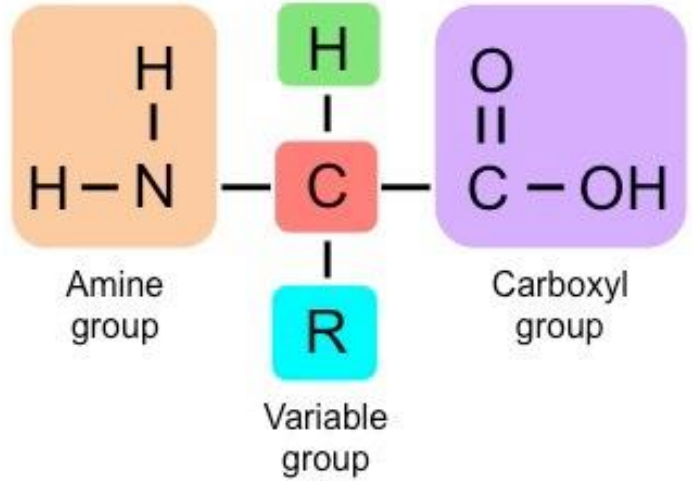
AMINO ACID METABOLISM



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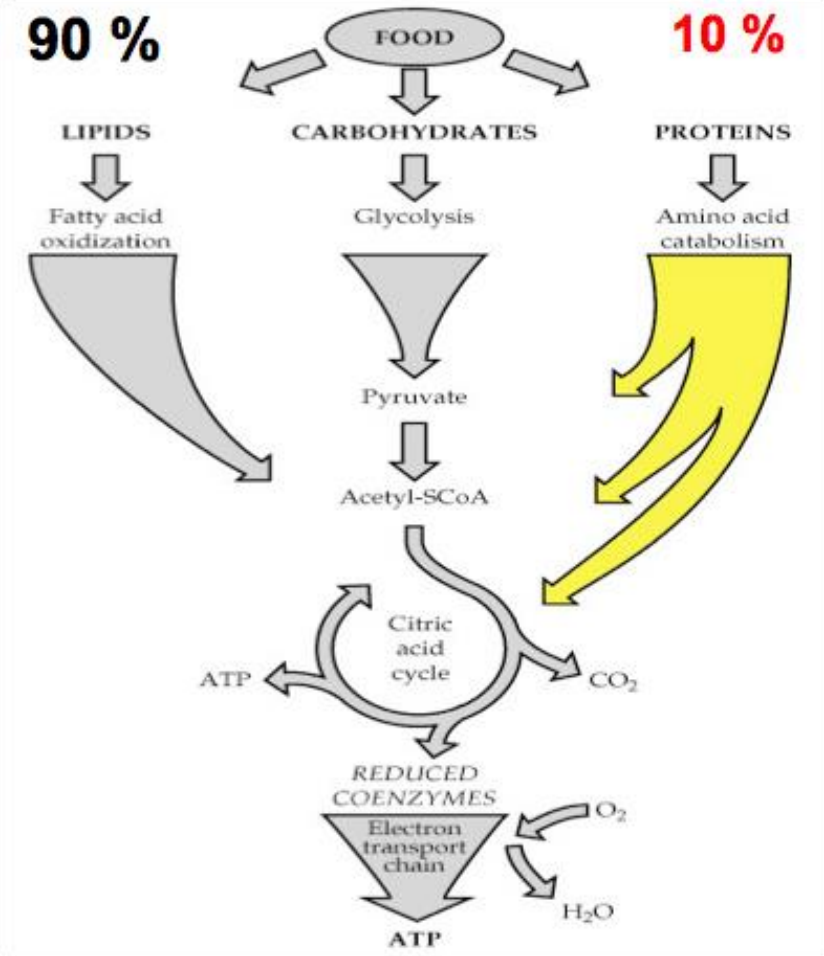
Learning objectives

At the end of this lecture, students should be able to understand:

1. Amino acid catabolism
2. Metabolism of ammonia
3. Urea cycle
4. Fate of carbon skeleton
5. Nitrogen balance
6. Diseases in the dysregulation of amino acid metabolism

Energy production from biomolecules



- 90% of energy is obtained from

degradation of carbohydrate and

triacylglecerol.

- Only 10% of energy is derived from

catabolism of protein.

Protein digestion and absorption in GI tract

- Proteases degrade ingested proteins.
- Initially synthesized proteases-zymogens include pepsinogen, trypsinogen, chymotrypsin and procarboxypeptidase.
- Free amino acids are absorbed through intestinal mucosa of jejunum, then blood and transported to liver.

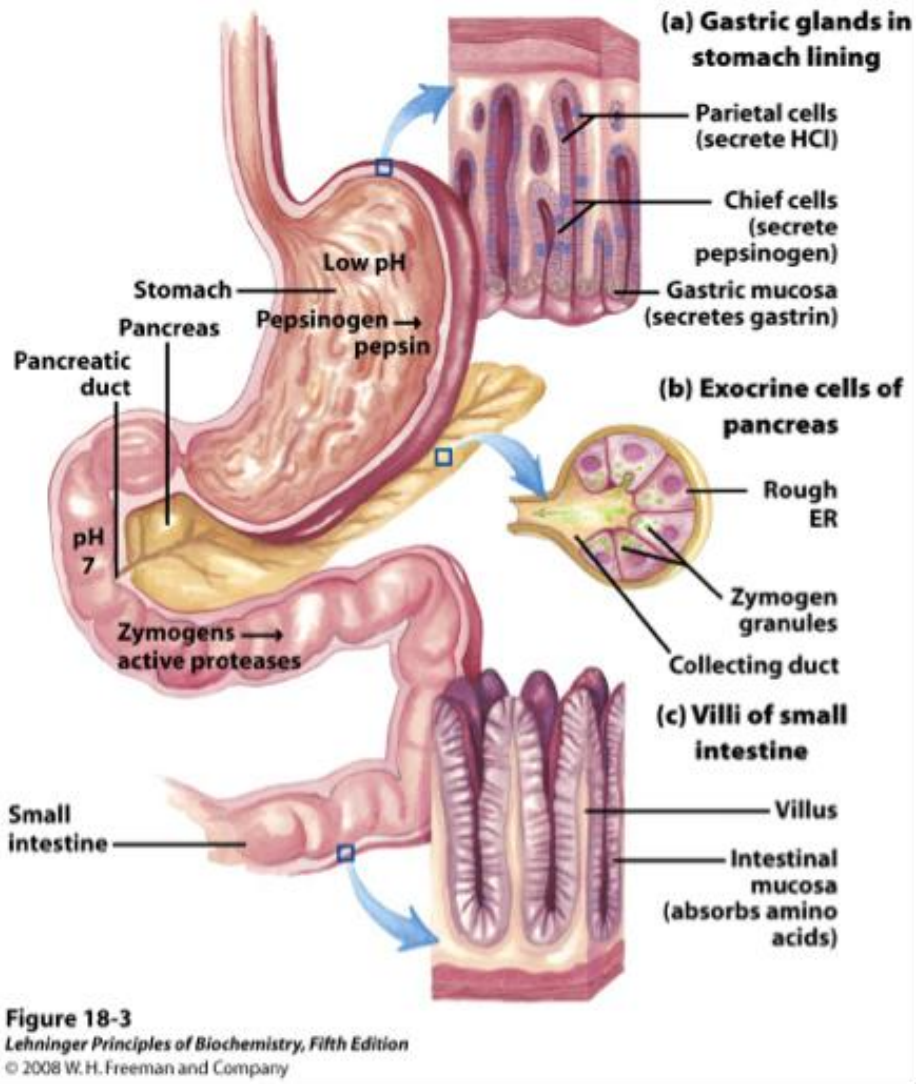


Figure 18-3
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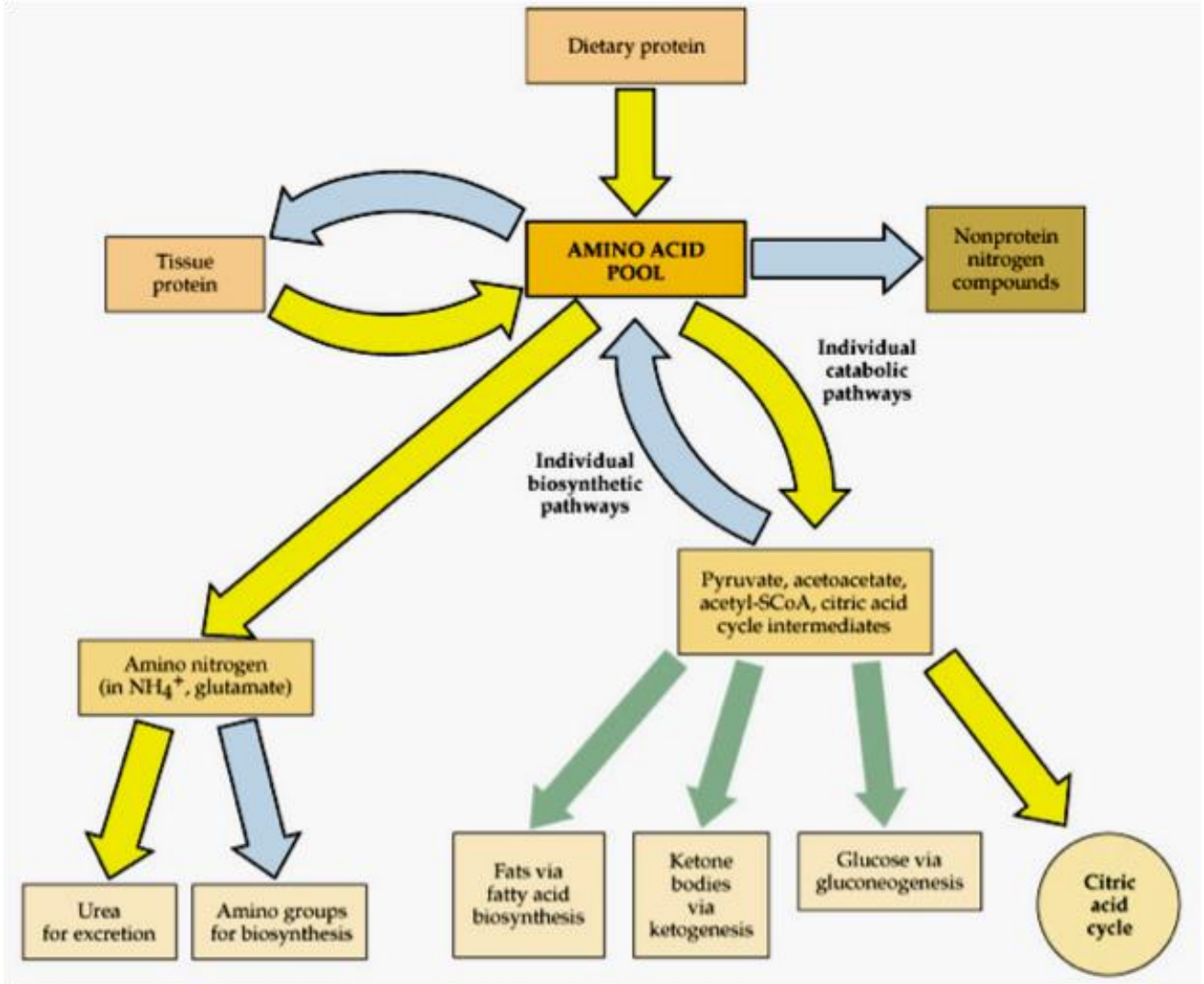
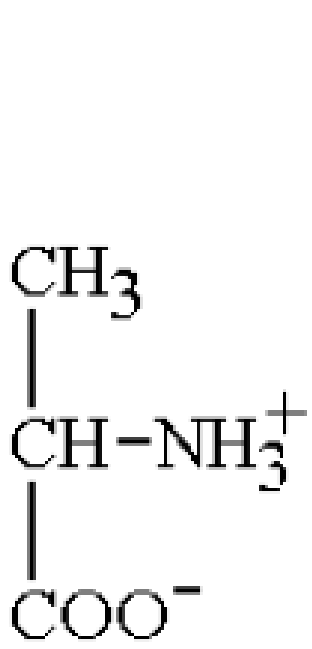
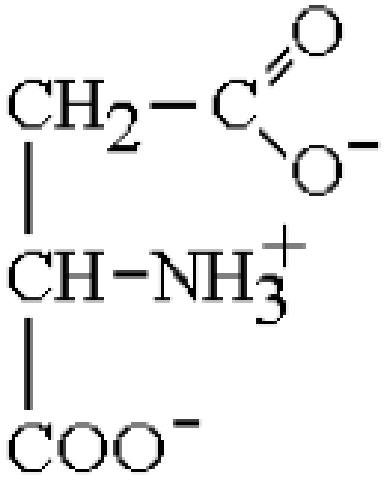
Overview of amino acid metabolism



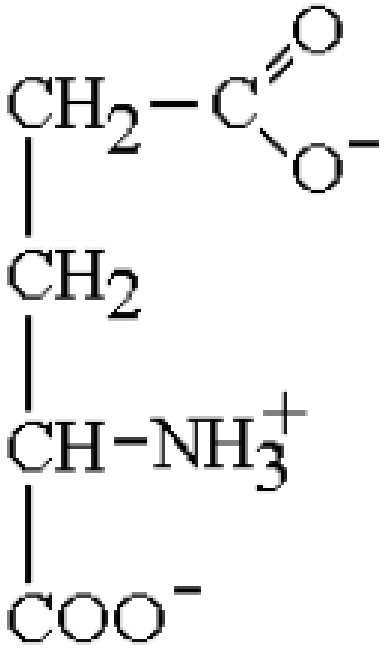
Amino acids mostly found in cells



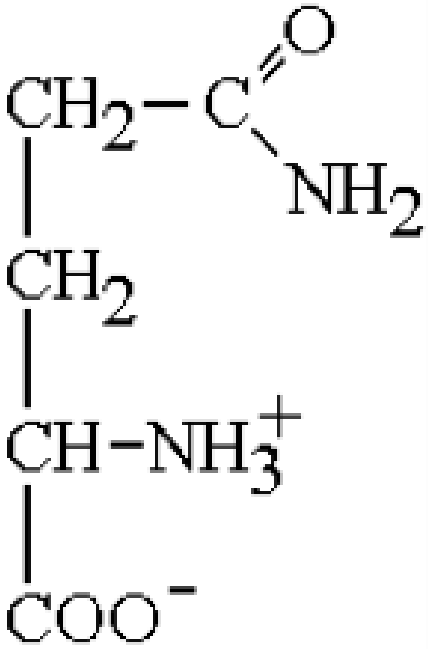
Alanine



Aspartate



Glutamate



Glutamine

Amino group catabolism

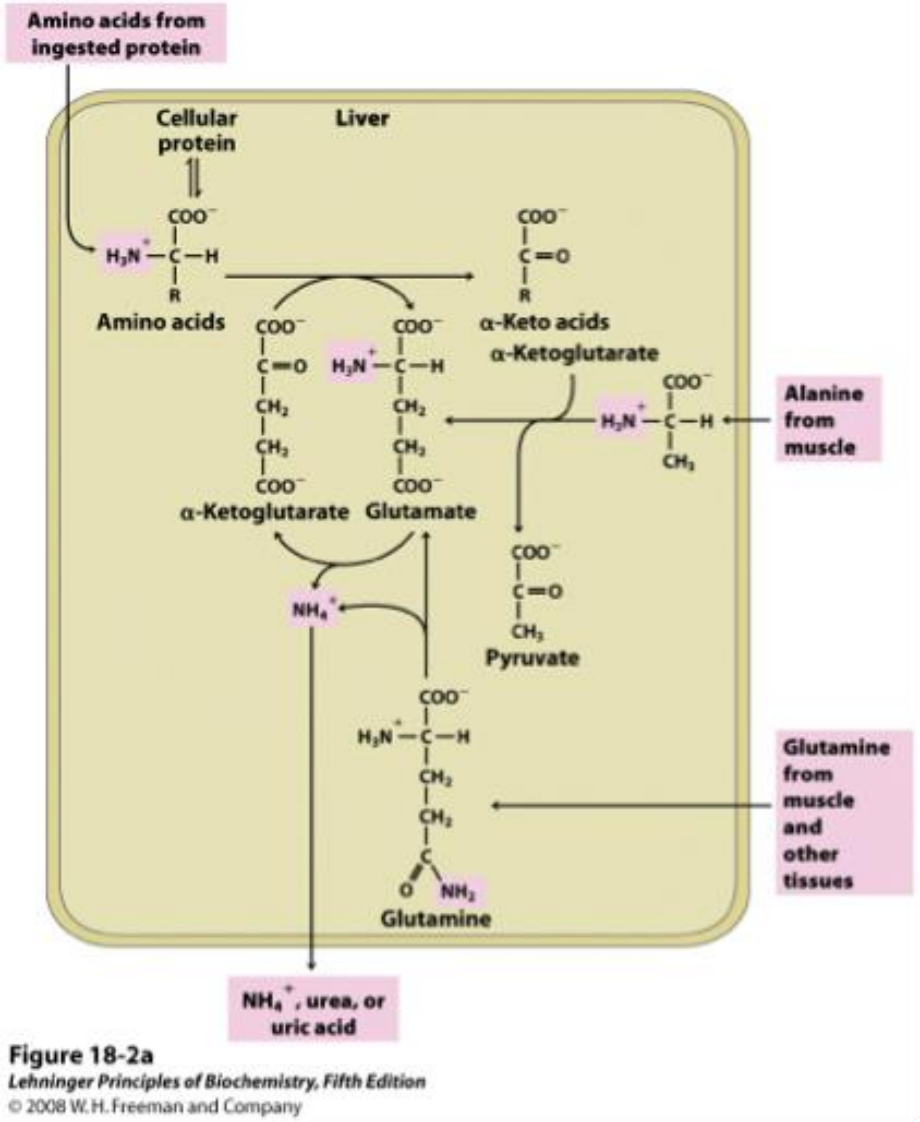


Figure 18-2a
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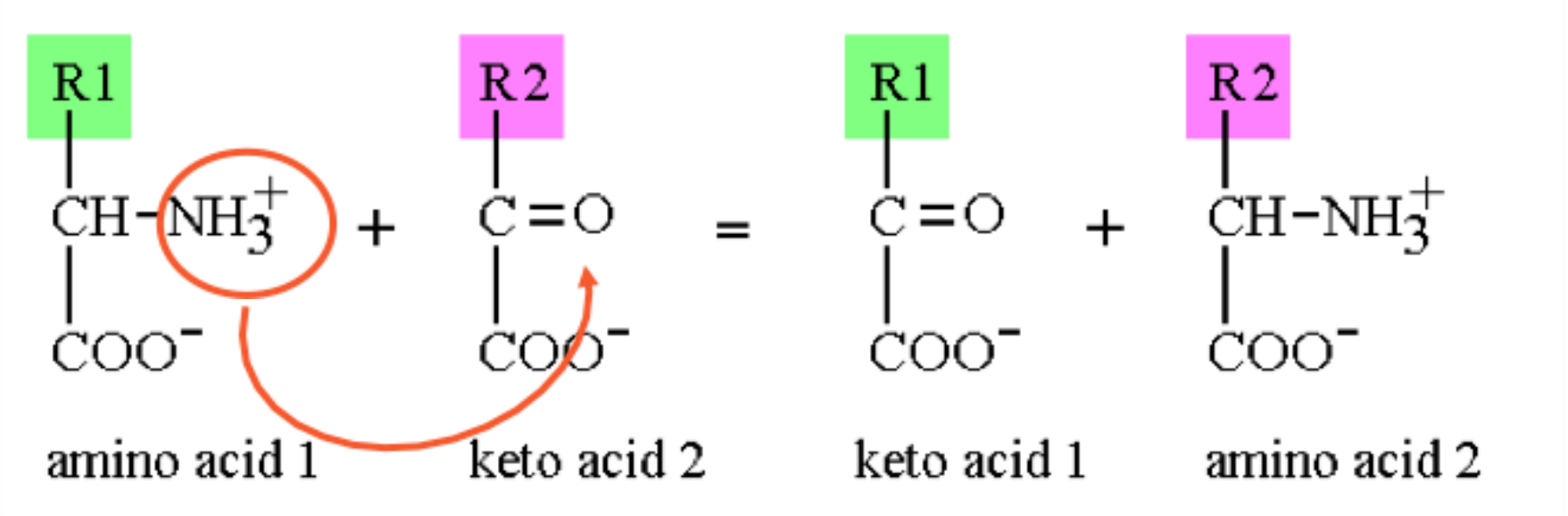
Amino acid catabolism

Removal of alpha- amino group from amino acid

Degradation of carbon skeleton

1. Transamination

- Alpha-amino group is transferred to alpha-carbon of alpha-ketoglutarate.
- Catalyzed by aminotransferase or transaminase
- Occurs in cytoplasm of liver cells



1. Transamination (cont.)

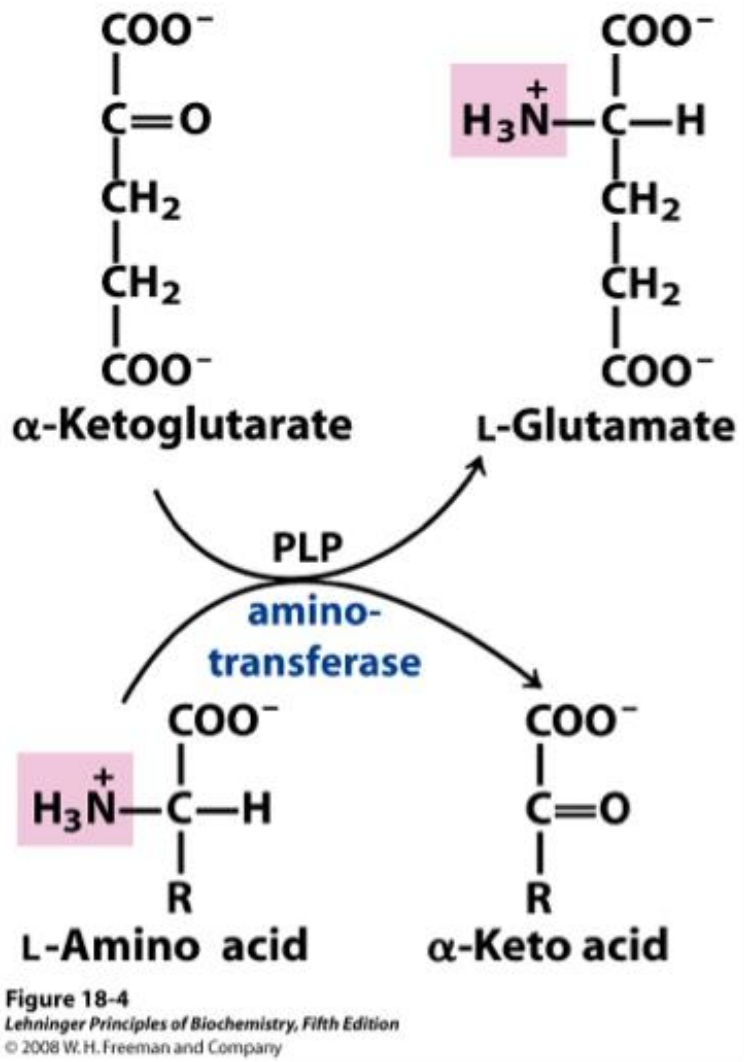


Figure 18-4
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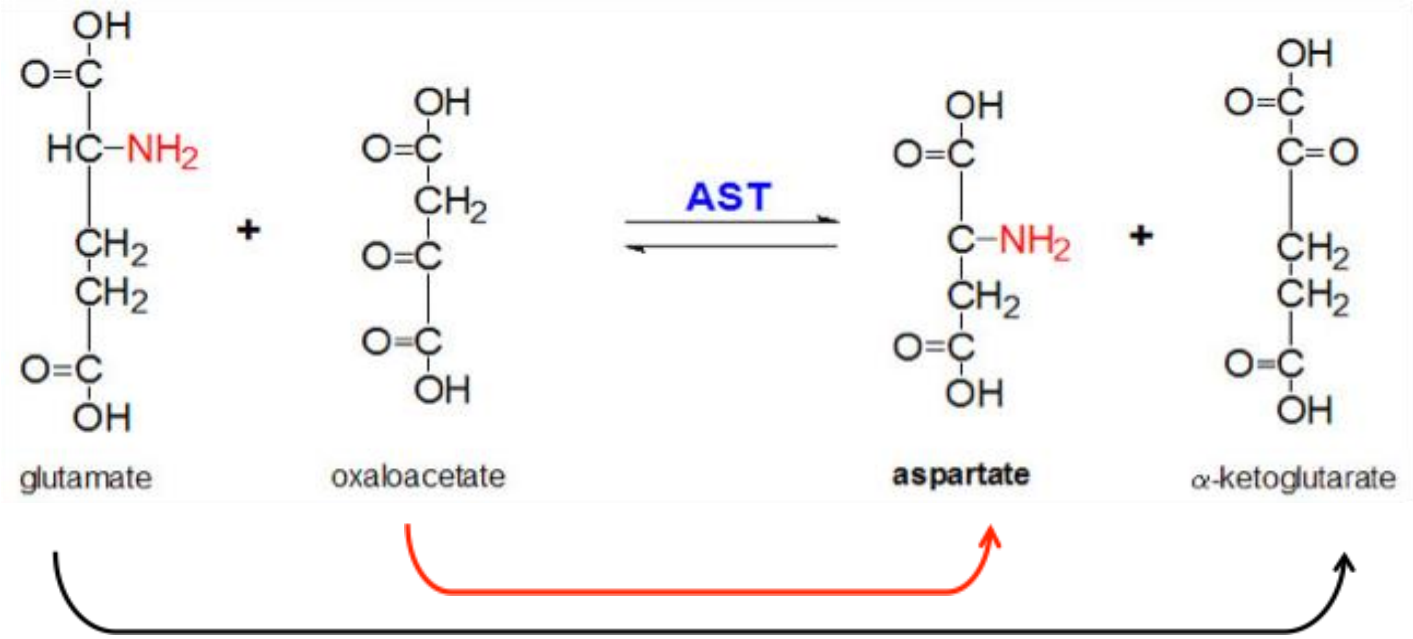
- Effect of transamination --> collect amino groups from as many as different amino acids in form of L-glutamate
- L-glutamate acts as amino group donor for biosynthetic pathways or for excretion pathways
- Aminotransferases are specific for alpha-ketoglutarate as donor but differ in their specificity for the L-amino acid

1. Transamination (cont.)

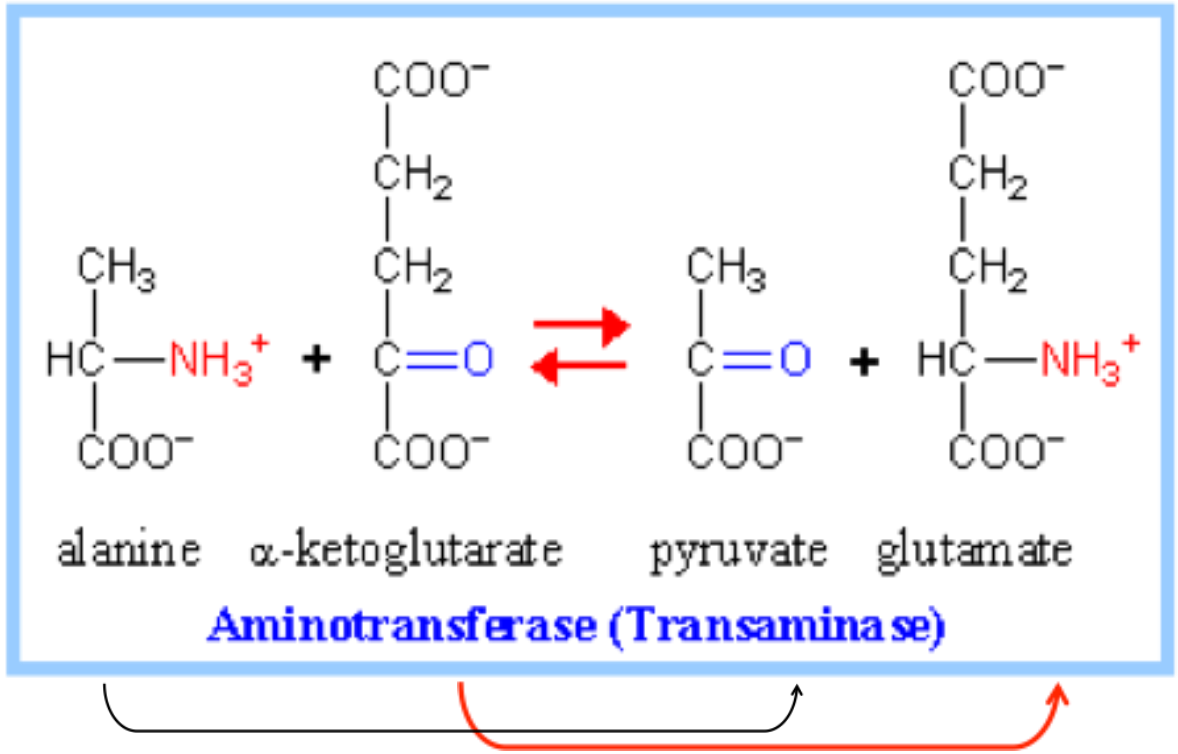
Aspartate Transaminase: AST

- Glutamate transfers α-NH_2 to keto acid (oxaloacetate).
- Glutamate is converted to be the keto acid (α-ketoglutarte)
- Oxaloacetate is converted to be amino acid (aspartate)



1. Transamination (cont.)

- alanine transfers α-NH_2 to the keto acid (α-ketoglutarate)
- alanine is converted to be the keto acid (pyruvate)
- α-ketoglutarate is converted to be the amino acid (glutamate)



1. Transamination (cont.)

Pyridoxal-5’-phosphate: PLP

- Prosthetic group of aminotransferase
- Co-enzyme form of pyridoxine or vitamin B6
- Intermediate carrier of amino group at active site of aminotransferases
- Reversible transformation between two forms; pyridoxal phosphate and pyridoxamine phosphate

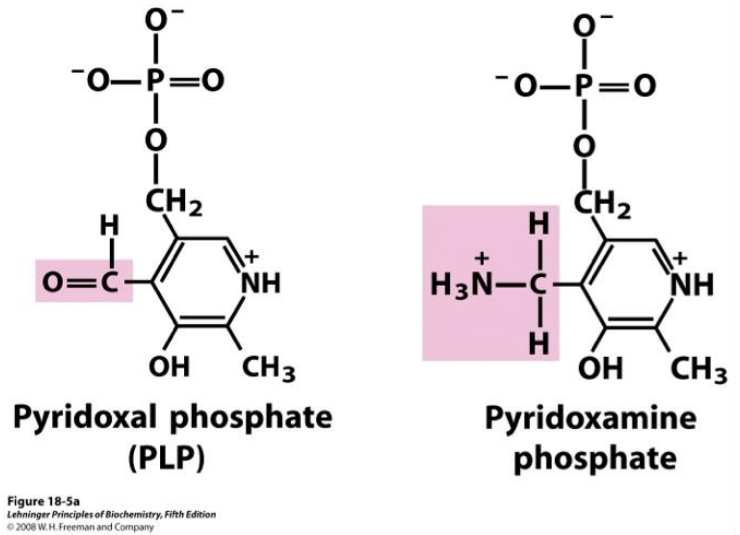


Figure 18-5a
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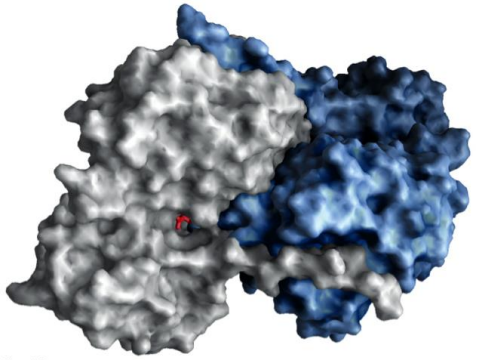

Figure 18-5c
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Take home message I

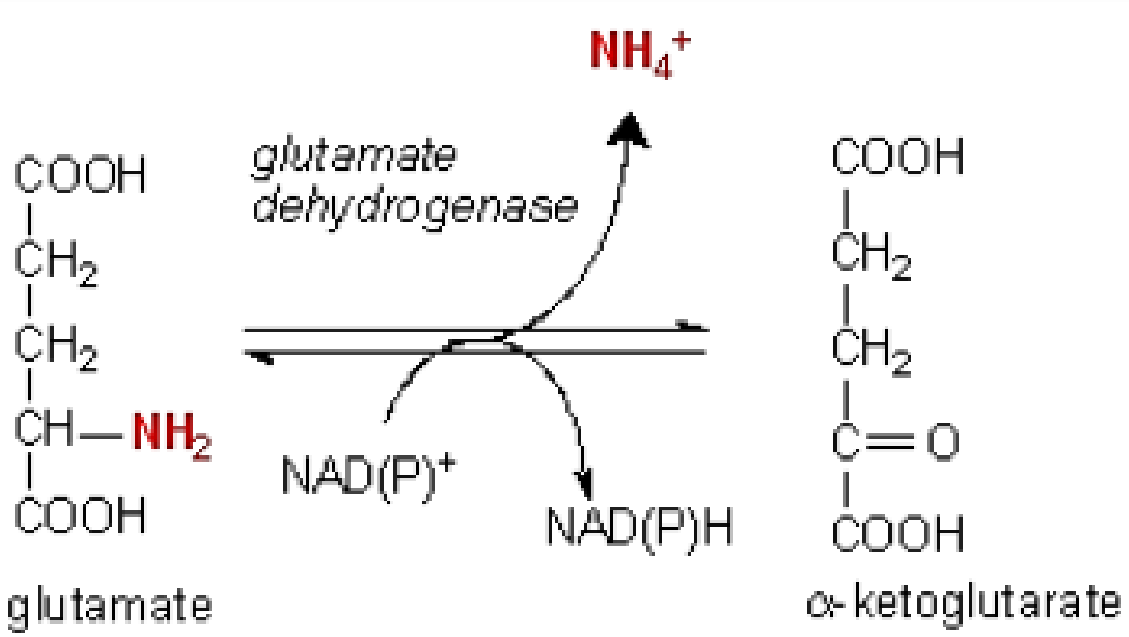
1. It is early step in amino acid catabolism to separate amino group from carbon skeleton & takes place in cytoplasm of hepatocytes.

2. In most cases, amino group is transferred to alpha-ketoglutarate to form glutamate.

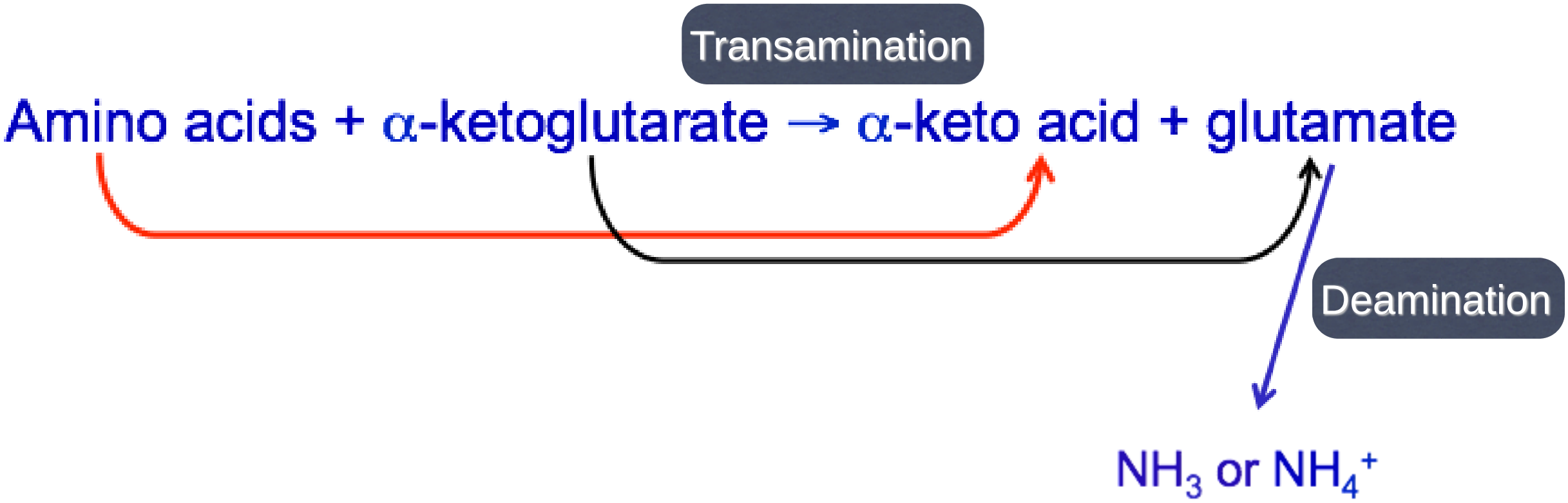
3. It requires enzyme namely aminotransferase or transaminase and vitamin B6 as co-enzyme in form of PLP.

2. Oxidative deamination

- To cleave alpha-amino group from glutamate
- Catalyzed by glutamate dehydrogenase (GDH)
- Requires NAD^+ or $NADP^+$ as an electron receptor
- NH^{4+} is released
- Occurs in mitochondria



3. Combination of transamination and deamination



Take home message II

1. Occurs in hepatocyte mitochondrial matrix

2. Catalyzed by GDH

3. Requires NAD^+ or $NADP^+$ as cofactor for GDH which is allosterically regulated by GTP and ADP

4. Products include alpha-ketoglutarate, NADH and NH_4

Why is ammonia toxic to body?

Ammonia

$NH3 \Leftrightarrow NH_4^+$

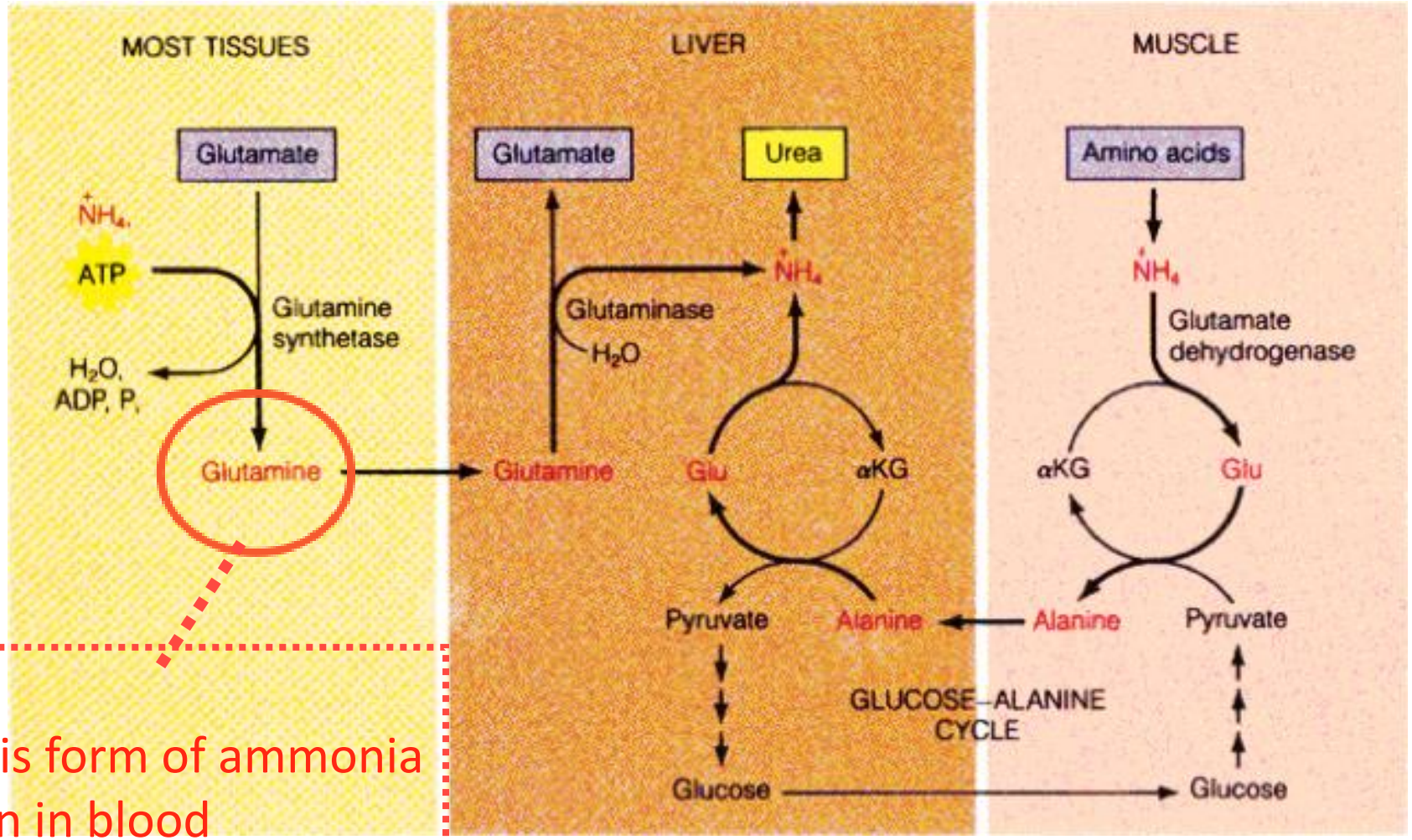
- pK of ammonia is high.
- 99% of ammonia in body is in the form of NH^{4+}.
- another 1% of ammonia is in the form of NH3, which can pass through blood brain barrier.

Glutamate dehydrogenase

NH_4^+ + alpha-ketoglutarate + NADPH + H^+ -------> Glutamate + $NADP^+$ + H2O

higher ammonia --> require more alpha-ketoglutarate
-->decrease in ATP --> affect brain's function (i.e. COMA)

Transportation of ammonia from body tissues to liver



High in blood
so glutamine is form of ammonia transportation in blood

Glucose-Alanine Cycle

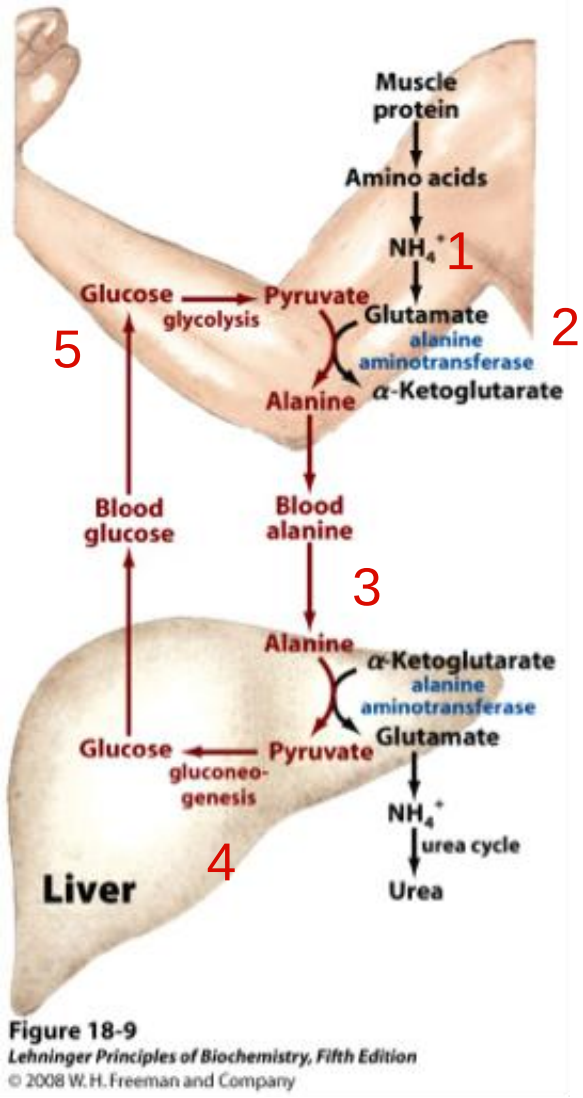


Figure 18-9
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1. Amino group is collected in form of glutamate by oxidative deamination.
2. Transfer of amino group from glutamate to pyruvate catalyzed by alanine aminotransferase --> alanine
3. Alanine is passed into blood and transported to liver.
4. Reverse of alanine aminotransferase takes place in liver and regenerated pyruvate forms glucose via gluconeogenesis.
5. Glucose returns to muscle through circulation system & glycolysis provides pyruvate.

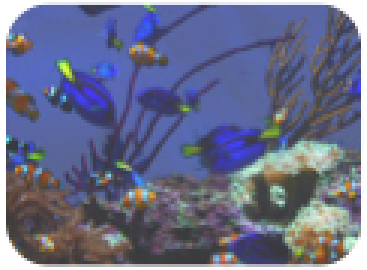
Take home message III

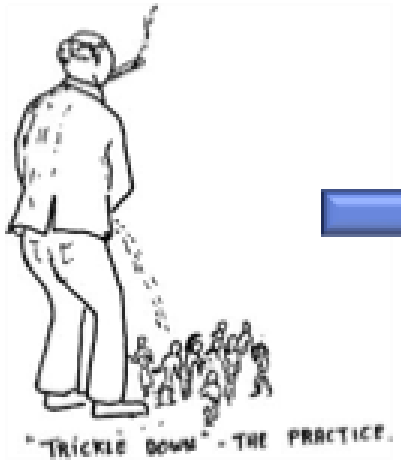
1.The glucose-alanine cycle enables pyruvate and glutamate to be removed from the muscle and find their way to the liver.

2. The energetic burden of gluconeogenesis is thus imposed on the liver instead of muscle.

3. All available ATP in muscle is devoted to muscle contraction.

Ammonia detoxification





amino acids

deamination

NH_3 + keto acids

NH_3 ammonia

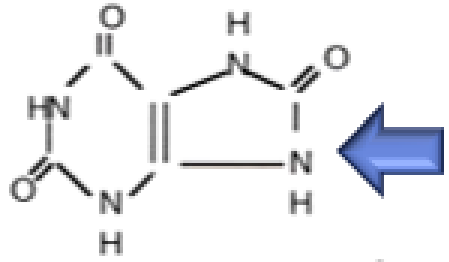
Urea cycle

15 reaction pathway

urea

$H_2N\text{-}C\text{-}NH_2$ (O)

uric acid



more energy needed to produce

more water needed to excrete

Urea cycle

- occurs only in hepatocyte

- spans two cellular compartments; both mitochondria and cytosol

- consists of four enzymatic steps

Urea cycle (cont.)

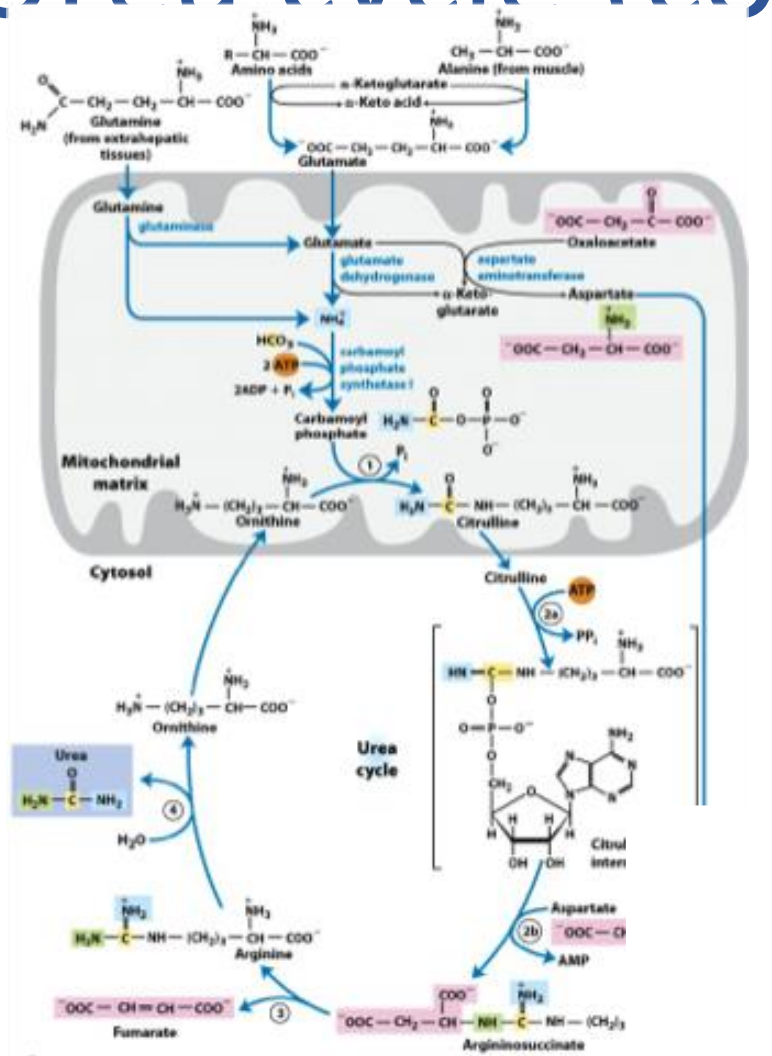


Figure 18-10
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Before step I

- NH_4^+ (generated in liver mitochondria) is used together with CO_2 as HCO_3^-, catalysed by carbamoyl phosphate synthase I (CPSI) to form carbamoyl phosphate in mitochondrial matrix.
- CPSI = rate-limiting enzyme

Step I

- Formation of citrulline from ornithine and carbamoyl phosphate (entry of the first amino group), catalyzed by ornithine transcarbamoylase
- Citrlline passes into cytosol

Step II

- Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of the second amino group), catalyzed by agininosuccinate synthase

Urea cycle (cont.)

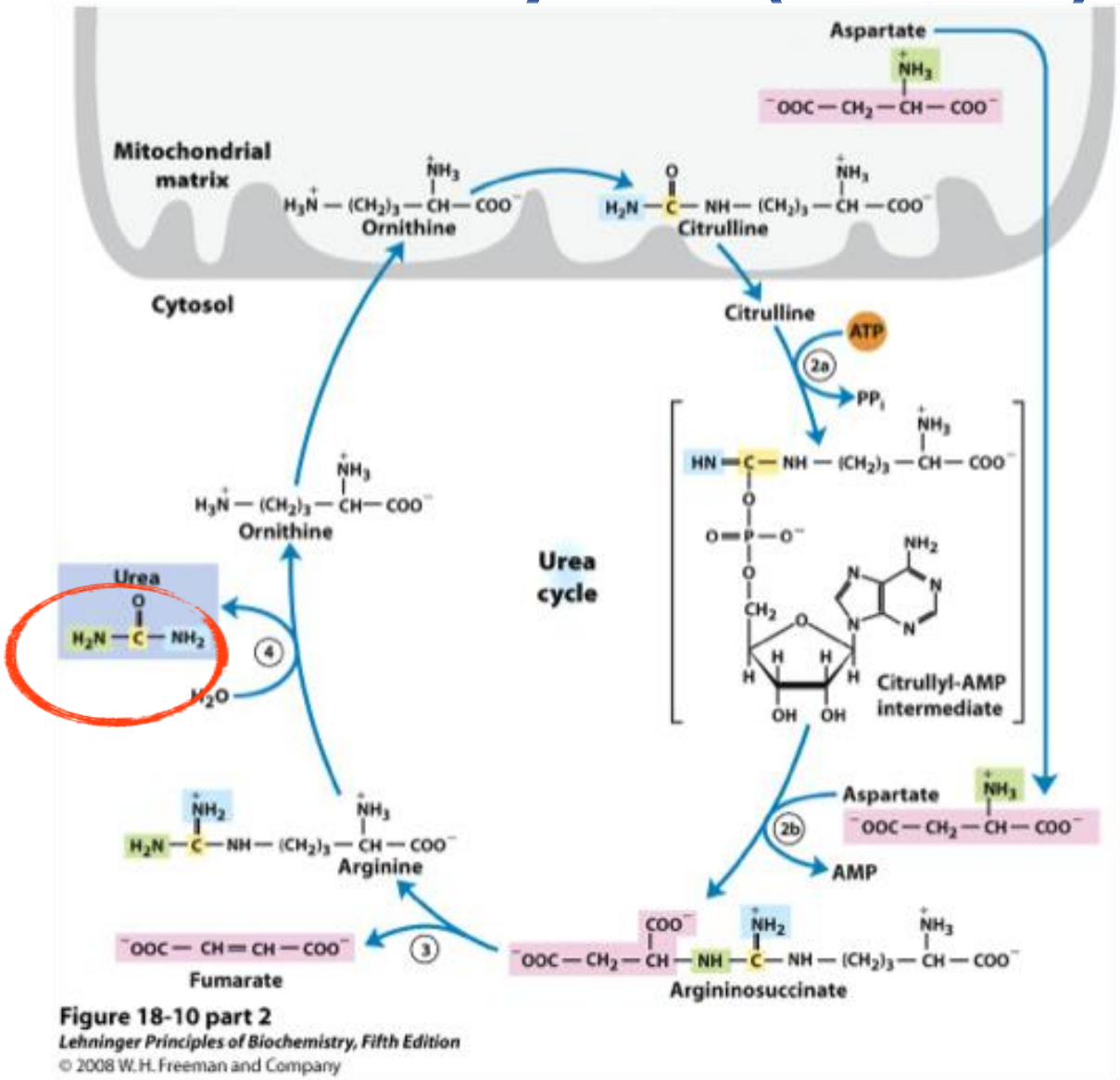

Figure 18-10 part 2
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Step III
- Cleavage of arginiosuccinate by <u>argininosuccinase</u> to form arginine and fumarate; only reversible step in urea cycle
- Fumarate can be converted into malate to join the pool of citric acid cycle intermediates.

Step IV
- Cleavage of arginine by <u>arginase</u> to yield urea and ornithine
- Ornithine is transported into mitochondrion to initiate another round of urea cycle.

Urea cycle is linked to Kreb's cycle

-Krebs Bicycle

- Citric acid cycle enzymes such as fumarase and malate dehydrogenase have both cytosolic and mitochondrial isozymes.

-Fumarate (produced in argininosuccinase reaction) can be converted to cytosolic malate or transported into mitochondria to enter citric acid cycle. These processes are intertwined with malate-aspartate shuttle

- Aspartate formed in mitochondria by transamination between oxaloacetate and glutamate can be transported to cytosol where it serves as nitrogen donor in urea cycle reaction catalysed by argininosuccinate synthetase = aspartate-argininosuccinate shunt

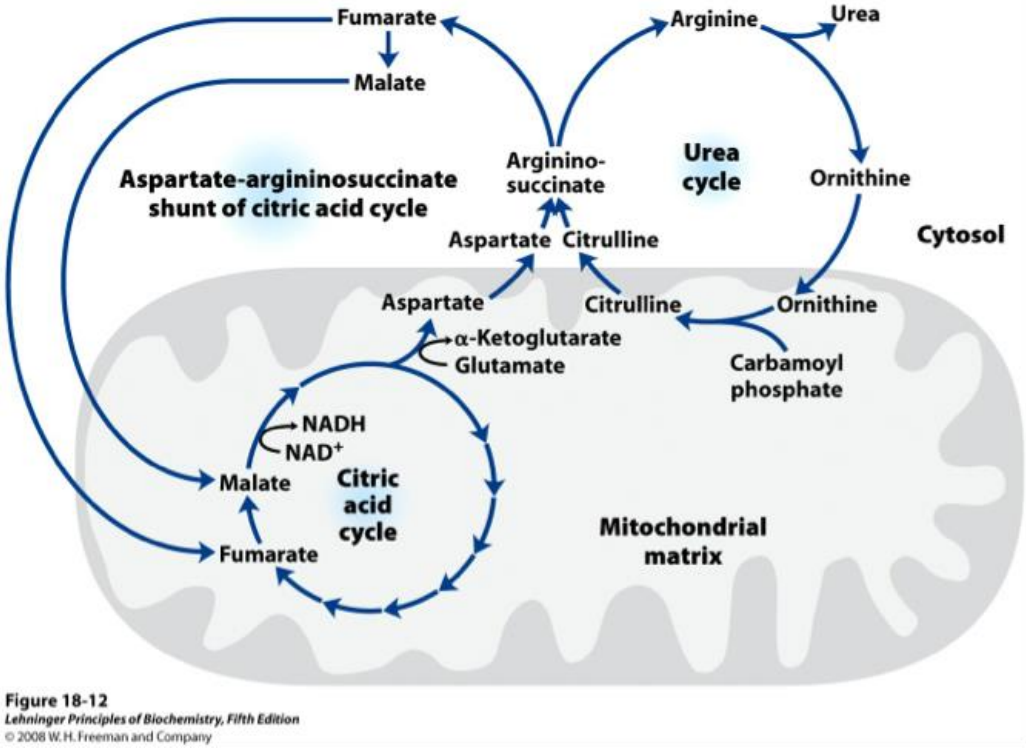

Figure 18-12
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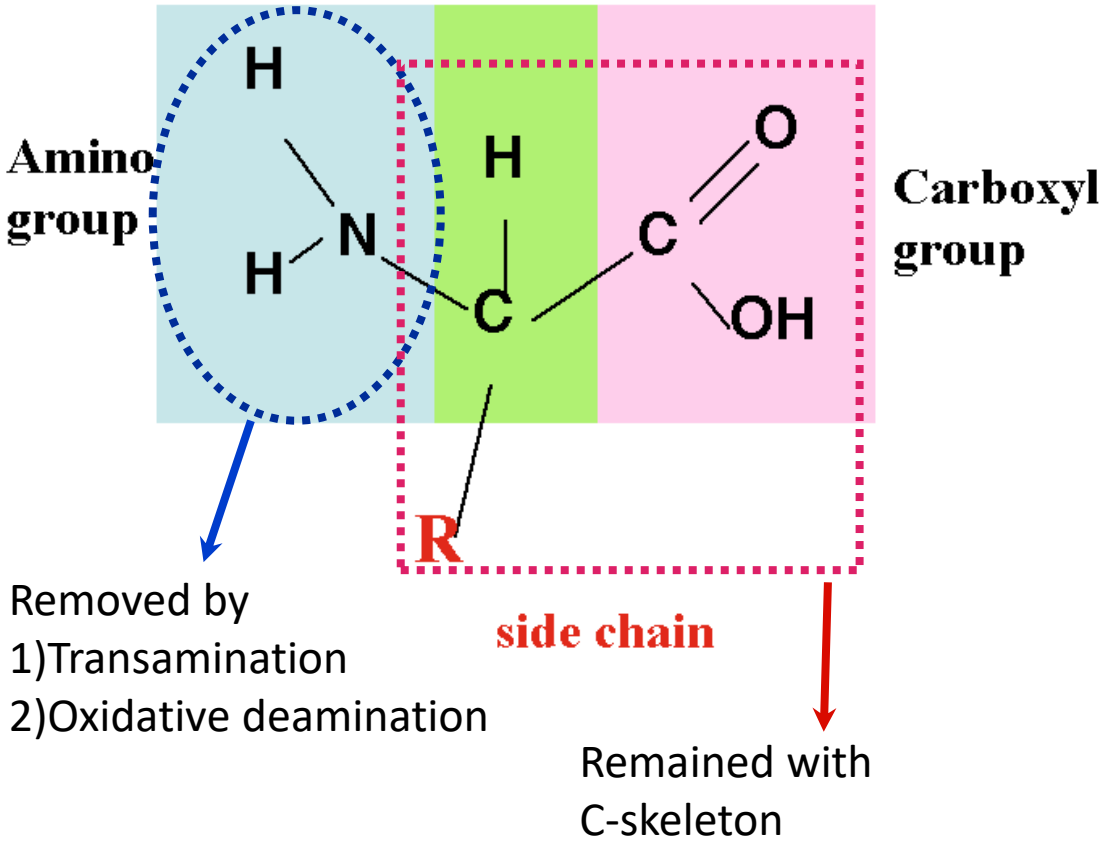
Take home message IV

1. Urea cycle takes place in liver (both cytoplasm and mitochondria) using ATP, CO_2, NH_3.

2. Ornithine combines with ammonia, in the form of carbamoyl phosphate, to form citrulline. A second amino group is transferred to citrulline from aspartate to form arginine - the immediate precursor of urea. Arginase catalyzes hydrolysis of arginine to urea and ornithine; thus ornithine is regenerated in each turn of the cycle.

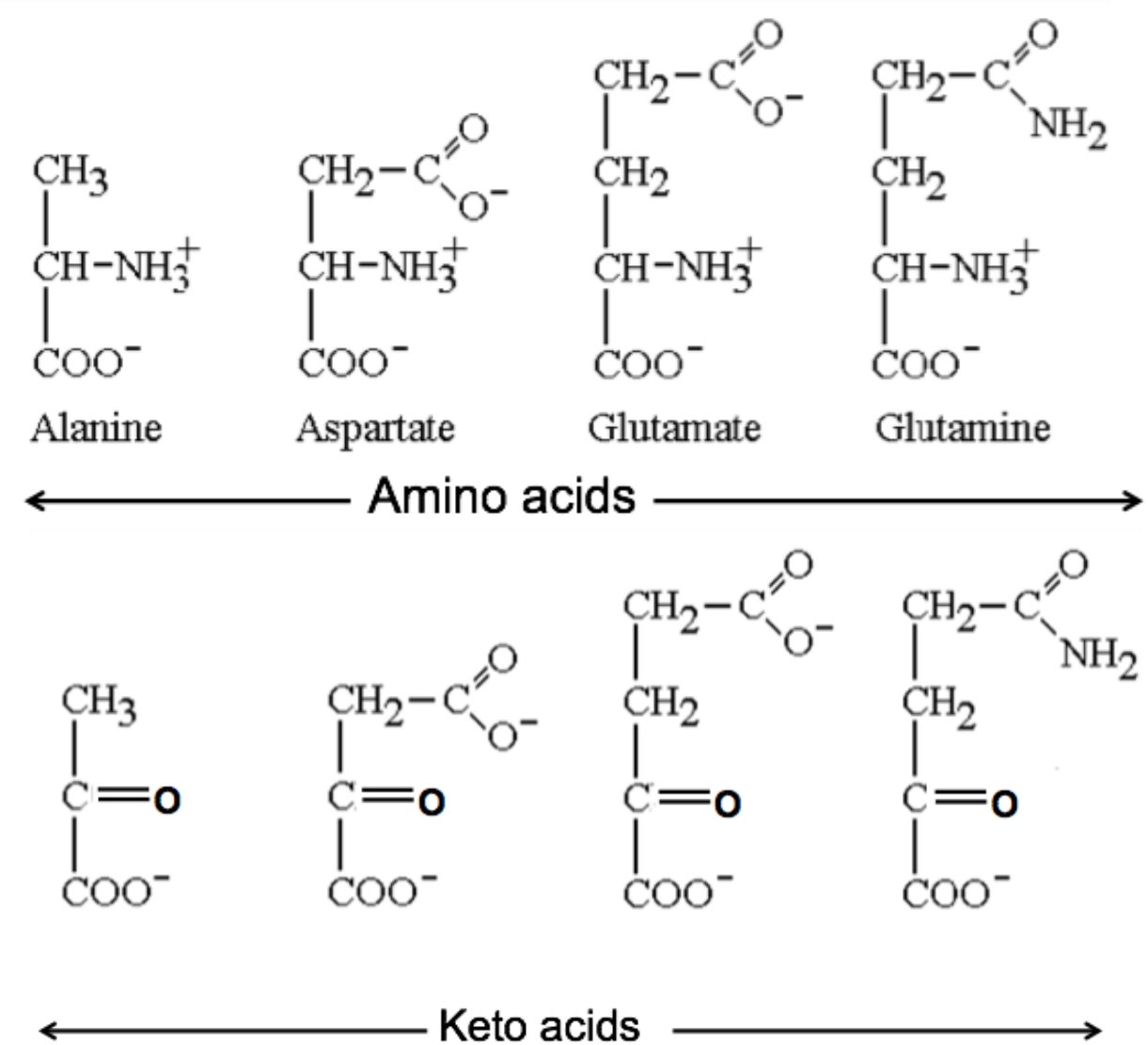
3. The urea cycle results in a net conversion of oxaloacetate to fumarate, both of which are intermediates in citric acid cycle. Thus, the two cycles are interconnected.

Degradation of carbon skeletons



- Following removal of alpha-amino group, the remaining is known as C-skeleton, most of which are in the form of alpha-keto acids.
- C-skeletons can be converted to intermediates of main metabolism pathways (glycolysis and Kreb's cycle). Ex: Conversion of C-skeletons yields pyruvate, alpha-ketoglutarate, oxaloacetate, fumarate, acetoacetyl CoA, succinyl CoA and acetyl CoA that can be either metabolized for energy or served as precursor for synthesis of molecules with other pathways.

Degradation of carbon skeletons (cont.)



Two classes of amino acids

1. Glucogenic amino acids:

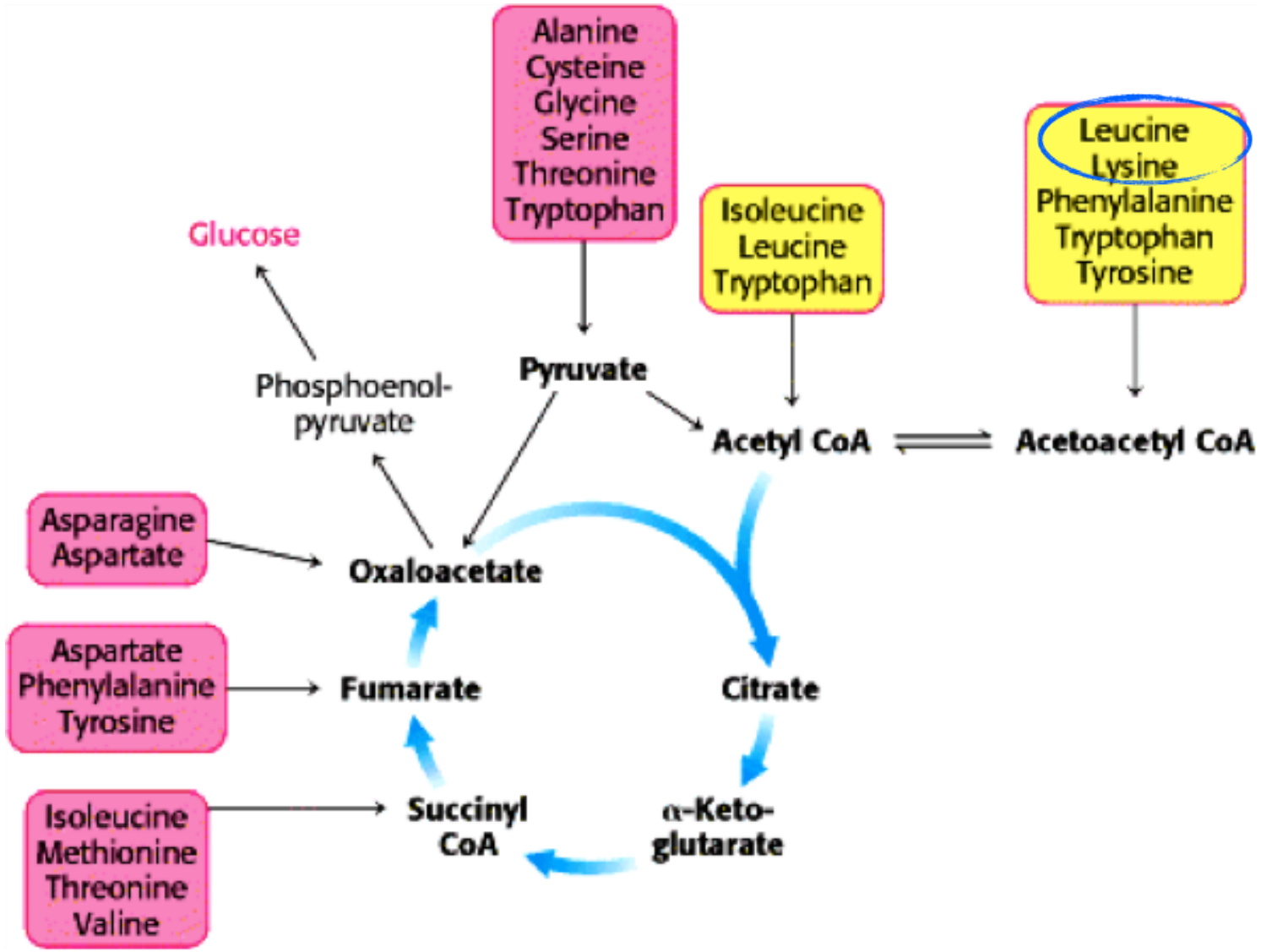
- Carbon skeletons are converted to pyruvate or other intermediates in Kreb's Cycle.
- Glucogenic amino acids act as a carbon source gluconeogenesis when Low blood glucose level
- Could be converted into the glycogen or fatty acids for Energy sources when starvation.

2. Ketogenic amino acids

- Carbon skeletons can be converted into acetyl CoA or acetoacetate.
- Carbon skeletons of ketogenic amino acids can be degraded and pass into the Kreb's cycle for energy production or converted into ketone bodies and fatty acids.

Degradation of amino acids

Glucogenic amino acids
Ketogenic amino acids



Take home message V

1. Removal of alpha-amino group results in remaining c-skeleton.

2. C-skeleton can be converted to several intermediates of main metabolic pathways.

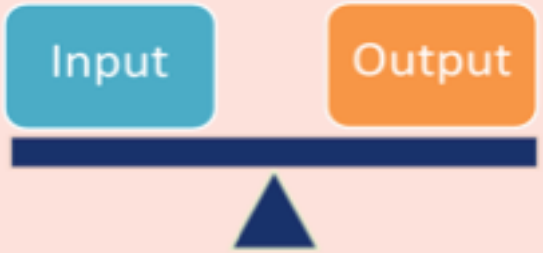
3. Amino acids are classified into 2 groups; glucogenic amino acids and ketogenic amino acids. Leucine and lysine are considered only as ketogenic acids whereas others can be both ketogenic and glucogenic.

Nitrogen balance

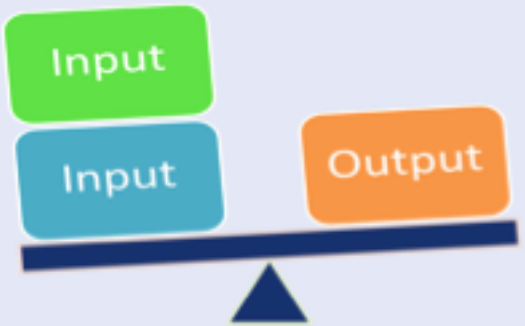
- Amino acid composition = C, H, O, N
- N = 16.5 % of protein weight
- **Nitrogen balance = Nitrogen input - Nitrogen output**
- **Nitrogen input = Dietary protein**
  (Protein requirement = 0.75-1 g/kg BW)
- **Nitrogen output = Urine nitrogen**
  (Urea 90%, Creatinine 5%, Uric acid 3%, Ammonium 2%)

Nitrogen balance (cont.)

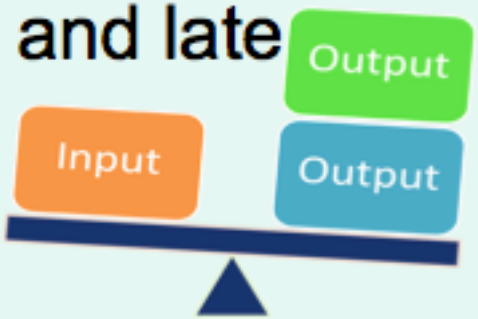
- **Balance** or **Equilibrium**
  eg, Healthy adult



- **Positive** nitrogen balance
  eg. Infants, Pregnancy,
  Recovery patients



- **Negative** nitrogen balance eg. Burn and late stage carcinoma patients,
  High dose glucocorticoid therapy



Take home message VI

1. Nitrogen equilibrium: nitrogen input = nitrogen output

2. Positive nitrogen balance: nitrogen input > nitrogen output

3. Negative nitrogen balance: nitrogen input < nitrogen output

Diseases caused by metabolic errors of amino acids

- Defects in enzyme involved in amino acid catabolism are RARE BUT still considered public health PROBLEMS.

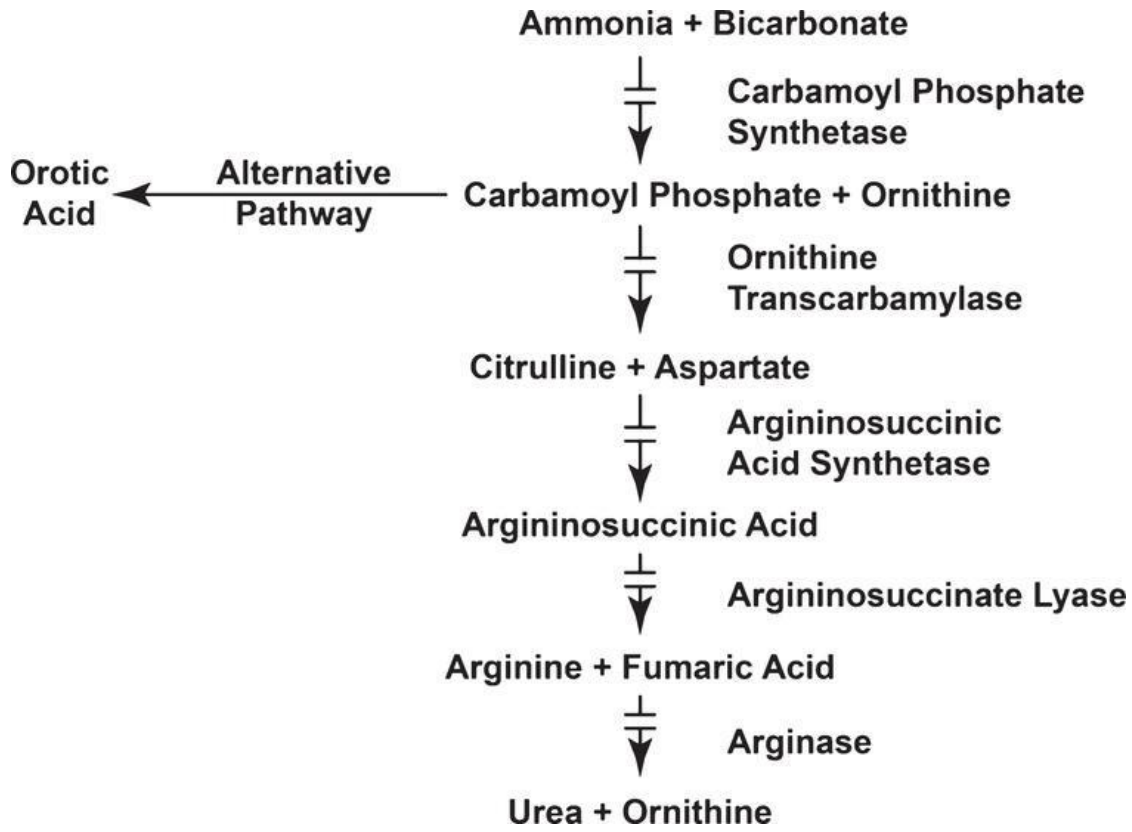
- In most cases, gene mutation is the cause of enzyme defects leading to ineffective enzyme --> accumulation of intermediates (that may result in nervous interference --> mental retardation)

- Absorption of amino acids
- Synthesis of amino acid derivatives
- Urea synthesis
- Degradation of amino acids
- Ex. Three main enzymes involved in degradations of phenylalanine and tyrosine are most common problems of three diseases; phenylketonuria (PKU), alkaptonuria and albinism.

Urea cycle defect

Metabolic disorders of Urea cycle: -

Disorders	Defective Enzymes	Product accumulated
Hyperammonemia-I	Carbamoyl Phosphate Synthase-I	Ammonia
Hyperammonemia-II	Ornithine Transcarbamoylase	Ammonia
Citrullinemia	Arginosuccinate Synthase	Citrulline
Arginosuccinic aciduria	Arginase	Arginosuccinate
Argininemia	Arginase	Arginine



Urea cycle defect (cont.)

- Main symptoms are related to high protein intake, increased catabolism, infections or stress.

Neonates:

* Poor feeding
* Lethargy
* Loss of reflexes
* Seizures
* Temperature lability
* Hyperventilation (respiratory alkalosis)
* Intracranial hemorrhages
* Progressive encephalopathy

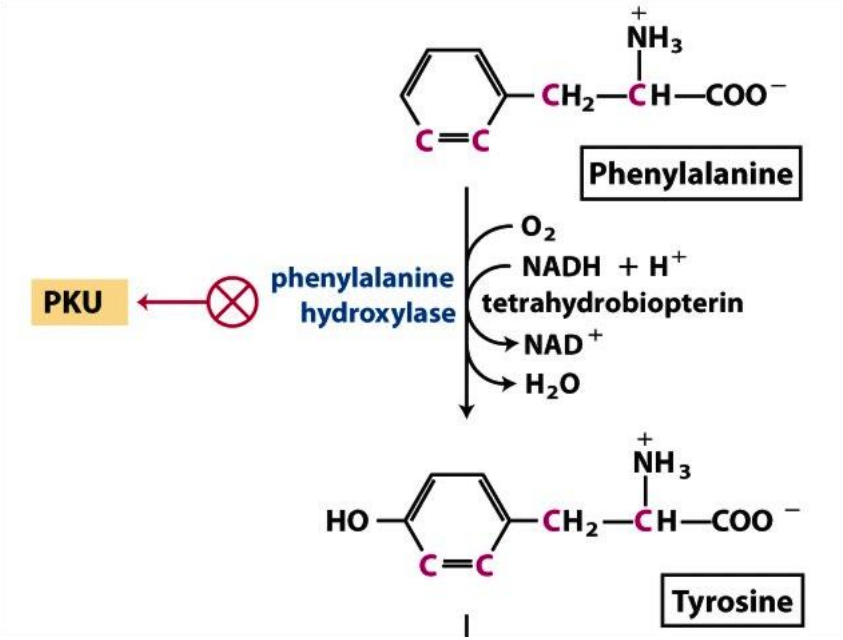
Infants and children

* Failure to thrive
* Feeding problems
* Nausea, vomiting
* Episodic encephalopathy
* Ataxia
* Convulsions

Adolescents and adults

* Chronic neurologic symptoms
* Chronic psychiatric symptoms
* Episodic encephalopathy
* Behavioral problems

Phenylketonuria (PKU)



Modified from Lehninger Principles of Biochemistry

- Normally, phenylalanine hydroxylase (PAH) catalyses the addition reaction of
-OH to phenylalanine to become tyrosine.

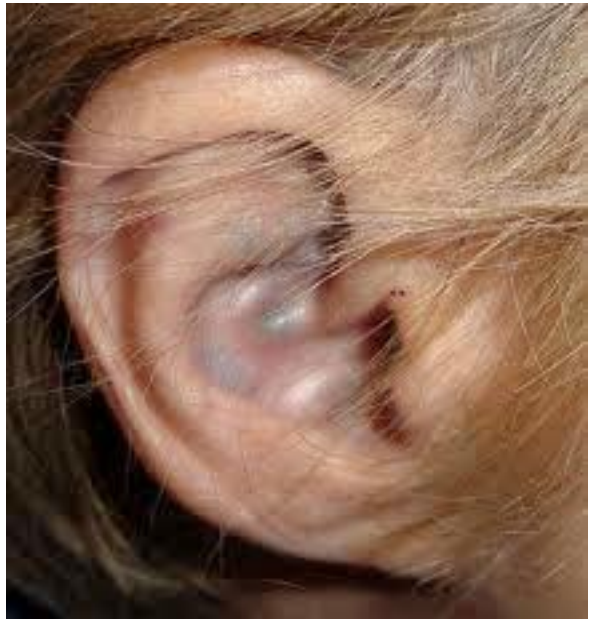
- PAH defect leads to the minor degradation pathway of phenylalanine (rarely occurs under normal condition) to form phenylpyruvate (difficult to be degraded) by transferring amino group to alpha-ketoglutarate.

- The consequence of PAH defect is accumulation of phenylpyruvate & phenylalanine in blood and tissues and excreted with urine --> phenylketonuria

- Mental retardation

- PKU investigation: level of phenylalanine or phenylpyruvate in blood or urine

Alkaptonuria



http://escholarship.org/uc/item/7rt3x92b/2.jpg

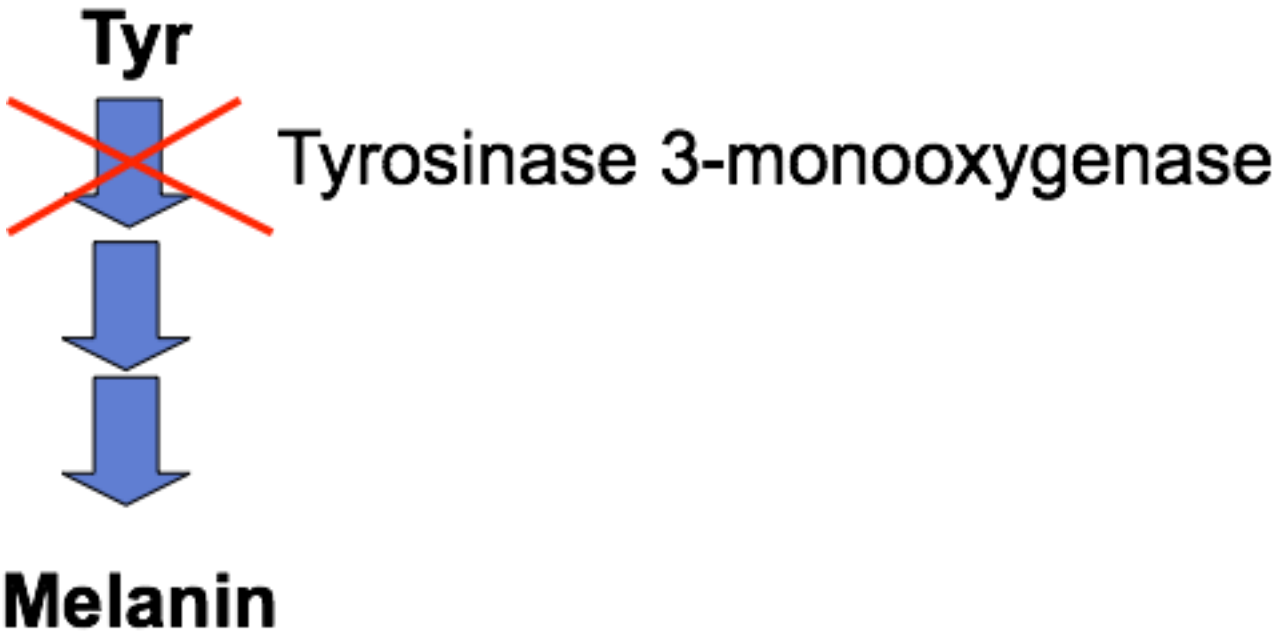
- Defects in homogentisate dioxygensase that catalyses catabolism of homogentisate (an intermediate in phenylalanine-tyrosine pathway)

- Defects in homogentisate dioxygensase that catalyses catabolism of homogentisate (an intermediate in phenylalanine-tyrosine pathway) results in accumulation of homogentisate

Dark Urine: urine with homogentisate --> urea degraded by air bacteria --> ammonia makes urine basic --> homogentisate is oxidised by oxygen --> melanin pigment

- If too much, accumulation of homogentisate in joints give rise dark appearance.

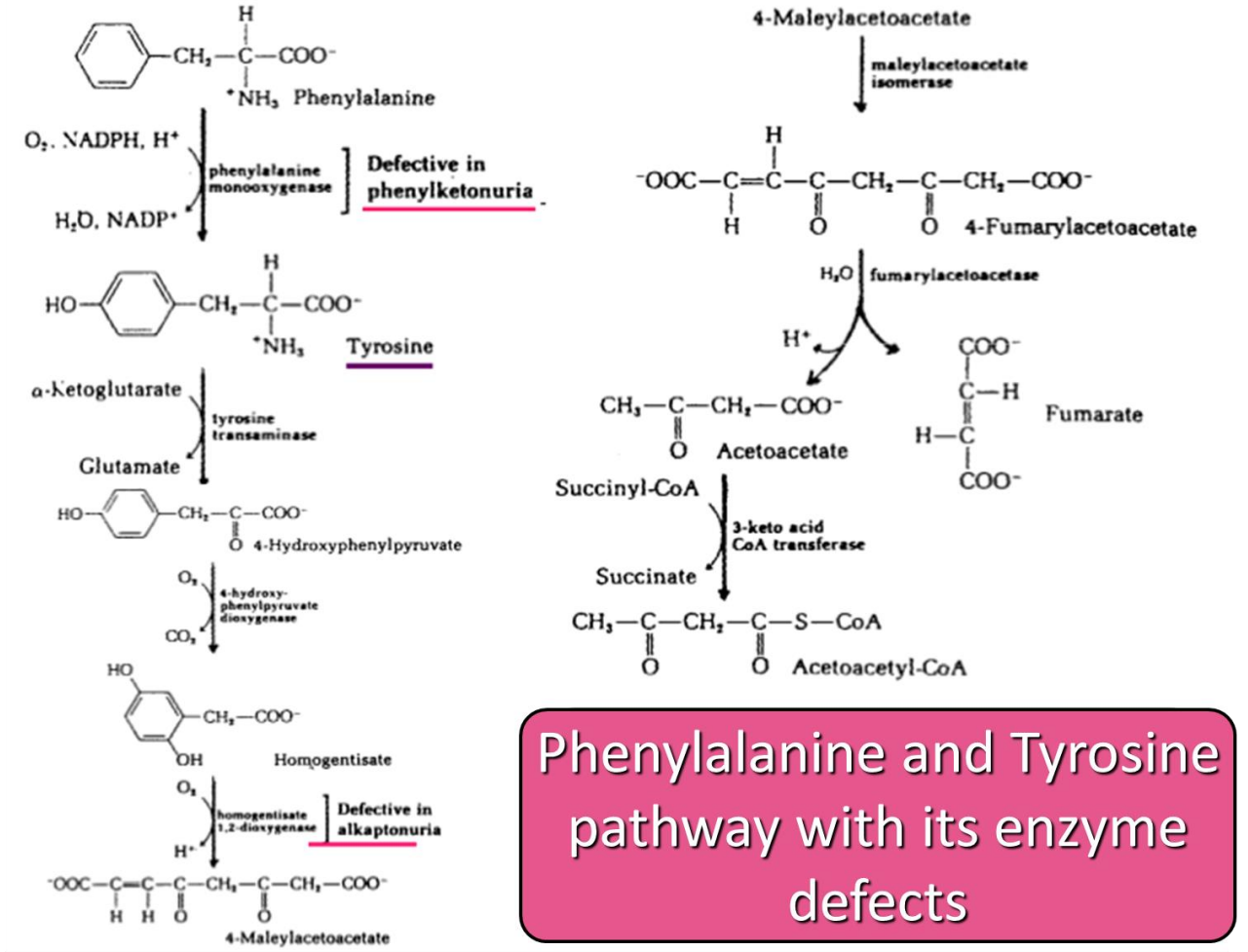
Albinism



Lack of melanin pigment in the eyes, skin and hair

http://laughingsquid.com/albinos-a-beautiful-photo-essay-of-people-with-albinism/

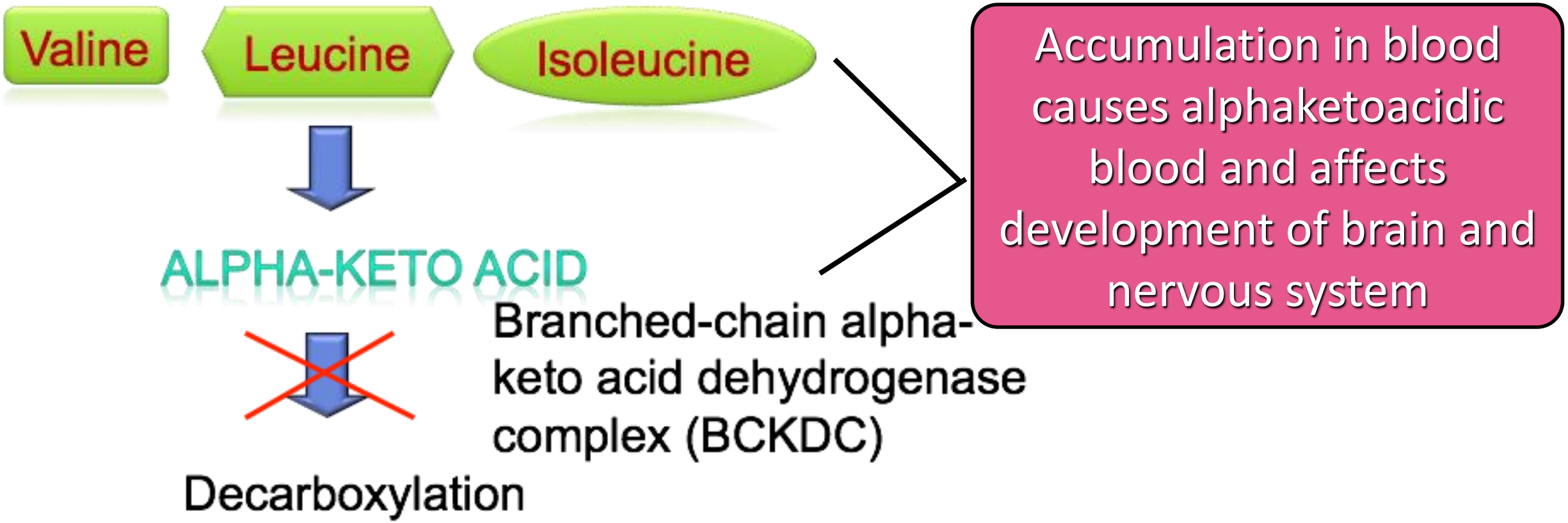
Diseases caused by metabolic errors of amino acids



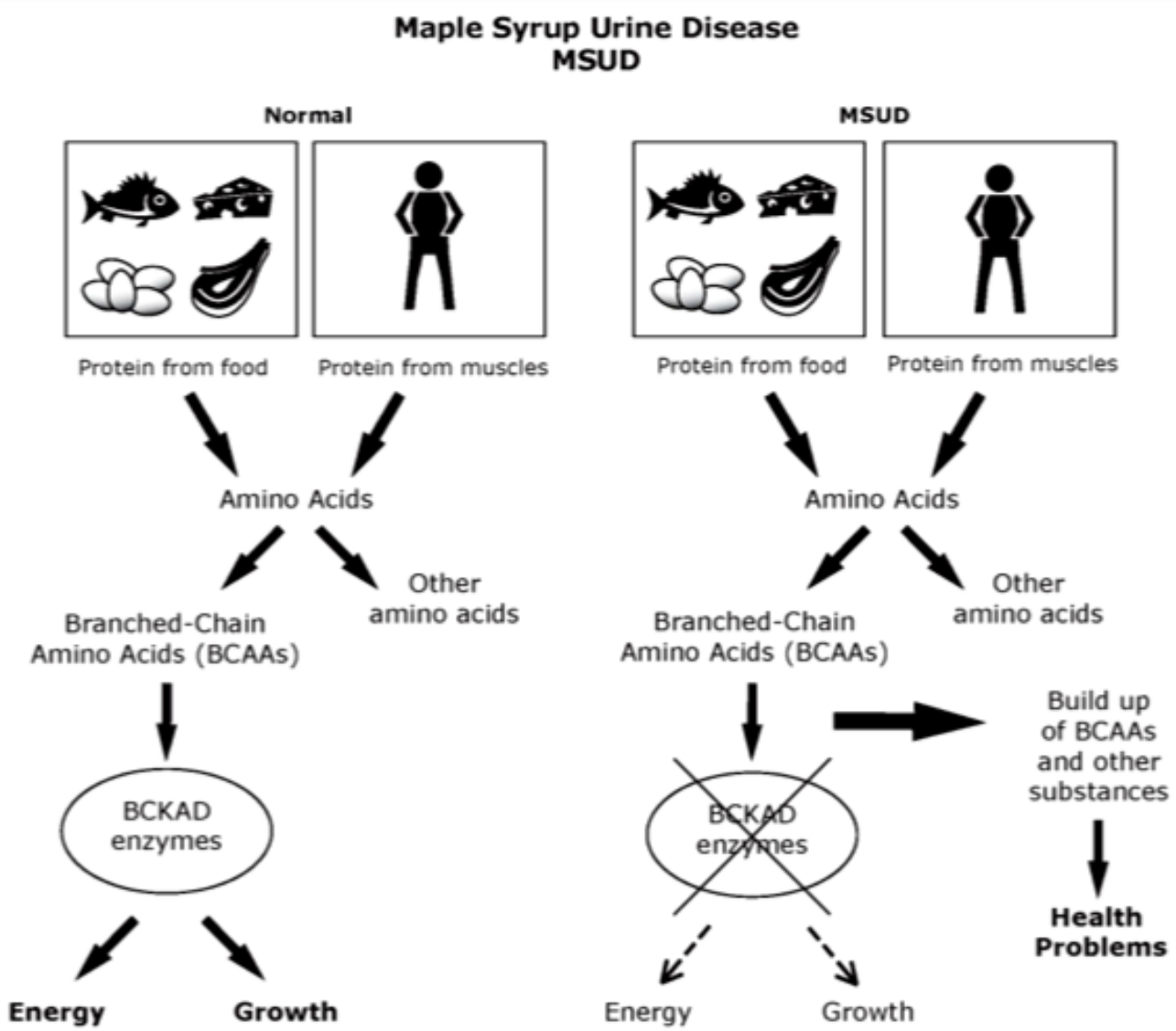
Phenylalanine and Tyrosine pathway with its enzyme defects

Figure from presentation of Somporn Katekaew

Branched-chain ketoaciduria (maple syrup urine)



Branched-chain ketoaciduria (maple syrup ui



Summary

- Protein digestion: Digestive enzymes
- Amino acid degradation : transamination, deamination
- Urea cycle: synthesized in the liver (cytoplasm and mitochondria) using ATP, CO_2, NH_3
- Metabolism of carbon skeleton : glucogenic amino acids and ketogenic amino acids
- Nitrogen balance: positive, negative, balance
- Genetic diseases

Practice questions

1. What are the conditions of amino acid catabolism to take place?
2. Amino acid catabolism involves 2 main steps; what are they, where and how does each reaction take place?
3. How is alpha-amino group processed?
4. How is ammonia generated, transported and metabolised?
5. What is the significance of glucose-alanine cycle in ammonia transportation from muscles to liver?