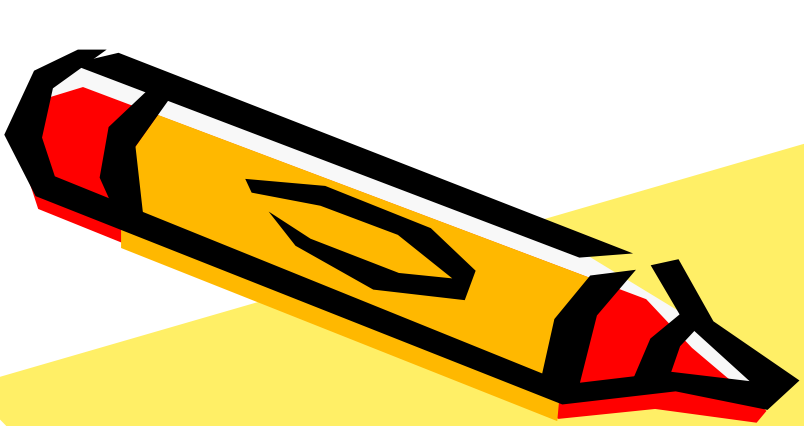
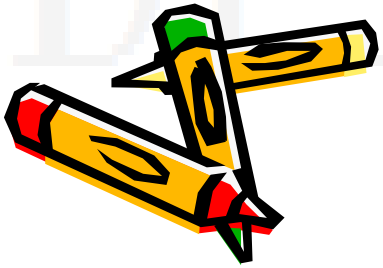


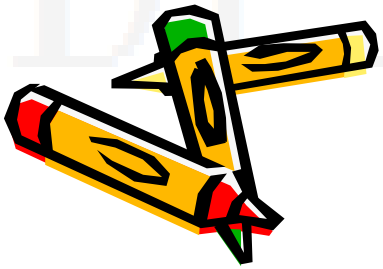
Protein & Amino Acid Metabolism AND The Urea Cycle



Dr Piyush B.Tailor
Department of Biochemistry
Govt.Medical College
Surat

Three sources of amino acids

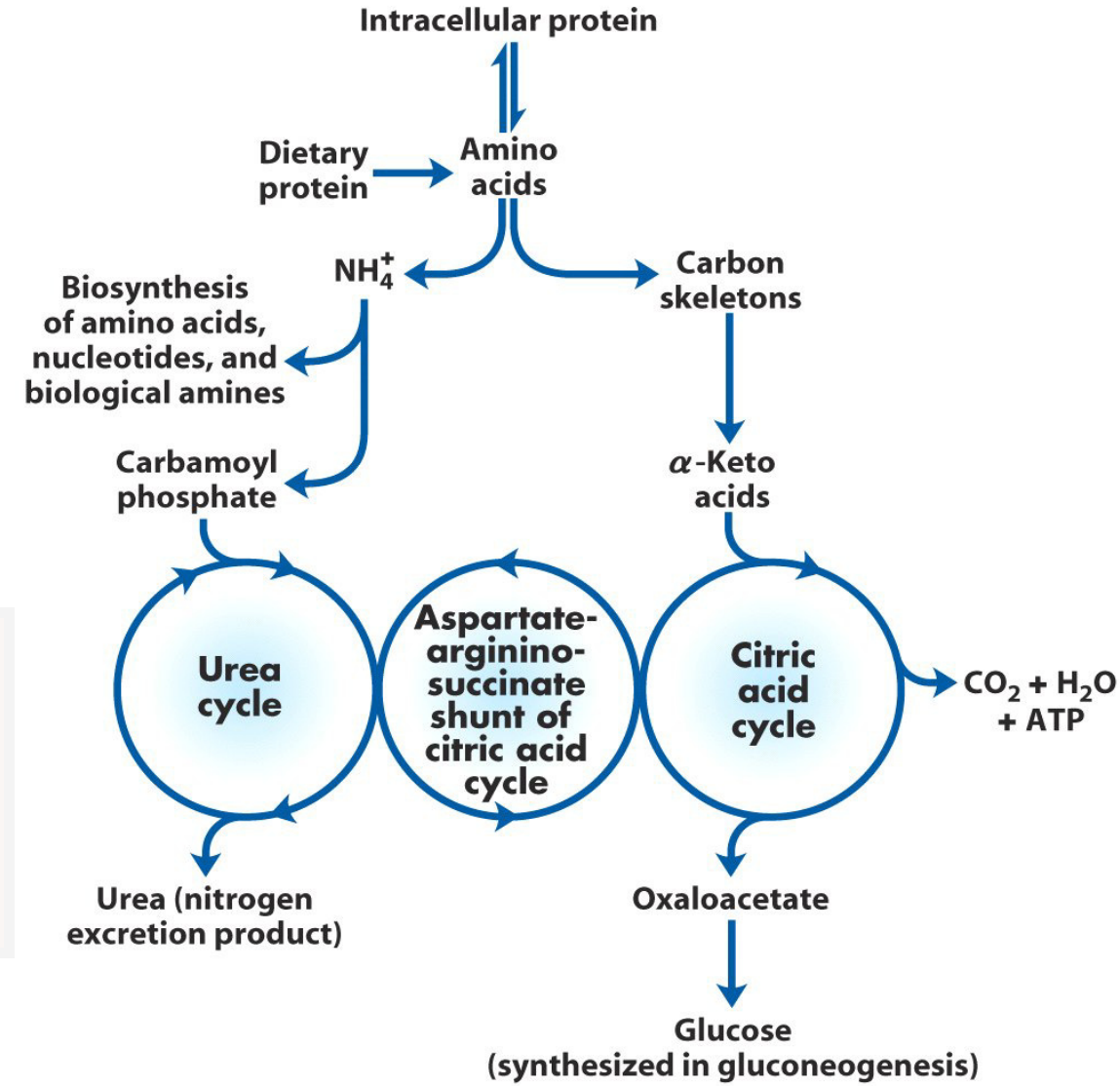
- Synthesis of Non-essential amino acid from metabolic intermediate.
- Breakdown of proteins.
- Amino acids derived from dietary protein.



Amino acid is depleted by three routes

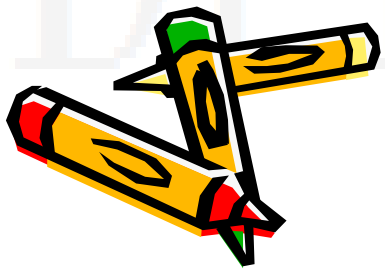
- Synthesis of body protein
- Amino acids consumed as precursors of essential nitrogen-containing small molecules
- Conversion of amino acids to glucose, glycogen, fatty acids or CO2

Amino Acid Degradation must account for an amino group



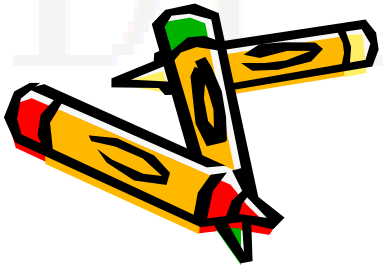
Protein Degradation

1. Ubiquitin – Proteasome Proteolytic enzyme
2. Chemical Signal for Protein Degradation



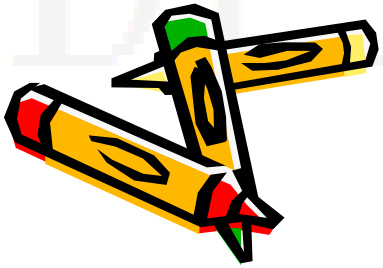
Ubiquitin – Proteasome Proteolytic enzyme

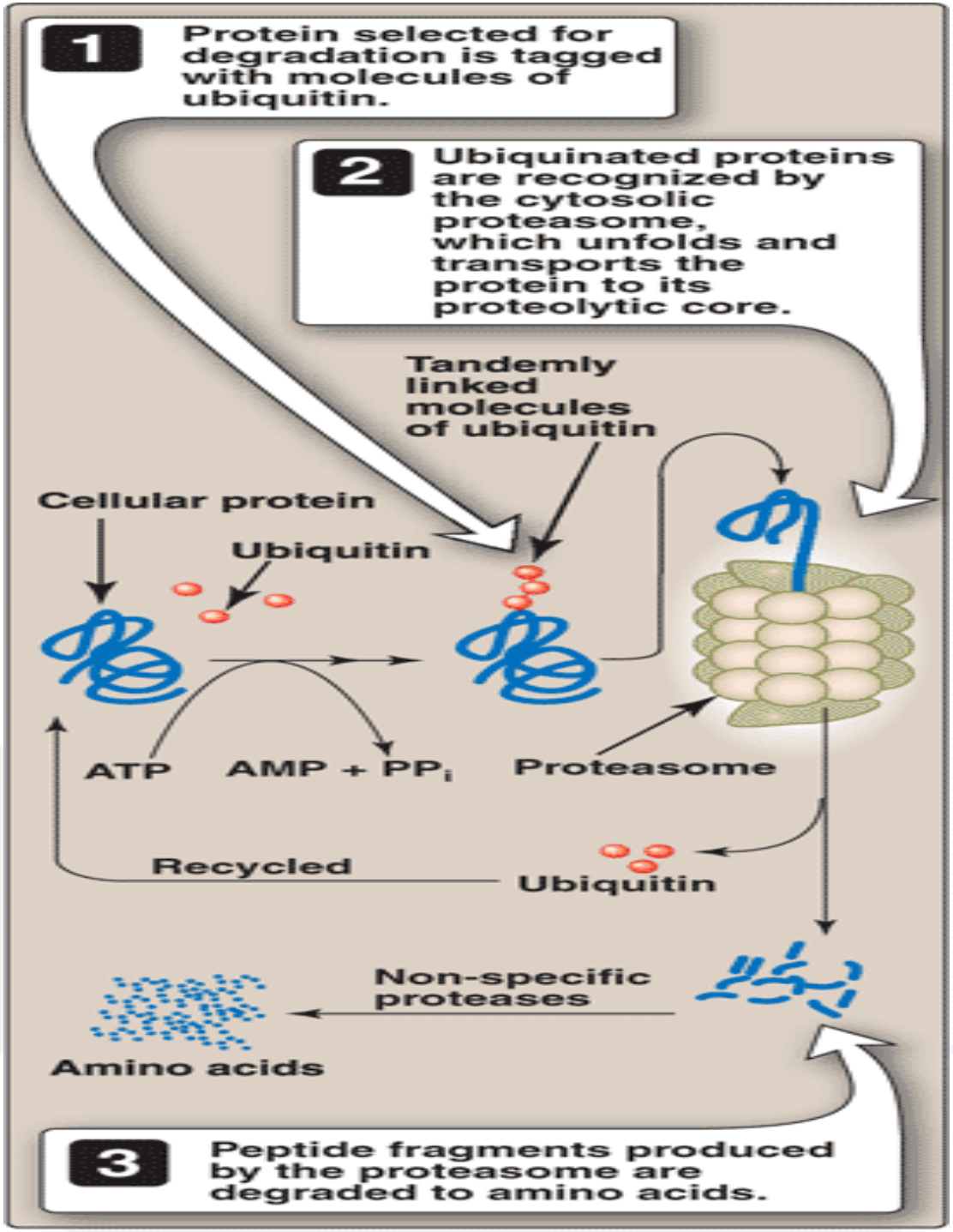
- first covalently attached to ubiquitin, a small globular protein.
- Through linkage of the Glycine of ubiquitin to a Lysine on protein substrate
- Proteins tagged with ubiquitin are targated by proteasome, which functions like a garbage disposal.
- The proteasome cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool.
- The ubiquitins are recycled.



Chemical Signal for Protein Degradation

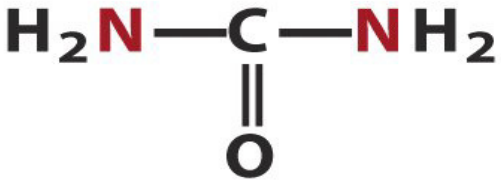
- Because proteins have different half-lives, it is clear that protein degradation cannot be random.
- But rather is influenced by some structural aspect of the protein.
- For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded.
- The half-life of a protein is influenced by the nature of the N-terminal residue.




1
Protein selected for degradation is tagged with molecules of ubiquitin.
2
Ubiquinated proteins are recognized by the cytosolic proteasome, which unfolds and transports the protein to its proteolytic core.
Tandemly linked molecules of ubiquitin
Cellular protein
Ubiquitin
ATP
AMP + PPi
Proteasome
Recycled
Ubiquitin
Non-specific proteases
Amino acids
3
Peptide fragments produced by the proteasome are degraded to amino acids.

The different forms of excreted nitrogen

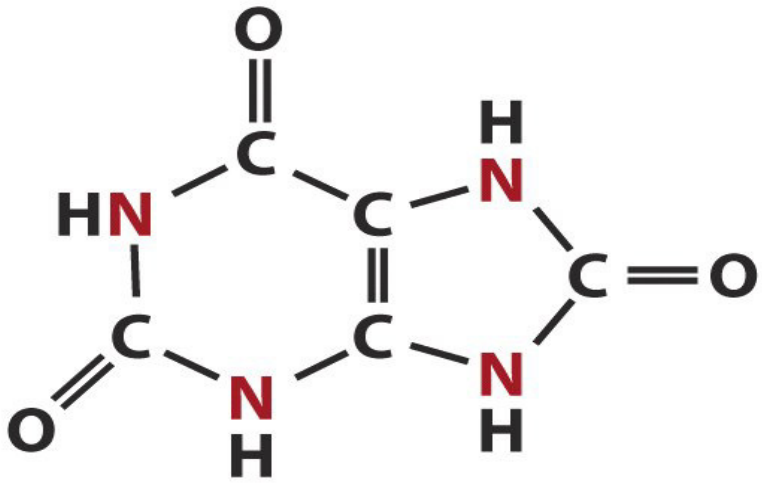
Ammonia (as ammonium ion)



Urea

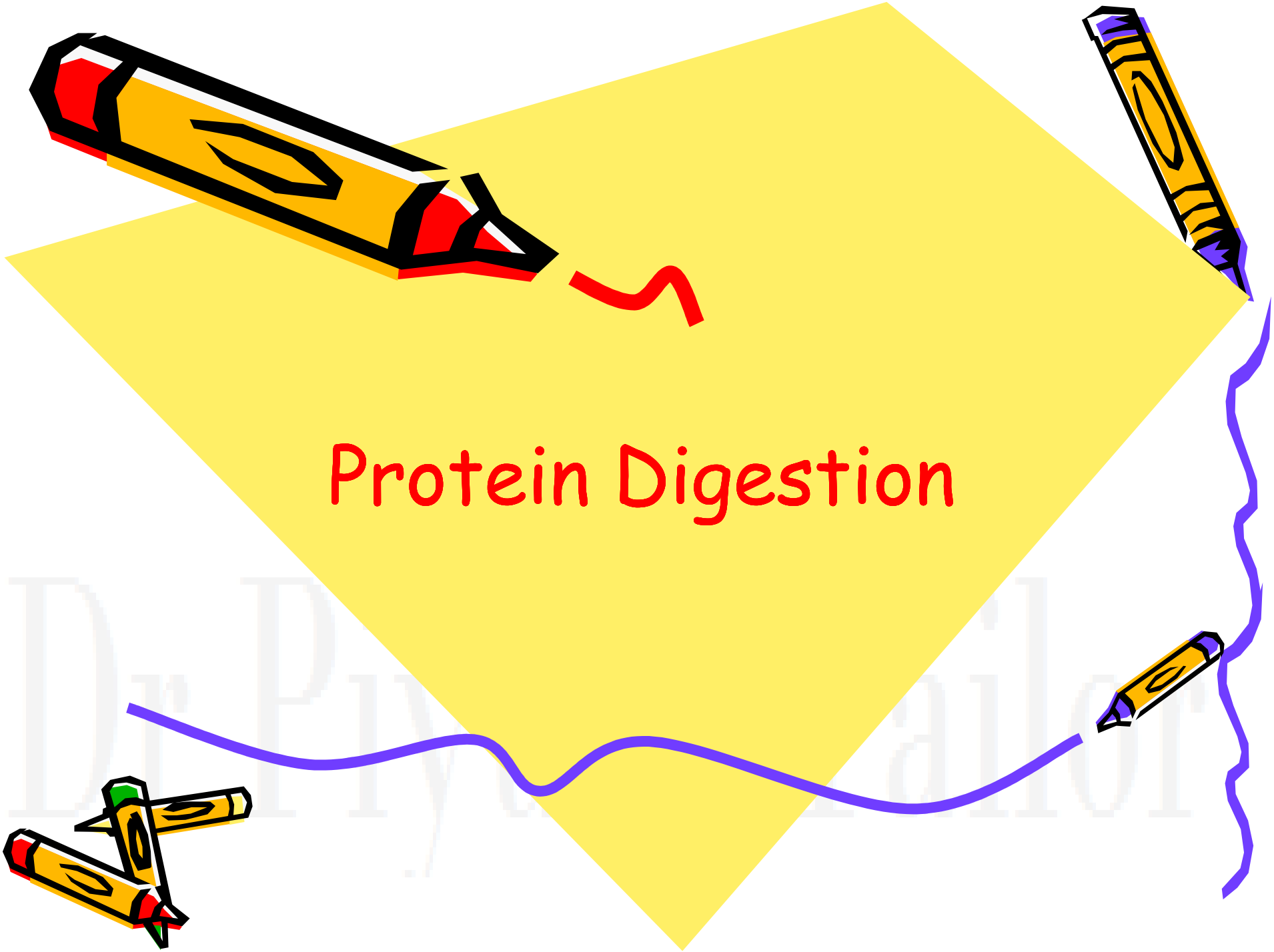
Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia

Ureotelic animals: many terrestrial vertebrates; also sharks



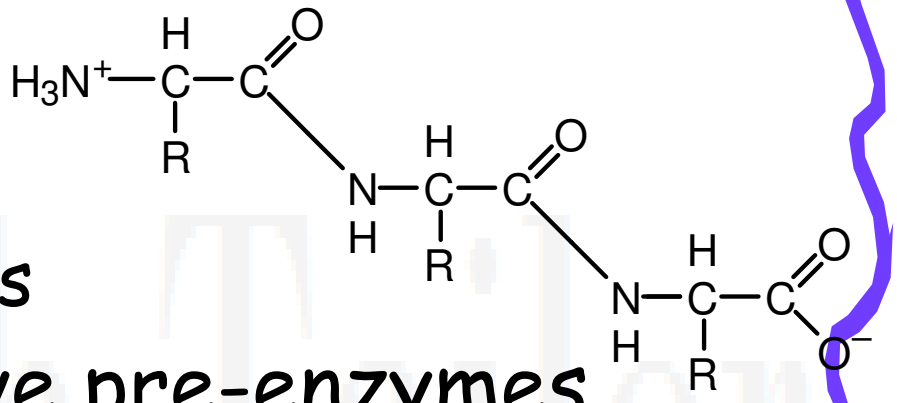
Uric acid

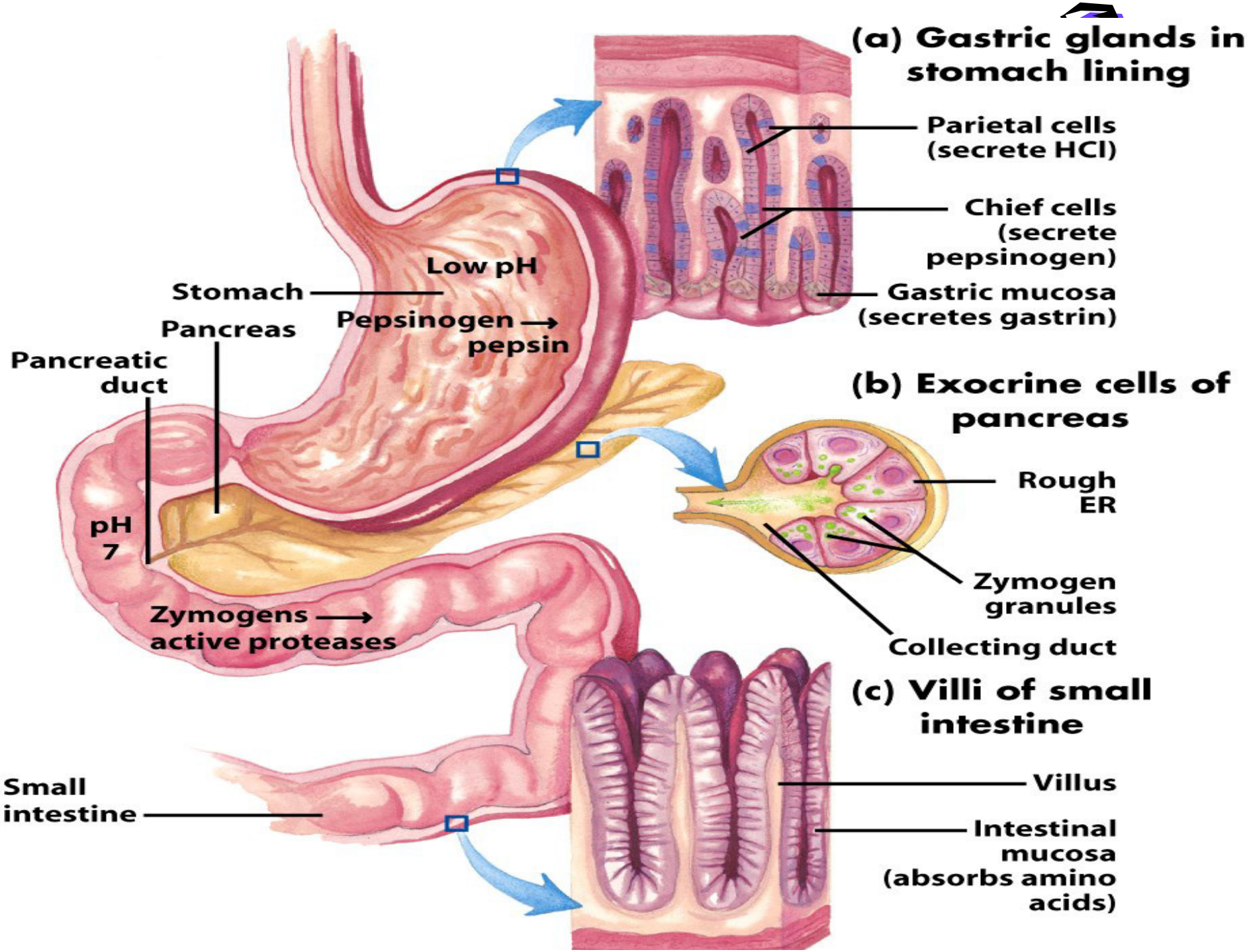
Uricotelic animals: birds, reptiles

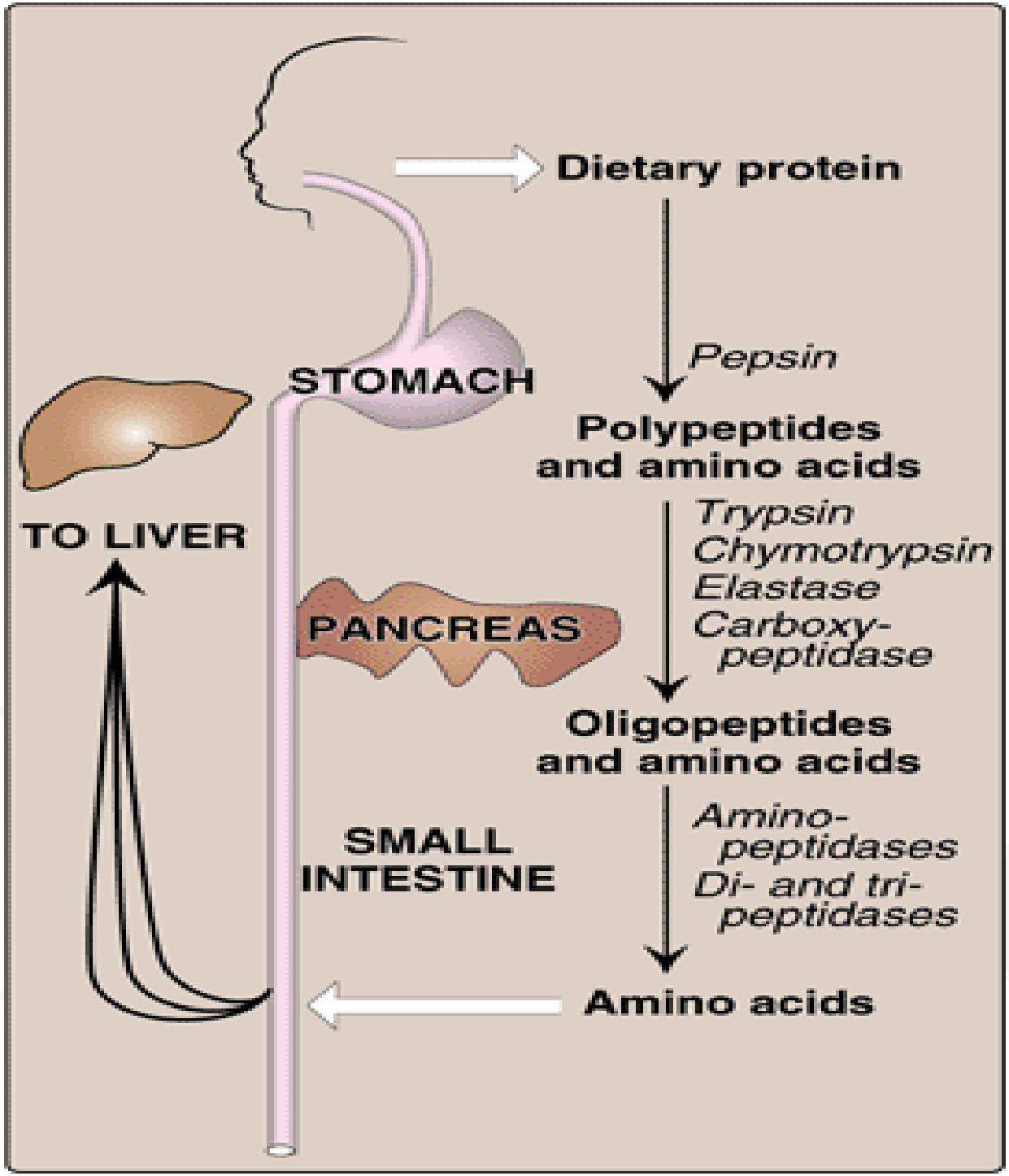

Protein Digestion

Monogastric Protein Digestion

- Whole proteins are not absorbed
  - Too large to pass through cell membranes intact
- Digestive enzymes
  - Break peptide bonds
- Secreted as inactive pre-enzymes
  - Prevents self-digestion




(a) Gastric glands in stomach lining
Parietal cells (secrete HCl)
Chief cells (secrete pepsinogen)
Gastric mucosa (secretes gastrin)
Low pH
Stomach
Pepsinogen → pepsin
Pancreas
Pancreatic duct
pH 7
Zymogens → active proteases
(b) Exocrine cells of pancreas
Rough ER
Zymogen granules
Collecting duct
(c) Villi of small intestine
Small intestine
Villus
Intestinal mucosa (absorbs amino acids)


Dietary protein
Pepsin
STOMACH
Polypeptides
and amino acids
TO LIVER
Trypsin
Chymotrypsin
Elastase
Carboxy-
peptidase
PANCREAS
Oligopeptides
and amino acids
Amino-
peptidases
Di- and tri-
peptidases
SMALL
INTESTINE
Amino acids

Monogastric Protein Digestion

- Initiated in stomach
  - –HCl from parietal cells
    - Stomach pH 1.6 to 3.2
    - Denatures 4^0, 3^0, and 2^0 structures
  - –Pepsinogen from chief cells

Pepsinogen $\xrightarrow{HCl}$ Pepsin

- Cleaves at phenylalanine, tyrosine, tryptophan

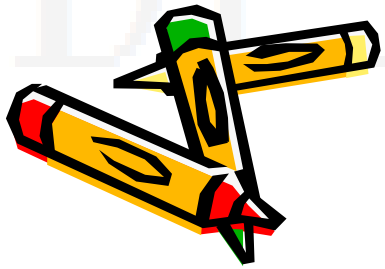
Aromatic amino acids

- Protein leaves stomach as mix of insoluble protein, soluble protein, peptides and amino acids

Protein Digestion – Small Intestine

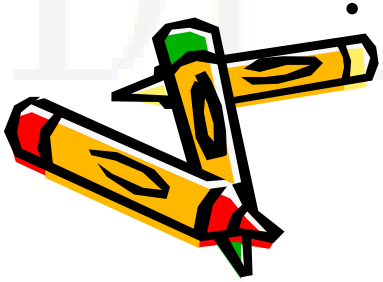
- Pancreatic enzymes secreted
  - Trypsinogen
  - Chymotrypsinogen
  - Procarboxypeptidase
  - Proelastase
  - Collagenase

Zymogens



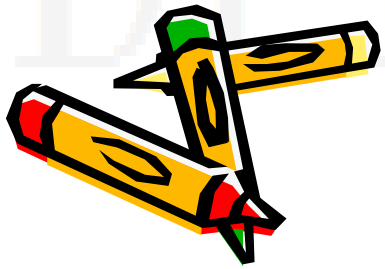
Digestion – Small Intestine

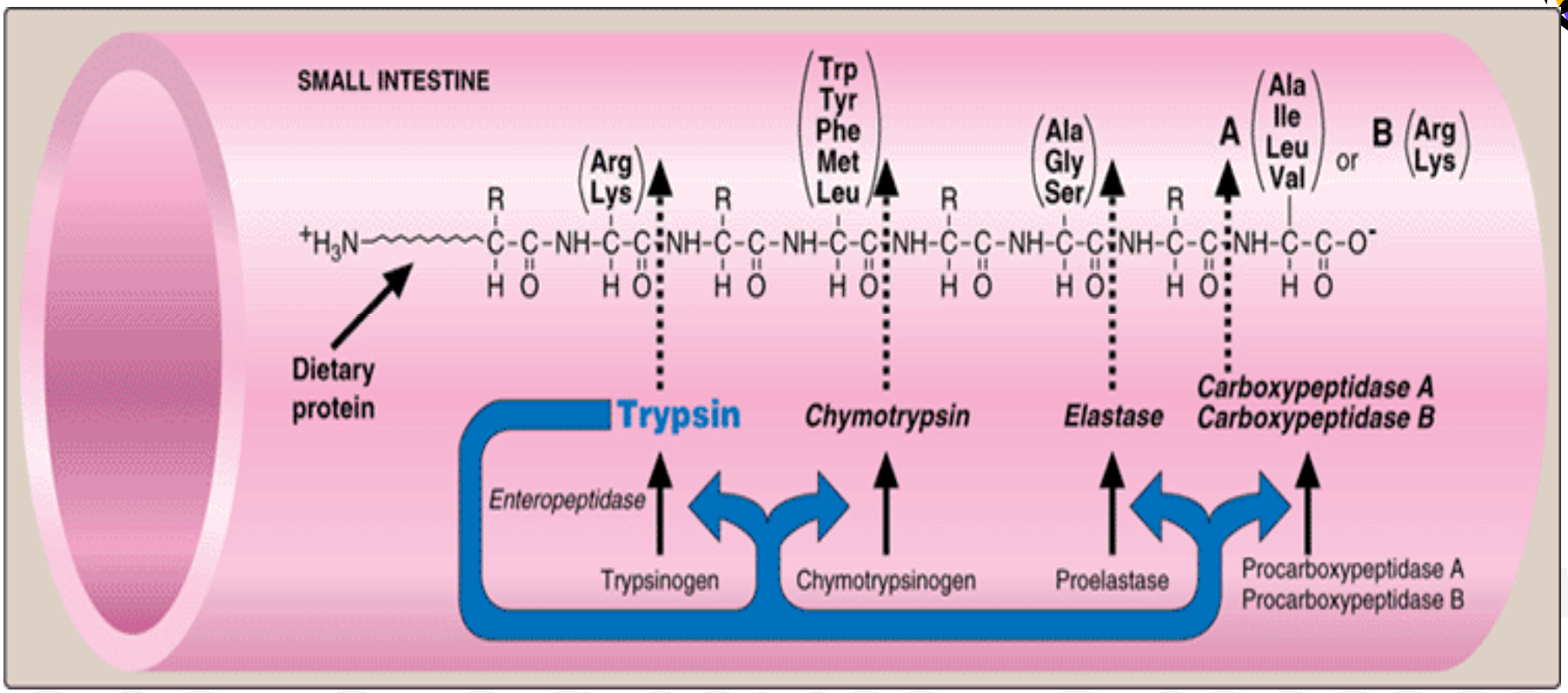
- Zymogens must be converted to active form
  - Trypsinogen $\xrightarrow{\text{Enteropeptidase/Trypsin}}$ Trypsin
    - Endopeptidase
      - Cleaves on carbonyl side of Lys & Arg
  - Chymotrypsinogen $\xrightarrow{\text{Trypsin}}$ Chymotrypsin
    - Endopeptidase
      - Cleaves carboxy terminal Phe, Tyr and Trp
  - Procarboxypeptidase $\xrightarrow{\text{Trypsin}}$ Carboxypeptidase
    - Exopeptidase
      - Removes carboxy terminal residues



Protein Digestion

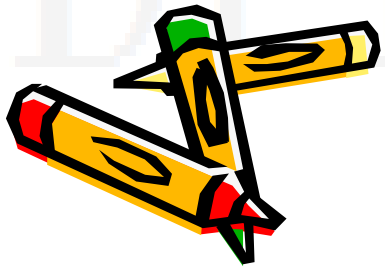
- Small intestine (brush border)
  - Aminopeptidases
    - Cleave at N-terminal *AA*
  - Dipeptidases
    - Cleave dipeptides
  - Tripeptidase
    - Cleave tripeptides
  - (Enterokinase or Enteropeptidase)
    - Trypsinogen → trypsin
    - Trypsin then activates all the other enzymes




SMALL INTESTINE
Arg
Lys
Trp
Tyr
Phe
Met
Leu
Ala
Gly
Ser
A
Ala
Ile
Leu
Val
or
B
Arg
Lys
Dietary protein
Trypsin
Chymotrypsin
Elastase
Carboxypeptidase A
Carboxypeptidase B
Enteropeptidase
Trypsinogen
Chymotrypsinogen
Proelastase
Procarboxypeptidase A
Procarboxypeptidase B

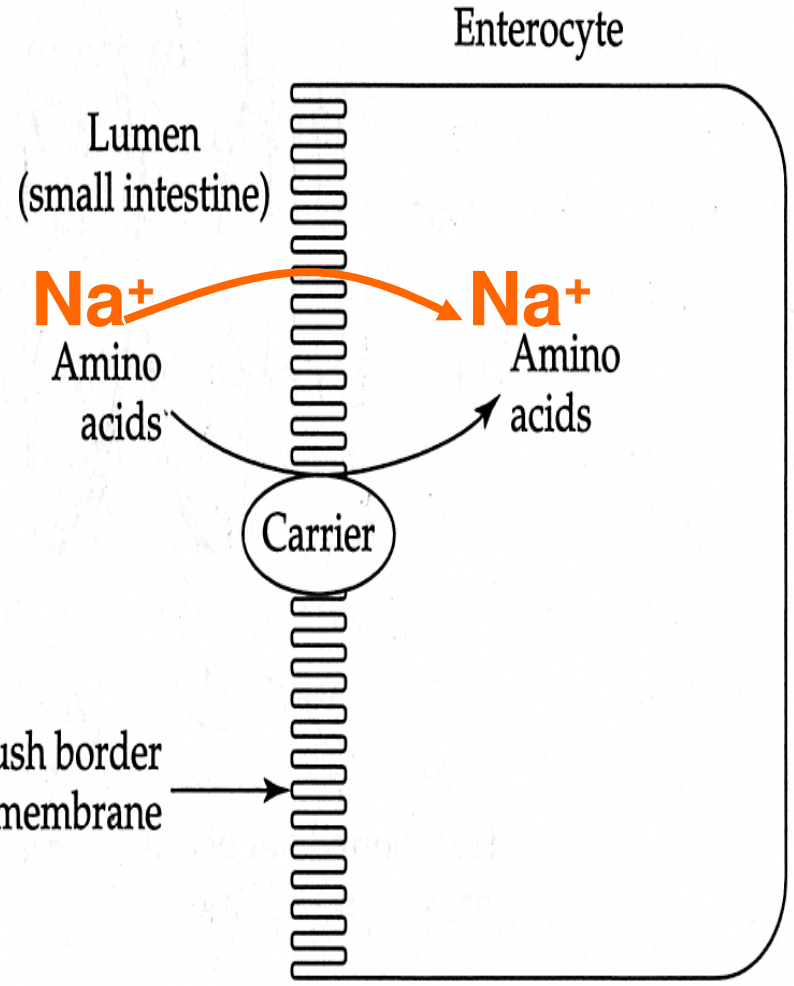
Protein Digestion

- Proteins are broken down to
  - Tripeptides
  - Dipeptides
  - Free amino acids



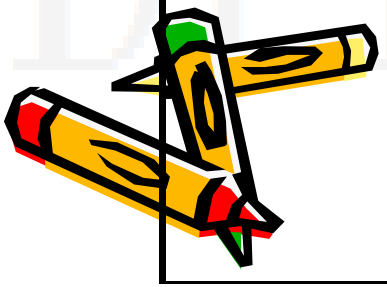
Free Amino Acid Absorption

- Free amino acids
  - Carrier systems
    - Neutral AA
    - Basic AA
    - Acidic AA
    - Imino acids
  - Entrance of some AA is via active transport
    - Requires energy



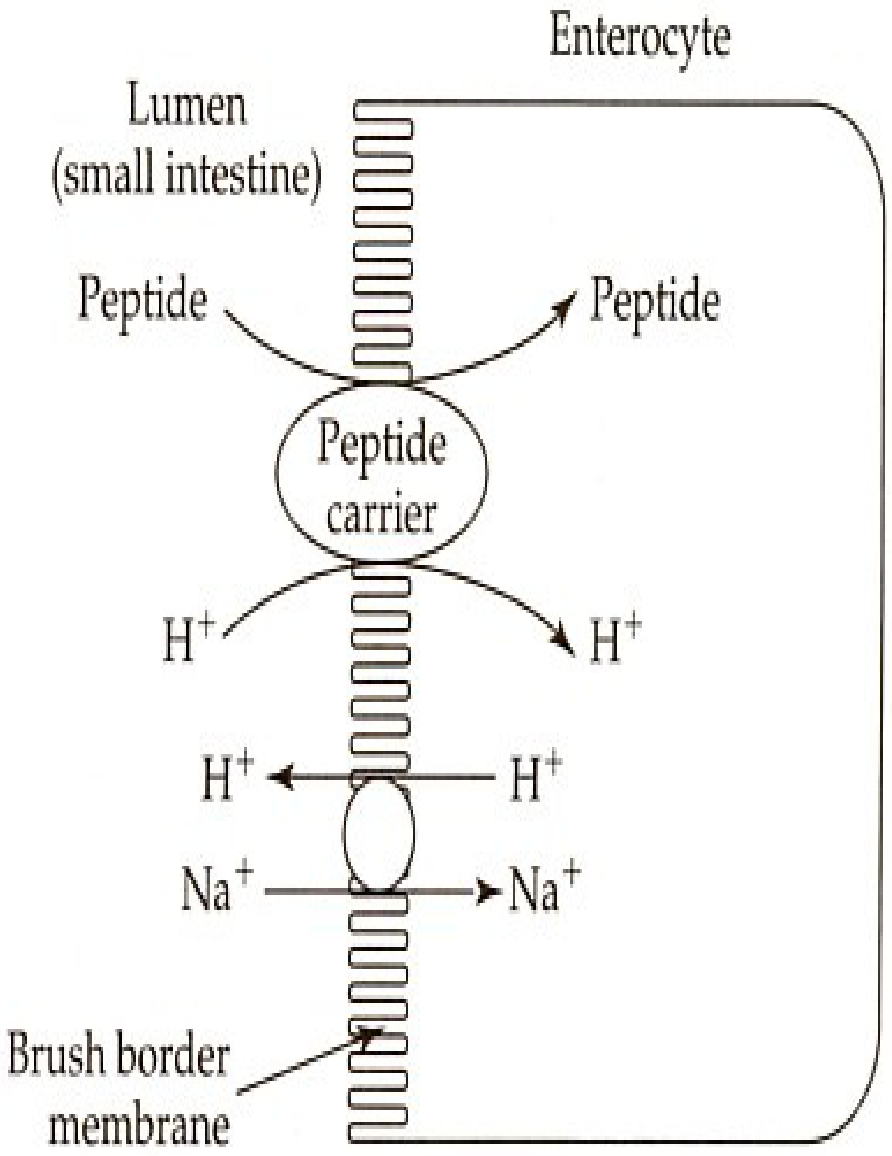
Amino Acid Transporters - Brush Border Membrane

Transport system	Energy required	Substrates carried
L	No	Leu, other neutral
B	Yes	Phe, Tyr, Trp, Ile, Leu, Val
IMINO	Yes	Pro, Gly
y^+	No	Basic amino acids
$B^{o,+}$	Yes	Most neutral and basic
$b^{o,+}$	No	Most neutral and basic

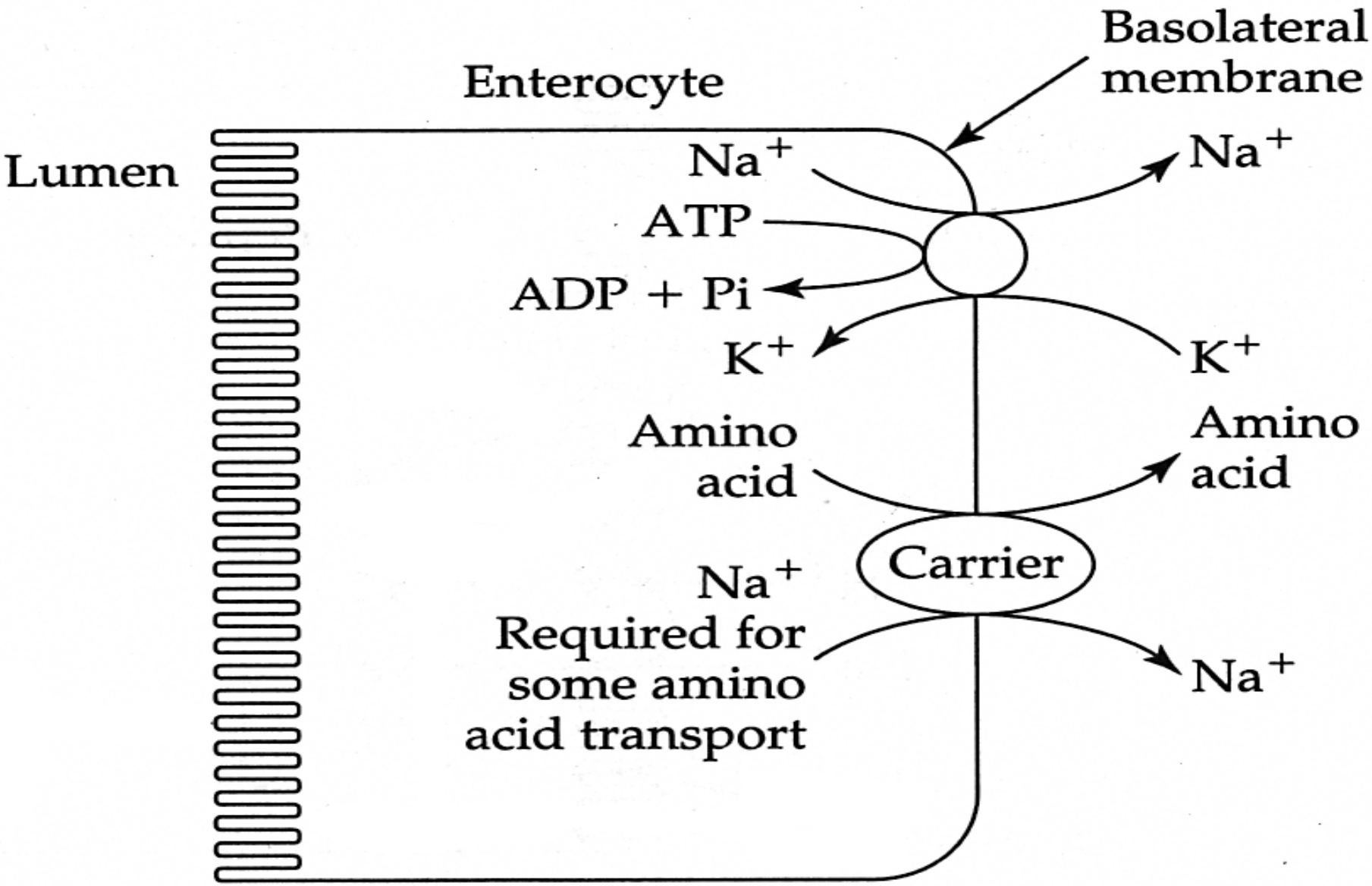


Peptide Absorption

- Form in which the majority of protein is absorbed
- More rapid than absorption of free amino acids
- Active transport
  - Energy required
- Metabolized into free amino acids in enterocyte
- Only free amino acids absorbed into blood



Basolateral Membrane



Absorption of Intact Proteins

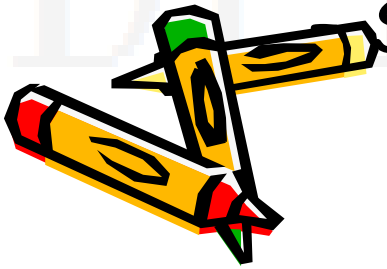
- In Newborns
  - First 24 hours after birth
  - Immunoglobulins get absorbed
    - Passive immunity
- In Adults
  - By Paracellular routes
    - Tight junctions between cells
  - By Intracellular routes
    - Endocytosis
    - Pinocytosis

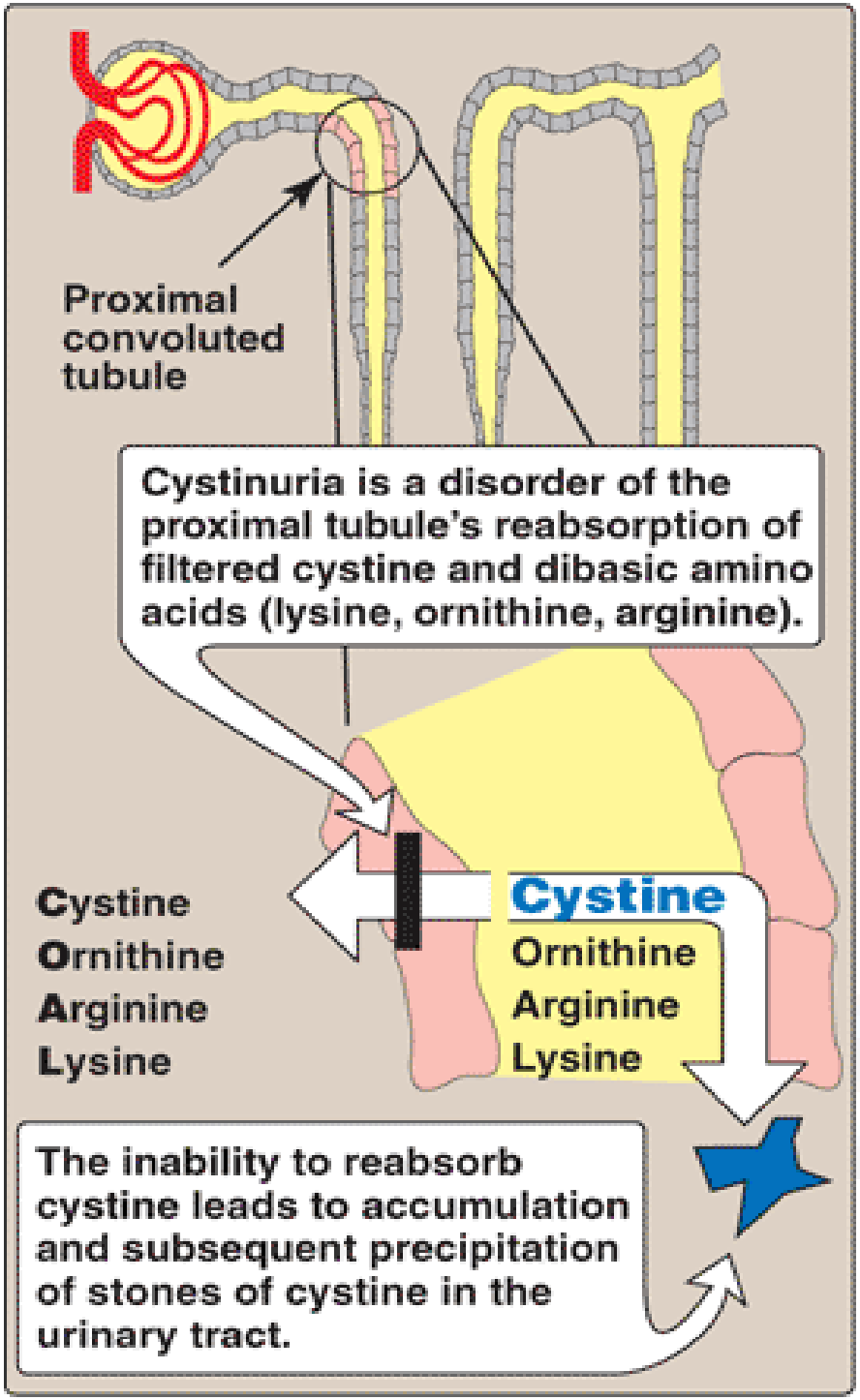
It has little nutritional significance...

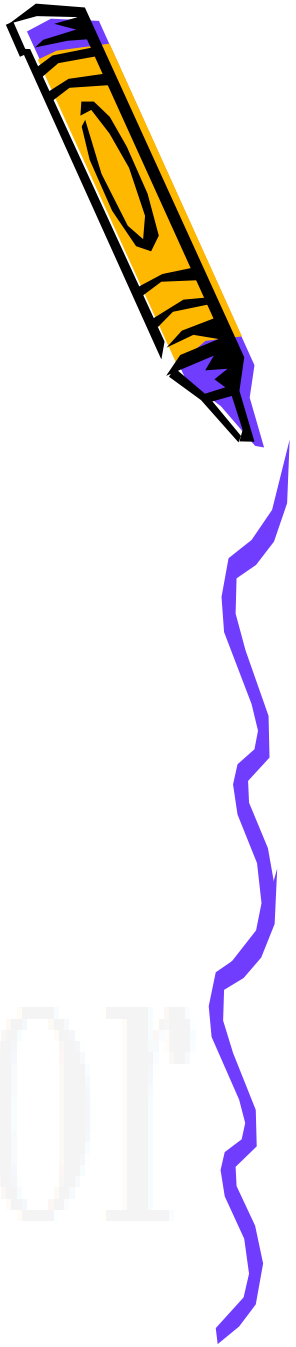
- Affects health (allergies and passive immunity)

Protein Transport in the Blood

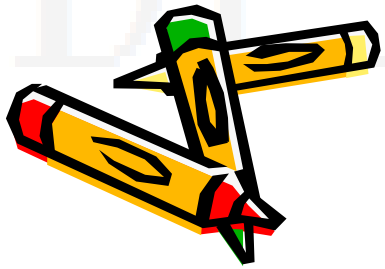
- Amino acids diffuse across the basolateral membrane
  - Enterocytes → portal blood → liver → tissues
  - Transported mostly as free amino acids
- Liver
  - Breakdown of amino acids
  - Synthesis of non-essential amino acids



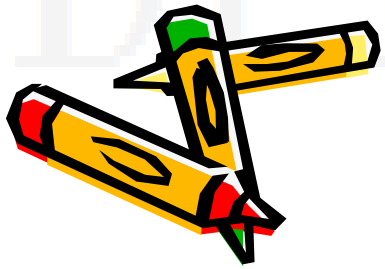

Proximal convoluted tubule
Cystinuria is a disorder of the proximal tubule's reabsorption of filtered cystine and dibasic amino acids (lysine, ornithine, arginine).
Cystine
Ornithine
Arginine
Lysine
Cystine
Ornithine
Arginine
Lysine
The inability to reabsorb cystine leads to accumulation and subsequent precipitation of stones of cystine in the urinary tract.



Catabolism of Amino acids

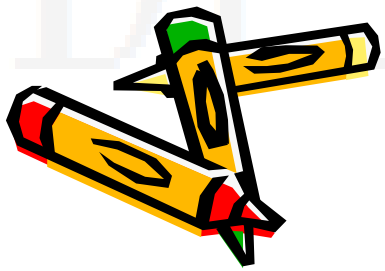


- Breakdown of Amino acid.
- Prodcuce CO2 and NH3
- NH3 need to be detoxify
  - Peripheral detoxification
  - Peripheral to liver transport
  - In Liver detoxification

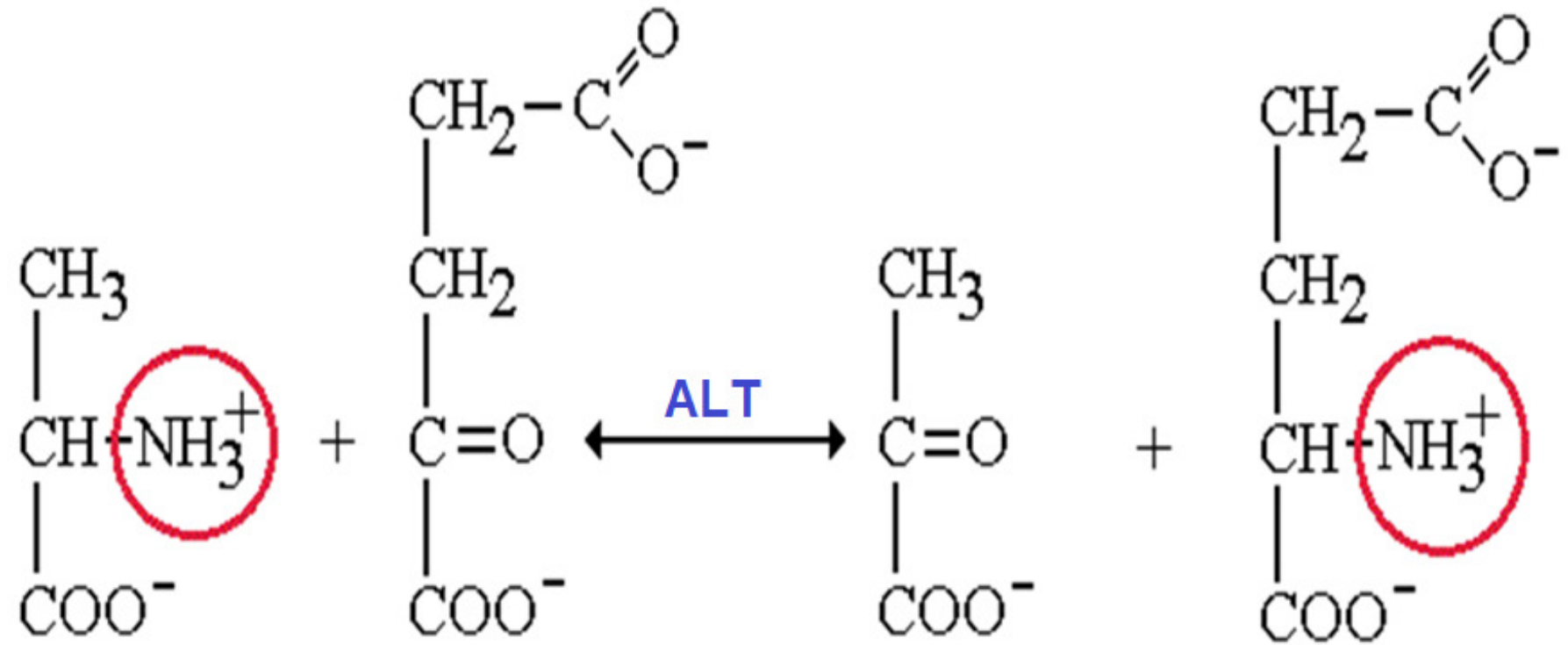


Peripheral detoxification

- First step is the removal of the α-amino group
  - by enzymes - **Amino-transferases or Transaminases.**

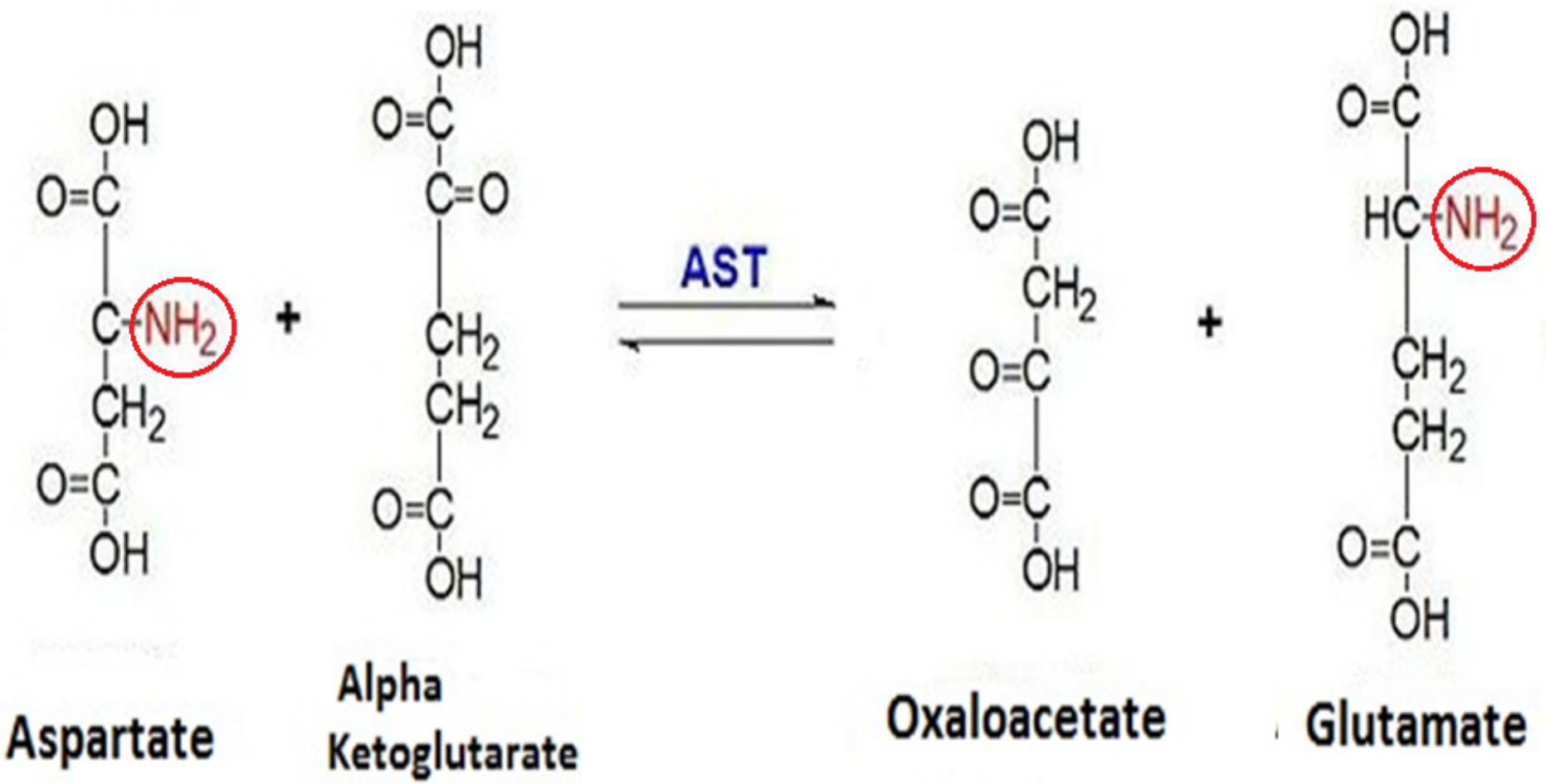


Alanine Amino-transferase
Alanine Transaminase (ALT)
Glutamate Pyruvate Transaminase (GPT)



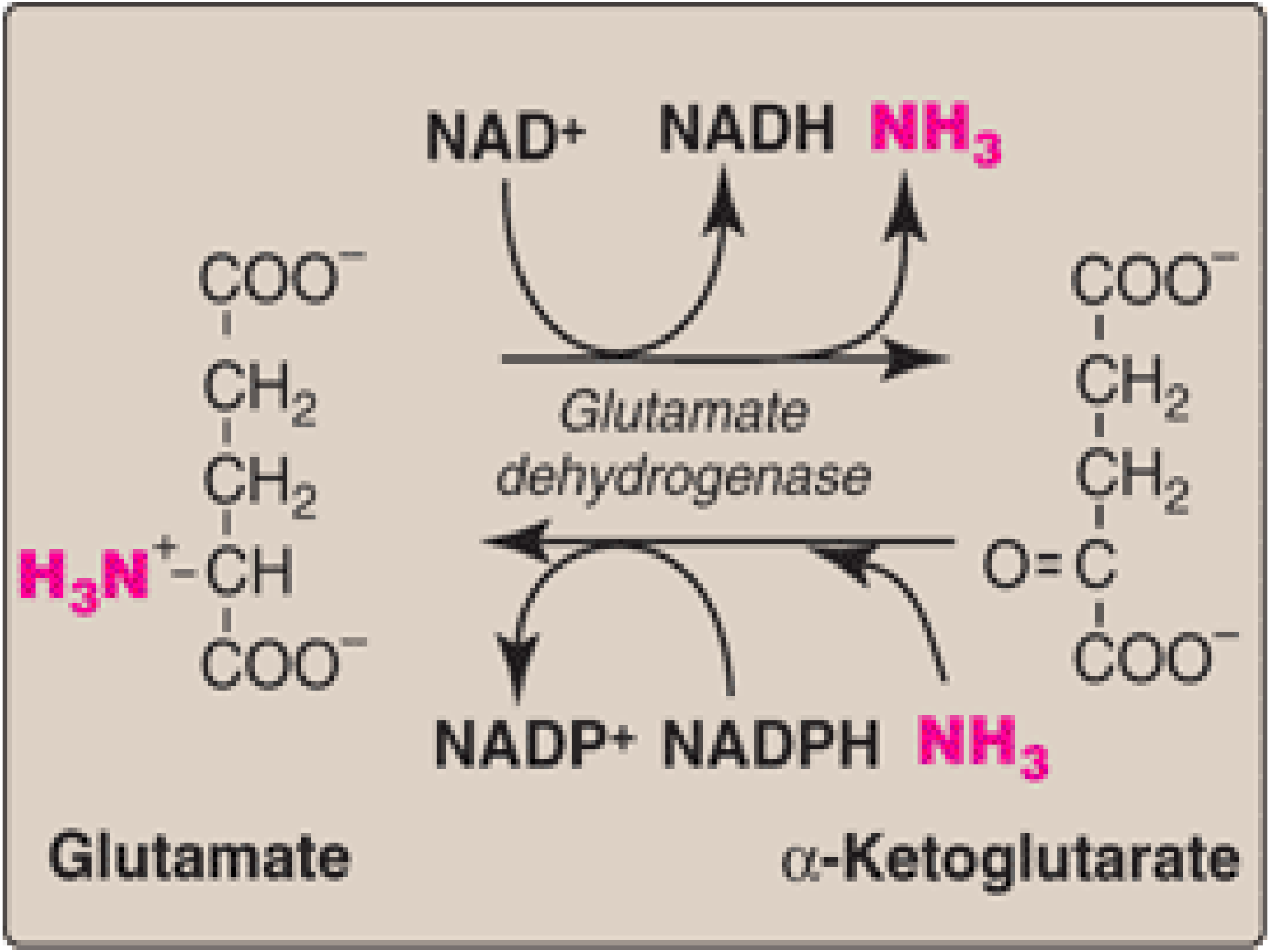
Alanine **Alpha Ketoglutarate** Pyruvate Glutamate

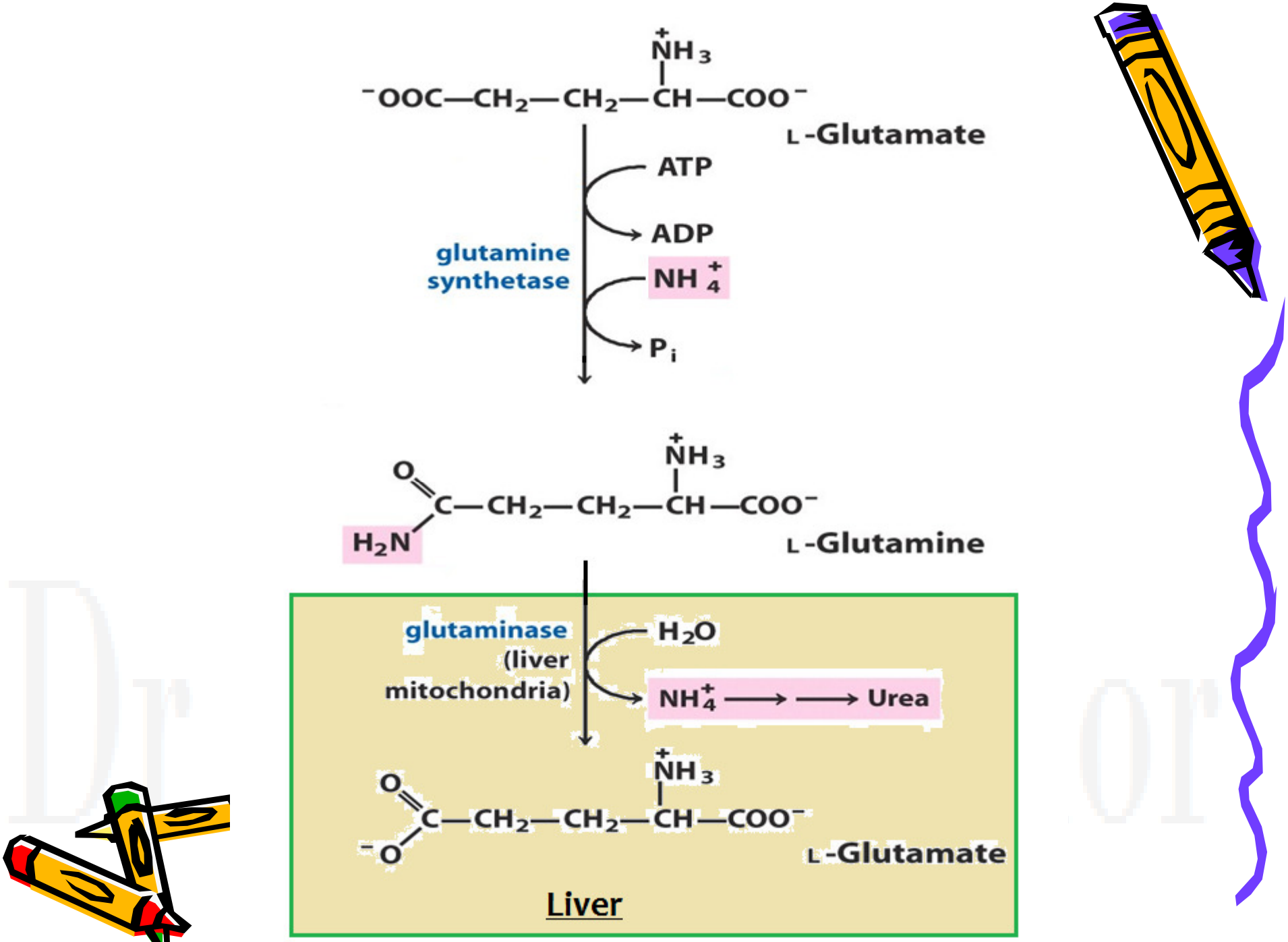
Aspartate Amino-transferase
Aspartate Transaminase (AST)
Glutamate Oxaloacetate Transaminase (GOT)



Transamination Reaction

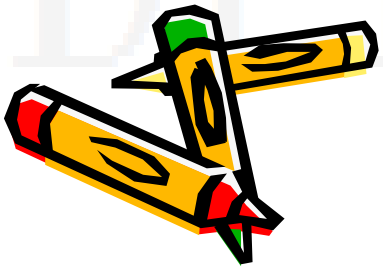
- The amino group is transferred to α-ketoglutarate to make glutamate.
- Formation of Non-essential amino acid
- Formation substrate of Gluconeogenesis
- Detoxification of amide group
-


NAD+
NADH
NH3
COO⁻
CH2
CH2
H3N+-CH
COO⁻
Glutamate
dehydrogenase
O=C
COO⁻
CH2
CH2
COO⁻
NADP+
NADPH
NH3
Glutamate
α-Ketoglutarate


NH3
⁻OOC—CH2—CH2—CH—COO⁻
L-Glutamate
ATP
ADP
glutamine synthetase
NH4+
Pi
NH3
O
C—CH2—CH2—CH—COO⁻
H2N
L-Glutamine
glutaminase (liver mitochondria)
H2O
NH4+ ⟶ ⟶ Urea
NH3
O
C—CH2—CH2—CH—COO⁻
⁻O
L-Glutamate
Liver

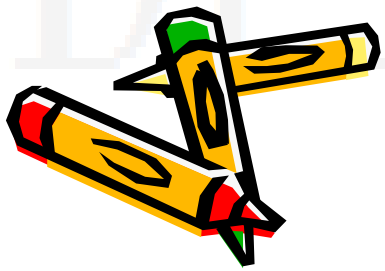
Glutamine transports NH_3 in the bloodstream

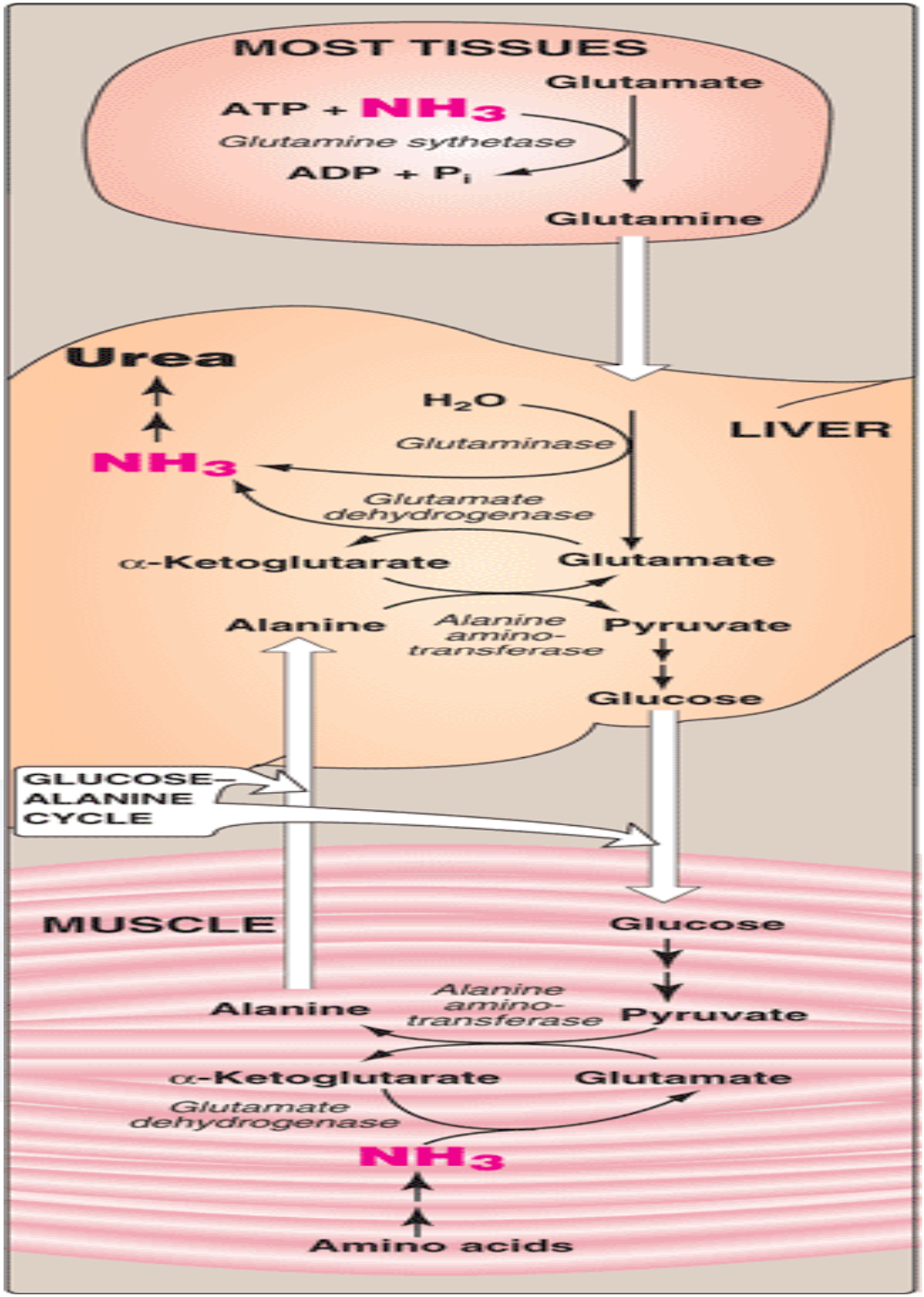
- Glutamate accepts the NH_3 by the action of Glutamine Synthetase.
- Glutamine transport to ammonia from periphery to liver



Urea

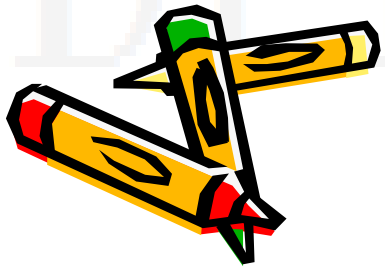
- If Ammonia is not used for production of new amino acids or other nitrogenous compounds, amino groups are transferred to the liver and converted to urea.
- Urea is produced in the cytosol via the urea cycle.
- Almost all urea is produced in the liver.
- Than Urea excreted in the urine.

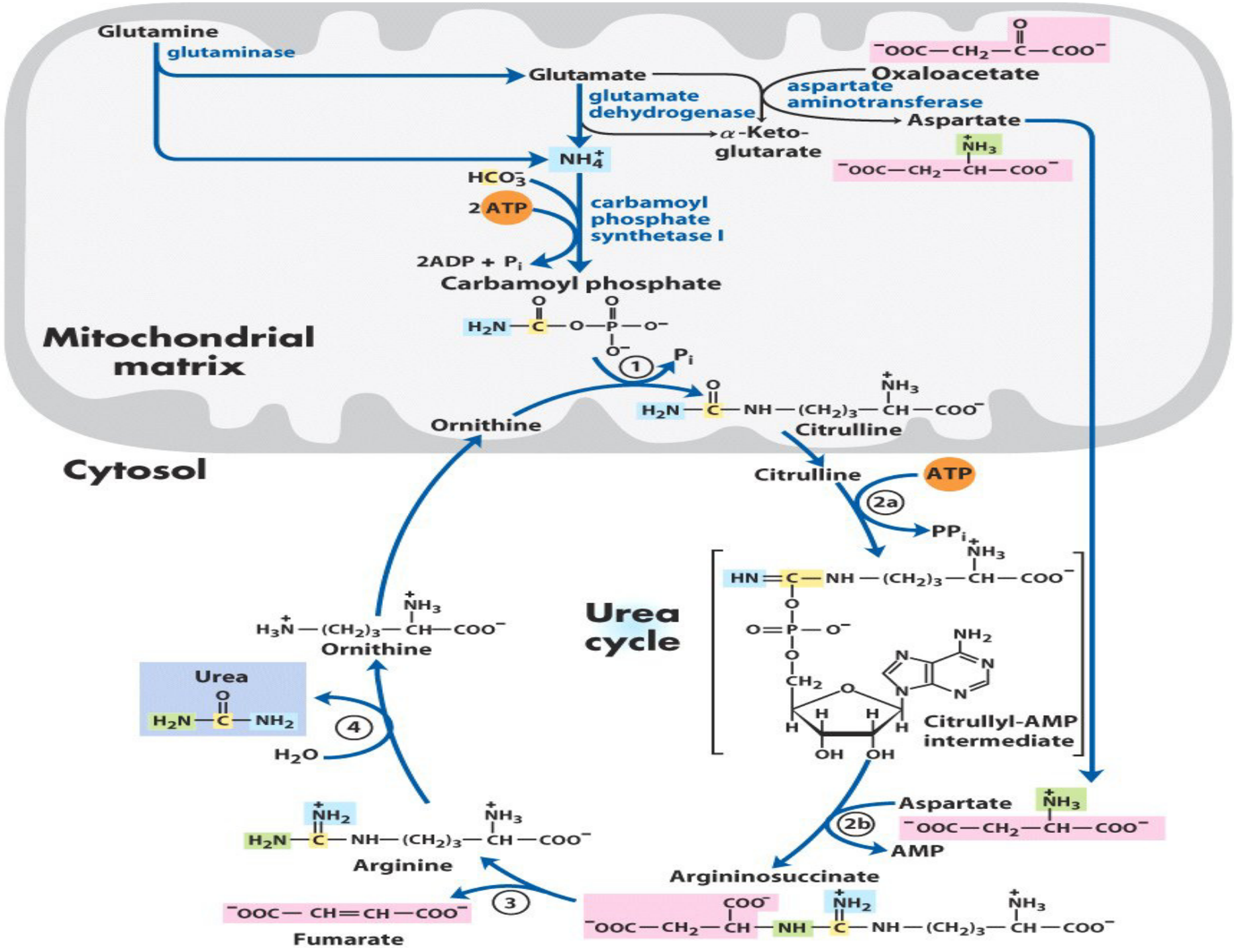


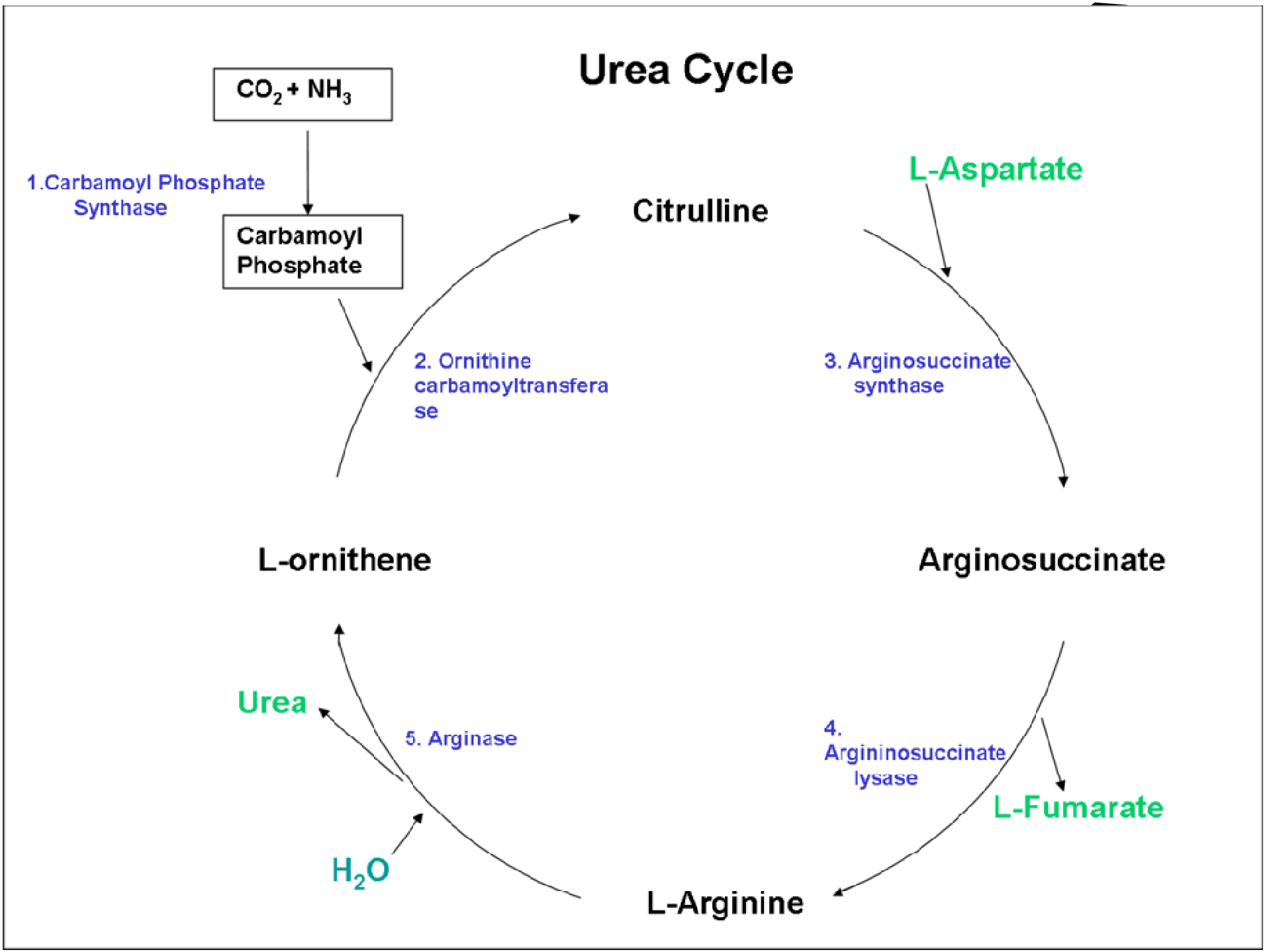

MOST TISSUES
Glutamate
ATP + NH3
Glutamine sythetase
ADP + Pi
Glutamine
Urea
LIVER
H2O
Glutaminase
NH3
Glutamate dehydrogenase
α-Ketoglutarate
Glutamate
Alanine
Alanine amino-transferase
Pyruvate
Glucose
GLUCOSE–ALANINE CYCLE
MUSCLE
Glucose
Alanine
Alanine amino-transferase
Pyruvate
α-Ketoglutarate
Glutamate
Glutamate dehydrogenase
NH3
Amino acids

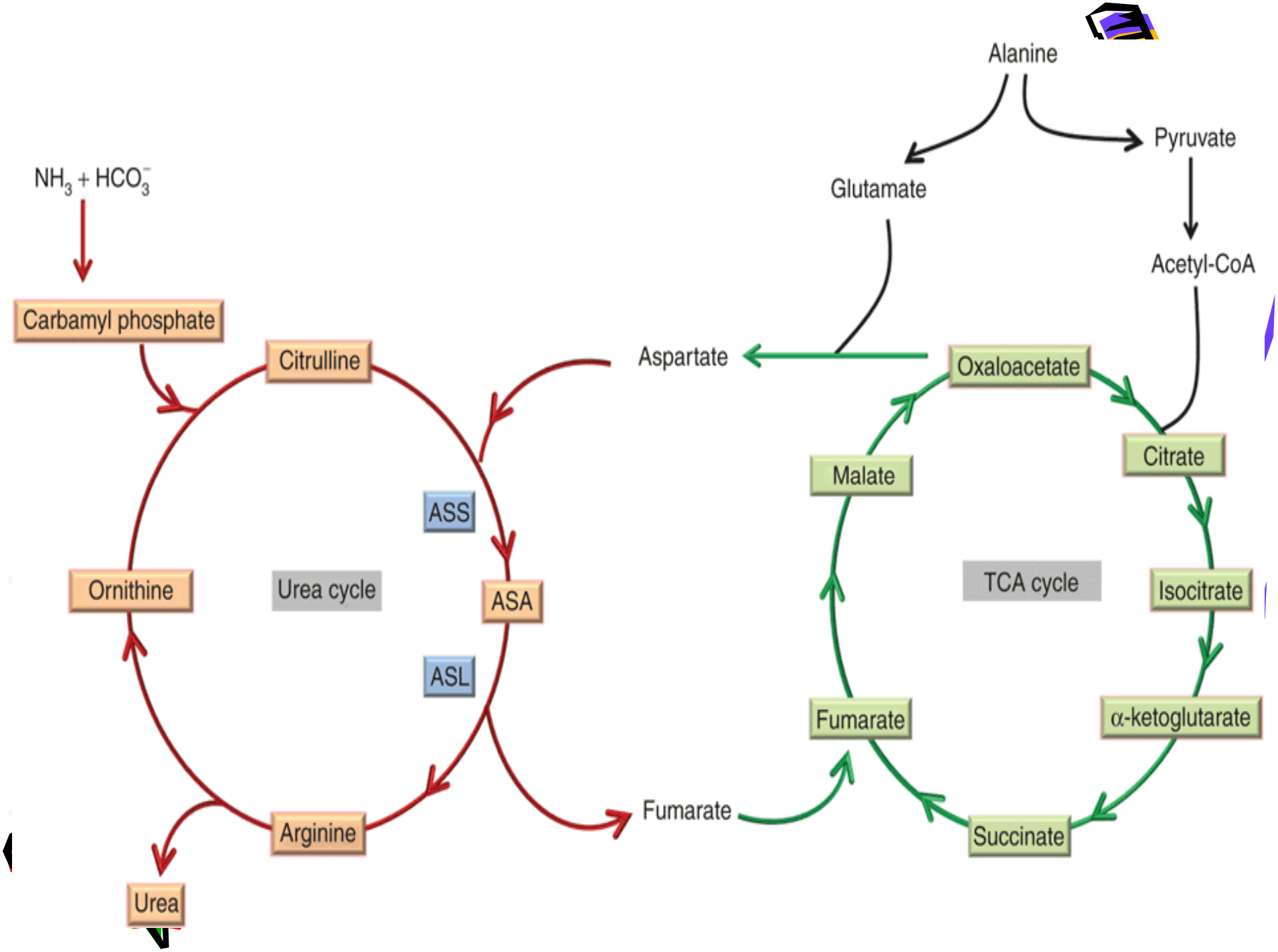
The Urea Cycle

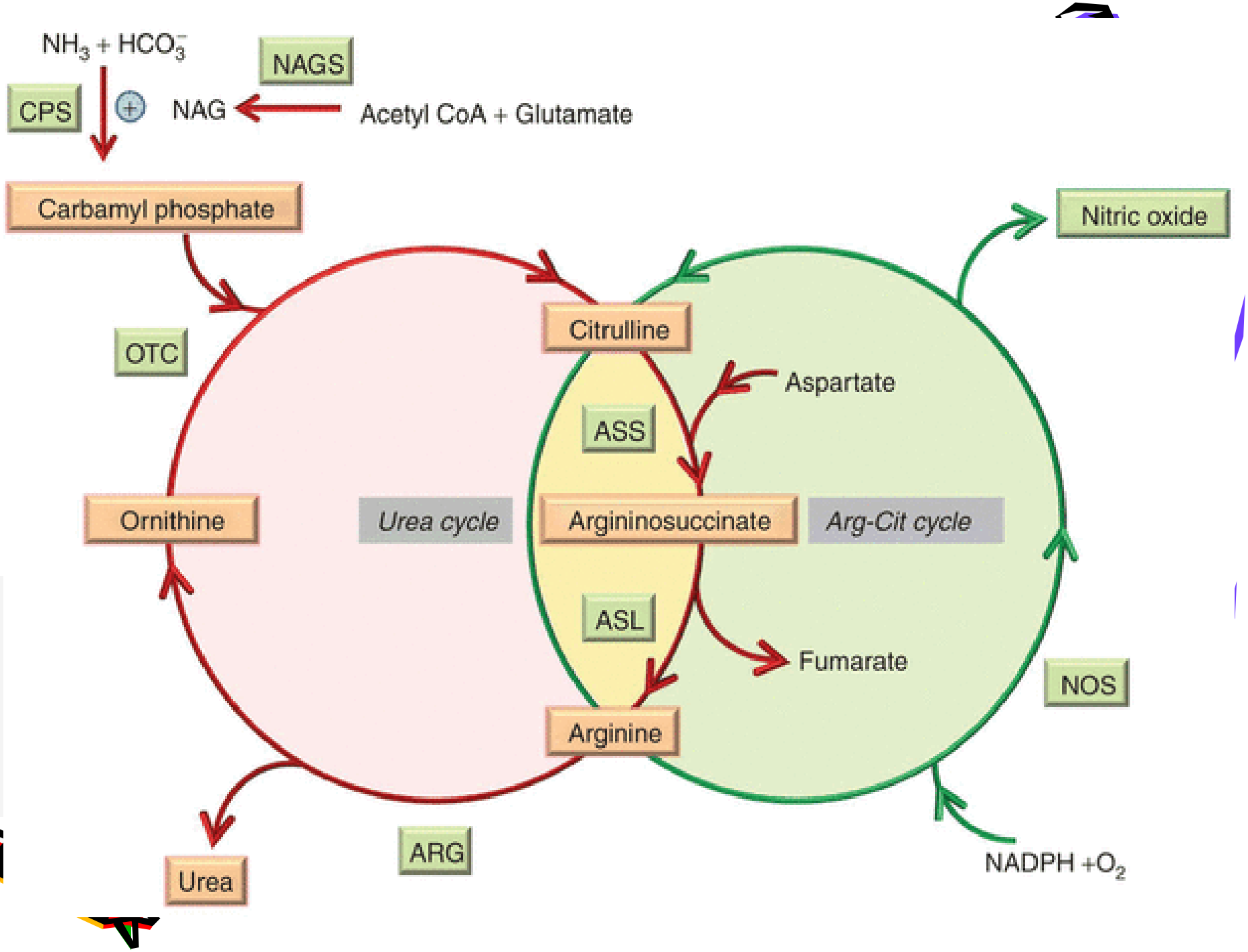
- The first two steps = mitochondrion.
- Remaining three = cytosol.



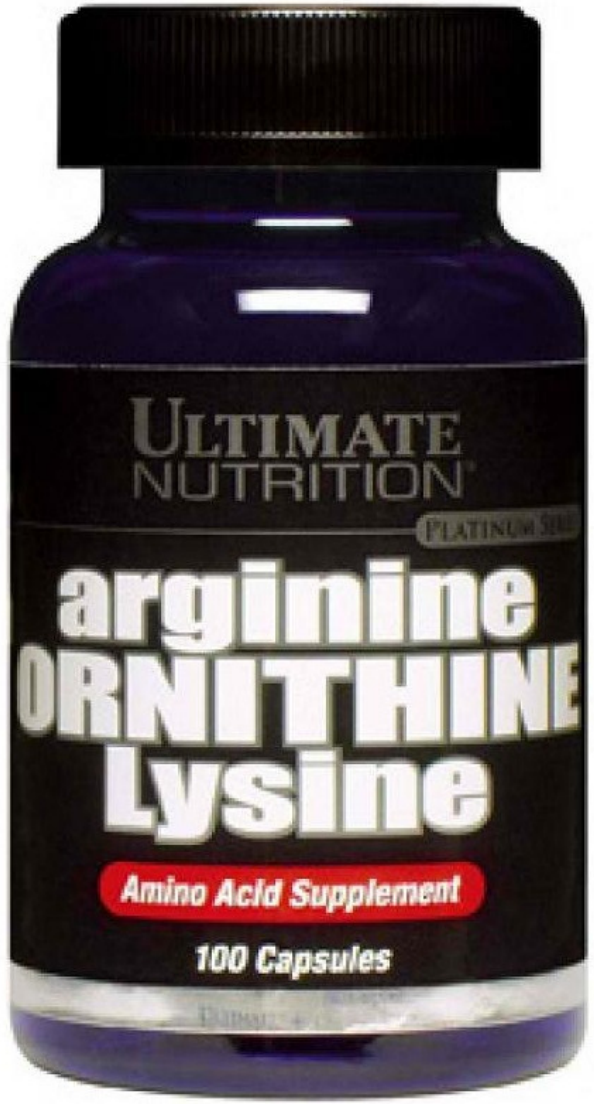

Glutamine
glutaminase
Glutamate
glutamate dehydrogenase
α-Keto-glutarate
Oxaloacetate
aspartate aminotransferase
Aspartate
NH4+
HCO3−
2 ATP
carbamoyl phosphate synthetase I
2ADP + Pi
Carbamoyl phosphate
Mitochondrial matrix
Pi
1
Ornithine
Citrulline
Cytosol
Citrulline
ATP
2a
PPi
Urea cycle
Citrullyl-AMP intermediate
Aspartate
2b
AMP
Argininosuccinate
3
Fumarate
Arginine
4
H2O
Urea
Ornithine

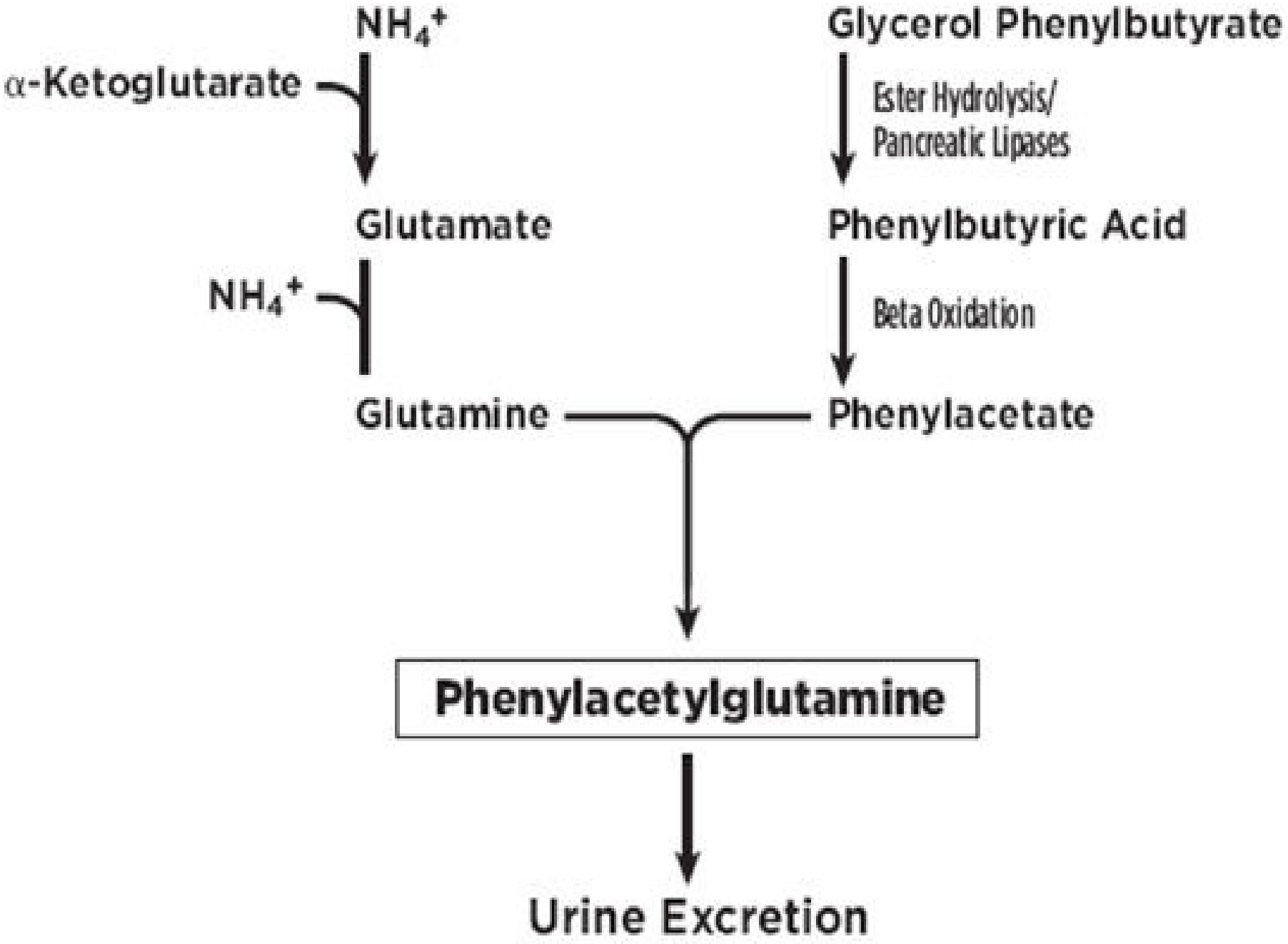

Urea Cycle
$CO_2 + NH_3$
1.Carbamoyl Phosphate Synthase
Carbamoyl Phosphate
2. Ornithine carbamoyltransfera se
Citrulline
L-Aspartate
3. Arginosuccinate synthase
Arginosuccinate
4. Argininosuccinate lysase
L-Fumarate
L-Arginine
5. Arginase
Urea
H_2O
L-ornithene

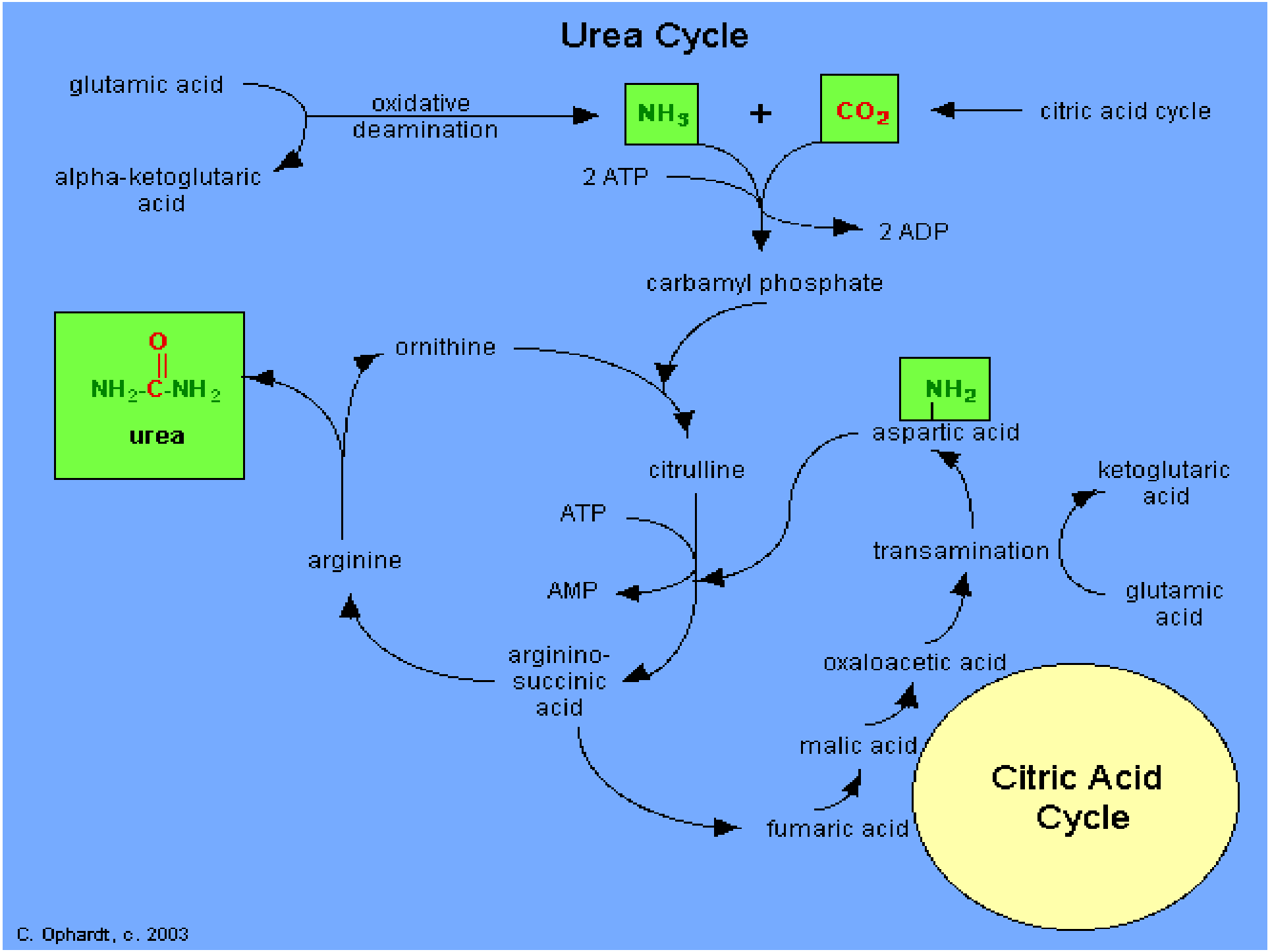

Alanine
Pyruvate
Glutamate
Acetyl-CoA
$NH_3 + HCO_3^-$
Carbamyl phosphate
Citrulline
Aspartate
Oxaloacetate
Citrate
Malate
ASS
Ornithine
Urea cycle
ASA
TCA cycle
Isocitrate
ASL
Fumarate
α-ketoglutarate
Fumarate
Arginine
Succinate
Urea


$NH_3 + HCO_3^-$
NAGS
CPS
NAG
Acetyl CoA + Glutamate
Carbamyl phosphate
Nitric oxide
OTC
Citrulline
Aspartate
ASS
Ornithine
Urea cycle
Argininosuccinate
Arg-Cit cycle
ASL
Fumarate
NOS
Arginine
ARG
Urea
NADPH +O_2

What can be use of this product?

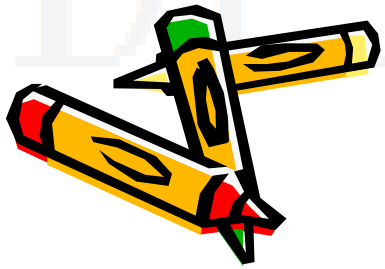



NH_4^+
α-Ketoglutarate
Glutamate
NH_4^+
Glutamine
Glycerol Phenylbutyrate
Ester Hydrolysis/
Pancreatic Lipases
Phenylbutyric Acid
Beta Oxidation
Phenylacetate
Phenylacetylglutamine
Urine Excretion


Urea Cycle
glutamic acid
oxidative deamination
alpha-ketoglutaric acid
NH3
+
CO2
citric acid cycle
2 ATP
2 ADP
carbamyl phosphate
ornithine
O
NH2-C-NH2
urea
citrulline
NH2
aspartic acid
ketoglutaric acid
ATP
AMP
arginine
transamination
glutamic acid
arginino-succinic acid
oxaloacetic acid
malic acid
fumaric acid
Citric Acid Cycle
C. Ophardt, c. 2003

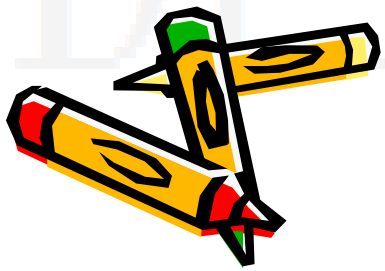
The enzymes catalyzing the urea cycle reactions

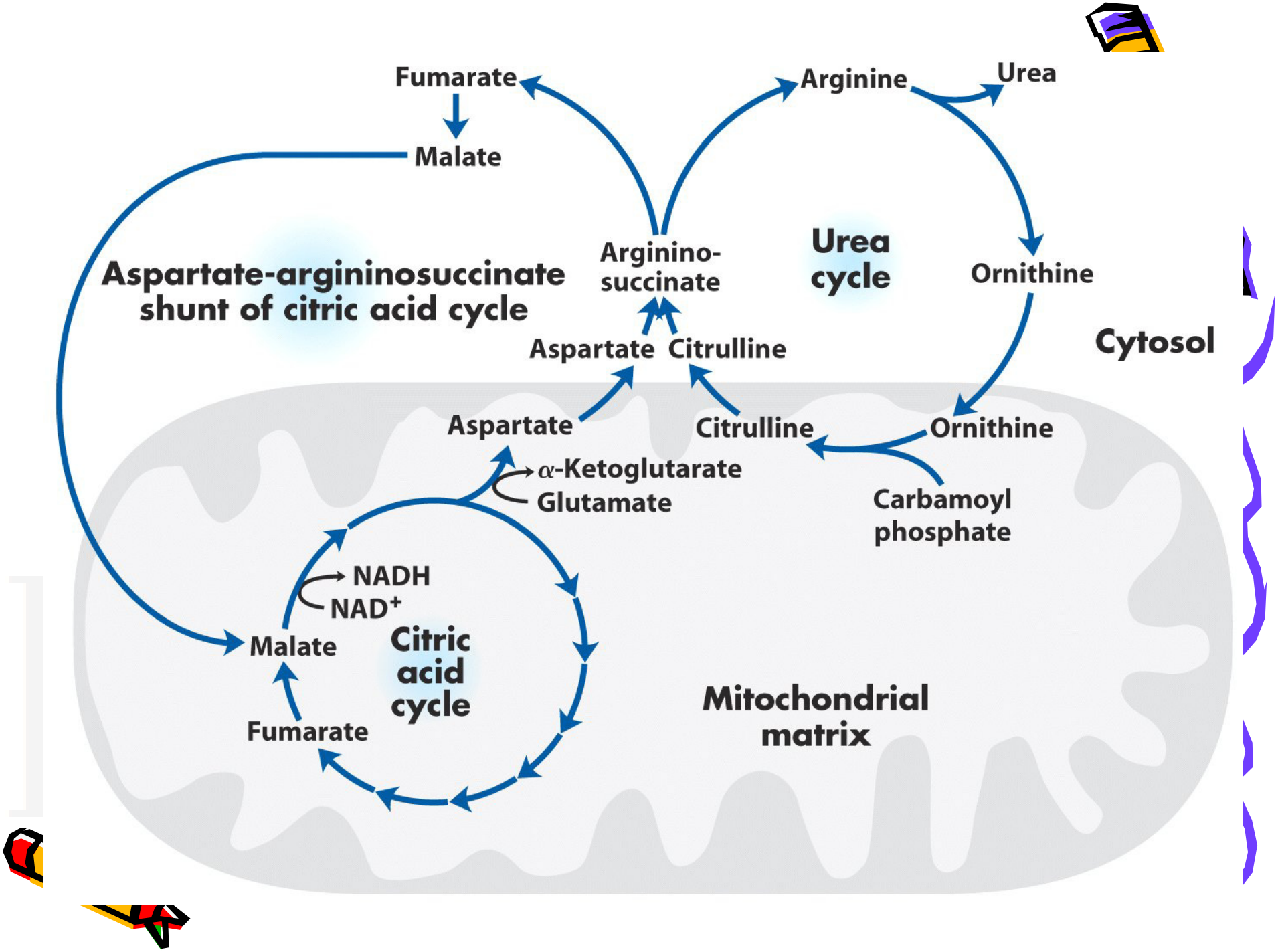
1. Ornithine transcarbamoylase
2. Argininosuccinate synthetase
3. Argininosuccinase
4. Arginase



The Urea Cycle and TCA Cycle are interconnected

- Cytosolic Isozymes of
  - Fumarase
  - Malate dehydrogenase.
- Malate enter in Mitochondria
- Than enter into the TCA cycle.

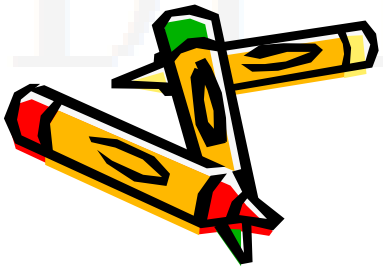


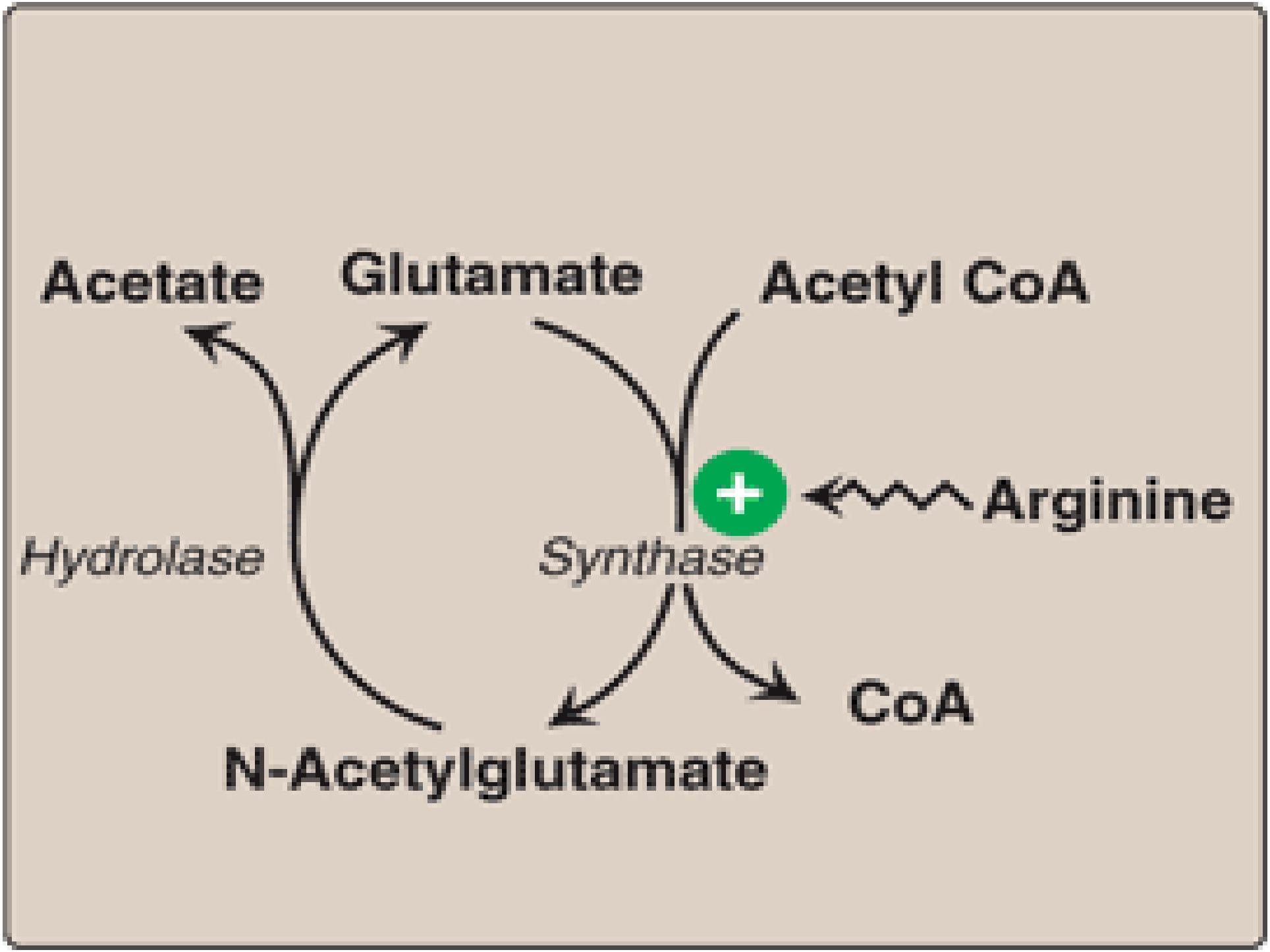

Fumarate
Arginine
Urea
Malate
Aspartate-argininosuccinate shunt of citric acid cycle
Arginino-succinate
Urea cycle
Ornithine
Cytosol
Aspartate
Citrulline
Aspartate
Citrulline
Ornithine
α-Ketoglutarate
Glutamate
Carbamoyl phosphate
NADH
NAD+
Malate
Citric acid cycle
Fumarate
Mitochondrial matrix

Regulation of the Urea Cycle

- Within an individual the movement of nitrogen through the cycle depends on diet.

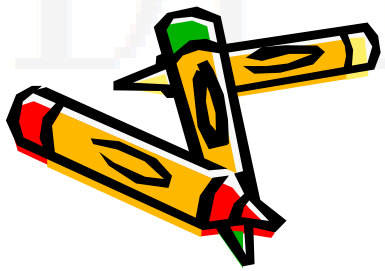
- Changes in diet will only affect urea cycle activity over the long term.




Acetate
Glutamate
Acetyl CoA
Arginine
Hydrolase
Synthase
CoA
N-Acetylglutamate

Regulation of the Urea Cycle

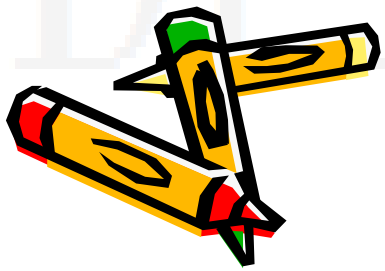
- Short term
  - **Carbamoyl Phosphate Synthetase.**
  - Allosteric regulation
  - N-acetylglutamate activates CPS-1
  - Arginine activates N-acetylglutamate synthase,



Energetic cost of The Urea Cycle

- $2NH_4^+ + HCO_3^- + 3ATP + H_2O \rightarrow$
  $urea + 2ADP + 4P_i + AMP + 2H^+$

- However, through linkage of the pathways the toll is not so bad. Some NADH is produced which regains about 2.5 ATP form respiration.

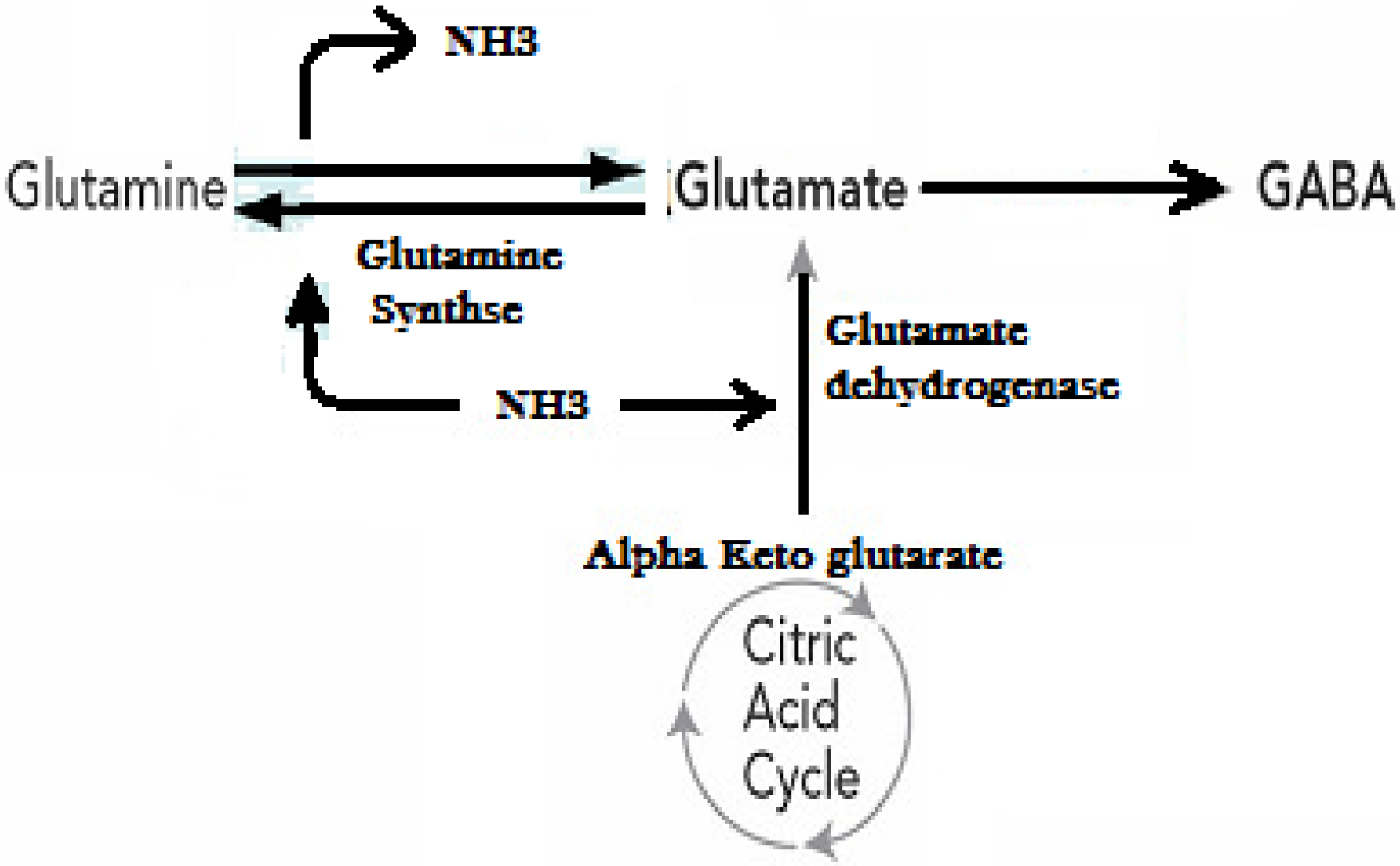


Hereditary deficiency of any of the Urea Cycle enzymes leads to **hyperammonemia** - elevated [ammonia] in blood.

Total lack of any Urea Cycle enzyme is lethal.

Elevated ammonia is toxic, especially to the brain.

If not treated immediately after birth, severe mental retardation results.


NH3
Glutamine
Glutamate
GABA
Glutamine
Synthse
NH3
Glutamate
dehydrogenase
Alpha Keto glutarate
Citric
Acid
Cycle

Mechanisms for toxicity of high Ammonia

1. High [NH_3] would drive **Glutamine Synthase**:

glutamate + ATP + NH_3 → glutamine + ADP + P_i

This would deplete glutamate – a neurotransmitter & precursor for synthesis of the neurotransmitter GABA.

2. Depletion of glutamate & high ammonia level would drive **Glutamate Dehydrogenase** reaction to **reverse**:

glutamate + $NAD(P)^+$ ← α-ketoglutarate + $NAD(P)H + NH_4^+$

The resulting depletion of α-ketoglutarate, an essential Krebs Cycle intermediate, could impair energy metabolism in the brain.

Mechanisms for toxicity of high Ammonia

3. Due to high ammonia, conc. of Glutamine remains high in brain cell.

Glutamine is co-transported outside from brain cell with tryptophan influx.

So, More Tryptophan get accumulated in brain cell if more glutamine goes out.

From accumulated Tryptophan, Serotonine synthesis & that have depressive effect on neurons.

Treatment of deficiency of Urea Cycle enzymes (depends on which enzyme is deficient):

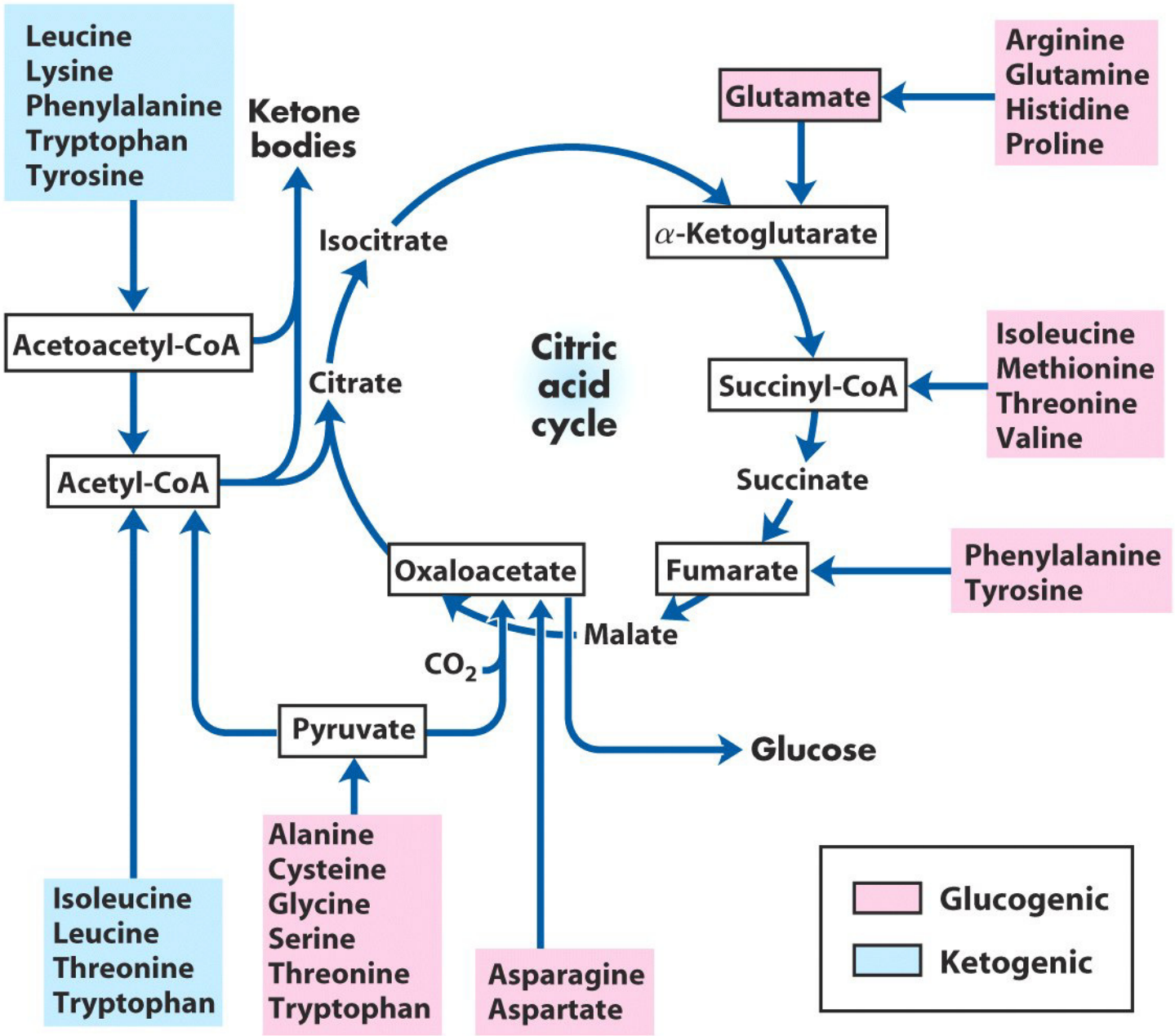
- **limiting protein intake** to the amount barely adequate to supply amino acids for growth, while adding to the diet the α-keto acid analogs of essential amino acids.
- **Liver transplantation** has also been used, since liver is the organ that carries out Urea Cycle.

One-carbon Transfer Reactions

- Cofactors for one-carbon transfer reactions in amino acid degradation.

- Tetrahydrofolate (H_4 folate) – Transfers carbon in intermediate oxidation states, sometimes methyl.

- S-adenosylmethione (SAM or adoMet) – Transfers carbon as methyl groups.


Leucine
Lysine
Phenylalanine
Tryptophan
Tyrosine
Ketone bodies
Isocitrate
Glutamate
Arginine
Glutamine
Histidine
Proline
α-Ketoglutarate
Acetoacetyl-CoA
Citrate
Citric acid cycle
Isoleucine
Methionine
Threonine
Valine
Succinyl-CoA
Acetyl-CoA
Succinate
Oxaloacetate
Fumarate
Phenylalanine
Tyrosine
Malate
CO2
Pyruvate
Glucose
Isoleucine
Leucine
Threonine
Tryptophan
Alanine
Cysteine
Glycine
Serine
Threonine
Tryptophan
Asparagine
Aspartate
Glucogenic
Ketogenic