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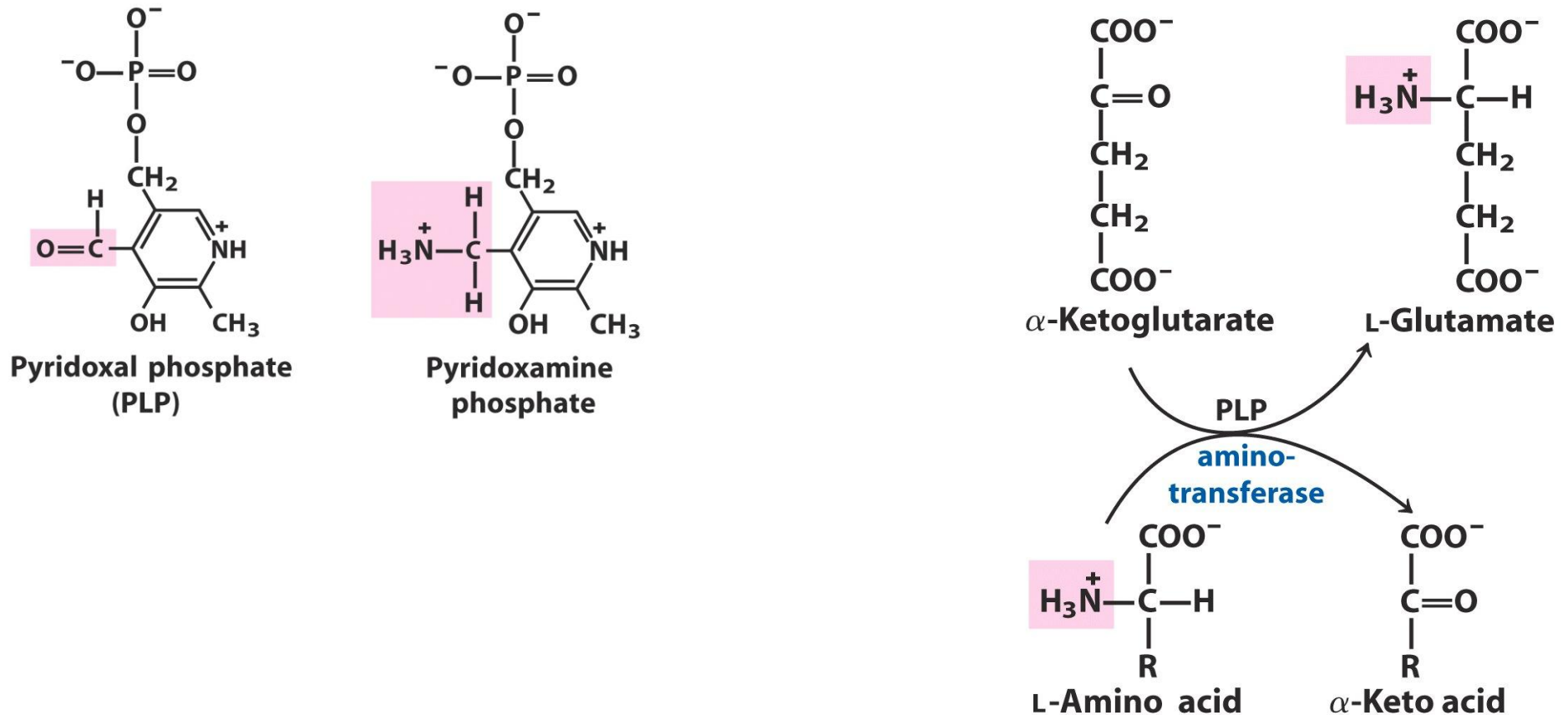
MCB 102, Spring 2008, Metabolism Lecture 11

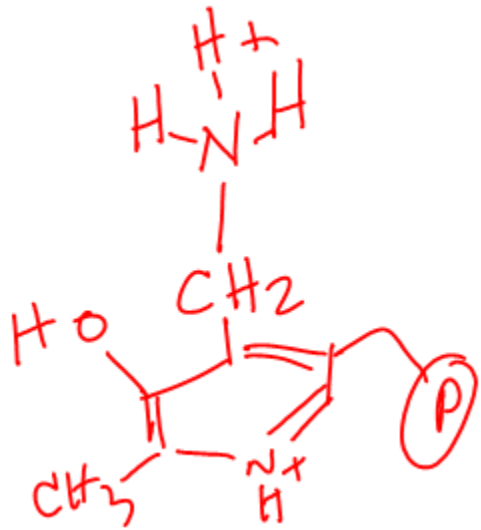
Reading: Chs. 18/19 of *Principles of Biochemistry*, “Amino Acid Degradation” & “Oxidative Phosphorylation & Photophosphorylation.”

Transaminase. There are many types for each amino acid. They are found in all tissue, but the general acceptor is α -ketoglutarate, which makes glutamate.



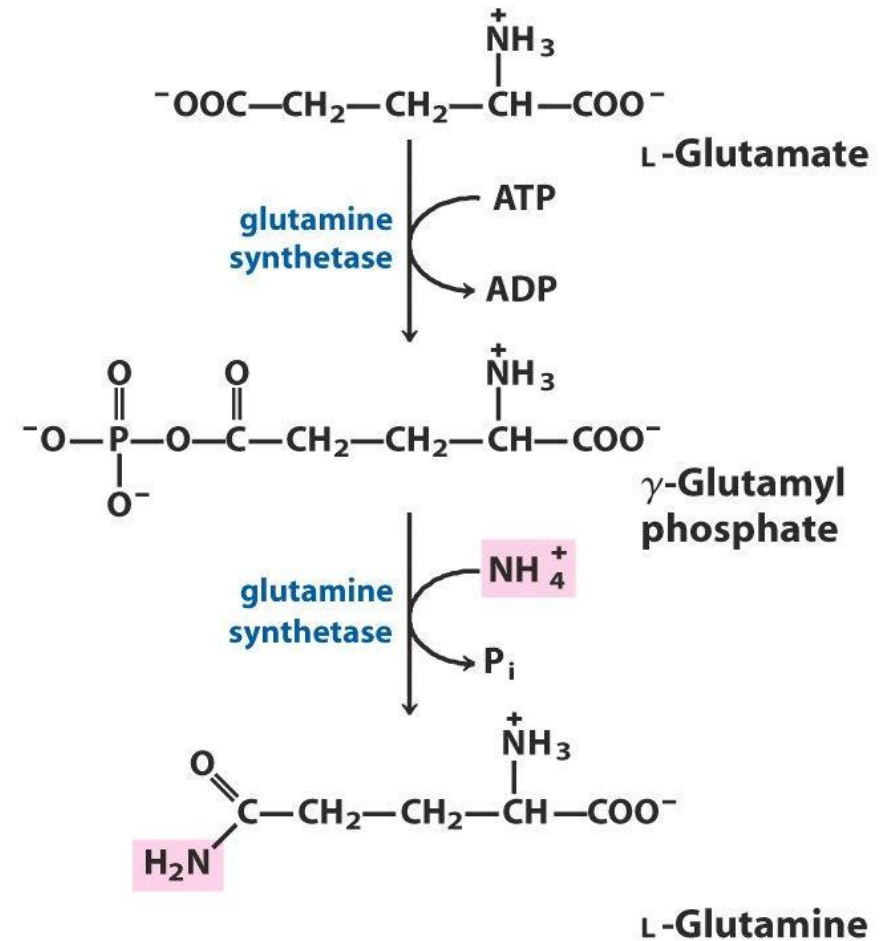
Transaminase Mechanism. Starting from pyridoxamine phosphate.





Glutamine synthetase.

- Excess **glutamate** → **glutamine** (via **glutamine synthetase** enzyme) then be transferred from tissues to liver. We introduced this ATP-dependent enzyme in the 1st lecture.
- Excess glutamine can then enter the blood and be processed by the liver to make urea (or kidneys to make ammonia).
- Muscles do the glucose/alanine cycle—a modified form of the Cori Cycle, when proteins are used as a fuel source using.



Glutaminase.

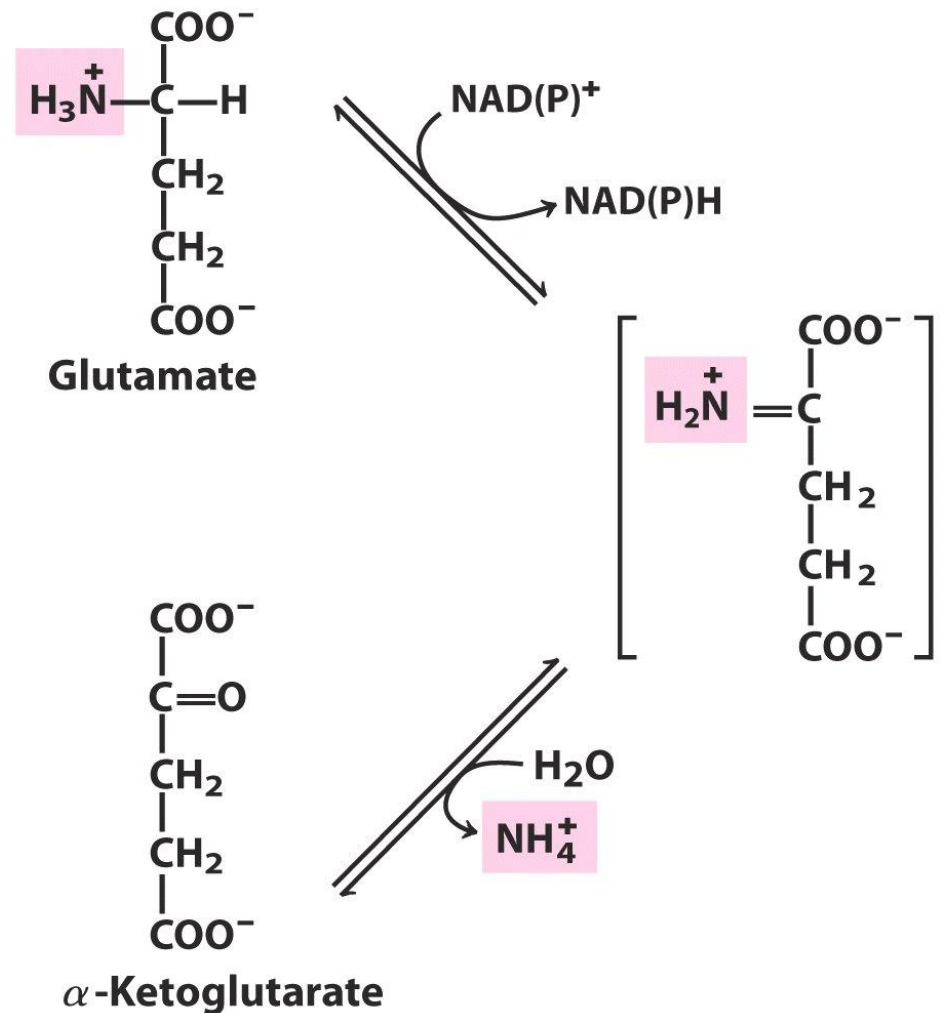
- In mitochondria of liver, **glutamine** → **glutamate** via **glutaminase**, releasing ammonia inside the liver.

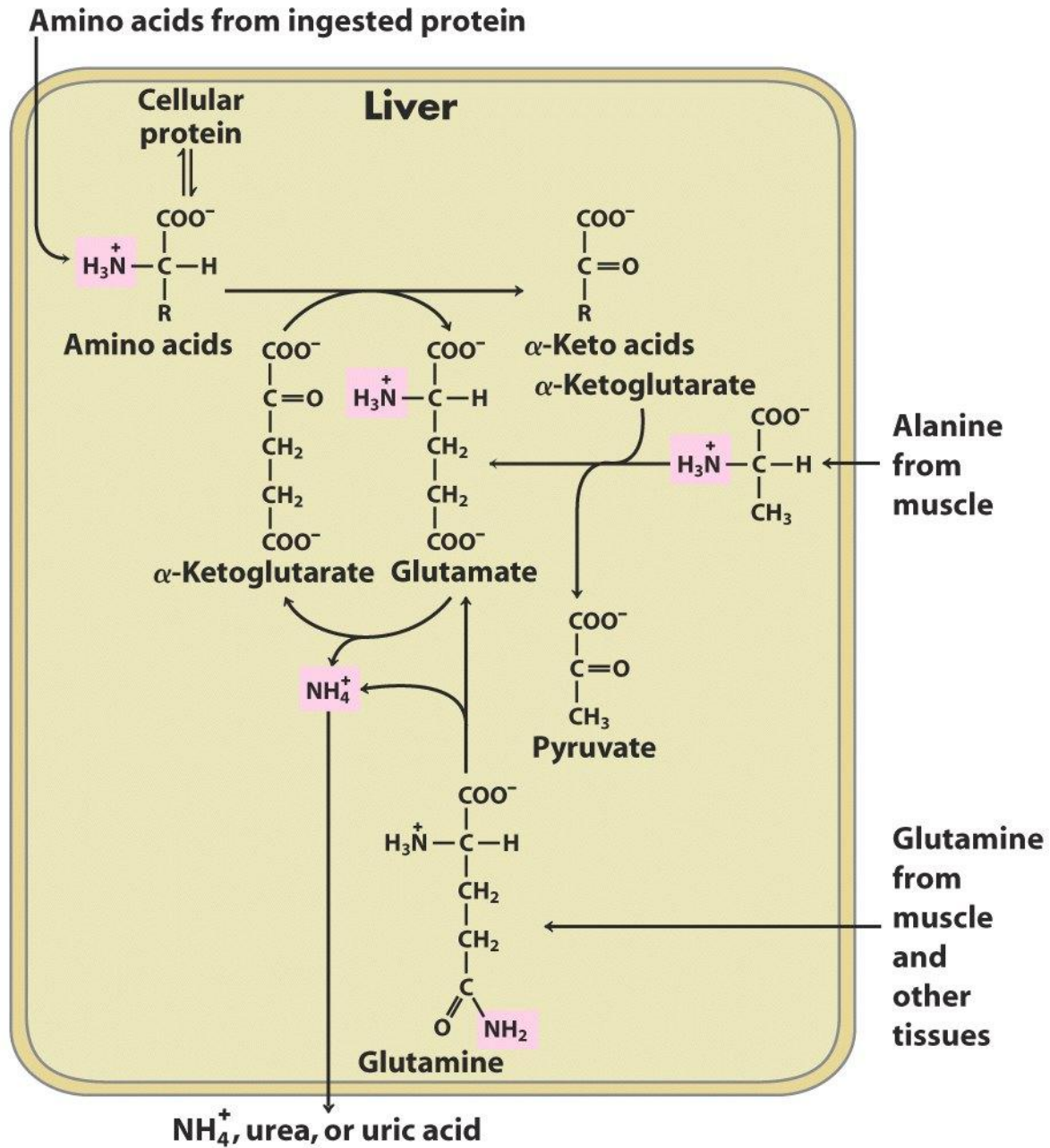
Glutamate & Glutamine transporters. The mitochondrion has a transporter for **E** and **Q**.

- In liver cells, **E** and **Q** enter the mitochondrion because it has a transporter.
- Alanine taken to the liver from muscle can generate glutamate using transaminase.

Glutamate dehydrogenase.

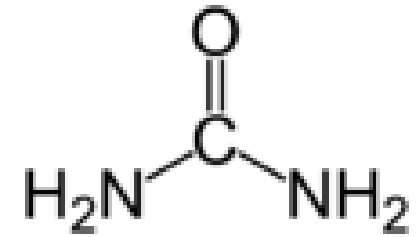
- In mitochondrion, the amino group gets released as ammonia by **glutamate dehydrogenase**.
- Within mitochondrion, toxicity issues regarding ammonium production are thought to be contained.





UREA CYCLE

Net equation of the urea cycle:



Interconnectedness with the Citric Acid Cycle



Why is it required?

- Amino acids were degraded and excess ammonium is present.
- Ammonia is toxin to cells for unknown reasons. High concentrations can cause brain swelling. Also ammonia can pass through membranes and raise the pH of acidic compartments in the cell.

Why urea?

- Urea is neither acidic nor basic, so it is a perfect vehicle for getting rid of nitrogen waste.
- Urea is used as an osmolyte by the kidney to reabsorb water and useful ions.
- Other organisms can secrete ammonium directly or make less soluble solid forms (like uric acid) to reduce overall weight (and is important for birds, for example).

Where?

- In the liver. Some steps occur in the liver mitochondria and others in the cytosol.
- Kidneys remove excess urea from the blood. Kidneys and intestine can only make ammonium.

Who? Hans Krebs discovered it in the 30s.

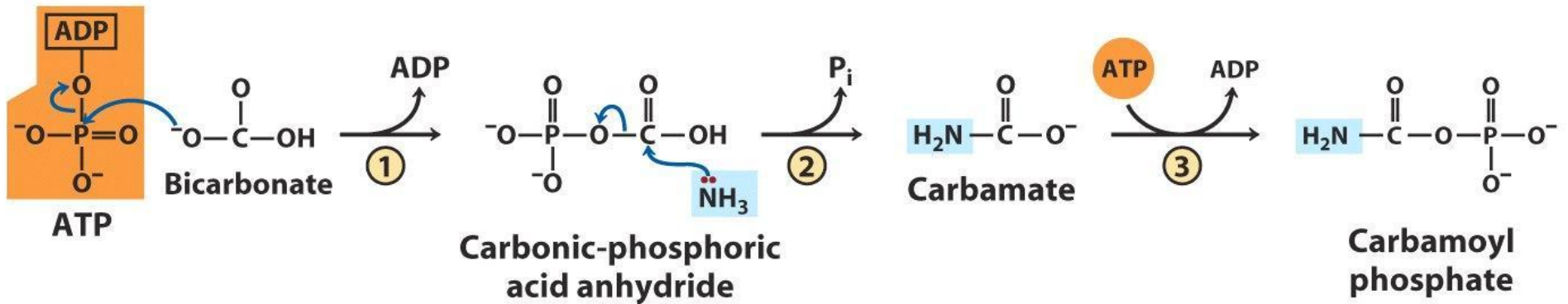


Hans Krebs, 1900-1981

[STEP 1] Carbamoyl Phosphate Synthetase I.

The ammonia that has been generated within the mitochondrion is converted into **carbamoyl-phosphate** by this **synthetase**.

The enzyme uses **two ATP** and **bicarbonate**.

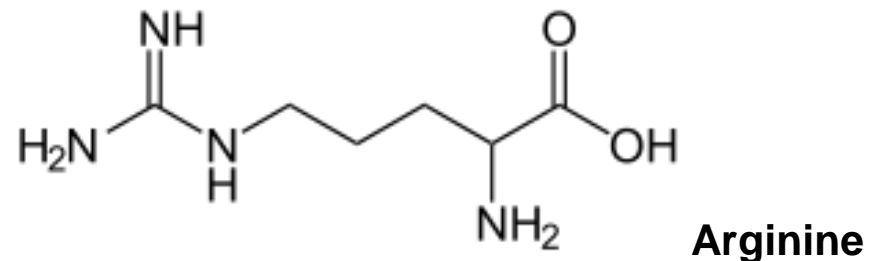
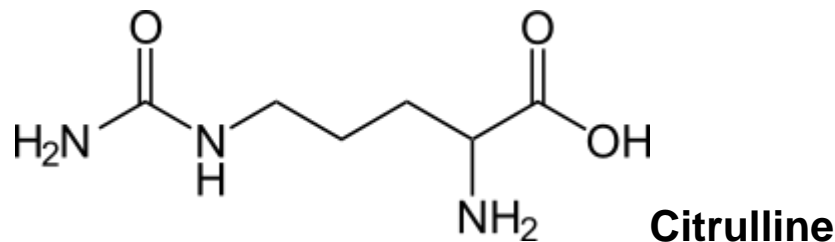


[STEP 2] Ornithine Transcarbamoylase.

Ornithine is almost like Lys, but is a methylene shorter: $NH_2-CH_2-CH_2-CH_2-CHNH_2-COOH$.



Citrulline is almost like Arg, but its R-group has a urea group on the end instead of a guanido group.

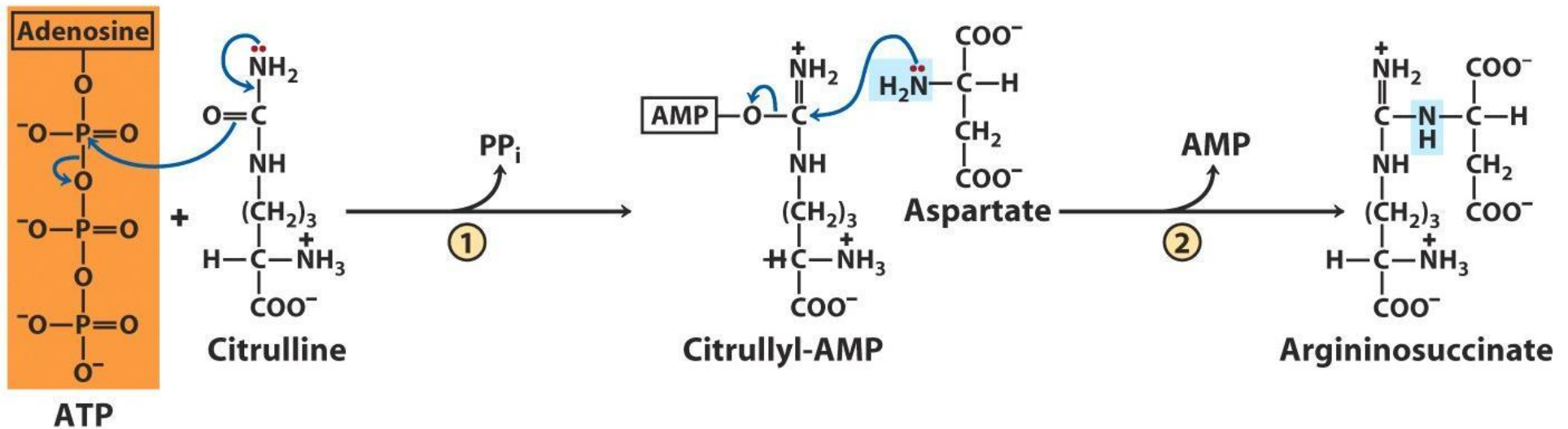


[STEP 3] Argininosuccinate Synthetase.

- The uninitiated may think that you could just cut off urea from citrulline. Not so fast.
- The urea cycle is a cycle. You must regenerate ornithine again, which is why everything is very complicated.
- We are now in the cytosol. Citrulline was exported through a mitochondrial transporter.



Mechanism. An unusual reaction takes place. Generally, a nucleophile attacks the carbonyl carbon. Here, the carbonyl oxygen acts as a nucleophile on the α-phosphorus of ATP. A **citrullyl-AMP intermediate** is made then that is attacked by the amino group of the Asp.



[STEP 4] Argininosuccinase.

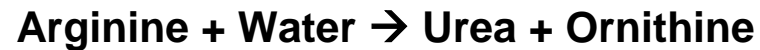
- Now fumarate is released—not succinate.

Arginine is produced.



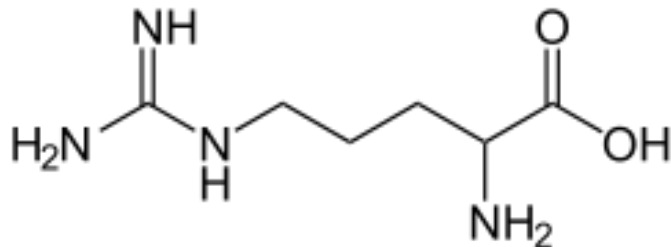
Mechanism. This is an elimination reaction. There is an abstraction of a proton and a C=C double bond is made. Fumarate is then liberated.

[STEP 5] Argininase.

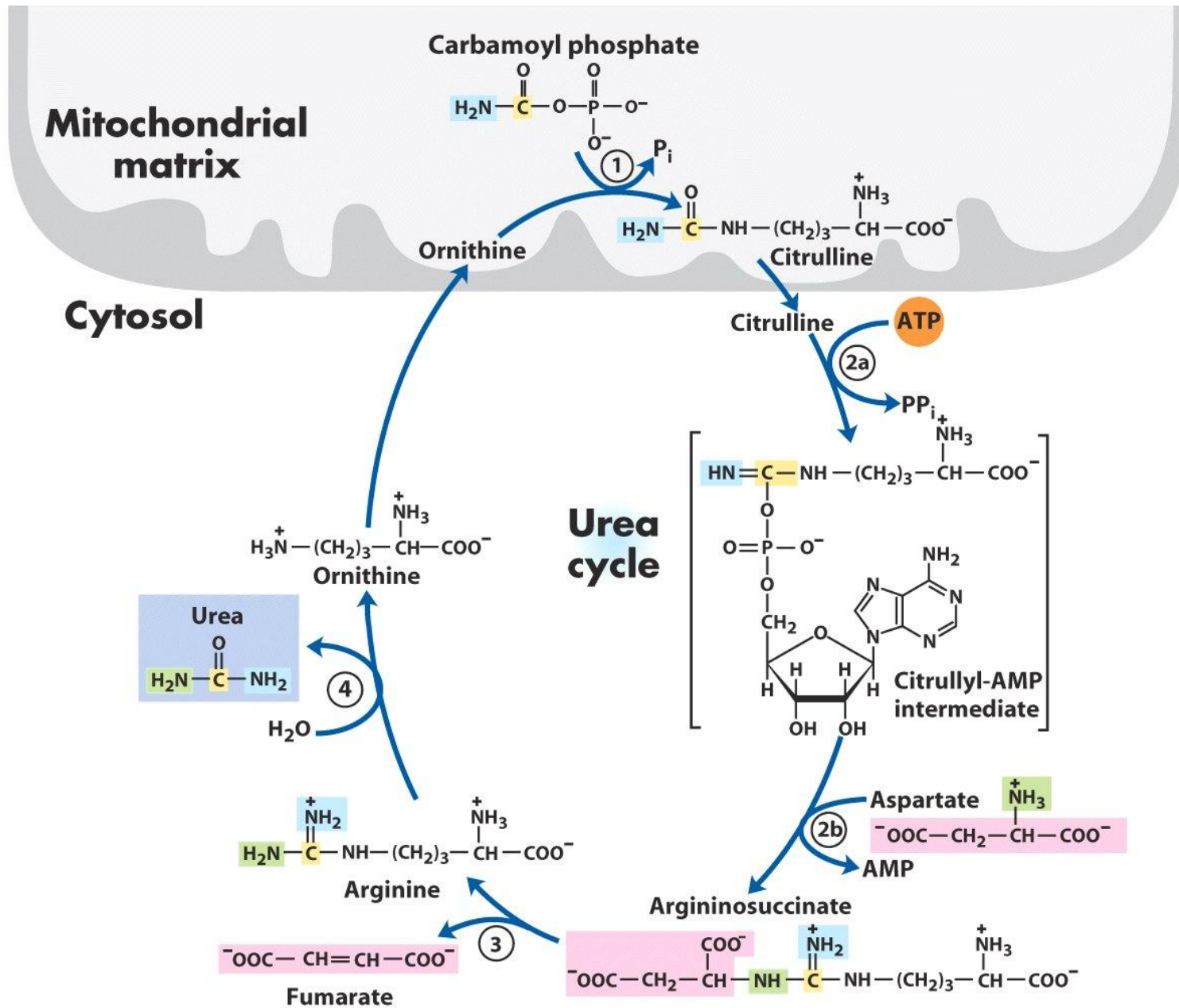


Mechanism. Hydroxyl ion attacks carbon in guanido group of Arg. Resonance stabilization in the guanido system withdraws electrons from the central carbon, inviting nucleophilic attack. Urea is liberated after removing the N-C-N structure of urea, making ornithine.

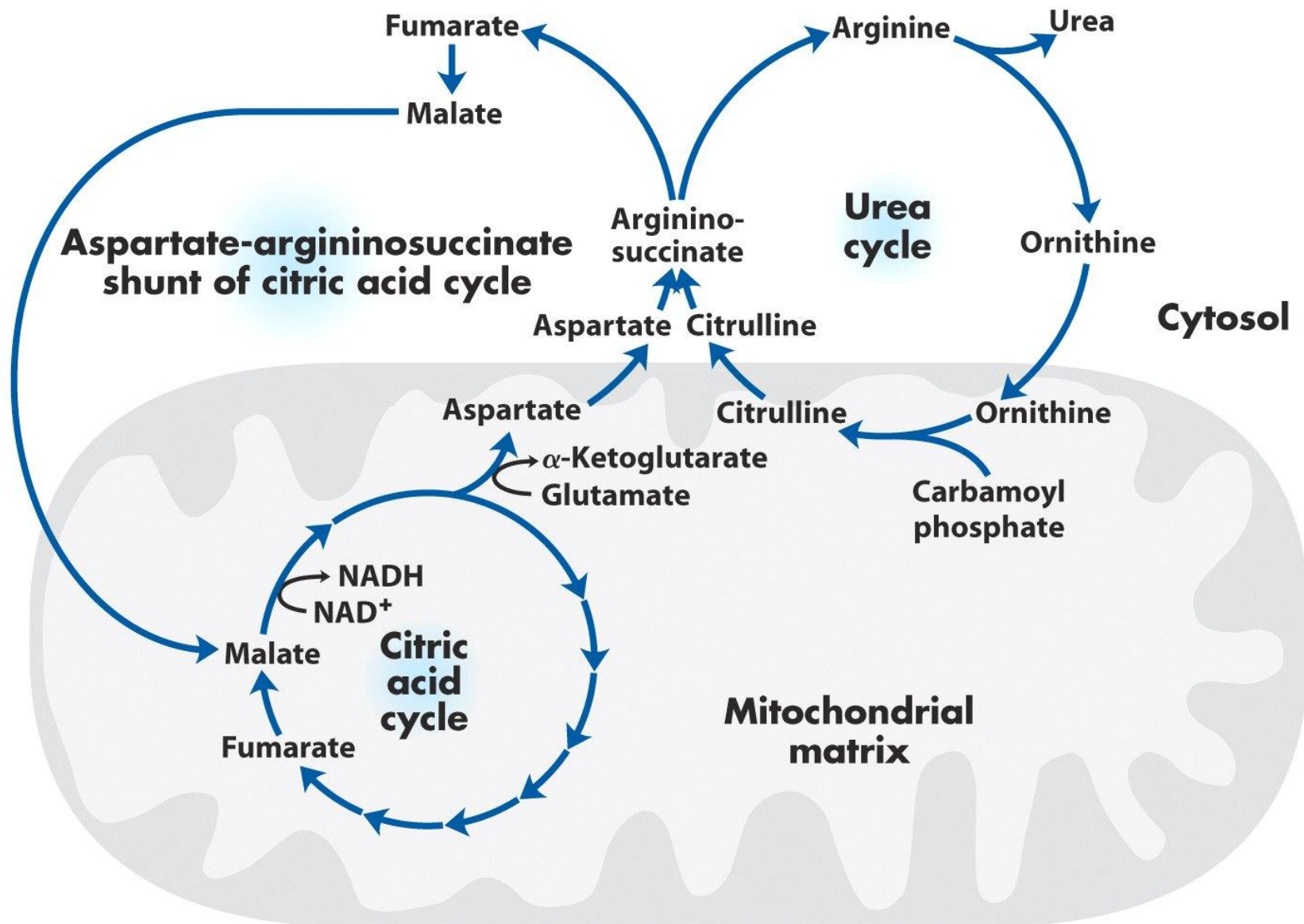
- The cycle is “complete” and ornithine was regenerated.



UREA CYCLE



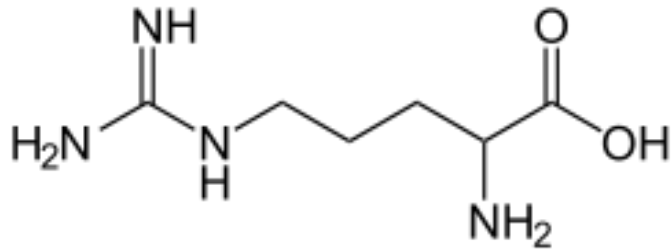
THE REST OF THE UREA CYCLE



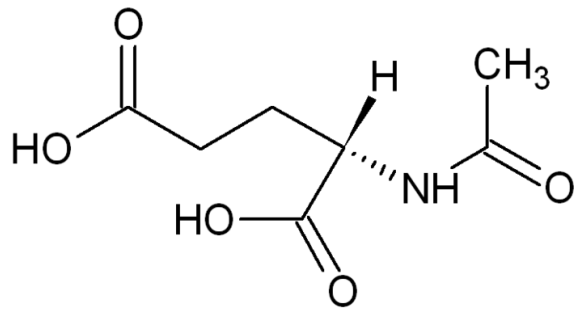
HOMEWORK PROBLEM: Determine the overall energy utilization of the pathway for the urea cycle alone and the in combination with the Asp-Argininosuccinate shunt.

Regulation.

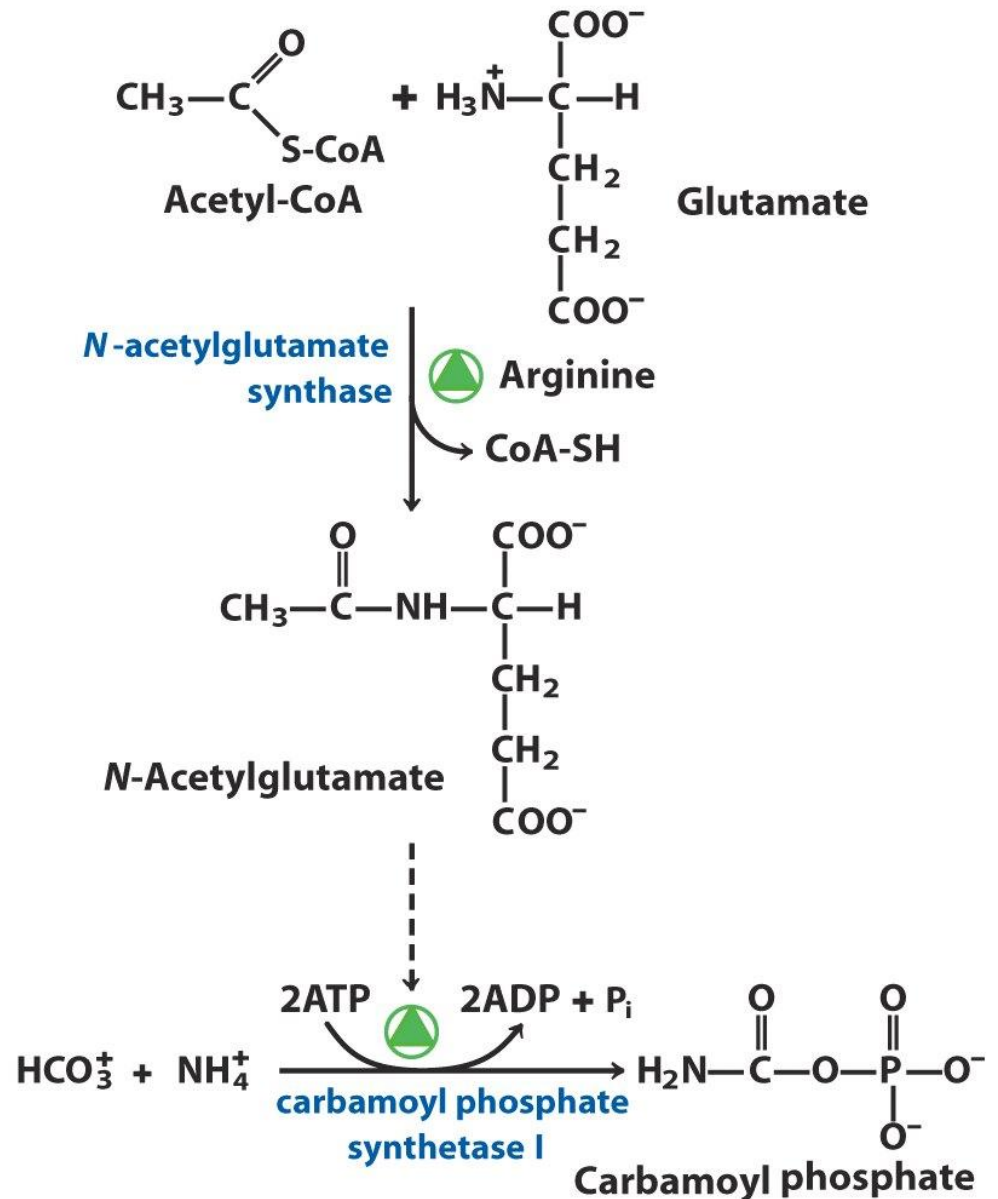
- The Urea Cycle is regulated allosterically by arginine (though indirectly.)



- Arginine stimulates the cycle to proceed via activation of a acetylation reaction of glutamate to make a pure signally molecule in man, called **N-acetylglutamate**.



- N-acetylglutamate allosterically stimulates Carbamoyl phosphate Synthetase I.



OXIDATIVE-PHOSPHORYLATION

- Reduced coenzymes, **FADH₂** or **NADH**, are made by many pathways (but they are intermediates).
- *How do these reducing compounds generate ATP?* The answer is **oxidative phosphorylation**.

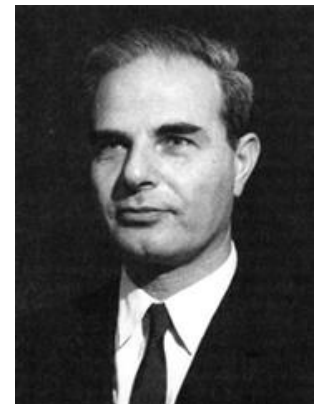
Oxidative phosphorylation is not substrate level phosphorylation, which we saw in glycolysis.

An example of substrate level phosphorylation is the pyruvate kinase step of glycolysis:



Oxidative phosphorylation (ox-phos). A fixed amount of ATP is generated depending on how much oxidation occurs. The **P/O ratio**, for example, is the molar amount of ATP generated per atom of oxygen that gets reduced. This ratio is ~3.

Herman Kalckar. He is attributed the discovery of ox-phos in 1938. He was born in Copenhagen, Denmark and went through medical school. He did not know what to do with his life, so he became an army doctor (not exactly the best doctors in Denmark). But at the age of thirty or so, he decided to become a graduate student in biochemistry. His professor was interested in the mechanism whereby glucose gets reabsorbed in the kidney.



Steps in the oxidative phosphorylation discovery

- Kalckar was incubated slices of rat kidney with some substrates that get oxidized as an energy source with glucose and discovered glucose-6-phosphate.
- Kalckar then discovered that the amount of glucose-6-phosphate made by the kidney slices was proportional to the amount of oxygen consumed in the oxidative process.

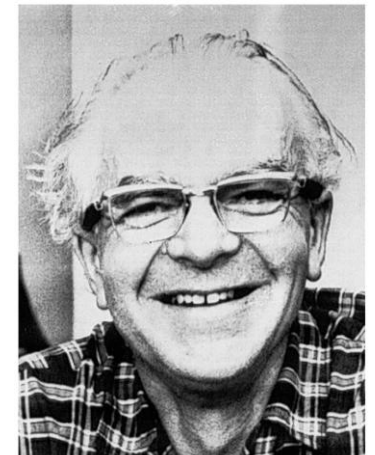
Instead of thinking of glucose-6-phosphate simply as an intermediate in the glucose reabsorption process of the kidney, he thought that maybe glucose-6-phosphate works as a sink for ATP.



CHEMIOSMOTIC HYPOTHESIS

The world was confused how ox-phos occurs. The very dominant thinking favored substrate level phosphorylation. In science, things get established; it is very difficult to overturn doctrine. Everybody thought that there were complicated and unstable intermediates in the phosphorylation process. Every year, people were reporting a new intermediate that was totally unstable. It all went nowhere.

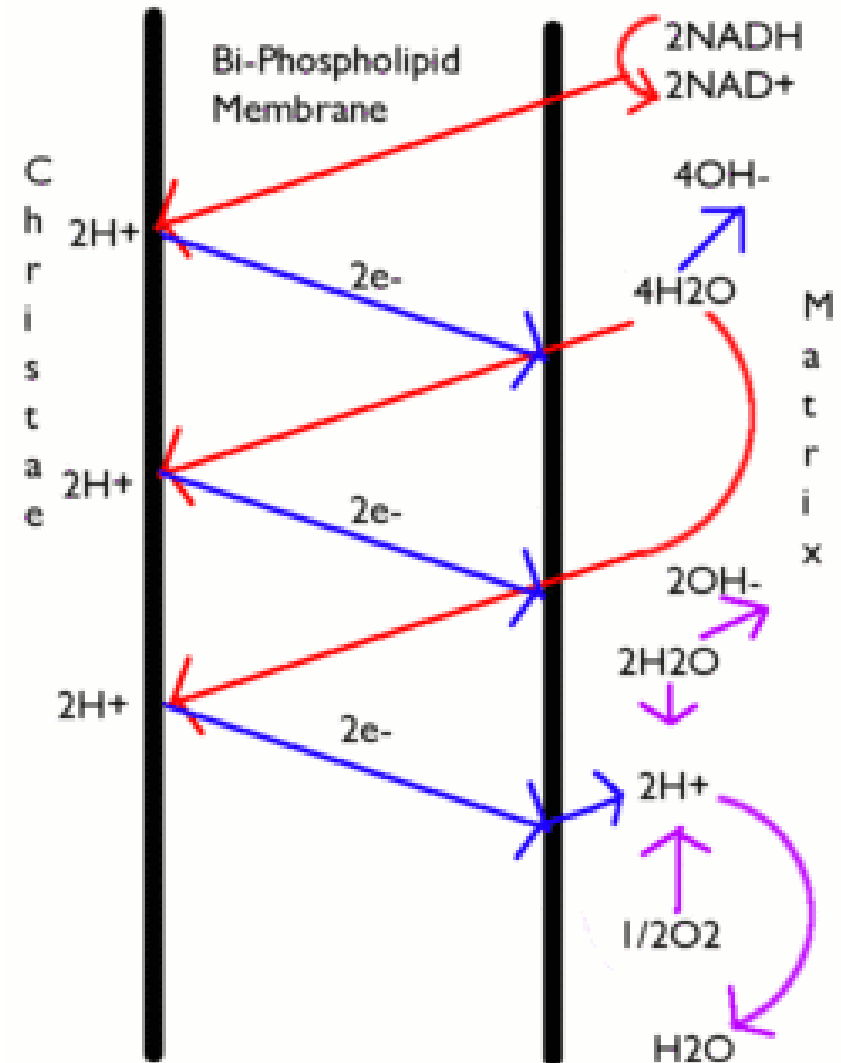
Peter Mitchell. In the meantime, **Peter Mitchell**, who was a mediocre student at Cambridge, who had difficulty getting a PhD, ultimately landed a Nobel Prize in 1978 for fighting the dogma and figuring out the way that protons could make ATP. His idea was the **chemiosmotic hypothesis**.



Peter Mitchell, 1920–1992

The Chemiosmotic Hypothesis

- Oxidative phosphorylation requires an organelle that is totally enclosed by a membrane.
- He argued that electron transport could occur by a succession of carriers fixed in the membrane in a fixed direction.
- If you start from a substrate in the mitochondria such as AH_2 that gets oxidized to A , then the first carrier is going to be a carrier of hydrogen.
- When the reducing equivalent reaches the outside surface of the membrane, the next carrier could be a carrier of electrons and cannot get reduced by using hydrogen. This e^- carrier may be a metal. The metal now becomes reduced.
- In order to reduce a metal, you have to come in with electrons. Two hydrogen atoms have to dissociate into two protons and two electrons. The electrons are going to reduce this metal carrier.
- The third carrier has to be a hydrogen carrier. This can go on and on. The next carrier is going to be a metal electron carrier.



Proton Motive Force

Proton gradient, ΔpH . In this way, you can produce a situation that sees the successive transfer of reducing power, which cause a net movement of protons to the outside surface of the membrane.

Membrane Potential, $\Delta\Psi$. At the same time, a positively charged ion moves across the membrane that generates a membrane potential.

Mitchell called this **proton motive force (PMF)**:
$$\text{PMF} = \Delta\Psi - 2.3(RT/F) \Delta\text{pH}$$

He argued that this is a form of energy storage, just like ATP. He argued that this is a way the energy gets stored through the electron transport process, by the oxidation of NADH or FADH₂. This PMF can be used as the source of energy to produce ATP.

PMF → ATP (proportionately)

Evidence. (i) Membrane system must remain intact. (ii) There must be proton gradient.

[Peter Mitchell quit his university lecturer post, because he could not stand the bureaucracy and paperwork. He bought a small farmhouse in the rural area of England, performing his research with basically pH paper.]

[1] Membrane integrity required. Disruption of organelles stopped acidification.

[2] pH changes. He stuck the pH paper into a mitochondrial suspension. He gave substrates to the mitochondria and found that the medium outside of the mitochondria became more acidic during the oxidation of various substrates.

[3] Uncouplers. The third thing that was very important in persuading everybody that his hypothesis was correct was through the use of **uncouplers**, which are fancier means to move protons across membranes less invasively.