Biochemistry

Unit 5 · Lesson 1 · Metabolism, enzymes and ATP

The notes for Lesson 1 of Unit 5: chapters 5.1 to 5.2 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 1 of 4 | 2 chapters | Chapters 5.1 to 5.2

About these notes

These are the notes for **Lesson 1 · Metabolism, enzymes and ATP** of the University free n easy video lessons on Biochemistry Unit 5, Bioenergetics and Carbohydrate Metabolism. They are chapters 5.1 to 5.2 of the unit book, unchanged, so a page here is the same page in the full book.

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.

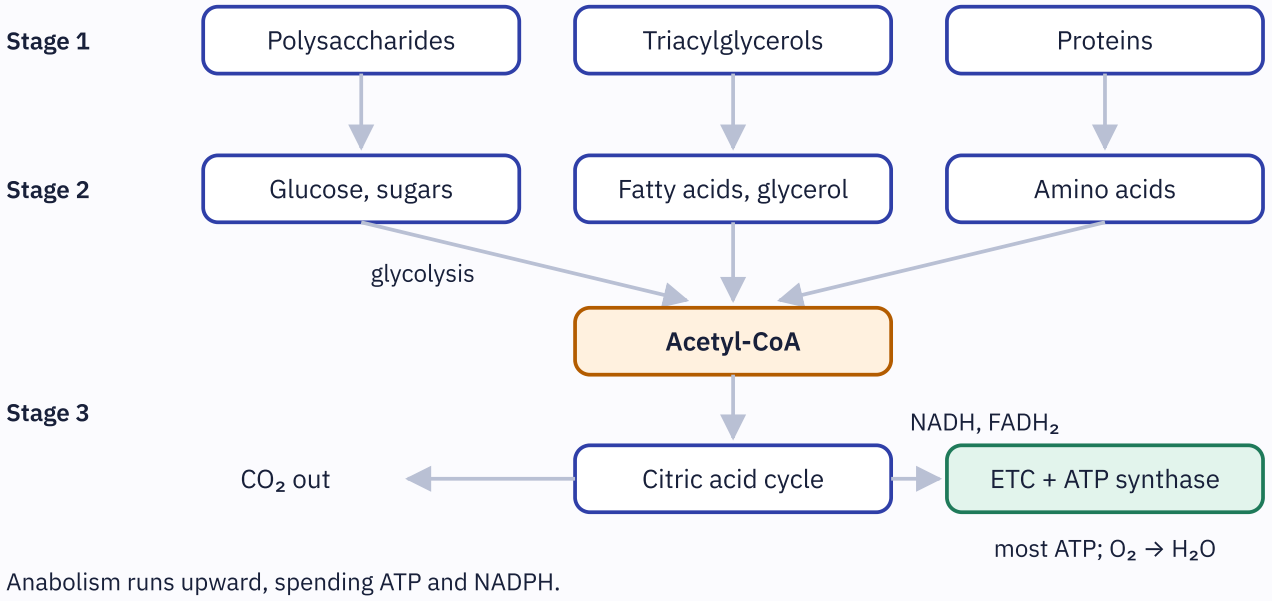


Figure 5.1.1 The three stages of catabolism. Many fuels funnel into one intermediate, acetyl-CoA, so a single citric acid cycle and one electron transport chain serve them all. Notice that most ATP is made in stage 3, not in the breakdown steps.

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: $k(enzyme)/k(none) = e^{(\Delta E_a/RT)}$, 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:

```
ΔG°' = −RT ln K'eq
ΔG   = ΔG°' + RT ln ( [products] / [reactants] )
```

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.

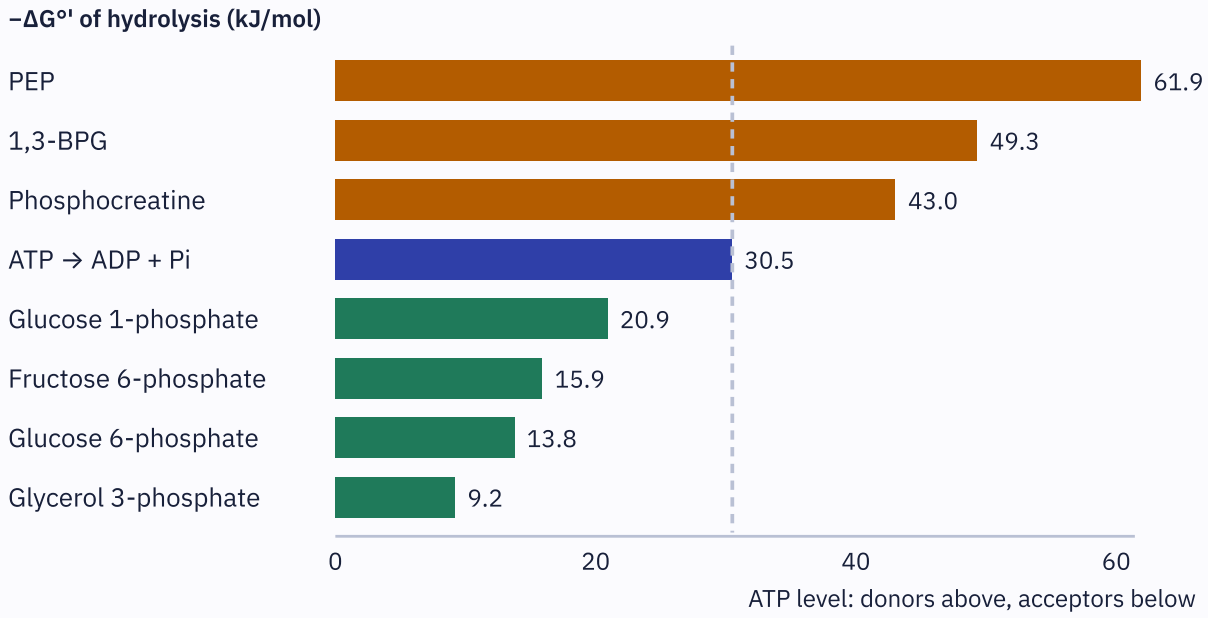


Figure 5.2.1 Phosphoryl group transfer potentials (standard values in common textbooks, e.g. Lehninger). Orange compounds can phosphorylate ADP to ATP; ATP (blue) can phosphorylate the green compounds. Notice that ATP is intermediate, which lets it act as the carrier between the two groups.

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. $\Delta G^{\circ\prime} = -30.5$ 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 $= 8.314 \times 310 = 2577$ 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. $\Delta G = -30.5 + (-22.2) = -52.7$ 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 $\Delta G^{\circ\prime}$ = +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; $\Delta G^{\circ\prime}$ = 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 ΔG°' 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 ΔG°'.** 2 marks

ΔG°' = −RT ln K'eq = −2.478 × 2.303 = −5.7 kJ/mol.

– APPLY **Find ΔG°' 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.

Key terms of this lesson

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

Glossary (terms used in this lesson)

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.

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

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

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

NADPH Reduced nicotinamide adenine dinucleotide phosphate, the electron donor of biosynthesis.

Phosphoryl transfer potential $-\Delta G^{\circ\prime}$ of hydrolysis of a phosphorylated compound.

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.