ V $= -219$ kJ/mol.
3. Energy kept in ATP = 2.5 × 30.5 = 76.3 kJ/mol.
4. Efficiency = 76.3 / 219 × 100 = 35 percent under standard conditions.

Answer. $\Delta G^{\circ\prime} \approx -219$ kJ/mol; about 35 percent is captured as ATP under standard conditions (more under cell conditions, where ATP synthesis costs about 50 kJ/mol).

WORKED EXAMPLE 2: ATP YIELD FROM ONE GLUCOSE

Question. Tabulate the ATP yield from complete oxidation of one glucose to CO_2 and H_2O in (a) liver and (b) skeletal muscle.

STAGE	DIRECT	CARRIERS	ATP (MODERN)	ATP (OLDER)
Glycolysis	2 ATP	2 NADH (cytosol)	2 + 5 or 3	2 + 6 or 4
Pyruvate dehydrogenase (×2)	–	2 NADH	5	6
TCA cycle (×2)	2 GTP	6 NADH, 2 $FADH_2$	2 + 15 + 3	2 + 18 + 4
Total			**32 (liver) / 30 (muscle)**	**38 / 36**

Answer. (a) Liver, malate–aspartate shuttle: 32 ATP. (b) Muscle, glycerol 3-phosphate shuttle: 30 ATP. With older P/O values, 38 and 36.

EXAM TRAPS

- $\Delta E^{\circ\prime}$ is acceptor minus donor; reversing it gives the wrong sign of $\Delta G^{\circ\prime}$.
- Uncouplers increase oxygen use; inhibitors of the chain stop it. Oligomycin stops both ATP synthesis and, because of respiratory control, oxygen use.
- Cytochrome c and ubiquinone are mobile carriers, not complexes.
- State which P/O values you use: 2.5/1.5 gives 30–32 ATP per glucose; 3/2 gives 36–38. Mixing them is the most common error.
- F_1 is in the matrix and makes ATP; F_o is in the membrane and carries protons. The "o" is for oligomycin, not zero.

CHECK YOURSELF

– REMEMBER **Name one inhibitor each for Complexes I, III and IV and for ATP synthase.** 4 marks

Rotenone (I), antimycin A (III), cyanide or CO (IV), oligomycin (ATP synthase).

– REMEMBER **What are the two components of the proton-motive force?** 2 marks

The membrane potential (charge difference) and the pH gradient across the inner membrane.

– UNDERSTAND **Why does $FADH_2$ give fewer ATP than NADH?** 3 marks

Its electrons enter at Q through Complex II, bypassing Complex I, so only 6 H^+ are pumped instead of 10: 1.5 ATP instead of 2.5.

– UNDERSTAND **Explain the binding change mechanism in four lines.** 4 marks

Proton flow turns the c ring and the γ stalk. γ turns inside the $\alpha_3\beta_3$ head, forcing each β into O, L and T states in turn. ADP + Pi bind in L, form ATP in T, and ATP is released in O. One turn gives 3 ATP.

– APPLY **Calculate ΔG°′ for electron transfer from succinate to O_2 (2 e^-).** 3 marks

$\Delta E^{\circ\prime} = 0.816 - 0.031 = 0.785$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 0.785 = -151.5$ kJ/mol.

– APPLY **Antimycin A is added to mitochondria oxidising succinate. Which carriers become fully reduced and which oxidised?** 3 marks

Carriers before the block (Complex II, Q) become reduced; those after it (cytochrome c, Complex IV) become oxidised.

– ANALYZE **2,4-dinitrophenol was once sold as a slimming drug. Explain why it causes weight loss and why it is dangerous.** 4 marks

It carries protons across the inner membrane, dissipating the gradient. Fuel is oxidised without making ATP, so the body burns more fat to meet its ATP needs and loses the energy as heat. The heat and ATP shortage can cause fatal hyperthermia.

BEYOND THE SYLLABUS

Cryo-electron microscopy has now shown Complexes I to IV and ATP synthase at near-atomic detail, and has found them grouped into "respirasomes" in the membrane. Defects in mitochondrial DNA genes for these complexes cause mitochondrial diseases that pass down from the mother. Bacteria such as *Mycobacterium tuberculosis* depend on their own ATP synthase, which is the target of the TB drug bedaquiline. Search "respirasome cryo-EM" and "bedaquiline ATP synthase". This is not for the exam.

5.6 Gluconeogenesis

Gluconeogenesis makes new glucose from non-carbohydrate precursors: lactate, glycerol and most amino acids. The brain and red blood cells need glucose every minute, and liver glycogen lasts less than a day of fasting, so after that the blood glucose depends on this pathway. It runs mainly in the liver, with the kidney cortex contributing more in long fasting. It uses seven reversible steps of glycolysis backwards and goes around the three irreversible ones by four different enzymes.

You need: the ten steps and the three irreversible enzymes from chapter 5.3; pyruvate carboxylase from chapter 5.4.

Precursors

- **Lactate** from working muscle and red cells is oxidised to pyruvate by lactate dehydrogenase in the liver (the Cori cycle).
- **Alanine** and other **glucogenic amino acids** (from muscle protein in fasting) give pyruvate or TCA cycle intermediates, which become oxaloacetate. Amino-group removal is in Unit 6, Amino acid catabolism.
- **Glycerol** from fat breakdown is phosphorylated by glycerol kinase and oxidised to dihydroxyacetone phosphate, which enters at the triose level.
- **Propionate**, from odd-chain fatty acids and from rumen fermentation in cattle, enters as succinyl-CoA.

Even-chain fatty acids cannot give net glucose in animals. They yield only acetyl-CoA; the pyruvate dehydrogenase step is irreversible, and the two carbons of acetyl-CoA that enter the TCA cycle are lost as two CO_2, so no net oxaloacetate is made. Plants and bacteria get around this with the glyoxylate cycle (chapter 5.7).

The three bypasses

1. **Pyruvate → phosphoenolpyruvate** (bypasses pyruvate kinase), in two steps. In the mitochondrion, **pyruvate carboxylase** (biotin coenzyme, activated by acetyl-CoA) makes oxaloacetate: pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + Pi. Oxaloacetate cannot cross the inner membrane, so it is reduced to malate, which leaves and is re-oxidised to oxaloacetate in the cytosol (this also carries reducing power out as NADH). Then **phosphoenolpyruvate carboxykinase (PEPCK)** decarboxylates and phosphorylates it: oxaloacetate + GTP → PEP + CO_2 + GDP. The CO_2 added in the first step is removed in the

second; carboxylation followed by decarboxylation is how the cell pays to climb back to the high-energy PEP.

2. **Fructose 1,6-bisphosphate → fructose 6-phosphate** (bypasses PFK-1): **fructose 1,6-bisphosphatase-1 (FBPase-1)** removes the phosphate by hydrolysis, no ATP made.
3. **Glucose 6-phosphate → glucose** (bypasses hexokinase): **glucose 6-phosphatase**, on the lumen side of the endoplasmic reticulum in liver and kidney. Muscle and brain lack it, so muscle cannot release free glucose into the blood.

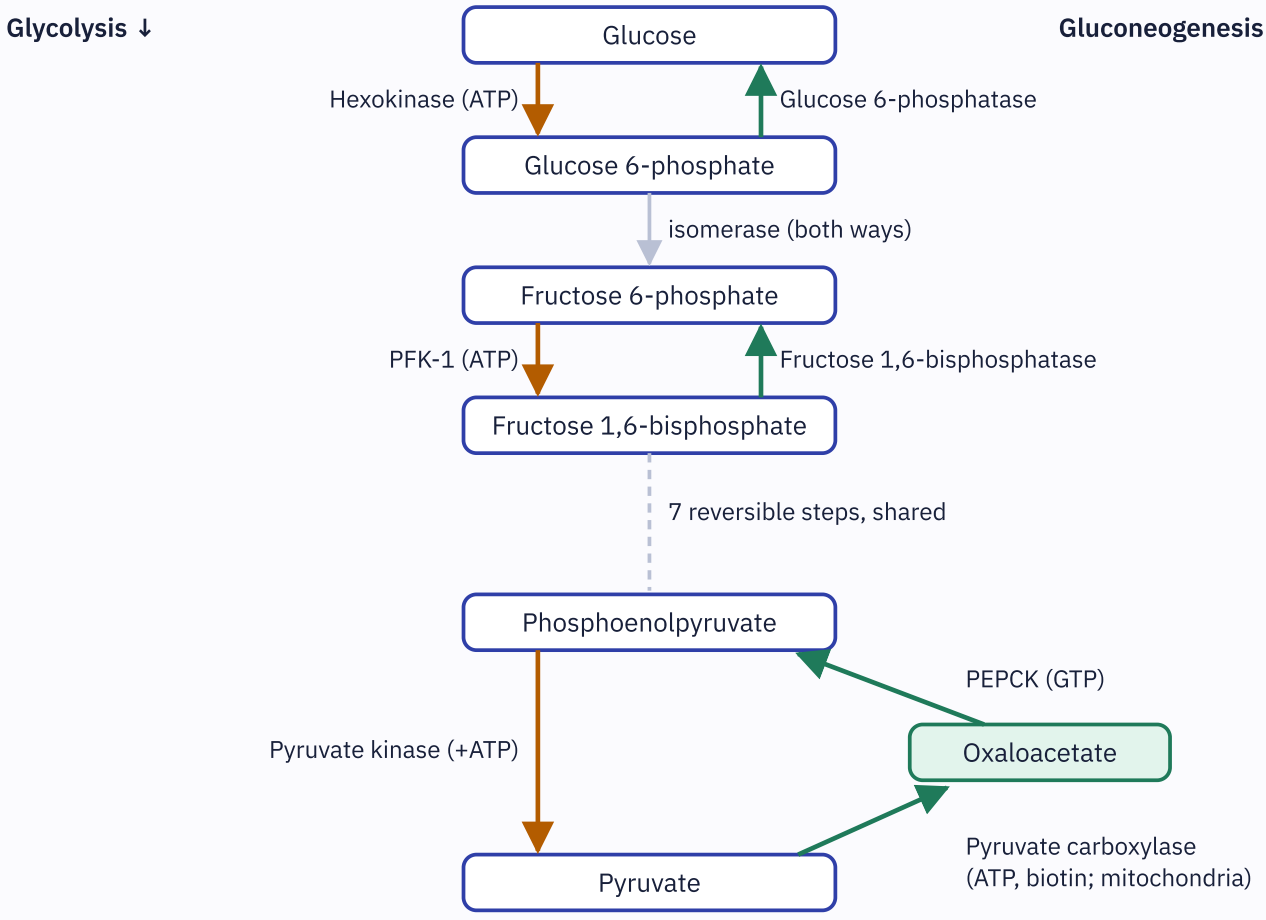


Figure 5.6.1 Glycolysis (orange, down) and gluconeogenesis (green, up) share seven reversible steps but use different enzymes at the three irreversible ones. Notice that the pyruvate kinase step needs a two-enzyme detour through oxaloacetate, and that pyruvate carboxylase works inside the mitochondrion.

Cost and regulation

$$2\ \text{Pyruvate} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ \text{H}^+ + 4\ \text{H}_2\text{O} \rightarrow \text{glucose} + 4\ \text{ADP} + 2\ \text{GDP} + 6\ \text{Pi} + 2\ \text{NAD}^+$$

Making one glucose from two pyruvate costs six high-energy phosphate bonds: 2 ATP at pyruvate carboxylase, 2 GTP at PEPCK and 2 ATP at phosphoglycerate kinase (run backwards). Glycolysis gives back only 2 ATP. The extra cost is what makes gluconeogenesis irreversible and strongly favourable in the cell.

Because glycolysis and gluconeogenesis run in the same cytosol, running both at once would be a **futile cycle** that only burns ATP. The cell prevents this by **reciprocal regulation**: the signals that switch one on switch the other off.

- **Acetyl-CoA** activates pyruvate carboxylase and inhibits pyruvate dehydrogenase, so in fasting (when fat breakdown makes acetyl-CoA) pyruvate goes to glucose, not to the TCA cycle.
- **AMP** inhibits FBPase-1 and activates PFK-1.
- **Fructose 2,6-bisphosphate** activates PFK-1 and inhibits FBPase-1. It is made and destroyed by one bifunctional enzyme, PFK-2/FBPase-2. In fasting, **glucagon** raises cAMP; protein kinase A phosphorylates the enzyme, which turns on its FBPase-2 activity, so fructose 2,6-bisphosphate falls and gluconeogenesis runs. After a meal **insulin** reverses this.
- Glucagon and cortisol also raise the amount of PEPCK by gene expression (slow, hours); insulin lowers it.

Cori cycle: lactate from muscle goes to the liver, becomes glucose, and returns to muscle. **Glucose–alanine cycle**: muscle transfers amino groups to pyruvate to make alanine, which goes to the liver and becomes glucose, while its nitrogen goes to urea (Unit 6, Urea cycle).

WORKED EXAMPLE

Question. During a 400 m sprint, muscle converts glucose to lactate, and the liver turns the lactate back to glucose. Calculate the net ATP cost of one turn of this Cori cycle per glucose, and say who pays.

1. Muscle, anaerobic glycolysis: glucose → 2 lactate gives **+2 ATP** (chapter 5.3).
2. Liver: 2 lactate → 2 pyruvate by lactate dehydrogenase, producing 2 NADH, which are exactly the 2 NADH needed at the GAPDH step run backwards. No net NADH.
3. Liver, 2 pyruvate → glucose: 2 ATP (pyruvate carboxylase) + 2 GTP (PEPCK) + 2 ATP (phosphoglycerate kinase) = **6 ATP equivalents**.
4. Net for the cycle = 2 − 6 = **−4 ATP** per glucose.

Answer. Each turn costs 4 ATP. The liver pays, using ATP from fatty acid oxidation, which shifts the metabolic burden away from the working muscle. This is part of why breathing stays fast after a sprint (the "oxygen debt").

EXAM TRAPS

- Gluconeogenesis is not glycolysis in reverse: four enzymes differ (pyruvate carboxylase, PEPCK, FBPase-1, glucose 6-phosphatase).
- Fatty acids (even-chain) cannot make net glucose in animals; glycerol from fat can.
- Muscle can make glucose 6-phosphate but not free glucose, because it lacks glucose 6-phosphatase.
- Count the cost correctly: 4 ATP + 2 GTP per glucose (6 high-energy bonds), not 2.
- Pyruvate carboxylase is mitochondrial and needs biotin; PEPCK uses GTP, not ATP, in animals.

CHECK YOURSELF

– REMEMBER **Name the four enzymes unique to gluconeogenesis.** 2 marks

Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase-1, glucose 6-phosphatase.

– REMEMBER **Which organs carry out most gluconeogenesis?** 1 mark

Liver mainly; kidney cortex, especially in prolonged fasting.

– UNDERSTAND **Why can glycerol but not palmitate be used to make glucose in humans?** 3 marks

Glycerol becomes dihydroxyacetone phosphate, a glycolytic intermediate. Palmitate gives only acetyl-CoA; PDH is irreversible and the TCA cycle loses two CO_2 per acetyl-CoA, so no net oxaloacetate forms.

– UNDERSTAND **Explain how fructose 2,6-bisphosphate prevents a futile cycle between PFK-1 and FBPase-1.** 4 marks

It activates PFK-1 and inhibits FBPase-1, so only one direction runs. Its level is high after a meal (insulin) and low in fasting (glucagon, via PKA phosphorylation of PFK-2/FBPase-2).

– APPLY **How many high-energy phosphate bonds are needed to make one glucose from two molecules of alanine (ignore urea cost)?** 2 marks

Alanine gives pyruvate by transamination, so the cost is the same as from pyruvate: 6 (4 ATP + 2 GTP).

– APPLY **Starting from glycerol, how many ATP are needed per glucose?** 3 marks

Two glycerol → two glycerol 3-phosphate (2 ATP) → two DHAP (makes 2 NADH) → one F1,6BP → glucose. Total 2 ATP; the costly pyruvate steps are skipped.

– ANALYZE **A child with deficiency of glucose 6-phosphatase becomes hypoglycaemic within hours of a meal. Explain, and predict what happens to liver glycogen.** 4 marks

Both gluconeogenesis and glycogen breakdown end in glucose 6-phosphate; without the phosphatase no free glucose leaves the liver, so blood glucose falls fast. G6P accumulates and is stored as glycogen, which builds up and enlarges the liver (von Gierke disease; Unit 6, Inborn errors of metabolism).

BEYOND THE SYLLABUS

In type 2 diabetes the liver keeps making glucose even when insulin is present, and too much gluconeogenesis is a major cause of the high fasting blood sugar. Metformin, the usual first-line drug for type 2 diabetes, lowers hepatic glucose output, though its exact molecular target is still debated. Researchers measure gluconeogenesis in people with stable-isotope tracers such as deuterated water. Search "hepatic glucose production metformin mechanism". This is not for the exam.

5.7 Pentose phosphate pathway and glyoxylate cycle

Two side roads of glucose and acetyl-CoA metabolism. The **pentose phosphate pathway** oxidises glucose 6-phosphate in the cytosol not for ATP but for two products: NADPH, the reducing power for biosynthesis and antioxidant defence, and ribose 5-phosphate for nucleotides. The **glyoxylate cycle**, found in plants, bacteria and fungi but not in vertebrates, skips the two CO_2-releasing steps of the TCA cycle so that acetyl-CoA from fat can become glucose.

You need: glycolysis intermediates from chapter 5.3; the TCA cycle from chapter 5.4; the reason animals cannot make glucose from fat in chapter 5.6.

Pentose phosphate pathway: the two phases

The pathway is also called the hexose monophosphate (HMP) shunt or phosphogluconate pathway. It is most active in tissues that make fatty acids or steroids (liver, adipose tissue, mammary gland, adrenal cortex) and in red blood cells and the lens, which need NADPH to keep glutathione reduced.

Oxidative phase (irreversible), three steps:

1. **Glucose 6-phosphate dehydrogenase (G6PD)**: glucose 6-phosphate + $NADP^+$ → 6-phosphoglucono-δ-lactone + NADPH. The rate-limiting, committed step.
2. **Lactonase**: the lactone is hydrolysed to 6-phosphogluconate.
3. **6-Phosphogluconate dehydrogenase**: 6-phosphogluconate + $NADP^+$ → ribulose 5-phosphate + CO_2 + NADPH (oxidative decarboxylation).

```
Glucose 6-P + 2 NADP⁺ + H₂O → ribulose 5-P + CO₂ + 2 NADPH + 2 H⁺
```

Non-oxidative phase (all reversible). Ribulose 5-phosphate is converted to ribose 5-phosphate by an **isomerase**, or to xylulose 5-phosphate by an **epimerase**. Then two enzymes shuffle carbons between sugars: **transketolase** (coenzyme thiamine pyrophosphate) moves a two-carbon unit, and **transaldolase** moves a three-carbon unit. The net effect turns three pentoses into two hexoses and one triose:

```
3 Pentose 5-P (15 C) ⇌ 2 fructose 6-P (12 C) + glyceraldehyde 3-P (3 C)
```

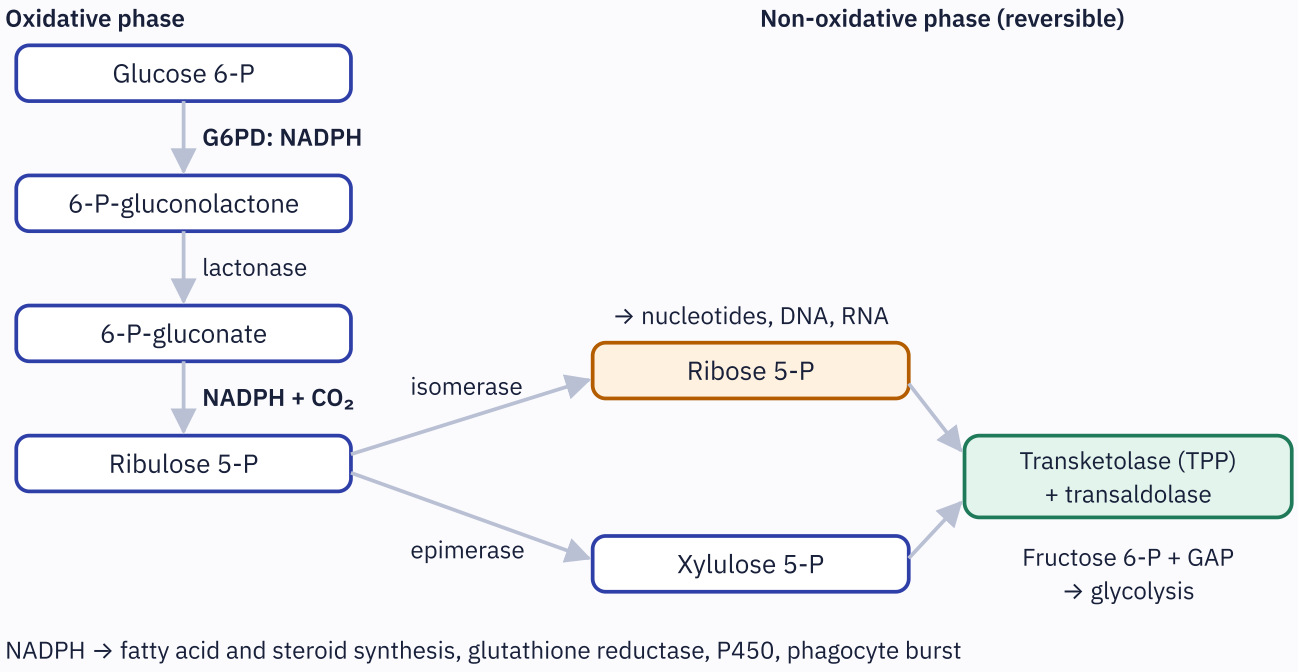


Figure 5.7.1 The pentose phosphate pathway. The oxidative phase makes 2 NADPH and 1 CO_2 per glucose 6-phosphate; the reversible non-oxidative phase either supplies ribose 5-phosphate or recycles extra pentoses back into glycolysis. Notice that no ATP is made or used anywhere.

Modes of operation and regulation

Because the non-oxidative phase is reversible, the cell can run the pathway to match its needs. If it needs **ribose more than NADPH** (dividing cells), fructose 6-phosphate and GAP from glycolysis run backwards through transketolase and transaldolase to make ribose 5-phosphate without the oxidative phase. If it needs **both equally**, the oxidative phase alone suffices. If it needs **NADPH more than ribose** (adipose tissue), the pentoses are converted back to fructose 6-phosphate and GAP, which are recycled to glucose 6-phosphate, so the glucose is completely oxidised to CO_2 in effect. The flux through G6PD is set mainly by the $NADP^+$/NADPH ratio: NADPH inhibits the enzyme, so the pathway speeds up when NADPH is used.

Clinical links. G6PD deficiency is an X-linked disorder, common in several Indian communities and in malaria regions. Red cells with low G6PD cannot make enough NADPH to keep glutathione reduced, so oxidant drugs (primaquine, some sulphonamides) or fava beans cause haemolysis. The trait gives some protection against falciparum malaria. Red-cell **transketolase activity** is used as a test of thiamine status, since thiamine pyrophosphate is its coenzyme (see Unit 4, Water-soluble vitamins).

Glyoxylate cycle

Germinating oil seeds (groundnut, castor, sunflower) have stored fat but no photosynthesis yet, so they must turn fat into sugar. They do it in **glyoxysomes**, specialised peroxisomes. The cycle

uses three TCA enzymes (citrate synthase, aconitase, malate dehydrogenase) and two of its own:

1. **Isocitrate lyase** splits isocitrate (6C) into succinate (4C) and glyoxylate (2C), instead of decarboxylating it.
2. **Malate synthase** joins glyoxylate with a second acetyl-CoA to make malate (4C).

$$2\ \text{Acetyl-CoA} + \text{NAD}^+ + 2\ \text{H}_2\text{O} \rightarrow \text{succinate} + 2\ \text{CoA} + \text{NADH} + 2\ \text{H}^+$$

Both CO_2-releasing steps of the TCA cycle are skipped, so two acetyl-CoA give one net four-carbon molecule. The succinate goes to the mitochondrion, becomes oxaloacetate through the TCA cycle, and oxaloacetate becomes glucose by PEPCK and gluconeogenesis. Vertebrates lack isocitrate lyase and malate synthase, which is the exact reason they cannot make net glucose from fatty acids.

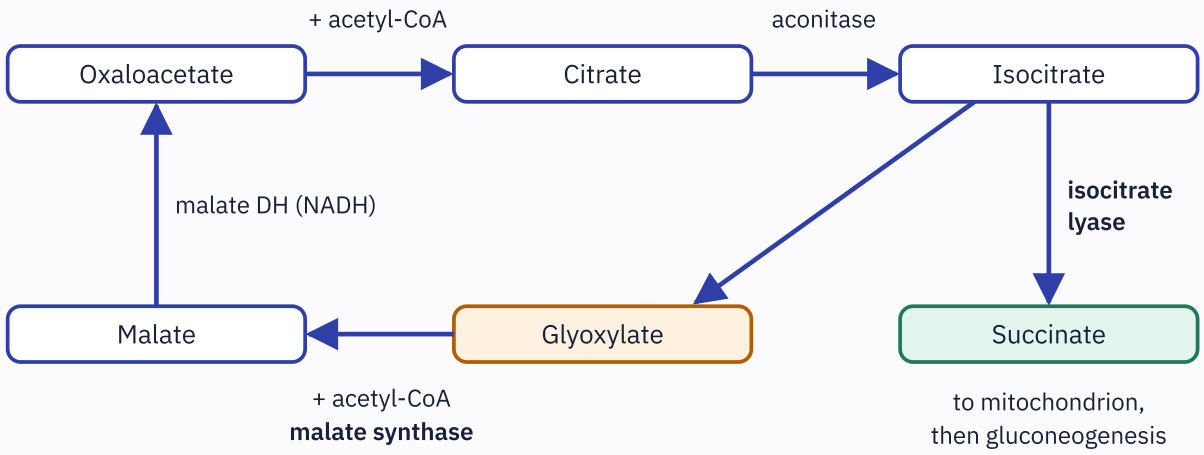


Figure 5.7.2 The glyoxylate cycle in a glyoxysome. Isocitrate lyase and malate synthase (bold) replace the two decarboxylation steps of the TCA cycle, so carbon from two acetyl-CoA leaves as succinate instead of CO_2. Notice that two acetyl-CoA enter per turn.

WORKED EXAMPLE

Question. An adipose cell needs 12 NADPH for fatty acid synthesis and no ribose. Show how the pentose phosphate pathway can supply this from glucose 6-phosphate and write the net equation.

1. Each glucose 6-phosphate through the oxidative phase gives 2 NADPH, so 6 G6P are needed: 6 G6P → 6 ribulose 5-P + 6 CO_2 + 12 NADPH.
2. Non-oxidative phase: 6 pentoses (30 C) → 4 fructose 6-P (24 C) + 2 GAP (6 C).
3. Recycling by the gluconeogenic enzymes: 4 fructose 6-P → 4 G6P; 2 GAP → 1 fructose 1,6-bisphosphate → 1 fructose 6-P (FBPase-1 releases Pi) → 1 G6P. Total 5 G6P regenerated.
4. Net: 6 G6P used, 5 regenerated, so 1 G6P is consumed.

Answer. G6P + 12 $NADP^+$ + 7 H_2O → 6 CO_2 + 12 NADPH + 12 H^+ + Pi. In effect one glucose 6-phosphate is completely oxidised to CO_2, with all its electrons going to NADPH instead of to oxygen.

EXAM TRAPS

- The pentose phosphate pathway makes NADPH, not NADH, and makes no ATP. Do not add it to an ATP count.
- Transketolase transfers 2 carbons and needs TPP; transaldolase transfers 3 carbons and needs no coenzyme.
- The oxidative phase is irreversible; the non-oxidative phase is reversible. Questions on "modes" depend on this.
- The glyoxylate cycle is absent in humans; isocitrate lyase and malate synthase are its marker enzymes.

CHECK YOURSELF

– REMEMBER **Name the enzyme of the committed step of the pentose phosphate pathway and its coenzyme.** 2 marks

Glucose 6-phosphate dehydrogenase; $NADP^+$.

– REMEMBER **Name the two enzymes unique to the glyoxylate cycle.** 2 marks

Isocitrate lyase and malate synthase.

– UNDERSTAND **Why do red blood cells depend on the pentose phosphate pathway though they make no fatty acids?** 3 marks

It is their only source of NADPH, needed by glutathione reductase to keep glutathione reduced. Reduced glutathione removes hydrogen peroxide and protects haemoglobin and the membrane from oxidation.

– UNDERSTAND **How does the glyoxylate cycle let seeds convert fat to sugar?** 4 marks

Isocitrate lyase and malate synthase bypass the two decarboxylations of the TCA cycle, so two acetyl-CoA give a net succinate instead of two CO_2. Succinate becomes oxaloacetate, then PEP and glucose by gluconeogenesis.

– APPLY **How many acetyl-CoA are needed to make one glucose through the glyoxylate cycle and gluconeogenesis?** 3 marks

Two acetyl-CoA give one succinate, then one oxaloacetate, then one PEP (one CO_2 lost at PEPCK). Glucose needs two PEP, so 4 acetyl-CoA.

– APPLY **A dividing cell needs ribose 5-phosphate but little NADPH. Which route supplies it?** 2 marks

The reversed non-oxidative phase: 2 fructose 6-P + 1 GAP from glycolysis → 3 ribose 5-P by transketolase, transaldolase, epimerase and isomerase, without the oxidative phase.

– ANALYZE **Why does a G6PD-deficient person stay healthy until exposed to primaquine or fava beans?** 4 marks

Normal oxidant load is handled even by low NADPH supply. Oxidant drugs and fava-bean compounds raise peroxide production sharply; older red cells, whose remaining G6PD is lowest, cannot regenerate enough reduced glutathione, so haemoglobin and membranes are damaged and the cells lyse.

BEYOND THE SYLLABUS

Fast-growing tumour cells push glucose through the pentose phosphate pathway to supply both nucleotides and NADPH against oxidative stress, so its enzymes are studied as cancer targets. Isocitrate lyase is essential for *Mycobacterium tuberculosis* to survive on host lipids during chronic infection, making it a long-studied TB drug target. Engineers also add glyoxylate-cycle genes to industrial microbes so they can grow on acetate. Search "isocitrate lyase tuberculosis persistence". This is not for the exam.

5.8 Biosynthesis of starch, sucrose, cellulose and peptidoglycan

Cells build polysaccharides from sugars that have first been "activated" by joining a nucleotide: ADP-glucose for starch, UDP-glucose for sucrose and cellulose, and UDP-N-acetylglucosamine for the bacterial wall. The nucleotide makes the glycosidic bond easy to form, and the release and hydrolysis of pyrophosphate drives the reaction forward. The peptidoglycan part matters for anyone heading to microbiology or antibiotic work.

You need: glucose 1-phosphate and the structures of starch, cellulose and the glycosidic bond from Unit 1, Polysaccharides and Disaccharides; ATP and pyrophosphate from chapter 5.2.

Activated sugars

Glucose 1-phosphate reacts with a nucleoside triphosphate:

```
Glucose 1-P + UTP → UDP-glucose + PPi      (UDP-glucose pyrophosphorylase)
Glucose 1-P + ATP → ADP-glucose + PPi      (ADP-glucose pyrophosphorylase)
PPi + H₂O → 2 Pi                            (inorganic pyrophosphatase)
```

The first reaction alone is near equilibrium; hydrolysis of PPi ($\Delta G^{\circ\prime} \approx -19$ kJ/mol) makes the pair irreversible. Different nucleotides label sugars for different pathways, so the cell can control each one separately. Animals use UDP-glucose to make glycogen; plants use ADP-glucose for starch.

Starch and sucrose in the plant leaf

Starch is made inside the chloroplast during the day (and in amyloplasts of storage tissues). **Starch synthase** transfers glucose from ADP-glucose to the non-reducing end of a growing chain, forming an α(1→4) bond. A **branching enzyme** moves a short piece of chain to make α(1→6) branches, giving amylopectin. ADP-glucose pyrophosphorylase is the control point: 3-phosphoglycerate (abundant when photosynthesis is fast) activates it and Pi inhibits it.

Sucrose is made in the cytosol, from triose phosphates exported from the chloroplast in exchange for Pi. They become fructose 6-phosphate and UDP-glucose, then two steps follow:

1. **Sucrose 6-phosphate synthase**: UDP-glucose + fructose 6-phosphate → sucrose 6-phosphate + UDP.
2. **Sucrose 6-phosphate phosphatase**: sucrose 6-phosphate + H_2O → sucrose + Pi.

Sucrose is the form in which the plant moves carbon through the phloem; starch is the form in which it stores carbon. The Pi released by sucrose synthesis returns to the chloroplast, and a shortage of it shifts carbon into starch.

Cellulose

Cellulose synthase sits in the plasma membrane as large rosette-shaped complexes. It takes glucose from UDP-glucose on the cytosolic side and adds it with a β(1→4) bond, pushing the growing chain out through the membrane. Many chains made side by side hydrogen-bond into a microfibril. Cortical microtubules under the membrane guide the direction the complexes move, so they set the orientation of the microfibrils in the wall.

Peptidoglycan of the bacterial wall

Peptidoglycan (murein) is a mesh of glycan chains of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), joined β(1→4), with a short peptide on each NAM that cross-links neighbouring chains. It is made in three places (Figure 5.8.1):

1. **Cytoplasm.** UDP-NAG gains a lactyl group from PEP (enzyme MurA, target of fosfomycin) and is reduced to UDP-NAM. Amino acids are added one by one, ending with the dipeptide D-Ala-D-Ala, to give UDP-NAM-pentapeptide (in *E. coli*: L-Ala, D-Glu, meso-diaminopimelic acid, D-Ala, D-Ala). D-Alanine is made by alanine racemase and joined by D-Ala-D-Ala ligase, both blocked by cycloserine.
2. **Membrane.** NAM-pentapeptide is transferred to the lipid carrier **bactoprenol** (undecaprenyl phosphate) to form lipid I; adding NAG gives lipid II, which is flipped to the outer face.
3. **Outside. Transglycosylases** join the disaccharide units into chains; **transpeptidases**, the penicillin-binding proteins, cross-link the peptides, cutting off the last D-Ala. The carrier returns as bactoprenol pyrophosphate and must be dephosphorylated; bacitracin blocks this step.

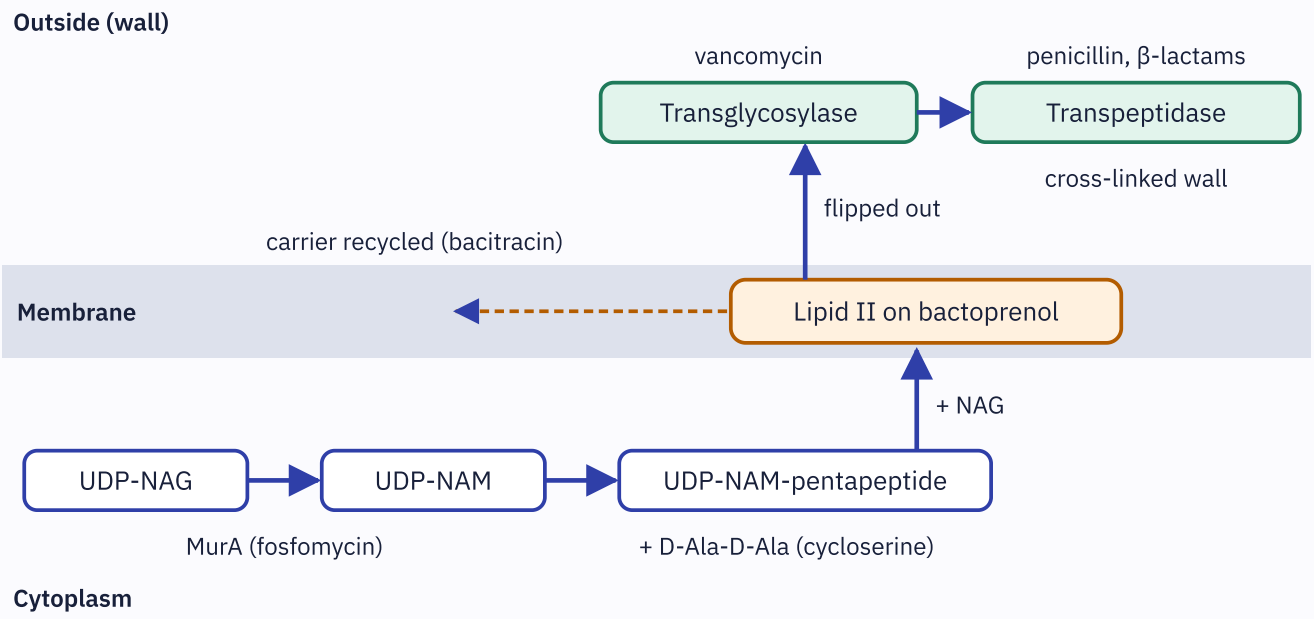


Figure 5.8.1 Peptidoglycan synthesis runs across three compartments, carried through the membrane on bactoprenol. Antibiotic names mark the step each one blocks; vancomycin acts by binding the D-Ala-D-Ala end of the substrate, not the enzyme. Notice that the final cross-linking happens outside the cell, where no ATP is available, so it is driven by cutting off the terminal D-Ala.

WORKED EXAMPLE

Question. How many high-energy phosphate bonds are spent to add one glucose from glucose 6-phosphate to a starch chain?

1. Glucose 6-P → glucose 1-P by phosphoglucomutase: no ATP.
2. Glucose 1-P + ATP → ADP-glucose + PPi: one phosphoanhydride bond of ATP is used.
3. PPi → 2 Pi: a second phosphoanhydride bond is hydrolysed.
4. Starch synthase transfers glucose and releases ADP: no further cost.

Answer. Two high-energy bonds per glucose added (one ATP becomes ADP, plus PPi hydrolysis). The same count applies to glycogen synthesis in animals with UTP.

EXAM TRAPS

- Plants make starch from ADP-glucose but sucrose and cellulose from UDP-glucose; do not mix them.
- Starch is made in the chloroplast; sucrose in the cytosol.
- Penicillin inhibits transpeptidation, the last step outside the cell, not the cytoplasmic steps; animal cells have no peptidoglycan, which is why it is selective.
- Lysozyme breaks the β(1→4) bond between NAM and NAG; it does not stop synthesis.

CHECK YOURSELF

– REMEMBER **Which activated sugar donates glucose for starch, sucrose and cellulose?** 3 marks

ADP-glucose for starch; UDP-glucose for sucrose and for cellulose.

– REMEMBER **Name the lipid carrier of peptidoglycan synthesis.** 1 mark

Bactoprenol (undecaprenyl phosphate).

– UNDERSTAND **Why does pyrophosphate hydrolysis matter in sugar activation?** 3 marks

The pyrophosphorylase reaction is near equilibrium. Removing PPi by hydrolysis (about –19 kJ/mol) pulls it forward and makes activation effectively irreversible.

– APPLY **A bacterium becomes resistant to vancomycin by making D-Ala-D-lactate ends. Explain.** 3 marks

Vancomycin binds D-Ala-D-Ala by hydrogen bonds; D-Ala-D-lactate loses one of them, so binding is much weaker while transpeptidases can still use the substrate.

– ANALYZE **Why do high 3-phosphoglycerate and low Pi in the chloroplast favour starch synthesis?** 4 marks

They signal active photosynthesis with carbon in excess of export. 3-PGA activates and Pi inhibits ADP-glucose pyrophosphorylase; low cytosolic Pi also slows triose phosphate export, so carbon stays in the chloroplast and is stored as starch.

BEYOND THE SYLLABUS

Lipid II is a target of new antibiotics such as teixobactin, found in 2015 in a soil bacterium grown with new culture methods. Cellulose synthase structures solved by cryo-EM are helping engineer biomass for second-generation ethanol, which India's biofuel policy encourages. Search "lipid II antibiotics" and "cellulose synthase structure". This is not for the exam.

Unit summary

KEY TERMS AND FORMULAS

TERM	MEANING OR FORMULA	CHAPTER
Catabolism / anabolism	Breakdown that releases energy / synthesis that uses ATP and NADPH	5.1
Arrhenius rate ratio	$k_2/k_1 = e^{(\Delta E_a/RT)}$; 5.9 kJ/mol lower barrier ≈ 10× faster at 37 °C	5.1
Standard free energy	$\Delta G^{\circ\prime} = -RT \ln K'_{eq}$	5.2
Actual free energy	$\Delta G = \Delta G^{\circ\prime} + RT \ln([\text{products}]/[\text{reactants}])$	5.2
ATP hydrolysis	$\Delta G^{\circ\prime} = -30.5$ kJ/mol; about −50 kJ/mol in cells	5.2
Glycolysis net	Glucose → 2 pyruvate + 2 ATP + 2 NADH	5.3
Irreversible glycolytic steps	Hexokinase, PFK-1, pyruvate kinase	5.3
PDH complex	Pyruvate → acetyl-CoA + CO_2 + NADH; TPP, lipoate, CoA, FAD, NAD^+	5.4
TCA cycle per acetyl-CoA	3 NADH + 1 $FADH_2$ + 1 GTP + 2 CO_2 = 10 ATP	5.4
Redox free energy	$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$, $\Delta E^{\circ\prime} = E^{\circ\prime}(\text{acceptor}) - E^{\circ\prime}(\text{donor})$	5.5
P/O ratios	NADH 2.5, $FADH_2$ 1.5 (older: 3 and 2)	5.5
ATP per glucose	30–32 (older 36–38)	5.5
Gluconeogenesis cost	4 ATP + 2 GTP + 2 NADH per glucose from 2 pyruvate	5.6
Fructose 2,6-bisphosphate	Activates PFK-1, inhibits FBPase-1	5.3, 5.6
Pentose phosphate oxidative phase	G6P + 2 $NADP^+$ → ribulose 5-P + CO_2 + 2 NADPH	5.7
Glyoxylate cycle net	2 acetyl-CoA → succinate + NADH	5.7
Activated sugars	ADP-glucose (starch), UDP-glucose (sucrose, cellulose), UDP-NAG (peptidoglycan)	5.8

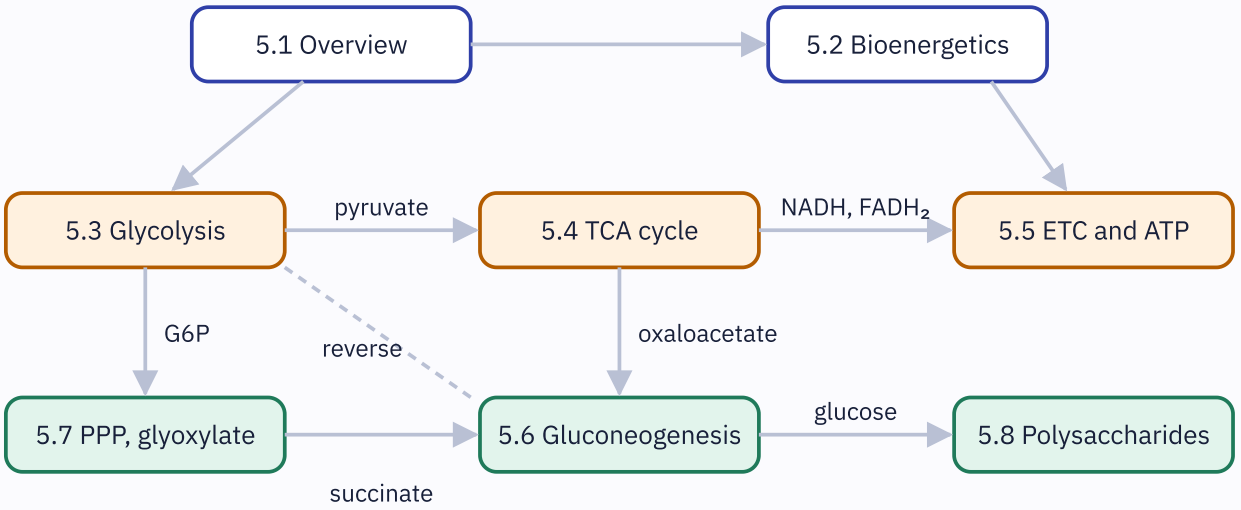


Figure 5.S.1 How the chapters connect. The orange row is the energy-capturing road from glucose to ATP; the green row builds glucose, NADPH and polysaccharides. Labels name the compound that links two chapters.

1. Catabolism funnels fuels into acetyl-CoA; most ATP is made in the last stage, oxidative phosphorylation.
2. Enzymes speed reactions by lowering activation energy; they never change ΔG or K'eq.
3. Only ΔG, not ΔG°', decides direction. Cells drive uphill steps by coupling them to ATP hydrolysis.
4. Glycolysis makes a net 2 ATP and 2 NADH per glucose and is controlled mainly at PFK-1.
5. The PDH complex and the TCA cycle oxidise pyruvate completely, yielding 12.5 ATP per pyruvate through NADH and $FADH_2$.
6. Electron flow from NADH to O_2 ($\Delta G^{\circ\prime} \approx -219$ kJ/mol) pumps 10 H^+; ATP synthase uses about 4 H^+ per ATP, giving 30–32 ATP per glucose.
7. Gluconeogenesis bypasses three irreversible glycolytic steps with four enzymes and costs 6 high-energy bonds per glucose; it is regulated reciprocally with glycolysis.
8. The pentose phosphate pathway makes NADPH and ribose, not ATP; G6PD deficiency shows why red cells need it.
9. The glyoxylate cycle lets plants and microbes, but not animals, turn fat into sugar.
10. Polysaccharides are built from nucleotide sugars; bacterial wall synthesis is the target of penicillin, vancomycin and other antibiotics.

Exam question bank

Remember and understand

- REMEMBER **Describe the reactions of glycolysis with enzymes, and state its energetics.** 16 marks

  Ten steps with substrates, products and enzymes; preparatory and payoff phases; three irreversible steps; net equation; 2 ATP net, 2 NADH; ΔG°' about −85 kJ/mol overall; fates of pyruvate (lactate, ethanol, acetyl-CoA); a neat diagram.

- UNDERSTAND **Explain the classification of high-energy compounds and why ATP hydrolysis is strongly exergonic.** 8 marks

  Groups with examples and ΔG°' values (PEP, 1,3-BPG, phosphocreatine, ATP, thioesters); phosphoryl transfer potential; ATP's intermediate place; four reasons (charge repulsion, resonance, ionisation, solvation); ATP cycle and substrate-level versus oxidative phosphorylation.

- UNDERSTAND **Describe the electron transport chain and the chemiosmotic theory of ATP synthesis.** 16 marks

  Complexes I–IV with prosthetic groups, Q and cytochrome c; E°' order; protons pumped (4, 0, 4, 2); proton-motive force; ATP synthase structure F_oF_1; binding change mechanism; P/O ratios; diagram of the membrane; inhibitors and uncouplers.

- UNDERSTAND **Write the reactions of the pentose phosphate pathway and explain its significance.** 8 marks

  Oxidative phase with G6PD and 6-phosphogluconate dehydrogenase; non-oxidative phase with isomerase, epimerase, transketolase, transaldolase; stoichiometry; modes; uses of NADPH and ribose; G6PD deficiency.

Apply

- APPLY **Prepare the balance sheet of ATP produced by complete oxidation of one glucose in liver and in skeletal muscle.** 16 marks

  Stage-wise table: glycolysis (2 ATP + 2 NADH via shuttle), PDH (2 NADH), TCA (6 NADH, 2 $FADH_2$, 2 GTP); P/O values stated; shuttles explained; totals 32 and 30 (or 38 and 36 with older values).

- APPLY **Calculate $\Delta G^{\circ\prime}$ for NADH oxidation by O_2 and by ubiquinone, and comment on where ATP is made.** 8 marks

  $\Delta E^{\circ\prime}$ 1.136 V → −219 kJ/mol; NADH to Q: 0.045 − (−0.320) = 0.365 V → −70.4 kJ/mol; large drops at Complexes I, III, IV are the proton-pumping sites; Complex II's small drop pumps no protons.

- APPLY **Calculate the ATP cost of synthesising glucose from (a) pyruvate, (b) glycerol, and (c) the net cost of the Cori cycle.** 8 marks

  (a) 6 high-energy bonds (4 ATP + 2 GTP) and 2 NADH; (b) 2 ATP; (c) 6 − 2 = 4 ATP per turn; organs involved; why liver pays.

- APPLY **Predict the effect of each on oxygen consumption and ATP synthesis in isolated mitochondria: rotenone, antimycin A, cyanide, oligomycin, 2,4-dinitrophenol, and succinate added after rotenone.** 12 marks

  Rotenone, antimycin, cyanide: both stop (with NADH substrates). Oligomycin: ATP synthesis stops, O_2 use falls through respiratory control. DNP: O_2 use rises, ATP synthesis stops. Succinate after rotenone: respiration and ATP synthesis resume (P/O 1.5) because electrons enter at Complex II.

Analyze and evaluate

- ANALYZE **Compare glycolysis and gluconeogenesis, and explain how the liver avoids futile cycling between them.** 16 marks

  Shared steps, bypass enzymes and their locations; energetics of each; reciprocal control by AMP, acetyl-CoA, fructose 2,6-bisphosphate (PFK-2/FBPase-2, glucagon, insulin, PKA); gene-level control of PEPCK; fed versus fasting state. Spans chapters 5.3 and 5.6.

– ANALYZE **"The citric acid cycle is amphibolic." Discuss, with its regulation and anaplerotic reactions.** 12 marks

Catabolic role and yield; intermediates drawn off for amino acids, haem, fatty acids and glucose; anaplerosis by pyruvate carboxylase; control at citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase by NADH, ATP, ADP and Ca^{2+}; link to PDH regulation.

– ANALYZE **Explain why humans cannot make net glucose from palmitate but a germinating groundnut seed can.** 12 marks

Fatty acids give acetyl-CoA; PDH irreversible; TCA cycle loses 2 CO_2 per turn so no net oxaloacetate; glyoxylate cycle enzymes isocitrate lyase and malate synthase in glyoxysomes bypass the decarboxylations; succinate to oxaloacetate to PEP to glucose; 4 acetyl-CoA per glucose. Spans chapters 5.4, 5.6 and 5.7.

– ANALYZE **Trace the fate of glucose 6-phosphate in a cell that needs (a) ATP, (b) NADPH only, (c) ribose only, and (d) a cell building its wall from glucose (plant or bacterium).** 16 marks

(a) Glycolysis, TCA, ETC; (b) pentose phosphate pathway with recycling, 12 NADPH per G6P fully oxidised; (c) reversed non-oxidative phase from F6P and GAP; (d) G6P → G1P → UDP-glucose → cellulose, or UDP-NAG → peptidoglycan. Spans chapters 5.3–5.5, 5.7 and 5.8.

Glossary

Activation energy — The energy barrier reactants must cross to reach the transition state; enzymes lower it.

Amphibolic — A pathway serving both catabolism and anabolism, such as the TCA cycle.

Anaplerotic reaction — A reaction that refills cycle intermediates, such as pyruvate carboxylase.

ATP synthase — The F_oF_1 enzyme of the inner mitochondrial membrane that makes ATP using proton flow.

Bactoprenol — Undecaprenyl phosphate, the lipid carrier that moves peptidoglycan units across the bacterial membrane.

Binding change mechanism — Boyer's model: each β subunit of F_1 cycles through open, loose and tight states as γ rotates.

Chemiosmotic theory	Mitchell's idea that a proton gradient couples electron transport to ATP synthesis.
Committed step	The first irreversible step unique to a pathway; usually the main control point.
Cori cycle	Lactate from muscle becomes glucose in liver and returns to muscle.
Endergonic / exergonic	Reaction with positive / negative ΔG.
Fructose 2,6-bisphosphate	Regulator that activates PFK-1 and inhibits FBPase-1.
Futile cycle	Two opposing reactions running together, wasting ATP as heat.
G6PD deficiency	X-linked lack of glucose 6-phosphate dehydrogenase causing drug- or food-triggered haemolysis.
Glucokinase	Liver hexokinase IV, low glucose affinity, not inhibited by glucose 6-phosphate.
Glyoxysome	Plant peroxisome that houses the glyoxylate cycle in germinating oil seeds.
High-energy compound	A compound with ΔG°' of hydrolysis more negative than about −25 kJ/mol.
Malate–aspartate shuttle	Carries cytosolic NADH electrons into mitochondrial NAD^+ (liver, heart).
NADPH	Reduced nicotinamide adenine dinucleotide phosphate, the electron donor of biosynthesis.
P/O ratio	ATP made per pair of electrons passed to one oxygen atom.
Phosphoryl transfer potential	−ΔG°' of hydrolysis of a phosphorylated compound.
Proton-motive force	The energy of the proton gradient: membrane potential plus pH difference.
Reduction potential (E°')	Tendency of a redox pair to accept electrons, in volts at pH 7.
Substrate-level phosphorylation	ATP (or GTP) formation by direct transfer from a high-energy compound.
Transketolase	TPP enzyme that transfers two-carbon units between sugars.
Uncoupler	A compound that lets protons leak back, so oxidation continues without ATP synthesis.

Sources and further reading

- D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 8th edition (2021): chapters on bioenergetics, glycolysis and gluconeogenesis, the citric acid cycle, oxidative phosphorylation, and carbohydrate biosynthesis in plants and bacteria (from memory; check).
- J. M. Berg, J. L. Tymoczko, G. J. Gatto and L. Stryer, *Biochemistry* (W. H. Freeman); the 5th edition is on the NCBI Bookshelf (from memory; check the edition you use).
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- U. Satyanarayana and U. Chakrapani, *Biochemistry* (Elsevier India), widely used in Indian colleges (from memory; check).
- J. L. Jain, S. Jain and N. Jain, *Fundamentals of Biochemistry* (S. Chand) (from memory; check).
- ExplorEnz, The Enzyme Database news item on the new EC 7 translocase class (August 2018): enzyme-database.org/news.php?id=5
- U.S. National Library of Medicine technical bulletin on adding Berg, Tymoczko and Stryer's *Biochemistry* (5th edition) to the Bookshelf: nlm.nih.gov technical bulletin

Standard ΔG°' and E°' values in this unit are the commonly tabulated textbook values (Lehninger tables; from memory; check against your prescribed book).

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