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Tutorial 4

Sugar Metabolism: Glycolysis and Gluconeogenesis

BMOL2201/6201

Tutorial 4 Aims

- Understand the basics of **metabolism**, including **enzyme clustering**, **regulation** and **energy charge**
- Describe how the body **breaks down glucose** to get useful energy through the **glycolysis** pathway, including how it's regulated
- Describe how cells are able to **store glucose** as **glycogen** and **the liver** is able to **make glucose** by **gluconeogenesis**

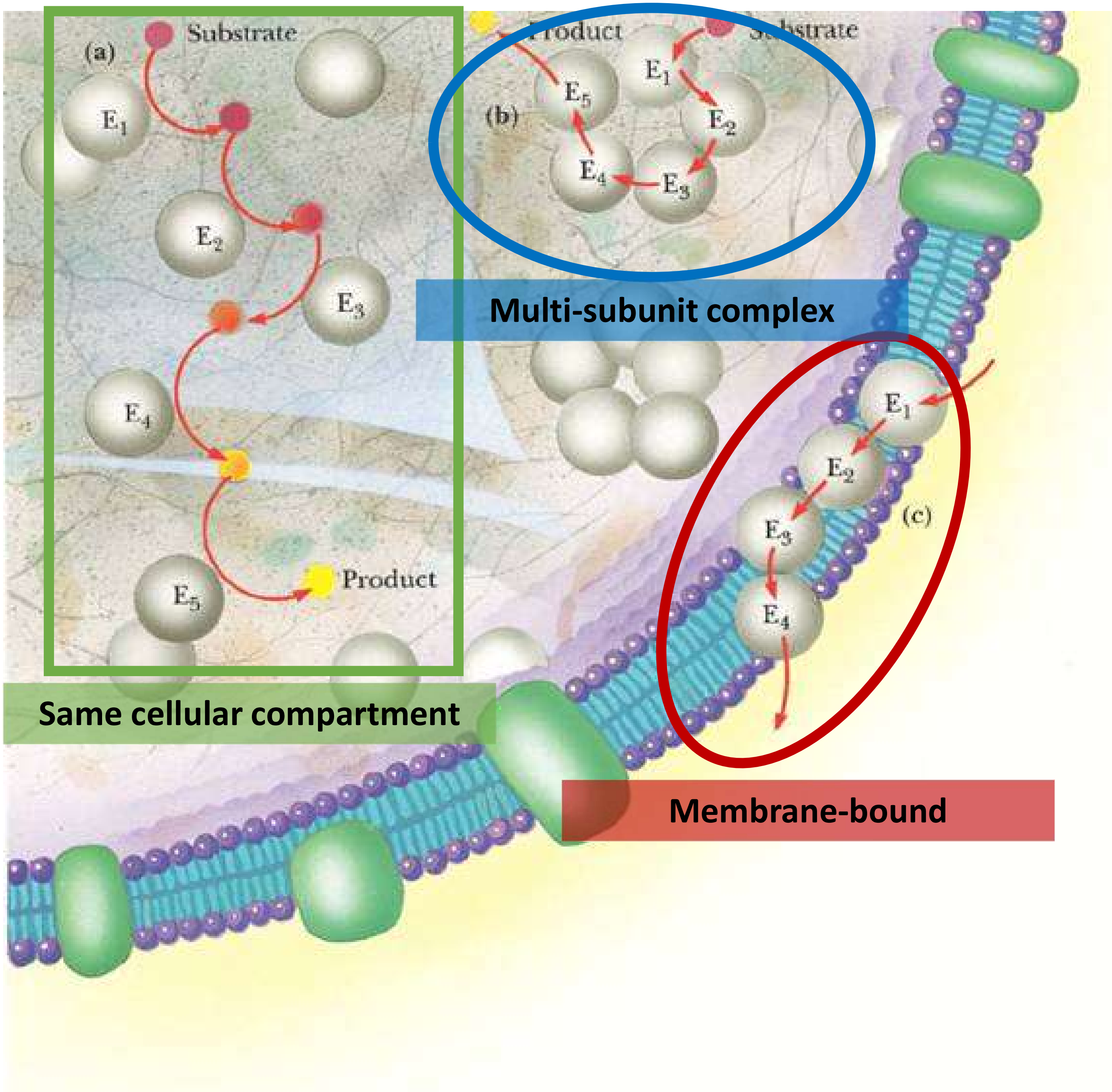
Basics of Metabolism

- Organisms create complex materials and break down unnecessary materials through metabolic processes
  - Anabolic = **MAKING** bigger molecules from smaller ones
  - Catabolic = **BREAKING** bigger molecules into smaller ones

- Anabolic and catabolic processes are interrelated – useful material doesn’t all go to waste!

Enzyme Clustering

- Enzymes in pathways are usually **clustered**, allowing intermediates to rapidly go from one enzyme to the next
- Three types of clustering:
  - **Same cellular compartment**: enzymes contained in same organelle or cytosol
  - **Multi-subunit complex**: enzymes are connected as a bigger protein complex
  - **Membrane-bound**: enzymes are bound to the same membrane (of the cell or organelle)



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Regulating Pathways

- Pathways can make or break biomolecules and many enzymes catalyse reversible reactions.
  - How do we make sure we don't get "futile cycling"?
- **Regulation** of key enzymes in a pathway occurs through 4 main methods:
  - **Allosteric control:** non-covalent interaction of small molecules with the enzyme
  - **Covalent modification:** Covalent changes to parts of key enzymes in the pathway
  - **Substrate cycles:** different enzymes catalyze the forward and reverse reactions
  - **Genetic control:** enzyme production is controlled at the transcription level
- Regulation can be either **positive** or **negative**

When do we want to regulate an enzyme?

- Regulation is only really required for **spontaneous** or **favourable** reactions
  - **ΔG negative**
- If the reaction will not proceed rapidly, then there is probably no point in regulating it
  - Analogy of a dam with varying water levels on each side
  - The flood gates are the regulatory mechanism that allows for the flow of water
- Good to regulate before “hard committing” to a pathway with a step with a big energy jump

Powering the pathways: Energy charge

- A major control over all pathways is the energy status of the cell
- ATP couples energy release with unfavourable reactions. So, if its concentration decreases, this will determine which pathways are active and which ones are not.
  - Both synthetic and breakdown pathways can require ATP
- **ATP + ADP + AMP** = **Total [ATP] attainable** in a cell
- Energy of a cell is equal to:

$$E = \frac{[ATP] + 0.5\,[ADP]}{[ATP] + [ADP] + [AMP]}$$

(energy carried by ADP is about half of ATP)

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Question 1: Basics of Metabolism

a) What are the three ways that enzymes cluster in a cell?

b) I have the following steps of a pathway, with their associated energy changes:

$$A \xrightarrow{\Delta G = -1 \text{ kJ/mol}} B \xrightarrow{\Delta G = -20 \text{ kJ/mol}} C \xrightarrow{\Delta G = -5 \text{ kJ/mol}} D$$

Which step is most likely to be regulated: A → B, B → C or C → D?

Sugars: Simple sources of energy

- 6-carbon (6C) sugars (hexoses) are most important for energy metabolism
  - Most important are D-glucose and D-fructose
- 5C sugars (pentoses) make nucleotides
- Polymers of glucose are called complex carbohydrates or polysaccharides
  - We make glycogen from glucose, plants make starch
- Sugars are an important source of energy for the body – how do we make it into a usable form?

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Glycolysis

- Glycolysis is the *catabolic* (breakdown) process by which **glucose** (6C) and other hexoses are converted to **pyruvate** (2 x 3C), producing energy in the process
  - Pyruvate can then be used by other pathways to produce more energy!
- Glycolysis occurs in almost **every** living cell
- Glycolysis **does not require oxygen**
- The glycolytic pathway was the **first** (major) metabolic pathway to be elucidated
  - Also known as the ***Embden-Meyerhof-Parnas*** pathway

The Reactions of Glycolysis

- 10 steps in glycolysis pathway
- Enzymes in this pathway catalyze **phosphorylation** reactions, **isomerizations**, carbon–carbon **bond cleavage**, and **dehydration**
- ATP is **consumed** in Steps 1 and 3 but **regenerated** in Steps 7 and 10, with a **net yield of 2 ATP** per glucose molecule.
- For each glucose, **2 NADH are produced** in Step 6.
- *Note*: metabolism requires **ATP** and reducing molecules such as **NADH** – so both these are important products from glycolysis.

First Stage of Glycolysis: Investment

- Three 6C sugar or hexose phosphates (from polysaccharides)
  - glucose-6-phosphate (G6P)
  - fructose-6-phosphate (F6P)
  - fructose-1,6-bisphosphate (FBP)
- First step **locks glucose in the cell** – free glucose can go across the membrane, but G6P can't
- Two ATPs **consumed**
- Two 3C sugar or triose phosphates generated
  - DHAP and GAP can change into each other

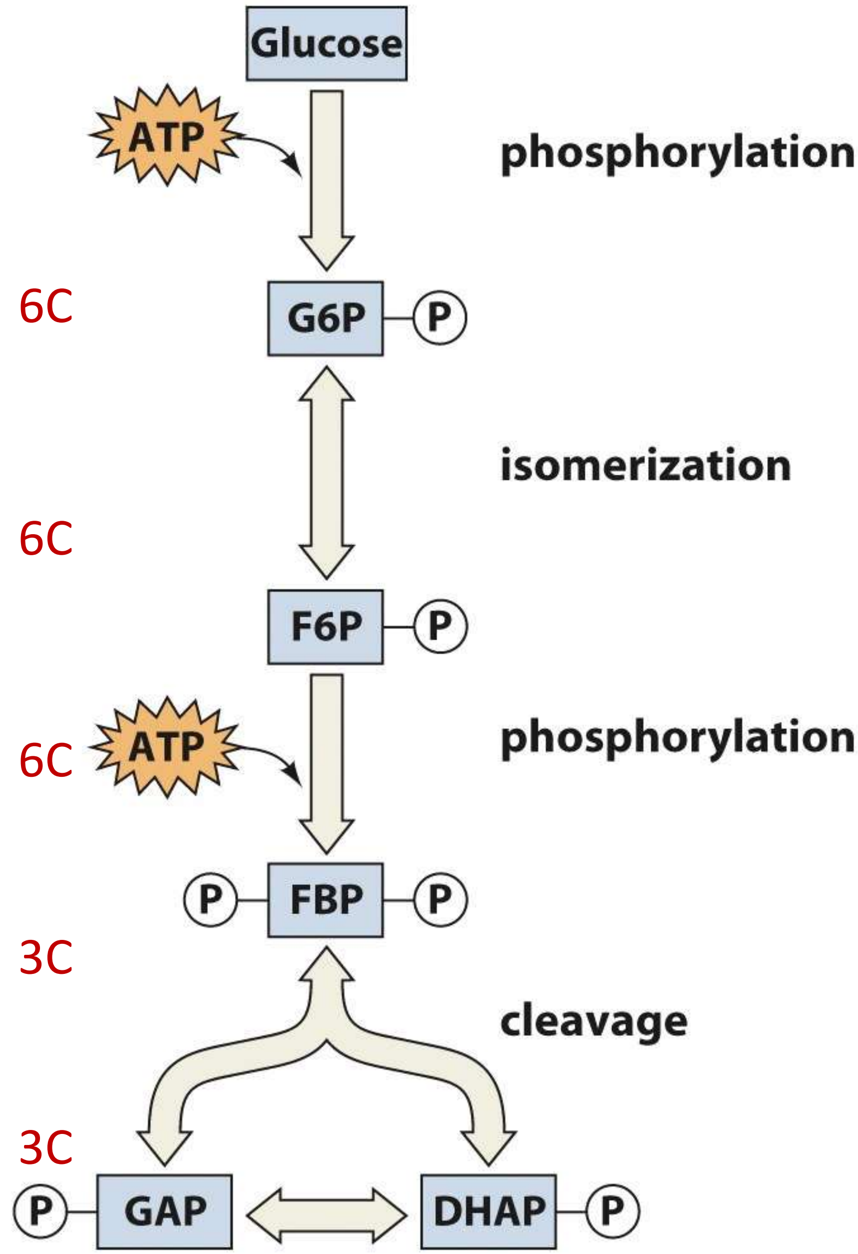

Figure 15-7

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Second Stage of Glycolysis: Recovery

From one molecule of glucose (=2 GAPs), we get:

- 2 NADH
- 2 pyruvates
- 4 ATPs

- Less 2 ATPs used up
- **Overall, 2 ATPs generated**

- NADH produced must be reconverted (by oxidation) to NAD^+ for glycolysis to continue
  - ➢ Under anaerobic conditions, this is done be fermentation (pyruvate to lactate)
  - ➢ Under aerobic conditions, this is done by the electron transport chain

NB: 2 GAPs from 1 glucose

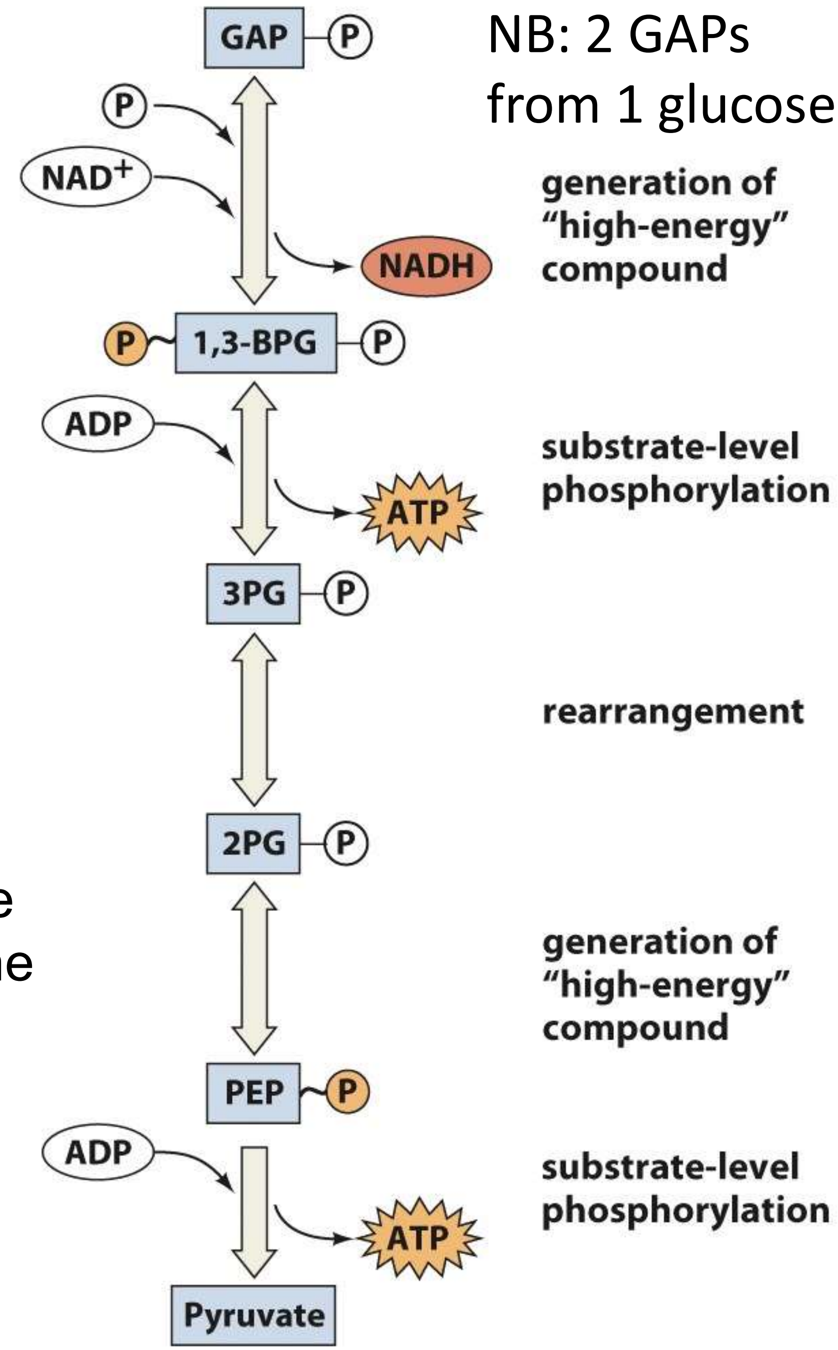

Figure 15-15

Glycolysis: Overall Gain

For each glucose molecule used:

- **Invest** 2 ATP, **get out** 4 ATP

NET ENERGY **GAIN**: 2 ATP

- Also gain:
  - **2 NADH** – reducing agent, important in other pathways
  - **2 pyruvate** – can be converted into **more** energy via the citric acid cycle

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What happens to pyruvate?

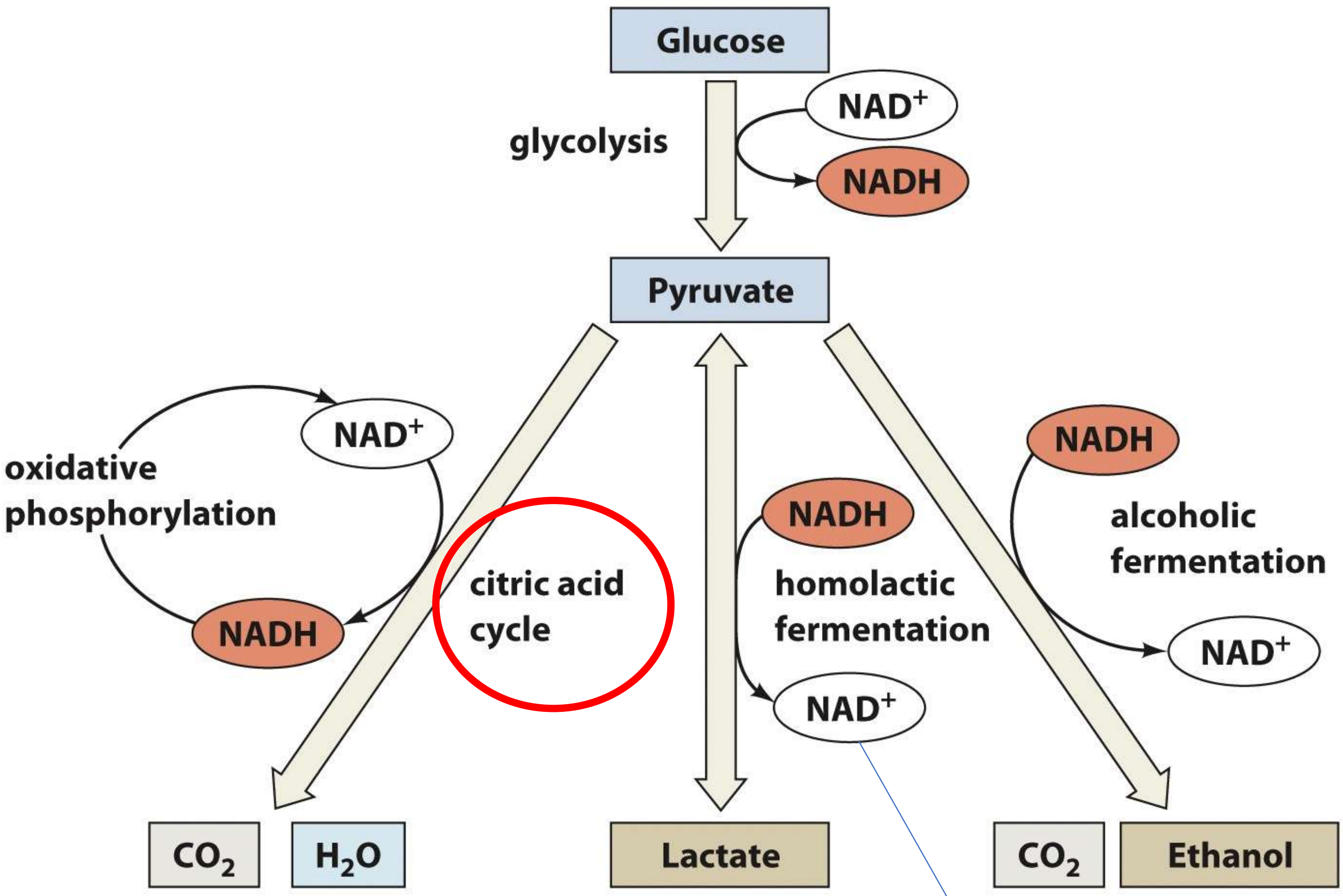

Figure 15-16

Back to glycolysis

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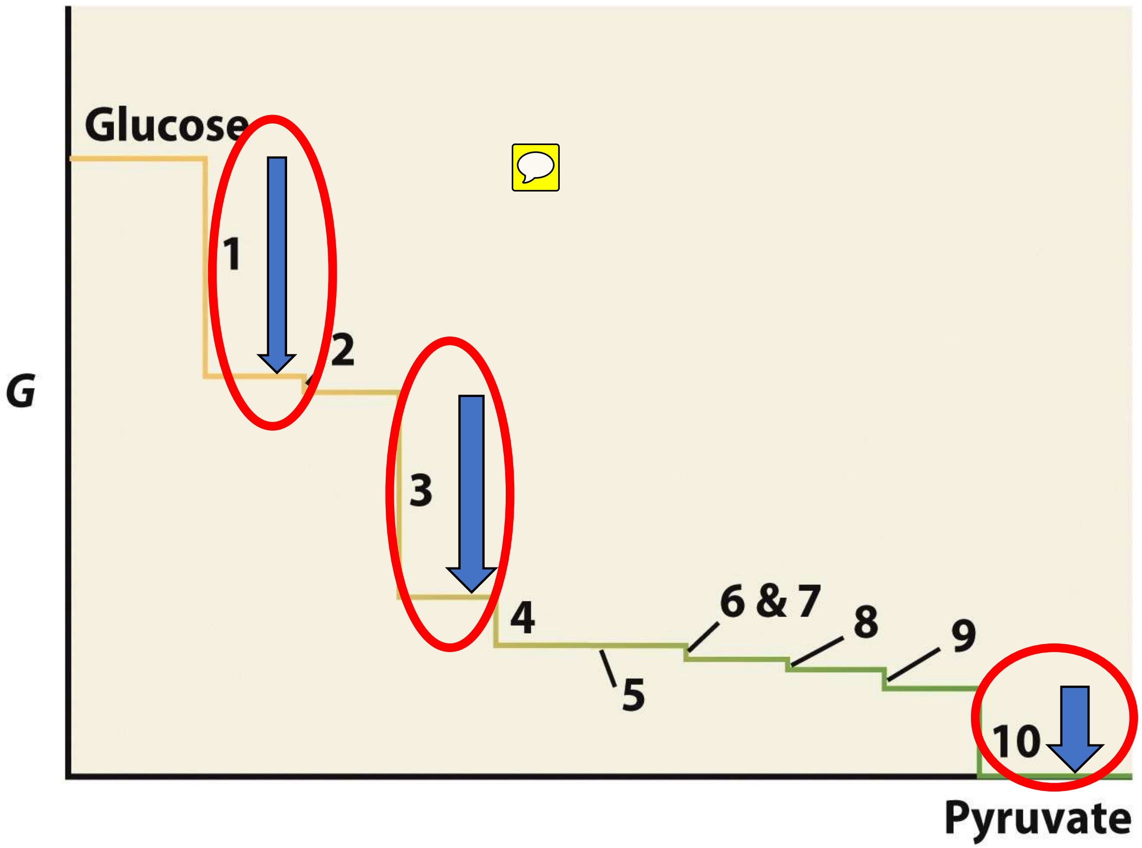
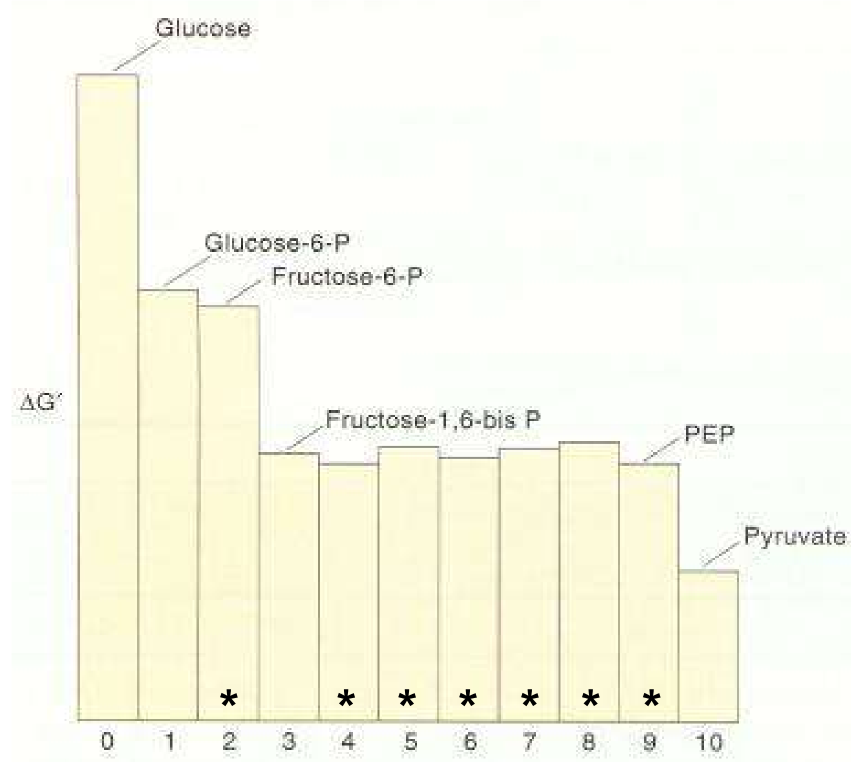
Regulating Glycolysis: Energy Changes





- Steps 2, 4-9 (* in figure) operate close to 0 free energy change in the cell and are therefore self-regulating equilibrium reactions
- Driving force for glycolysis comes from the **big "leaps":** **STEP 1**. Hexokinase; **STEP 3.** Phosphofructokinase, and **STEP 10**. Pyruvate kinase
  - Regulating these alone will control the entire pathway.

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Question 2: Glycolysis

a) Which steps of the glycolysis pathway are regulated? Why these ones?

b) If I put in one molecule of glucose into the pathway, what's produced? How much of each?

Case Study 4: Sugars!

Time to apply theory!

Monday at the Metabolic Clinic

Adventures in Glycolysis

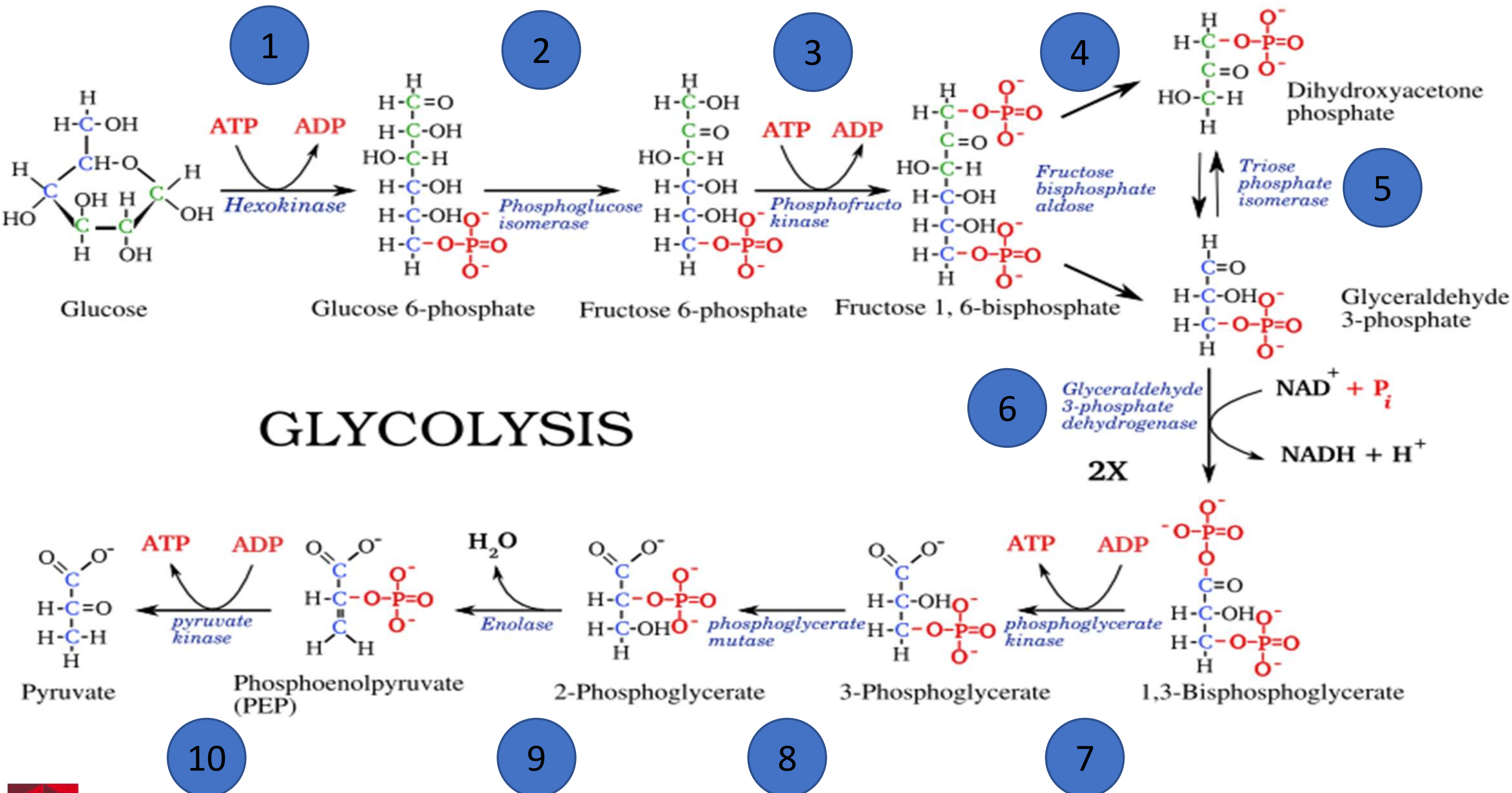
by
Nancy Boury
Iowa State University

Background

You have been chosen for a very competitive paid clinical internship position (CLIP). You spent your first weeks becoming a certified nurse assistant (CNA). You will be paid to work as a CNA, assisting the nurses on the suspected **metabolic disorder's acute-care floor**.

As part of the internship, you also will be allowed to follow Dr. Saccharo as he sees clinic patients. Dr. Saccharo is an expert in **enzyme deficiency disorders** relating to **glycolysis**. Because you hope to one day become a family physician, you are excited about the opportunity to learn about these **rare metabolic disorders**. In order to prepare you for you first day on the metabolic ward, Dr. Saccharo requests that you research **normal sugar metabolism, particularly glycolysis**.

Glycolysis Pathway:
10 steps with 10 enzymes



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Jamie, one of your fellow interns, is bothered because he knew the clinic frequently treats people with **enzyme deficiency disorders**. He asks Dr. Saccharo:

"If glycolysis is needed to use glucose, how can a person live without all the enzymes needed for glycolysis?"

For one reaction, we can have many slightly different enzymes

- **Isoenzymes** use the ***same reactants*** to produce the ***same products***
- They may have:
  - Different genes
  - Different tissue expression
  - Different developmental timing of expression
- They will likely have slightly different affinities for their substrates.

Example: Phosphofructokinase (catalyzing reaction 3)

- Three different forms and three different genes:
  - **PFK-L** is expressed in the liver and the gene for this isoenzyme is found on chromosome 21
  - **PFK-M** is expressed in the muscle and the gene is found on chromosome 12
  - **PFK-P** is expressed in platelets and the gene is found on chromosome 10
- This is the same reaction!
  - Adds a 2nd phosphate to fructose-6-phosphate (F6P) to form fructose-1,6-bisphosphate (FBP)

CS-Q1: If a person would be deficient in all 3 forms of PFK, this person would ___.

A. Have lower than normal glycogen stores.
B. Have more mitochondrial activity than normal.
C. Likely be dead.
D. Produce more energy per glucose molecule because glycolysis would be unregulated.

Time for the clinic to open…

Dr. Saccharo asks the interns, “Any questions before we get started seeing patients?”

Please keep your notes handy to use as a reference as we discuss the patients.

Patient #1: **Tom** is a teenaged golfer who was referred to the clinic after being refused at the blood drive, and *tiring easily* on the high school golf course during practice.

Levels (+ = normal, - = decreased levels, ++ = increased levels)

Blood Metabolic Panel	
Blood Glucose	+
Glucose-6-phosphate	++
Fructose-6-phosphate	++
Fructose-1,6,bisphosphate	++
Glyceraldehyde-3-phosphate	++
1,3-bisphosphoglycerate	++
Phosphoenolpyruvate	++
Pyruvate	-
ATP	-
Red Blood Cell Concentration	-

1. Which product is Tom not producing?
2. What is reaction number?
3. Which enzyme is responsible for this step?

CS-Q2: Assuming that Dr. Saccharo is correctly assuming that Tom has a defect in glycolysis, what is the most likely defect based on the blood metabolic profile?

A. Hexokinase
B. Phosphofructokinase
C. Triosephosphate Isomerase
D. Pyruvate kinase
E. Aldolase

Patient #2: Marie is a 32-year-old mother of three, complaining of **fatigue and muscle cramps with exercise**. She had always blamed her intolerance to exercise on her sedentary lifestyle and recently joined a gym. After a week of aerobics classes, she went to her physician, who ordered a series of blood tests. The **blood** work came back with **abnormal results**, leading to her muscle biopsy and referral to the metabolic clinic.

Marie’s Blood Test Results

Levels (+ = normal, - = decreased levels, ++ = increased levels)

Blood Metabolic Panel	
Blood Glucose	+
Glucose-6-phosphate	++
Fructose-6-phosphate	++
Fructose-1,6,bisphosphate	-
Glyceraldehyde-3-phosphate	-
1,3-bisphosphoglycerate	-
Pyruvate	-
ATP	-
Red Blood Cell Concentration	-

1. Which product is Marie not producing?
2. What is reaction number?
3. Which enzyme is responsible for this step?

CS-Q3: What is the most likely enzyme deficiency?

A. Pyruvate kinase
B. Hexokinase
C. Phosphofructokinase
D. Triosephosphate Isomerase
E. Aldolase

CS-Q4: If you wanted to test red blood cells for their ability to complete glycolysis, what compound would you try to detect?

A. Fructose-6-phosphate
B. Aldolase
C. Pyruvate
D. Phosphofructokinase
E. Dihydroxyacetone phosphate

Back to Tutorial 4

Gluconeogenesis: Making Glucose in Emergencies

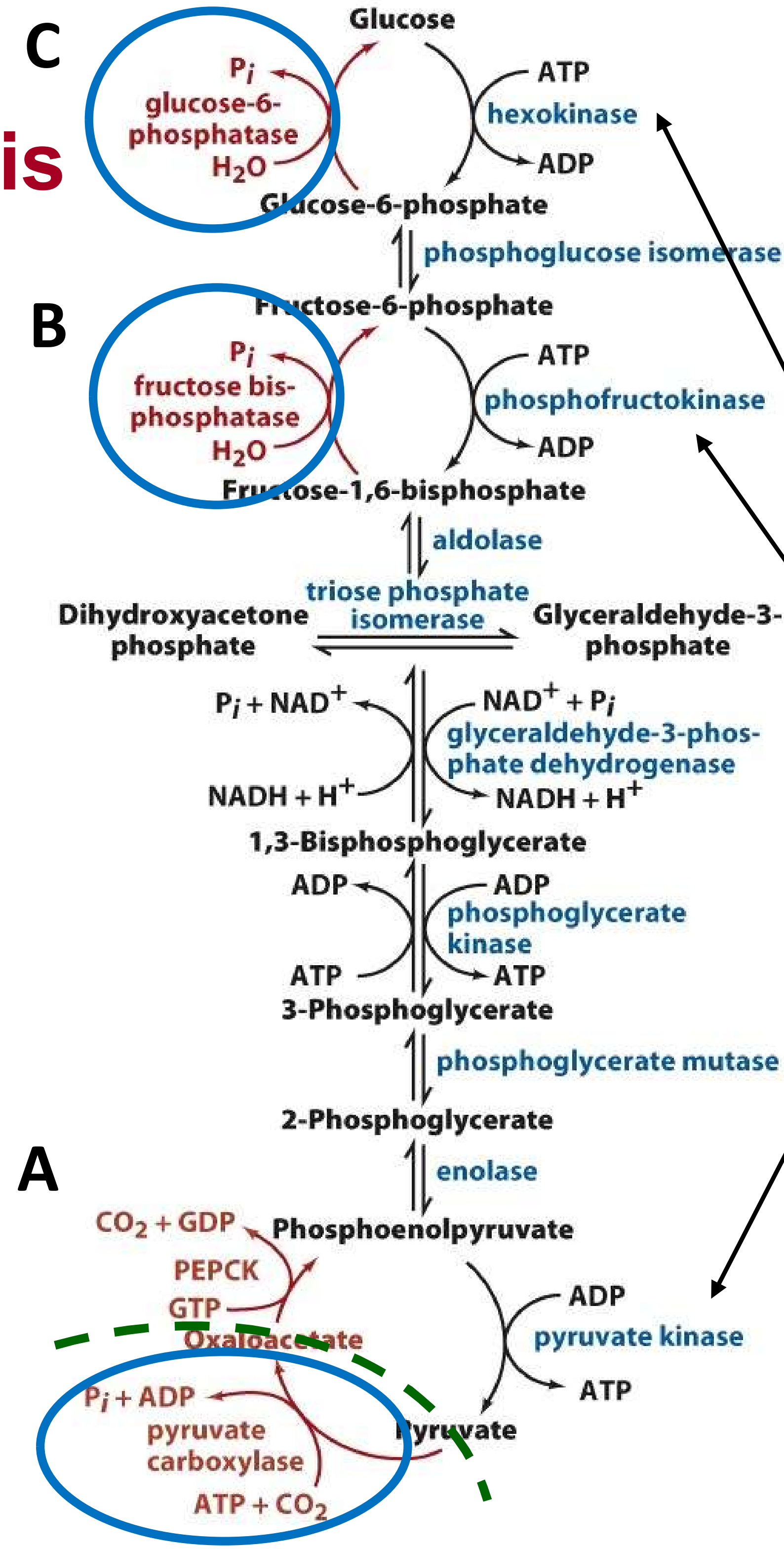
- In order to make glucose out of small molecules, we need a way to **reverse** the glycolysis pathway

- To go back, need to change only **3 enzymes** – steps 1, 3 and 10 – the steps with the big energy jumps!

- **ONLY HAPPENS IN EMERGENCIES!**
  - When the brain absolutely NEEDS glucose to survive, and there's none present

Gluconeogenesis Pathway

- Conversion of pyruvate (3C) to glucose (6C)
- Most reactions are glycolytic reactions in reverse
- Only the 3 steps with large negative ΔG need to be modified (**A, B, C**)



Glycolysis Pathway

Need new enzymes for:

- *hexokinase* (Step 1),
- *phosphofructo-kinase* (Step 3) and
- *pyruvate kinase* (Step 10)

Mitochondria (dotted line)

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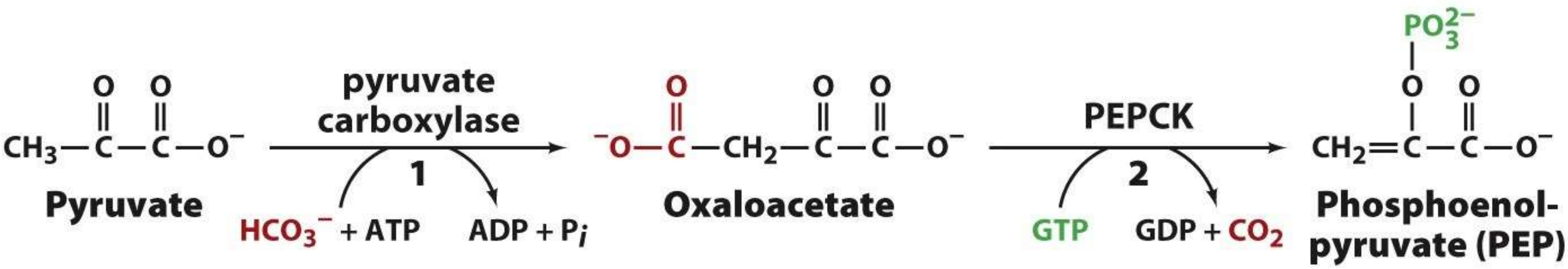
A: Pyruvate Conversion to PEP (reverse of Step 10 of Glycolysis)



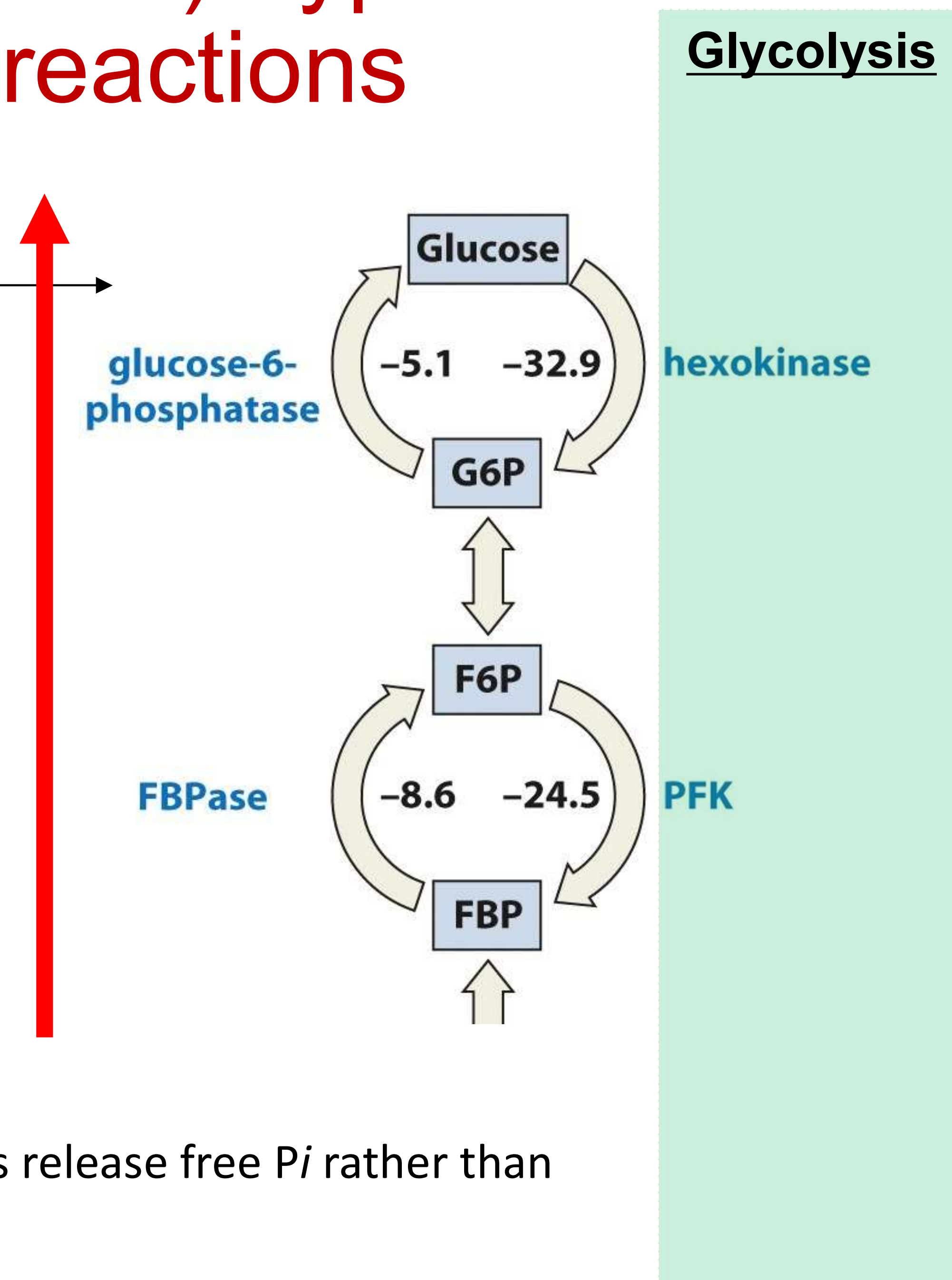
- Pyruvate carboxylase is in the mitochondria of the liver – *separate compartment!*
- Requires biotin as a prosthetic group and ATP.
- Biotin functions as a CO_2 carrier – Vitamin B_7
- Oxaloacetate (OAA) is considered "activated" pyruvate with CO_2

- PEPCK: PEP carboxy-kinase requires **GTP** – *in mitochondria as well as cytosol*
- CO_2 added in step 1 is the one that is removed.
- **GTP is regenerated from GDP by ATP (similar to UTP in glycogen metabolism)**

Hydrolytic steps (B and C) bypass irreversible glycolytic reactions

- **Glucose-6-phosphatase** (present only in liver and kidneys) hydrolyses G6P to G (Step 1 in Glycolysis)

- FPBase: **fructose-1,6-bisphos-phatase** hydrolyses FBP to F6P (Step 3 in Glycolysis)



NB: These two reactions release free P*i* rather than convert ADP to ATP.

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Energy cost of making 1 glucose molecule in gluconeogenesis

- 2 molecules of pyruvate require 2 **ATP** + 2 **GTP** (so equivalent to 4 **ATP**) in new step A
- Reversible step catalysed by phosphoglycerate kinase requires 2 **ATP** for 2 3-phopho-glycerate molecules (Step 7 in Glycolysis)
- Total: **6 ATP equiv. consumed**– expensive!
    - *Compare to 4 ATP generated in glycolysis.*

Question 3: Making and Storing Glucose

Which molecule provides the energy to convert glucose to glycogen?

a) ATP
b) CTP
c) GTP
d) UTP

Glycogen: Sugar Storage

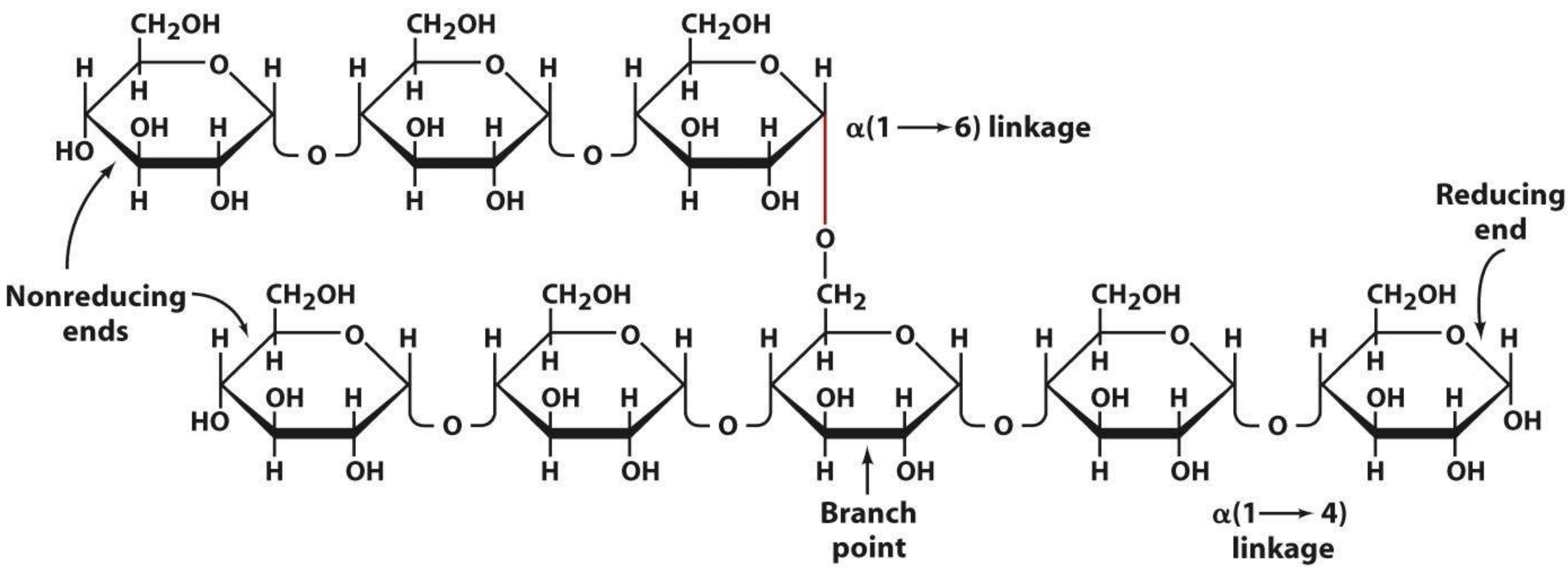
- What do we do with sugar that we don't want to use up right now? How do we store sugar for later?

- The body stores glucose as **glycogen** in the liver

- Hormones control whether glycogen is **made from** glucose or **broken down into** glucose

Structure of Glycogen

- **Glycogen** (*<u>like starch</u>*) is a **polymer of glucose** residues linked by **α linkages**



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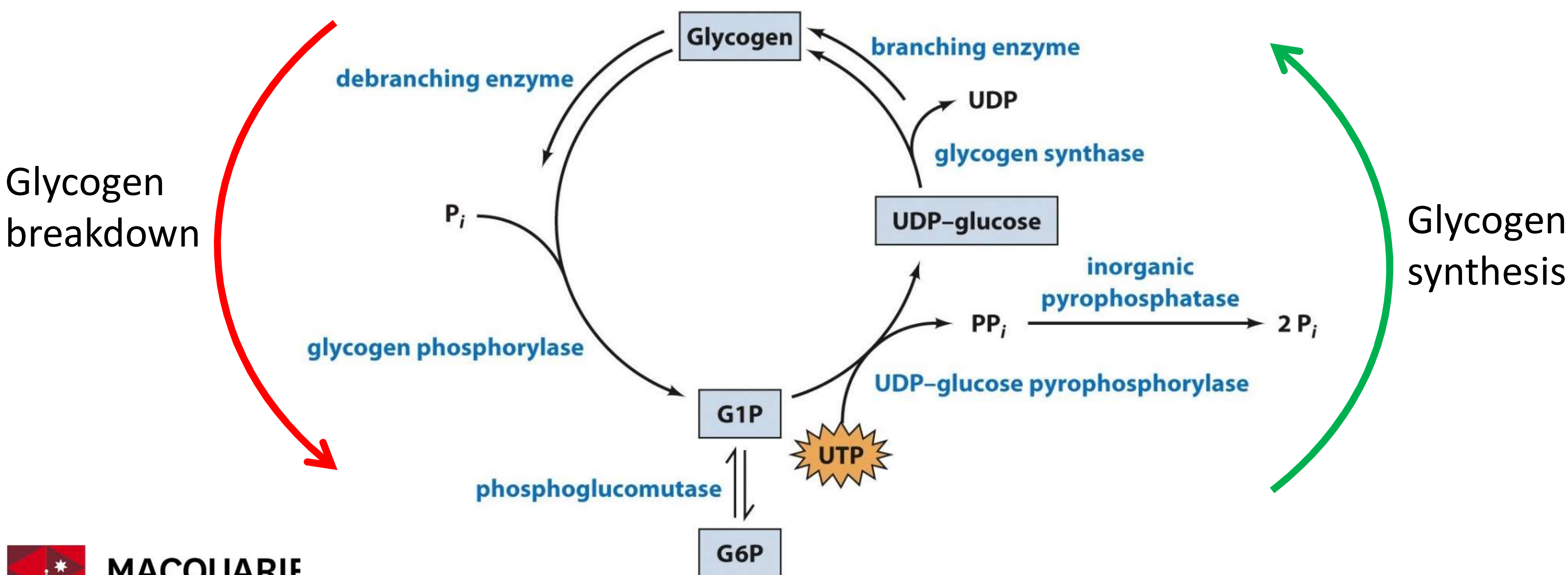
Glycogen Synthesis

- Glycogen synthesis involves a series of conversions from glucose to glucose-6-phosphate, to UDP–glucose, and finally to glycogen. **UTP** provides energy for this process
  - Like ATP, but with a uracil instead of an adenine
- Glycogen is extended by the protein glycogenin



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Overview of Glucose Metabolism

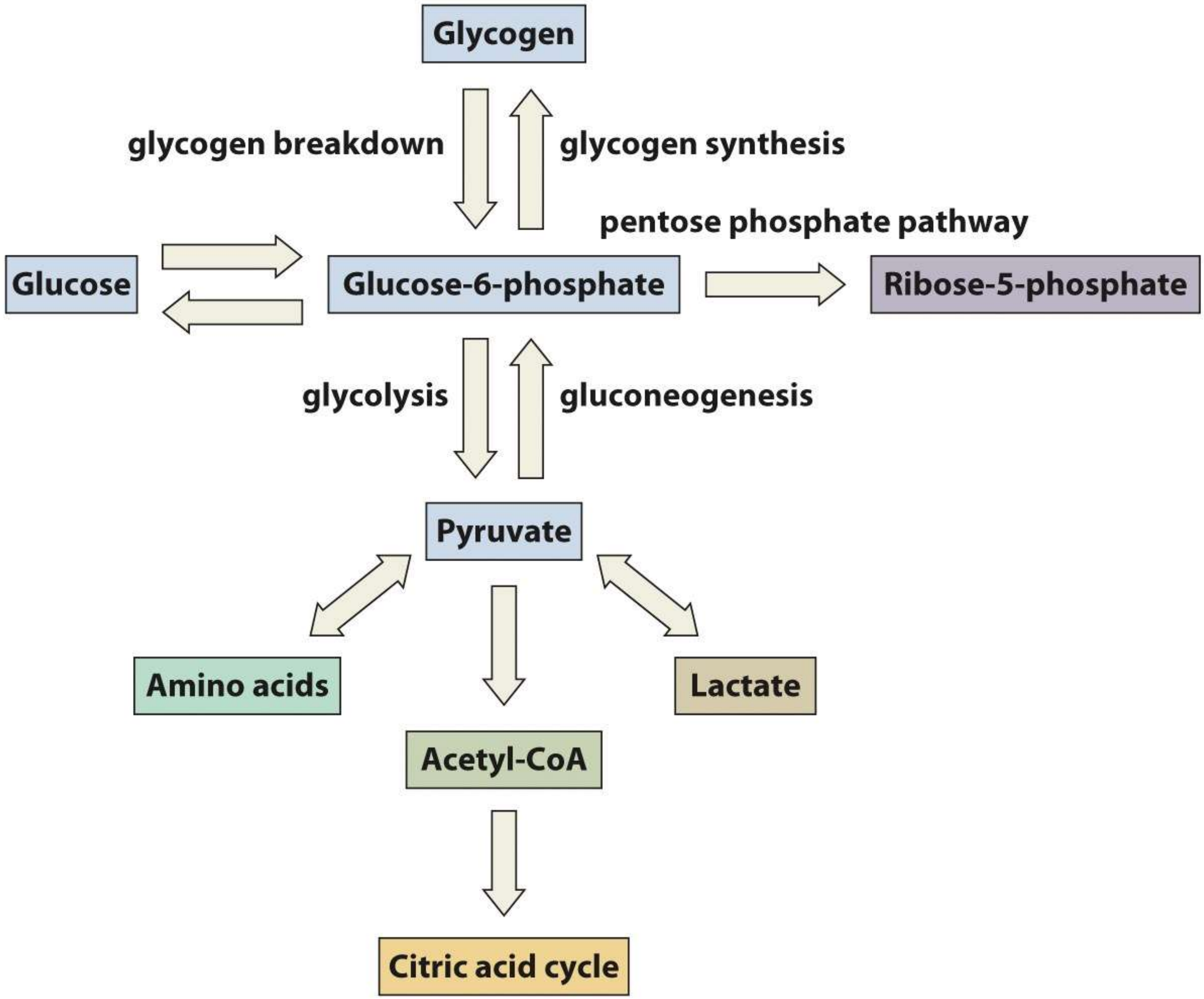


Figure 16-1

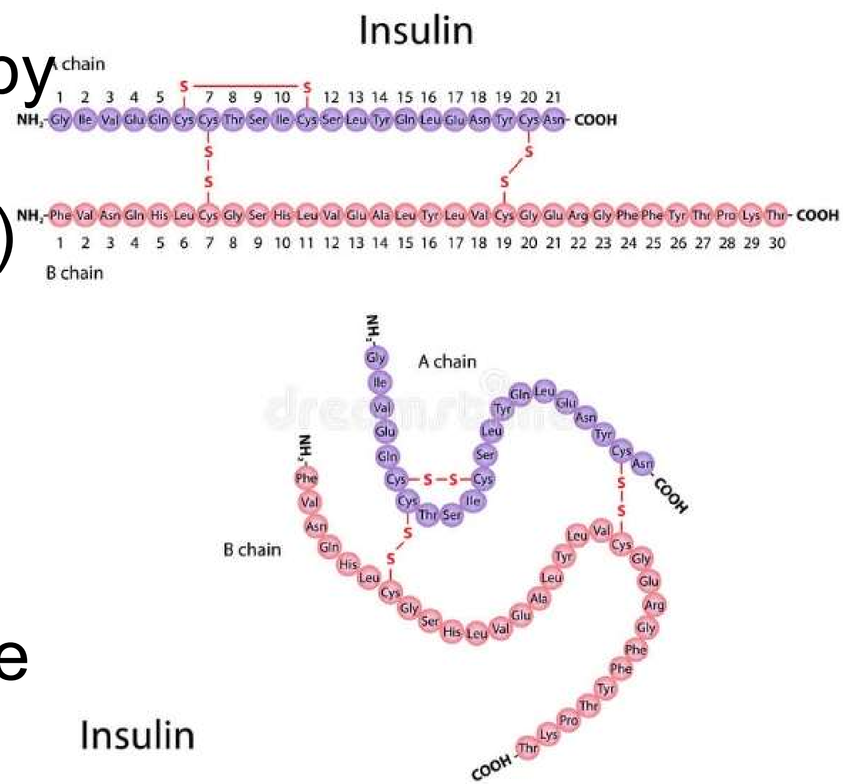
Pancreatic Islet Hormones Control Fuel Metabolism

Polypeptide hormones released by the Islet cells

1. α cells: **glucagon** (29 residues)
2. β cells: **insulin** (51 residues)
3. δ cells: **somatostatin** (14 residues)

Glucagon and insulin have opposite effects on sugar metabolism.

Somatostatin inhibits the release of both glucagon and insulin.



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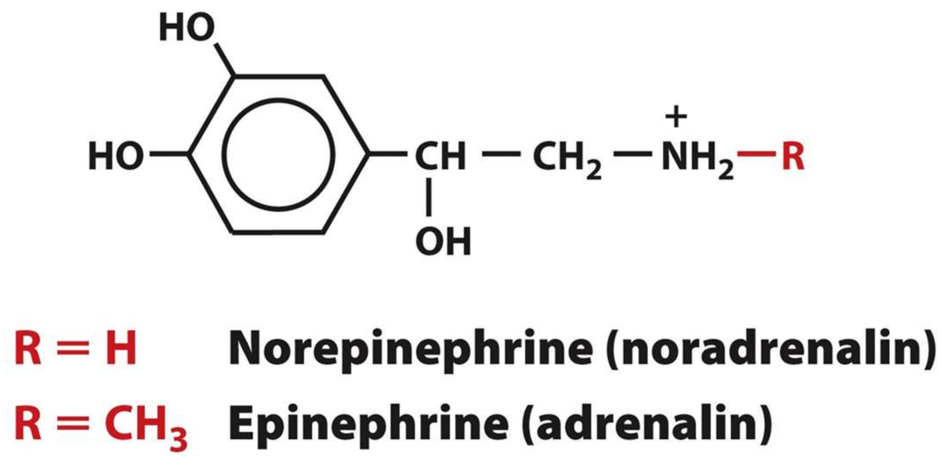
Epinephrine and Norepinephrine Prepare the Body for Action



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- The **medulla** (core) of the **adrenal glands** makes two ***catecholamine hormones***:
  1. **norepinephrine (noradrenalin)** and
  2. its methyl derivative **epinephrine (adrenalin)**
- These bind to membrane-bound α- and β-adrenergic receptors on the different tissues, with different biological responses.
- **The main function of these hormones is to overcome normal regulation for "flight-or-fight" responses.**

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Insulin, Epinephrine and Glucagon

Muscle and liver:

- **High glucose**: **insulin** ⇧ → glycogen synthesis (immediately after a meal)
- "Fight and flight" hormones: **epinephrine** is generated in response to stress and overrides cellular control: **spurt of energy**

  → cAMP ⇧ → glycogen breakdown

Liver alone:

- **Low glucose** levels (exercise or many hours after a meal): **glucagon** is released:

  → cAMP ⇧ → glycogen breakdown

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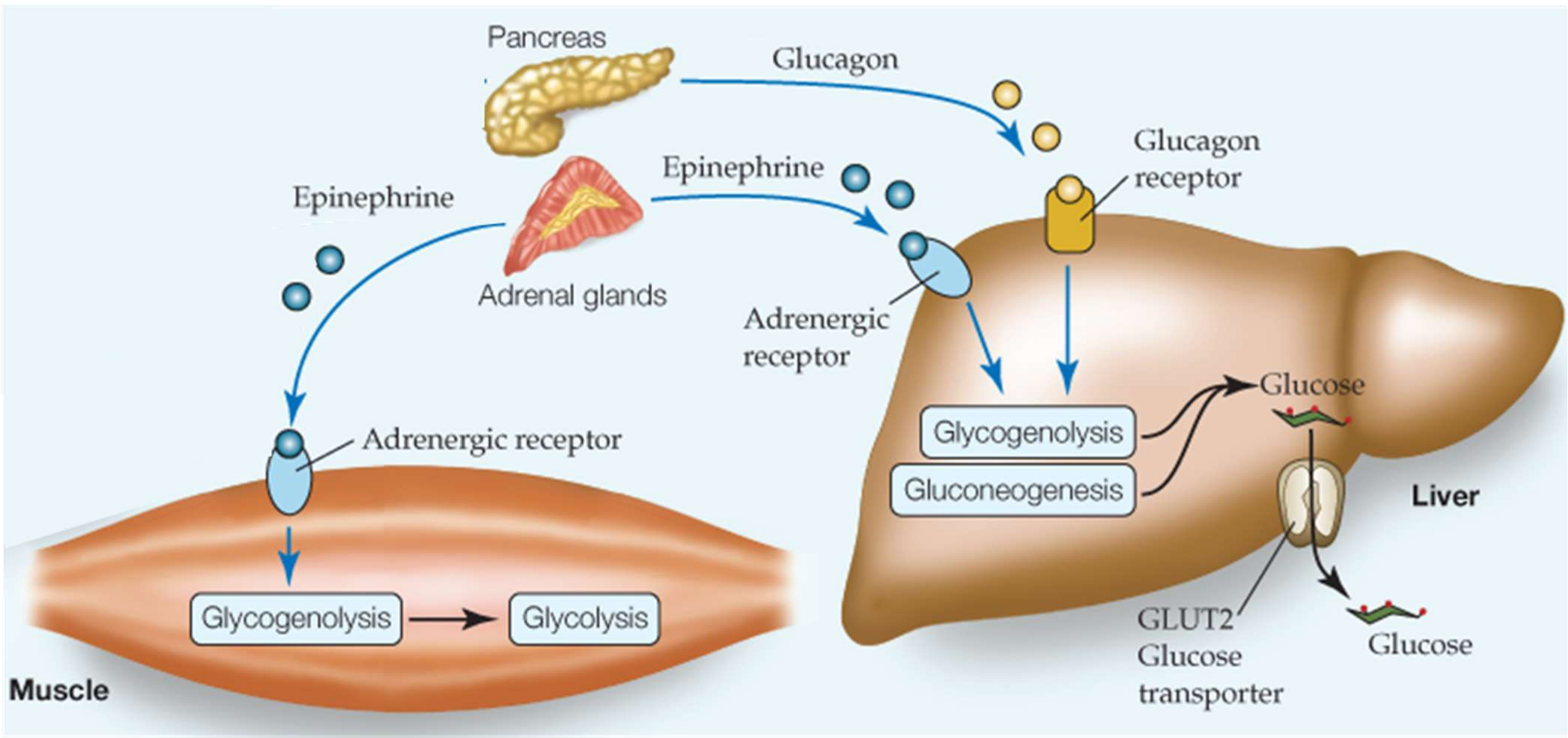
Hormones and sugar metabolism

TABLE 22-1 Hormonal Effects on Fuel Metabolism

Tissue	Insulin	Glucagon	Epinephrine
Muscle	↑ Glucose uptake ↑ Glycogen synthesis	No effect	↑ Glycogenolysis
Liver	↑ Glycogen synthesis ↑ Lipogenesis ↓ Gluconeogenesis	↓ Glycogen synthesis ↑ Glycogenolysis	↓ Glycogen synthesis ↑ Glycogenolysis ↑ Gluconeogenesis

- Glycogenolysis = breakdown of glycogen, for glycolysis
- When there is increase in glycogen breakdown, glycolysis is increased: “fight-or-flight”

Hormonal control of Glycogenolysis (and indirectly Glycolysis) and Gluconeogenesis



Question 4:
Effect of hormones

a) You have just eaten something very sweet. Which hormone will be released?

i. Glucagon
ii. Insulin
iii. Somatostatin
iv. Adrenalin

b) Which pathway will get activated?

i. Glycolysis
ii. Gluconeogenesis
iii. Glycogen synthesis
iv. Glycogen breakdown

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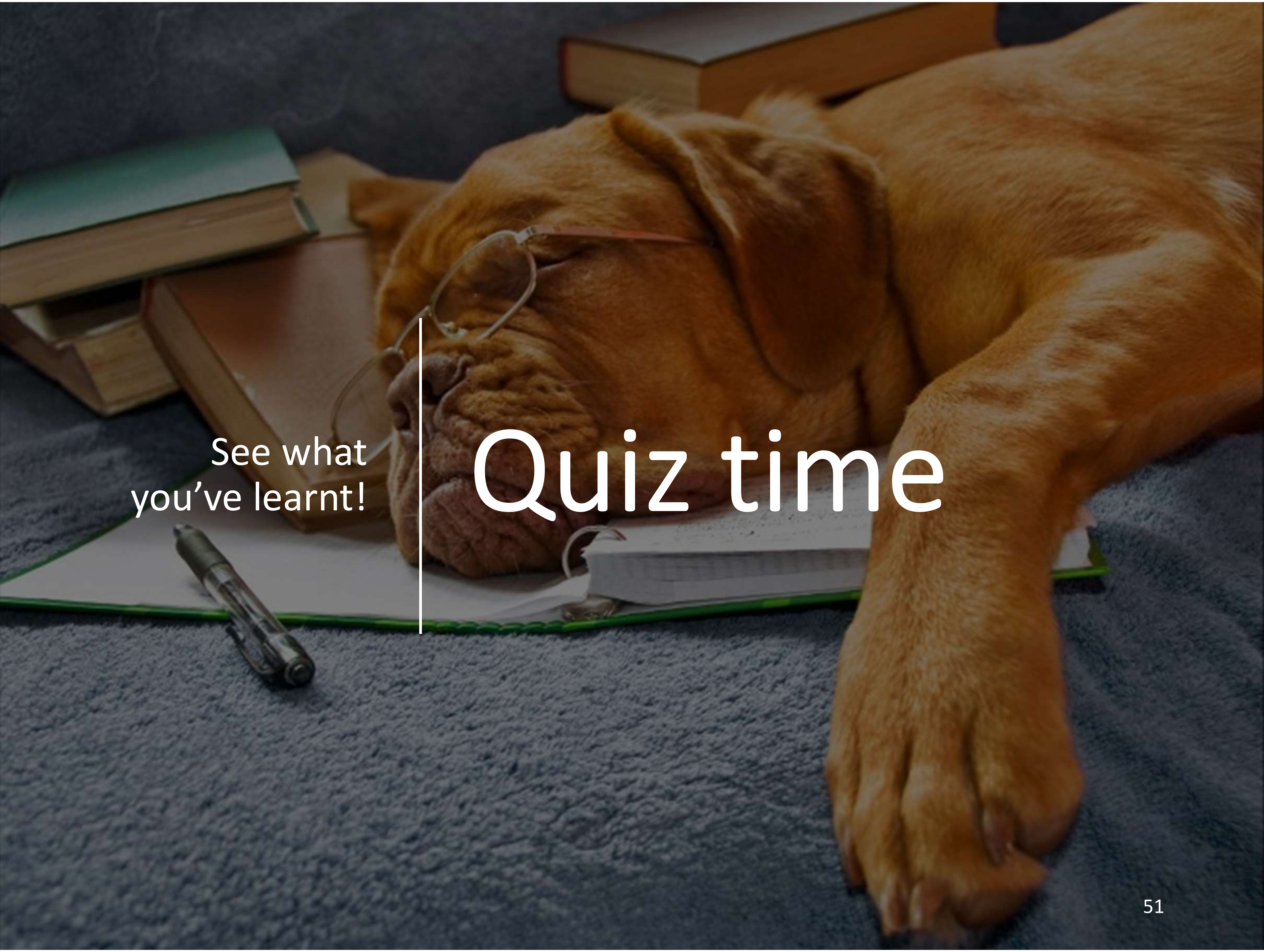

See what you’ve learnt!

Quiz time