Metabolism of N-Molecules

- Amino acid catabolism/degradation
  - Amino group
  - C-skeleton
- Amino acid anabolism/biosynthesis
  - Non-essential amino acids
  - Essential amino acids
- Other N containing molecules
- Nucleotide synthesis and degradation
  - de novo synthesis and Salvage pathway
  - N-containing waste

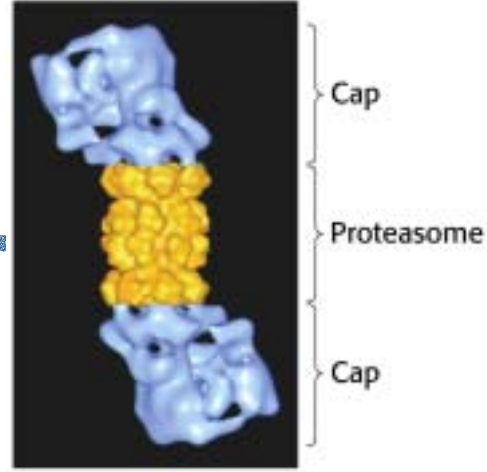
Amino acids catabolism

In animals

1) Protein turnover
   - ✓ Normal cellular protein degradation
     - ATP-independent process in lysosomes
     - Ubiquitin-tag + ATP → proteasome (p. 1066)
2) Dietary protein surplus
   - ✓ Amino acids can not be stored
     - Positive N balance (excess ingestion over excretion)
       - Growth and pregnancy
     - Negative N balance (output exceeds intake)
       - After surgery, advanced cancer, and **kwashiorkor** or **marasmus**
3) Starvation or diabetes mellitus
   - ✓ Protein is used as fuel

p. 623

Protein turnover



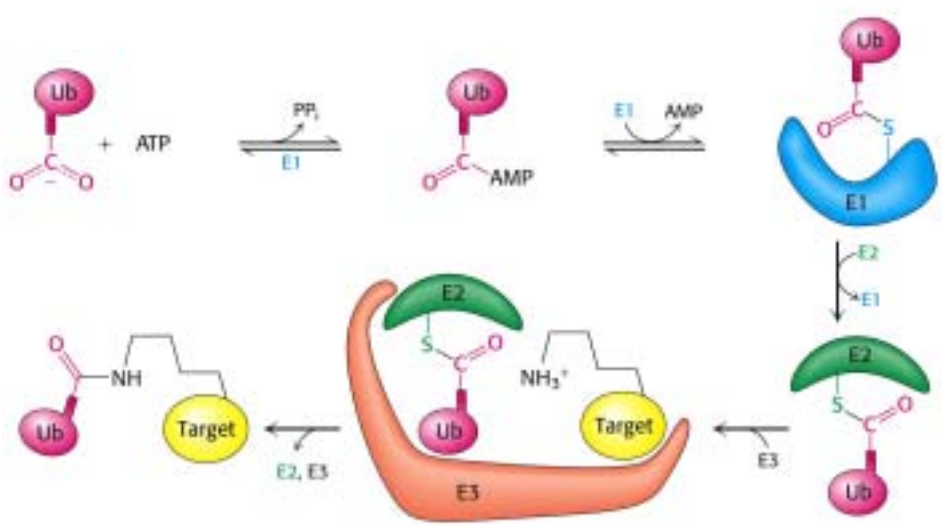
Stryer 5th Fig 23.6

- Membrane associated protein
  - ✓ Lysosome
- Cellular protein
  - ✓ Abnormal, damaged, or regulatory proteins.
  - ✓ Ubiquitin (Ub) and proteasome
    - Ub: the death signal, covalently attached to the target protein
      - N-terminal rule: (Table 27-10)
        - Destabilizing residue: Arg, Leu
        - Stabilizing: Met, Pro
      - Cyclin destruction boxes
        - A.a. sequences that mark cell-cycle proteins for destruction
      - PEST
        - Proteins rich in Pro, Glu, Ser, and Thr.
    - Proteasome: executioner
      - ATP-driven multisubunit protease complex.
      - Proteasome product: Ub + peptides of 7-9 a.a.
      - Peptides are further degraded by other cellular proteases.

Biological function

- Human papilloma virus (HPV)
  - ✓ Encodes a protein that activates a specific E3 enzyme in ubiquitination process.
  - ✓ E3 Ub the tumor suppressor p53 and other proteins that control DNA repair, when are then destroyed.
  - ✓ E3 activation is observed in 90% of cervical carcinoma.
- Inflammatory response
  - ✓ NF-κB (transcription factor) initiates the expression of a number of the genes that take part in this process.
  - ✓ NF-κB normally remains inactivated by binding to an inhibitory protein, I-κB. (NF-κB - I-κB complex)
  - ✓ Signal → I-κB phosphorylated → I-κB – Ub → release NF-κB → immune response.

Stryer 5th Fig 23.3



Stryer 5th

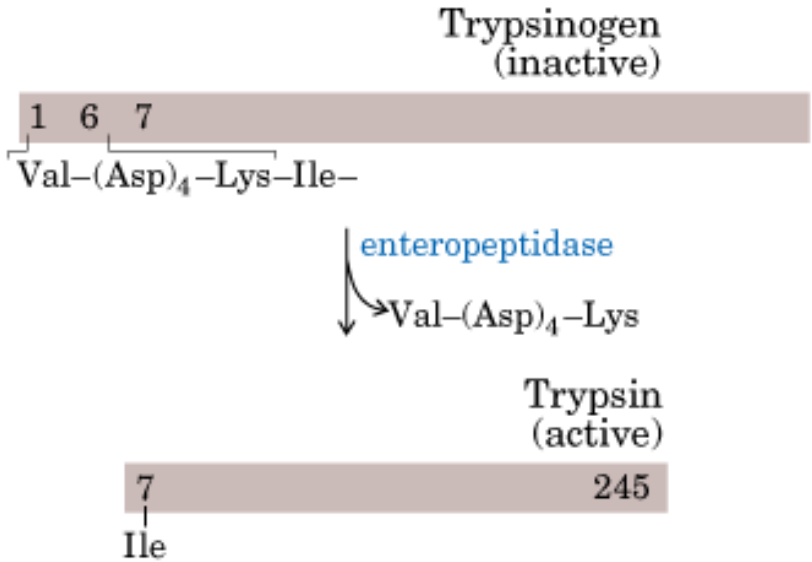
TABLE 23.2 Processes regulated by protein degradation

Gene transcription
Cell-cycle progression
Organ formation
Circadian rhythms
Inflammatory response
Tumor suppression
Cholesterol metabolism
Antigen processing

Regulatory enzymes (Review)

Zymogen or
Proprotein or
Proenzyme

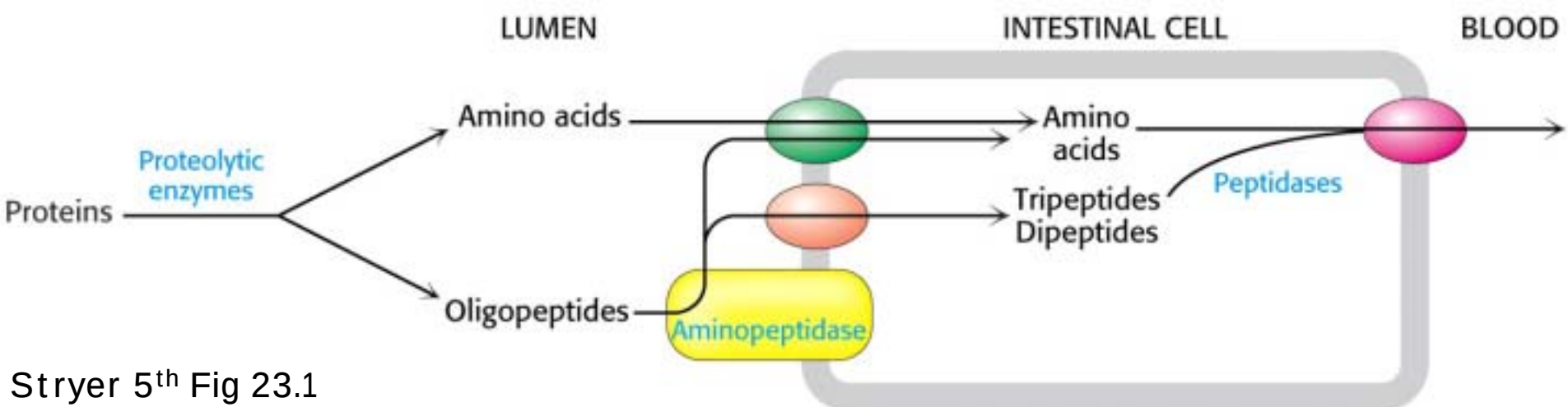
Fig 8-31



- Polypeptide cleavage : inactive → active
  - ✓ Pepsinogen → pepsin
  - ✓ Chymotrypsinogen → chymotrypsin
  - ✓ Trypsinogen → trypsin
  - ✓ Procarboxypeptidase A(B) → carboxypeptidase A(B)
- Irreversible activation → inactivate by inhibitors
  - ✓ Pancreatic trypsin inhibitor (binds and inhibits trypsin)

Protein Digestion

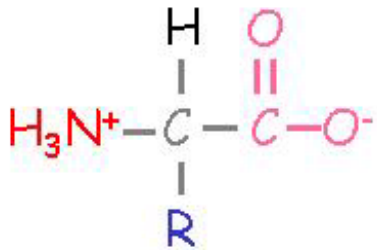
- In stomach
  - ✓ Pepsinogen + HCl → Pepsin
    - HCl : denaturing protein exposing peptide bonds
    - Pepsin cleaves peptide bond before aromatic residues (Table 5-7)
  - ✓ Peptide fragments (7-8 residues)
- Pancreas and small intestine
  - ✓ Trypsin (C of Lys, Arg)
  - ✓ Chymotrypsin (C of aromatic a.a.)
  - ✓ Carboxypeptidase, and aminopeptidase ➔ free a.a. for absorption
  - ◆ Acute pancreatitis
    - Obstruction of pancreatic secretion
    - Premature enzymes attack the pancreatic tissue



Stryer 5th Fig 23.1

Amino acid catabolism

- Amino acid = NH_3^+- + C skeleton
- “Bookkeeping”



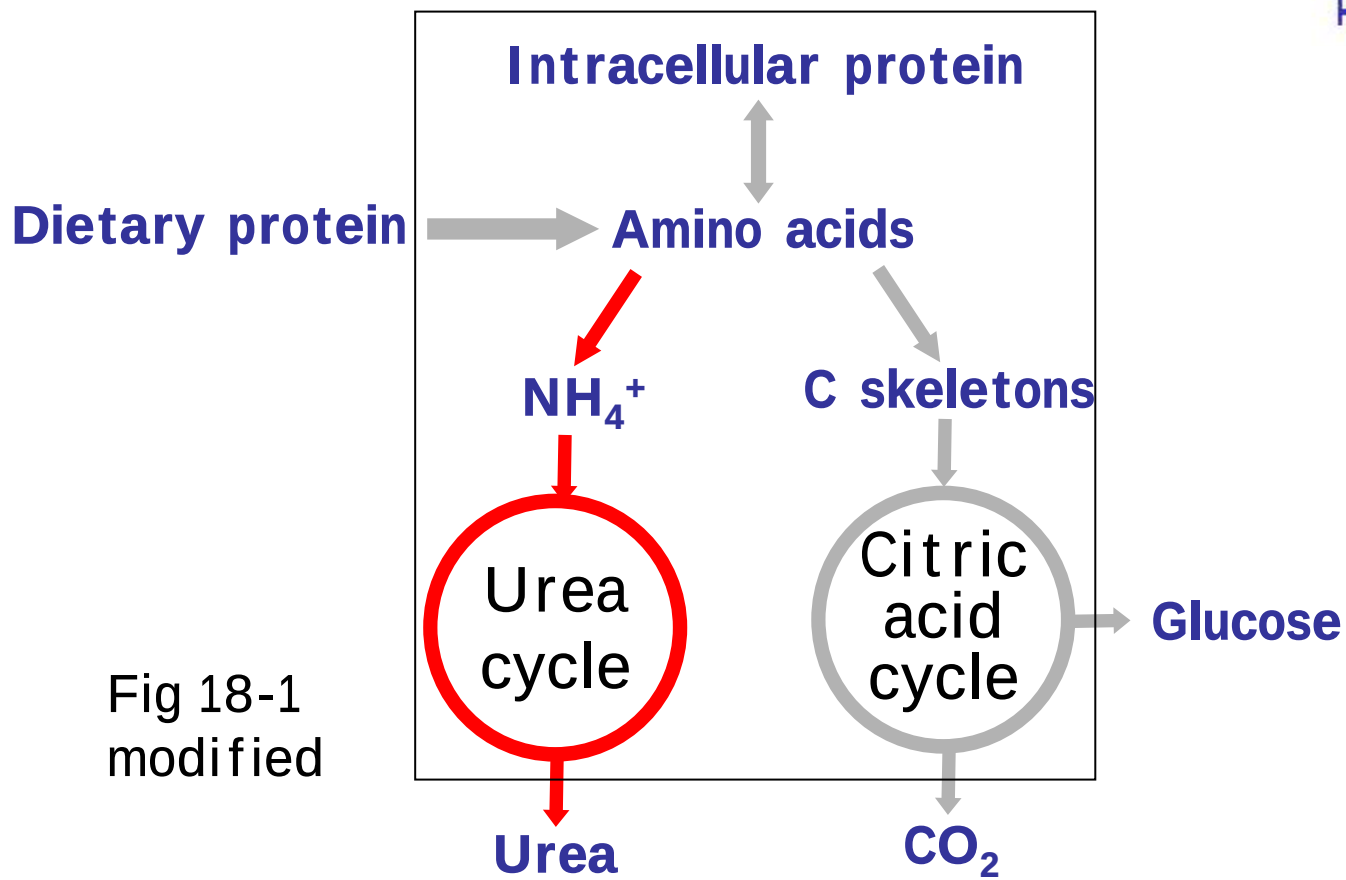
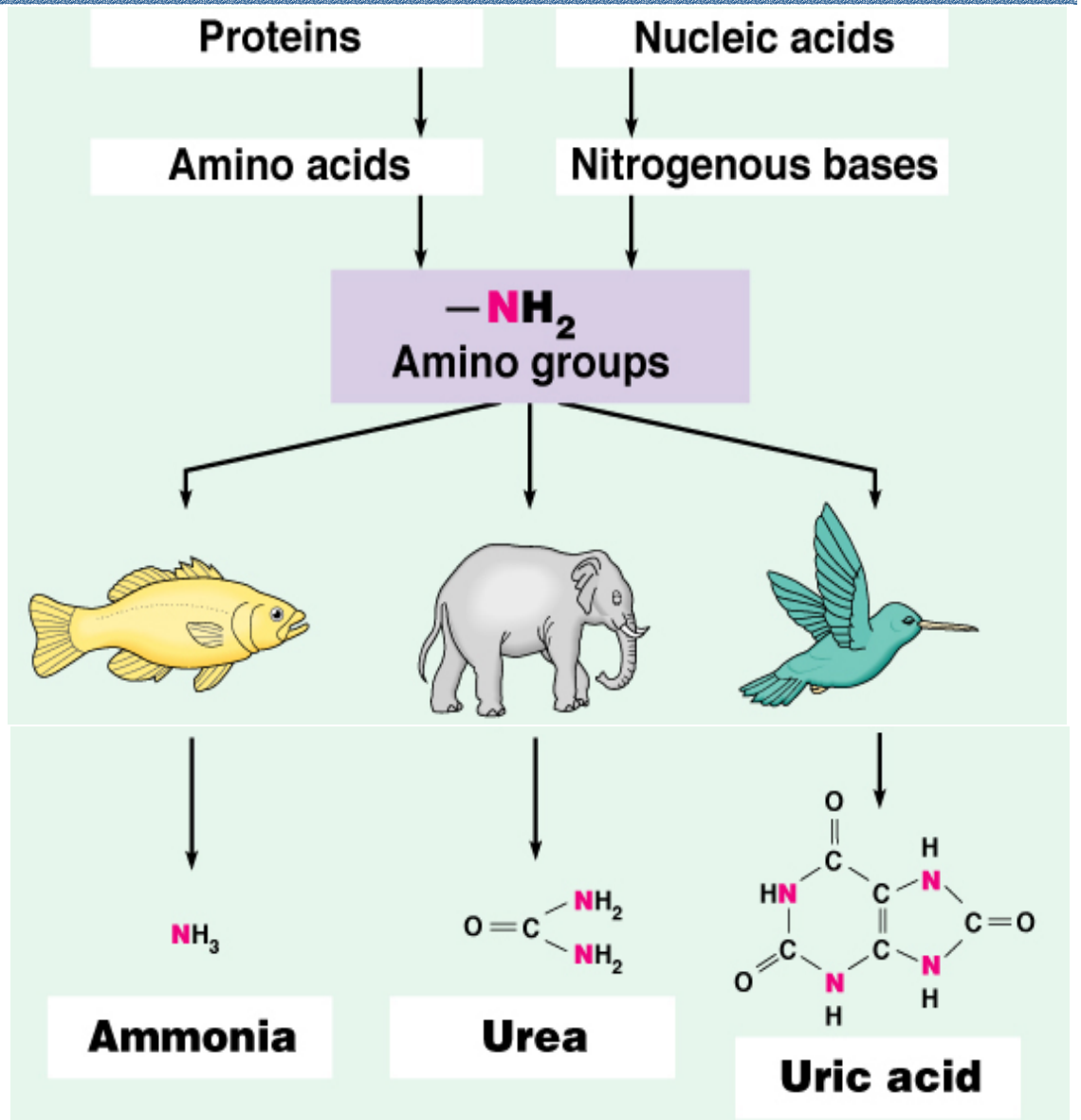


Fig 18-1 modified

N-containing wastes (p. 634)

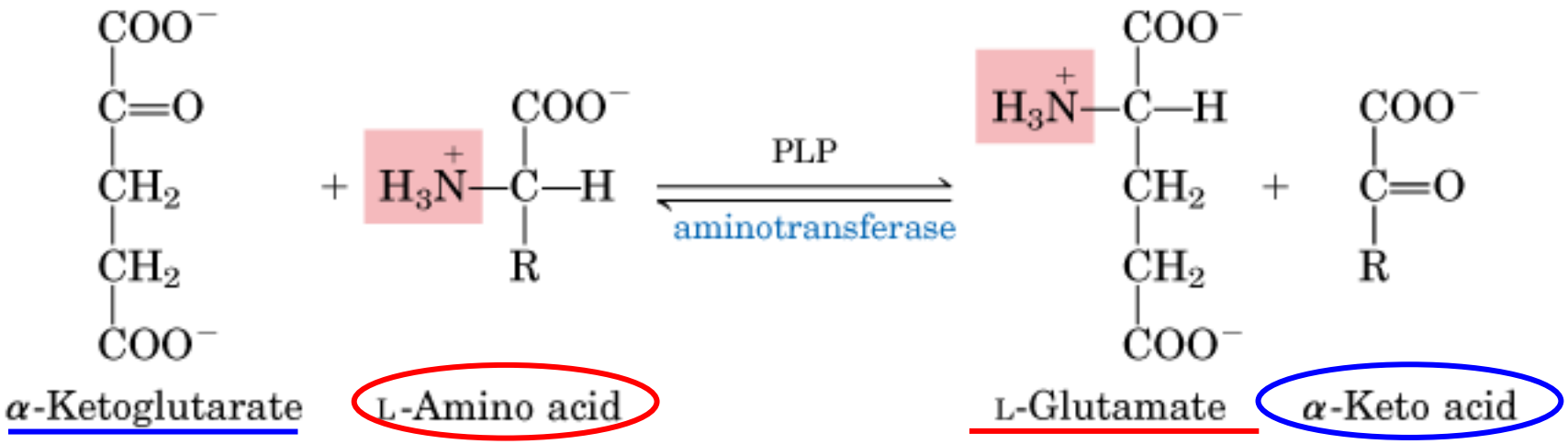


p. 625,
Fig 18-2(b)

Remove α-amino group

- 1st step in liver: **transamination**
  - ✓ Aminotransferase or transaminase
  - ✓ Exception: proline, hydroxyproline, threonine, and lysine
- Collect amino group in **glutamate** form

Fig 18-4



✓ Classic example of enzyme catalyzing bimolecular Ping-Pong reactions.

Aminotransferase

- A family of enzymes with different specificity for the amino acids.
  - ✓ Alanine aminotransferase
  - ✓ Aspartate aminotransferase
- A common *prosthetic group* (*coenzyme*):
  - ✓ PLP (pyridoxal phosphate)
    - Derived from Vit B_6
      - Transamination
        - As a carrier of amino group (accept ↔ donate)
      - Decarboxylation
      - Racimization
    - Forms enzyme-bound Schiff base intermediate.
- Medical diagnoses (Box 18-1)
  - ✓ A variety of enzymes leak from the injured cells into the bloodstream
    - Heart and liver damages caused by heart attack, drug toxicity, or infection.
    - Liver damages caused by CCl_4, chloroform, and other industrial solvent.
  - ✓ ↑ [Enz] in blood serum
    - SALT test (alanine aminotransferase, or GPT)
    - SAST test (aspartate …, or GOT)
    - SCK test (serum creatine kinase)

Glu releases NH_4^+ in liver

- In hepatocytes, Glu is transported from cytosol into the mitochondria.
- Glutamate dehydrogenase catalyze the oxidative deamination in mitochondria to release NH_4^+.
- Trans-deamination

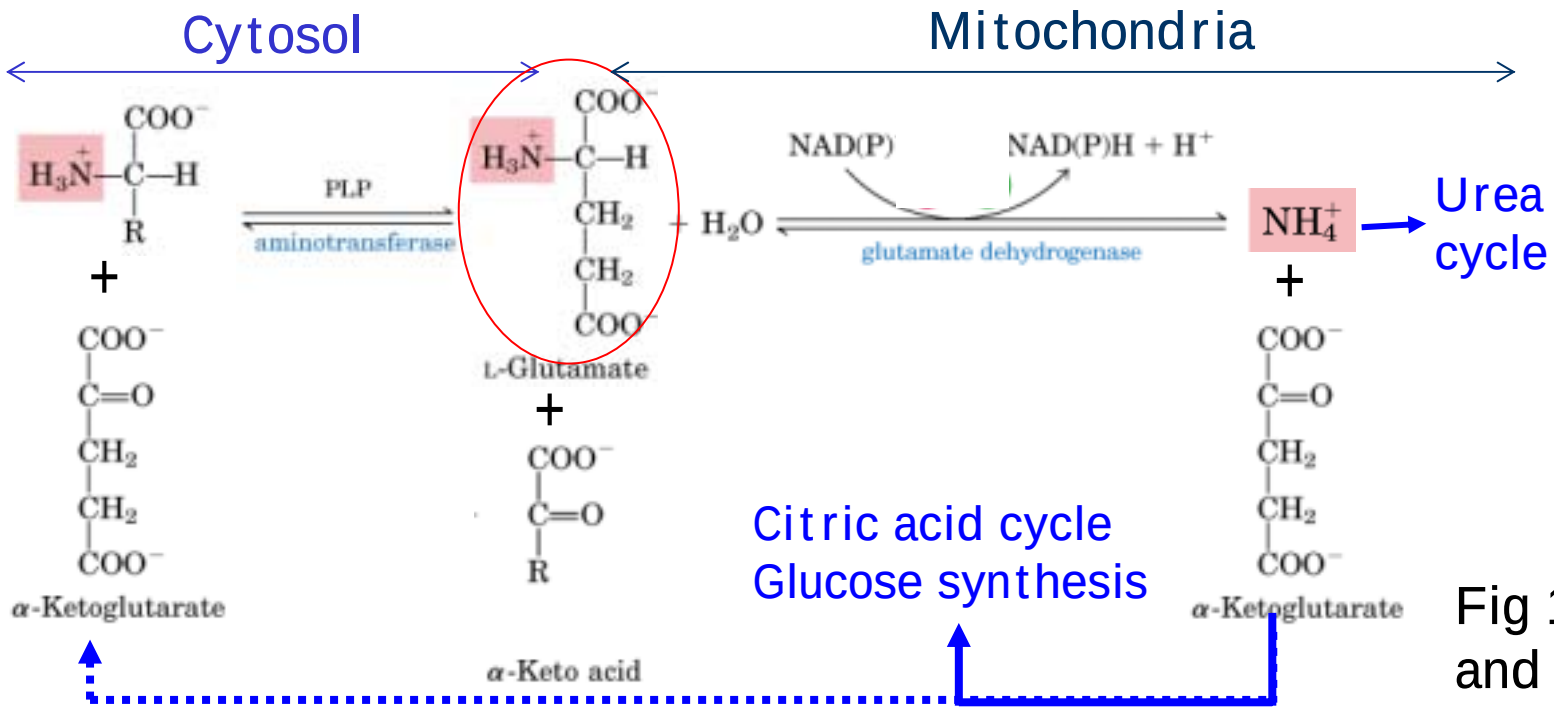
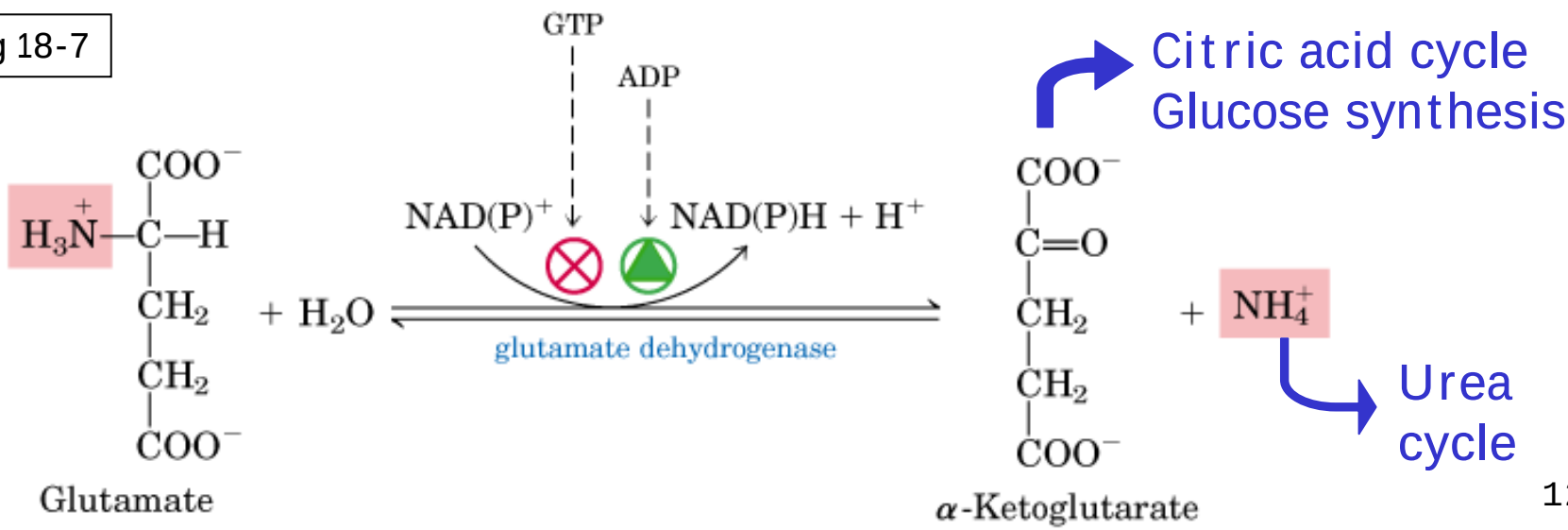


Fig 18-4 and 18-7

Glutamate dehydrogenase

- Operates at the intersection of N- and C- metabolism
  - ✓ Present only in hepatic mitochondria matrix
  - ✓ Requires NAD^+ or $NADP^+$
  - ✓ Allosterically regulated
    - Inhibitor: [GTP] and [ATP]
    - Activator: [GDP] and [ADP]
    - ✓ *A lowering of the energy charge accelerates the oxidation of a.a.*
    - ✓ Hyperinsulinism-hyperammonemia syndrome:
      - ✓ mutation in GTP binding site, permanently activated.

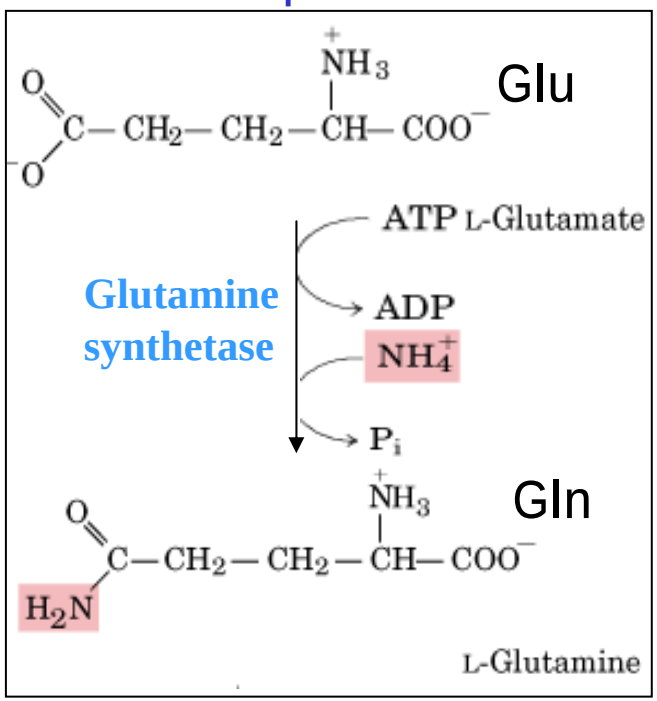
Fig 18-7



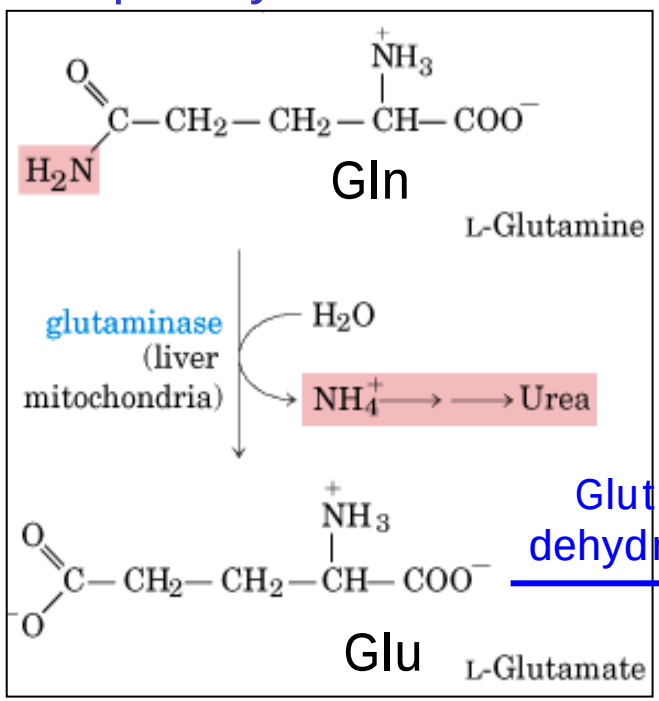
NH_4^+ transport in blood (I)

- NH_4^+ is toxic to animal tissues
- Gln is a nontoxic transport form of NH_4^+
- Gln releases NH_4^+ in liver and kidney mitochondria by **glutaminase**

In extrahepatic tissues



In hepatocyte mitochondria



α-ketoglutarate
+
NH_4^+

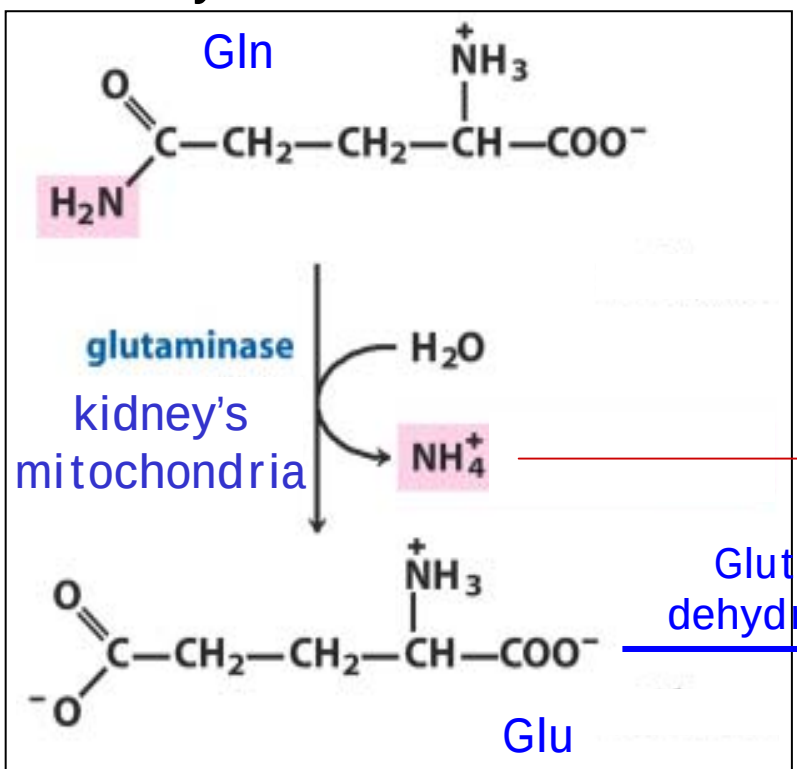
Glutamate dehydrogenase

p. 632

Metabolic acidosis (p. 663)

- Kidney extracts little Gln from bloodstream normally
- Acidosis ***increases*** glutamine processing in kidney
  - ✓ NH_4^+ + metabolic acids → salts (excreted in urine)
  - ✓ α-ketoglutarate → bicarbonate (HCO_3^-, buffer)

In kidney



α-ketoglutarate → (TCA cycle) → HCO_3^- (buffer)

+

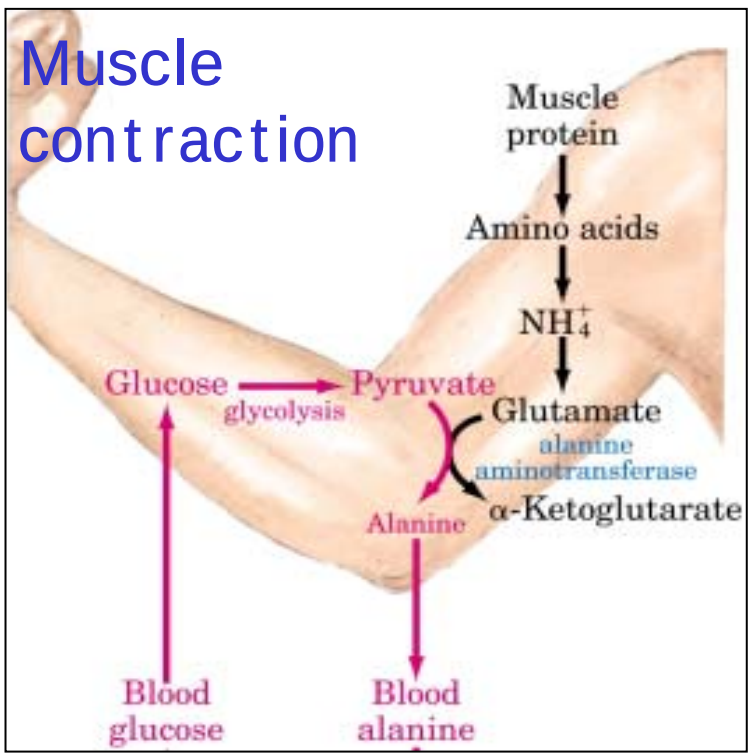
NH_4^+ + acids → Salts (excreted)

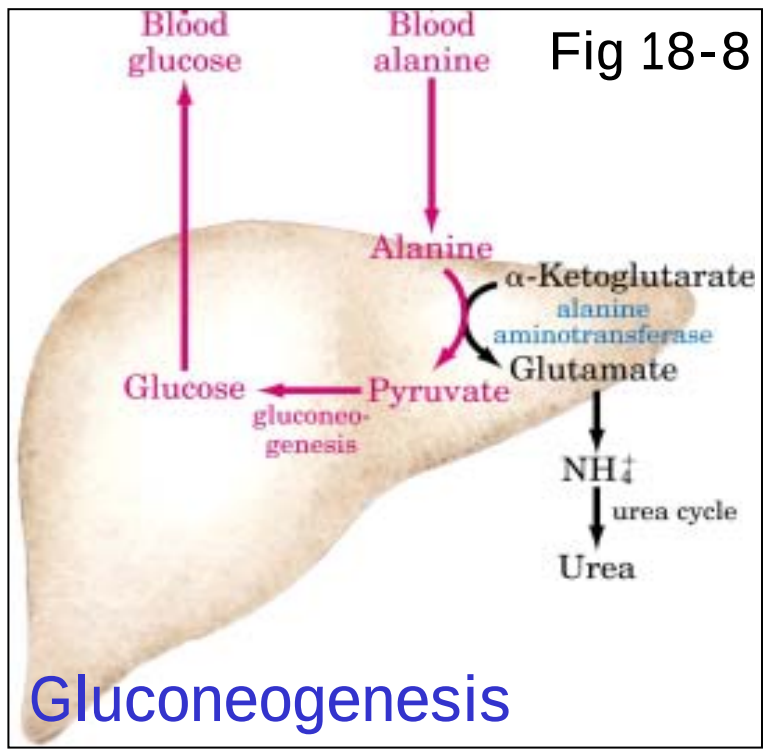
Glutamate dehydrogenase

Lehninger 4th ed.
Fig 18-8 modified

NH_4^+ transport in blood (II)

- Glucose-alanine cycle
  - ✓ Ala transports NH_4^+ from skeletal muscle to liver
  - ✓ Pyruvate is recycled to glucose in liver and then returned to muscle
- Economy in energy use
  - ✓ Tissue cooperation
  - ✓ Cori cycle (glucose-lactate cycle)





N excretion

Most terrestrial animals:

- Almost exclusively in liver:
  - ✓ NH_4^+ → urea (urea cycle)
  - ✓ 5 enzymatic steps (4 steps in urea cycle)
  - ✓ 2 cellular compartments involved
  - ✓ Urea → bloodstream → kidney → excreted into urine
- Urea cycle and citric acid (TCA) cycle
- Regulation of urea cycle
- Genetic defect and NH_4^+ intoxication
  - ✓ Urea cycle defect and protein-rich diet
    - Essential a.a. must be provided in the diet.
      - A.A. can not be synthesized by human body.

Ch 22
Biosynthesis

table 18–1

Nonessential and Essential Amino Acids for Humans and the Albino Rat

Nonessential	Essential
Alanine	Arginine*
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine	Methionine
Glycine	Phenylalanine
Proline	Threonine
Serine	Tryptophan
Tyrosine	Valine

*Essential in young, growing animals but not in adults.

Urea cycle

- Sources of N and C in synthesized $(NH_2)_2CO$

In the **mitochondria** and **cytoplasm** of liver cells

1. Carbamoly phosphate synthetase I
2. Ornithine transcarbamoylase
3. Argininosuccinate synthetase
4. Argininosuccinate lyase
5. Arginase

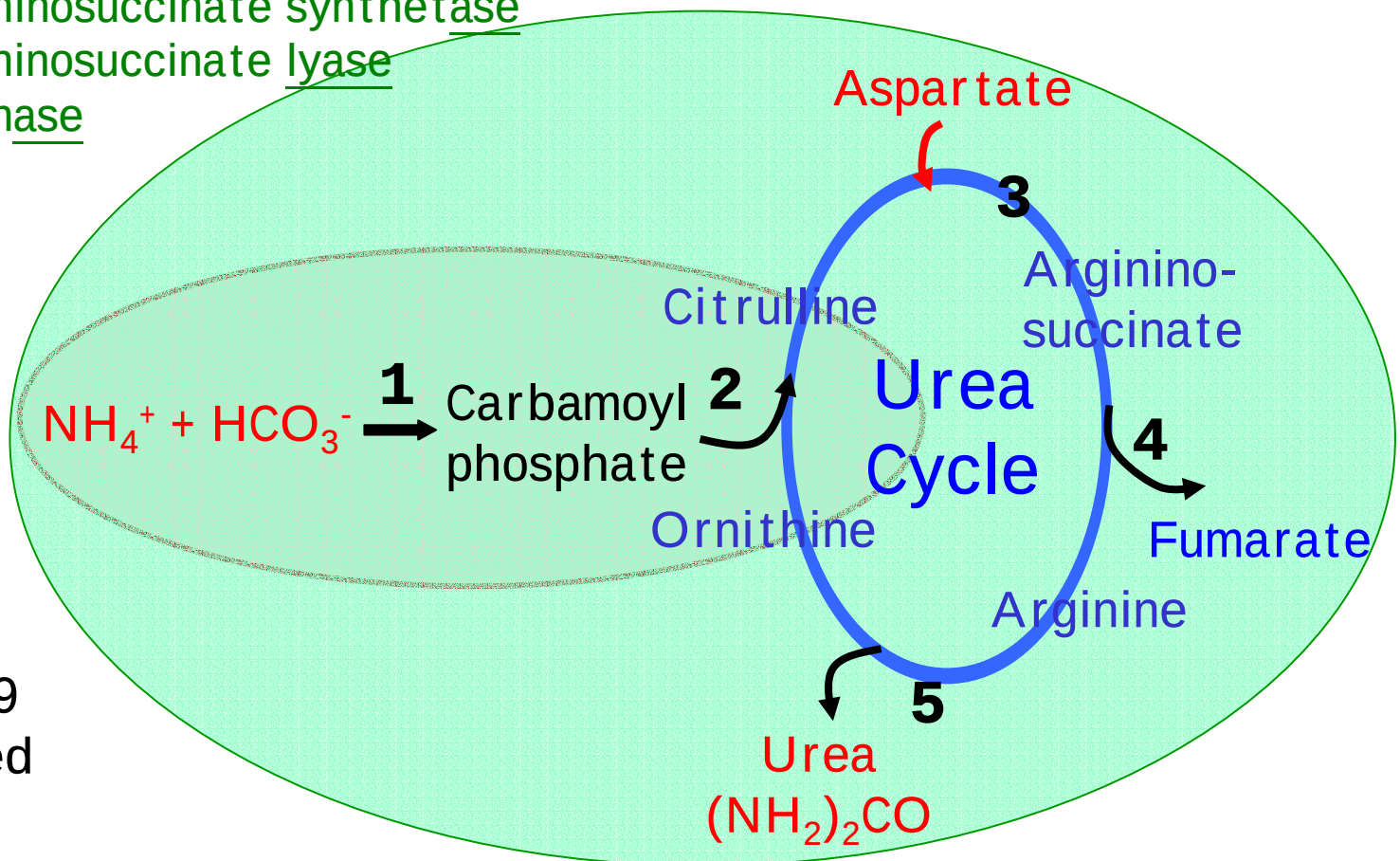


Fig 18-9
modified

Sources of NH_4^+

- **Glu** and **Gln** release **NH_4^+** in the mitochondria of hepatocyte
- **Asp** is generated in mitochondrial matrix by transamination and transported into the cytosol of hepatocyte

- Refer to Fig 19-26 p. 685
- **Malate-Asp shuttle**
  - ✓ OAA cannot cross membrane
  - ✓ Malate-αKG transporter
  - ✓ Glu-Asp transporter

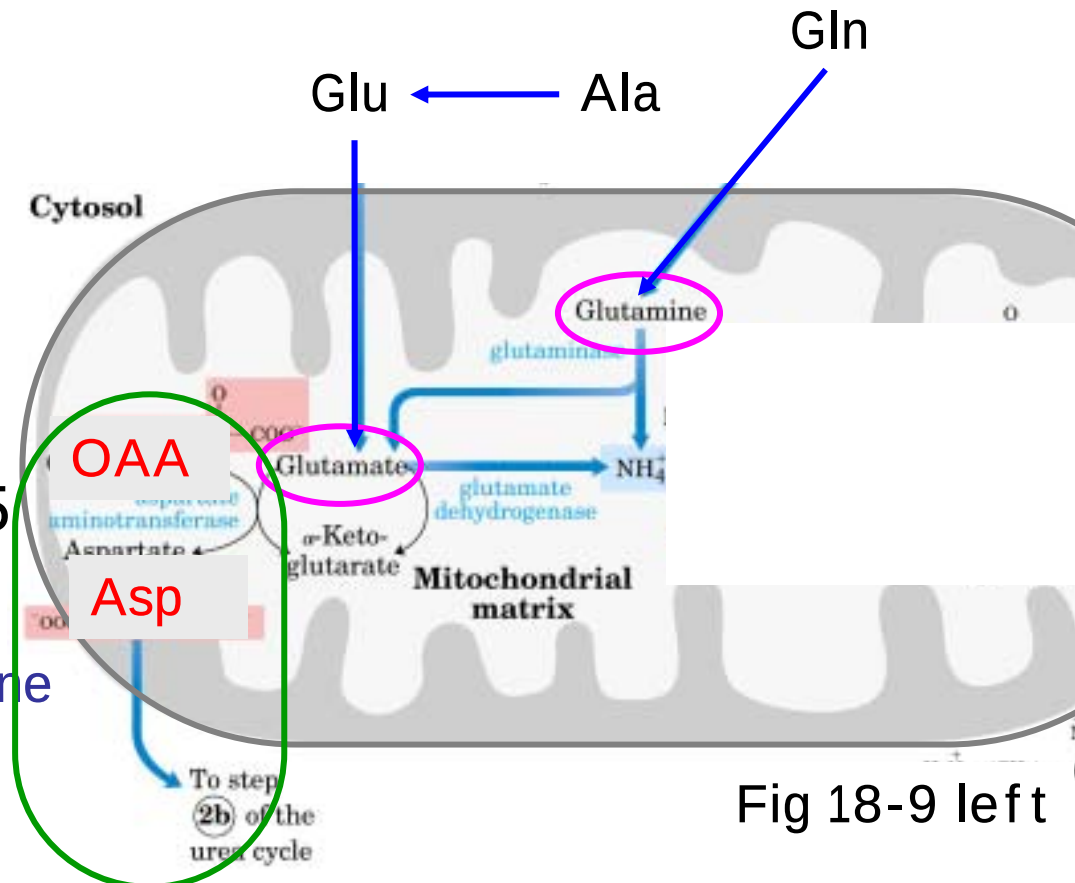


Fig 18-9 left

Regulation of urea cycle

Fig 18-12

p. 636

- Protein-rich diet and prolonged starvation:
  - ✓ ↑ urea production.
- Long term:
  - ✓ Rate of synthesis of the 4 urea cycle Enz. and carbamoyl phosphate synthetase I in the liver.
- Short term:
  - ✓ Allosteric regulation of carbamoyl phosphate synthetase I
  - ✓ Activator: N-acetylglutamate, enhances the affinity of synthetase for ATP.

Carbamoyl phosphate synthetase I

- Properties
  - ✓ The 1st enzyme for NH_4^+ → urea
  - ✓ Mitochondria matrix isoform
    - Type II in cytosol for pyrimidine synthesis (p. 667, and Ch 22)
  - ✓ High conc. than type II in cytosol
    - Greater need for urea production
- Activator:
  - ✓ N-acetylglutamate
    - acetyl-CoA + Glu
  - ✓ Arginine
- Urea cycle defect
  - ✓ N-acetylglutamate synthase deficiency
    - Supplement with carbomylglutamate (p. 670)

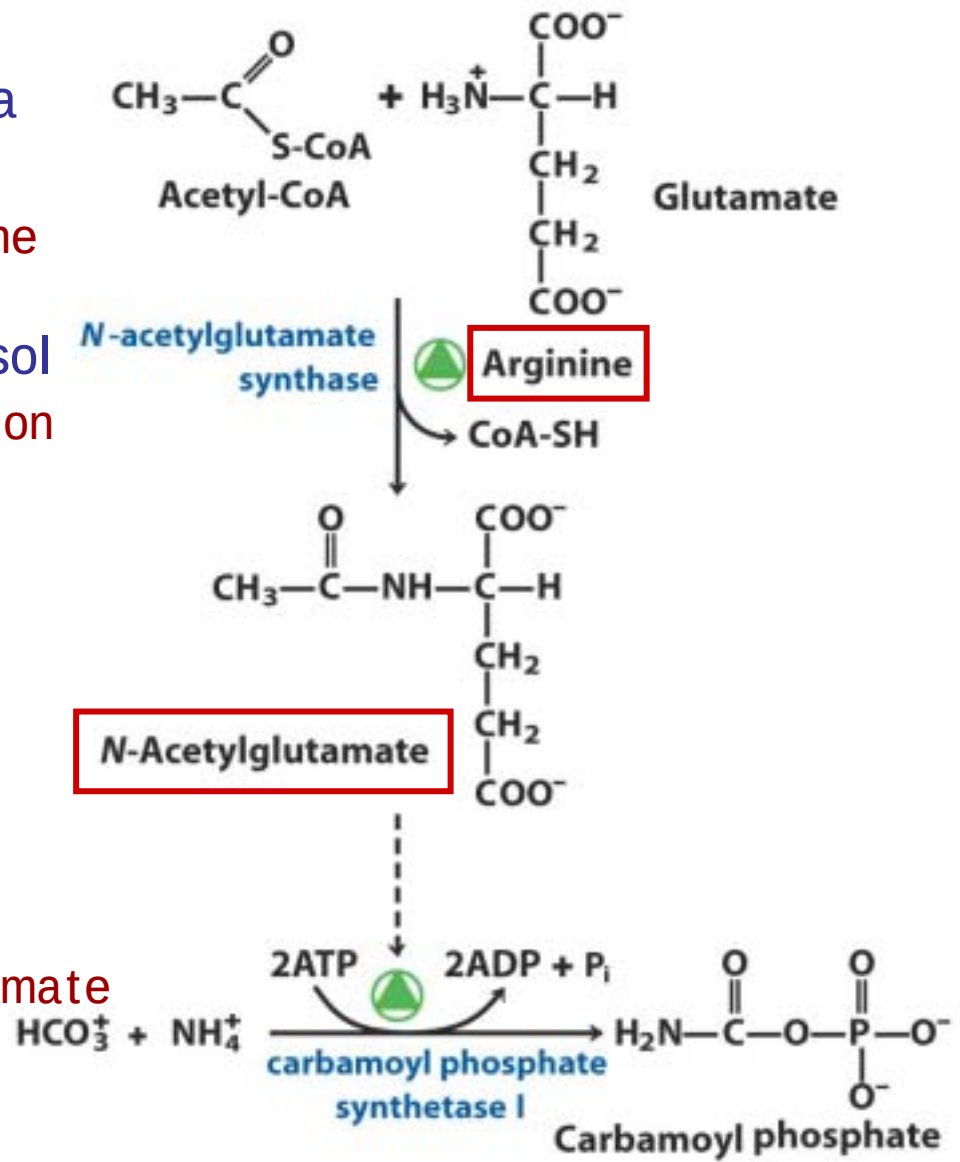
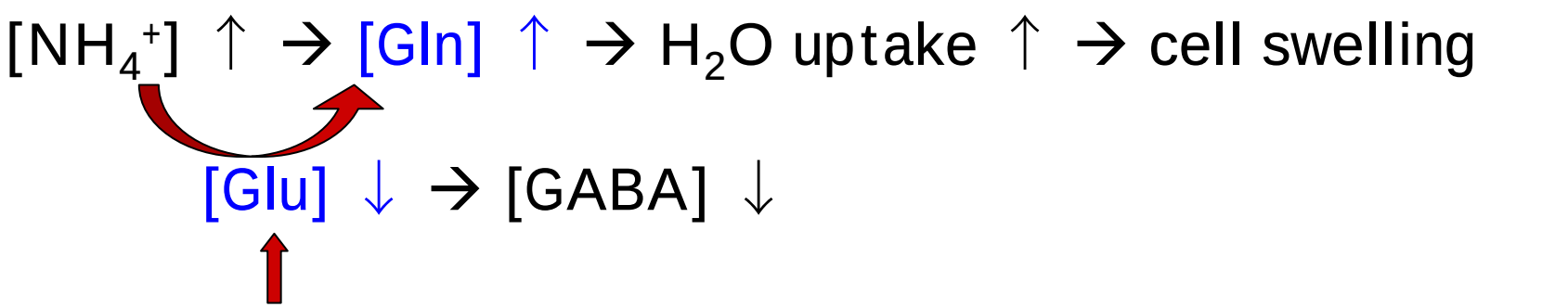


Fig 18-13

NH_4^+ intoxication (p.665)

- Symptoms
  - ✓ Coma
  - ✓ Cerebral edema
  - ✓ Increase cranial pressure
- Possible mechanisms
  - ✓ Depletion of ATP in brain cells
  - ✓ Changes of cellular osmotic balance in brain
  - ✓ Depletion of neurotransmitter
- Remove excess NH_4^+
  - ✓ Glutamate dehydrogenase: $NH_4^+ + \alpha$-KG → Glu
  - ✓ Glutamine synthetase: NH_4^+ + Glu → Gln



Defect in urea cycle enzymes

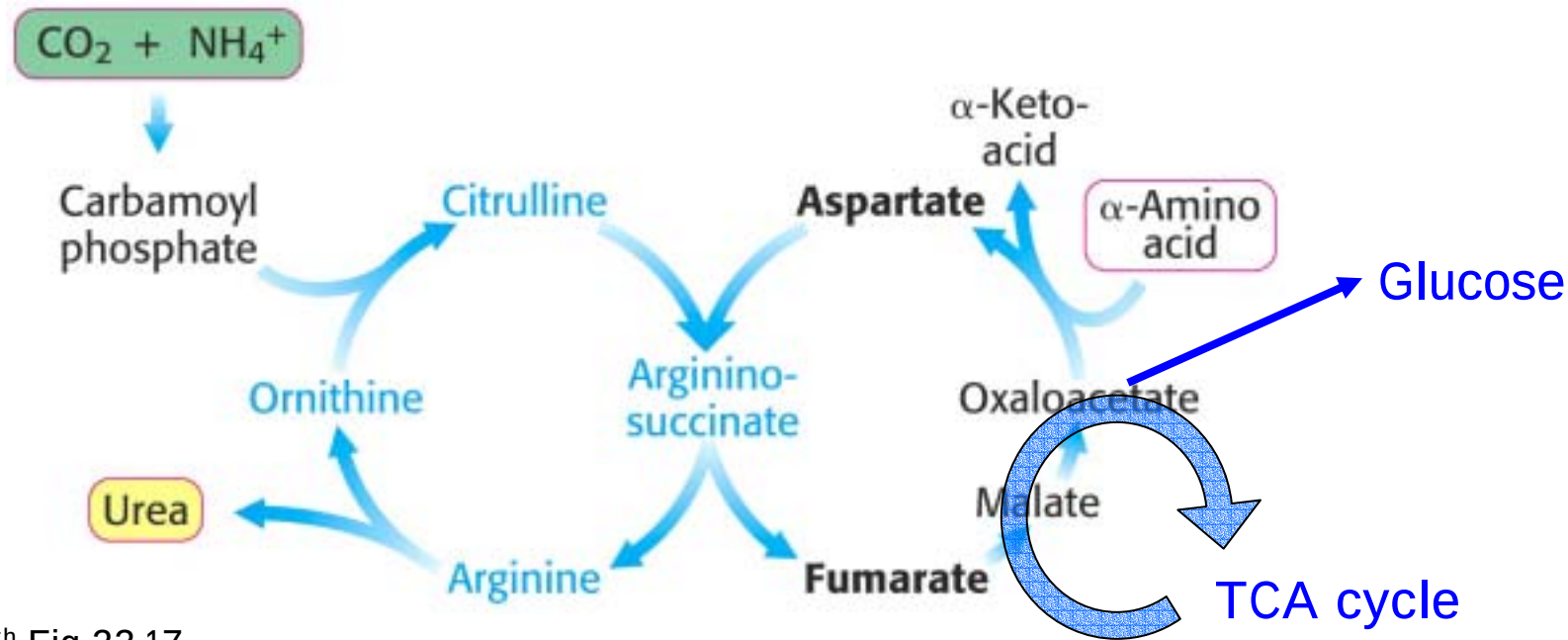
- Build-up of urea cycle intermediates
- Treatments

Lehninger 4th ed.
p. 669-670

  - ✓ Strict diet control and supplements of essential a.a.
  - ✓ With the administration of :
    - Aromatic acids (Fig 18-14)
      - Lower NH_4^+ level in blood
        - Benzoate + Gly + … → hippurate (left)
        - Phenylbutyrate + Glutamine + … → phenylacetylglutamine (right)
    - BCAA derived keto acids
    - Carbamoyl glutamate (N-acetylglutamate analog)
      - Deficiency of N-acetylglutamate synthase
    - Arginine
      - Deficiency of ornithine transcarbamoylase
      - Deficiency of argininosuccinate synthetase
      - Deficiency of argininosuccinase

Energy cost of urea cycle

- Urea synthesis costs energy… p. 637
  - ✓ 4 high energy phosphate groups from 3 ATP
- Oxaloacetate (OAA) regenerate produces NADH (Fig 18-11)
  - ✓ 1 NADH → 2.5 ATP
- Pathway interconnections reduce the energetic cost of urea synthesis
  - ✓ Argininosuccinate shunt



Stryer 5th Fig 23.17

Metabolism of C skeleton

Fatty acids oxidation (Ch 17)

Acetone
Acetoacetate
D-β-hydroxybutyrate

- Amino acid = NH_3^+- + C skeleton
  - ✓Oxidized to CO_2 and H_2O
  - ✓Glucose (glucogenic a.a.)
  - ✓Ketone bodies (ketogenic a.a.)

Entering citric acid cycle

- 20 a.a. enter TCA cycle:
  - ✓ Acetyl-CoA (10)
  - ✓ α-ketoglutarate (5)
  - ✓ Succinyl-CoA (4)
  - ✓ Fumarate (2)
  - ✓ Oxaloacetate (2)
- Some a.a. yields more than one end product
  - ✓ Different C fates

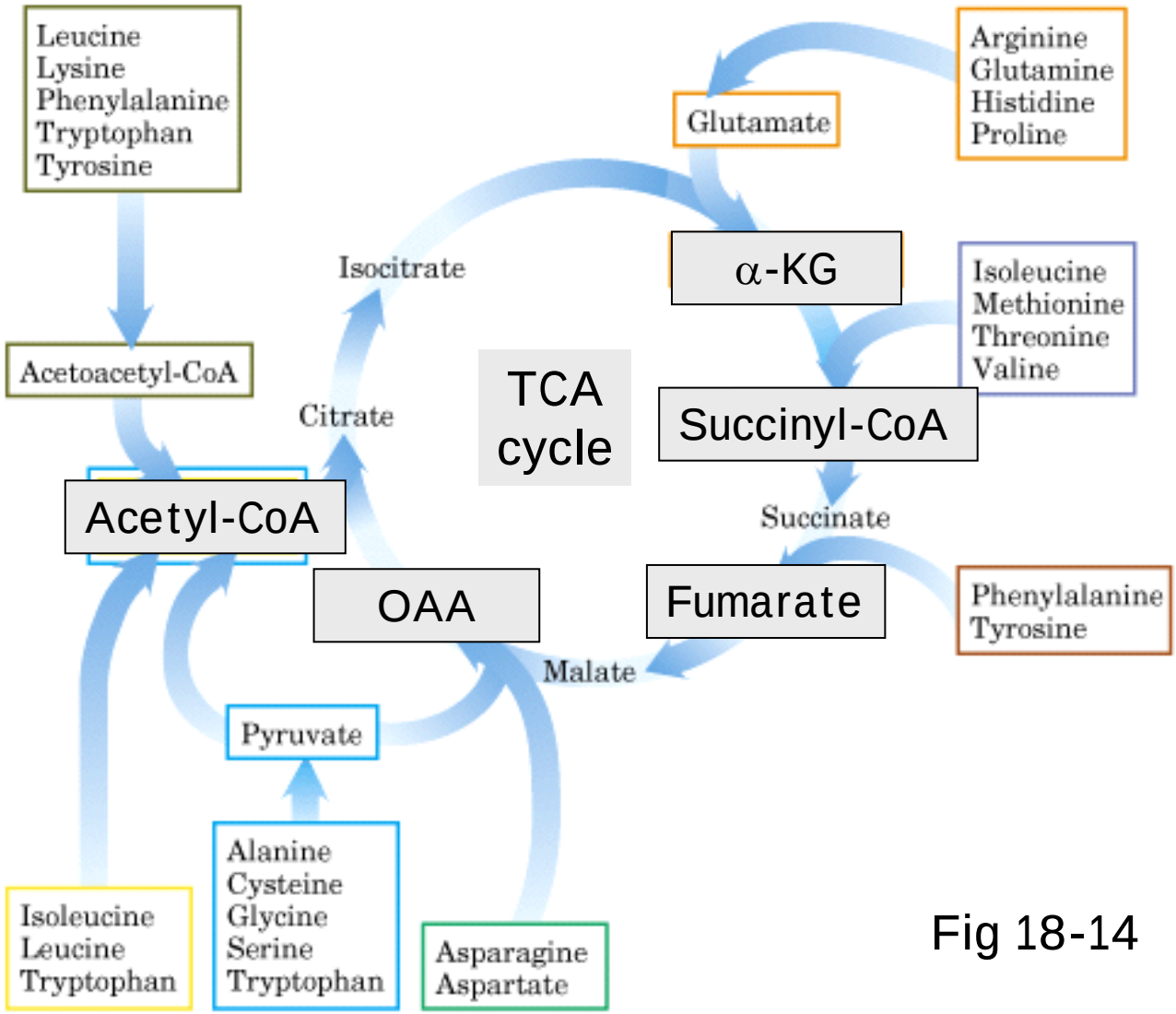


Fig 18-14

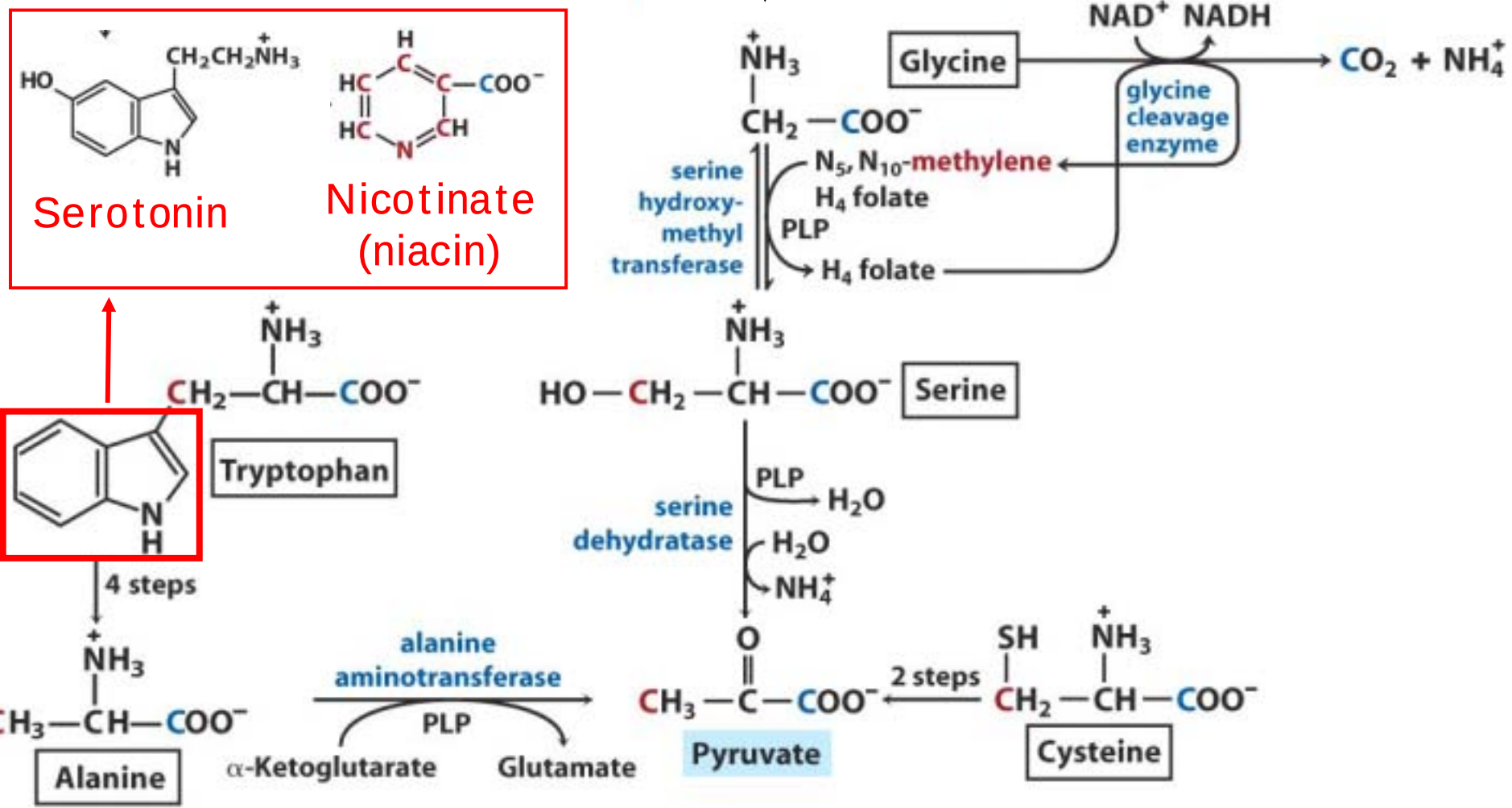
One-carbon transfer

p.640-643

- Transfer one-carbon groups in different oxidation states.
- Some enzyme cofactors involved (Fig 18-15):
  - ✓ Biotin
    - Transfer CO_2
  - ✓ Tetrahydrofolate (H_4 folate)
    - Transfer –HC=O, -HCOH, or $–CH_3$
  - ✓ S-adenosylmethionine (adoMet, SAM)
    - Transfer $–CH_3$

Ala, Trp, Cys, Thr, Ser, Gly → Pyruvate

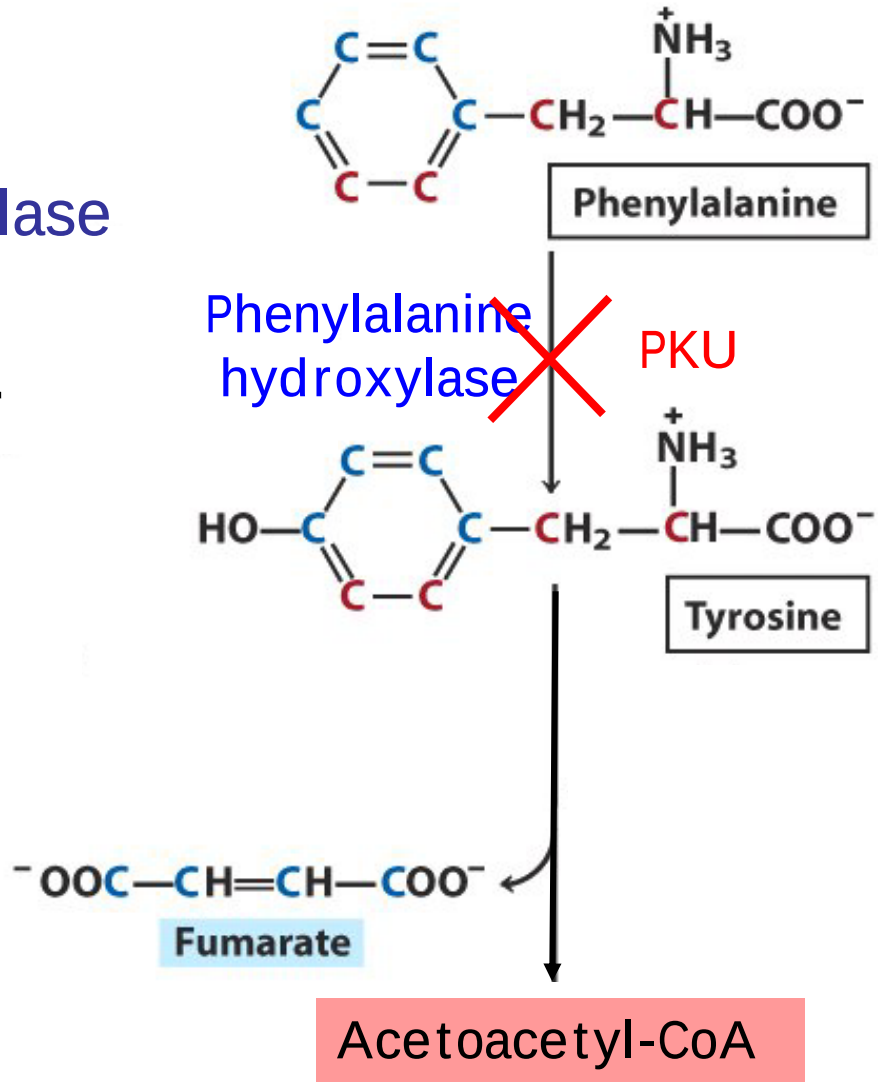
Lehninger 4th ed.
Fig 18-19 modified



Phe and Tyr

Fig 18-21 Top right

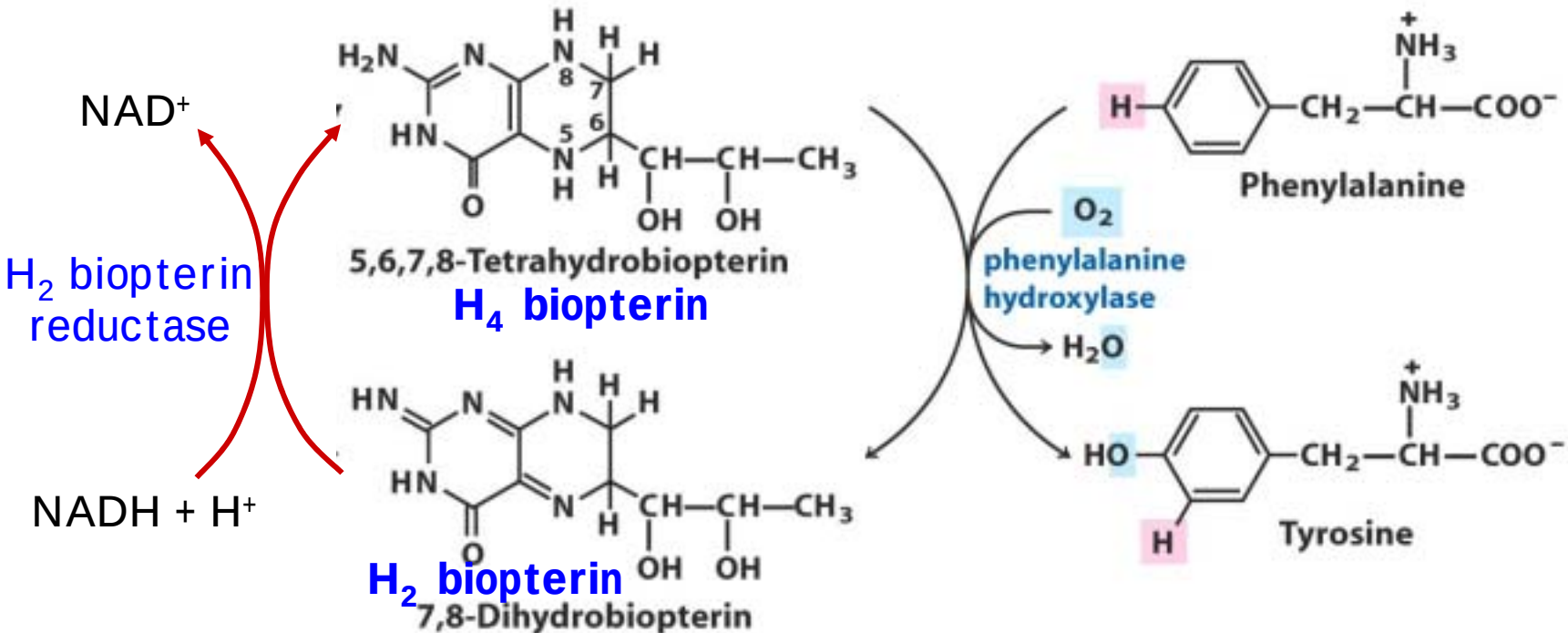
- Phe + -OH → Tyr
  - ✓ Phenylalanine hydroxylase
  - ✓ Phenylketonuria (PKU)
- Phe, Tyr as precursor
  - ✓ Fig 22-29, p. 860
    - Dopamine
    - Norepinephrine
    - Epinephrine
- Tyr as precursor
  - ✓ Melanin



H_4 biopterin

- Phenylalanine hydroxylase
  - ✓ Mixed-function oxidase
  - ✓ Cofactor: tetrahydrobiopterin (H_4 biopterin)
- Dihydrobiopterin reductase is required to regenerate H_4 biopterin
  - ✓ Defect in dihydrobiopterin (H_2 biopterin) reductase
    - PKU, norepinephrine, serotonin, L-dopa deficiency, …
    - Supplement with H_4 biopterin, as well as 5-OH-Trp and L-dopa

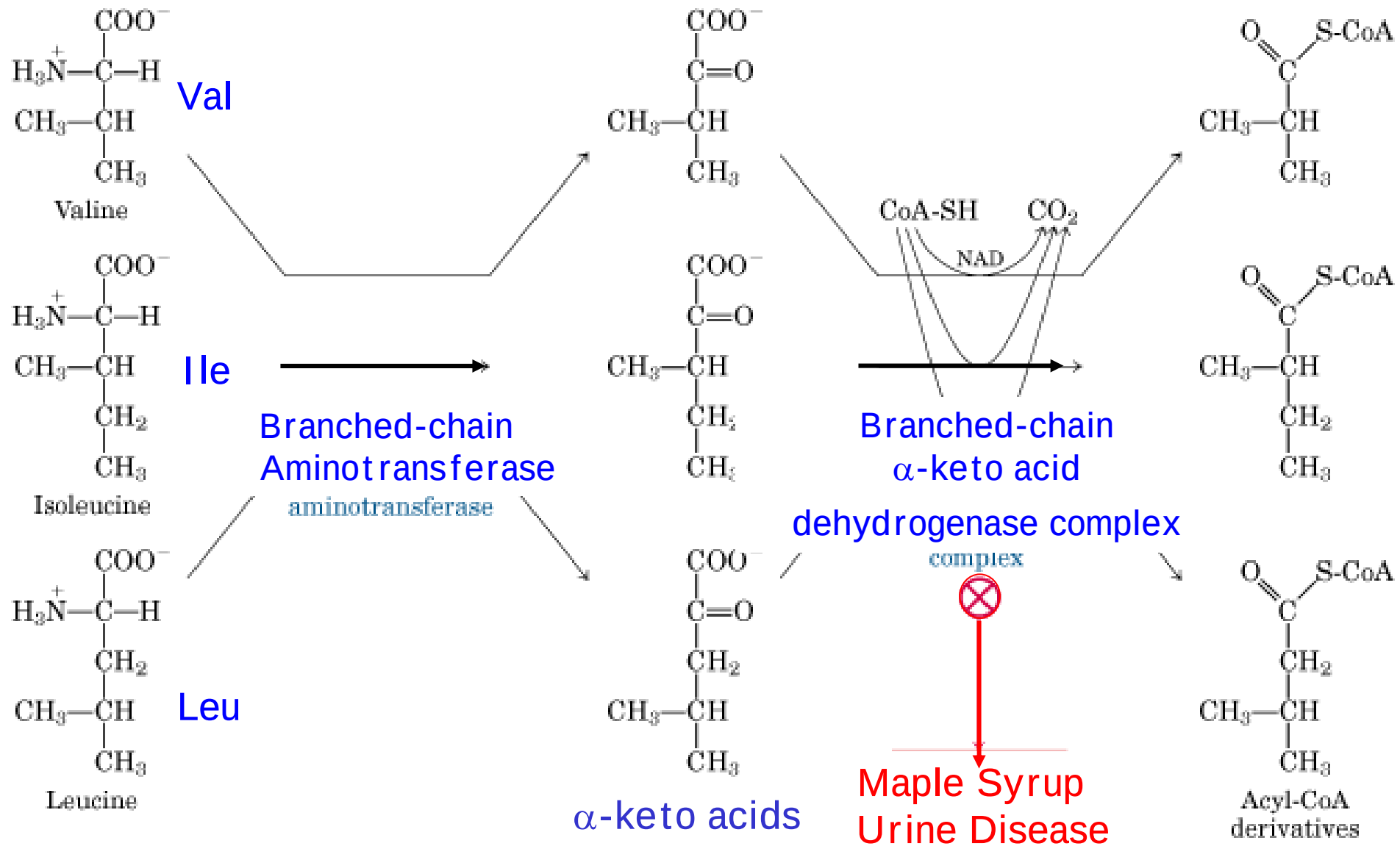
Lehninger 4th ed.
Fig 18-24



Branched-chain a.a. (p. 651)

- BCAA: Val, Ile, Leu
  - ✓ Not degraded in the liver
  - ✓ Oxidized as fuels in extrahepatic tissues
    - Muscle, adipose, kidney and brain
- The 3 a.a. share the first 2 enzymes for catabolism
  - ✓ Fig 18-27
  - ✓ Branched-chain aminotransferase → α-keto acids
  - ✓ Branched-chain α-keto acid dehydrogenase complex → acyl-CoA derivatives
    - Closely resemble pyruvate dehydrogenase
      - Inactivated by phosphorylation
      - Activated by dephosphorylation

Val, Ile, and Leu (Fig 18-27)



Maple syrup urine disease

p. 652

- MSUD
  - ✓ Branched-chain ketonuria
- Defective branched-chain α-keto acid dehydrogenase complex
- α-keto acids (odor) derived (Val, Ile and Leu) accumulate in blood and urine
  - ✓ Abnormal brain development
  - ✓ Mental retardation
  - ✓ Death in infancy
- Rigid diet control
  - ✓ Limit the intake of Val, Ile, Leu to min. requirement for normal growth

Genetic disorders

- Caused by defective catabolic enzymes

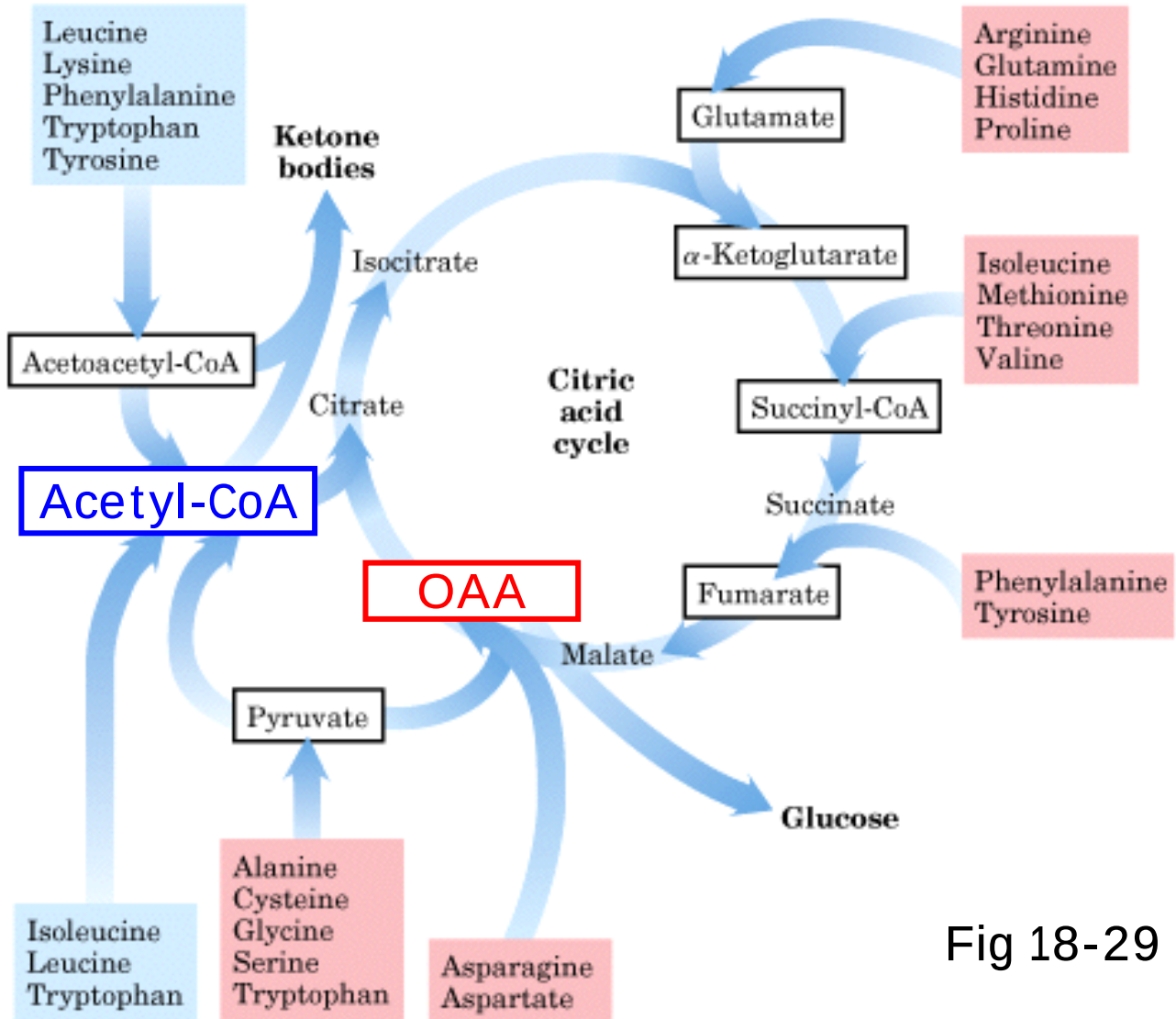
table 18–2

Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	Approximate incidence (per 100,000 births)	Defective process	Defective enzyme	Symptoms and effects
Albinism	3	Melanin synthesis from tyrosine	Tyrosine 3-mono-oxygenase (tyrosinase)	Lack of pigmentation; white hair, pink skin
Alkaptonuria	0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	1.5	Urea synthesis	Argininosuccinate lyase	Vomiting, convulsions
Carbamoyl phosphate synthetase I deficiency	>0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy, convulsions, early death
Homocystinuria	0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development, mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting, convulsions, mental retardation, early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting, convulsions, mental retardation, early death
Phenylketonuria	8	Conversion of phenyl-alanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

Ketogenic vs. glucogenic a.a.

- Acetyl-CoA
  - ➢ Ketone bodies
- OAA
  - ✓ α-ketoglutarate
  - ✓ Succinyl-CoA
  - ✓ Fumarate
  - ➢ Gluconeogenesis



- Ketogenesis
- Glucogenesis

Fig 18-29

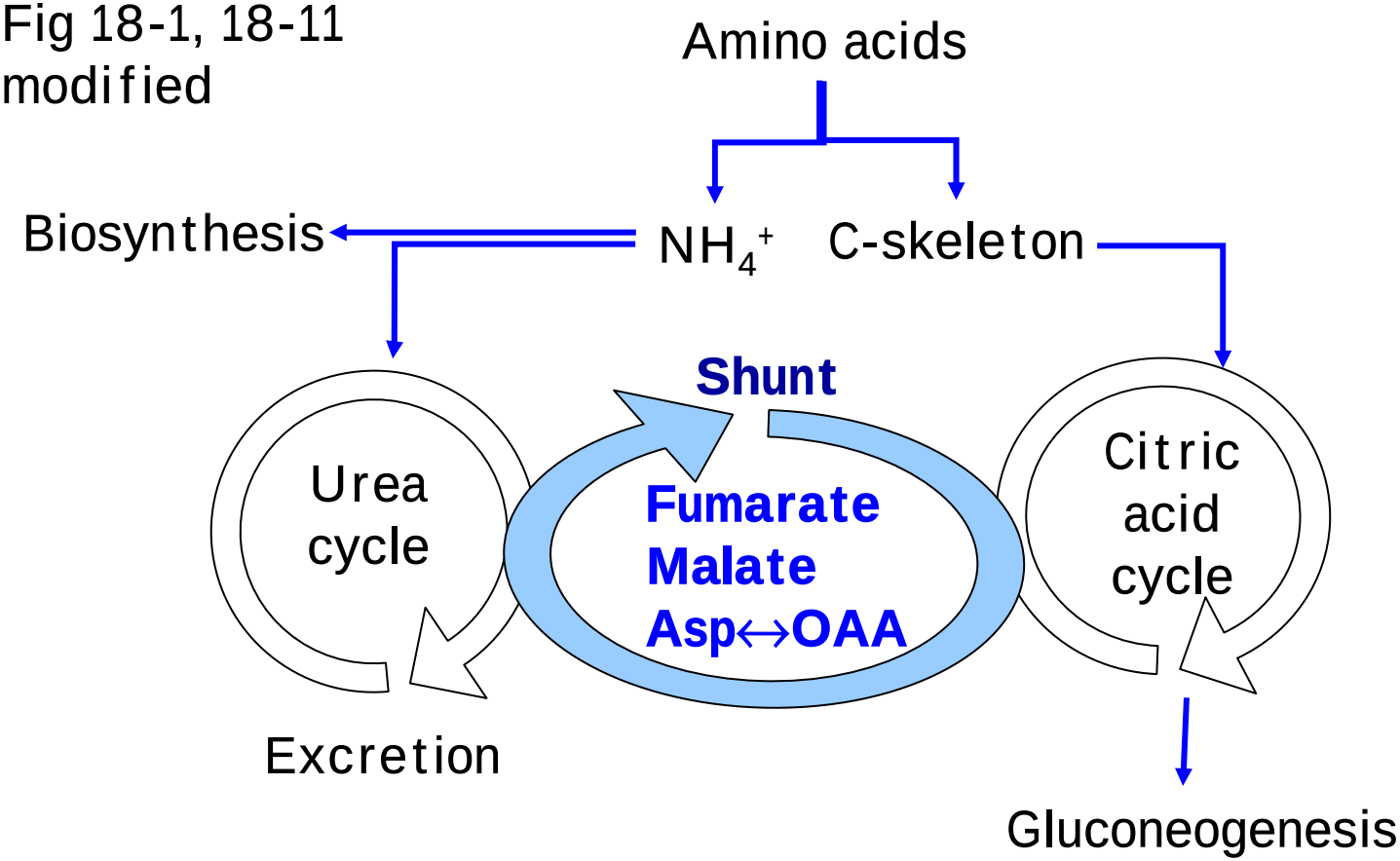
Ketogenesis vs. glucogenesis

- Ketogenesis
  - ✓ A.A. degraded to acetoacetyl-CoA and or acetyl-CoA (6 a.a.)
  - ✓ Yield **ketone bodies** in the liver
  - ✓ In untreated **diabetes mellitus**, liver produces large amounts of ketone bodies from both fatty acids and the ketogenic a.a.
  - ✓ Exclusively ketogenic: **Leu and Lys**
- Glucogenesis
  - ✓ A.A. degraded to pyruvate, a-ketoglutarate, succinyl-CoA, fumarate, and/or oxaloacetate
  - ✓ Converted into **glucose** and **glycogen**.
- Both ketogenic and glucogenic
  - ✓ Phe, Tyr, Trp, and Ile

On p. 588, read the 1st paragraph under "The Glyoxylate Cycle"

Catabolism of a.a. in mammals

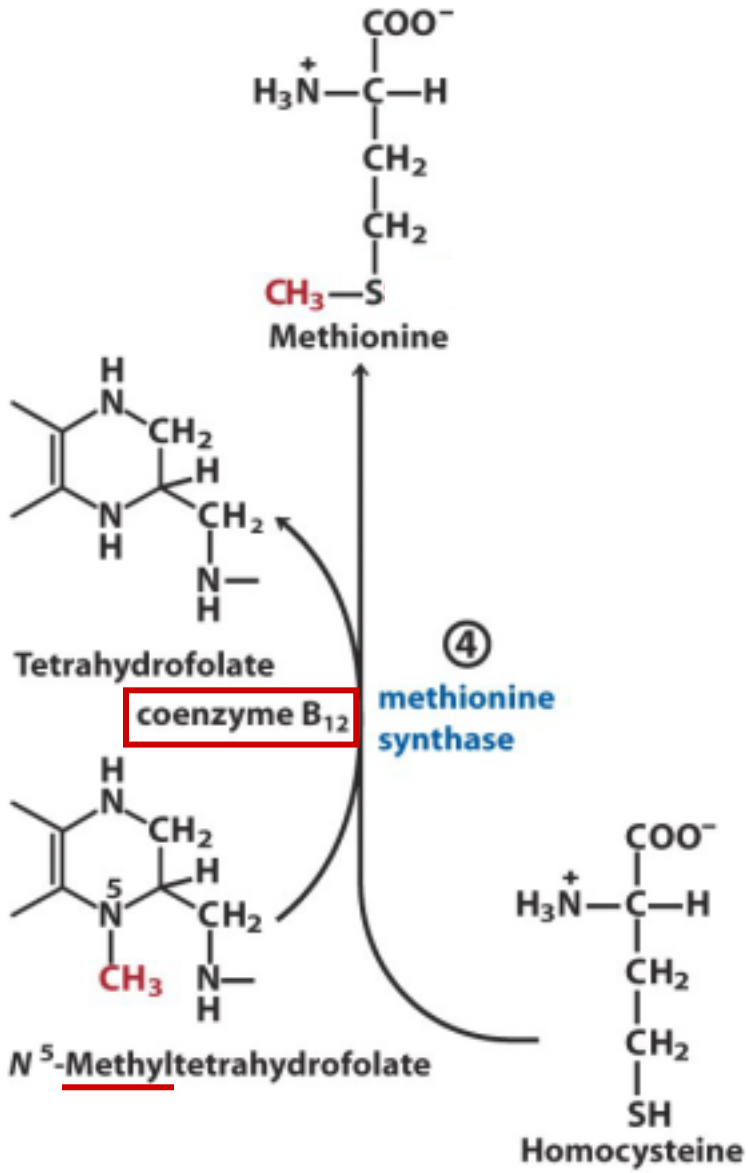
Fig 18-1, 18-11 modified



- The NH_3^+ and the C skeleton take separate but interconnected pathways

Vit B_{12} and folate (p. 674)

- Met synthesis in mammal
  - ✓ N^5-methyl H_4 folate as C donor
    - C is then transferred to Vit B_{12}
    - Vit B_{12} as the final C donor
- Vit B_{12} deficiency
  - ✓ H_4 folate is trapped in N^5-methyl form (formed irreversibly)
  - ✓ Available folate ↓
    - e.g. pernicious anemia



Lehninger 4th ed.
Fig 18-18 left