Glycolysis & Gluconeogenesis

Introduction

- A **sequence of reactions** that **metabolizes one molecule** of **glucose** to **two molecules** of **pyruvate** with the **net concomitant** production of **two molecules** of **ATP**.
- This process is **anaerobic**, having **evolved** before the **accumulation** of $\mathbf{O_2}$ in the **atmosphere**.
- **Hans** and **Eduard Buchner** in **1897** discovered that **fermentation** was **not inextricably linked** to the **cell**. This opened up the study of **metabolism** as **chemistry**.

Glucose

- We typically **consume** large amounts of **starch** and **glycogen**. These **complex molecules** are **converted** into **simpler carbohydrates**
  - **Pancreatic** and **salivary α-amylase** cleaves the **α-1, 4 bonds** but not the **α-1, 6 bonds** of **starch** and **glycogen**. The products are **maltose** and **maltriose**. The products that **cannot** be **digested** because of the **α-1, 6 bonds** are called the **limit dextrin**.
  - **Maltase cleaves maltose** into **two glucose** molecules, and **α-glucosidase** digests **maltriose** and any other **oligosaccharides** that have **escaped** digestion with **amylase**. [These are on the **surface** of the **intestinal cells**].
  - **Sucrase** degrades **sucrose** contributed by **vegetables** to **fructose** and **glucose**.
  - **Lactase** degrades **lactose** into **glucose** and **galactose**.
  - **α-dextrinase** digests the **limit dextrin**.
- **Glucose** is an **important fuel** in most organisms. Reasons could include:
  - **Glucose** is one of several **monosaccharides** formed from **formaldehyde** under **prebiotic conditions**, and so it might have been available to **primitive biochemical systems**.

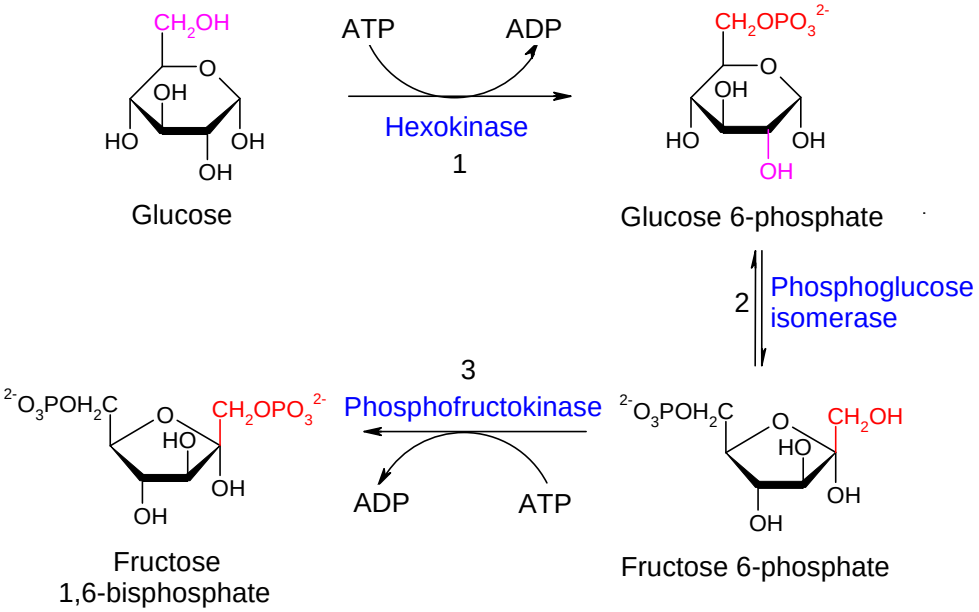
- **Glucose** tends to exist in a **ring**. This means that it does not **expose** a **carbonyl group**, susceptible to **nonselectively glycosylate** and thereby **deactivate proteins** (via the amino group) to form **Schfiff bases**.

Glycolysis

- In **eukaryotic cells**, glycolysis takes place in the **cytoplasm**.
- It comprises **three stages**.

Stage I

- This consists of a **phosphorylation**, an **isomerisation** and a further **phosphorylation**.
- The strategy of this step is to **trap glucose into the cell** and **form** a **compound** that is **readily cleaved** into **phosphorylated three-carbon units**.



A couple of notes on each of the reactions:

- **Reaction 1**

  The **phosphorylation** of **glucose** is **notable** for two reasons:

  - **Glucose 6-phosphate** can no longer **pass through the membrane**, because it is not a **substrate** for the **glucose transporters**.

- The **phosphoryl** group **destabilises** glucose, **facilitating** its **further metabolism**.

The process is **catalysed** by **hexokinase**, which, like other kinases, requires the presence of **Mg^{2+}** or another **divalent metal ion**, which forms a **complex** with **ATP**.

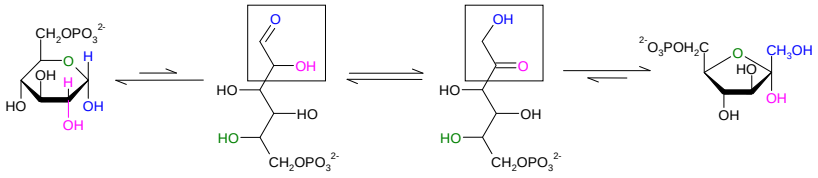
The enzyme contains **two lobes** which **closes** on glucose when it binds, leaving exposed only the **hydroxyl group** about to be **phosphorylated**. This has two consequences:

- It makes the **environment** around the glucose **more nonpolar** – this **favours** the donation of the **terminal phosphoryl group** of **ATP**.
- It enables the kinase to **discrimate against H_2O** as a substrate. **Closing** the **clef** keeps **H_2O away** from the **active site**. An **H_2O** in the **active site** of a **kinase** would **attack** the **γ-phosphoryl** group of **ATP**, forming **ADP + P_i**.

This is a **general feature** of **kinases**.

- **<u>Reaction 2</u>**

The enzyme must first **open** the **six-membered ring** of **glucose**, **catalyze** its **isomerisation**, and then **promote the formation** of the **five-membered ring** of **fructose 6-phosphate**.



Note that this is the **conversion** of an **aldose** (glucose has an **aldehyde** group at **carbon 1) into** a **ketose** (fructose has a **ketone** group at **carbon 2**).

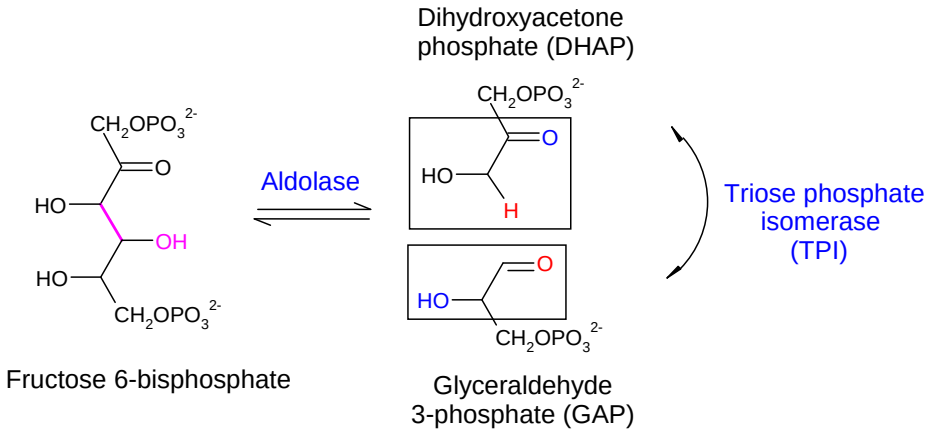
- **Reaction 3**

  The prefix **"bis"** indicates that two **separate monophosphoryl groups** are present. The prefix **"di"** indicates that two phosphoryl groups are present, but **linked** by an **anhydride bond**.

  Note that **phosphofructokinase** (**PFK**) is an **allosteric enzyme** and it **sets the pace** of **glycolysis**, as we shall discuss later.

Stage II

This consists of the **cleavage** of **fructose 1,6-bisphosphate** into **two three-carbon fragments**.

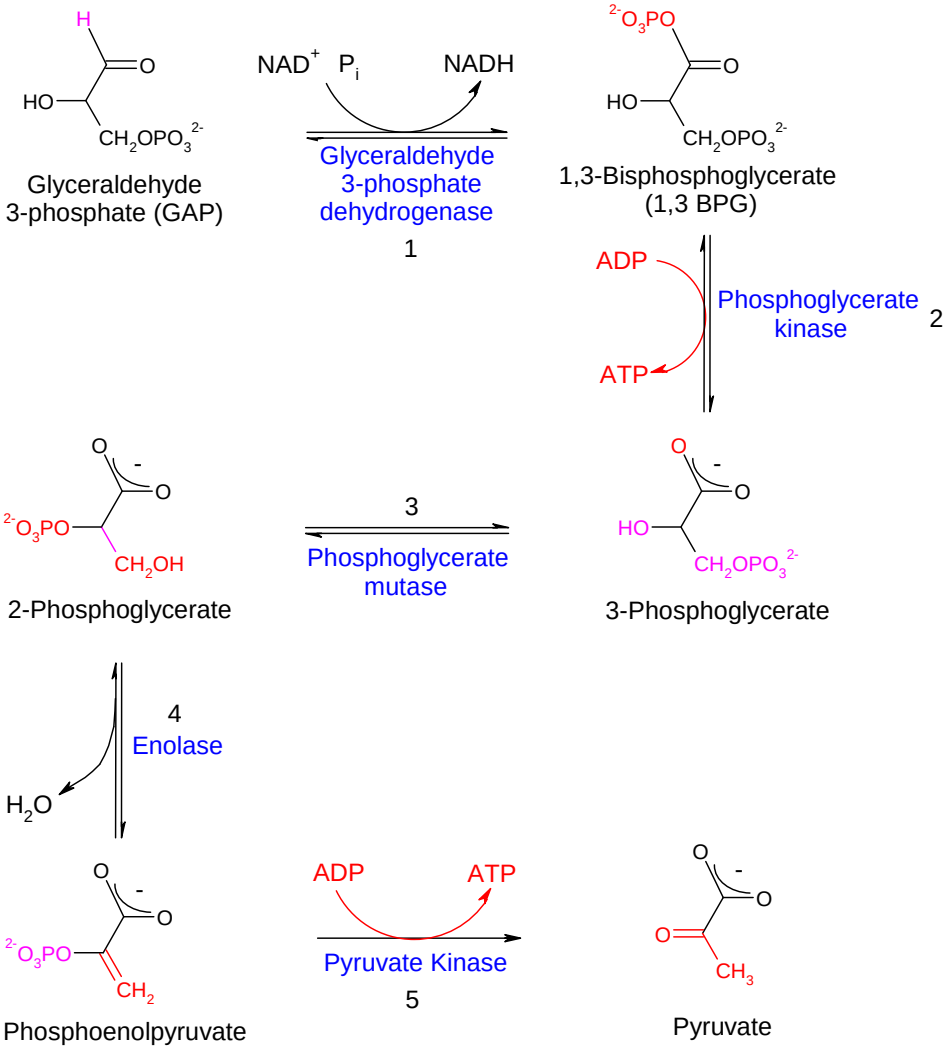


A few notes

- The reaction catalysed by **triose phosphate isomerase** is very similar to that catalysed by **phosphoglucose isomerase** – it is the conversion of a **ketose** to an **aldose**.
- The conversion of **DHAP** to **GAP** is **reversible**, and at **equilibrium**, **96%** of the **triose phosphate** is **DHAP**. However, since **GAP** is **quickly removed**, the reaction occurs **fairly fast**.
- We now see the significance of **Step I**. Had the **cleavage** occurred in **glucose directly**, we would have ended up with **one two-carbon sugar** and one **four-carbon sugar**, each requiring **different metabolic pathways**.
- The mode of action of **TPI** is well known – see Stryer, p439.

Stage III (Occurs Twice)

The **three-carbon fragments** are **oxidised** to **pryuvate**, and **ATP** is **harvested**.



A couple of notes on each of the reactions:

- **Reaction 1**

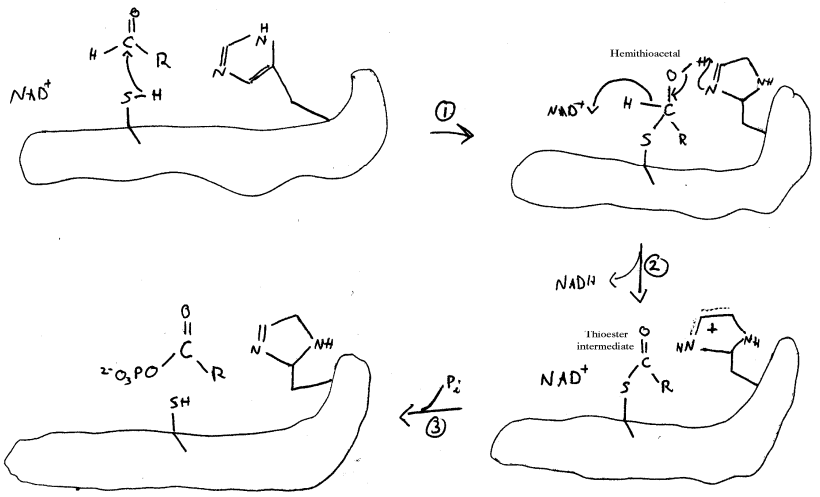
  The product of this reaction is a **mixed anhydride** of **phosphoric acid** and **carboxylic acid**. Such compounds have **high phosphoryl-transfer potential**.

  This reaction can be thought of as two reactions in succession:

- The **oxidation** of the **aldehyde** group to a **carboxylic acid** (**oxidising power** provided by **NAD^+** and **oxygen** provided by **H_2O**).
- The **joining** of the **acid** and the **phosphate** to form the final compound.

Now, the *first* step is **quite favourable**. The *second* step is about **equally unfavourable**.

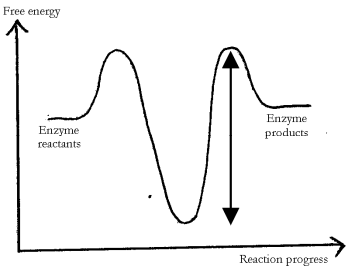
The two steps are therefore **coupled** by the **formation** of a **thioester intermediate**, which is **comparable in energy** to the reagents and products:

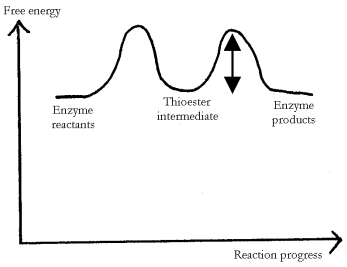


Comments:

- **Step (2)** is **aided** by the **His 176** residue **accepting** a **proton**.
- In **step (3)**, the new **NAD^+ polarises** the **thioester intermediate** to make **attack** by the **P_i easier**.
- This illustrates the **essence** of **energy transformations** and **metabolism** – energy from the **oxidation of carbon** is **converted** into **high phosphoryl-transfer potential**.

The **energy diagrams** with and without this **intermediate** therefore are:





- **Reaction 2**

  The **1,3-BPG**, as was mentioned above, has a **greater phosphoryl transfer potential** than **ATP**. It therefore **produces ATP** from **ADP**. This is referred to as **substrate-level phosphorylation**.

  In essence, the **energy** from the **oxidation** of **glyceraldehyde-3-phosphate** is **trapped** as **1,3-bisphosphoglycerate**. The powers the production of ATP.

- **Reaction 3**

  The mechanism of **3-phosphoglycerate mutase** is in Stryer, p445.

- **Reaction 4**

  This is a **dehydration reaction**, and it significantly **elevates** the **transfer potential** of the **phosphoryl group**. This is because the **phosphoryl group traps** the molecule in its **unstable enol form** – once the group has been **donated to ATP**, however, the enol is free to undergo a **conversion** into a **more stable ketone** – **pyruvate**. The **ketone** form is **more stable** because the **negative charge** can be **delocalised** onto the **oxygen atom**. Because this process is so **energetically favourable**, it is **practically irreversible**.

Stage IV (Occurs Twice)

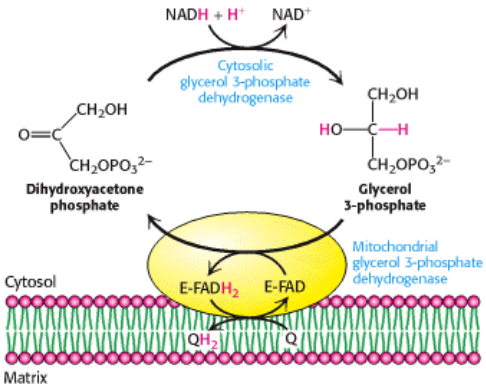
Redox balance is not maintained in the cycle above – **NAD^+** is only present in **limited amounts** in the cell (derived from the **vitamin niacin**). Consequently, it needs to be **regenerated** through the **metabolism of pryuvate**.

There are three possible fates for **pyruvate**

1. **Further oxidation** in the **Krebs cycle**, which eventually leads to an **electron transport chain**. We shall look at this process in more detail later.

   Even though the electron transport chain **regenerates NAD$^+$**, it does so in the **mitochondria**, and the **inner mitochondrial membrane** is **impermeable** to **NADH** and **NAD$^+$**. There must therefore be another way for NAD$^+$ to be **regenerated** for the use of the glycolytic pathway.

   This is done by means of **shuttles** – for example, the **glycerol 3-phosphate** shuttle, which works as follows:

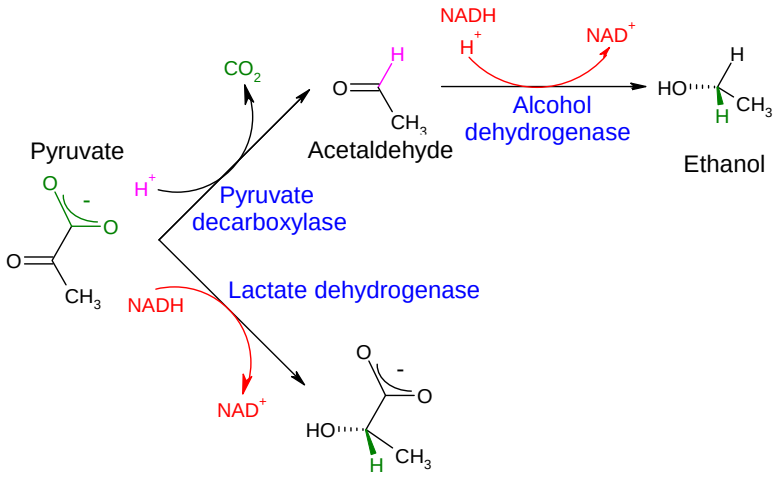


   The resulting QH_2 is fed into the electron transport chain. As a result, we do not obtain the usually **2.5 ATP** per **NADH**, but only **1.5 ATP** per **NADH**, because NADH is now fed into the chain in the same way $FADH_2$ is fed into it. We can think of this as energy expended to *pump* NADH **into** the matrix, against its concentration gradient. Thus, **oxidative glycolysis** produces **5 ATP** (2 directly and three through 2 $\times$ NADH).

   Another example of such a shuttle is the **malate-aspartate shuttle**, active in the heart and liver.

2. **Reduction** to **lactate**, in a process called *lactic acid fermentation.*
3. **Reduction** to **ethanol**, in a process called **fermentation**.

This first involves the **decarboxylation** of **pyruvate**, catalysed by **pyruvate decarboxylase**, requiring the **coenzyme thiamine pyrophosphate**, derived from the **vitamin thiamine** ($\mathbf{B_1}$).



Miscellaneous points

- **Three** of the steps in **glycolysis** involve a large change in **energy**, and are therefore **nearly irreversible**.
  - **Glucose + ATP → glucose 6-Phosphate** [$\Delta G = -33$ kJ mol^{-1}] (this, as we mentioned above, is part of a **chemical priming process**, whereby the molecule can no longer cross the membrane).
  - **Fructose 6-phosphate + ATP → fructose 1,6-bisphosphate** [$\Delta G = -22$ kJ mol^{-1}].
  - **Phosphoenolpyruvate → pyruvate + ATP** [$\Delta G = -17$ kJ mol^{-1}].
- Even though **fermentation** yields only a **fraction** of the energy available from glucose, it is **extensively used** because it is **anaerobic**. This is important in
  - Organisms living in **anaerobic conditions**.
  - Tissues **lacking mitochonria** (such as **red blood cells** and the **retina**)
  - Situations in which a **burst in activity** is required. In such a case, the **ATP needs exceed** the **ability to provide** $\mathbf{O_2}$. For example, in

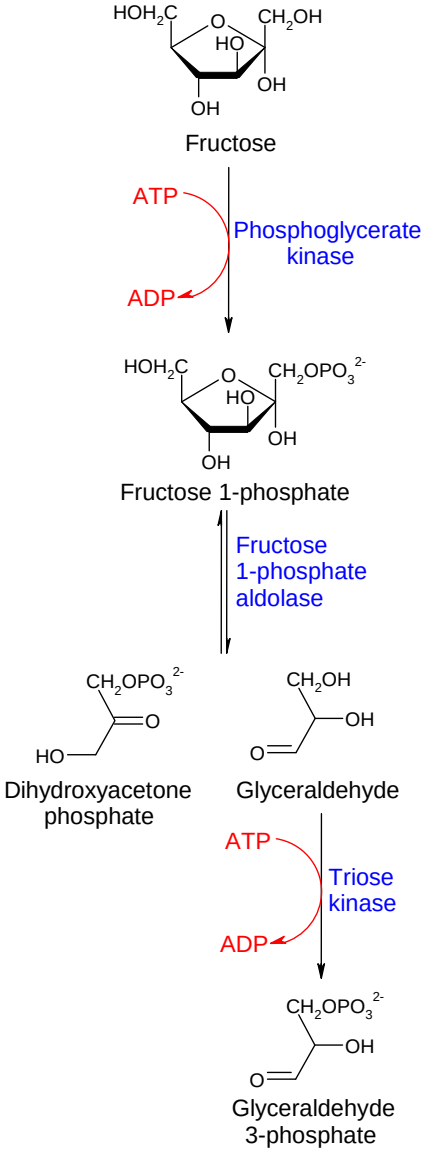
fast-twich muscle (also called **white muscle**, due to the few blood vessels there[1]).

In such a case, however, the **oxygen debt** must be **repaid** by **increasing** the **citric acid cycle rate** to **oxidise** the **lactate produced**. If this is not done and blood lactate **concentrations** increase above about **5 mM** (usual conc: **1mM**, fully dissociated [pKa = 3.86]), the **buffering capacity** of the blood is **overpowered**, and the **pH** drops from about **7.4** to **7**. This is part of the **burning sensation** that we feel.

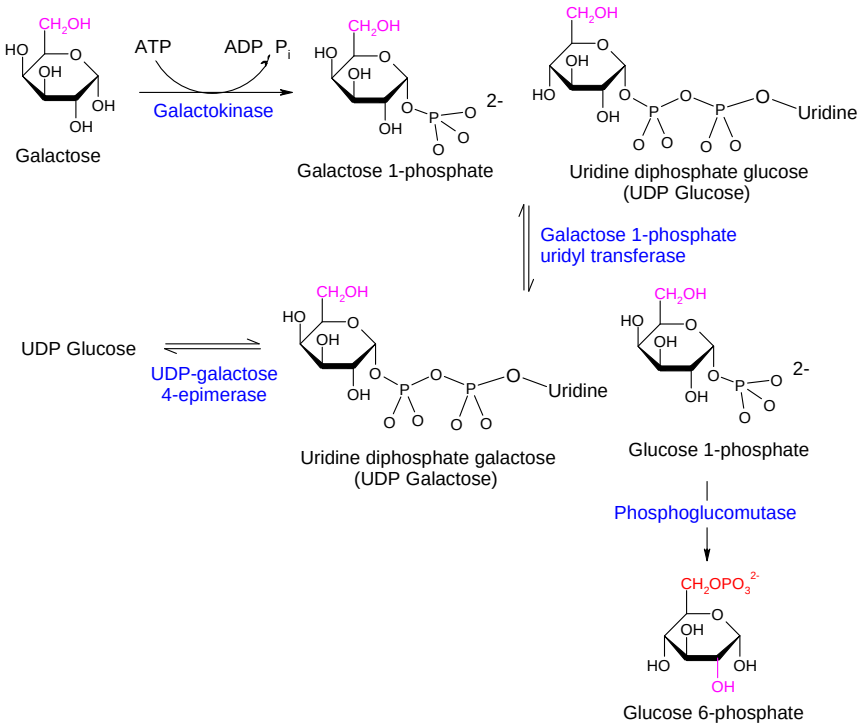
- The **binding site** for **NAD^+** is **similar** in all the **dehydrogenases**. It consists of up to **four α-helices** and a **sheet** of **six parallel β-strands**. This **common structural domain** is called the **Rossmann fold**.

[1] It is interesting to note how different organisms have different proportions for slow and fast twictch muscles, depending on how much they do. Pigeons, who fly long distances, have large amounts of red muscle (ie: red meat). Chickens, on the other hand, have more white meat.

- **Fructose** can be **metabolised** by **conversion** into a **glycolytic intermediate**:



- So can **galactose**



Notes:

- This reaction is **reversible**. In fact, the conversion of **UDP-glucose** into **UDP-galactose** is **essential** for the **synthesis** of **galactosyl residues** in **complex polysaccharides** and **glycoproteins** if the amount of **galactose** in the **diet** is **inadequate**.
- **Classic galactosemia** is an **inherited deficiency** in **galactose 1-phosphate uridyl transferase activity**. Afflicted infants fail to thrive, vomit or have diarrhea after consuming milk and form cataracts. Enlargement of the liver and jaundice are also common, and so are lethargy and retarted mental evelopment. The most common treatment is a diet low in galactose, though this does not prevent central nervous system malfunction or ovarian failure, for reasons that are not well understood.

  The formation of **cataract**, however, is understood. If the **transferace** is not active in the lens of the eye, the presence of **aldose reductase** causes the **accumulating galactose** to be reduced to **galactitol** (Stryer, p452). This is **osmotically active** and water will **diffuse into the lens**, instigating the formation of cataracts.

The "Turbo" Design of Glycolysis

In some organisms, **trypanosomes** andb other **Kinetoplastida**, the larger part of glycolysis takes place in a specialised organelle called the **glycosome**.

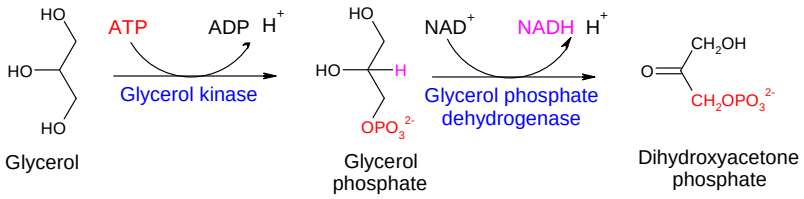
Gluconeogenesis

Maintaining levels of glucose in the body is important, because the **brain** depends on glucose as its **primary fuel** and **red blood cells** use glucose as their **only fuel**.

The body needs about **160g** of glucose a day (**120g** of which are used by the **brain**). The amount of glucose present in **body fluids** s about **20g**, and that **readily available** from **glycogen** is about **190g**. Thus, the direct reserves are **sufficient** for **about a day**. **Gluconeogensis** becomes particularly important during **longer periods of fasting** or **starvation**.

A few notes:

- **Gluconeogenesis** mostly occurs in the **liver**, and to a certain extent in the **kidney**. Very little takes place in the **brain**, **heart muscle** or **skeletal muscles**. It is the **liver** and **kidney** that help **maintain** the **glucose level** in the **blood**, so that the **brain** and **muscle** can obtain **sufficient glucose**.
- Other **noncarbohydrate** biological molecules can also be **converted to glucose** by **gluconeogensis**. They are first converted into **pyruvate** or one of the **intermediates** of **gluconeognesis**. For example:
  - **Lactate** (see above) is readily converted to **pyruvate** by the action of **lactate dehydrogenase**.
  - **Amino acids** are fed in as **pyruvate** or **oxaloacetate**.
  - **Glycerol** can enter both the **glycolytic** and **gluconeogenic** pathway as **dihydroxiacetone phosphate**:



The **net effect** of **gluconeogenesis** is the **reverse** of **glycolysis**. However, **several reactions** must **differ**, because the **equilibrium** of **glycolysis** lies **far** on the side of **pyruvate formation**.

The **actual ΔG** for the formation of **pyruvate** from **glucose** is about **–84 kJ mol^{-1}**. **Most** of this **decrease in energy** takes places in three **essentially irreversible steps**:

1. **Glucose + ATP → glucose 6-Phosphate** [$\Delta G = -33$ kJ mol^{-1}].
2. **Fructose 6-phosphate + ATP → fructose 1,6-bisphosphate** [$\Delta G = -22$ kJ mol^{-1}].
3. **Phosphoenolpyruvate → pyruvate + ATP** [$\Delta G = -17$ kJ mol^{-1}].

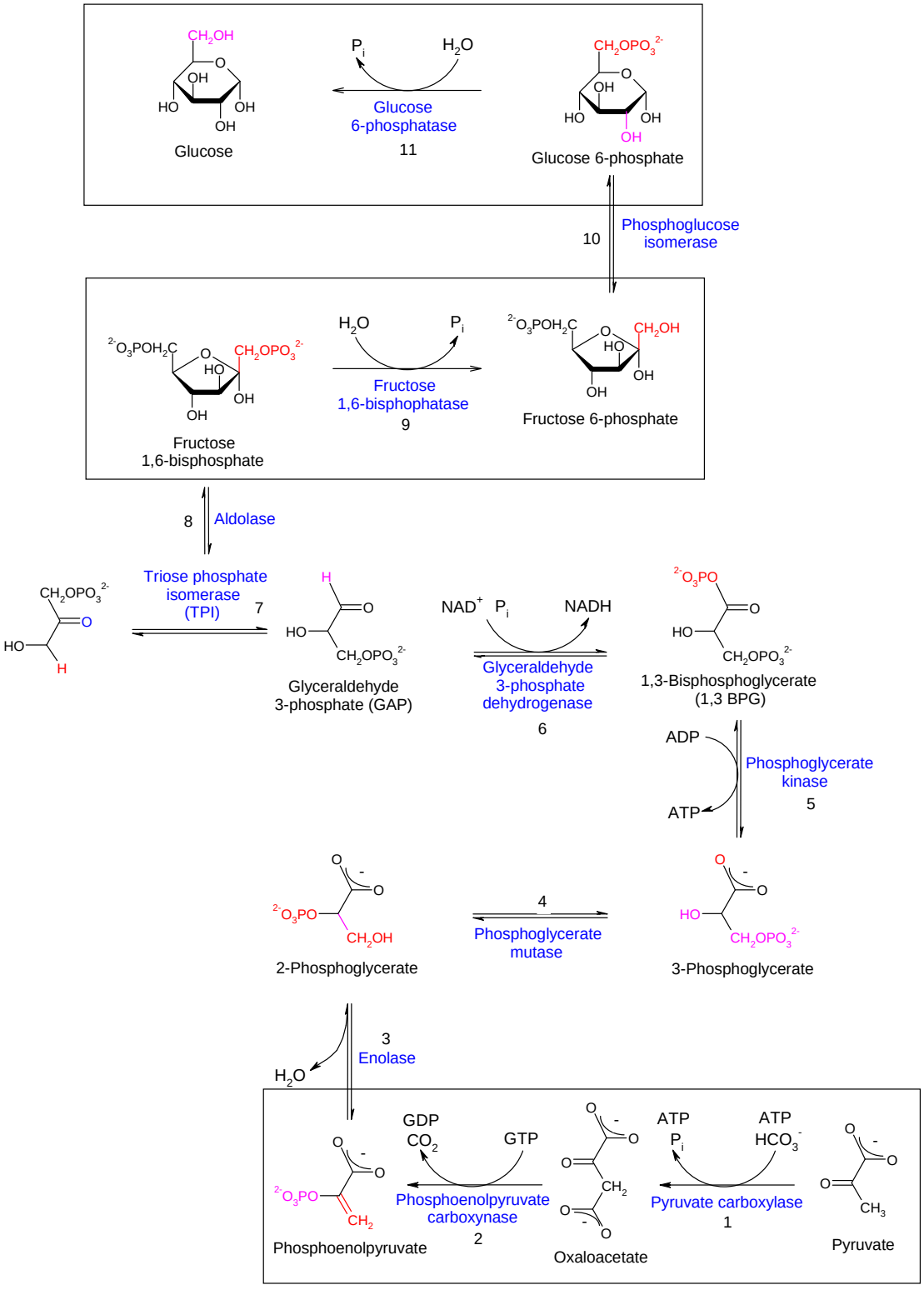
There are reactions in **gluconeogenesis** that **bypass** these three reactions.

Note, however that **six nucleoside triphosphate** molecules are **hydrolysed** to synthesise **glucose** from **pyruvate**, whereas only **two molecules** of **ATP** are

generated by **glycolysis**. These **four additional molecules** are needed to turn an **energetically unfavourable reaction** into a **favourable one**.

The process of gluconeogenesis is as follows. The **reactions** that are **not** the **reverse** of **glycolysis** (ie: those that **bypass** the **three reactions above**) are indicated by a **dotted rectangle**.

Most of these enzyme are located in the **cytoplasm**, except for **pyruvate carboxylase** (in the **mitochondria**) and **glucose 6-phosphatase** (**membrane bound** in the **ER**).



We now examine these three different reactions in detail

- **<u>Reaction 1</u>**

The first reaction involves the conversion of **pyruvate** to **oxaloacetate** by **pyruvate decarboxylase**. A few points on this **important enzyme**:

- The **N-terminal 300** to **350 amino acids** form an **ATP-grasp domain**, which **surrounds ATP** and **holds it** in an **orientation** suitable for **nucleophilic attack** at the **γ-phosphoryl group**. This is a **widely used ATP-activating domain**.
- The **C-terminal 80 amino acids** constitute a **biotin binding domain**. **Biotin** is a **covalently attached prosthetic group** which serves as a **carrier of activated CO_2**. The **carboxylate group** of **biotin** is linked to the **ε-amino group** of a **specific lysine residue** by an **amide bond**. It turns out that **biotin** is linked to the **pyruvate carboxylase** molecule by a **long, flexible chain**.
- The **central domain** of the enzyme binds to **pyruvate**.

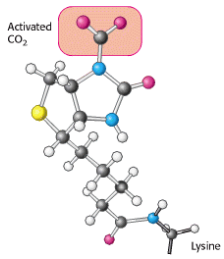
The actual carboxylation occurs in **three stages**

a) $HCO_3^- + ATP \rightleftharpoons HOCO_2PO_3^{2-} + ADP$

- In **solution**, **CO_2 exists** primarily as **HCO_3^-**, thanks to **carbonic anhydrase**.
- In effect, **HCO_3^-** is **activated** to **carboxyphosphate**.

b) $\text{Biotin-enzyme} + HOCO_2PO_3^{2-} \rightleftharpoons CO_2\text{-biotin-enzyme} + P_i$

- The **activated carboxyphosphate** is **bonded** to the **N-1 atom** of the **biotin ring** to form a **carboxybiotin-intermediate**:



- This **CO_2** is still **quite activated**. The **$\Delta G^{\circ\prime}$** for its **cleavage** is **–20 kJ mol^{-1}**. This is **negative**, and indicates that **carboxybiotin** is able to transfer **CO_2** to **acceptors** without the **input** of **additional free energy**.

- Note that **reaching this stage** depends on the **presence** of **Acetyl-CoA**. **Biotin** is *not* **carboxylated** unless **Acetyl-CoA** is **bound** to the **enzyme**. This **allosteric activation** is an important **physiological control mechanism**.

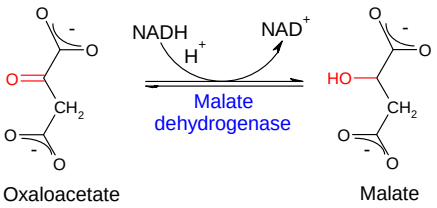
c) CO_2-biotin-enzyme + pyruvate $\rightleftharpoons$ biotin-enzyme + oxaloacetate

- The **activated carboxyl group** is transferred to **pyruvate**.
- This is **possible** thanks to the **long, flexible link** between the **enzyme** and **biotin**, which enables it to **rotate** from one **active site** of the enzyme (the **ATP-bicarbonate binding site**) to **another** (the **pyruvate-binding site**).

- **<u>Transport of oxaloacetate</u>**

  Pyruvate carboxylase exists in the **mitochondria**. The enzyme for **reaction 2**, however, exists in the **cytoplasm**. **Oxaloacetate** must therefore be **shuttled** from the **mitochondria** into the **cytoplasm** before **reaction 2** can take place.

  It is **not**, however, **transported** as **oxaloacetate**. In fact, the **oxaloacetate** is **reduced** to **malate**, leaves the mitochondrion by a **specific transport system** and is **reoxidised** back into **oxaloacetate**:



  This process is also **useful** in that it **releases NADH** into the **cytoplasm**, where it can be used for **later steps** of **gluconeogenesis**.

- **<u>Reaction 2</u>**

  The **oxaloacetate** is now **simultaneously decarboxylated** and **phosphorylated**. The **phosphoryl donor** is **GTP**, and the **CO_2** that was **added** to **pyruvate** is **removed**.

The reason why **carboxylation** was **necessary** in the first place was because the **phosphorylation of pyruvate** is such an **unfavourable reaction**
($\Delta G = +31$ kJ mol^{-1}). The **decarboxylation** of **pyruvate**, however, is a very **favourable** reaction. This is, in fact, a **common mechanism** – **decarboxylations** often **drive** reactions that are otherwise **highly endorgonic**.

- **Reaction 9**

  This is the next **irreversible reaction** in **gluconeogenesis**.

- **Reaction 11**

  In **most tissues**, this reaction **does not occur**. **Glucose** is **not produced**, and the **glucose 6-phosphate** is **processed** in some **other way**. One of the **advantages** of this is that glucose 6-phosphate **cannot cross membranes** and thus **remains inside the cell**.

  The reaction is **controlled** in two ways:

  a) **Glucose 6-phosphatase** is only **present** in tissues whose **metabolic duty** is to **maintain blood-glucose homeostasis** – in other words, tissues that **release glucose** into the **blood**. These tissues are the **liver**, and, to a lesser extent, the **kidneys**.

  b) Even when present, **glucose 6-phosphatase** is **regulated**.

     In fact, **glucose 6-phosphatase** is **contained** in the **membrane** of the **endoplasmic reticulum**, and no less than **five proteins** take part in its conversion:

     - One **transporter** is needed for each of **glucose 6-phosphate**, **glucose** and **P_i**.
     - **Glucose 6-phosphatase** is needed.
     - An **associated Ca^{2+}-binding proteins** is essential for phosphatase activity.

The Cori Cycle

Some organs have **little oxidative capacity** – for example, **red blood cells** posses **no mitochondira** and thus **cannot fully oxidise glucose**. They must rely on **glycolysis**. **Fast-twitch** (white) **muscle** do posses mitochondria, but the **rate** at which **pyruvate** is **oxidised** is far inferior to the rate at which it is **produced**.

In these cells, **lactate dehydrogenase** convert this **pyruvate** to **lactate** to **restore the redox balance**. However:

- Even though **lactate** still contains a fairly **large amount of energy**, it is a **dead-end** in **metabolism**. It must be **converted back** to **pyruvate** before it can be **metabolised**.
- A **build-up** of **lactate** in **muscle tissues** can cause **acidosis** if it is not **exported into the blood**.

The **lactate**, therefore is **exported into the blood**, and the **burden** of **metabolising it** is **shifted** to **other organs**. These molecules in the bloodstream have two fates:

- The **plasma membranes** of **some cells** (particularly those in cardiac muscle) contain **carriers** that make the cell cells **highly permeable** to **lactate** and **pyruvate**. Once inside these **well-oxygenated** cells, **lactate** can be **reverted** back to **pyruvate** and **metabolised** in the **citric acid cycle**. This makes more **circulating glucose** available for **muscle cells**.
- **Excess lactate** enters the **liver** and is converted **back to glucose** by the **gluconeogenetic pathway**. Thus, the liver **restores** the **level of glucose** necessary for **active muscle cells**. These reactions constitute the **Cori cycle**.

A consequence of this is that (under the assumption that most of the lactate enters the liver) cells like **red blood cells** do not **drain blood glucose**. The brain, however, does.