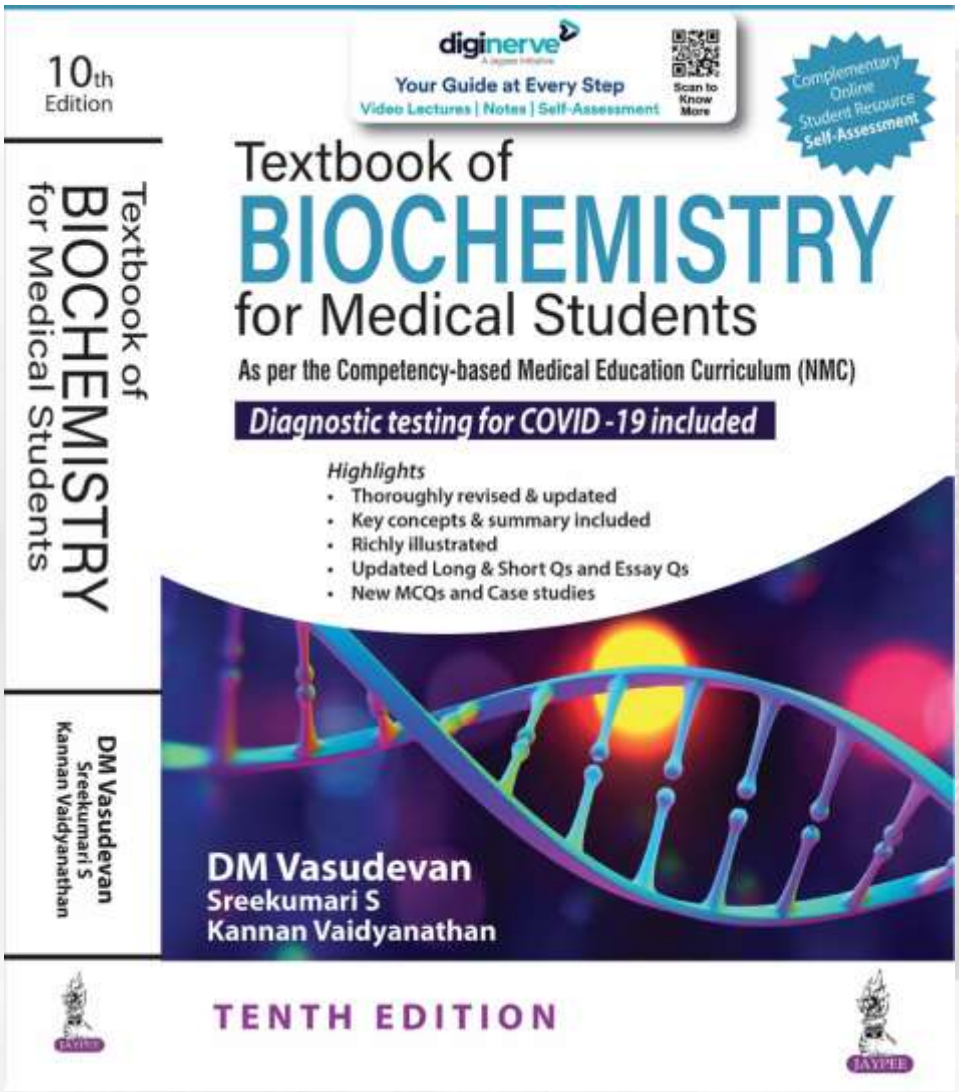


Chapter 16B:

Metabolism of Aliphatic Amino Acids (Glu, Asp, Lys, Arg, NO, Val, Leu, Ile)

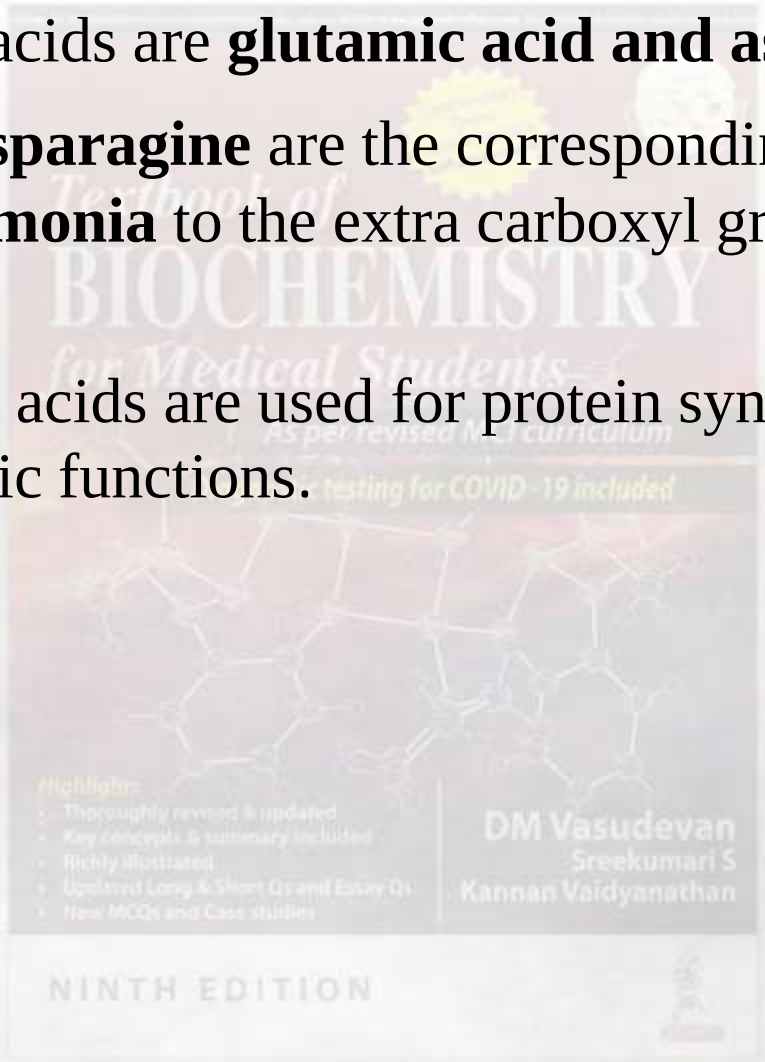
Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

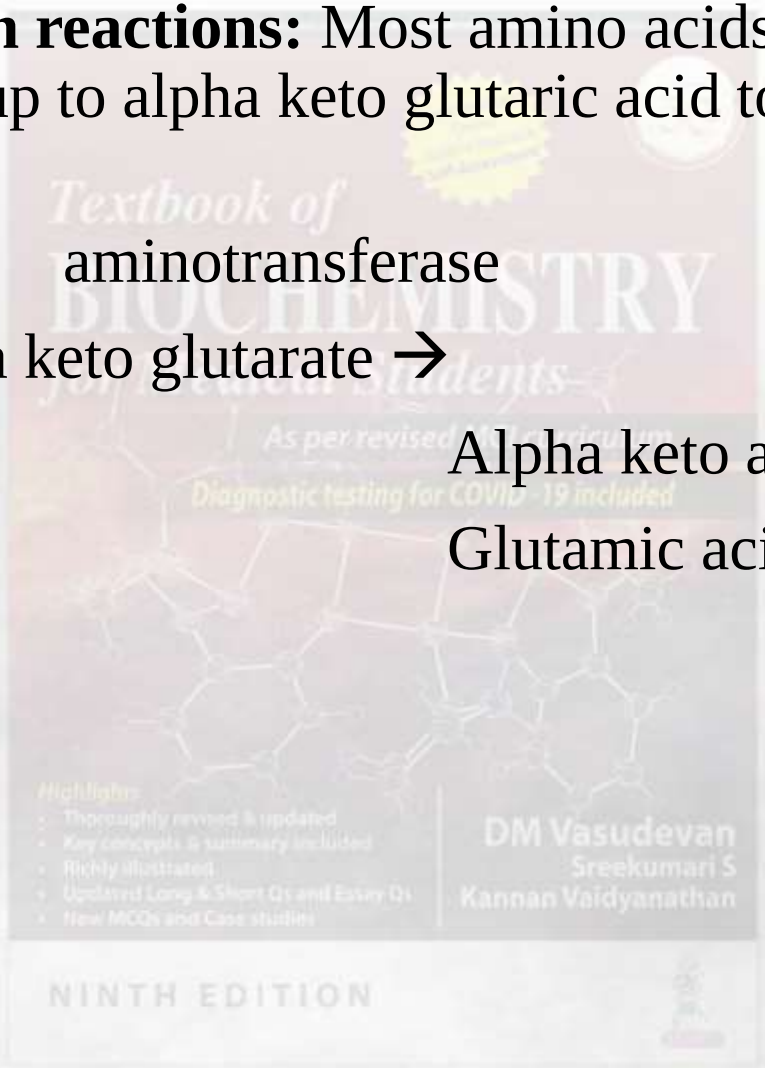
Acidic Amino Acids

- The acidic amino acids are **glutamic acid and aspartic acid.**
- **Glutamine and asparagine** are the corresponding **amides** formed by addition of **ammonia** to the extra carboxyl group to form an amide.
- All the four amino acids are used for protein synthesis and serve important metabolic functions.



GLUTAMIC ACID (GLU) (E)

1. **Transamination reactions:** Most amino acids transfer their amino group to alpha keto glutaric acid to form glutamic acid.



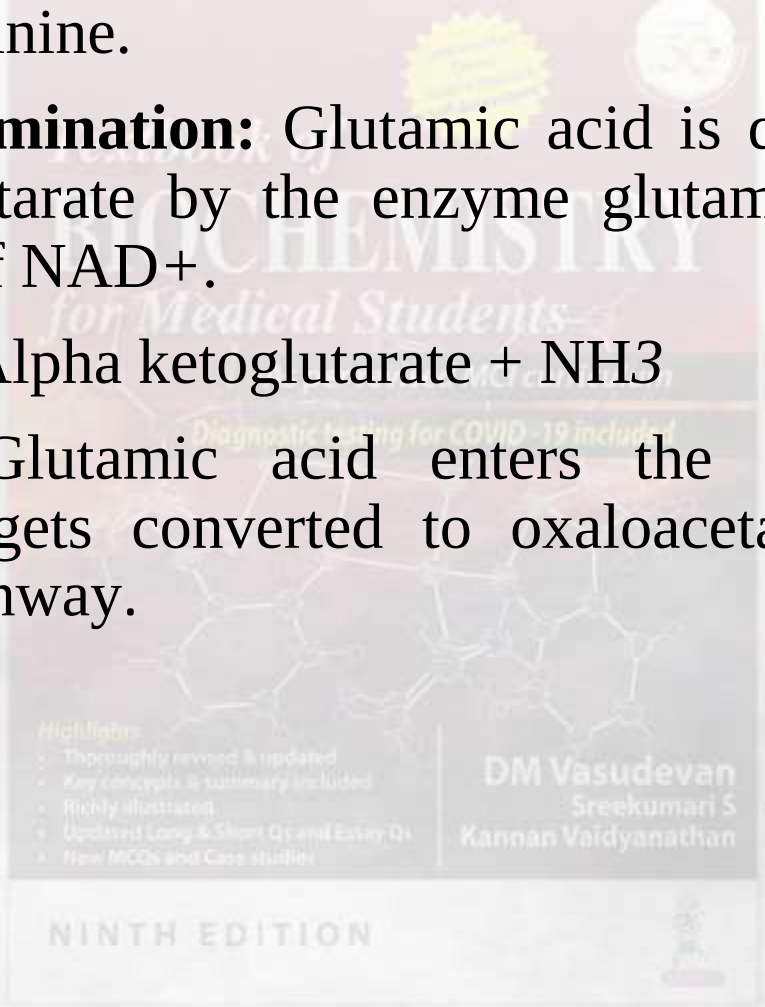
aminotransferase

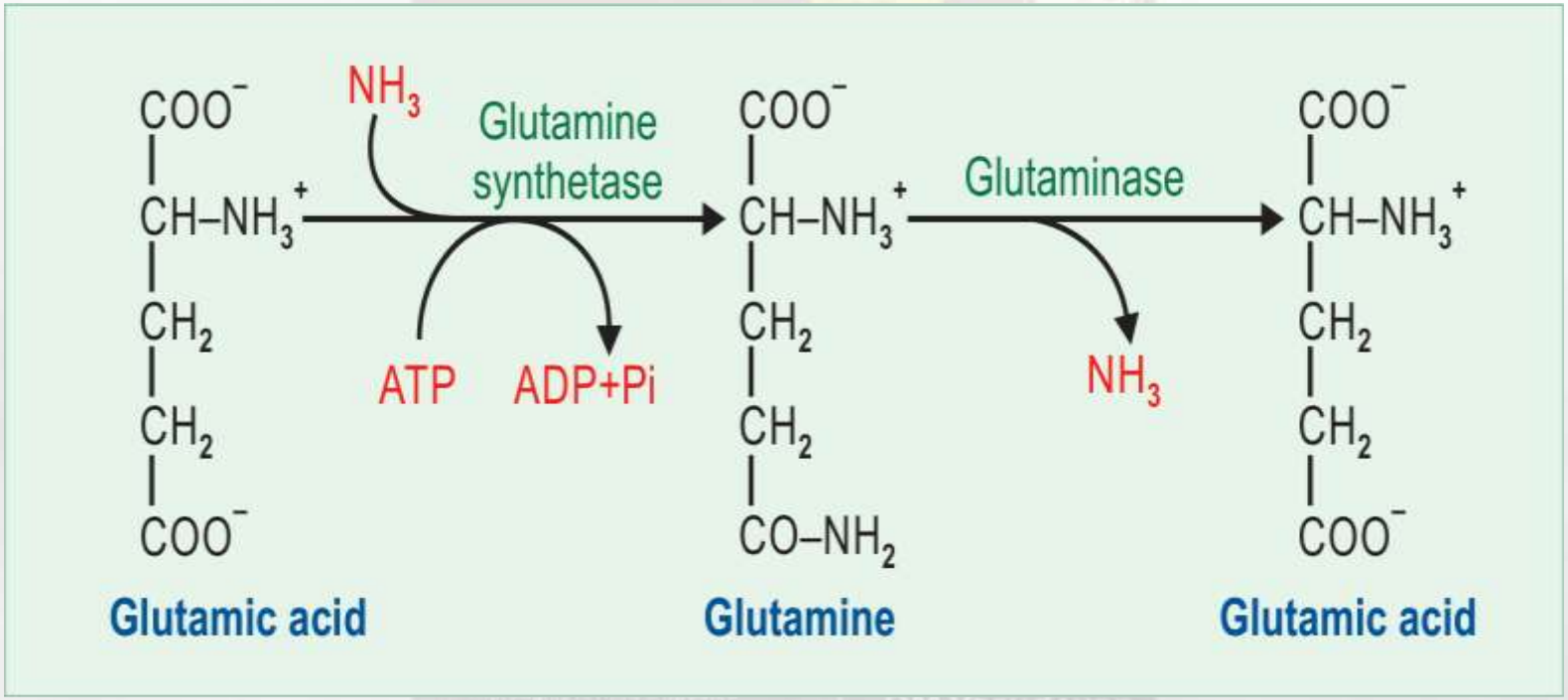
Amino acid + Alpha keto glutarate → Alpha keto acid + Glutamic acid

2. Glutamic acid is also formed during the metabolism of histidine, proline and arginine.
3. **Oxidative deamination:** Glutamic acid is deaminated to form alpha keto glutarate by the enzyme glutamate dehydrogenase with the help of NAD+.

Glutamic acid -→Alpha ketoglutarate + NH3

4. **Glucogenic:** Glutamic acid enters the TCA cycle as α ketoglutarate, gets converted to oxaloacetate and enters the **glucogenic** pathway.




NH3
Glutamine synthetase
ATP
ADP+Pi
Glutaminase
NH3
Glutamic acid
Glutamine
Glutamic acid

5. **N-acetyl glutamate** (NAG) is a positive modifier of carbamoyl phosphate synthetase-I in the mitochondria.

Glutamic acid + Acetyl-CoA

→ NAG + CoASH

6. **Gamma carboxy glutamic acid** (GCGA) is present in prothrombin.

- The gamma carboxyl group is added as a post-translational modification, which needs vitamin K

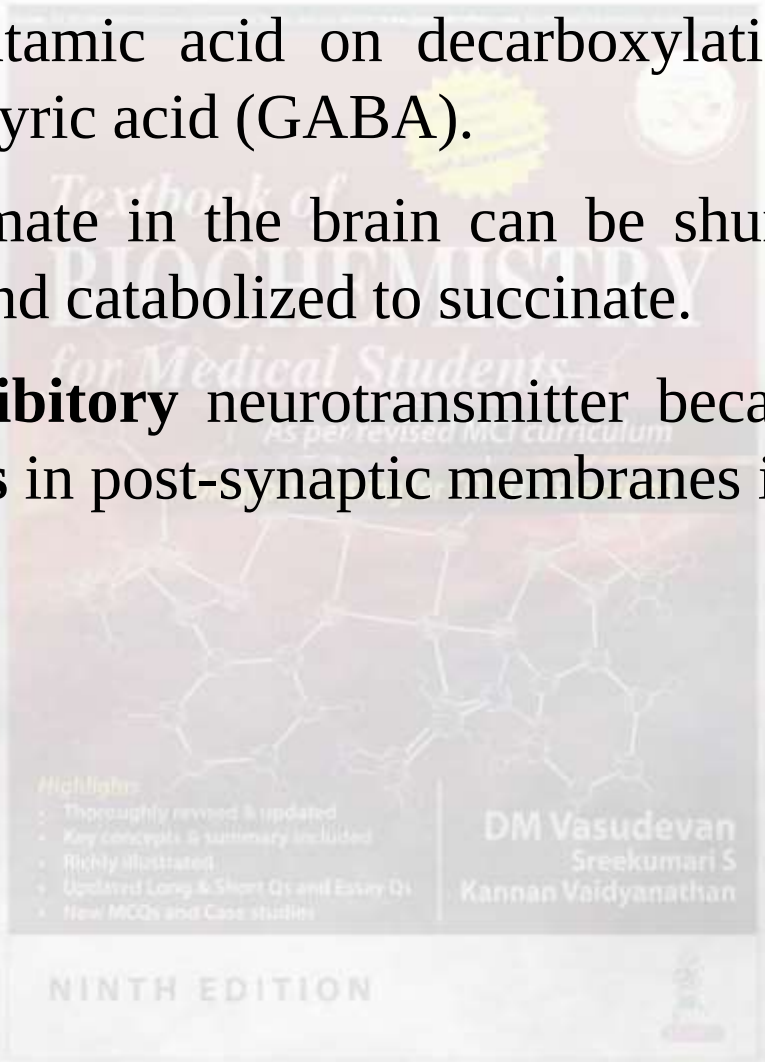
8. **Excitatory neurotransmitter**. Neurons contain NMDA (N-methyl-D-aspartate) receptor.

- Stimulation of NMDA receptors by glutamate opens calcium channel, leading to stimulation of NOS (nitric oxide synthase).
- This in turn, results in transient production of NO• (**nitric oxide**).
- This raises the cellular level of cyclic GMP and neurons are excited.

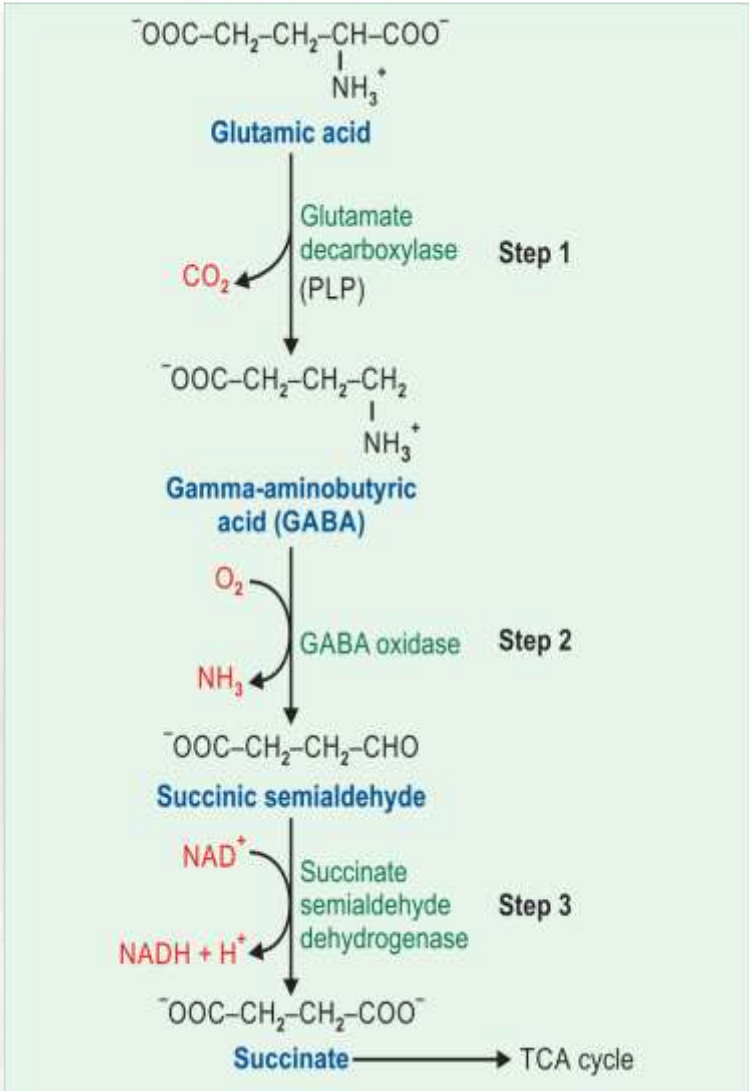
9. **Glutathione:** Glutamate is a constituent of the tripeptide glutathione.
10. Glutamic acid is decarboxylated to GABA.

Gamma-Amino Butyric Acid (GABA)

- **Metabolism:** Glutamic acid on decarboxylation gives rise to gamma-amino butyric acid (GABA).
- Part of the glutamate in the brain can be shunted through the GABA pathway and catabolized to succinate.
- **GABA is an inhibitory** neurotransmitter because it opens the **chloride channels** in post-synaptic membranes in CNS.



- **Pyridoxal phosphate:** Both the formation and catabolism of GABA requires pyridoxal phosphate as coenzyme.
- Therefore, in pyridoxine deficiency, GABA formation is reduced.
- Since GABA is an inhibitory transmitter, a low level of GABA or deficiency of pyridoxal phosphate would lead to **convulsions**.
- Sodium **valproate** which inhibits GABA oxidase is used in the treatment of epilepsy.
- Congenital **deficiencies** of GABA amino transferase and succinic semialdehyde dehydrogenase (leading to hydroxy butyric aciduria) are reported, but are very rare.


$^-OOC–CH_2–CH_2–CH–COO^-$
NH_3^+
Glutamic acid
Glutamate decarboxylase (PLP)
CO_2
Step 1
$^-OOC–CH_2–CH_2–CH_2$
NH_3^+
Gamma-aminobutyric acid (GABA)
O_2
NH_3
GABA oxidase
Step 2
$^-OOC–CH_2–CH_2–CHO$
Succinic semialdehyde
NAD^+
$NADH + H^+$
Succinate semialdehyde dehydrogenase
Step 3
$^-OOC–CH_2–CH_2–COO^-$
Succinate
TCA cycle

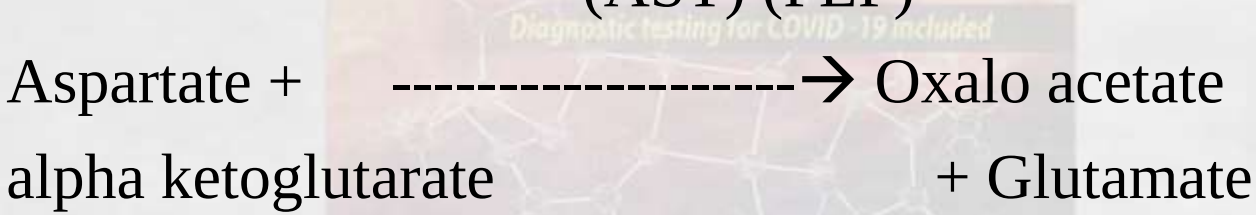
GLUTAMINE (GLN) (Q)

- It is a **glucogenic** amino acid.
- It is synthesized from glutamic acid.
- The amidation of glutamic acid to glutamine is catalyzed by **glutamine synthetase**.
- Glutamic acid can react with a molecule of NH*3* in presence of ATP.
- This reaction is important in ammonia trapping in brain as well as for transport of ammonia in a nontoxic form.
- Glutamine is hydrolyzed to glutamate and NH*3* by the enzyme glutaminase.
- This reaction is seen in the renal tubular cells.
- This **ammonia reacts with H+** to form NH4+ to excrete hydrogen ions in urine.

- Major fate of glutamine is to be hydrolyzed to glutamate and NH3.
- Glutamic acid is then deaminated to alpha ketoglutarate and enters TCA cycle for further catabolism.
- The N atoms **3 and 9 of purines** are derived from glutamine.
- Glutamine is the source of **3rd N of pyrimidine**.
- Glutamine is the source of NH2 group of **guanine** and **cytosine**.
- Glutamine is a **conjugating agent**, e.g. production of phenyl acetyl glutamine.
- Glutamine donates the amino group of amino sugars and amide group of nicotinamide.

ASPARTIC ACID (ASP) (D)

- It is a non-essential, **glucogenic** amino acid.
- Aspartate, on transamination gives rise to oxaloacetate which initiates the TCA cycle.
- Aspartate amino transferase (AST) transfers the amino group of aspartate to alpha ketoglutarate to form oxaloacetate.

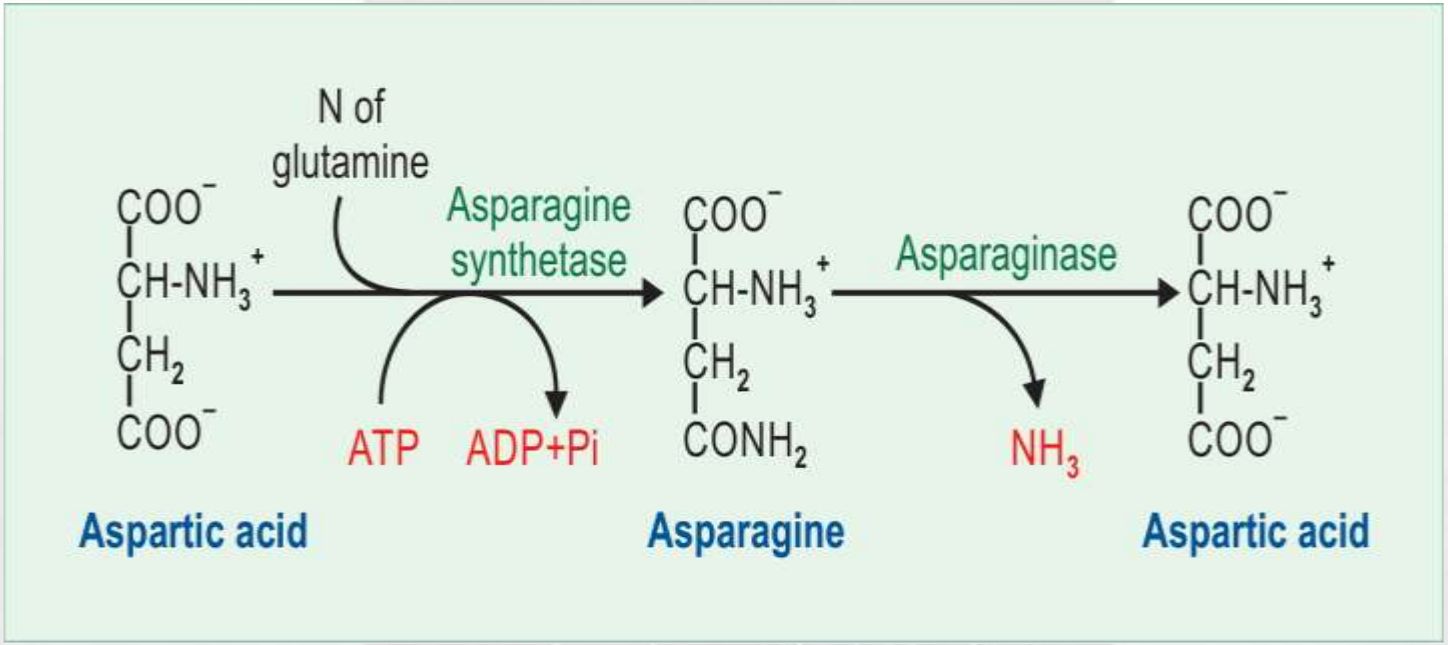


- AST is increased in cardiac ischemia and in hepatic diseases.
- **Malate aspartate shuttle** transfers the cytoplasmic NADH into mitochondria for oxidation in the electron transport chain.
- Aspartic acid is an important member of **urea cycle**.
- It directly contributes its **alpha amino group** to the urea molecule.
- The carbon skeleton of aspartate can also enter the glucogenic pathway as fumarate.

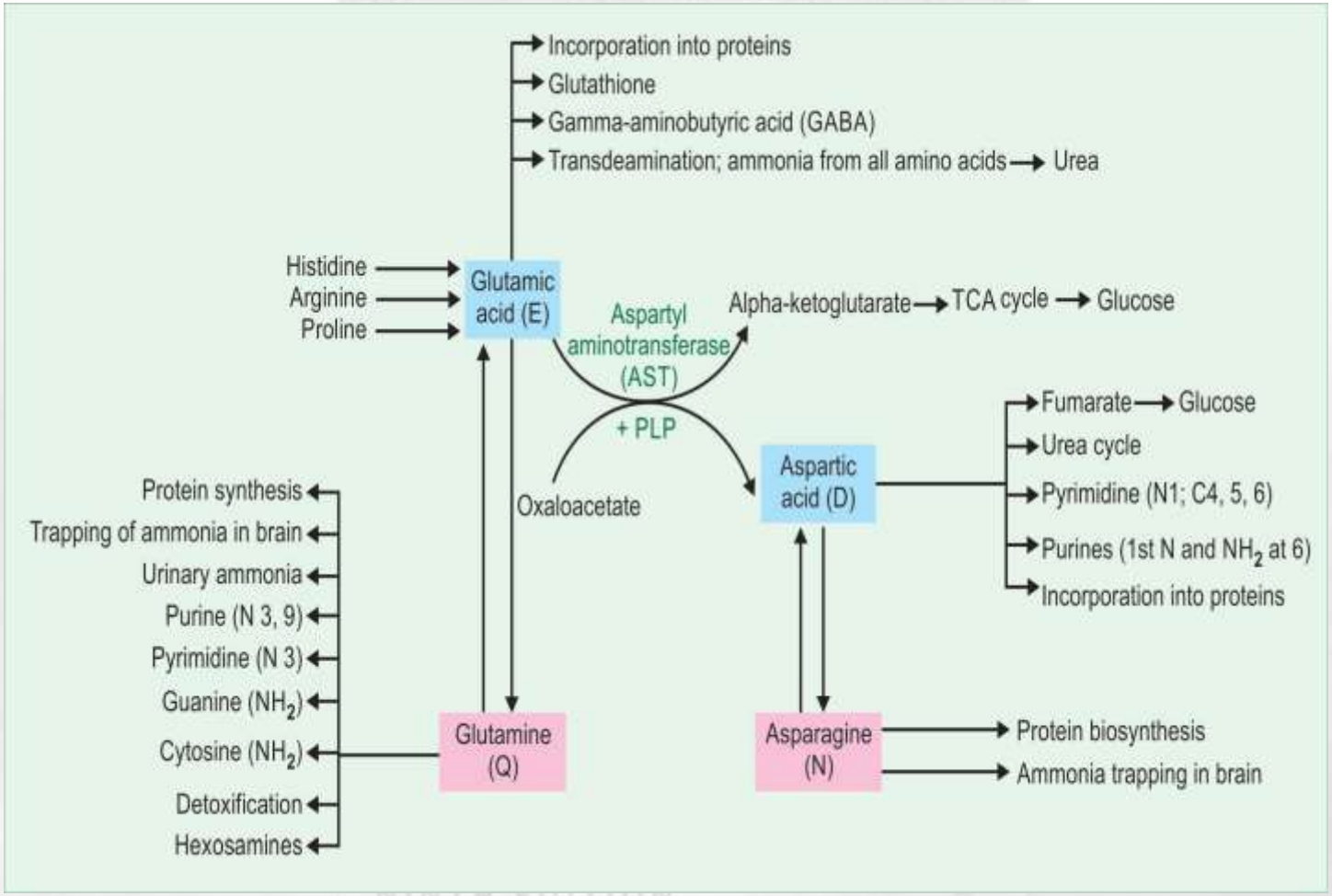
Citrulline + Aspartate → Arginine + Fumarate

ASPARAGINE (ASN) (N)

- Aspartate reacts with ammonia to form asparagine.
- This is a reaction similar to formation of glutamine.
- Asparagine can be hydrolyzed to aspartate and NH3 by asparaginase.
- L **asparaginase** is an anticancer drug against leukemias and lymphomas, because those cells cannot synthesize asparagine; the enzyme will destroy the available asparagine in the blood; so the cancer cells will die.
- Asparagine is a **glucogenic** amino acid.



Asparagine synthesis and breakdown.


Incorporation into proteins
Glutathione
Gamma-aminobutyric acid (GABA)
Transdeamination; ammonia from all amino acids → Urea
Histidine
Arginine
Proline
Glutamic acid (E)
Aspartyl aminotransferase (AST)
+ PLP
Alpha-ketoglutarate → TCA cycle → Glucose
Oxaloacetate
Aspartic acid (D)
Fumarate → Glucose
Urea cycle
Pyrimidine (N1; C4, 5, 6)
Purines (1st N and NH2 at 6)
Incorporation into proteins
Protein synthesis
Trapping of ammonia in brain
Urinary ammonia
Purine (N 3, 9)
Pyrimidine (N 3)
Guanine (NH2)
Cytosine (NH2)
Detoxification
Hexosamines
Glutamine (Q)
Asparagine (N)
Protein biosynthesis
Ammonia trapping in brain

Basic Amino Acids Lysine (Lys) K)

- Lysine is an essential basic amino acid.
- It is **deficient in cereals**.
- It does not undergo transamination.
- Lysine is predominantly **ketogenic**.
- In the catabolic pathway, lysine is converted to saccharopine, which finally enters into the pathway of odd numbered fatty acids.
- **Hyperlysinemias** result from congenital deficiency of any of the enzymes of the above pathway.
- Mental retardation and cortical degeneration are seen in these conditions.

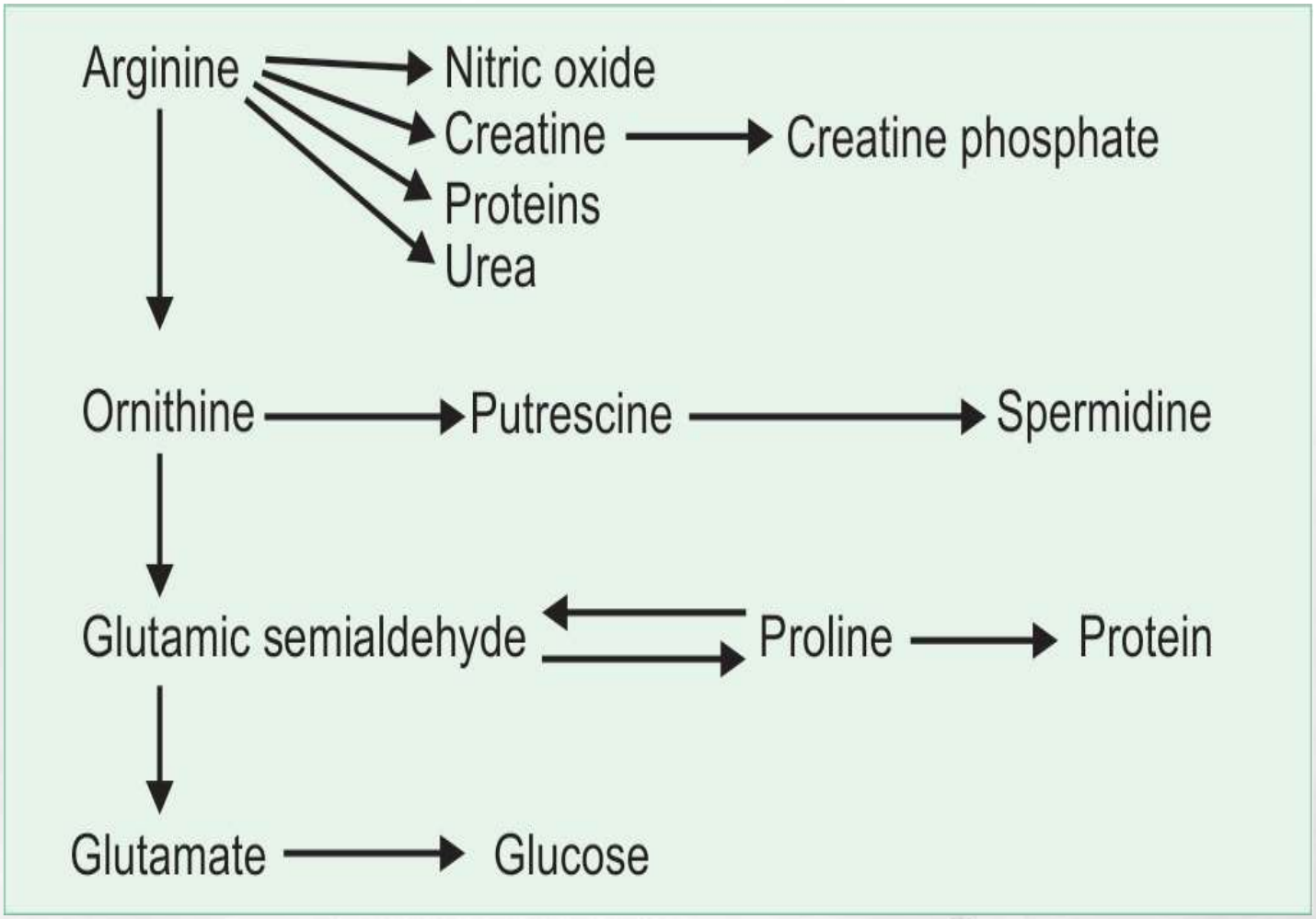
- Lysine serves the following functions:

a. Lysine and **hydroxylysine** residues of collagen and elastin are important in crosslinking.

b. The epsilon amino group of lysine can form Schiff bases, thus **linking to proteins**, e.g. Pyridoxal phosphate with transaminases.

c. Lysine is the precursor of **carnitine**.

d. Bacterial putrification (decarboxylation) of lysine in the intestine gives rise to **cadaverine**.



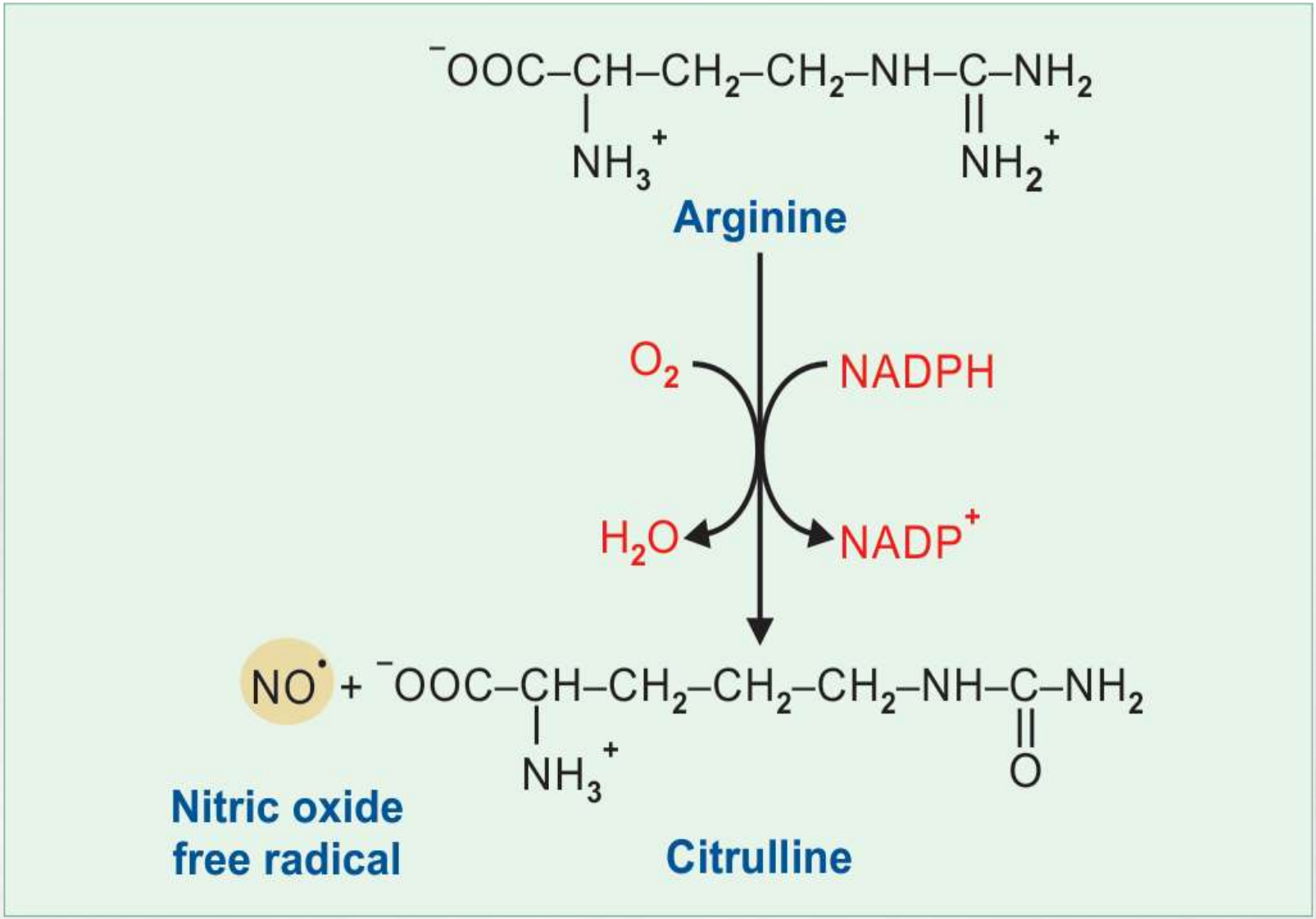
Metabolism of arginine and ornithine

ARGININE (ARG) (R)

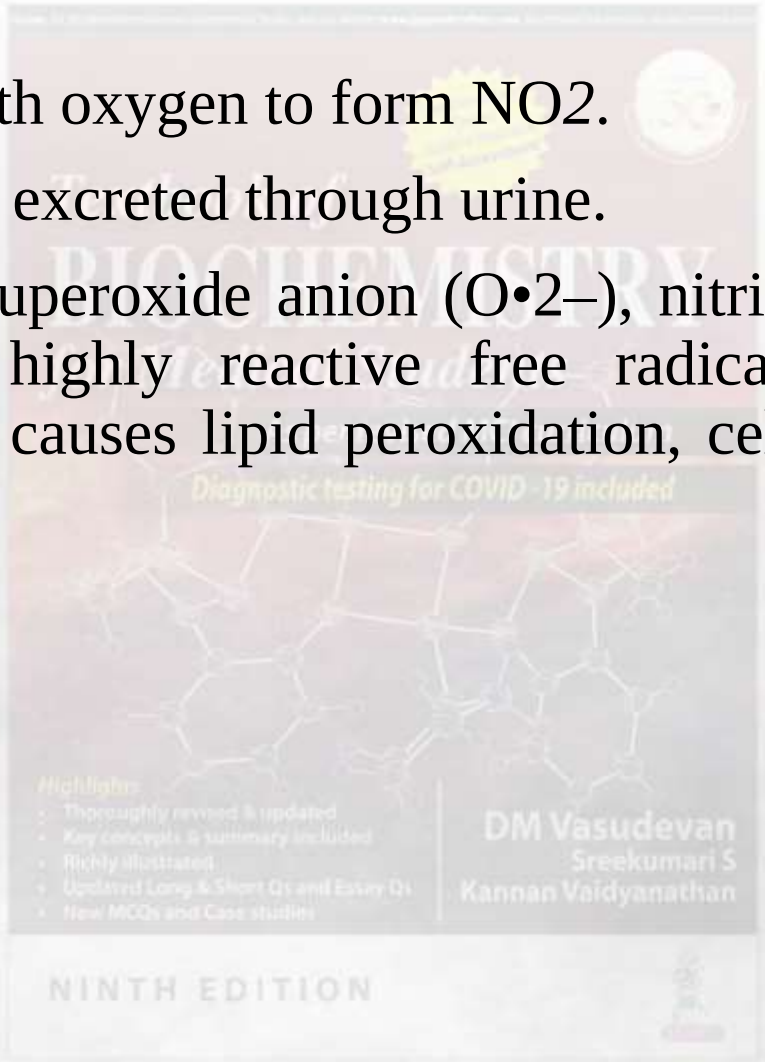
- Arginine contains guanidinium group.
- It is a highly basic, **semi-essential** amino acid.
- Arginine is **glucogenic**.
- In the urea cycle, where arginase splits arginine into urea and **ornithine.**
- Arginine is necessary for the synthesis of creatine.
- Arginine is the precursor of **nitric oxide** which is an important signal molecule in the body.

NITRIC OXIDE (NO˙)

- It is a toxic pollutant of air and automobile exhausts.
- But now it is shown to possess more potential biological functions than any other known molecule.
- The vasodilatory effect of nitroglycerine is due to the release of NO˙.
- "Endothelium derived relaxing factor" (EDRF) is required for arterial dilatation.
- EDRF is chemically NO˙.

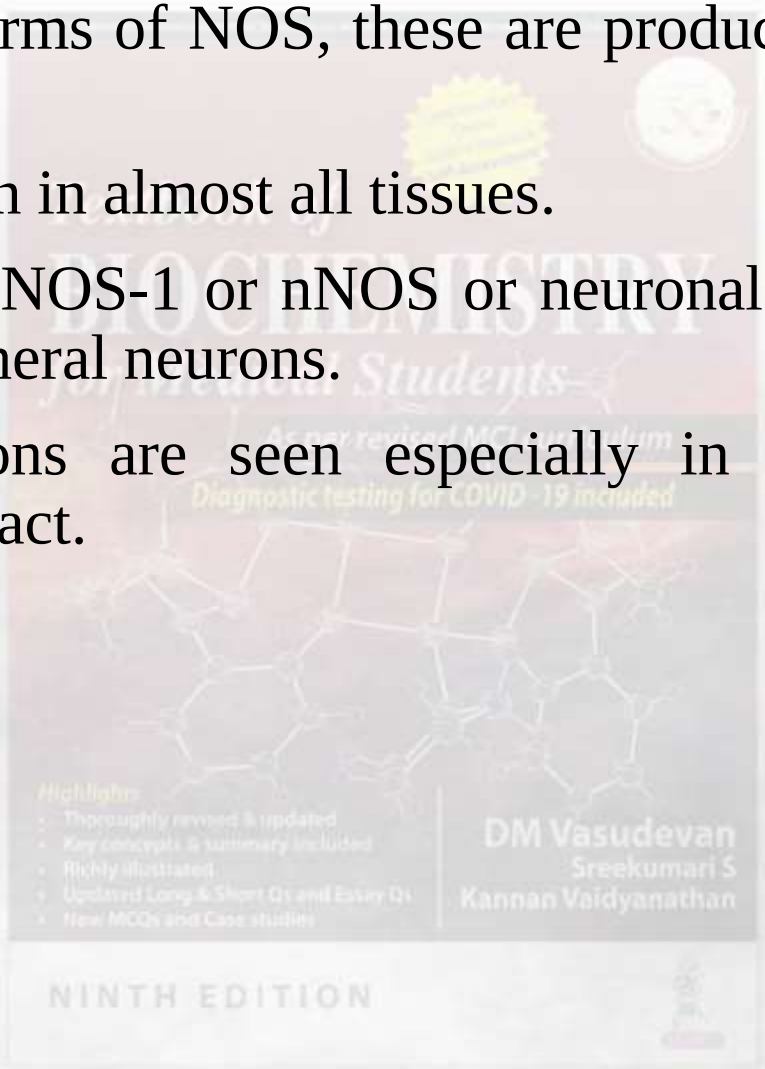

Arginine
O2
NADPH
H2O
NADP+
NO•
Nitric oxide free radical
Citrulline

- **Metabolic Fate**
- NO• combines with oxygen to form NO2.
- These **nitrites** are excreted through urine.
- On exposure to superoxide anion (O•2–), nitric oxide (NO•) is converted to a highly reactive free radical, **peroxynitrite** (OONO•), which causes lipid peroxidation, cell injury and cell death.



ISO-Enzymes of NOS

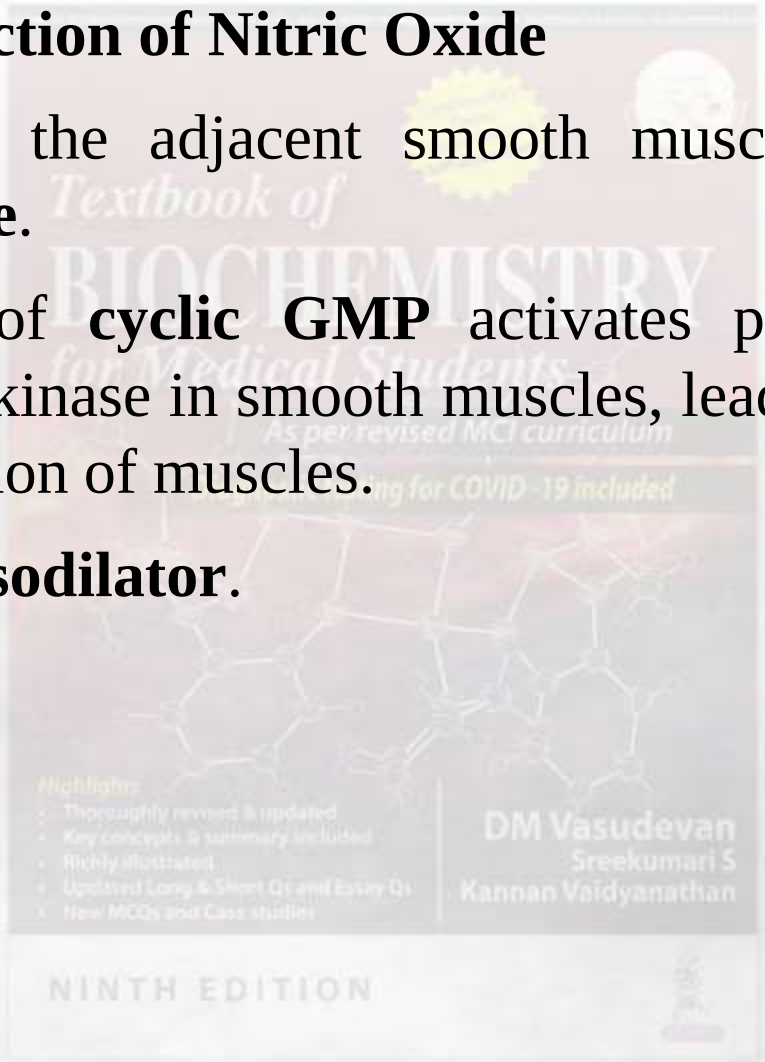
- There are 3 isoforms of NOS, these are products of 3 different genes.
- All forms are seen in almost all tissues.
- **Neuronal NOS**: NOS-1 or nNOS or neuronal NOS is seen in central and peripheral neurons.
- Nitrogenic neurons are seen especially in cerebellum and gastrointestinal tract.



Iso-enzymes of NOS Continuued

- **Macrophage NOS**: NOS-2 or iNOS or inducible NOS or macrophage NOS is mainly seen in macrophages and neutrophils.
- It is induced by cytokines (interleukin-1 and tumor necrosis factor).
- **Endothelial NOS**: NOS-3 or eNOS or endothelial NOS is seen in endothelial cells, platelets, endocardium and myocardium.
- In these sites, the NO• is constantly produced and released, so as to have arterial relaxation.

- **Mechanism of Action of Nitric Oxide**
- NO• diffuses to the adjacent smooth muscle and activates **guanylate cyclase**.
- Increased level of **cyclic GMP** activates protein kinase in smooth muscles, kinase in smooth muscles, leading to relaxation leading to relaxation of muscles.
- Thus NO• is a **vasodilator**.



Physiological Actions of Nitric Oxide

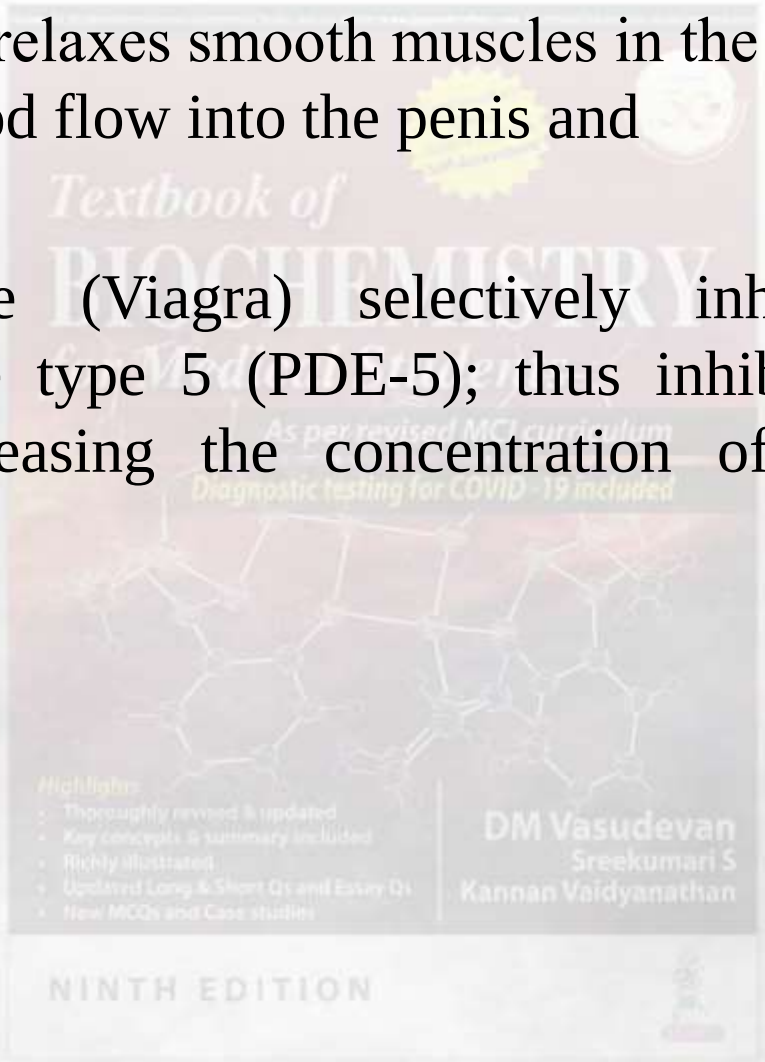
- **Blood vessels:** NO• is a potent vasodilator.
- The normal blood pressure is maintained by the NO• liberated by endothelial NOS (NOSe).
- NO• causes cerebral, coronary, renal and muscle arteries to dilate.
- A deficiency of NO˙ is associated with hypertension.
- Excessive production of NO• results in refractory hypotension, which may be seen in patients with septicemic shock.
- **Central nervous system:** In CNS, NOSn isoform is present.
- NO• stimulates the releasing hormones (CRH, GHRH and LHRH).

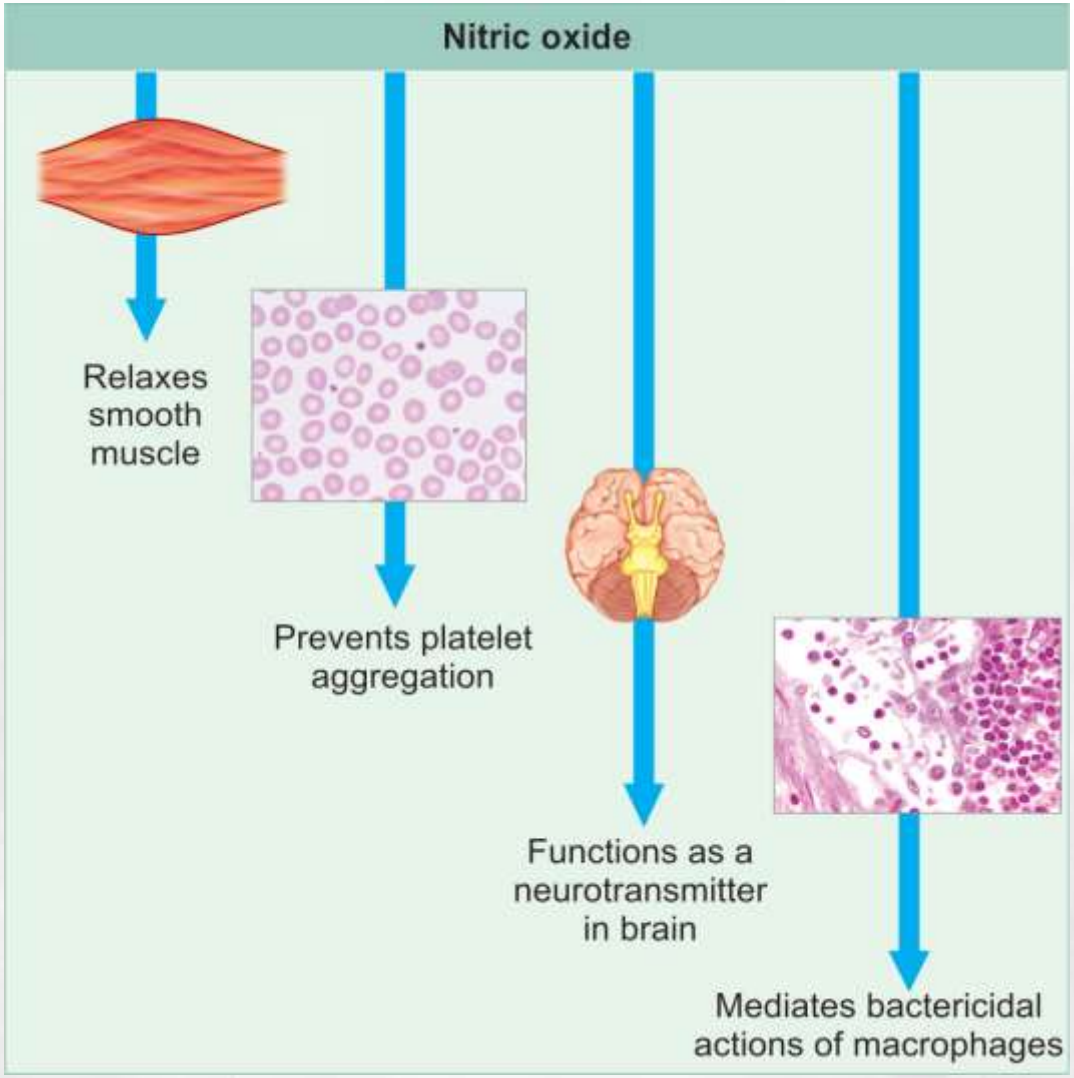
- **Macrophages:** Macrophages contain the isoform NOSi (i stands for inducible).
- This enzyme produces $NO^{\cdot}$ and peroxy nitrite; which are lethal to **micro-organisms**.
- NO• production in macrophage is induced by interleukin and tumor necrosis factor.
- **Platelets:** NO• inhibits adhesion of platelets and so depresses platelet functions.
- **Intestinal system:** NO• is a non-adrenergic and noncholinergic (NANC) neurotransmitter, especially in gastrointestinal tract and urogenital tract.
- It relaxes smooth muscles and leads to reduced gastrointestinal motility and relaxation of sphincters.

Nitric Oxide in Diseases and Treatment

- **Angina pectoris:** Nitroprusside can directly release NO•.
- Nitroglycerin (glyceryl trinitrite) requires glutathione to produce NO•.
- These will dilate coronary arteries, and are beneficial in treating angina pectoris.
- **Pulmonary hypertension:** Inhalation of NO• is useful in the treatment of pulmonary hypertension and high altitude pulmonary edema.
- NO• produces pulmonary vasodilatation, without lowering systemic blood pressure

- **Impotence:** NO• relaxes smooth muscles in the corpus cavernosum and increases blood flow into the penis and makes it erect.
- **Sildenafil** citrate (Viagra) selectively inhibits the specific phosphodiesterase type 5 (PDE-5); thus inhibiting hydrolysis of cGMP, and increasing the concentration of cGMP in corpus cavernosum.

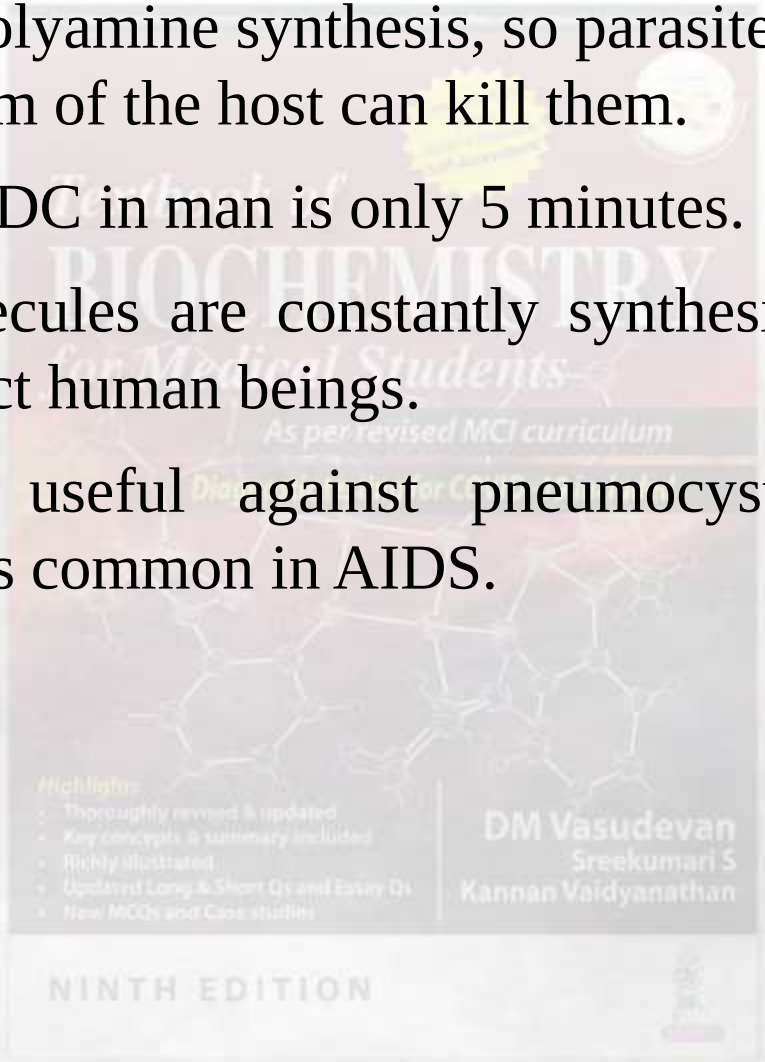



Nitric oxide
Relaxes smooth muscle
Prevents platelet aggregation
Functions as a neurotransmitter in brain
Mediates bactericidal actions of macrophages

POLYAMINES

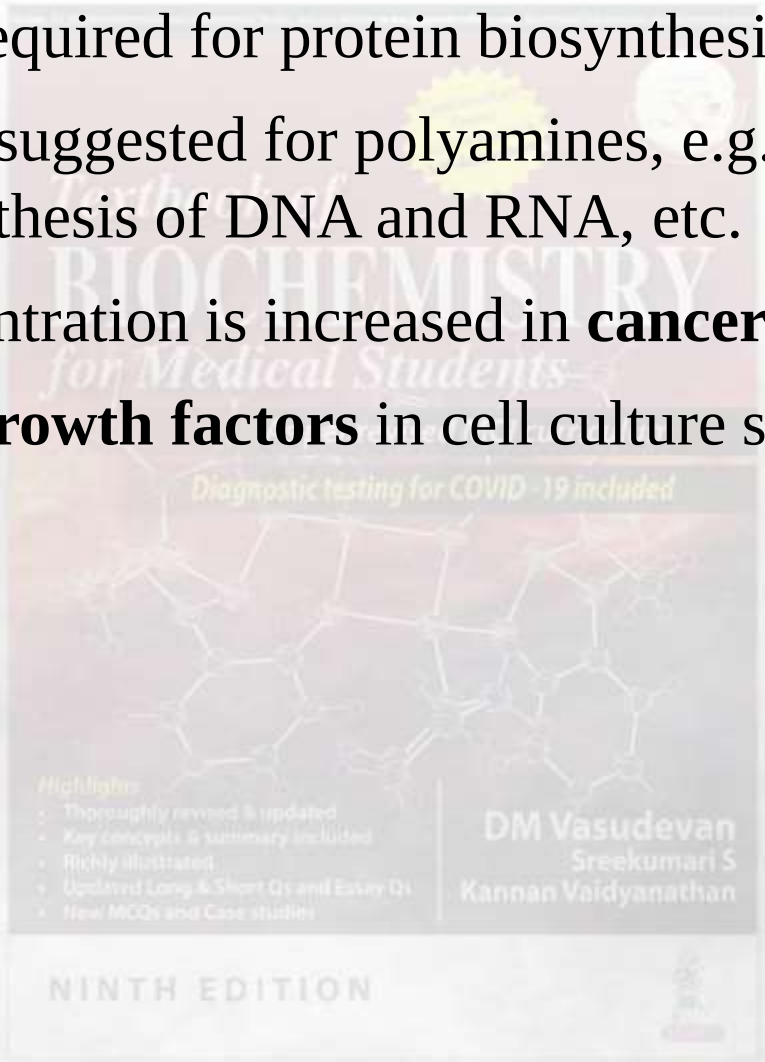
- Polyamines are **putrescine, spermidine and spermine.**
- They are synthesized from **Ornithine** with the help of Methionine.
- **DFMO** (difluromethyl ornithine) is a powerful inhibitor of polyamine synthesis.
- It is an example of **suicide inhibition**.
- African sleeping sickness and Indian Kala-azar are produced by parasites, trypanosomes.
- In these parasites, the half-life of ODC is many hours.

- DFMO inhibits polyamine synthesis, so parasites cannot divide, and the immune system of the host can kill them.
- The half-life of ODC in man is only 5 minutes.
- So, enzyme molecules are constantly synthesized, and hence the drug will not affect human beings.
- DFMO is also useful against pneumocystis carinii parasite infection, which is common in AIDS.



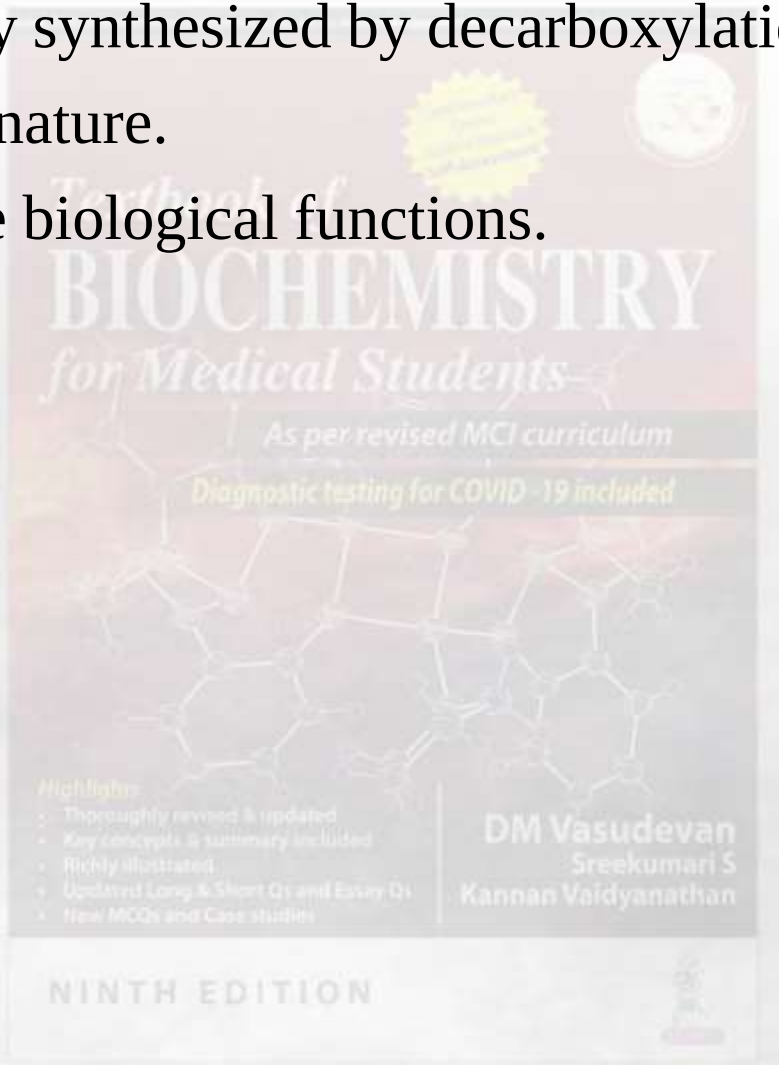
Biochemical Functions of Polyamines

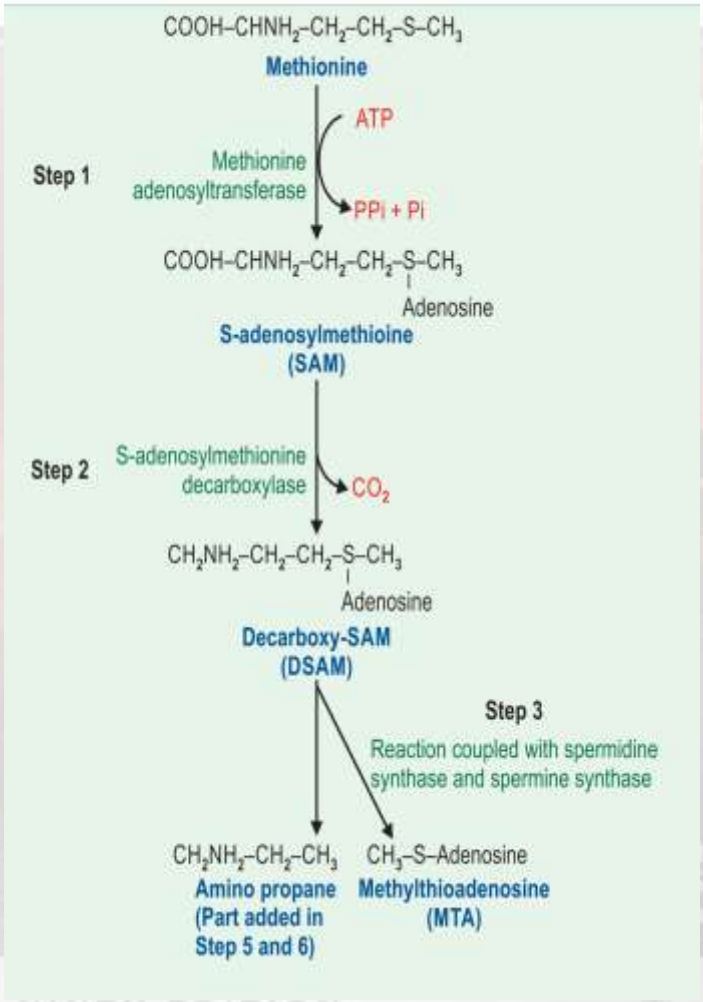
- Polyamines are required for protein biosynthesis.
- Several roles are suggested for polyamines, e.g. cell proliferation, synthesis of DNA and RNA, etc.
- Polyamine concentration is increased in **cancer** tissues.
- Polyamines are **growth factors** in cell culture systems.



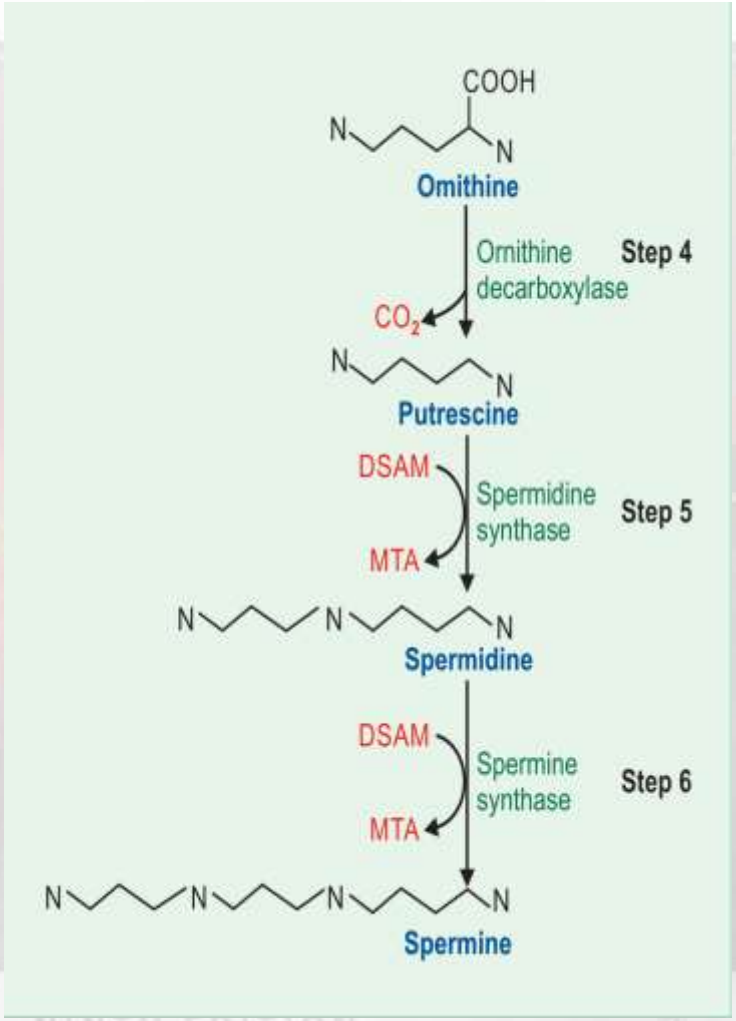
Biogenic Amines

- They are generally synthesized by decarboxylation of amino acids.
- They are basic in nature.
- They have diverse biological functions.





Polyamine synthesis



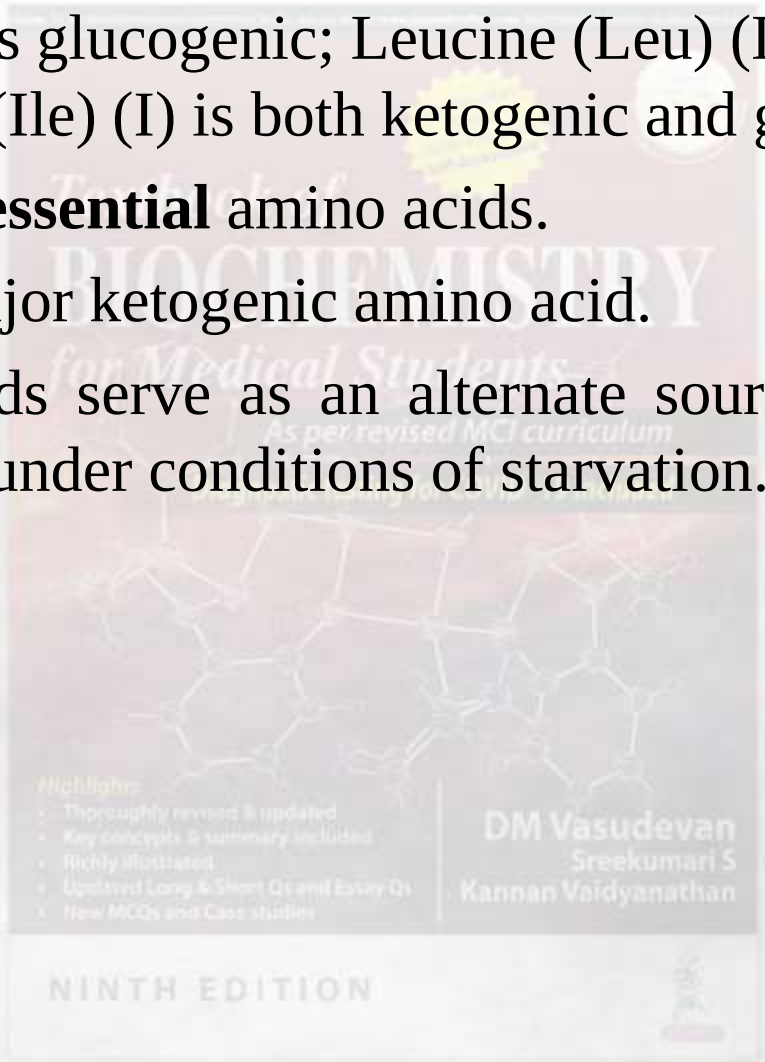
Polyamine synthesis, continued

Biogenic Amines

Substrate	Decarboxylated product, amine
Serine	Ethanol amine → Choline
Tyrosine	Tyramine
DOPA	Dopamine
Tryptophan	Tryptamine
5-OH-tryptophan	Serotonin
Histidine	Histamine
Ornithine	Putrescine
Lysine	Cadaverine
Cysteine	Taurine

BRANCHED CHAIN AMINO ACIDS (BCAA)

- Valine (Val) (V) is glucogenic; Leucine (Leu) (L) is ketogenic while Isoleucine (Ile) (I) is both ketogenic and glucogenic.
- All the three are **essential** amino acids.
- Leucine is the major ketogenic amino acid.
- These amino acids serve as an alternate source of **fuel for the brain** especially under conditions of starvation.



Catabolism of Branched Chain Amino Acids

No.	Reaction and co-enzymes	Valine	Leucine	Isoleucine
1.	Transamination to produce branched chain α-keto acid	Alpha keto isovaleric acid	Alpha keto isocaproic acid	Alpha keto beta methyl valeric acid
2.	Oxidative decarboxylation with the help of CoA, NAD+ and branched chain alpha keto acid dehydrogenase (lacking in maple syrup urine disease)	Iso butyryl CoA	Isovaleryl CoA	Alpha methyl butyryl CoA
3.	FAD dependent dehydrogenation	Methyl acrylyl CoA	β-methyl crotonyl CoA	Tiglyl CoA

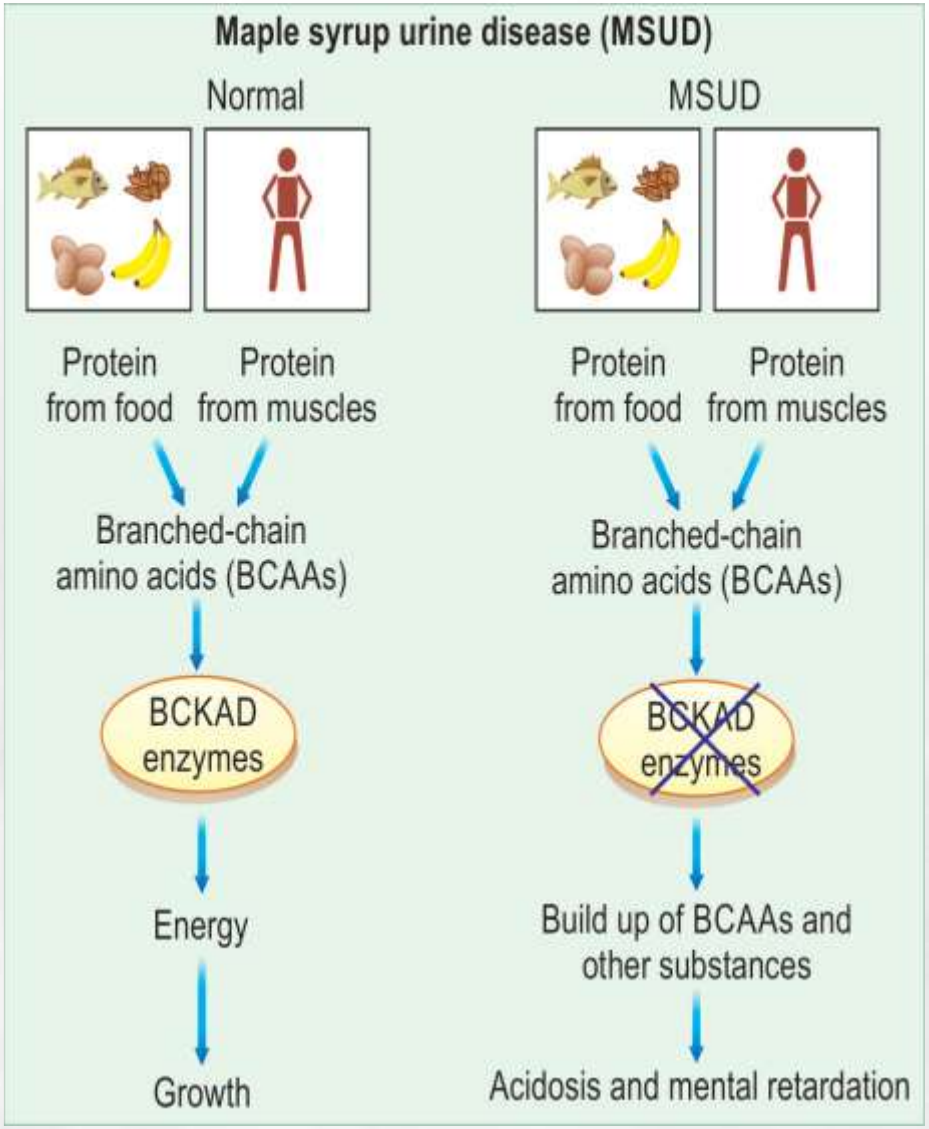
No.	Reaction and co-enzymes	Valine	Leucine	Isoleucine
4.	Individual reactions	+ H2O; remove CoA to form beta-hydroxy isobutyrate	+ CO2 with the help of biotin to form beta methyl glutaconyl CoA	+ H2O to form alpha methyl beta hydroxyl butyryl CoA
5.	Individual reactions	NAD dependent dehydro-genase; to form malonyl CoA	Hydrolysis; beta hydroxy beta methyl glutaryl CoA(HMG CoA)	NAD dependent dehy-drogenation; Methyl alpha methyl acetoacetyl CoA

No.	Reaction and co-enzymes	Valine	Leucine	Isoleucine
6.	End-products	B12-Coenzyme to form succinyl CoA	HMG CoA lyase to form acetoacetate and acetyl CoA	Cleavage to form acetyl CoA and propionyl CoA
7.	Final metabolic pathway	Glucogenic only	Ketogenic only	Ketogenic and glucogenic

Maple Syrup Urine Disease (MSUD)

- It is also called branched chain ketonuria.
- The incidence is 1 per 1 lakh births.
- The name originates from the characteristic smell of urine (similar to burnt sugar or maple sugar) due to excretion of branched chain keto acids.
- The basic biochemical defect is **deficient decarboxylation** of branched chain keto acids (BKA).
- **Clinical findings**: Disease starts in the first week of life.
- It is characterized by convulsions, **severe mental retardation**, vomiting, acidosis, coma and death within the first year of life.
- **Laboratory findings**: Urine contains **branched chain keto acids,** valine, leucine and isoleucine.

- Rothera's test is positive, but unlike in cases of ketoacidosis, even boiled and cooled urine will give the test.
- Diagnosis depends on enzyme analysis in cells.
- Diagnosis should be done prior to 1 week after birth.
- **Treatment**: Giving a diet low in branched chain amino acids.
- Mild variant is called **intermittent** branched chain ketonuria.
- This will respond to high doses of thiamine.
- This is because the decarboxylation of the BKA requires **thiamine.**


Maple syrup urine disease (MSUD)
Normal
MSUD
Protein from food
Protein from muscles
Protein from food
Protein from muscles
Branched-chain amino acids (BCAAs)
Branched-chain amino acids (BCAAs)
BCKAD enzymes
BCKAD enzymes
Energy
Build up of BCAAs and other substances
Growth
Acidosis and mental retardation

Isovaleric Aciduria

- Here leucine catabolism is affected.
- Severe metabolic acidosis and neurological deficit are seen.
- It is often fatal in early childhood.
- The characteristic offensive odor of urine is the first sign of the abnormal excretion of this metabolite.
- The defect lies in reaction No. 3 (FAD-dependent dehydrogenation).