Biochemistry

Unit 5 · Lesson 2 · Glycolysis and the citric acid cycle

The notes for Lesson 2 of Unit 5: chapters 5.3 to 5.4 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 4 | 2 chapters | Chapters 5.3 to 5.4

About these notes

These are the notes for **Lesson 2 · Glycolysis and the citric acid cycle** of the University free n easy video lessons on Biochemistry Unit 5, Bioenergetics and Carbohydrate Metabolism. They are chapters 5.3 to 5.4 of the unit book, unchanged, so a page here is the same page in the full book.

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.

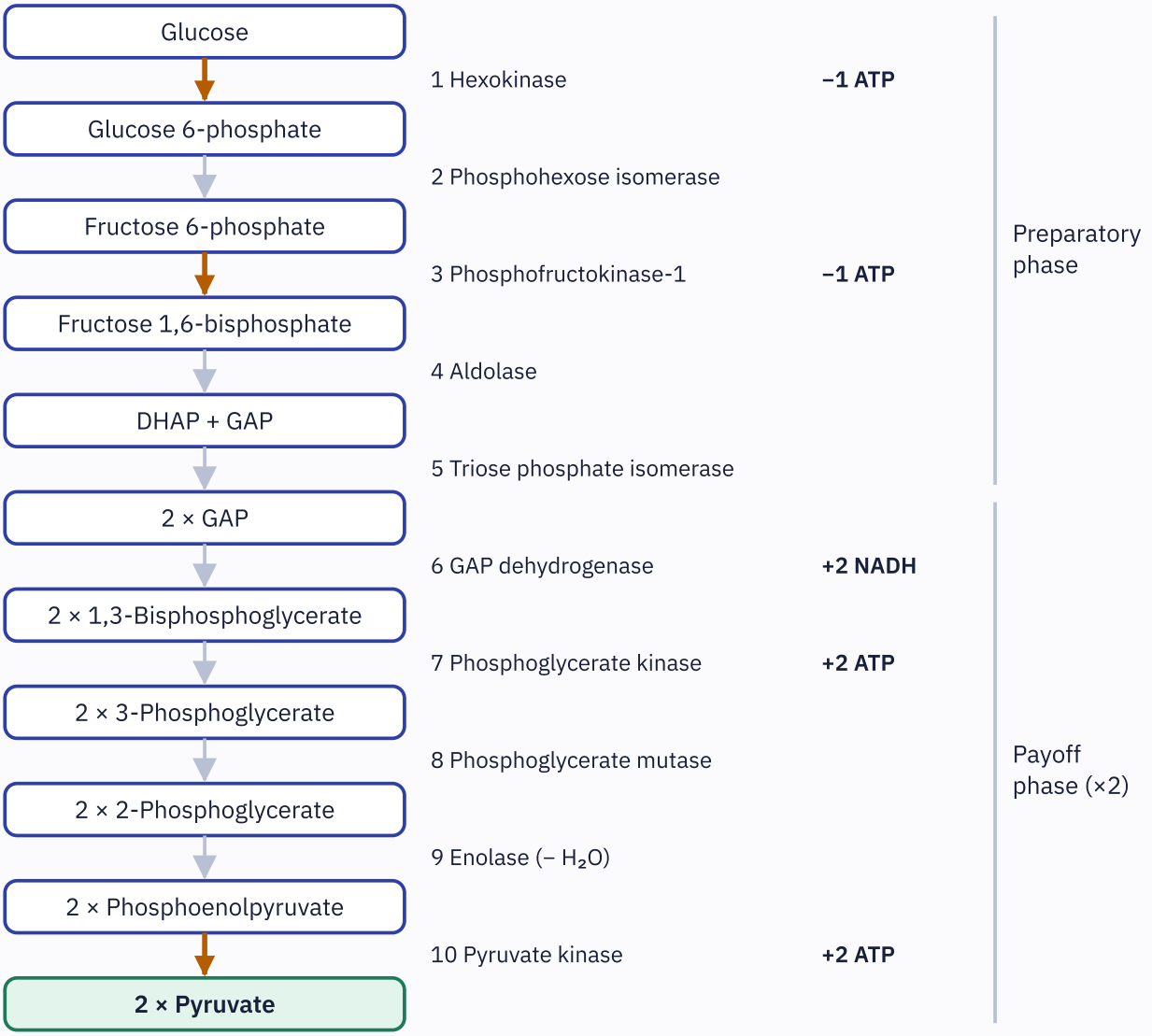


Figure 5.3.1 Glycolysis. Thick orange arrows mark the three irreversible steps (1, 3, 10), which are the control points and the steps gluconeogenesis must bypass. Notice that ATP is spent before the split and made after it, and that every step after the split happens twice per glucose.

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:

```
Pyruvate + CoA + NAD⁺ → acetyl-CoA + CO₂ + NADH + H⁺      ΔG°' ≈ −33.4 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.

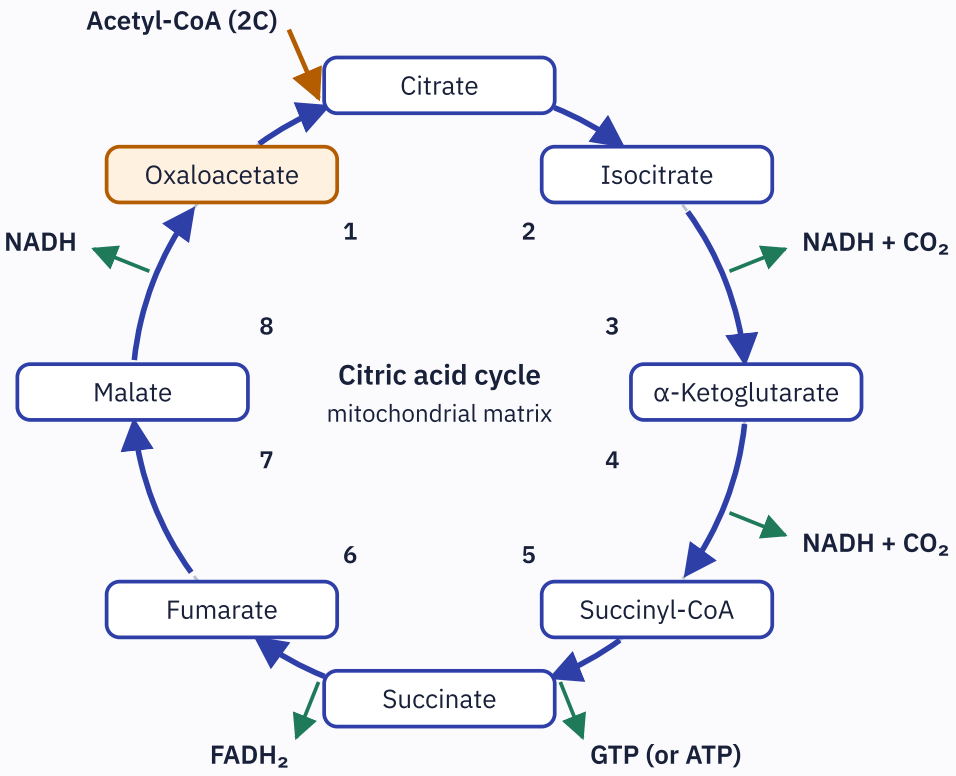


Figure 5.4.1 One turn of the citric acid cycle; numbers match the steps above. Acetyl-CoA (2 carbons) joins oxaloacetate (4 carbons), two carbons leave as CO_2 at steps 3 and 4, and oxaloacetate is regenerated. Notice the four oxidations (steps 3, 4, 6, 8) that load NADH and $FADH_2$.

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.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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
Fructose 2,6-bisphosphate	Activates PFK-1, inhibits FBPase-1	5.3, 5.6

Glossary (terms used in this lesson)

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

Committed step The first irreversible step unique to a pathway; usually the main control point.

Fructose 2,6-bisphosphate Regulator that activates PFK-1 and inhibits FBPase-1.

Glucokinase Liver hexokinase IV, low glucose affinity, not inhibited by glucose 6-phosphate.

High-energy compound A compound with $\Delta G^{\circ\prime}$ of hydrolysis more negative than about −25 kJ/mol.

P/O ratio ATP made per pair of electrons passed to one oxygen atom.

Substrate-level phosphorylation ATP (or GTP) formation by direct transfer from a high-energy compound.

The full unit book

Every chapter of Unit 5, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 5 · Bioenergetics and Carbohydrate Metabolism (one file, works

offline).

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).
- D. Voet and J. G. Voet, *Biochemistry* (Wiley) (from memory; check).
- 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).

Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

Built for the University free n easy YouTube channel from the universal syllabus of Biochemistry (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.