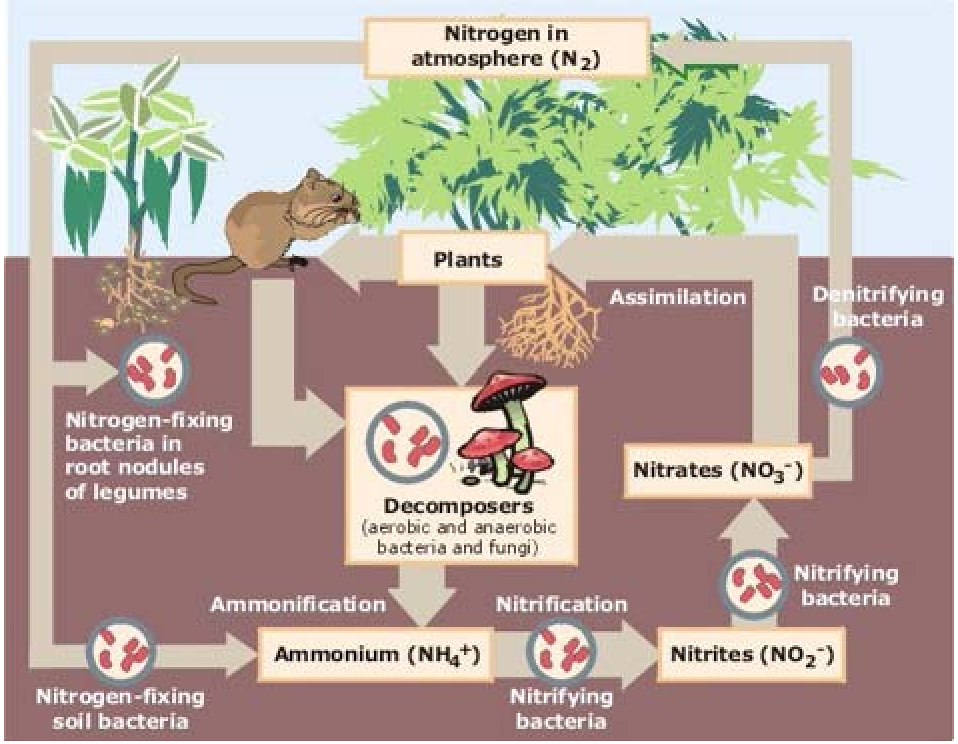
Amino acid metabolism
Global & Cellular

Nitrogen fixation

Chapter 20 Opener Fundamentals of Biochemistry, 2/e

The nitrogen cycle

Interconversion of nitrogen in the biosphere



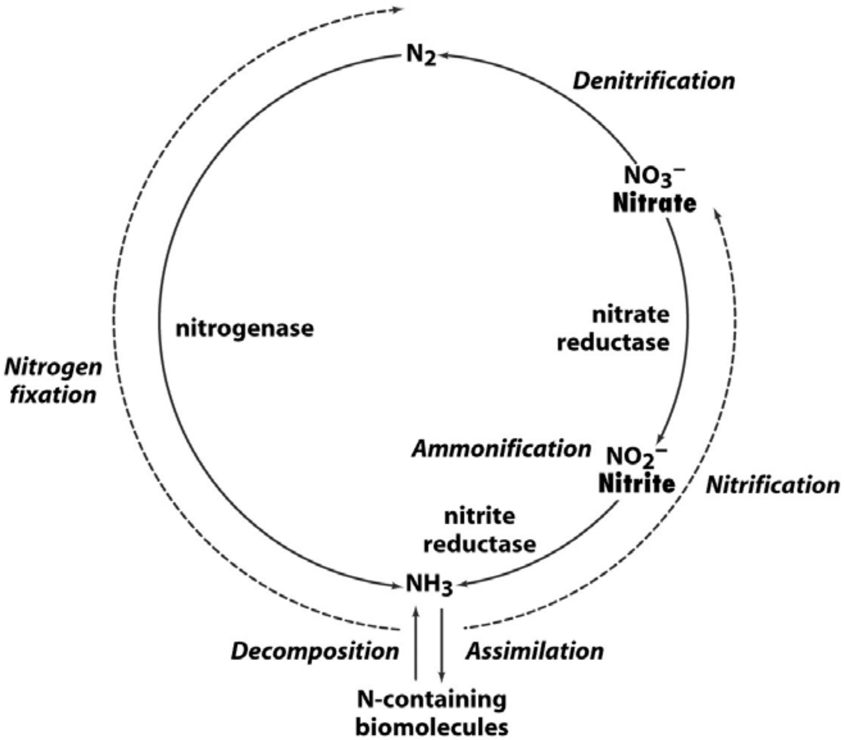
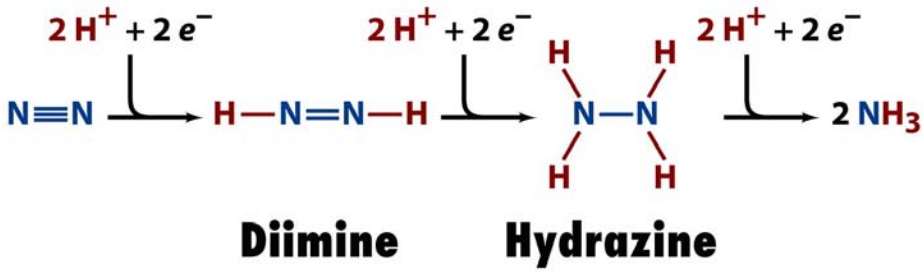


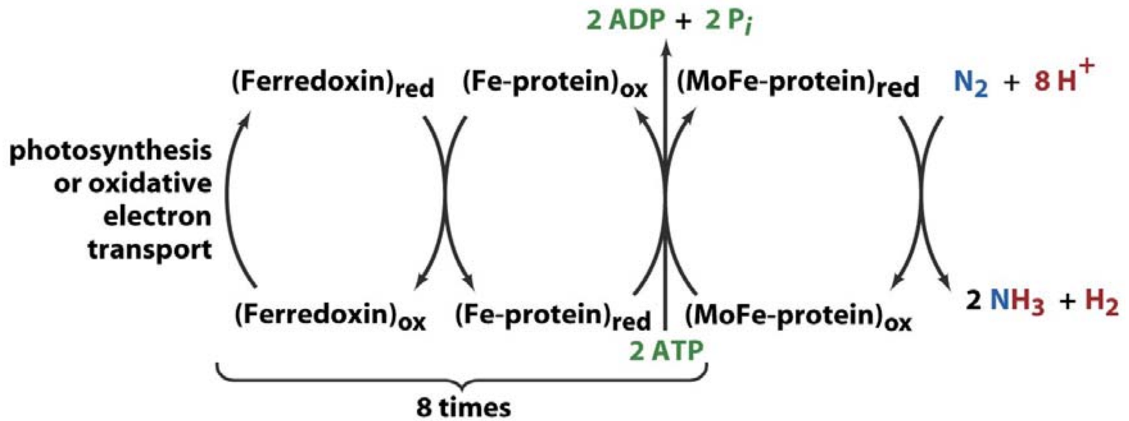
Figure 20-44 Fundamentals of Biochemistry, 2/e

N_2 reduction is energetically costly



6 times of electron transfer per N2 fixed: 12 ATP
Futile cycle: diimine to nitrogen, 2 electrons transfer and 4 ATP
Total: 16 ATP

The flow of electrons in the nitrogenase-catalyzed reduction of N_2

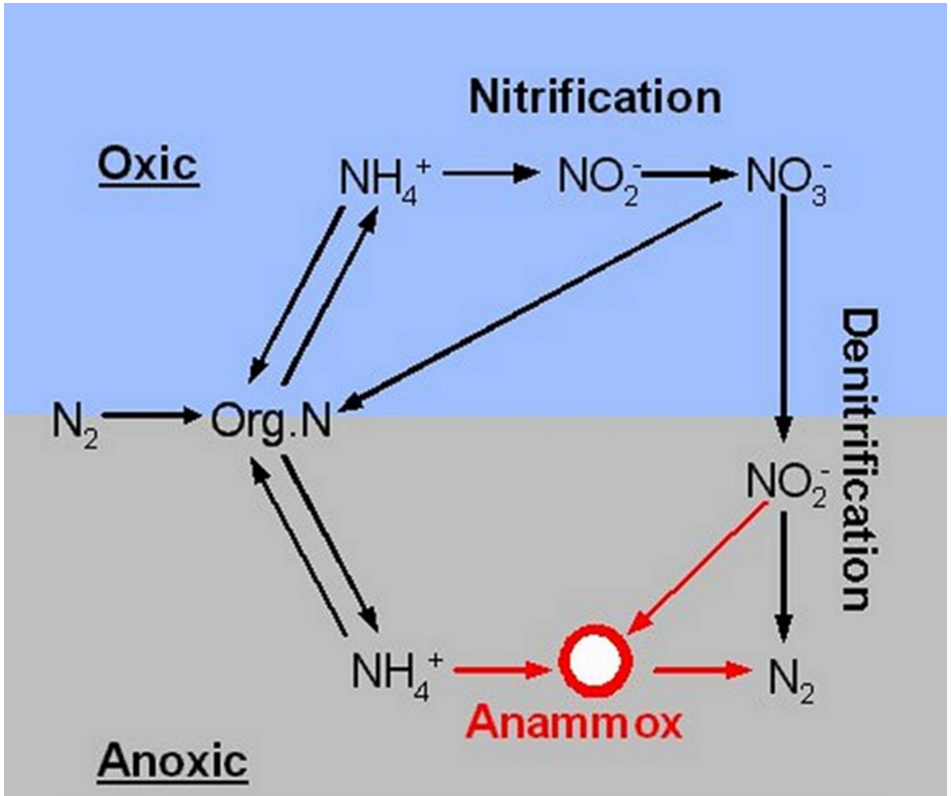


Anammox bacteria

Anaerobic oxidation of ammonia to N2 in the anaerobic environment

Anammox reaction in anammoxosome

$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$



The simplified marine nitrogen cycle including the potential anammox 'sink'; Org. N: organic nitrogen.

Picture: M. Kuypers

Protein degradation

Constant turning over of proteins

(1) *E* storage: muscle

(2) Elimination of abnormal proteins

(3) Regulation of cellular metabolism

Regulatory role → (short-lived enzymes)

Constant catalytic activity → (long-lived enzymes)

Table 20-1 Half-Lives of Some Rat Liver Enzymes

Enzyme	Half-Life (*h*)
Short-Lived Enzymes	
Ornithine decarboxylase	0.2
RNA polymerase I	1.3
Tyrosine aminotransferase	2.0
Serine dehydratase	4.0
PEP carboxylase	5.0
Long-Lived Enzymes	
Aldolase	118
GAPDH	130
Cytochrome *b*	130
LDH	130
Cytochrome *c*	150

Source: Dice, J.F. and Goldberg, A.L., *Arch. Biochem. Biophys.* **170,** 214 (1975).

Table 20-1 Fundamentals of Biochemistry, 2/e

Lysosomal degradation

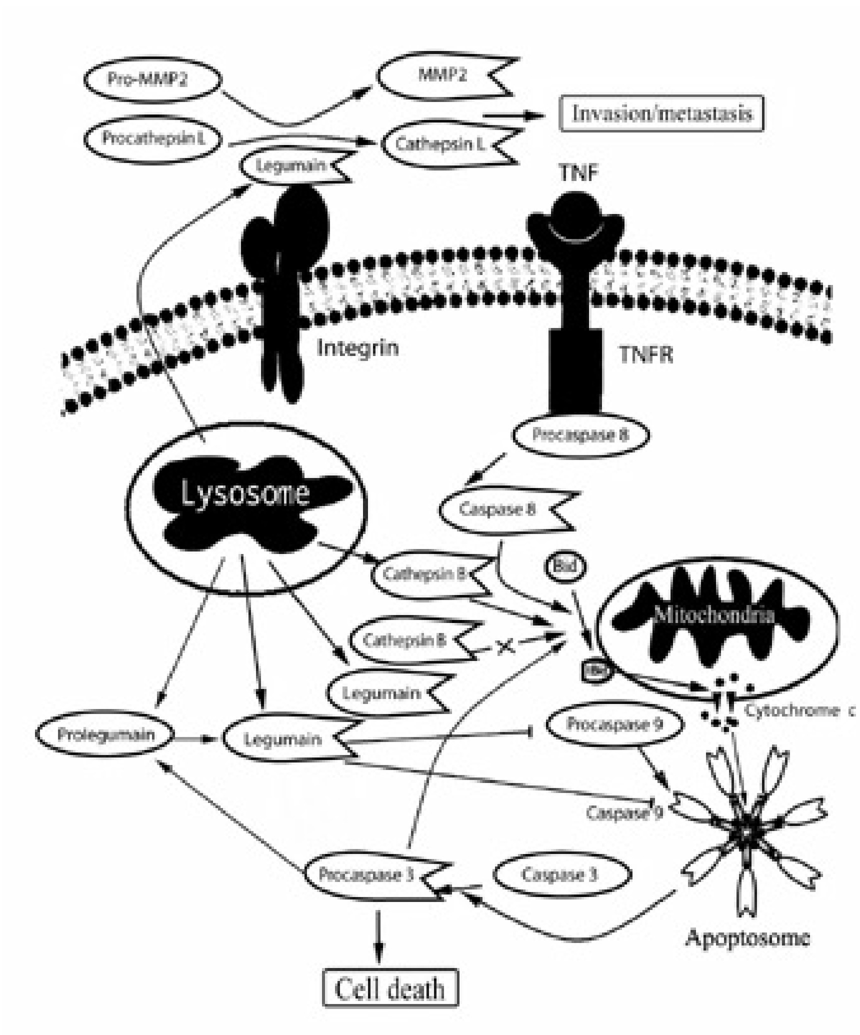
Lysosomes: ~50 hydrolytic enzymes
Proteases (cathepsins)

Cathepsins are usually characterised as members of the lysosomal cysteine protease family. In actuality, the cathepsin family also contains members of the serine protease (cathepsin A,G) and aspartic protease (cathepsin D,E) families as well.

Elevated cathepsin enzyme activity in serum or the extracellular matrix often signifies a number of gross pathological conditions.

Selective degradation of cytosolic proteins
KFERQ proteins: under fasting conditions

The Cysteine Protease Network in Tumor Progression and Therapy



Legumain (a cysteine protease) promotes tumor cell invasion and metastasis by binding to cell-surface integrins and activates both matrix metalloproteinase 2 (MMP2) and cathepsin L. It also protects cells from programmed cell death by catalytically inactivating caspase 9. It prevents Bid activation by cathepsin B by binding to and modulating the activity of the cathepsin.

<u>Ubiquitin:</u> highly conserved 76 a.a. proteins

Ubiquitin involving protein breakdown

- ATP-requiring
- Independent of lysosomes

<u>Proteins are marked for degradation</u>

E1: ubiquitin-activating enzymes

E2: ubiquitin-conjugating enzymes

- 11 in yeast, >20 in mammals

E3: ubiquitin-protein ligase

- Many species of E3 specific to a set of proteins
- 2 families containing HECT domain or RING finger
- Each E3 is served by one or a few specific E2s

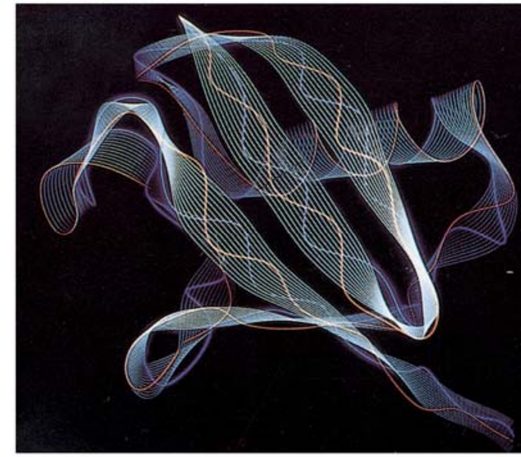


Figure 20-1 Fundamentals of Biochemistry, 2/e

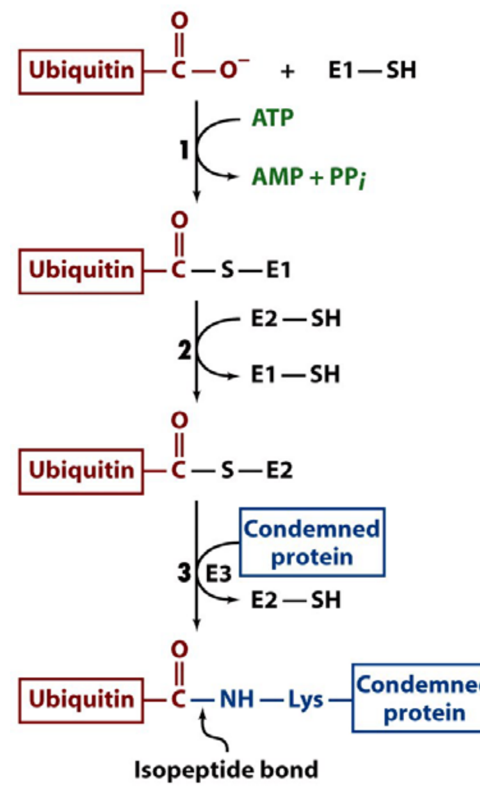
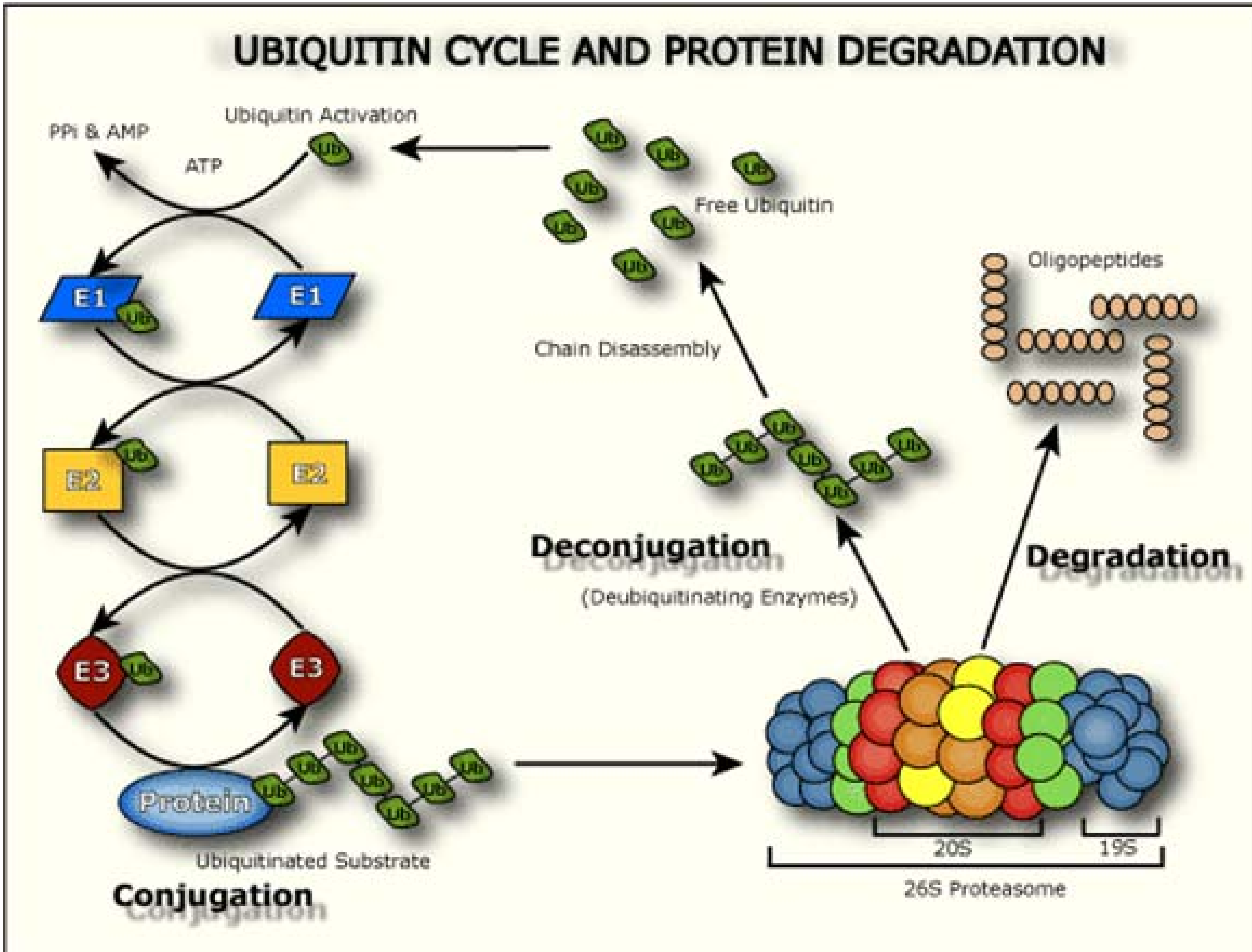


Figure 20-2 Fundamentals of Biochemistry, 2/e



UBIQUITIN CYCLE AND PROTEIN DEGRADATION
PPi & AMP
Ubiquitin Activation
ATP
Ub
E1
E2
E3
Free Ubiquitin
Chain Disassembly
Oligopeptides
Deconjugation
(Deubiquitinating Enzymes)
Degradation
Protein
Ubiquitinated Substrate
Conjugation
20S
19S
26S Proteasome

Ubiquitin system has both housekeeping and regulatory functions

The N-end rule

- Half-lives of many proteins depend on their N-terminal residues
- Conserved in both prokaryotes and eukaryotes
- Destabilizing residues: D R L K F, half-lives of 2~3 min
- Stabilizing residues: A G M S T V, half-lives of >10 hrs (in pro) or >20 (in Eu)

Destabilizing signal in eukaryotes

- Ubiquitination of E3α (Ring finger E3)
- Variety of ubiquitination signal by more E3s
- PEST proteins are rapidly degraded

The proteasome
Degradation of ubiquinated proteins
Multiprotein complex: ~2100 kD (26S proteasome)
7 different types of α-like and β-like subunits

EM-image of 26S proteasome

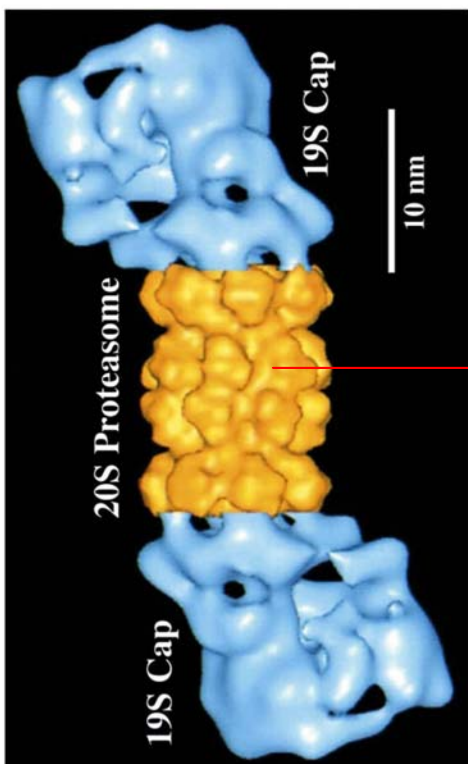


Figure 20-3 Fundamentals of Biochemistry, 2/e

X-ray structure of 20S proteasome
C2 & pseudo-sevenfold rotational symmetry

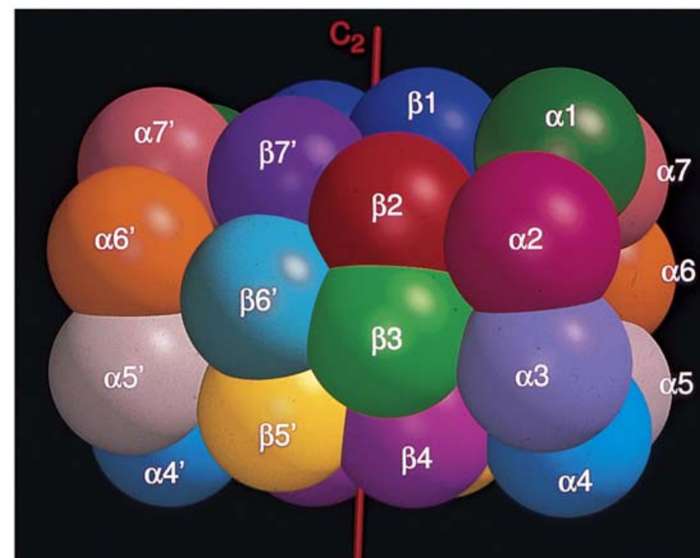


Figure 20-4a Fundamentals of Biochemistry, 2/e

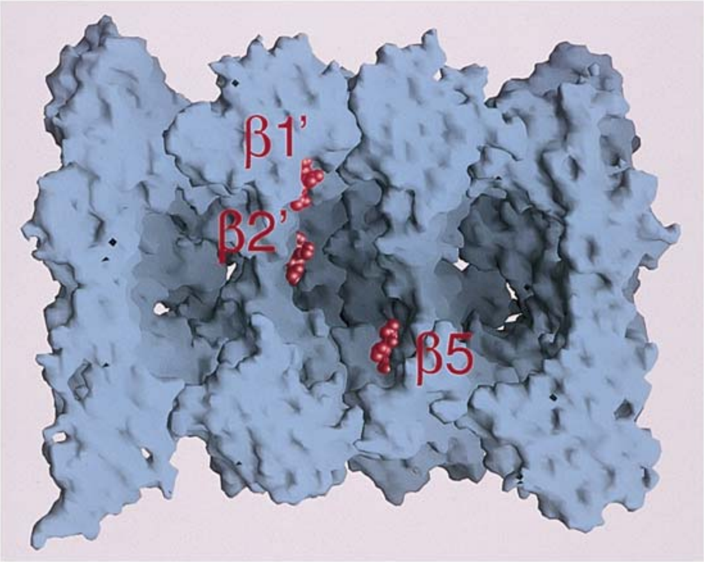


Figure 20-4b Fundamentals of Biochemistry, 2/e

Three proteolytic sites

- β1 subunit: cleaving after acidic residue
- β2 subunit: basic residue
- β5 subunit: hydrophobic
- Yielding fragments of ~8 residues

Amino acid deamination

Amino group to ammonia and to urea
Carbon skeleton (α-keto acid)

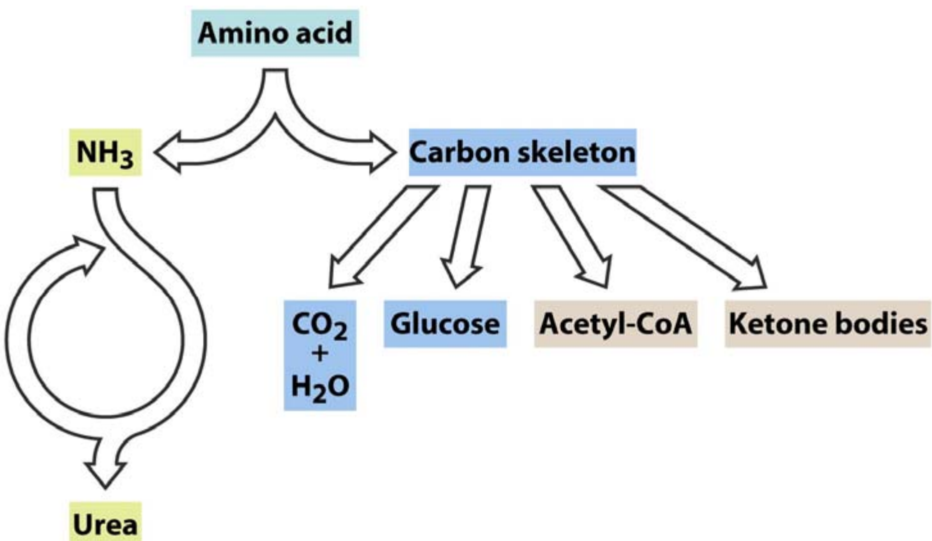
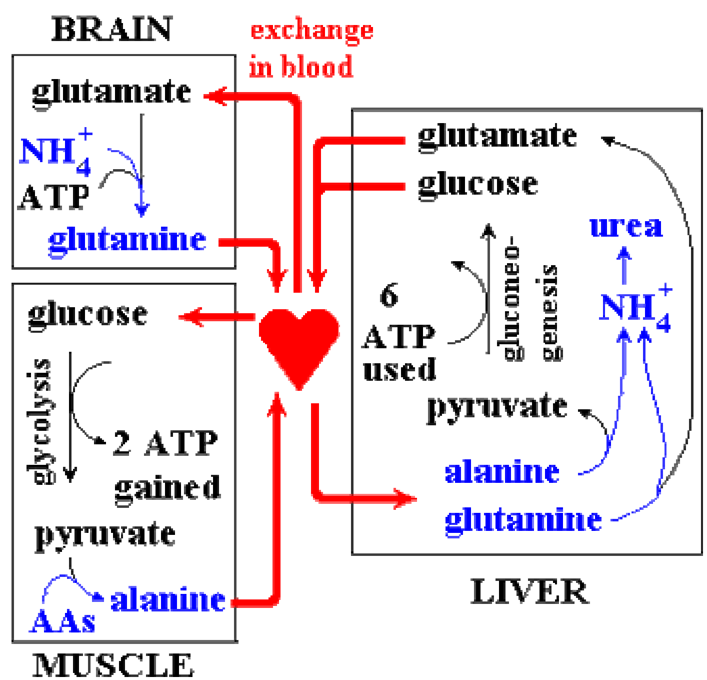


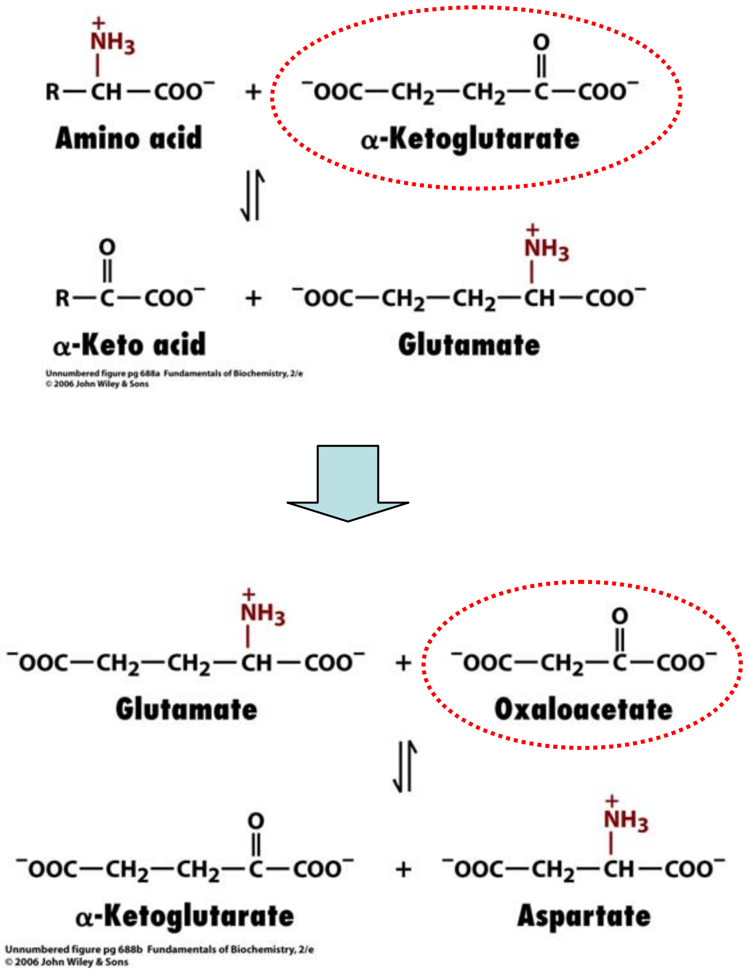
Figure 20-6 Fundamentals of Biochemistry, 2/e

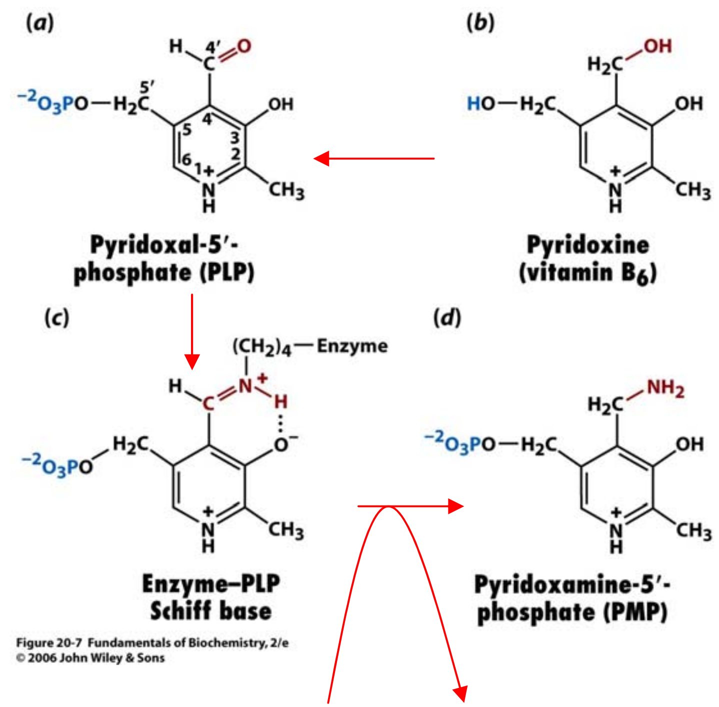


Transamination

The transfer of amino group to an α-keto acid

Aminotransferases (transaminases)





α-amino acid α-keto acid

Transaminases are freely reversible in rxn

Participate in both degradation and synthesis

Transaminases as a clinical marker

SGOT (serum glutamate-oxaloacetate transaminase)

= AST (aspartate transaminase)

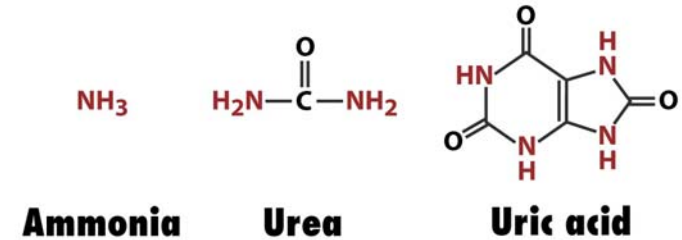
SGPT (serum glutamate-pyruvate transaminase)

= ALT (alanine transaminase)

Heart or liver damage: increase of SGOT and SGPT

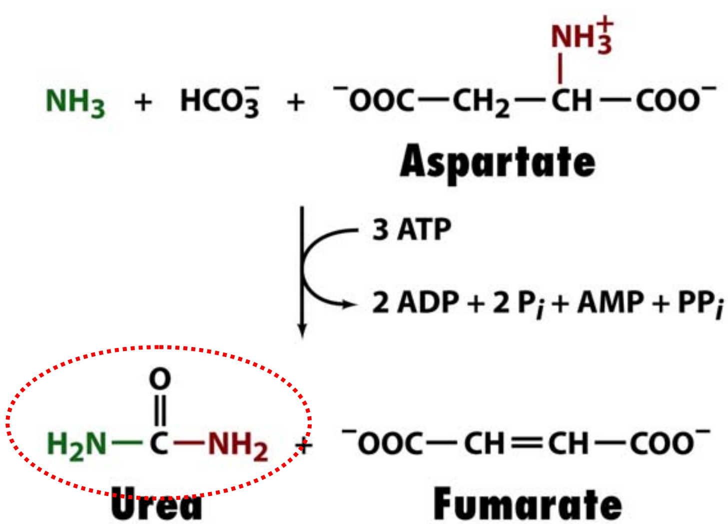
The urea cycle

Excess nitrogen to ammonia, urea, uric acid



synthesized in liver
secreted into the blood
sequestered by the kidney for excretion in the urine

The overall reaction



<u>The urea cycle</u>

Two mitochondrial reactions
Three cytosolic reactions

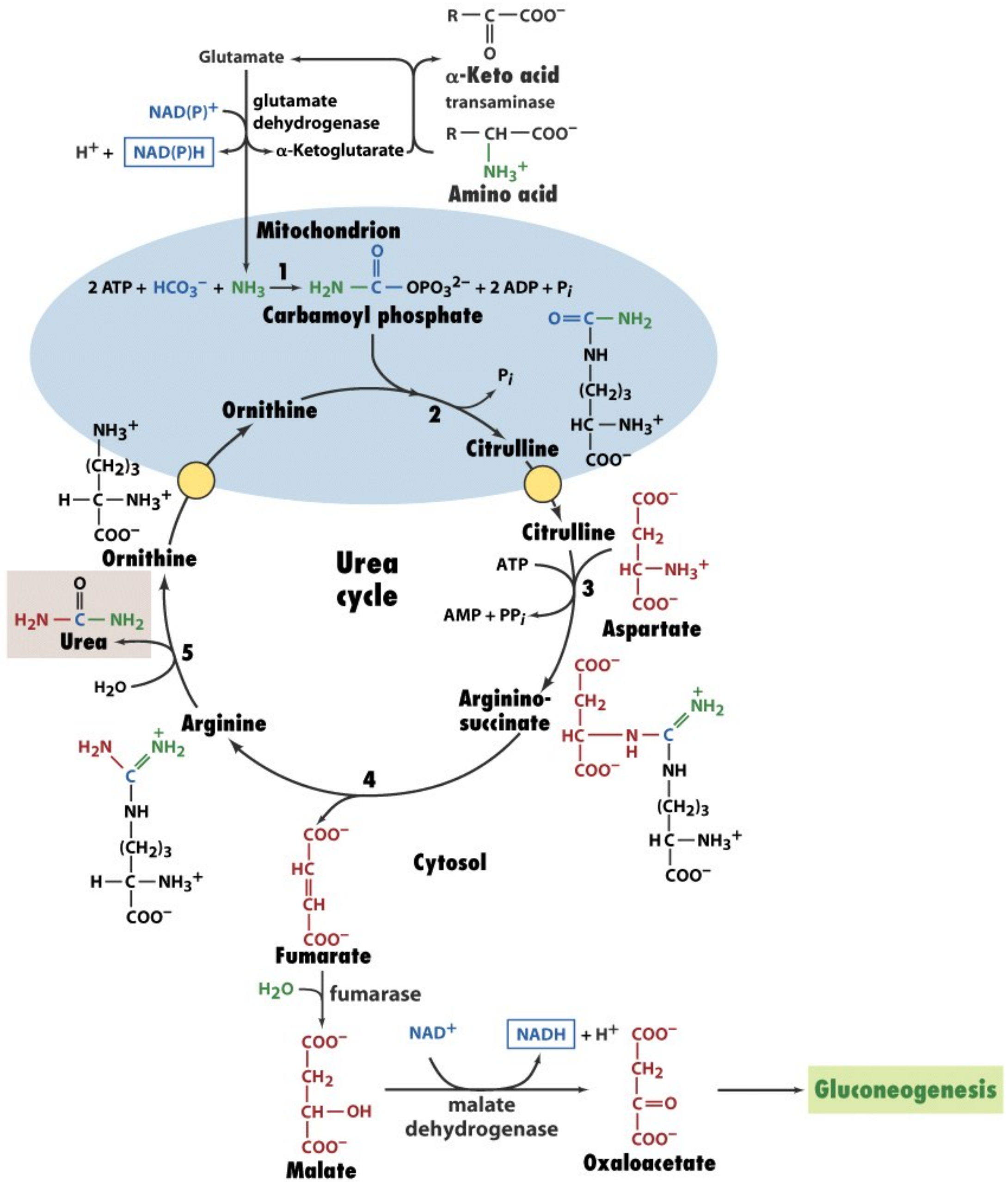


Figure 20-9 Fundamentals of Biochemistry, 2/e

Breakdown of amino acids

Glucogenic amino acids

Glucose precursor

Ketogenic amino acids

Precursors of fatty acids or ketone bodies

Purely ketogenic: Lys, Leu

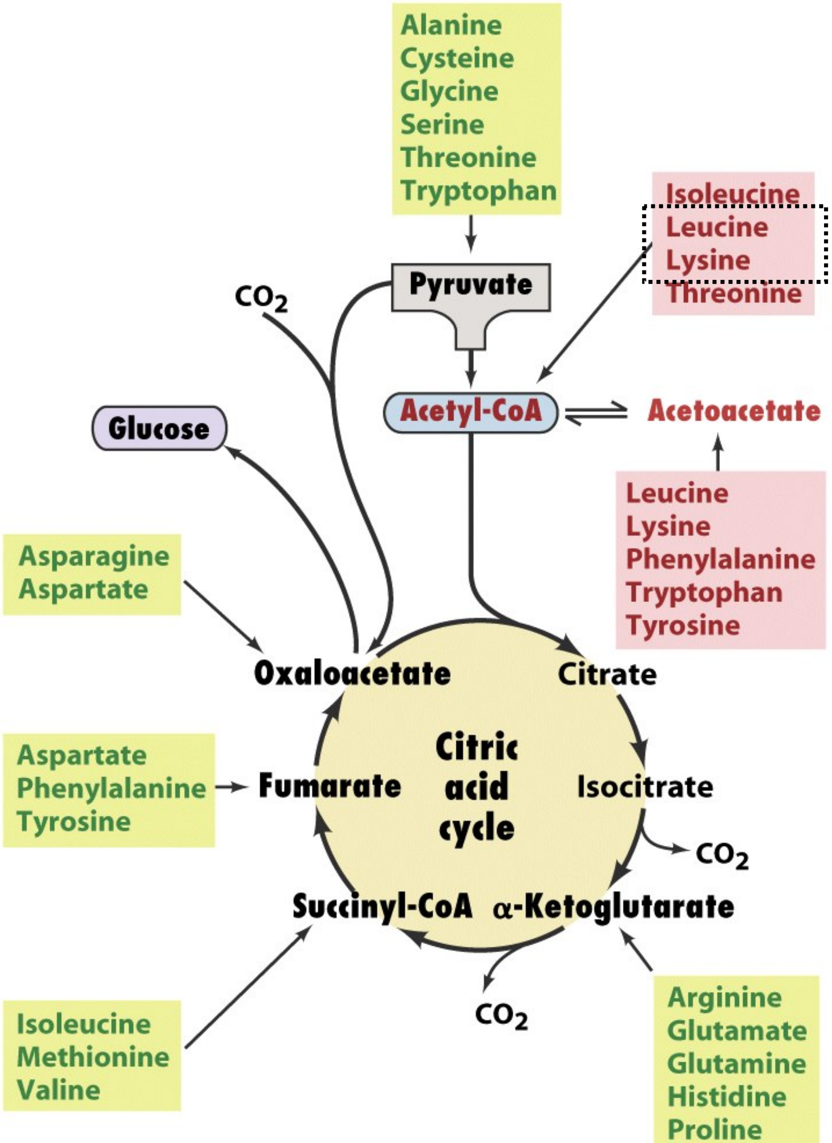


Figure 20-13 Fundamentals of Biochemistry, 2/e

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/aacarbon.htm

Degradation to pyruvate

ACGST

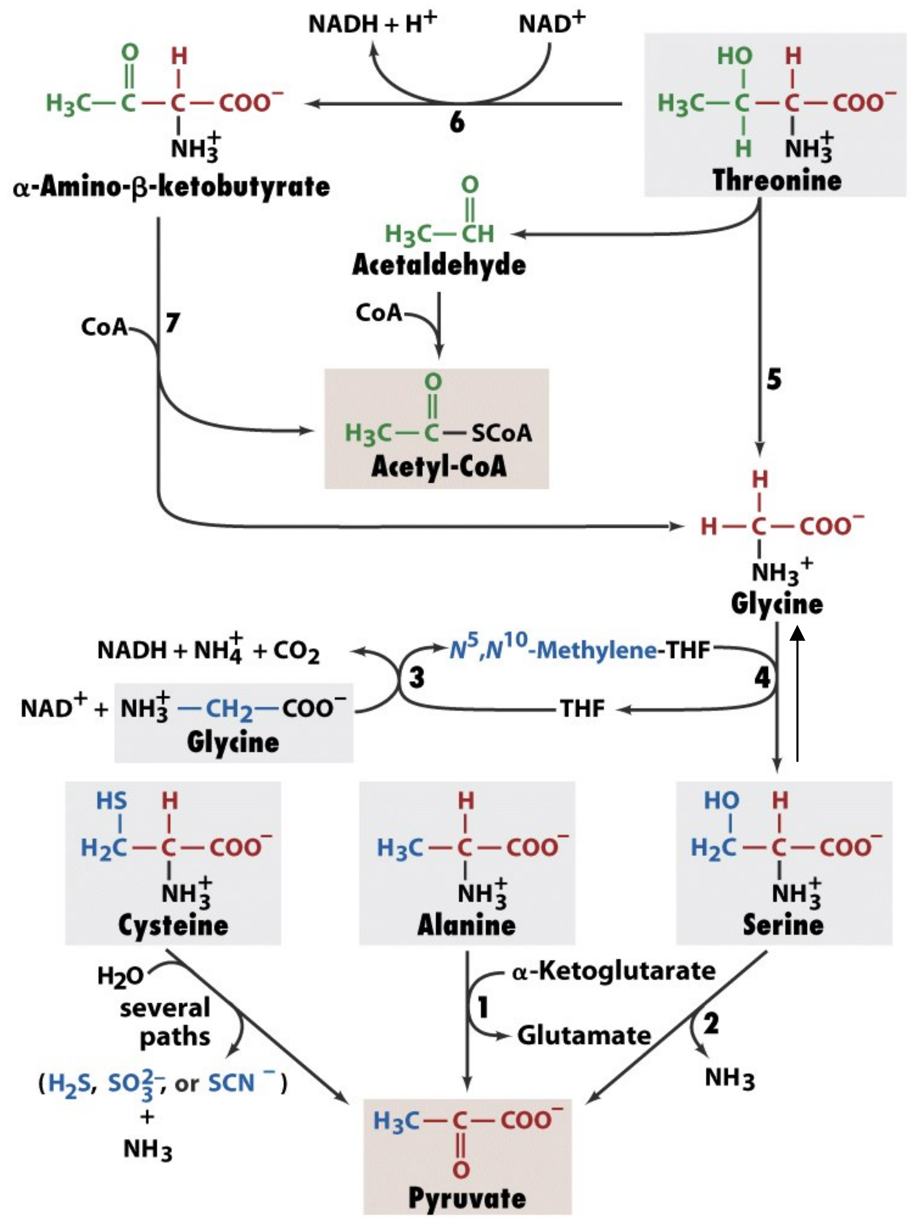


Figure 20-14 Fundamentals of Biochemistry, 2/e

PLP containing enzyme

Serine dehydratase: 2

Serine hydroxymethyltransferase: 4

Glycine cleavage system (rxn 3)

A major route of glycine degradation in mammals

Inherited deficiency: nonketotic hyperglycinemia (glycine encephalopathy)

Degradation to α-ketoglutarate
REQHP

Gln acts as an ammonia transport system between
the liver (synthesis) and
the kidney (hydrolyzed by glutaminase)

During metabolic acidosis
Glutaminase eliminate excess acid
By combining ammonia with a proton

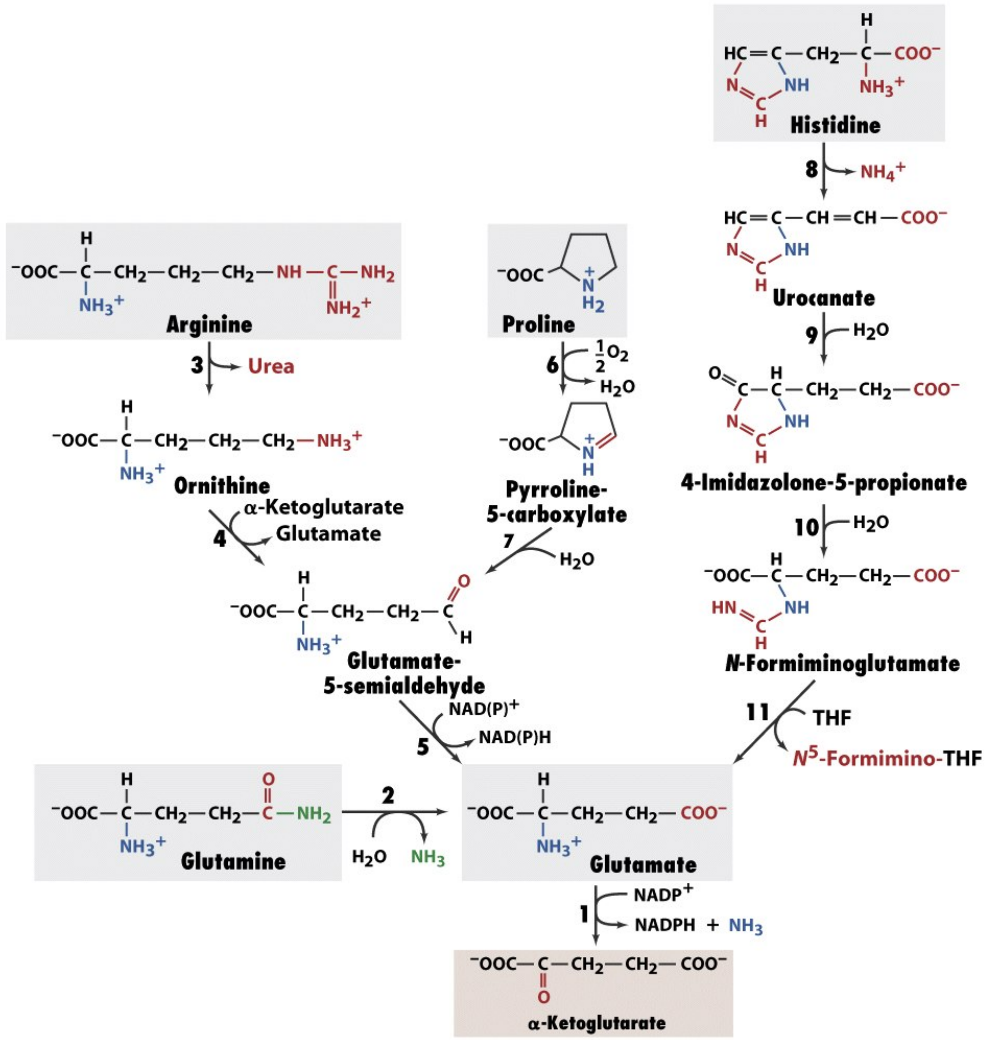


Figure 20-17 Fundamentals of Biochemistry, 2/e

Degradation to succinyl-CoA

IMV

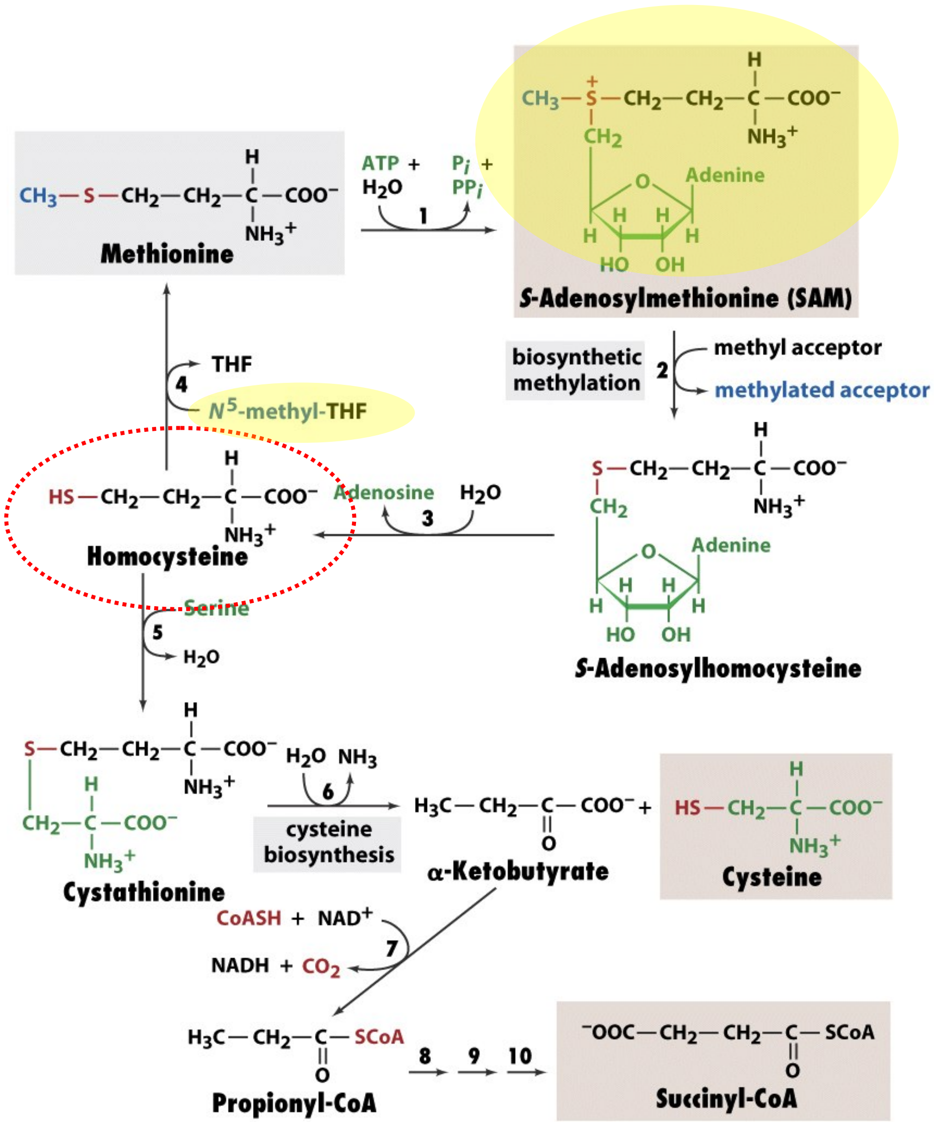


Figure 20-18 Fundamentals of Biochemistry, 2/e

Homocysteine is a marker of atherosclerosis

Homocysteine conc is determined by the rates of
  rxn 2,3,4 and rxn 5

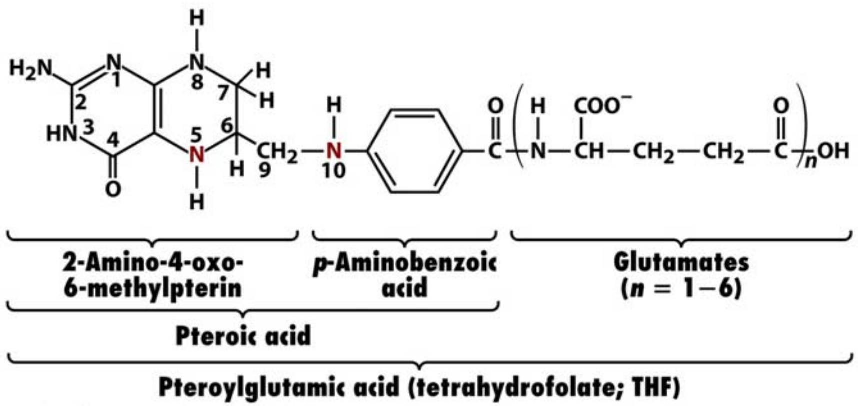
Hyperhomocysteinemia (homocysteinuria)
  associated with cardiovascular disease
  due to oxidative damage to endothelial cells
  (deficiency of folate or vit. B12)

Associated with neural tube defects
  Spina bifida
  Anencephaly (http://www.path.sunysb.edu/neuropath/developmental.htm)
  High incidence

MTHFR mutations ($q^2 = 0.01$)

N^5,N^{10}-methylene-THF to N^5-methyl-THF (cofactor for step 4)

Tetrahydrofolates (THFs): one-carbon carriers



Unnumbered figure pg 704 Fundamentals of Biochemistry, 2/e

Dihydrofolate reductase (DHFR)

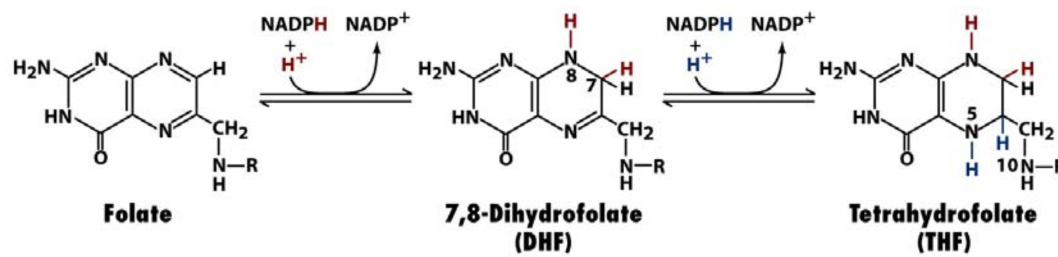


Figure 20-19 Fundamentals of Biochemistry, 2/e

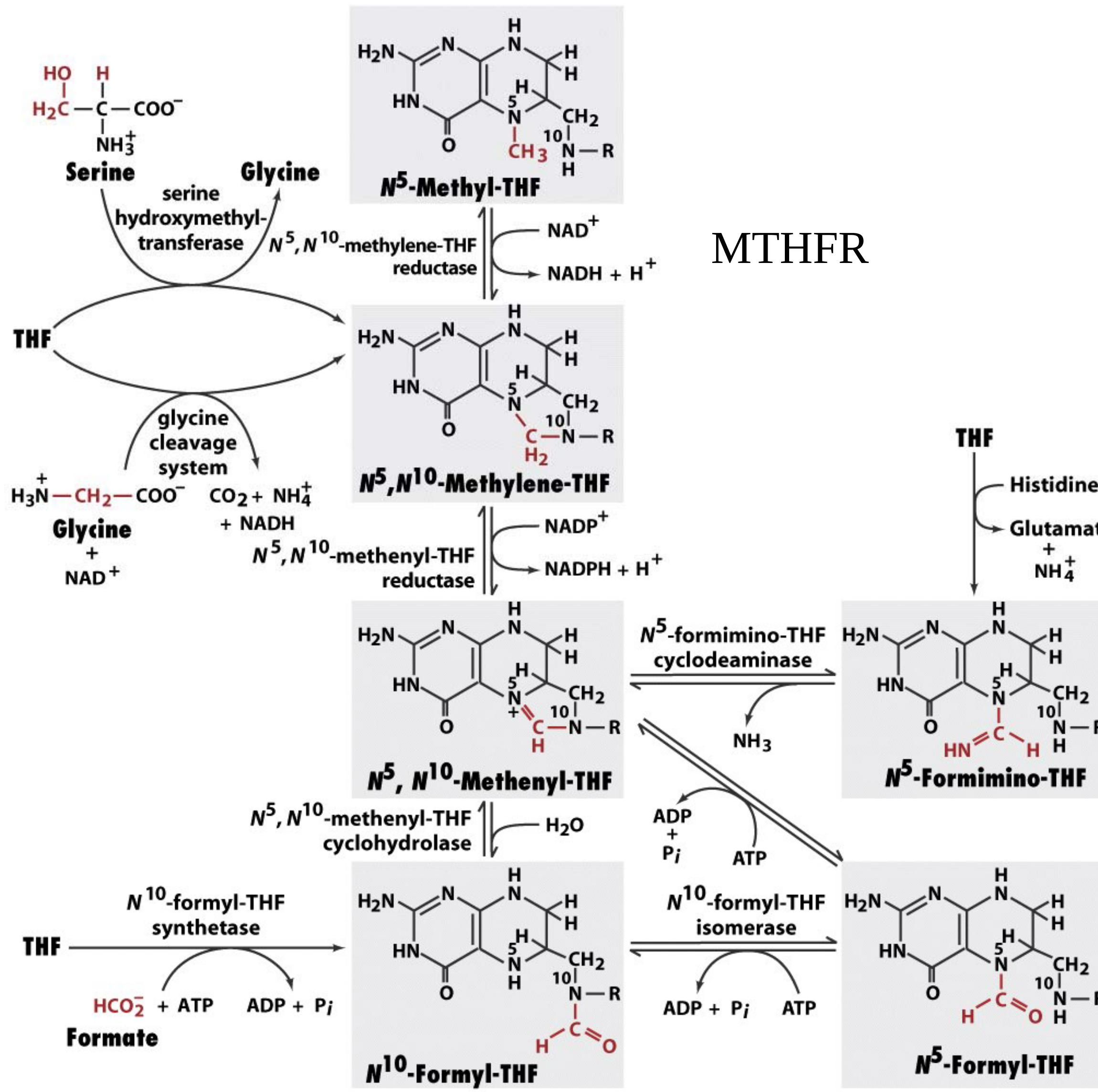
Biotin: CO_2
SAM: CH_3-
THF: various C1 groups

Table 20-2 Oxidation Levels of C_1 Groups Carried by THF

Oxidation Level	Group Carried	THF Derivative(s)
Methanol	Methyl ($—CH_3$)	N^5-Methyl-THF
Formaldehyde	Methylene ($—CH_2—$)	N^5,N^{10}-Methylene-THF
Formate	Formyl (—CH═O)	N^5-Formyl-THF, N^{10}-formyl-THF
	Formimino (—CH═NH)	N^5-Formimino-THF
	Methenyl (—CH═)	N^5,N^{10}-Methenyl-THF

Table 20-2 Fundamentals of Biochemistry, 2/e

Interconversion of the C1 units carried by THF

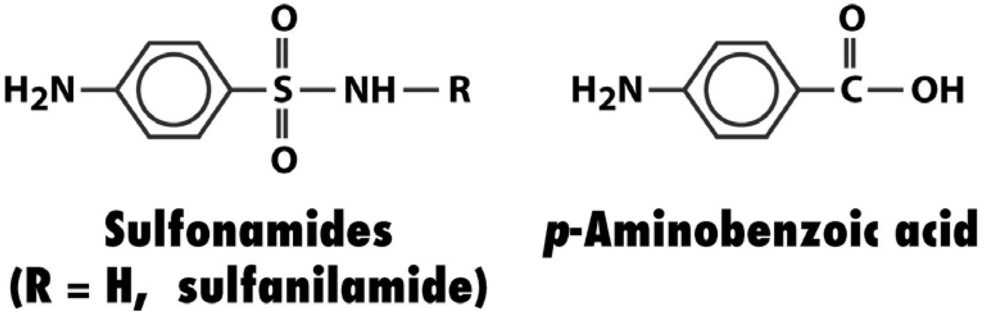


MTHFR

Figure 20-20 Fundamentals of Biochemistry, 2/e

Sulfonamides are antibiotics

Analog of the *p*-aminobenzoic acid of THF
Inhibits folic acid synthesis
Mammals lack folic acid synthesis



Unnumbered figure pg 706 Fundamentals of Biochemistry, 2/e

Degradation of the branched chain amino acids

Branched-chain α-keto acid dehydrogenase (BCKDH)

A genetic deficiency: maple syrup urine disease

a fatal disease

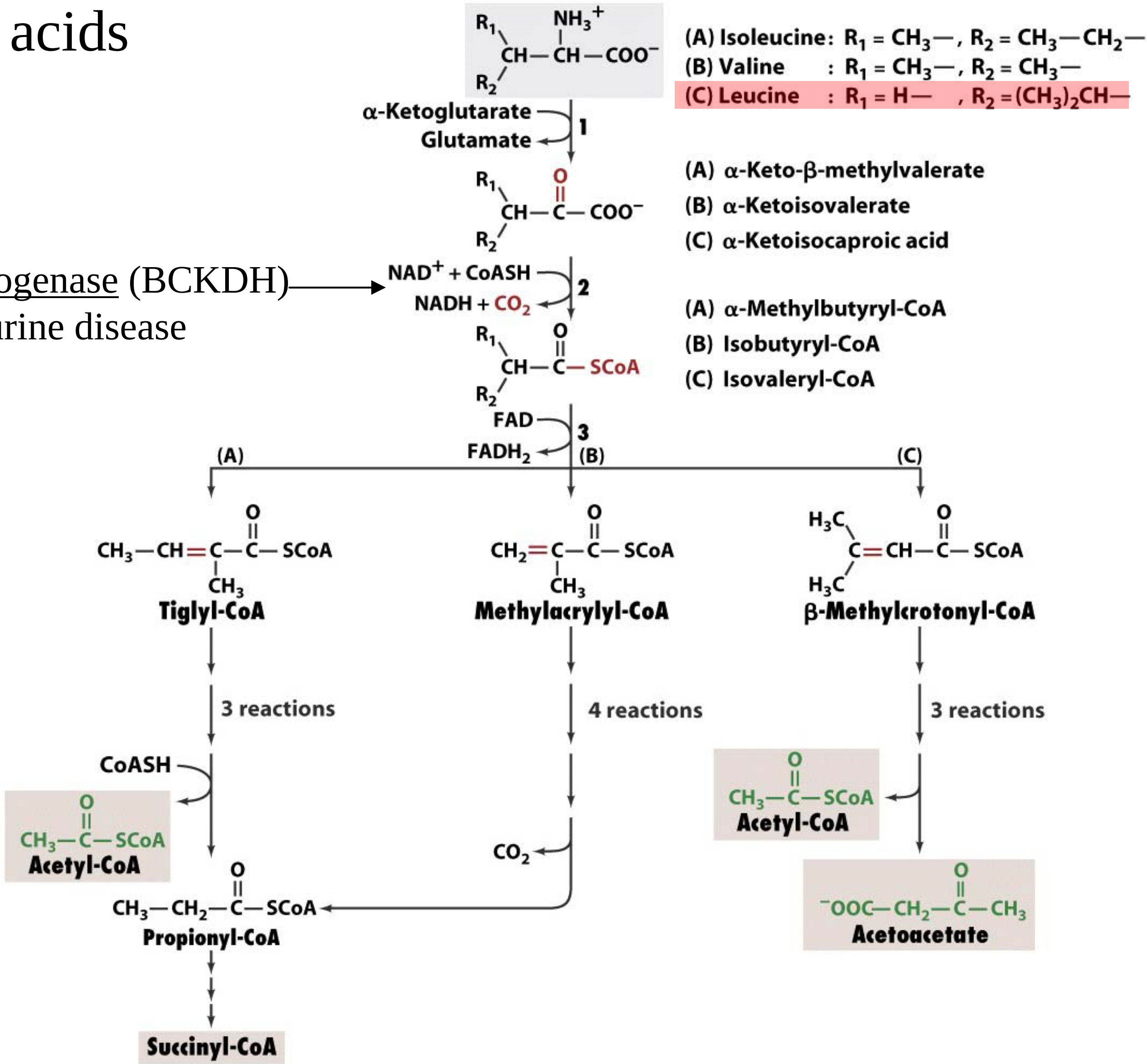


Figure 20-21 Fundamentals of Biochemistry, 2/e

Lysine degradation in mammalian liver

7 reactions were encountered previously (rxn 4,5,6,8-11)

<u>Deficiency in rxn 1</u>

Hyperlysinemia (in blood)

Hypelysinuria (in urine)

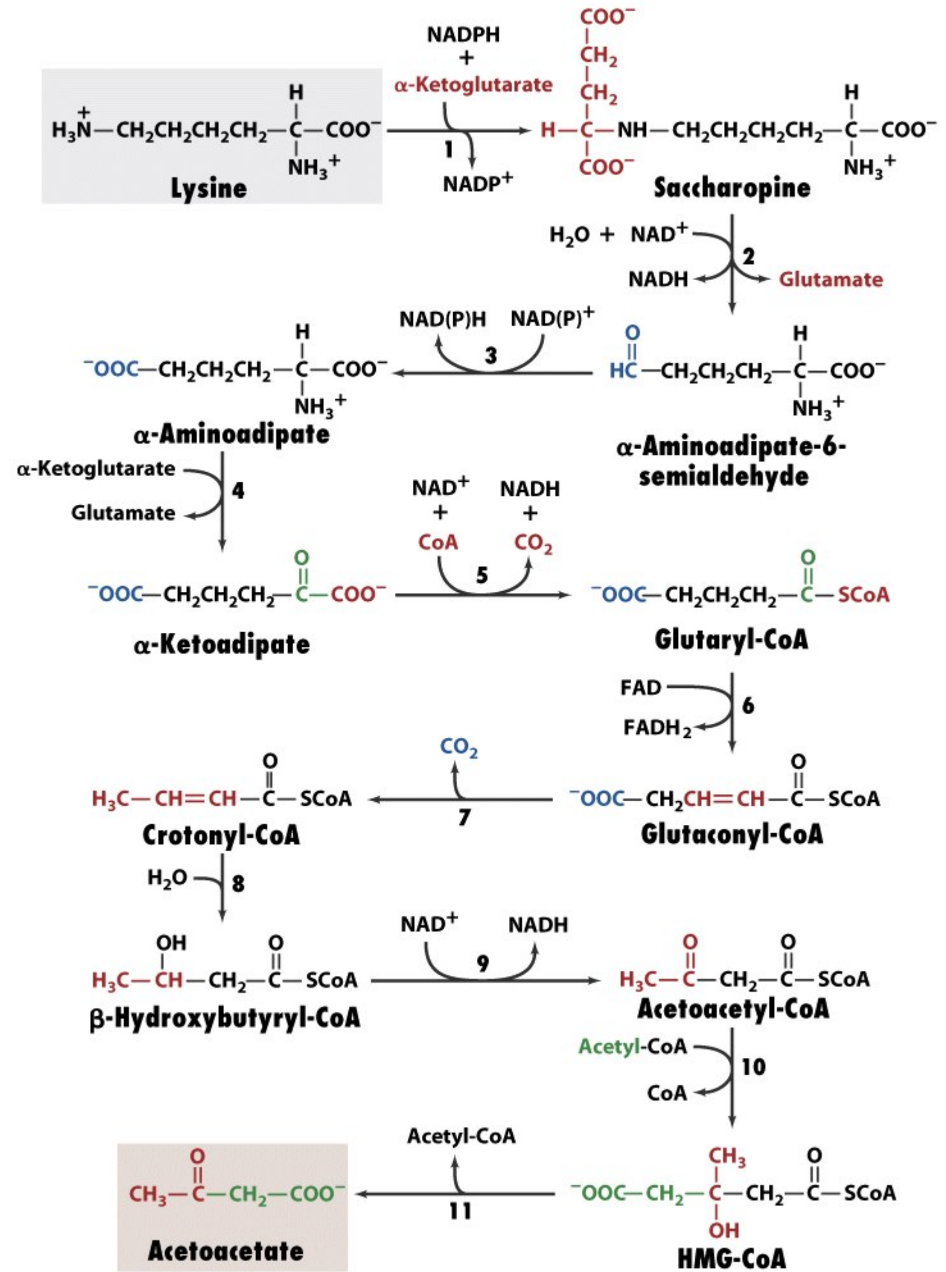


Figure 20-22 Fundamentals of Biochemistry, 2/e

Tryptophan degradation

Kynureninase: rxn 4, PLP-dependent rxn
IDO (indoleamine 2,3-dioxygenase)

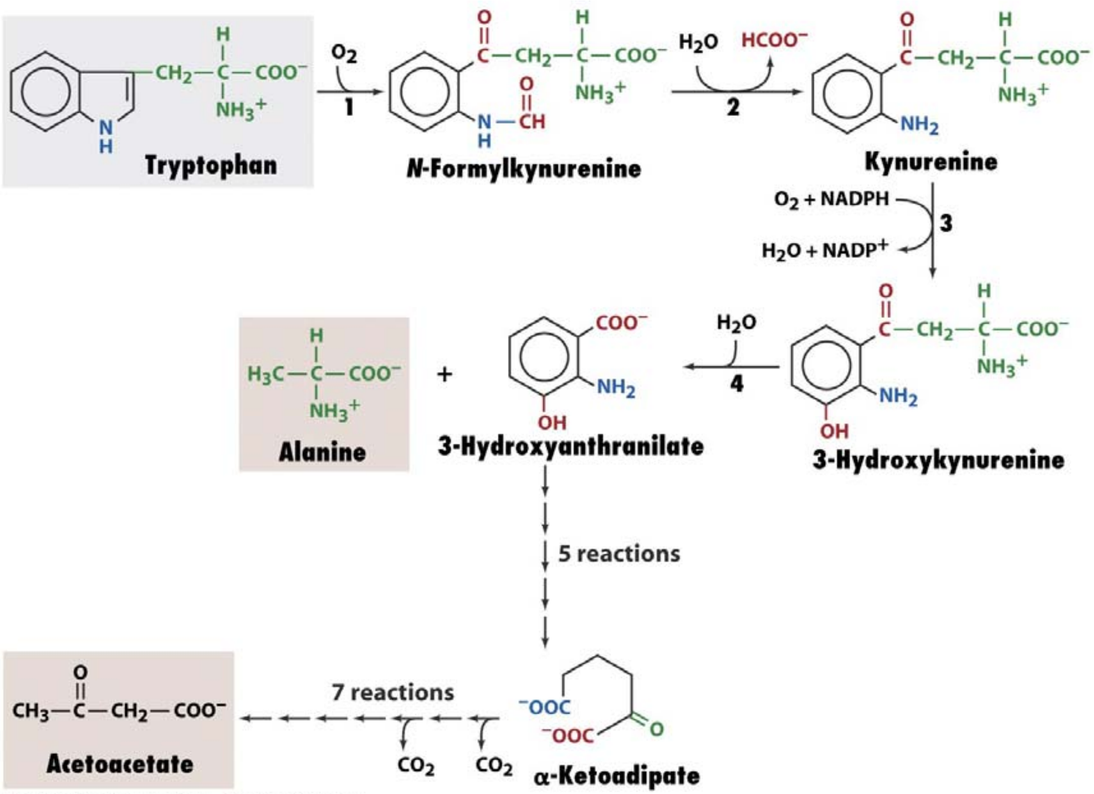
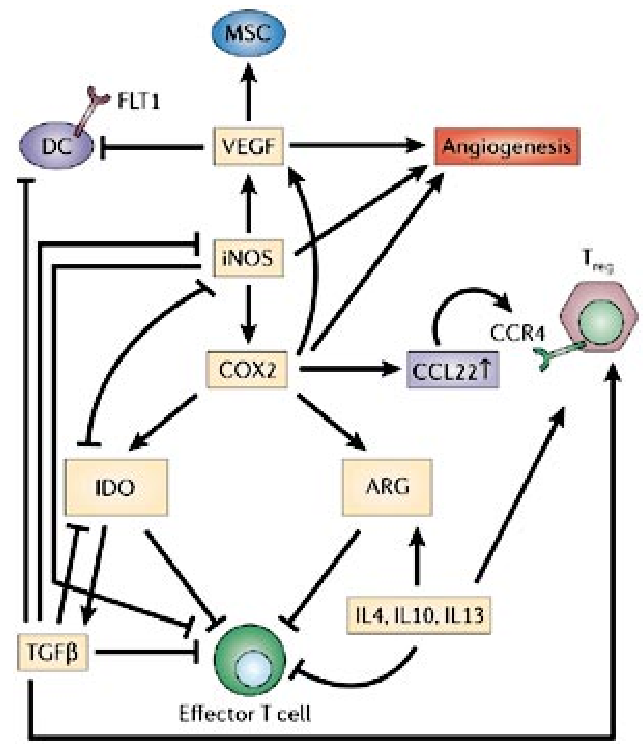


Figure 20-23 Fundamentals of Biochemistry, 2/e

Immunosuppression activity of IDO



Nature Reviews Cancer **6**, 613–625 (2006)

Phenylalanine degradation

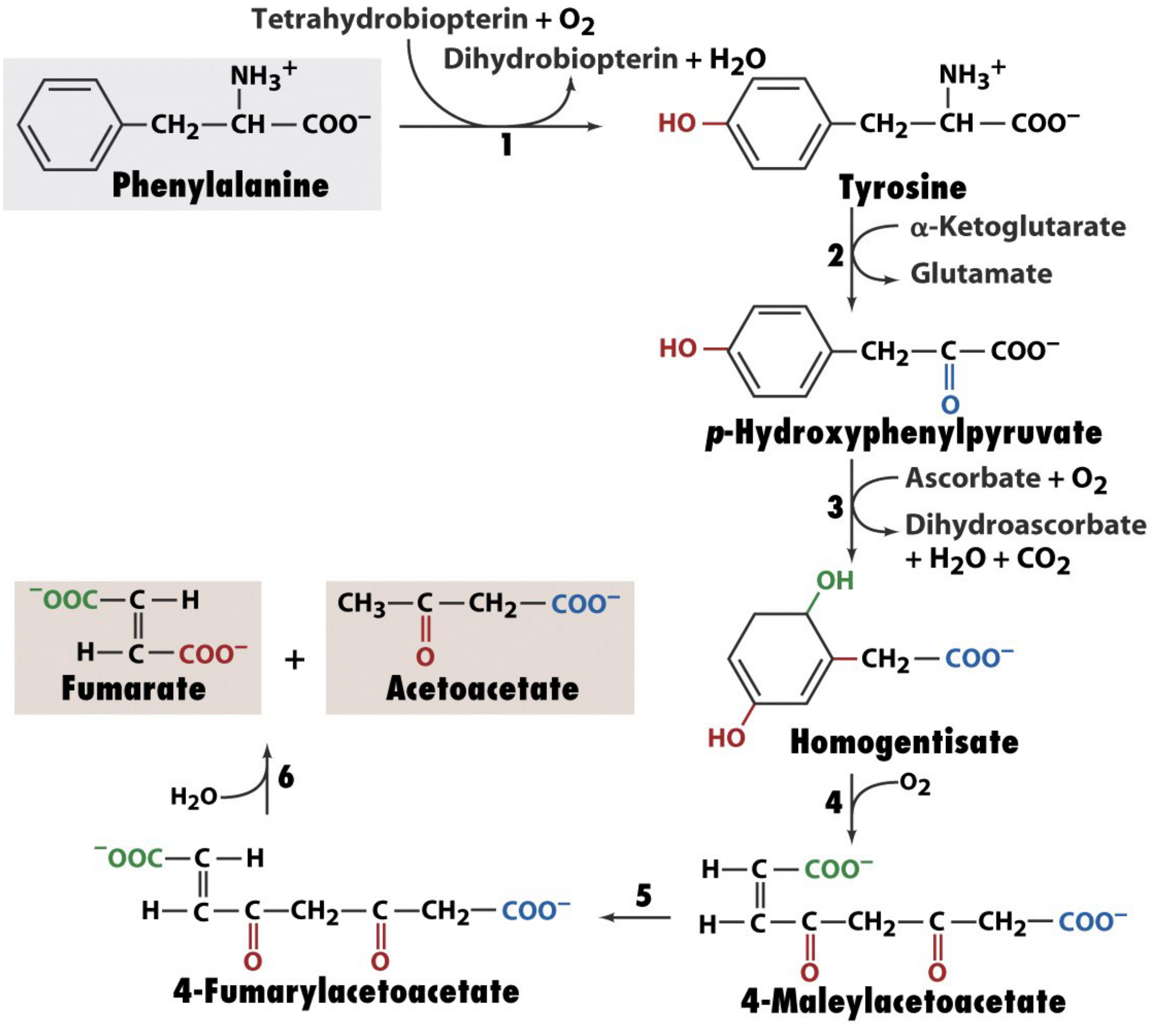


Figure 20-24 Fundamentals of Biochemistry, 2/e

Pteridine ring nucleus of biopterin and folate
Pterins are redox cofactors

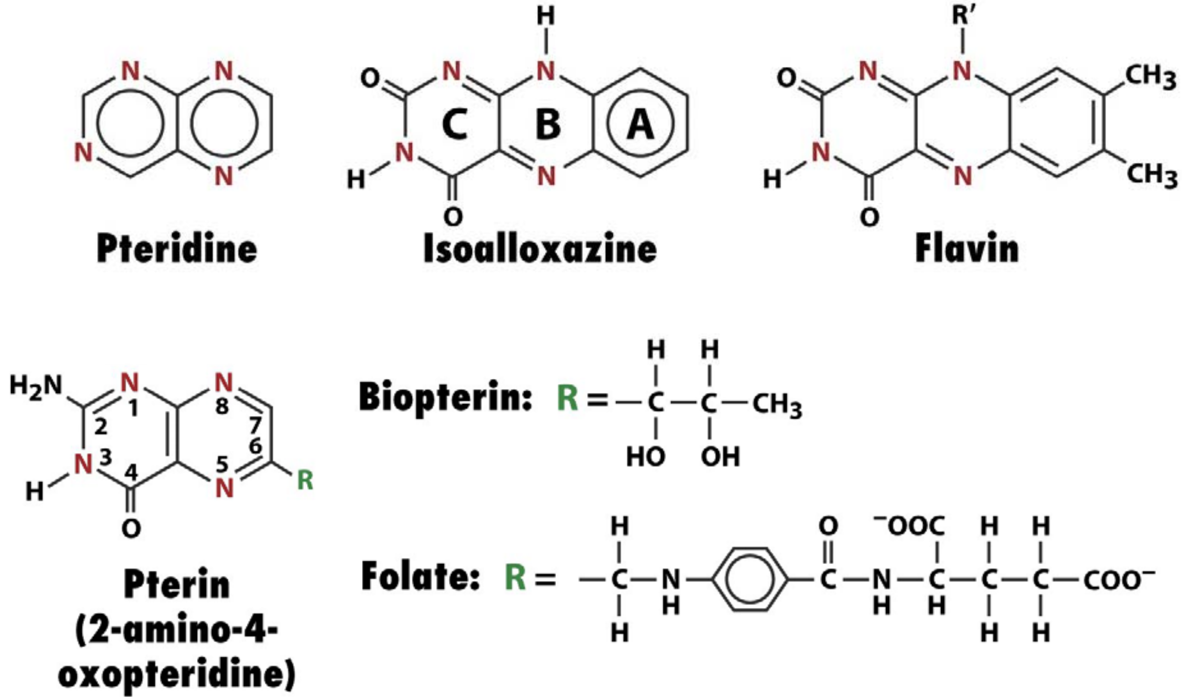


Figure 20-25 Fundamentals of Biochemistry, 2/e

Tetrahydrobiopterin
In PAH reaction

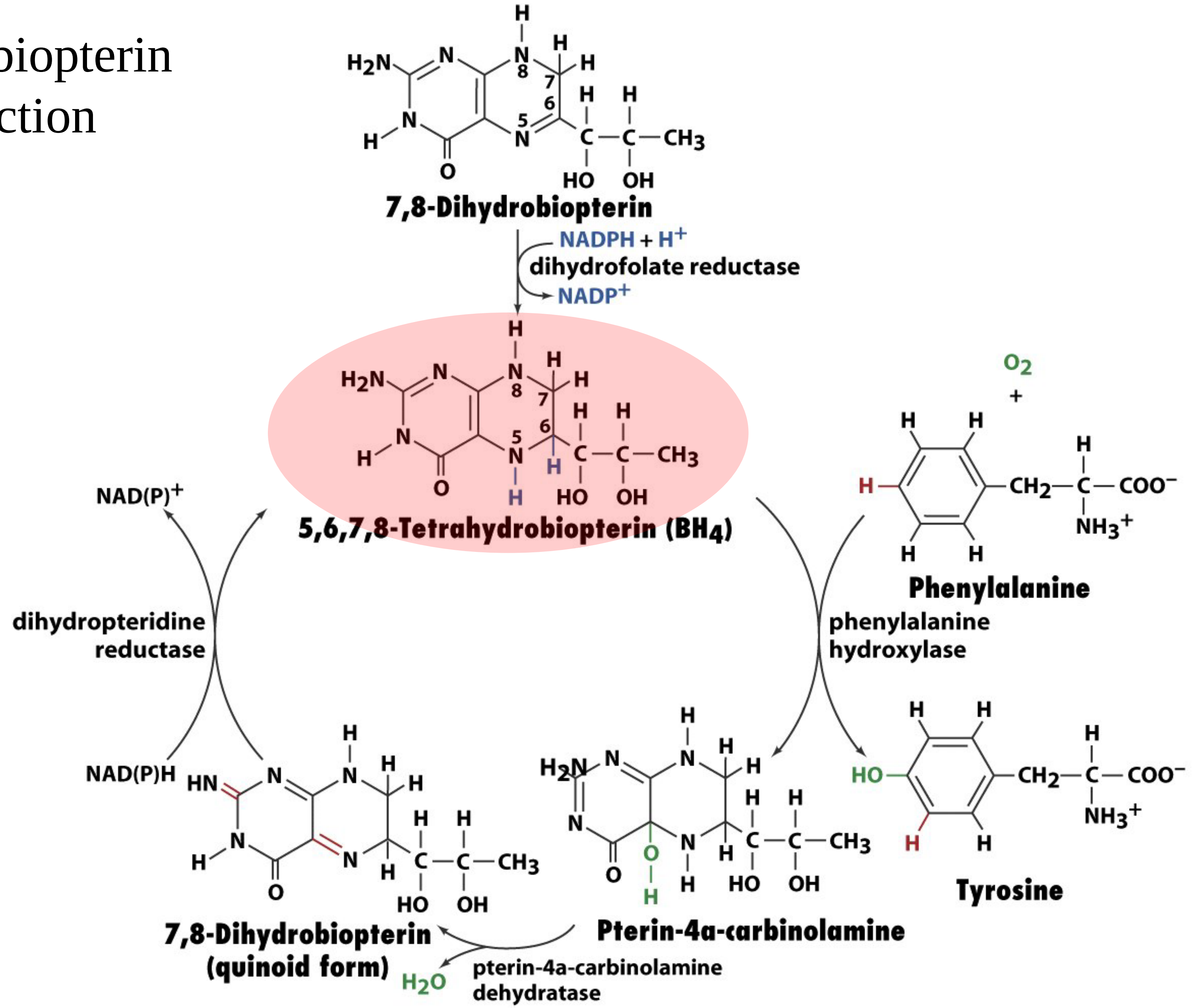


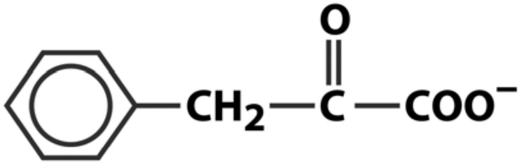
Figure 20-26 Fundamentals of Biochemistry, 2/e

Phenylketonuria and alkaptonuria

Alkaptonuria: deficiency of homogentisate dioxygenase
excretion of homogentisic acid

Phenylketonuria (PKU)
hyperphenylalaninemia: converted to phenylketo compounds
high phe inhibits tyrosine hydroxylation: reduced melanin
high phe saturates LNAAT and blocks transport of LNAA into brain

BH4 synthesis deficiencies



Phenylpyruvate

Amino acid biosynthesis

- Essential amino acids
- Nonessential amino acids

Table 20-3 Essential and Nonessential Amino Acids in Humans

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

[a] Although mammals synthesize arginine, they cleave most of it to form urea (Section 20-3A).

Table 20-3 Fundamentals of Biochemistry, 2/e

Heme biosynthesis and degradation

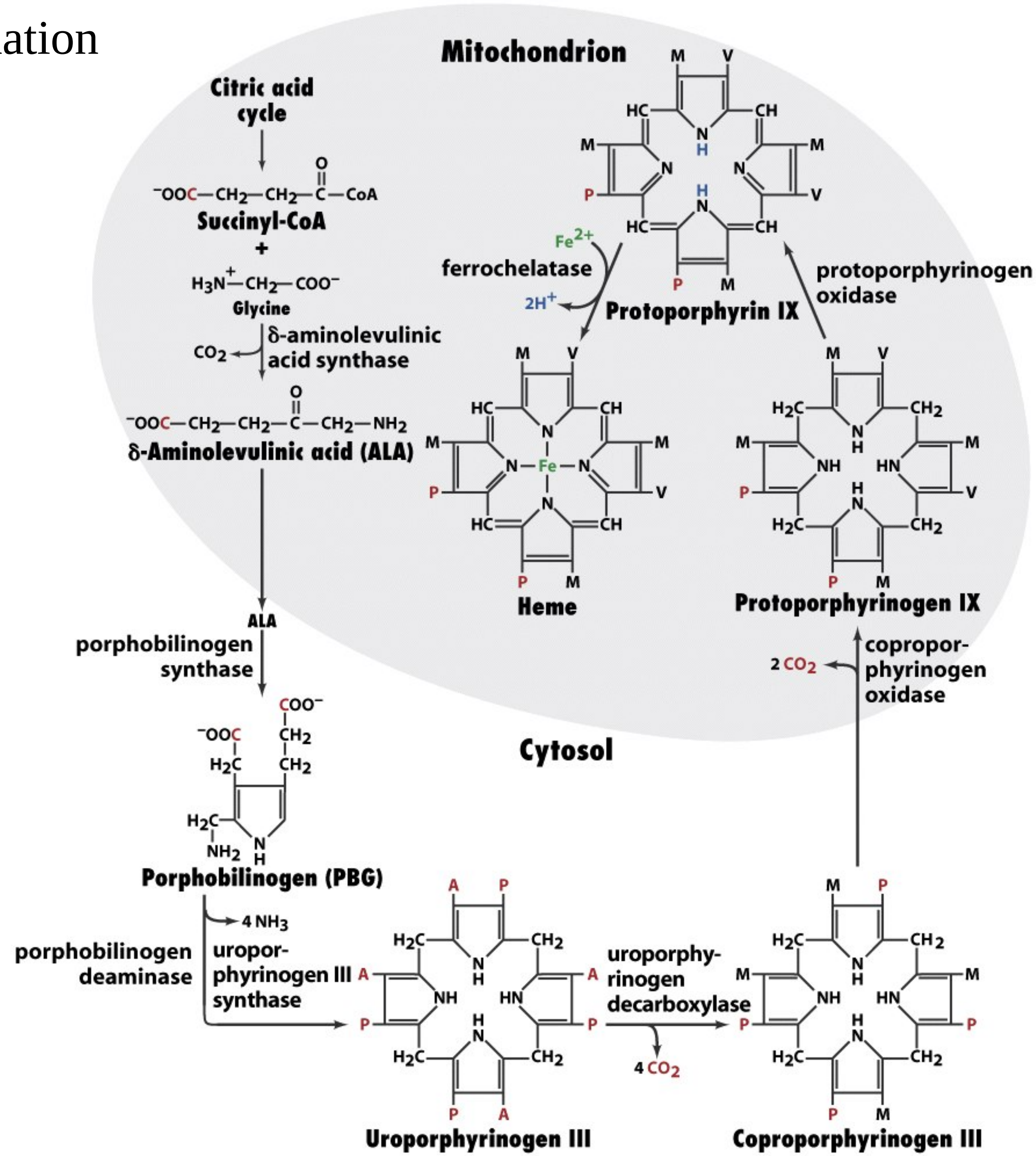
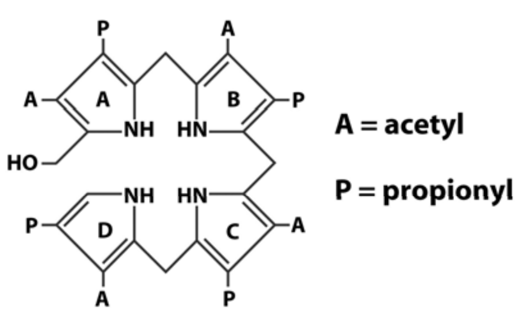


Figure 20-37 Fundamentals of Biochemistry, 2/e



Hydroxymethylbilane

Heme degradation

Jaundice: excess amount of bilirubin (insoluble) signals RBC destruction, liver dysfunction, and bile duct obstruction

green

red orange

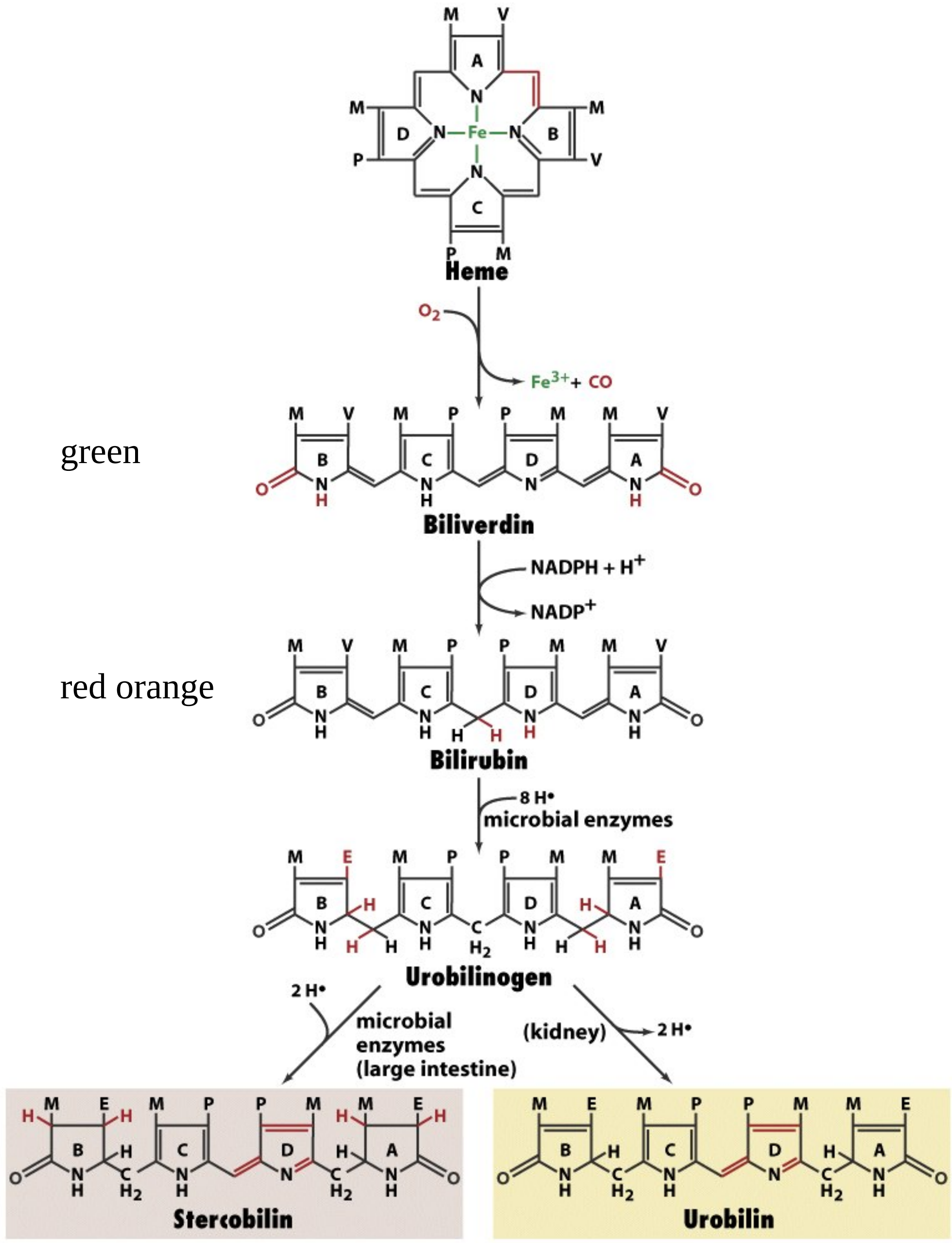


Figure 20-38 Fundamentals of Biochemistry, 2/e

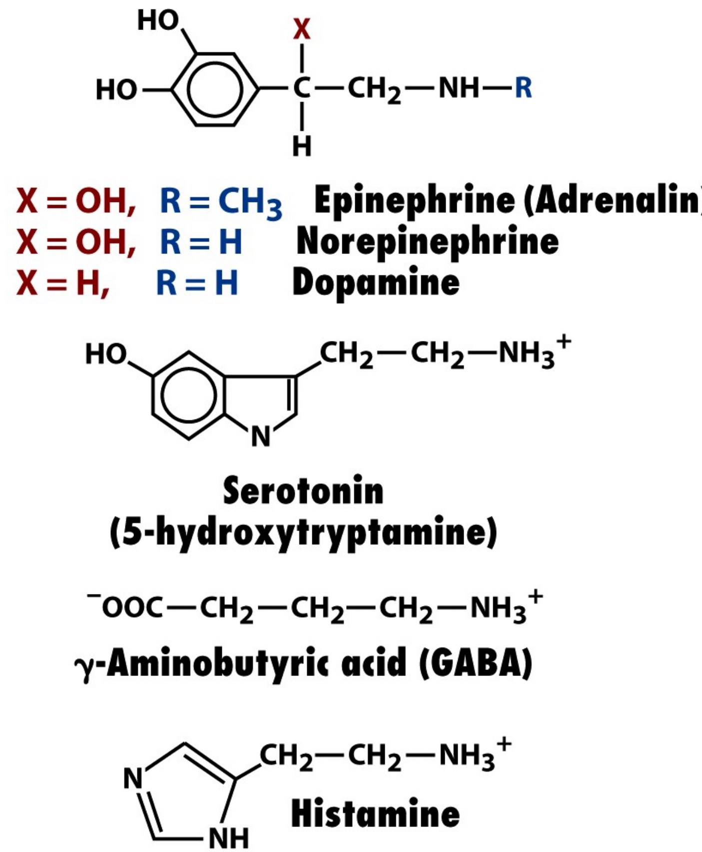
<u>Biosynthesis of physiologically active amines</u>

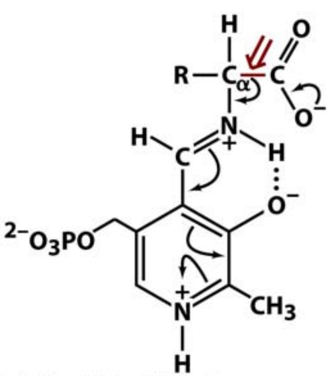
Epinephrine (adrenalin), norepinephrine, dopamine, serotonin (5-hydroxytryptamine), ɣ-aminobutyric acid (GABA), and histamine

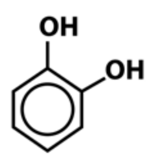
Hormones and/or neurotransmitters

Catechol amines & Indole amines

Amino acid decarboxylase (PLP-dependent rxn)







Catechol

The sequential synthesis of L-DOPA, dopamine, norepinephrine, and epinephrine

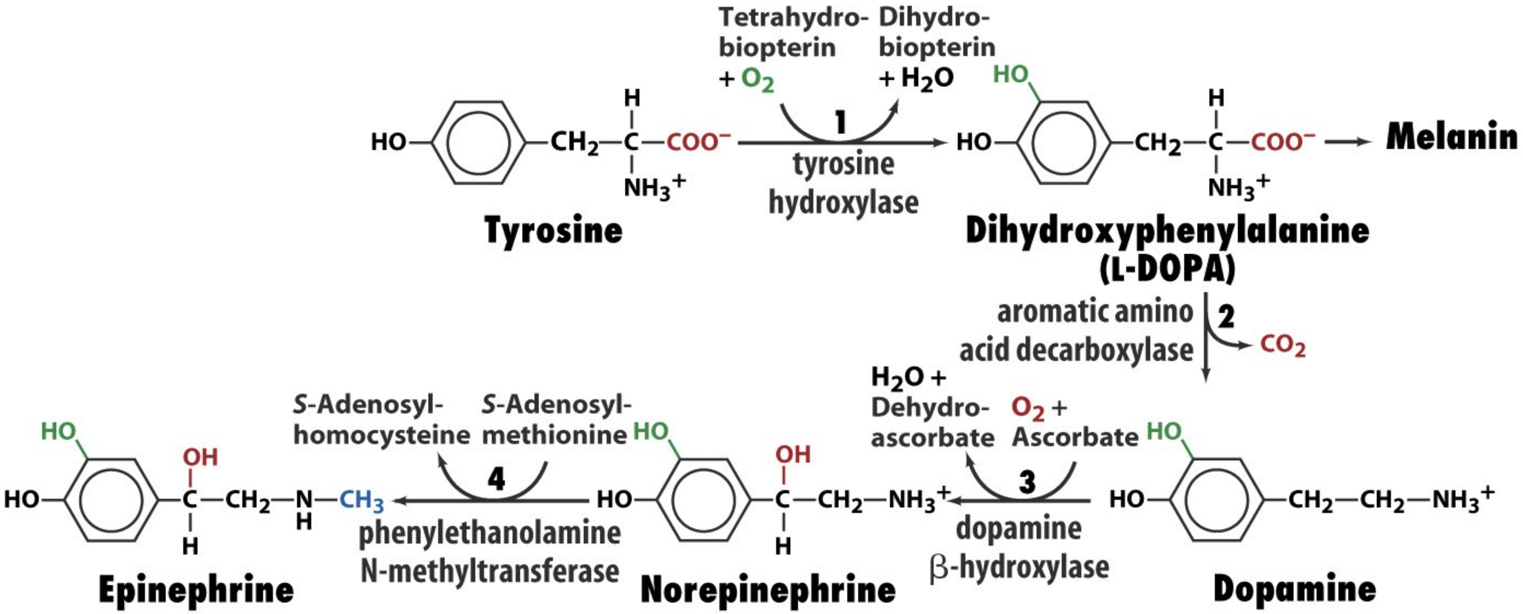


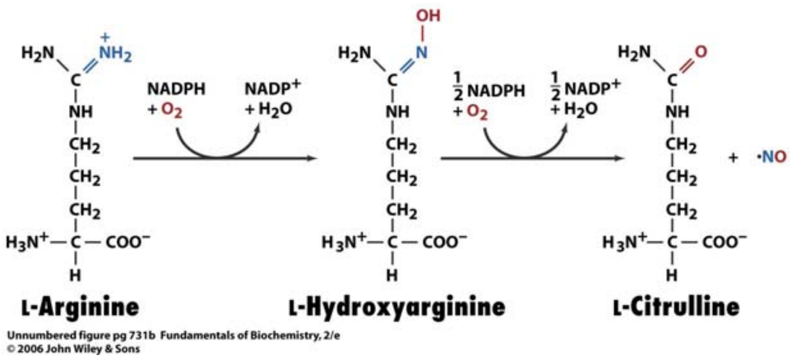
Figure 20-39 Fundamentals of Biochemistry, 2/e

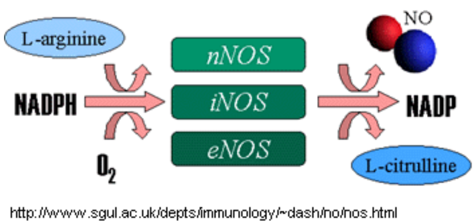
Nitric oxide

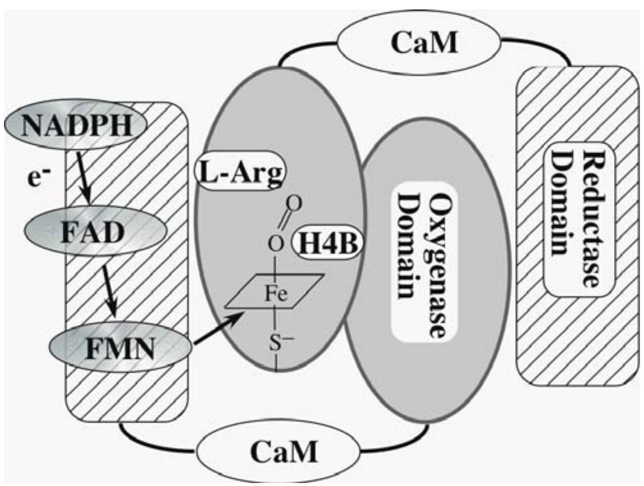
Arginine: endothelium-derived relaxing factor (EDRF)
making underlying smooth muscle relax

NOS: a homodimeric protein, 3 isoforms

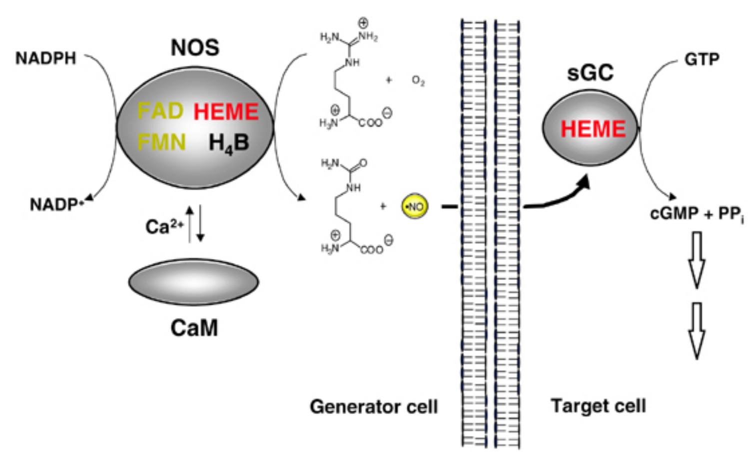
Angina pectoris
(협심증)





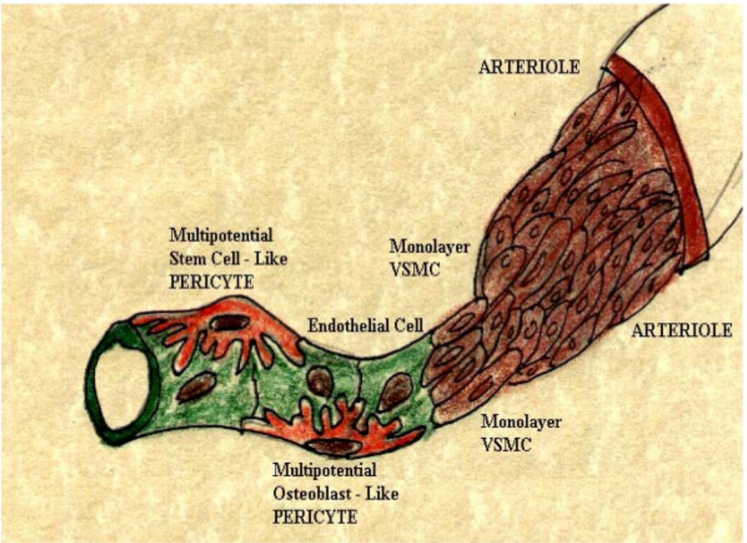


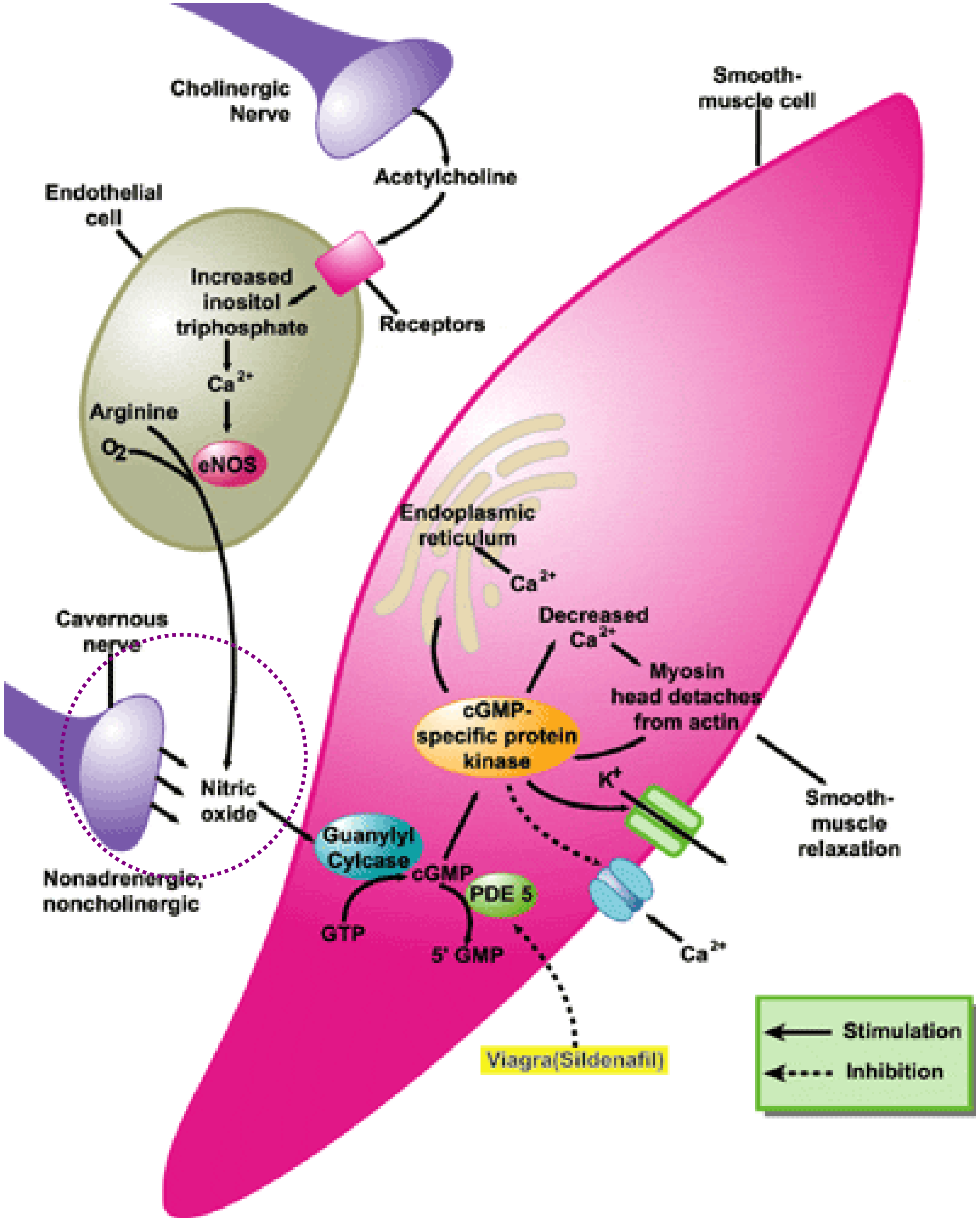
NO and cGMP



NO & cGMP

NO: half life of ~5 s, diffusion of ~1 mm

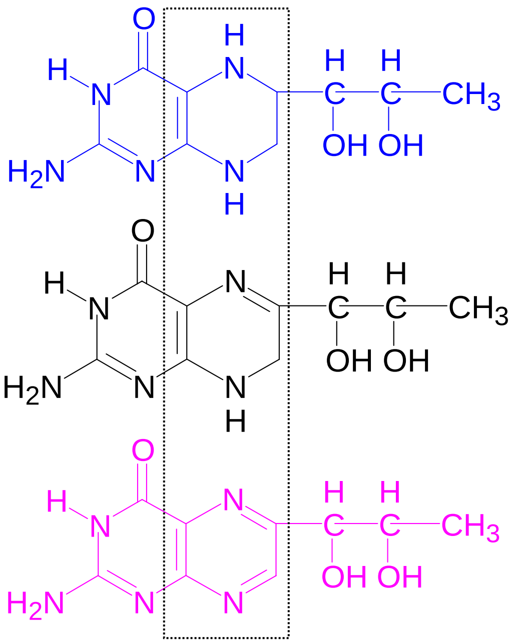




http://www.wiley.com/legacy/college/boyer/0470003790/cutting_edge/viagra/viagra.htm

Tetrahydrobiopterin (BH4)
in endothelial dysfunction

Chemical properties of BH4



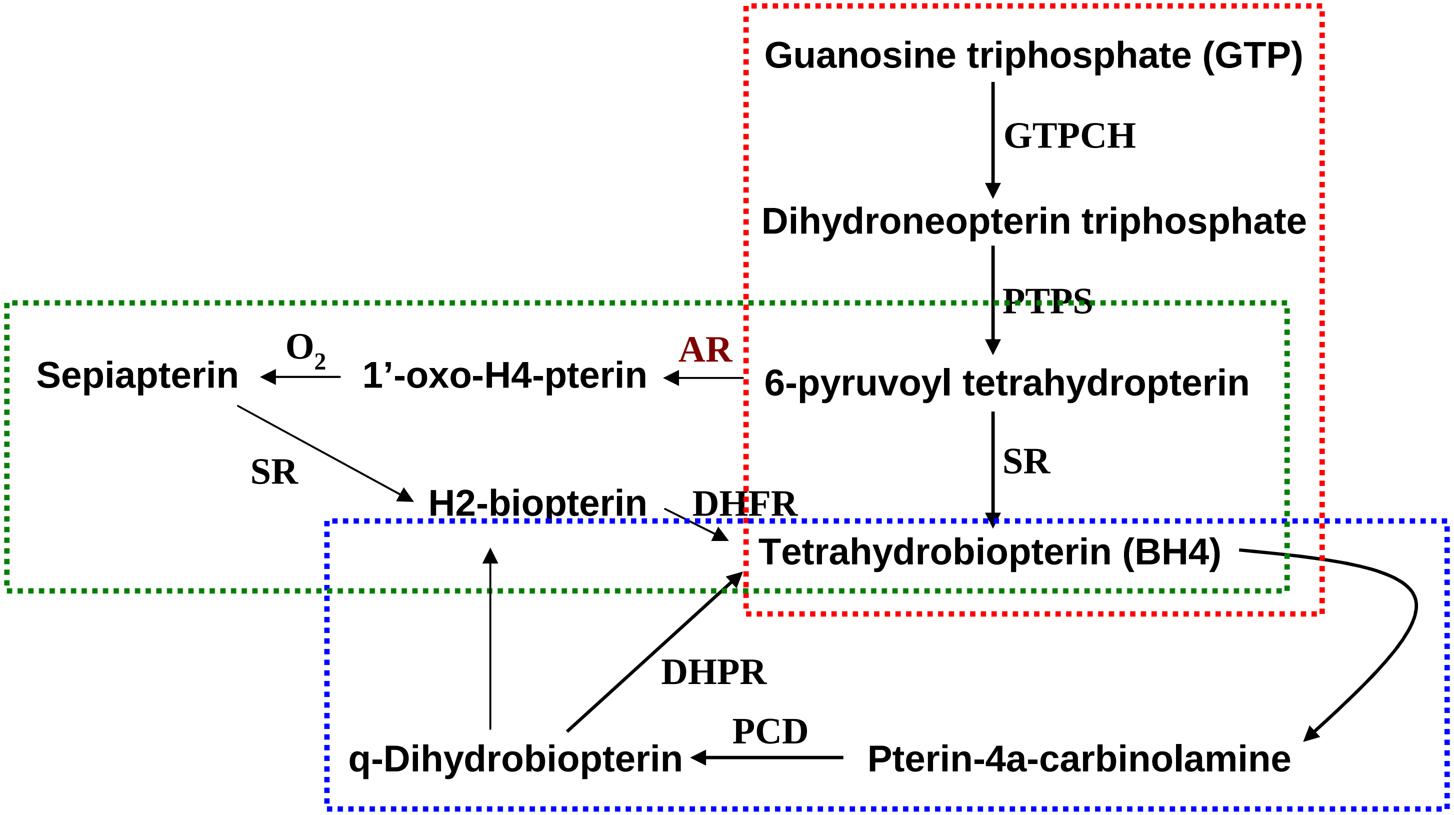
Tetrahydrobiopterin (BH4)

Dihydrobiopterin (BH2)

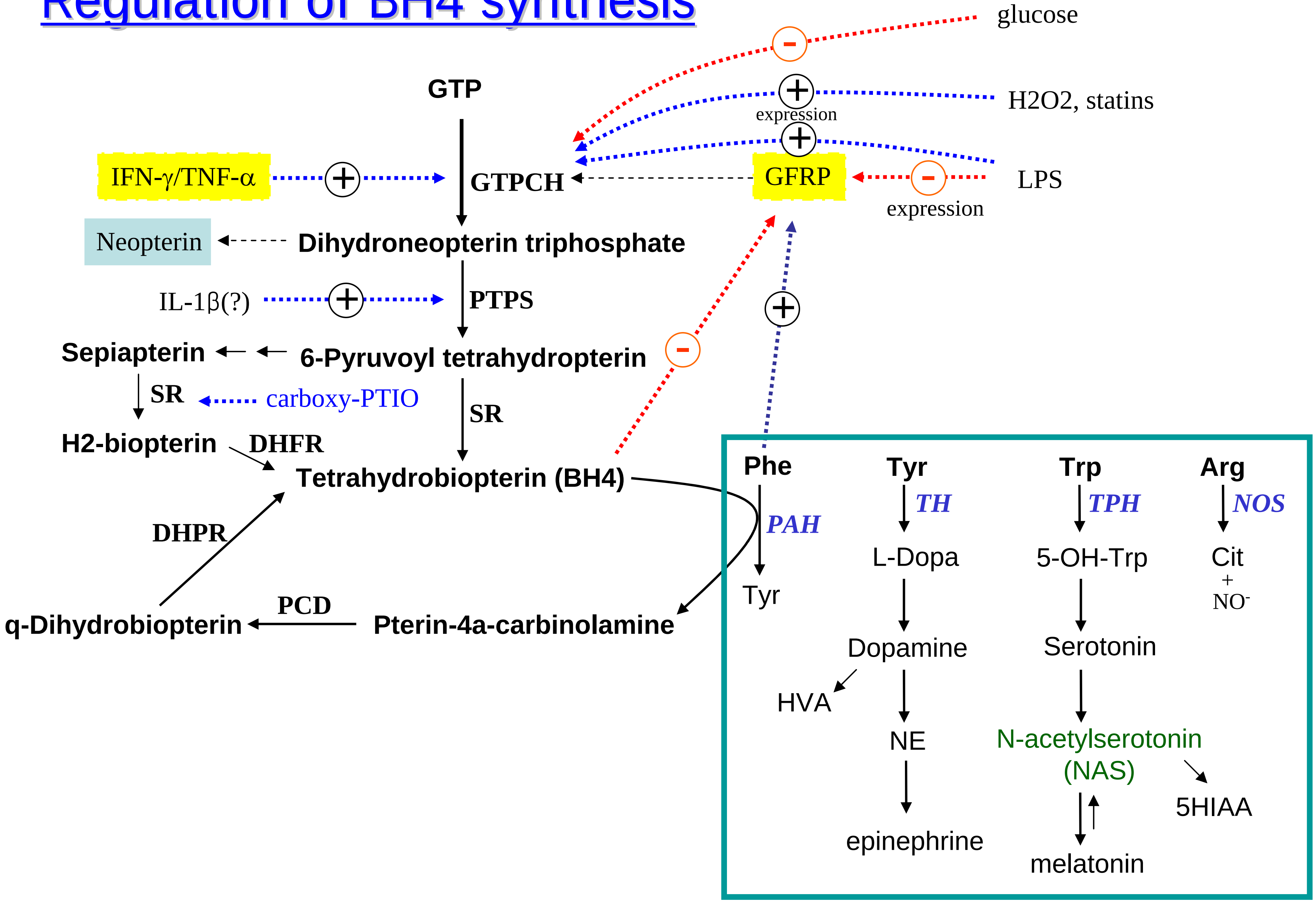
Biopterin

Biosynthesis of BH4

de novo synthesis from GTP: GTPCH, PTPS, SR
regeneration: PCD, DHPR
salvage pathway: AR, SR, DHFR



Regulation of BH4 synthesis



Human disorders related to BH4

Phenylketonuria (PKU): inborn error in phenylalanine hydroxylase NCBI OMIM

BH4 deficiency: genetic defects in BH4 biosynthesis

BH4 website, NCBI OMIM

BH4 implicated neuropsychiatric diseases

Parkinson's

Depression, Schizophrenia, Autism

Endothelial dysfunction

BH4 decrease under conditions of atherosclerosis, hypercholesterolemia, diabetes, and ischemia-reperfusion

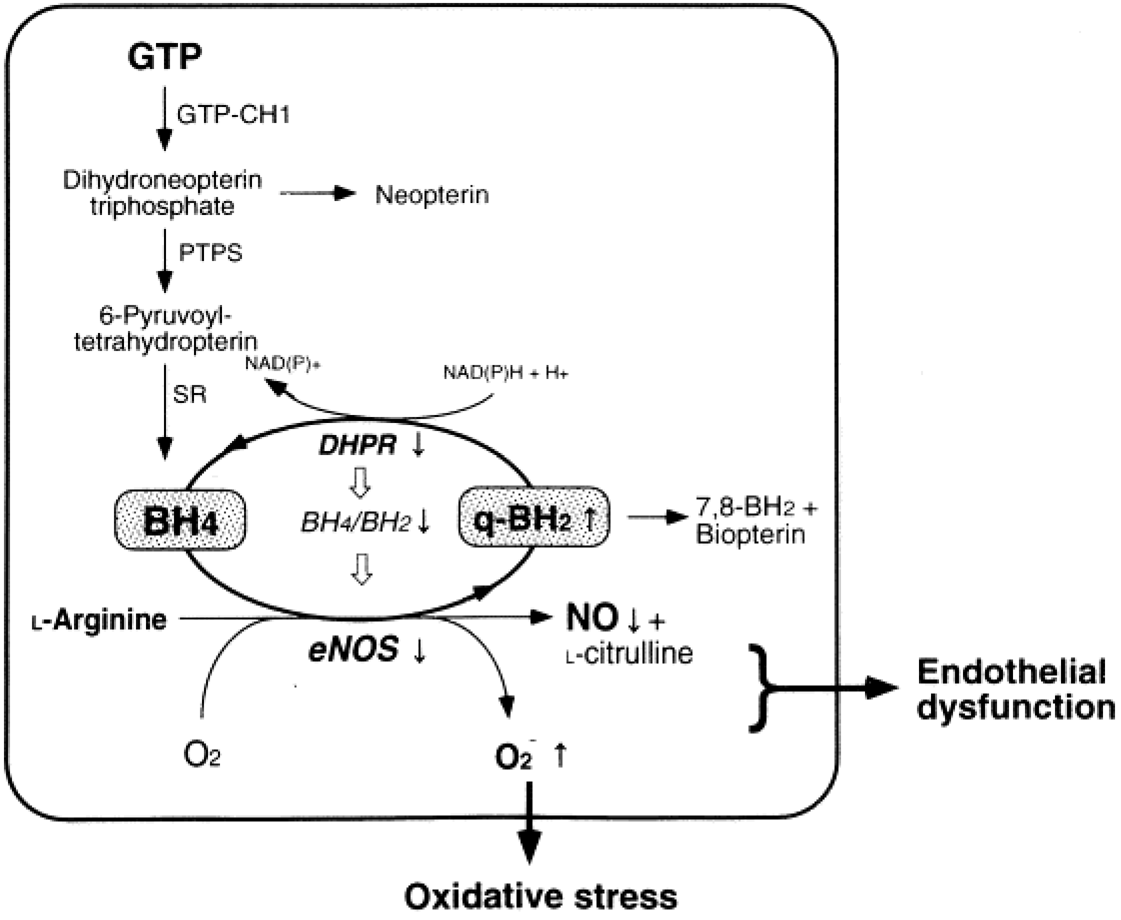
Septic shock

Increased plasma BH4 in septic: a possible therapeutic target using analogs

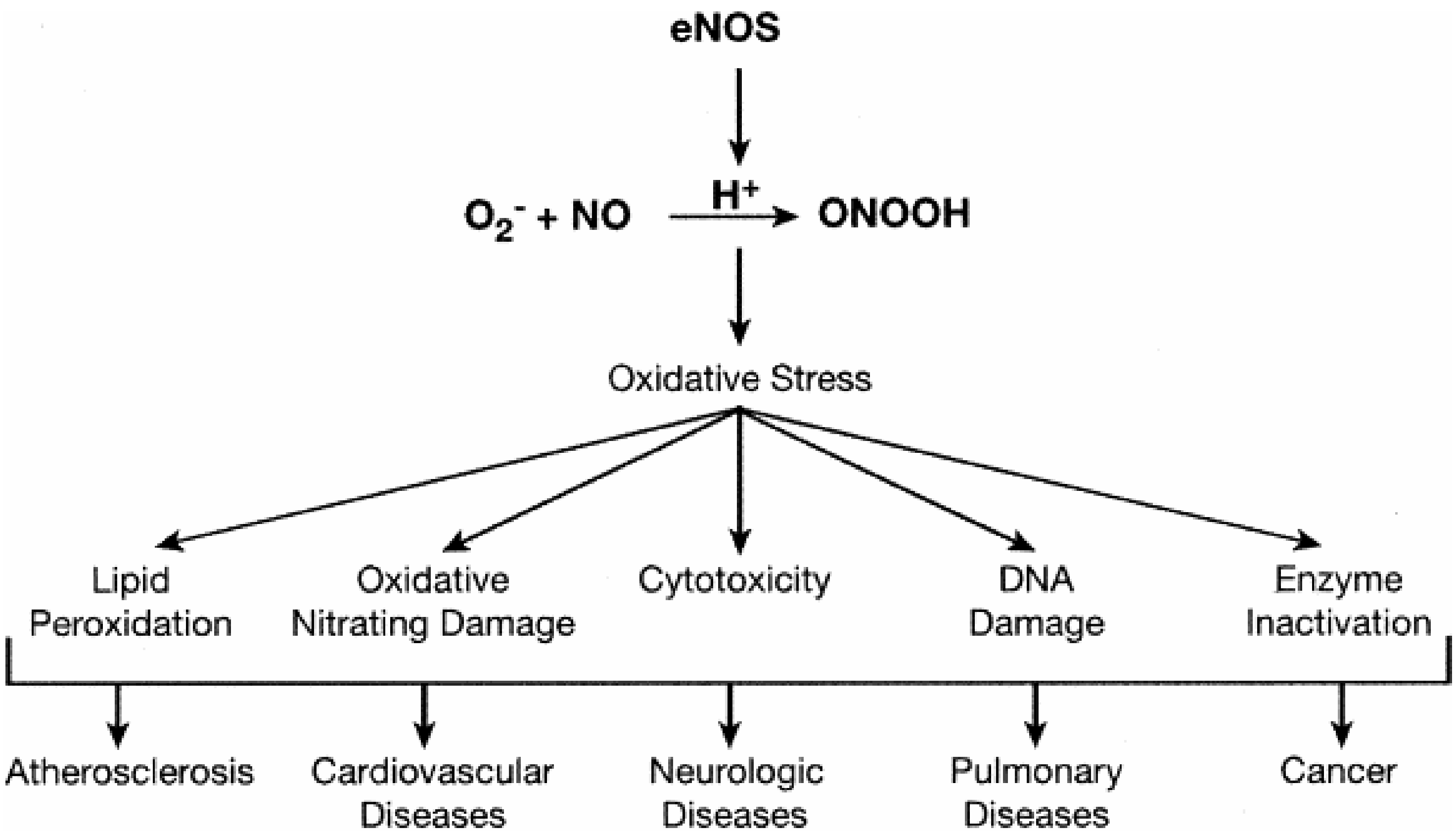
Pathophysiology. 2001 Mar;7(4):275-281

Vitiligo: DHPR inhibition by excess H_2O_2 (J Invest Dermatol 122:307 –313, 2004)

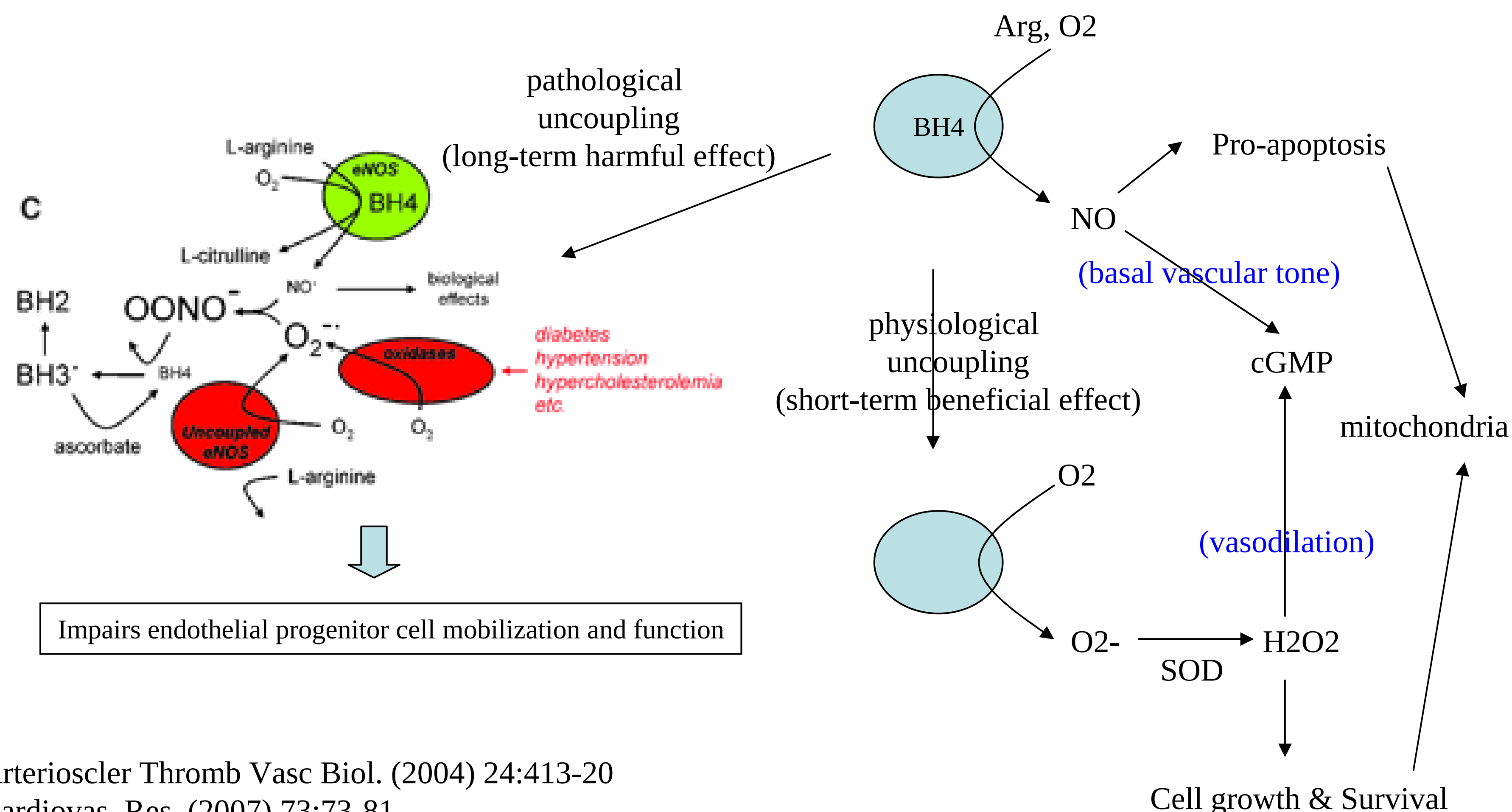
eNOS uncoupling



possible mechanism of impaired endothelial function in the insulin-resistant state
(J Am College Cardiol. 2001, 38:1821-1828)


eNOS
$O_2^- + NO \xrightarrow{H^+} ONOOH$
Oxidative Stress
Lipid Peroxidation
Oxidative Nitrating Damage
Cytotoxicity
DNA Damage
Enzyme Inactivation
Atherosclerosis
Cardiovascular Diseases
Neurologic Diseases
Pulmonary Diseases
Cancer

Role of BH4 in ED (NOS uncoupling)



Arterioscler Thromb Vasc Biol. (2004) 24:413-20
Cardiovas. Res. (2007) 73:73-81
Diabetes (2007) 56(3):666-74