Biochemistry

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Metabolism of aminoacids

Unlike FA and CH there is no storage form of AA in the body.
Average turnover rate of proteins in the 70 kg male – 400g per day.

Essential AA are derived from foods.
Non-essential AA are derived from food AND synthesized in the body

METABOLISM 1

The dietary proteins are denatured on cooking and therefore more easily digested.
All these enzymes are hydrolases (class 3 enzymes) in nature.
Proteolytic enzymes are secreted as inactive zymogens which are converted to their active form in the intestinal lumen. This would prevent autodigestion of the secretory acini.
The proteolytic enzymes include:

Enzyme	Hydrolysis of bonds formed by carboxyl groups of
Pepsin	Phe, Tyr, Trp, Met
Trypsin	Arg, Lys
Chymotrypsin	Phe, Tyr, Trp, Val, Leu
Elastase	Ala, Gly, Ser
Carboxypeptidase A	C-terminal aromatic amino acid
Carboxypeptidase B	C-terminal basic amino acid

1. Endopeptidases. They act on peptide bonds inside the protein molecule, so that the protein becomes successively smaller and smaller units. This group includes Pepsin, Trypsin, Chymotrypsin, and Elastase.

2. Exopeptidases, which act at the peptide bond only at the end region of the chain. This group includes: carboxypeptidase (acts on the peptide bond only at the carboxy terminal end of the chain), aminopeptidase, which acts on the peptide bond only at the amino terminal end of the chain.

Metabolism of aminoacids

METABOLISM 1

A. Gastric Digestion of Proteins

In the stomach, hydrochloric acid is secreted. It makes the pH optimum for the reaction of pepsin and also activates pepsin. The acid also denatures the proteins.

Enzymes: Rennin

Rennin otherwise called Chymosin, is active in infants and is involved in the curdling of milk. It is absent in adults. Milk protein, casein is converted to paracasein by the action of rennin. This denatured protein is easily digested further by pepsin.

Enzymes: Pepsin

It is secreted by the chief cells of stomach as inactive pepsinogen. The conversion of pepsinogen to pepsin is brought about by removal of 44 amino acids from the N-terminal end, by the hydrochloric acid. The optimum pH for activity of pepsin is around 2. Pepsin catalyses hydrolysis of the bonds formed by carboxyl groups of Phe, Tyr, Trp and Met.

Metabolism of aminoacids

METABOLISM 1

B. Pancreatic Digestion of Proteins

The optimum pH for the activity of pancreatic enzymes (pH 8) is provided by the alkaline bile and pancreatic juice. The secretion of pancreatic juice is stimulated by the peptide hormones, Cholecystokinin and Pancreozymin. Pancreatic juice contains the important enzymes, namely Trypsin, Chymotrypsin, Elastase and Carboxypeptidase.

These enzymes are also secreted as zymogens (trypsinogen, chymotrypsinogen and pro-elastase), so that the pancreatic acinar cells are not autolysed. All the three are serine proteases, i.e. the active centers of these enzymes contain serine residues.

Enzymes: Trypsin

Trypsinogen is activated by enterokinase (enteropeptidase) present on the intestinal microvillus membranes. Once activated, the trypsin activates other enzyme molecules. Trypsin is activated by the removal of a hexapeptide from N-terminal end. Trypsin catalyses hydrolysis of the bonds formed by carboxyl groups of Arg and Lys.

Enzymes: Chymotrypsin

Trypsin will act on chymotrypsinogen, in such a manner that A , B and C peptides are formed. These 3 segments are approximated, so that the active site is formed. Thus, selective proteolysis produces the catalytic site.

Enzymes: Carboxypeptidases

Small peptides generated by Trypsin and chymotrypsin are further hydrolysed into dipeptides and tripeptides by carboxypeptidases present in the pancreatic juice. The procarboxy peptidase is activated by trypsin. They are metalloenzymes requiring zinc.

Metabolism of aminoacids

METABOLISM 1

C. **Intestinal Digestion of Proteins**

Complete digestion of the small peptides to the level of amino acids is brought about by enzymes present in intestinal juice. The luminal surface of intestinal epithelial cell contains the following enzymes:

Enzymes: Leucine aminopeptidase

Releases the N-terminal basic amino acids and glycine.

Enzymes: Proline amino peptidase

It removes proline from the end of polypeptides.

Enzymes: Dipeptidases and tripeptidases

They will bring about the complete digestion of proteins

Metabolism of aminoacids

METABOLISM 1

D. **Absorption of AA**

The absorption of amino acids occurs mainly in the small intestine. It is an energy requiring process. These transport systems are carrier mediated and or ATP sodium dependent symport systems. There are 5 different carriers for amino acids:

1. Neutral amino acids (Alanine, Valine, Leucine, Methionine, Phenylalanine, Tyrosine, Isoleucine)
2. Basic amino acids (Lys, Arg) and Cysteine
3. Imino acids and Glycine
4. Acidic amino acids (Asp, Glu)
5. Beta amino acids (beta alanine).

Food Allergy

Dipeptides and tripeptides can enter the brush border of mucosal cells; they are immediately hydrolysed into single amino acids. They are then transported into portal vein. Rarely, larger molecules may pass paracellularly (between epithelial cells) and enter blood stream. These are immunogenic, causing antibody reaction, leading to food allergy. Caveolae mediated transcytosis has been shown to transport IgA molecules intact across the mucosal cell.

Metabolism of aminoacids

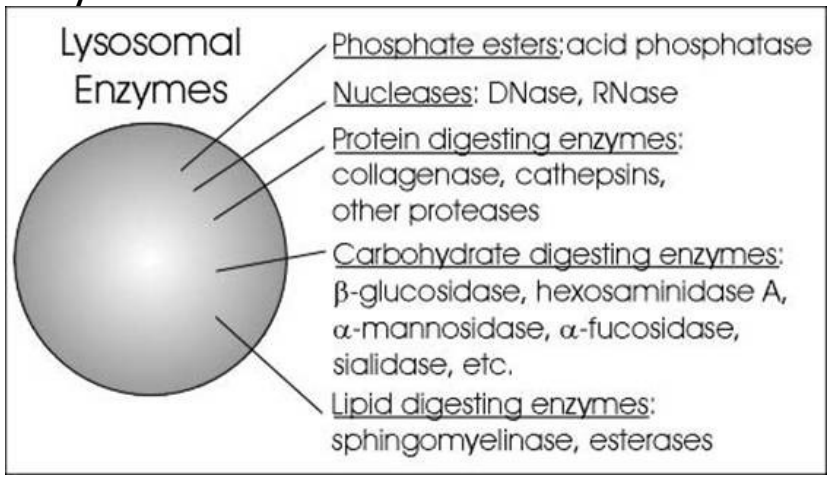
METABOLISM 1

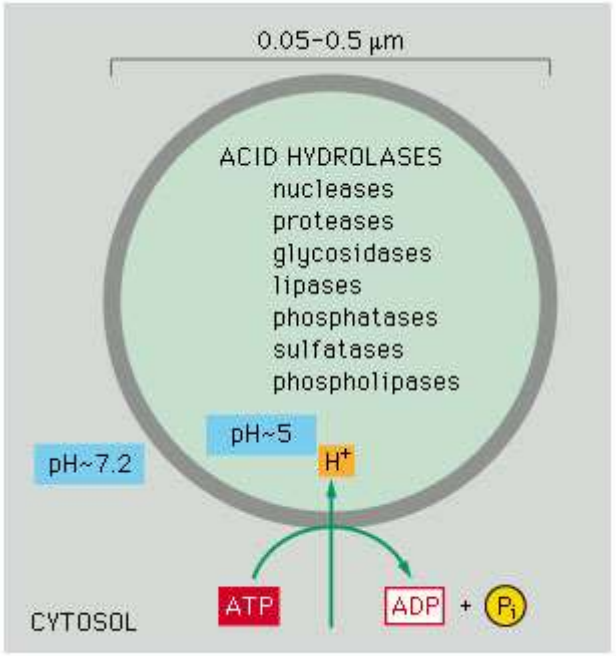
E. **All proteins in the body are constantly being degraded.**

The half-life of proteins is highly variable. Ornithine decarboxylase has only 11 minutes. Half life of hemoglobin depends on the life span of RBCs. The lens protein, Crystallin remains unchanged throughout the life of the organism. Damaged or defective proteins are prematurely degraded.

Intracellular Protein Degradation

Half-life of a protein is the time taken to lower its concentration to half of the initial value. General tissue proteins have halflives of few hours. Key enzymes have half lives usually of about few minutes only. PEST sequence (areas rich in proline, glutamate, serine and threonine) on a protein will give an inherent message to breakdown that protein very quickly. Extracellular particles or proteins are taken by endocytosis and are fused with Lysosomes.





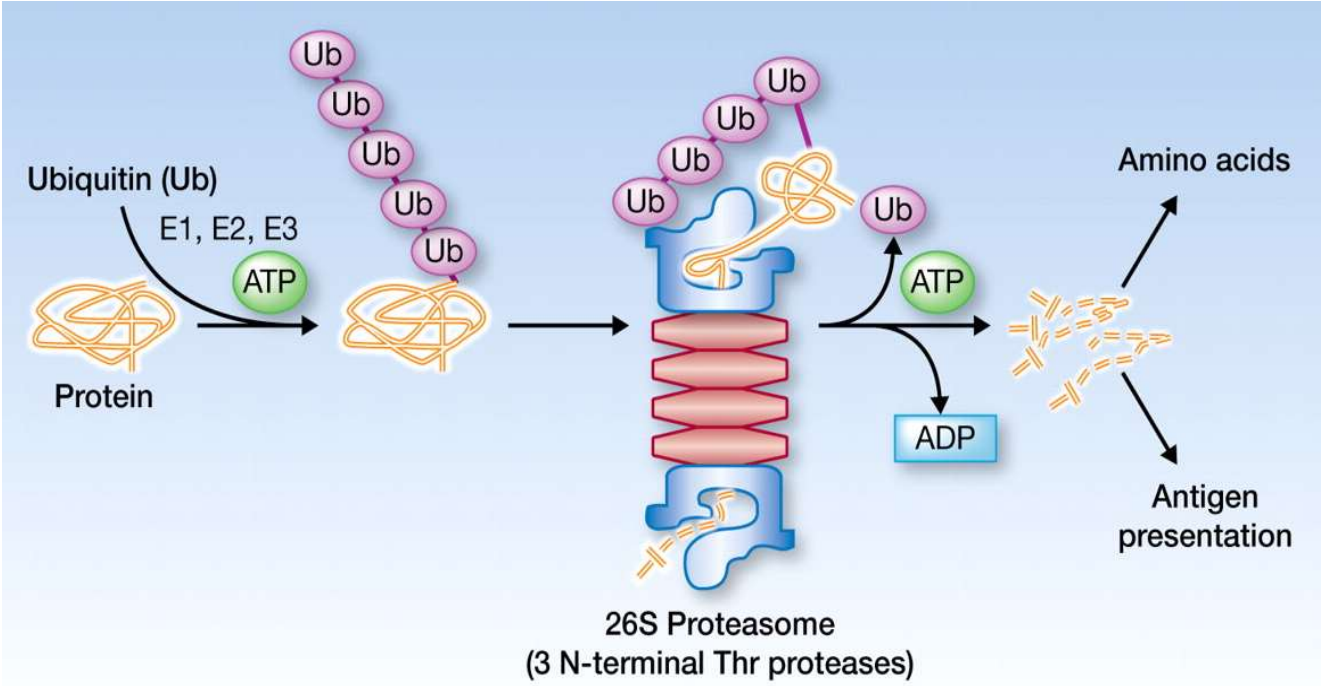
Metabolism of aminoacids

METABOLISM 1

E. **All proteins in the body are constantly being degraded.**

Ubiquitin-Proteasomes

Intracellular protein breakdown also occurs independent of lysosomes. This involves ubiquitin. It is a small protein with 76 residues (mol.wt., 8.5 kDa). Ubiquitin is attached with proteins with the help of 3 enzymes, E1 (activating enzyme), E2 (ligase) and E3 (transferase). Ubiquitin attached proteins are immediately broken down inside the proteasomes of the cells. The proteasome assembly has a central cylindrical hollow core. Ubiquitin-tagged proteins are taken into this barrel, and surrounding proteolytic enzymes digest the protein into small oligopeptides of 5-6 amino acids. (*Ciechanover, Hershko and Rose Nobel Prize in 2004)*



Metabolism of aminoacids

F. **Inter-organ Transport of Amino Acids**

In plasma, all amino acids are seen at a level roughly of 1 mg/dl, except glutamic acid and glutamine, which are present in higher concentrations (each about 10 mg/dl). Breakdown of muscle protein is the source of amino acids for tissues while liver is the site of disposal

In the Fed State

Amino acids absorbed from the diet are taken up by different tissues. Both muscle and brain take up *branched chain amino acids*, and release *glutamine* and *alanine*.

The *glutamine* is delivered to kidneys to aid in regulation of acid–base balance.

Alanine is taken up by liver.

In Fasting State

The muscle releases mainly *alanine and glutamine. Alanine* is taken up by liver and *glutamine* by kidneys. Liver removes the amino group and converts it to urea and the carbon skeleton is used for gluconeogenesis. (also refer glucose-alanine cycle under gluconeogenesis). The brain predominantly takes up branched chain amino acids.

GENERAL METABOLISM OF AMINO ACIDS

1. The anabolic reactions where proteins are synthesized.
2. Synthesis of specialized products such as heme, creatine, purines and pyrimidines.
3. The catabolic reactions where dietary and body proteins are broken down to amino acids.
4. Transamination: amino group is removed to produce the carbon skeleton (keto acid). The amino group liberated as ammonia is detoxified and excreted as urea.
5. The carbon skeleton is used for synthesis of nonessential amino acids.
6. It is also used for gluconeogenesis or for complete oxidation.
7. Other minor metabolic functions like conjugation, methylation, amidation, etc.

Metabolism of aminoacids

TRANSAMINATION

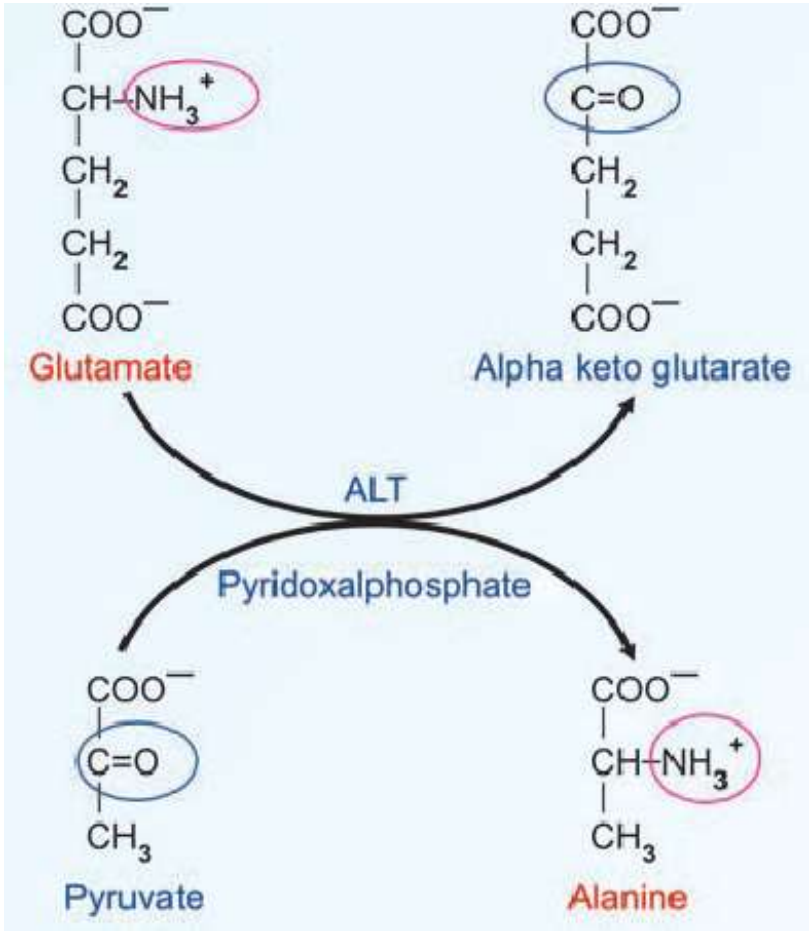
Transamination is the exchange of the alpha amino group between one alpha amino acid and another alpha keto acid, forming a new alpha amino acid.

amino acid 1 + keto acid 2 → amino acid 2 + keto acid 1

In almost all cases, the amino group is accepted by alpha ketoglutaric acid so that glutamic acid is formed.

The enzymes catalysing the reaction as a group are known as amino transferases (ALT = alanine AT).

These enzymes have pyridoxal phosphate as prosthetic group. The reaction is readily reversible.



Exceptions

Lysine, threonine and proline are not transaminated. They follow direct degradative pathways.

Clinical Significance of Transamination

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) are induced by glucocorticoids which favor gluconeogenesis. AST and ALT are markers of liver injury

Metabolism of aminoacids

TRANSAMINATION

Transamination is the exchange of the alpha amino group between one alpha amino acid and another alpha keto acid, forming a new alpha amino acid.

Biological Significance of Transamination

1. *First step of catabolism*

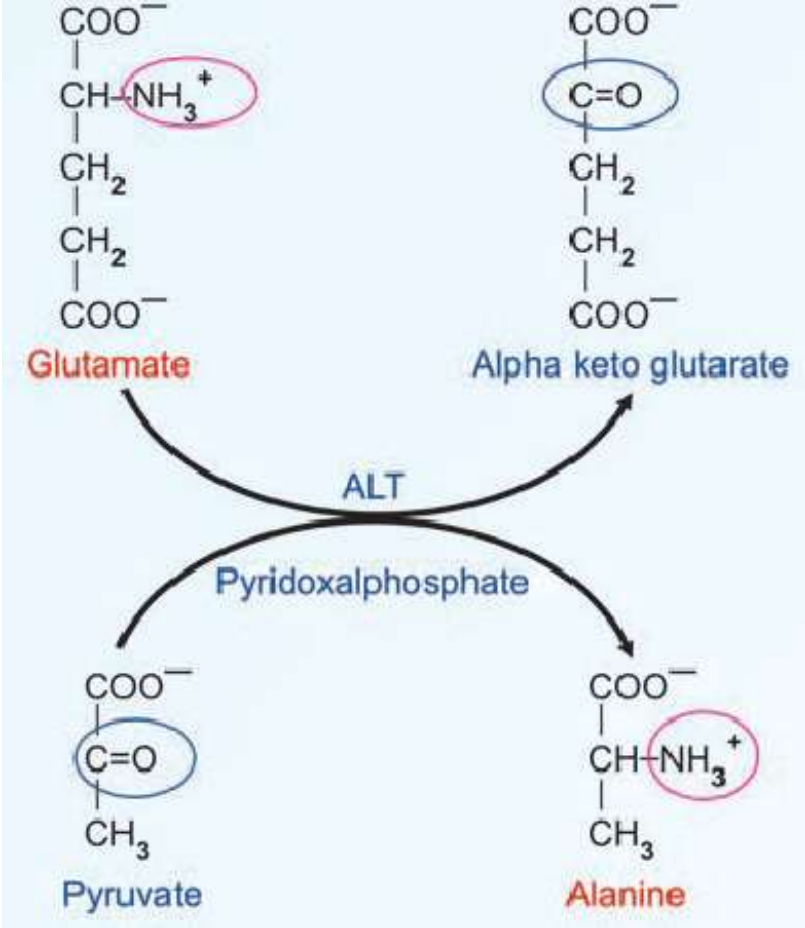
Removal of ammonia – generation of **carbon skeleton** of the amino acid that enters catabolic pathway.

2. *Synthesis of nonessential amino acids*

All nonessential amino acids can be synthesized by the body from keto acids available from other sources. For example, **pyruvate** can be transaminated to alanine. Similarly **oxaloacetate to** aspartic acid. **Alpha keto glutarate** to glutamic acid. Those amino acids, which cannot be synthesized in this manner, are therefore essential

3. *Interconversion of amino acids*

If amino acid no.1 is high and no.2 is low; the amino group from no.1 may be transferred to a keto acid to give amino acid no. 2 to equalize the quantity of both.



Metabolism of aminoacids

TRANSDEAMINATION

Trans-deamination = transamination followed by deamination and liberation of ammonia (by oxidative deamination).

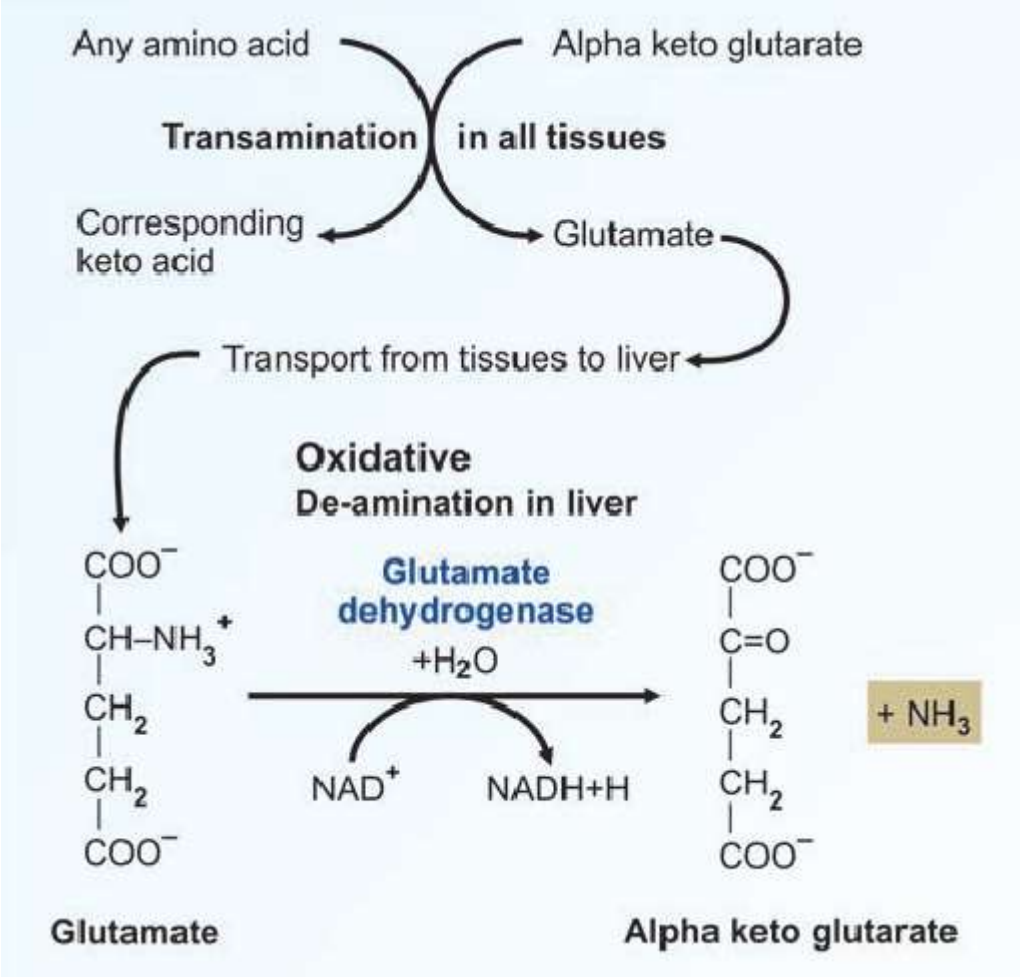
Biological Significance of Transdeamination

Removal of ammonia – generation of **carbon skeleton** of the amino acid that enters catabolic pathway.

! Transamination occurs in cytoplasm of all cells, the aminogroup is transferred by glutamate to liver, where it is deaminated in mitochondria.

ONLY liver mitochondria contains GDH (glutamate dehydrogenase)

Deamination rate = 50-70 g per day



Metabolism of aminoacids

DEAMINATION

There are several other pathways of deamination –

All AA but hydroxy/dicarboxylic can be converted from L-amino to L-imino acids with formation of peroxide (from O2). This peroxide is decomposed in peroxisomes by catalase, while L-iminoacids (COOH-C(R)=NH) are converted to keto acids (COOH-C(R)=O). ENZYME = **L-amino acid oxidase**! (FMN – coenzyme)

Glycine and D-aminoacids of bacterial metabolism can be oxidized by ENZYME **D-amino acid oxidase** (FAD – coenzyme).

Dehydratases –
Hydroxy-aminoacids (serine -> -H2O + C=C -> isomerization to imino acid -> –hydrolysis-> pyruvate, threonine -> alpha-keto-butyric acid)

Desulfhydrase –
Cysteine (deamination + simultaneous trans-sulphuration -> pyruvate)

Metabolism of aminoacids

DEAMINATION What's then??

DISPOSAL/DETOXIFICATION OF AMMONIA

1. **First line of Defense (Trapping of ammonia)**

Being highly toxic, ammonia should be eliminated or detoxified, as and when it is formed. The intracellular ammonia is immediately trapped **by glutamic acid** to form **glutamine**, especially in brain cells (!!). The glutamine is then transported to liver, where the reaction is reversed by the enzyme glutaminase. The ammonia thus generated is immediately detoxified into urea. Aspartic acid may also undergo similar reaction to form asparagine.

2. **Transportation of Ammonia**

Inside the cells of almost all tissues, the transamination of amino acids produce glutamic acid. The final deamination and production of ammonia is taking place in the liver (GDH). Thus, glutamic acid acts as the link between amino groups of amino acids and ammonia. **Glutamine** is the transport forms of ammonia from brain and intestine to liver; while **alanine** is the transport form from muscle.

3. **Final disposal**

The ammonia from all over the body thus reaches liver. It is then detoxified to urea by liver cells, and then excreted through kidneys. Urea is the end product of protein metabolism. Fishes excrete ammonia as such while birds and reptiles as uric acid. Although ammonia is toxic and has to be immediately detoxified, in kidney cells, ammonia is purposely generated from glutamine with the help of glutaminase. This is for buffering the acids, and maintaining acid-base balance.

Metabolism of aminoacids

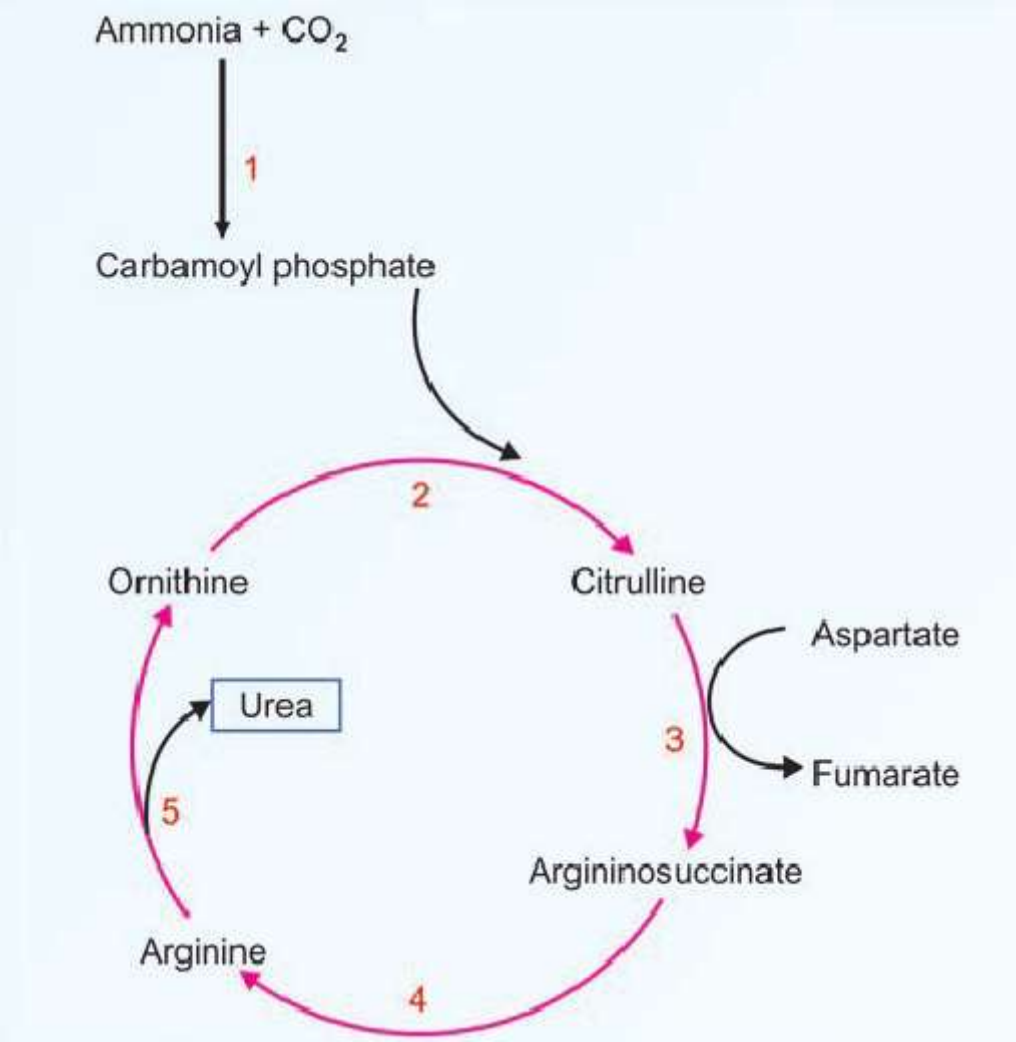
DEAMINATION

UREA CYCLE

In 1773, Rouelle isolated urea from urine. The urea cycle is the first metabolic pathway to be elucidated (1932). The cycle is known as Krebs–Henseleit urea cycle. As ornithine is the first member of the reaction, it is also called as Ornithine cycle.

SUMMARY:

The two nitrogen atoms of urea are derived from two different sources, one from ammonia and the other directly from the alpha amino group of aspartic acid.



Metabolism of aminoacids

DEAMINATION

UREA CYCLE

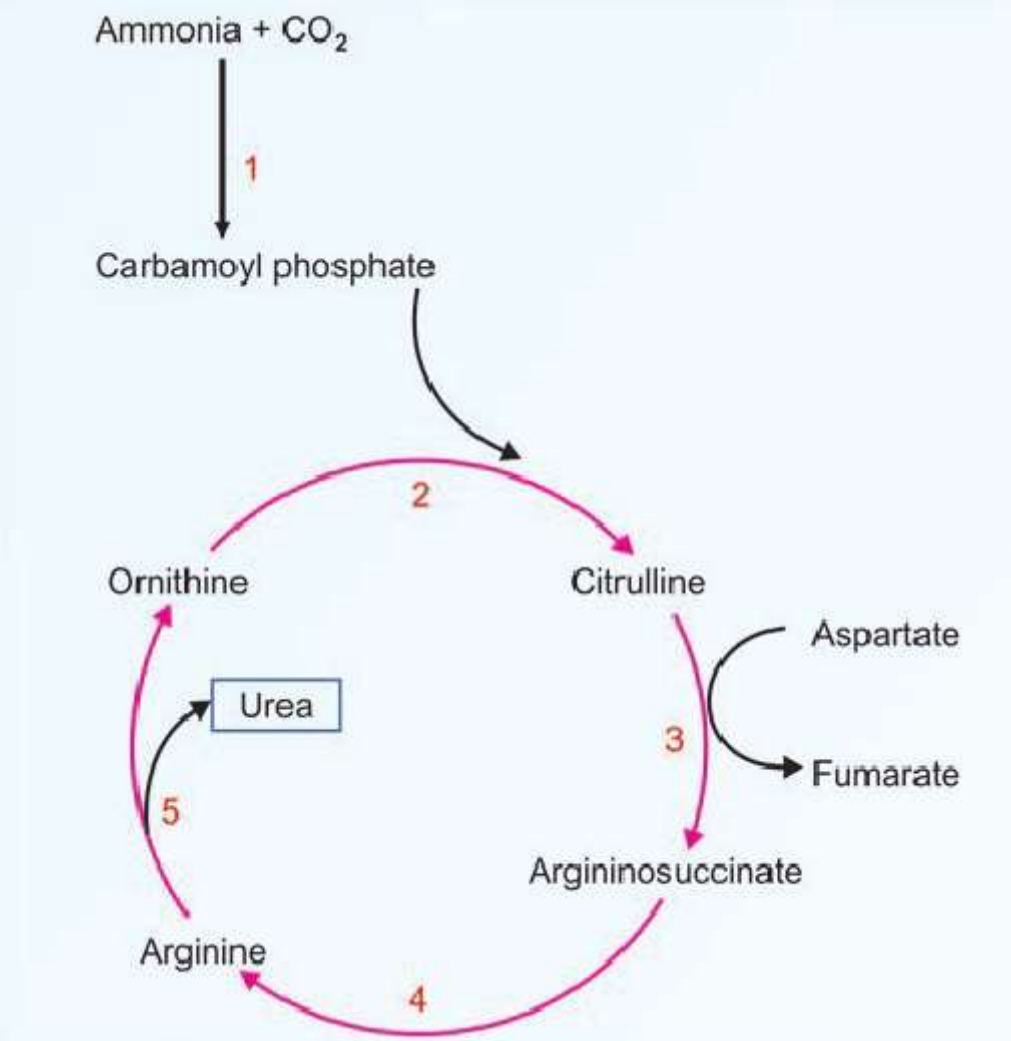
Step 1. Formation of Carbamoyl Phosphate
(**rate-limiting**, mitochondrial, requires energy)

Step 2. Formation of Citrulline (mitochondrial, transfer of NH3-CO-pi to ornithine, pi leaves)

Step 3. Formation of Argininosuccinate (cytoplasm, another C-N bond formation, -C(N-)3, requires energy)

Step 4. Formation of Arginine (cytoplasm, cleavage, release of fumarate)

Step 5. Formation of Urea (hydrolysis of arginine)



Metabolism of aminoacids

DEAMINATION

UREA CYCLE

Step 1. Formation of Carbamoyl Phosphate
(**rate-limiting**, mitochondrial, requires energy)

N (NH3) OCO (CO2) H2O ATP (Appp) -> N-CO-pi

Step 2. Formation of Citrulline
(mitochondrial, transfer of NH3-CO-pi to ornithine, pi leaves)

AA-CCC-N + N-CO-pi -> AA-CCC-N-CO-N

Step 3. Formation of Argininosuccinate
(cytoplasm, another C-N bond formation, -C(N-)3, requires energy)

AA-CCC-N-CO-N + N-C(COOH)-C-COOH ->
AA-CCC-N-C(N)-N-C(COOH)-C-COOH

Step 4. Formation of Arginine
(cytoplasm, cleavage, release of fumarate)

AA-CCC-N-C(N)-N-C(COOH)-C-COOH ->
AA-CCC-N-C(N)=N + (COOH)C=C(COOH)

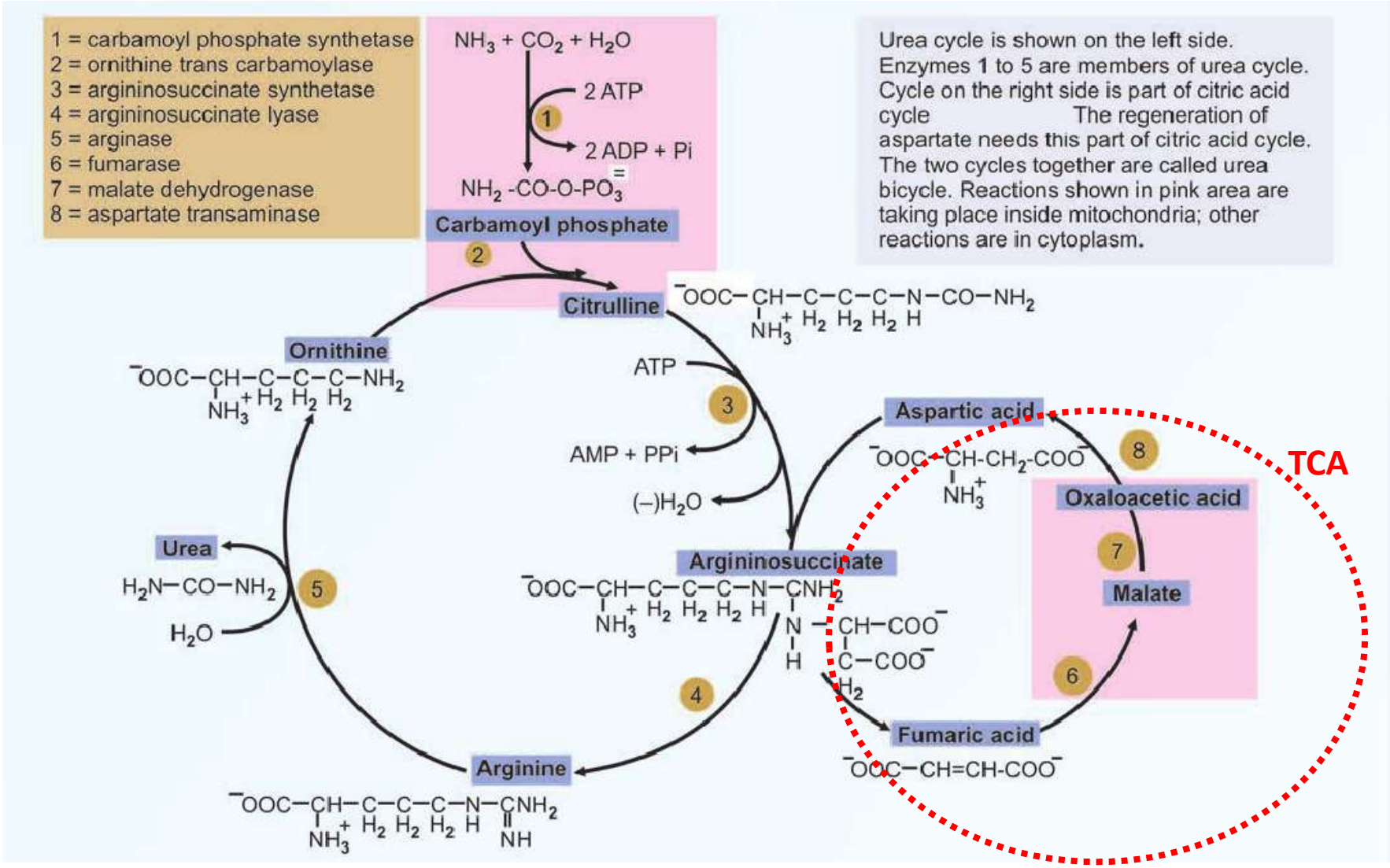
Step 5. Formation of Urea
(hydrolysis of arginine)

AA-CCC-N-C(N)=N + H2O -> AA-CCC-N + N-CO-N

Metabolism of aminoacids

DEAMINATION

UREA CYCLE



Metabolism of aminoacids

DEAMINATION

UREA CYCLE

2 ATPs are used in the 1st (preparative) reaction.
Another ATP is converted to AMP + PPi in the 3rd step (equivalent to 2 ATPs).
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Total = 4 high energy phosphate bonds.

However, fumarate may be converted to malate. Malate when oxidised to oxaloacetate (OAA) produces 1 NADH equivalent to 3 ATP. So net energy expenditure is only 1 high energy phosphate.

Disorders of Urea Cycle

Deficiency of any of the urea cycle enzymes would result in hyperammonemia. When the block is in one of the earlier steps, the condition is more severe, since ammonia itself accumulates. Deficiencies of later enzymes result in the accumulation of other intermediates which are less toxic and hence symptoms are less. As a general description, disorders of urea cycle are characterized by hyperammonemia, encephalopathy and respiratory alkalosis. Low protein diet with sufficient arginine and energy by frequent feeding can minimize brain damage since ammonia levels do not increase very high

Metabolism of aminoacids

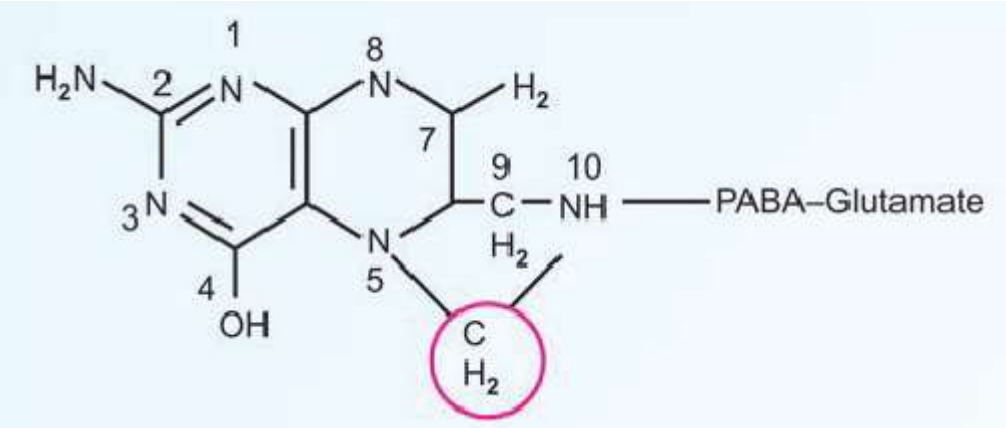
DEAMINATION

UTILIZATION of glycine

Glycine undergoes oxidative deamination (reversal of glycine synthase) to form NH3, CO2 and the one carbon unit methylene THFA. This pathway is the major catabolic route for glycine. The glycine cleavage system is a multi-enzyme complex consisting of:

A. Glycine decarboxylase with pyridoxal phosphate

B. Lipoamide containing amino methyl transferase

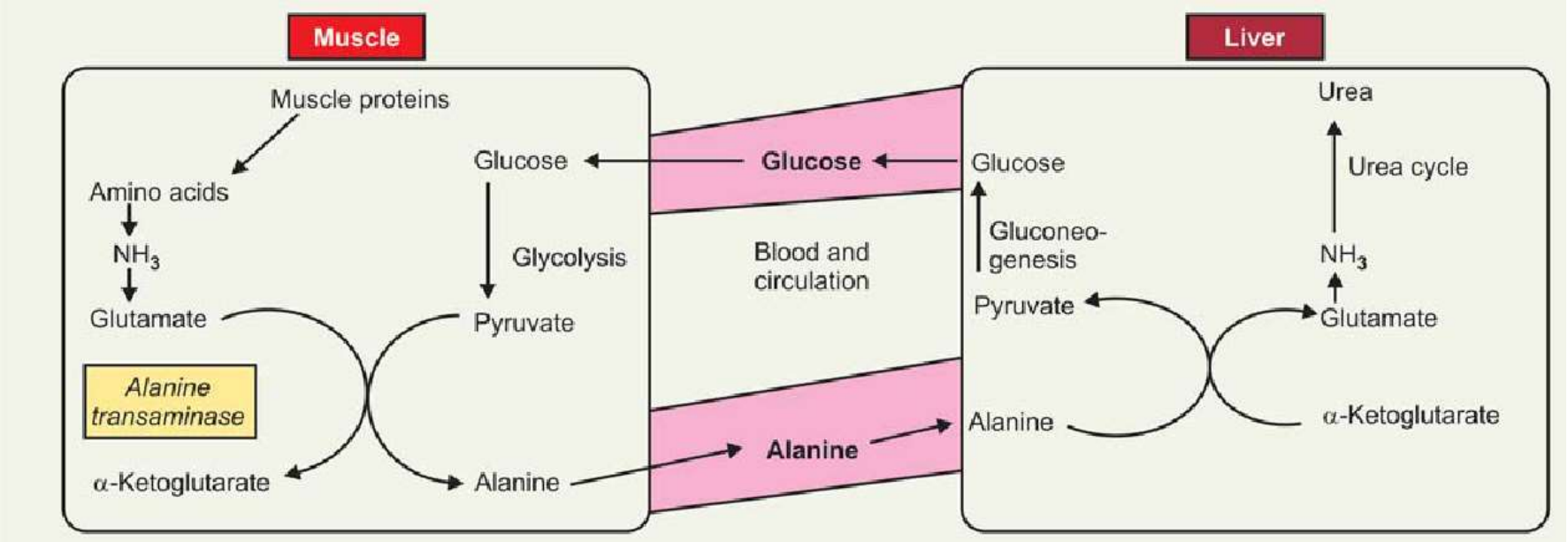


Metabolism of aminoacids

TRANSPORT OF NH3 from muscles

GLUCOSE-ALANINE CYCLE

1. Skeletal muscle transports NH3 to the Liver in the form of the amino acid ‘alanine’. The alanine is formed in the muscle by a transamination between pyruvate (PA) and glutamate.
2. The alanine is transported to the liver. It reacts with α-ketoglutarate to reform PA + glutamate.
3. The nitrogen originating from the glutamate is processed by the urea cycle.
4. When the blood glucose is low, the Pyruvate in liver is used to make glucose.
5. The glucose can be returned to the skeletal muscle to supply quick energy.
6. Cycle is proportional to the muscular activity of the organism. It is to be noted that active muscle tissue operates anaerobically, producing large quantities of PA and consuming large quantities of glucose.

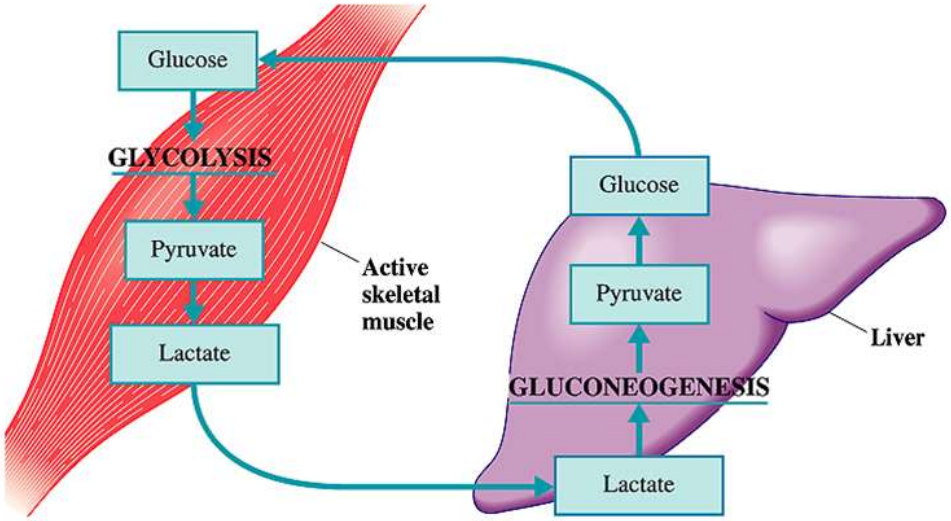


Bioenergetics

GLUCONEOGENESIS

It is the process by which glucose molecules are produced from non-carbohydrate precursors. These include lactate, glucogenic amino acids, glycerol part of fat and propionyl CoA derived from odd chain fatty acids.

Gluconeogenesis occurs mainly in the liver, and to a lesser extent in the renal cortex. The pathway is partly mitochondrial and partly cytoplasmic.



CORI'S CYCLE OR LACTIC ACID CYCLE

It is a process in which glucose is converted to lactate in the muscle; and in the liver this lactate is re-converted into glucose.

Bioenergetics

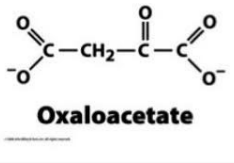
GLUCONEOGENESIS (GNG)

Gluconeogenesis involves several enzymes of glycolysis, but it is not a reversal of glycolysis. The irreversible steps in glycolysis are circumvented by four enzymes which are designated as the key enzymes of gluconeogenesis

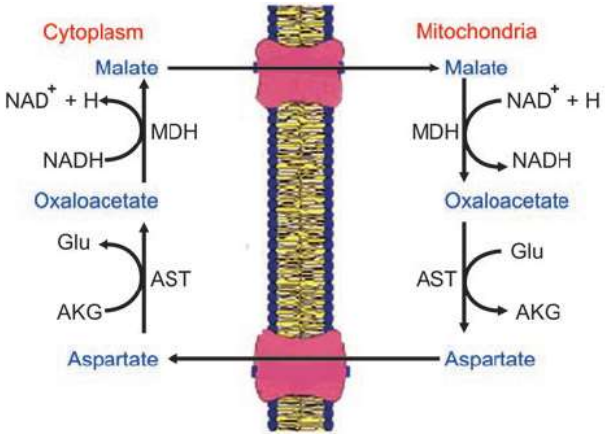
1. Pyruvate Carboxylase Reaction

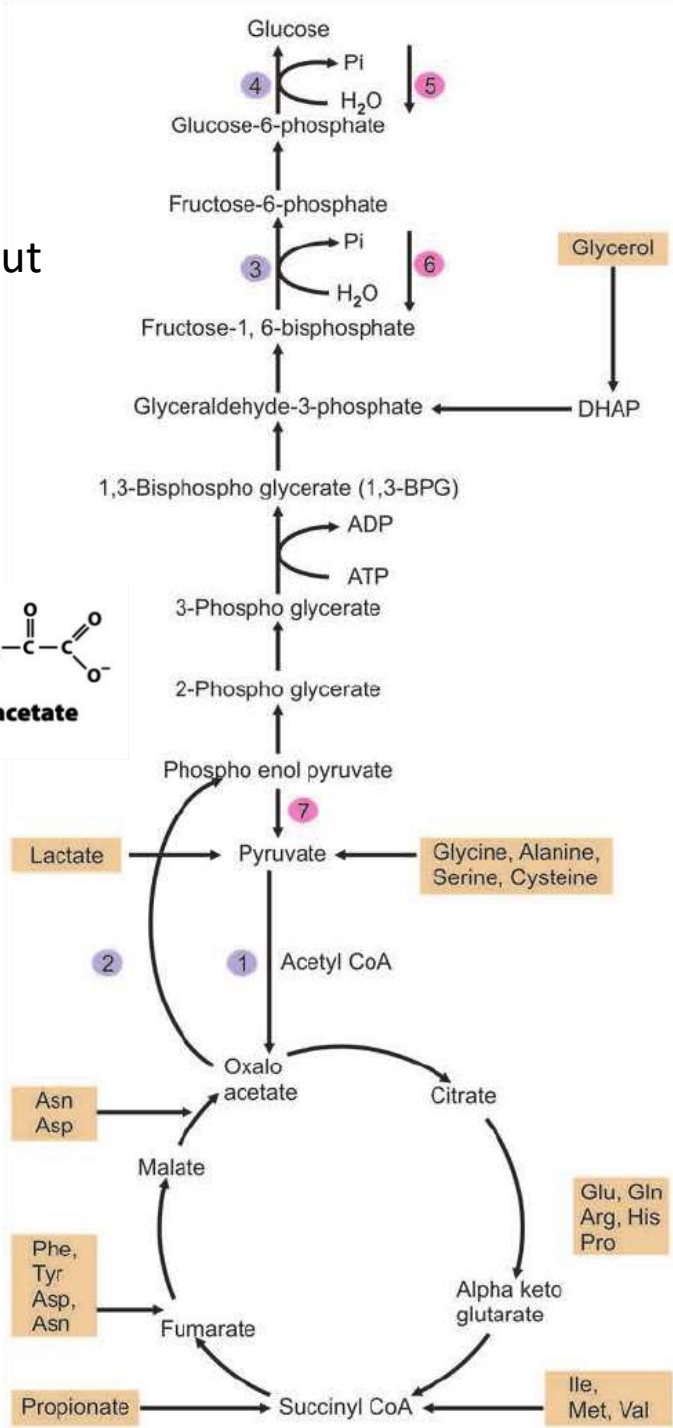
Pyruvate: $H_3C-C(=O)-C(=O)-O^-$

Pyruvate in the cytoplasm enters the mitochondria. Then, carboxylation of pyruvate to oxaloacetate is catalysed by a mitochondrial enzyme, pyruvate carboxylase (uses CO2, co-enzymes - biotin and ATP).



1.5. OAA is transferred to cytoplasm by Malate-Aspartate shuttle (the transfer reactions on the picture are reversible). If alanine is used - then malate is used (since NADH is also required in the cytoplasm for the GNG); if lactate – then aspartate shuttle (theres enough NADH in cytoplasm)



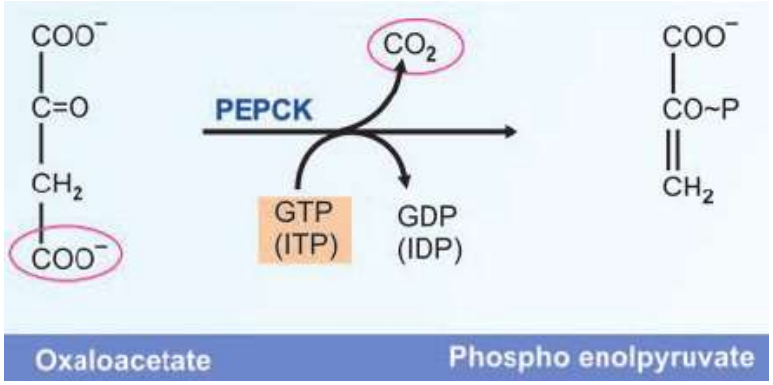


Bioenergetics

GLUCONEOGENESIS (GNG)

2. Phosphoenol Pyruvate Carboxy Kinase

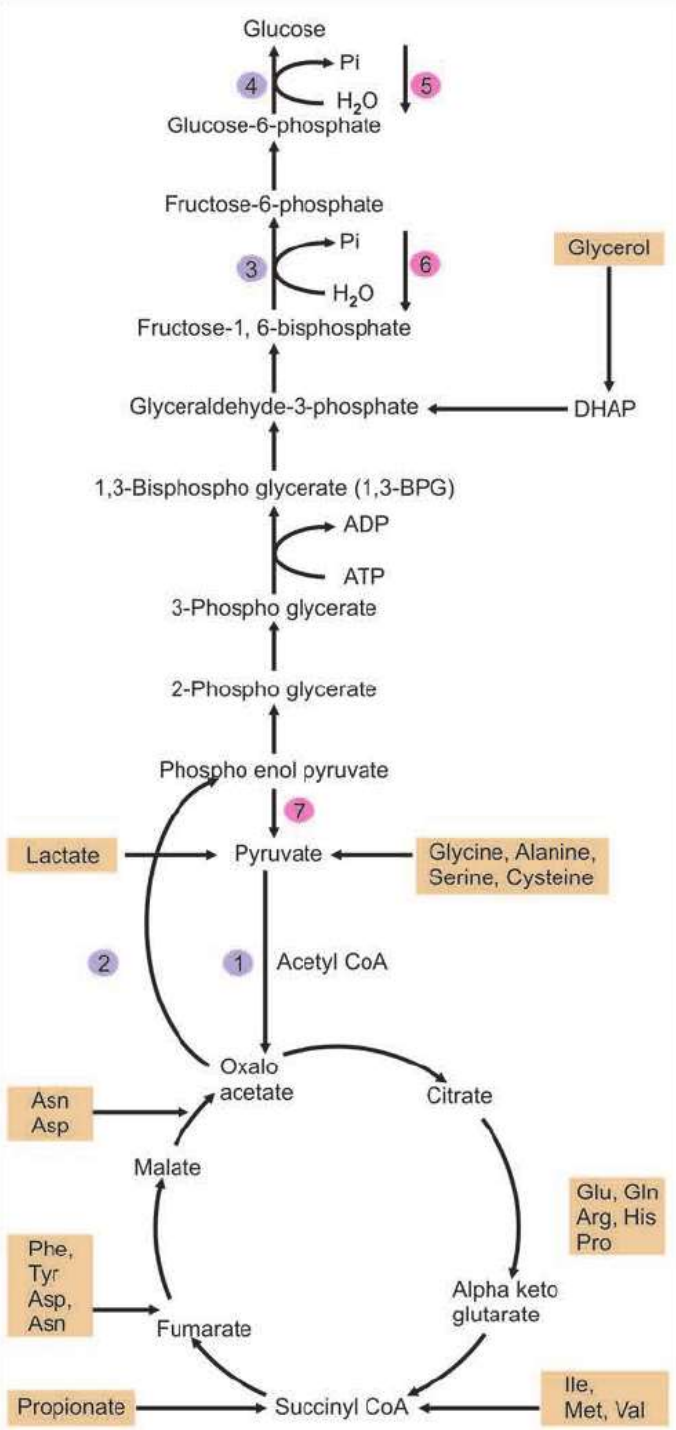
In the cytoplasm, PEPCK enzyme then converts oxaloacetate to phosphoenol pyruvate by removing a molecule of CO2. GTP or ITP donates the Phosphate.



So far - The net effect of two reactions is the conversion of pyruvate to phosphoenol pyruvate. This circumvents the irreversible step in glycolysis catalyzed by pyruvate kinase.

Then Partial Reversal of Glycolysis

The phosphoenol pyruvate undergoes further reactions catalyzed by the glycolytic enzymes to form fructose-1,6-bisphosphate. All these reactions are freely reversible.



Bioenergetics

GLUCONEOGENESIS (GNG)

3. Fructose-1,6-bisphosphatase

Fructose-1,6-biphosphate is then converted to fructose-6-phosphate. This will bypass the step of PFK reaction.

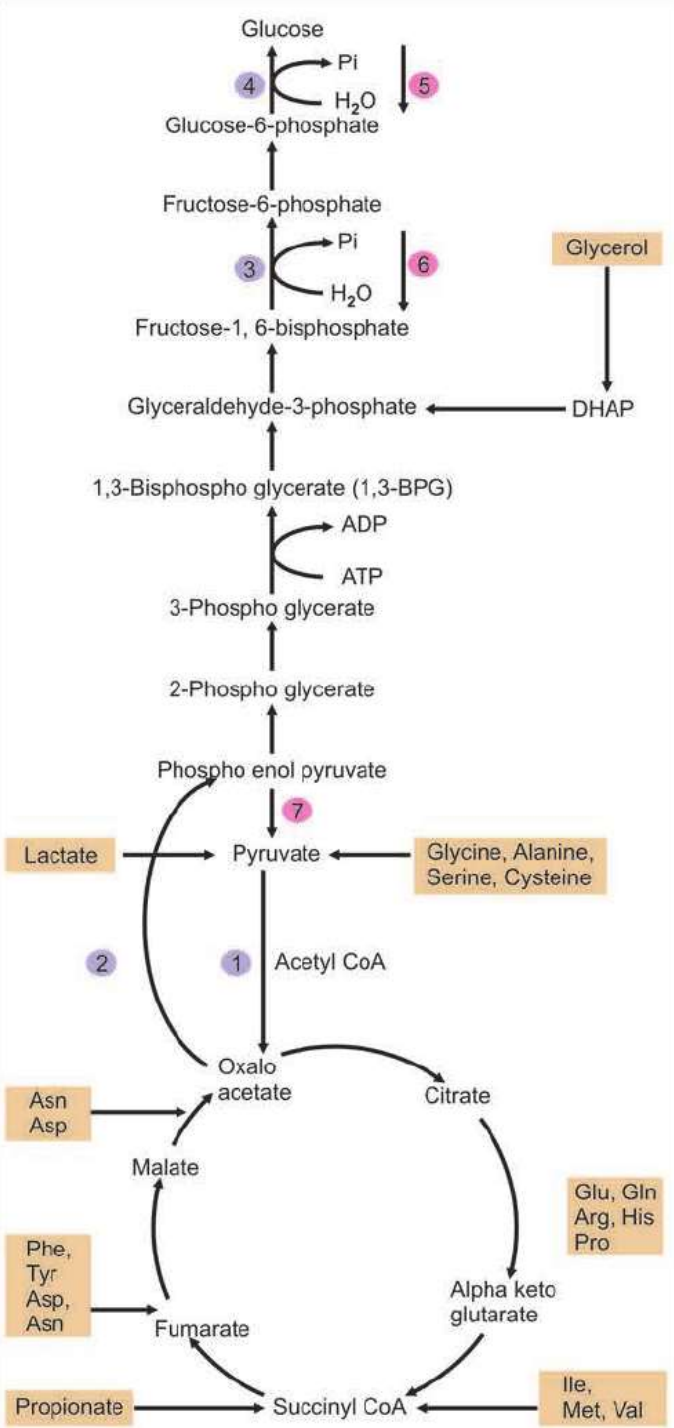
Then fructose-6-phosphate is isomerized to glucose-6-phosphate by the freely reversible reaction catalyzed by hexosephosphate isomerase

4. Glucose-6-phosphatase Reaction

The glucose 6-phosphate is hydrolysed to free glucose by glucose-6-phosphatase which is active in liver. It is present in kidney and intestinal mucosa to a lesser extent, but is absent in muscle.

Essential steps of gluconeogenesis have irreversible "twin reactions" in glycolysis catalazid by:

5. hexokinase
6. phosphofructokinase
7. Pyruvate kinase



Bioenergetics

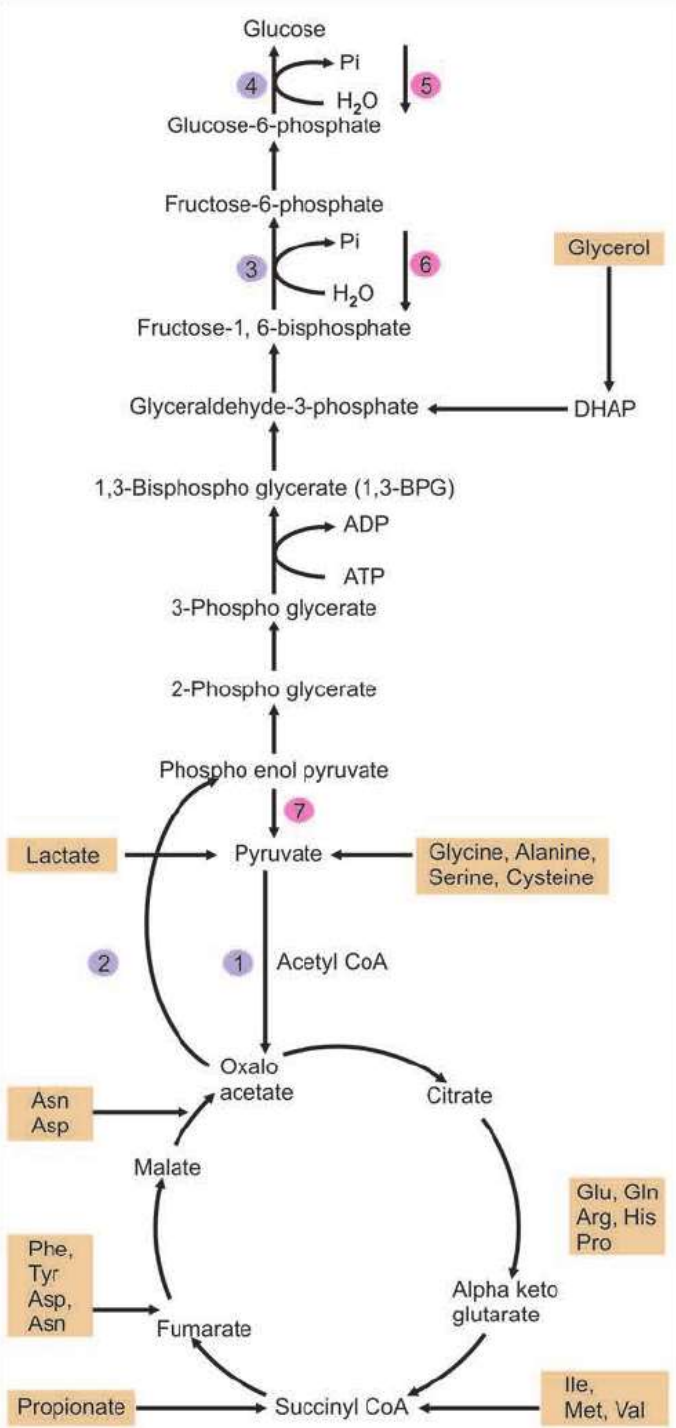
GLUCONEOGENESIS (GNG)

Significance of Gluconeogenesis

1. Only liver can replenish blood glucose through gluconeogenesis, because glucose-6-phosphatase is present mainly in liver. So liver plays the major role in maintaining the blood glucose level.

2. During starvation gluconeogenesis maintains the blood glucose level. The stored glycogen is depleted within the first 12-18 hours of fasting. On prolonged starvation, the gluconeogenesis is speeded up and protein catabolism provides the substrates, namely glucogenic amino acids.

Energy Requirement: The reactions catalyzed by pyruvate carboxylase, phosphoenol pyruvate carboxy kinase and phospho glycerate kinase require one ATP each; so 6 ATPs are required to generate one glucose molecule.
Glycolysis of this glucose in muscles w/o O2 will give 2ATP. Deficit of energy (energy debt) result in increased catabolism and oxygenation (oxygen debt).

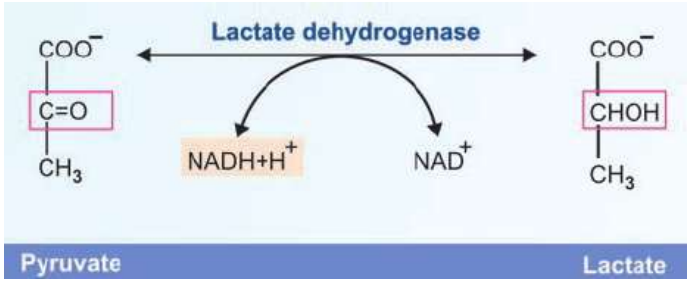


Bioenergetics

GLUCONEOGENESIS (GNG)

INPUT SOURCES TO GNG

Lactate

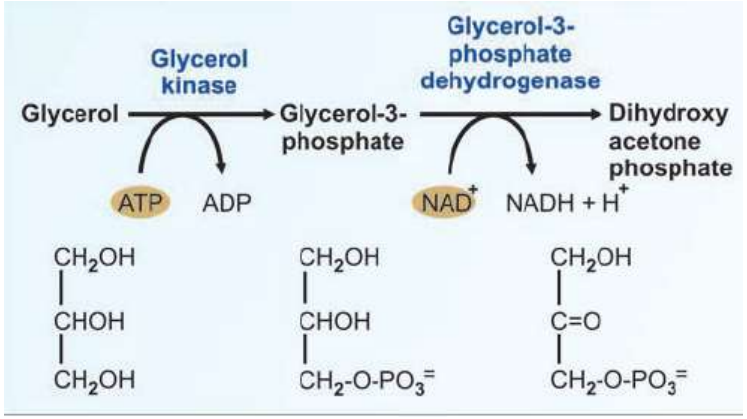


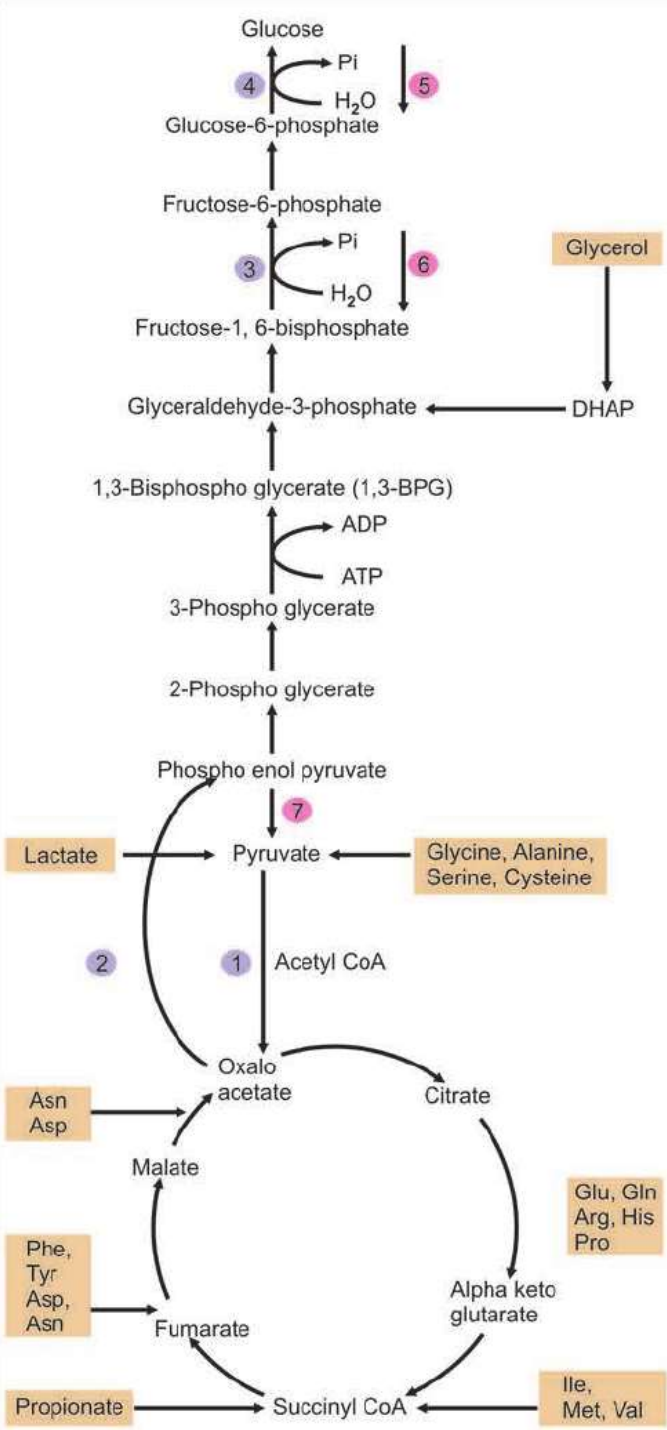
Glucogenic amino acids

W/o glucose (starvation/diabetes) alanine, glutamic acid, aspartic acid etc. are transaminated to carbon skeletons. They enter TCA and result in OAA/pyruvate. (Alanine influx from muscles! Muscle waste during diabetes).

Glycerol

Is phosphorylated in liver





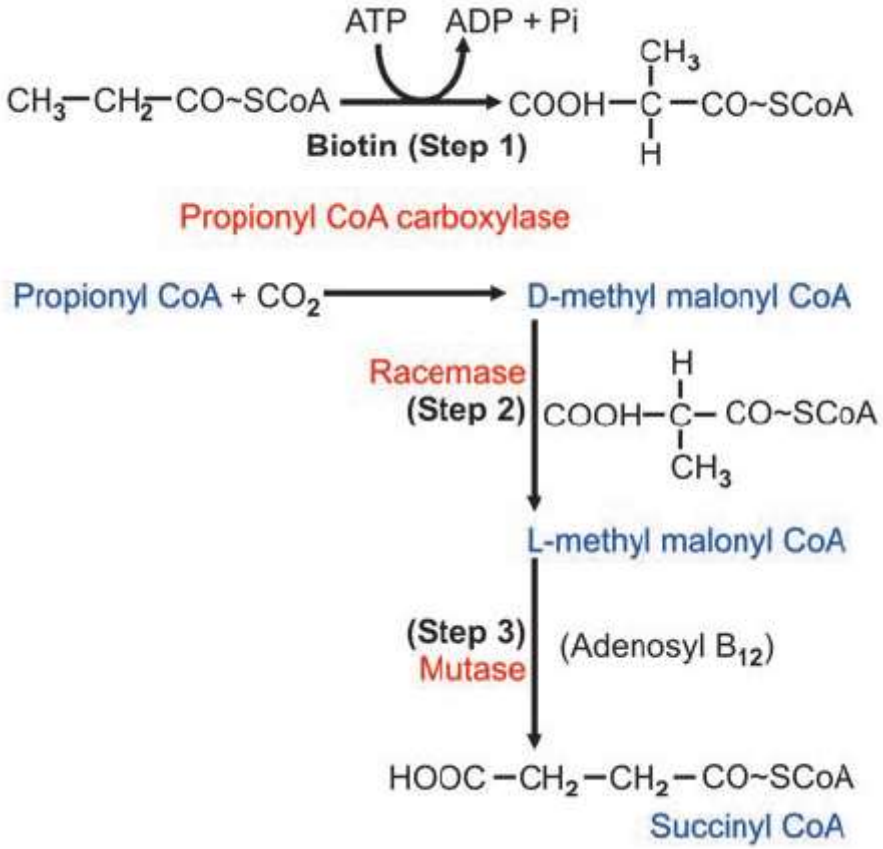
Bioenergetics

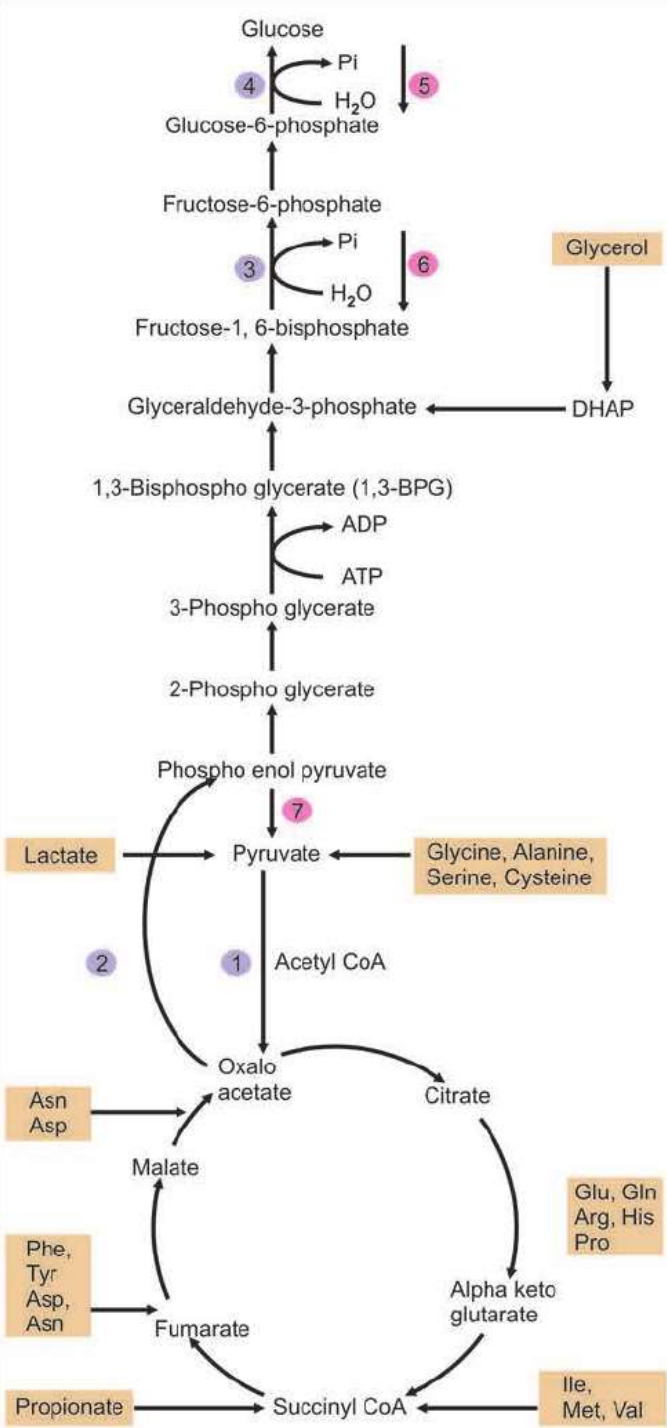
GLUCONEOGENESIS (GNG)

INPUT SOURCES TO GNG

Propionyl-CoA

From odd chain FA and some carbon skeletons of AA





Bioenergetics

GLUCONEOGENESIS (GNG)

Regulation

Gluconeogenesis and glycolysis are reciprocally regulated so that one pathway is relatively inactive when the other is active. Enzymes to control:

1. Pyruvate Carboxylase

It is an allosteric enzyme. Acetyl CoA is an activator of pyruvate carboxylase so that generation of oxaloacetate is favored when acetyl CoA level is sufficiently high

2. Fructose-1,6-bisphosphatase

Citrate is an activator while fructose-2,6-bisphosphate and AMP are inhibitors. All these three effectors have an exactly opposite effect on the phospho fructo kinase (PFK)

3. ATP

Gluconeogenesis is enhanced by ATP.

4. Hormonal Regulation of Gluconeogenesis

i. The hormones glucagon and gluco corticoids increase gluconeogenesis

ii. Glucocorticoids induce the synthesis of hepatic amino transferases thereby providing substrate for gluconeogenesis.

iii. The high glucagon-insulin ratio also favors induction of synthesis of gluconeogenic enzymes (PEPCK, Fructose-1,6-bisphos-phatase and glucose-6-phosphatase).

iv. At the same time, synthesis of glycolytic enzymes HK, PFK and PK are depressed.

v. Insulin inhibits the process

Literature biochemistry

1. Lehninger Principles of Biochemistry (Nelson D.L., Cox M.M.)
2. Principles and Techiniques of Biochemistry and Molecular Biology (Wilson K., Walker J.)