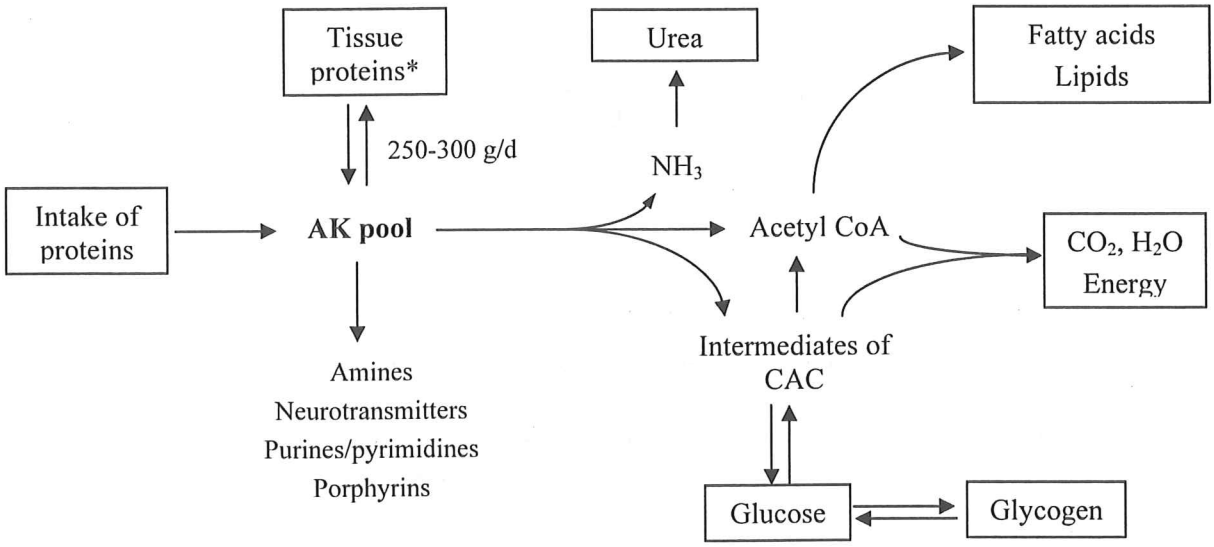
Metabolism of Proteins & Amino Acid Nitrogen 7

Amino acids – structure, properties. Intracellular degradation of proteins – proteasome, ubiquitin, lysosome. Transamination. Deamination. Ureosynthesis.

Metabolism of Proteins – Overview



* proteins with various half-life time (minutes – several days)

Intracellular Degradation of Proteins

a) Lysosomal Protein Turnover

- Proteins degraded: extracellular (accepted by endocytosis),
  membrane bonded,
  intracellular under the stress (autophagy)

b) Ubiquitin – Proteasome Pathway (cytoplasm, nucleus)

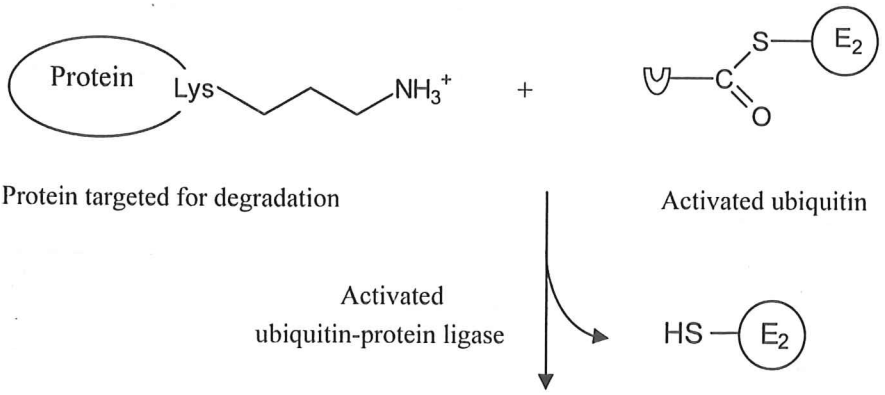
- Proteins degraded: damaged or misfolded intracellular proteins
  proteins coded by viruses and other intracellular parasites
  transcription factors
  cyclins and other regulation proteins
  proteins with the short half-life

Proteasome

- Important for cell processes (growth, differentiation, signal transduction, apoptosis).

1. Describe the steps in extracellular glycoproteins degradation.
2. What is the consequence of plasmatic glycoproteins desialization?
3. What is the function of ubiquitin in cells?
4. Describe the structure and function of proteasome.

Activation of target protein by ubiquitin



.....

(*complete*)

Target protein-ubiquitin complex bonded by amide bond

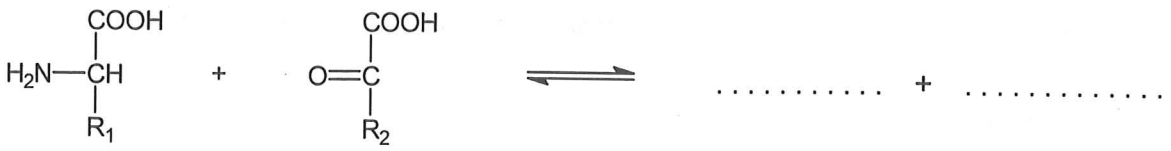
Regulation of protein degradation – levels: - activation of target protein by ubiquitin
- activation of ubiquitin-protein ligase

5. It was described more then 300 of different ubiquitin ligases in the cell. Each of them has targets a different kind structurally aberrant protein, protein with the short half-life time or regulatory proteins. What will be the consequence of missing of certain ubiquitin ligase in the cell?
6. Cell cycle is coordinated by cyclin-dependent kinases. Most of cyclins has half-life time about 0.5–1 hour. Which of degradation processes are involved in degradation of cyclins and what significance does it have.
7. There is known only one proteasome inhibitor used in therapy. It is synthetic tripeptide containing boron (bortezomib). It is approved in the U.S. for treating relapsed multiple myeloma. Try to explain the principle of its effect on the myeloma cells.
8. Which of protein degradation processes is ATP dependent?

Elimination of α-Amino Nitrogen from Amino Acids

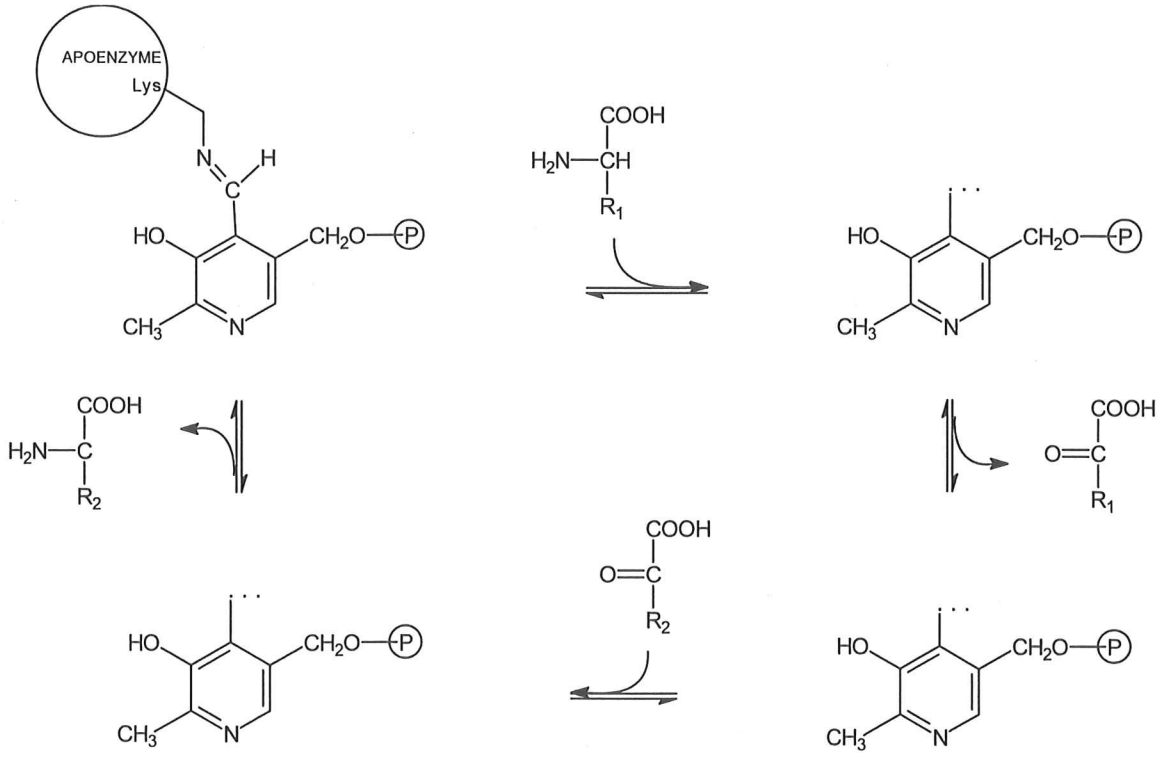
1) Transamination – most of amino acids except: Arg, Lys, Met, Thr, Trp, Pro, His

- General equation of transamination reaction (*complete*):



9. Which of the 2-oxo acids is most common acceptor of amino group?
10. What cofactor is used by amino transferases?

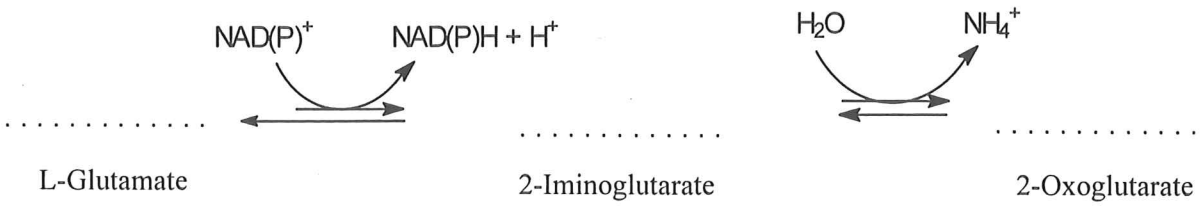
11. Write the equation of a reaction catalyzed by alanine aminotransferase (ALT).
12. Write the equation of a reaction at which is formed Asp in a reaction catalyzed by aspartate aminotransferase (AST).
13. What is the fate of amino acid that is formed in both the reaction catalyzed by ALT, AST?
14. Explain the general significance of aminotransferases in amino acid degradation.
15. Complete the steps in transamination reaction:



2) Deamination – only some amino acids

a) Deamination Associated with Dehydrogenation

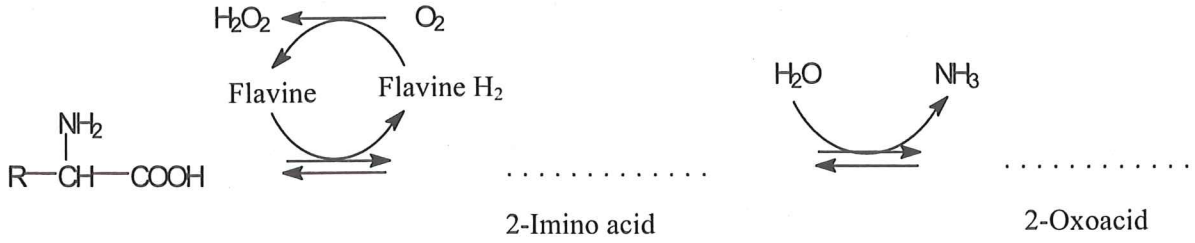
- Glutamate dehydrogenase (GMD)



Reaction is reversible, NAD^+ is utilized mainly at deamination reaction, while $NADP^+$ at the synthesis.

16. At which conditions will the reaction occur in opposite direction (formation of L-glutamate)?
17. Formation of glutamate in glutamate dehydrogenase catalyzed reaction is associated with decrease of energy production, especially in brain. Explain it.
18. Where in the cell is GMD found?

- L-Aminooxidases (FMN); D-aminooxidases (FAD, kidney, liver)



19. What significance D-aminooxidases have for human organism?

b) Histidine Deamination Associated with Desaturation

20. The product of histidine deamination is urocanic acid (double bond between α- and β-carbon). Draw the structure.

Detoxification of Ammonia

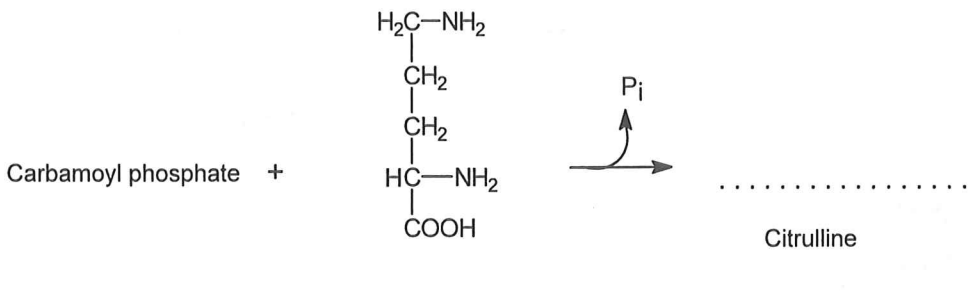
- Plasmatic concentration of (NH_4^+ and NH_3): usually 6–12 μmol/L (limit < 50 μmol/L)

a) Ureosynthesis (liver)

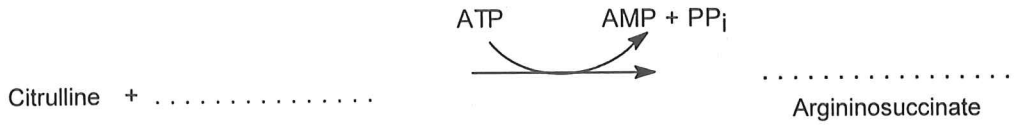
21. Ammonia is transported from muscle into the liver mainly in form of alanine a glutamine. Describe reactions at which is ammonia in liver released from these amino acids.
22. Write the equation summarizing the formation of 1 mol of urea.
23. What is the origin of two nitrogen atoms in the structure of urea?
24. What is the ATP consumption at the synthesis of 1 mol of urea?
25. What parts of a cell are processes of urea synthesis located in?
26. Partial reaction of ureosynthetic cycle (*complete*):

- Carbamoyl phosphate synthesis

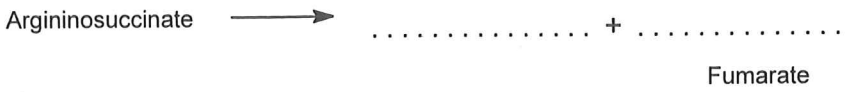
- Citrulline formation



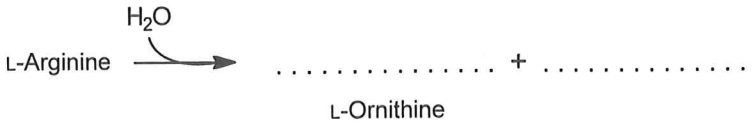
- Argininosuccinate formation



- Cleavage of argininosuccinate

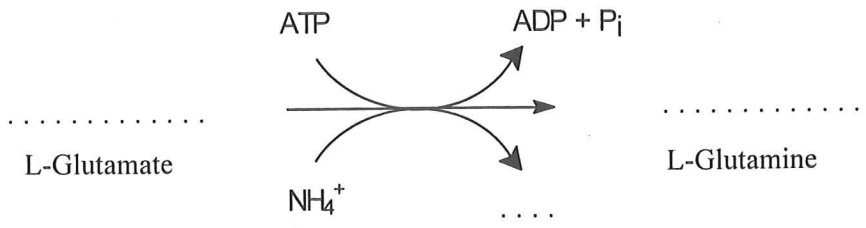


- Arginine cleavage



27. By which reaction can fumarate convert to L-aspartate?
28. Draw the structure of a compound that serves as allosteric activator of carbamoyl phosphate synthase I.
29. L-aspartate is consumed during the urea synthesis. How can it resynthetized from fumarate that is formed during urea synthesis?
30. Urea synthesis is acidifying process. Explain, why.
31. Urea is bonded to *N*-ends of proteins in non-enzymic reaction (carbamylation of proteins). Draw the structure of products that are formed by carbamylation of haemoglobin amino end Hb-Val-NH_2 (limit up to 1.6 % of the total Hb(4Fe)).

b) Glutamine Synthetase (muscle, brain, liver, mitochondria)



32. Detoxification of ammonia in brain occurs mainly by its binding to glutamate. What reaction supplies the consumed glutamate?

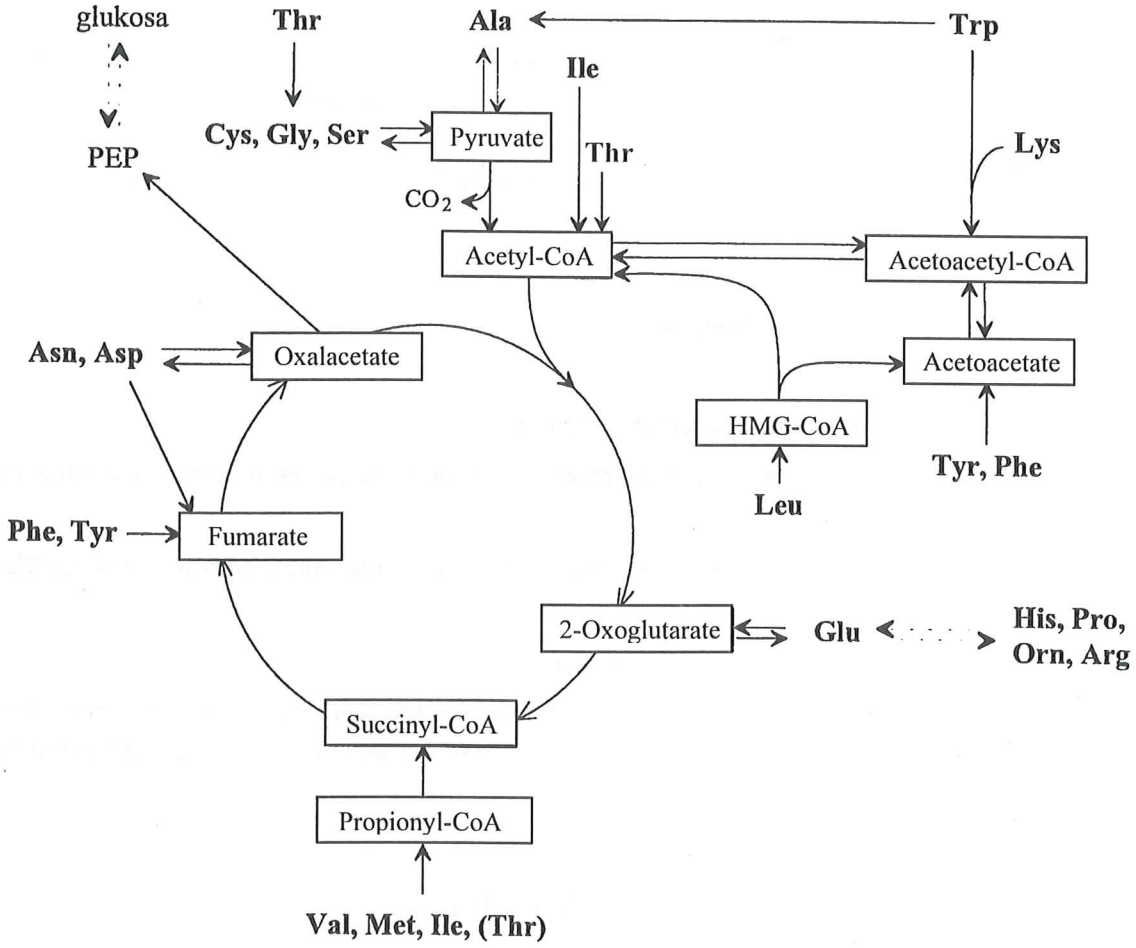
c) Glutamate Dehydrogenase

33. Give the name of substrate that binds ammonia in tissues in reaction catalyzed by GMD. Does this reaction require energy? Is it oxidation or reduction?

Metabolism of Amino Acids 8

Structure and names of amino acids, main pathways of their metabolism. Cofactors of transamination, decarboxylation, transfer one-carbon units, oxidation, reduction.

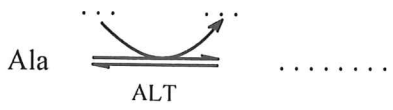
Overview of Catabolism of Amino Acid Carbon Skeletons



1. Which of amino acids are only ketogenic, keto- and glucogenic, only glucogenic?
2. Which of amino acids give acetyl-CoA by their metabolism?
3. Which of amino acids give propionyl-CoA by their metabolism? In which way is this compound metabolized? What cofactor is necessary?
4. What is the cofactors of: a) aminotransferases; b) decarboxylases.
5. Give the examples of decarboxylases of amino acids and their products.

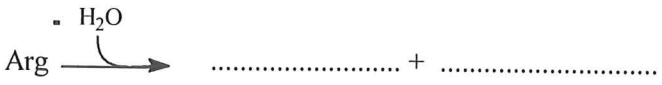
Complete the missing formulas and names in the following diagrams:

Alanine

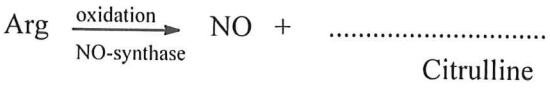


Arginine

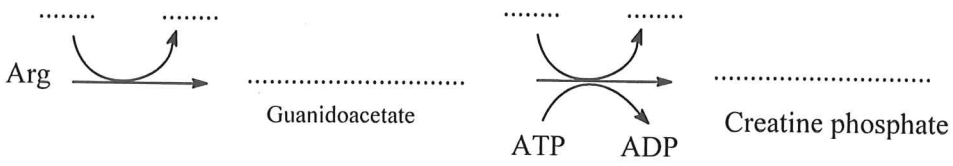
A. Urea cycle



B. NO formation



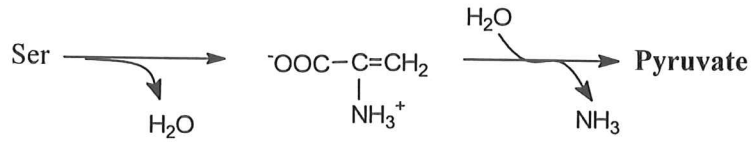
C. Creatine phosphate formation



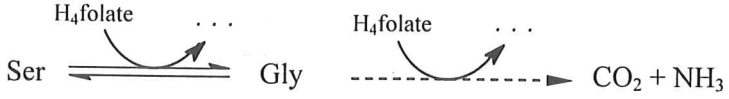
6. What amino acids are necessary for creatine synthesis?
7. Arg does not have specific aminotransferase. How is nitrogen from Arg eliminated?

Serine

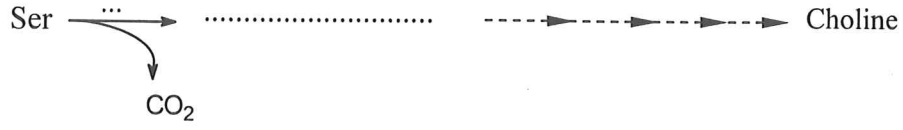
A. Dehydratation and deamination



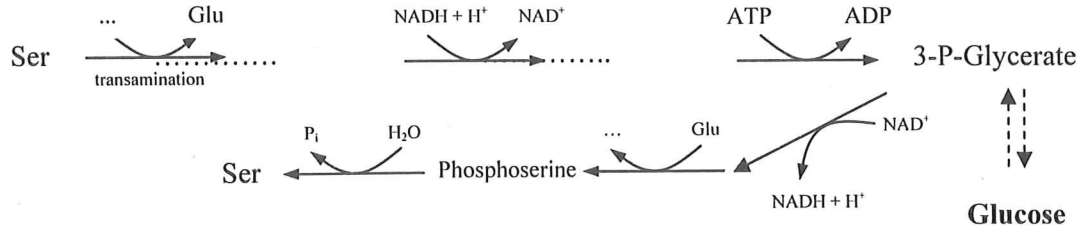
B. Conversion to glycine



C. Decarboxylation



D. Conversion to 3-P-glycerate (important for gluconeogenesis)

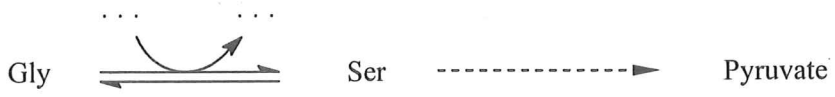


The reaction is important for glucose synthesis, the reaction proceeding in opposite direction is the main way of serine synthesis.

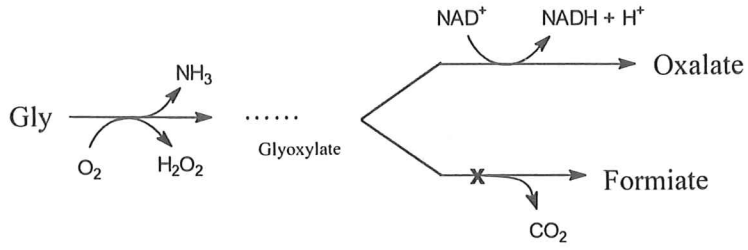
Glycine

A. The main catabolic pathway

B. Conversion to serine

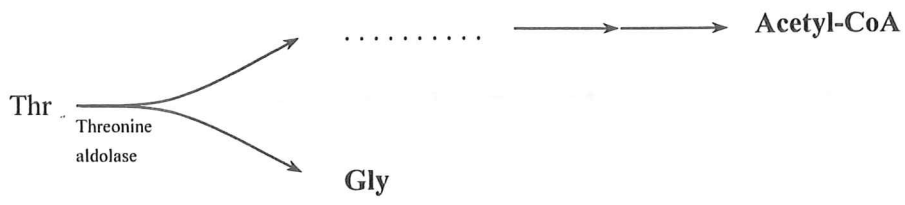


C. Side pathway of catabolism

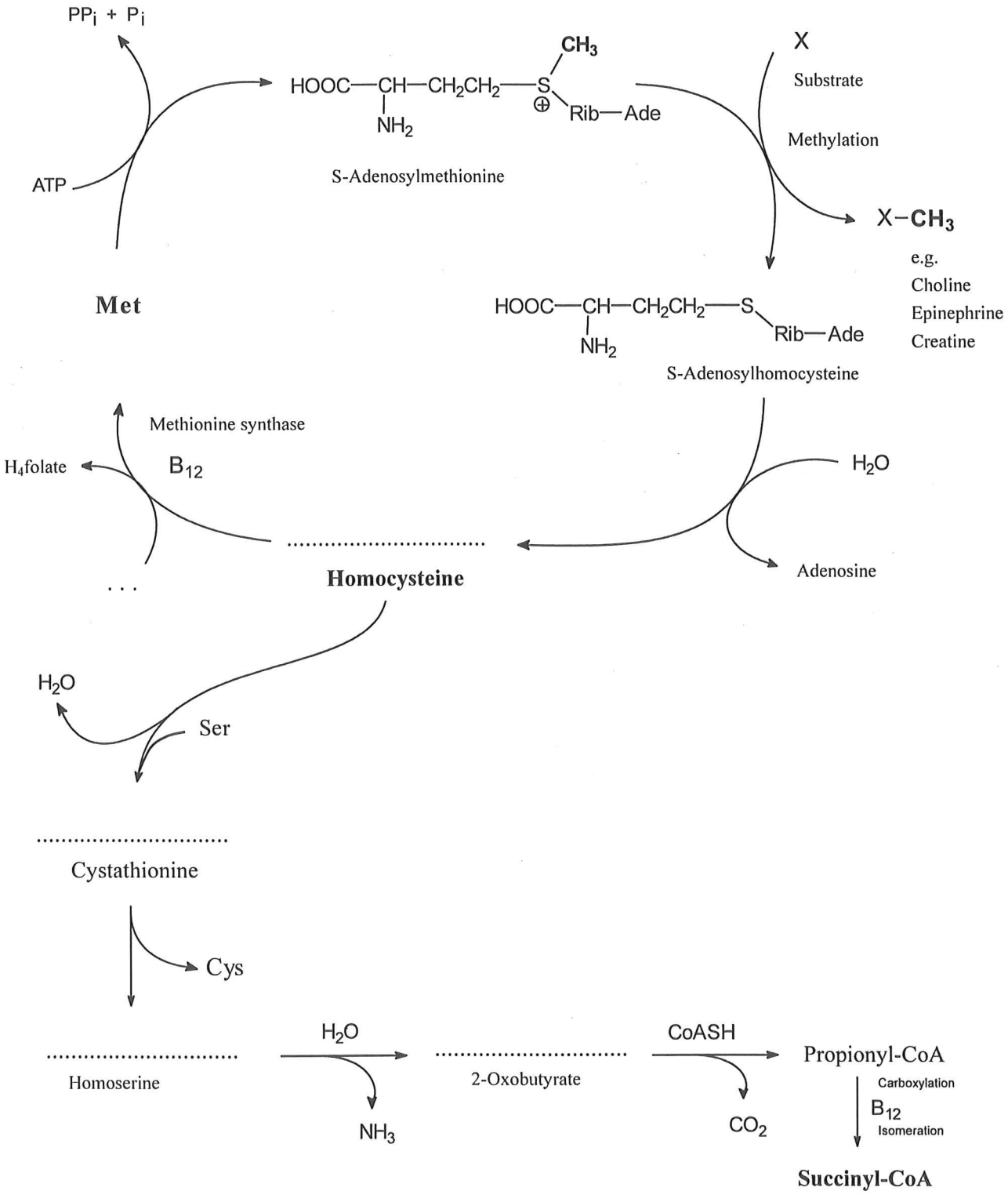


x ... primary hyperoxaluria, calcium oxalate urolithiasis

Threonine



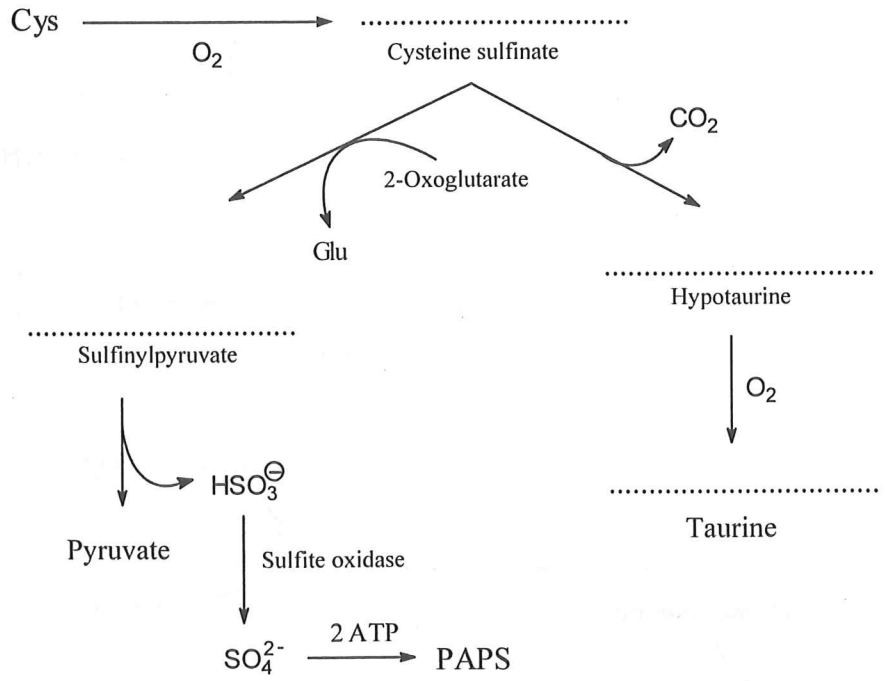
Methionine



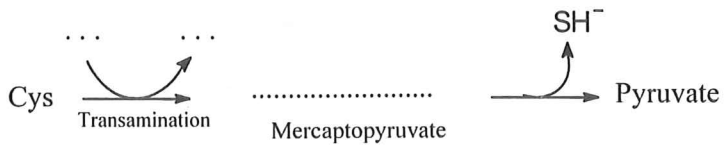
8. Hyperhomocysteinemia is a consequence of B_{12} vitamin deficiency. What other compound will be formed in the increased amount and what other compound will be missing at B_{12} deficiency?
9. What metabolite is accumulated in blood at methylene tetrahydrofolate reductase deficiency?
10. Met does not have specific aminotransferase. How is nitrogen from Met eliminated?

Cysteine

A. The main catabolic pathway



B. Side pathway of catabolism



11. What amino acids are necessary for conjugation reaction of bile acids?
12. What cofactor is a component of sulfite oxidase?
13. Why cysteine and phenylalanine are not essential when the intake of methionine a tyrosine is sufficient?
14. What is the role of taurine in metabolism?
15. What is significance of PAPS in metabolism?
16. What compound is formed by decarboxylation o cysteine? What cofactor includes this compound in its structure?

Selenocysteine

.....

It arises co-translationally from serine charged on selenocysteine t-RNA. Seryl-tRNA is converted to the selenocysteyl-tRNA in the reaction with selenophosphate. In the presence of a specific elongation factor that recognizes selenocysteyl-tRNA can be incorporated into proteins. The codon for its recognition is UGA that normally signals STOP.

Formation of selenophosphate

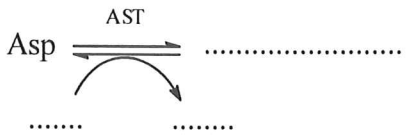
Se^{2-} + ATP ⟶ AMP + P_i + ...
Selenophosphate

Examples of enzymes containing selenocysteine: thioredoxin reductase
glutathione peroxidase
deiodinase

17. What is the function of glutathione peroxidase in the cells?

Aspartate

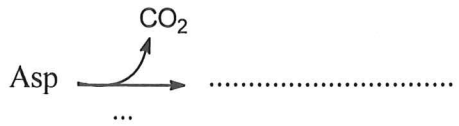
A. Transamination



B. Urea cycle

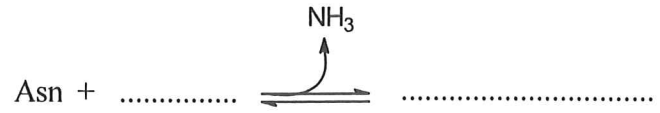
Asp + Citrulline $\xrightarrow{ATP}$ ..... ⟶ ..... + ...

C. Decarboxylation



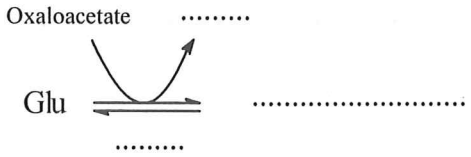
Asparagine

Deamidation

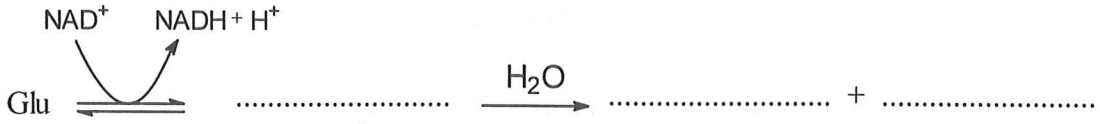


Glutamate

A. Transamination



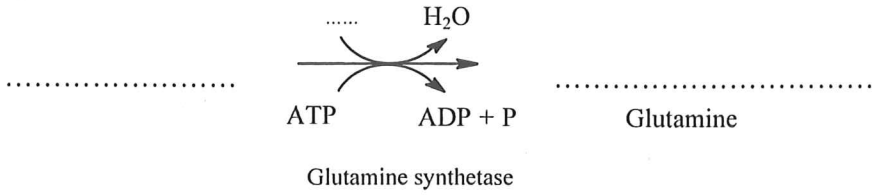
B. Oxidative deamination



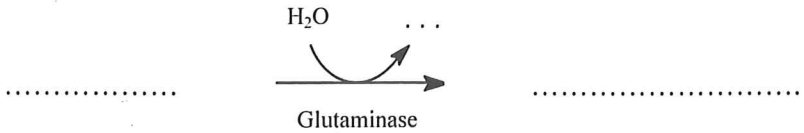
C. Decarboxylation

Glutamine

A. Synthesis

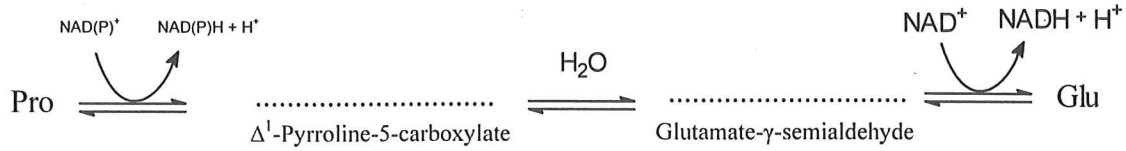


B. Deamidation

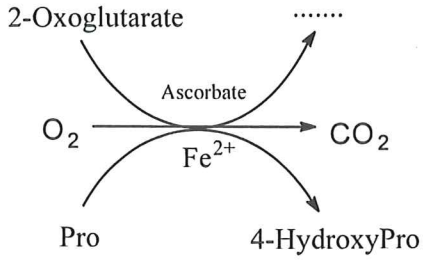


Proline

A. Catabolism



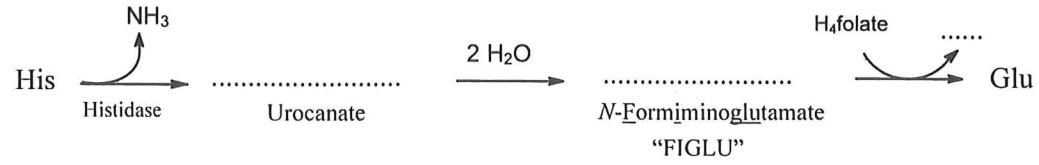
B. Hydroxylation of proline



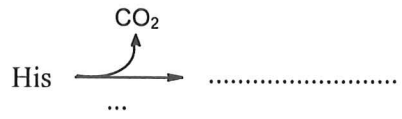
18. What is the general name of the enzyme catalyzing the proline hydroxylation?
19. Proline does not have specific aminotransferase. How is nitrogen from Pro eliminated?

Histidine

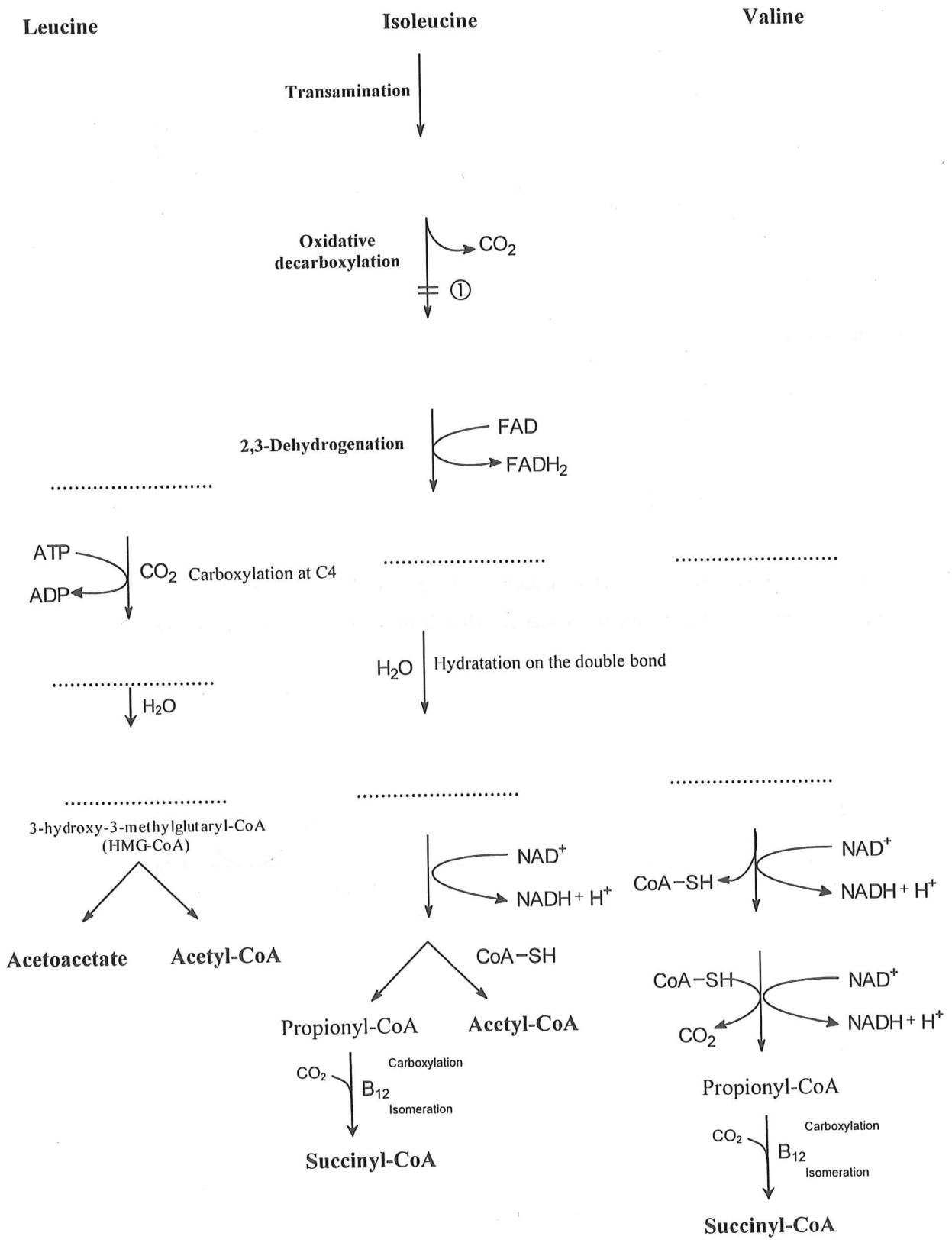
A. Catabolism



B. Decarboxylation



20. What heterocycle is comprised in histidine structure?
21. What cofactor is a component of histidine decarboxylase?
22. Histidine load serves as a test of tetrahydrofolate deficiency. Formiminoglutamate (FIGLU) is excreted into the urine when tetrahydrofolate is deficient. Explain.
23. His does not have specific aminotransferase. How is nitrogen from His eliminated?

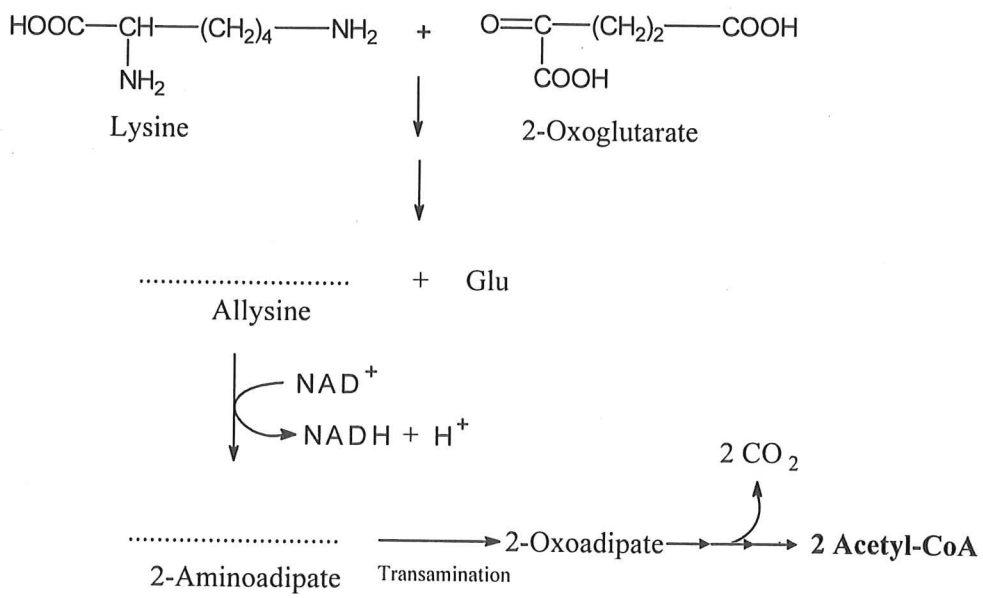


① (Deficiency of branched-chain 2-oxoacid dehydrogenases) Ketonuria with occurrence of branched chain 2-oxoacids in urine (an inborn error of metabolism called "maple syrup urine disease")

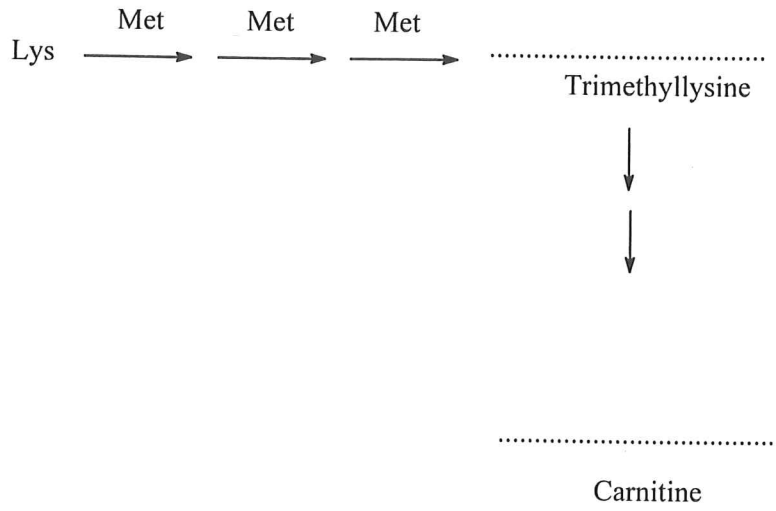
24. What is the cause of methylmalonyl aciduria? Metabolism of what amino acids can display this defect.

Lysine

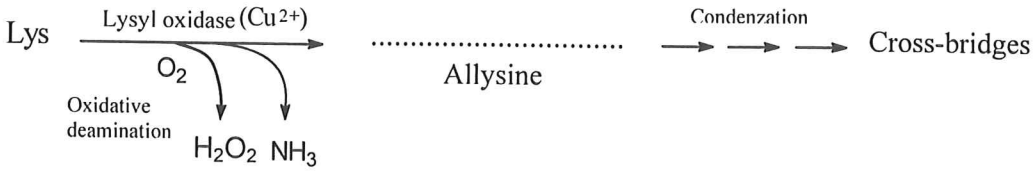
A. Catabolism



B. Conversion to carnitine



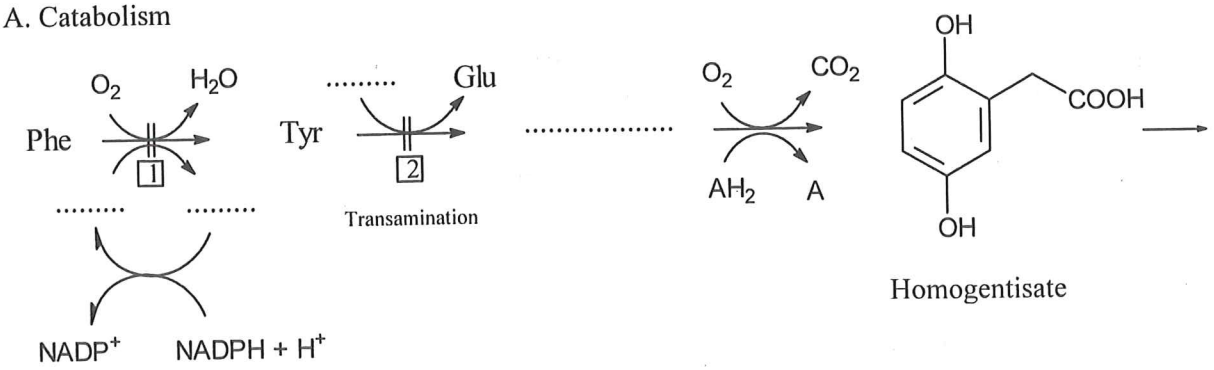
C. Formation of cross-bridges in collagen

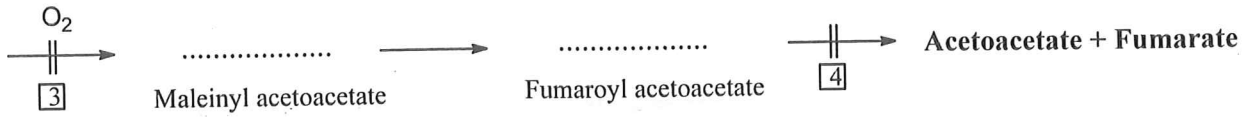


25. Lysine does not have specific aminotransferase. How is nitrogen from Lys eliminated?

Phenylalanine **Tyrosine**

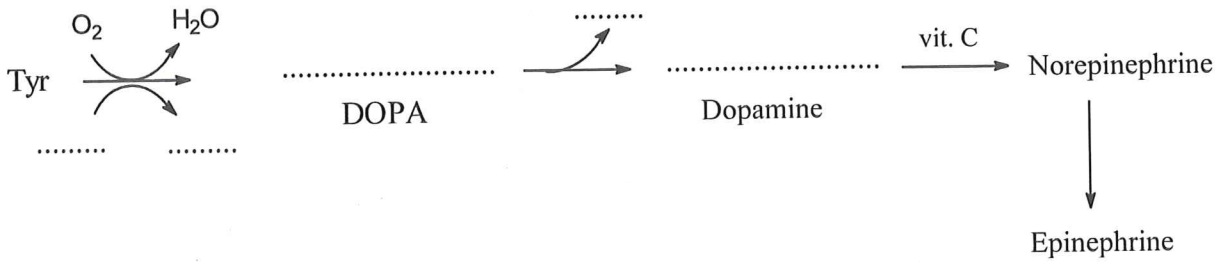
A. Catabolism





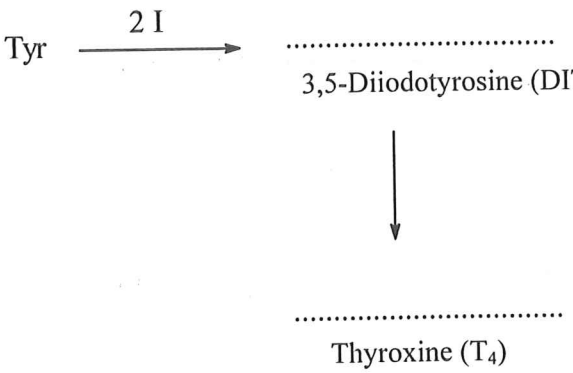
26. What is the general name of an enzyme that catalyzes the phenylalanine and tyrosine hydroxylation?
27. What compound is H-donor in the process homogentisate formation?
28. What products are accumulated and consequently excreted in urine when enzyme phenylalanine hydroxylase is deficient? What is the name of this disease?

B. Conversion to catecholamines

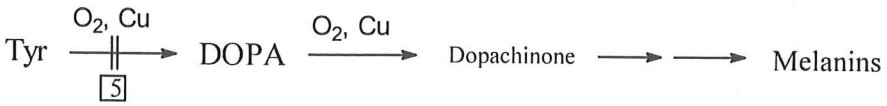


29. In which type of reaction is DOPA converted to dopamine?
30. What compound is formed from dopamine by the action of dopamine-β-hydroxylase?

C. Conversion to thyroidal hormones



D. Conversion to skin pigments



Disturbances in phenylalanine and tyrosine metabolism

Name of the disease	**Enzyme disturbance**	**Products that accumulates in urine**	**Symptoms**
Hyperphenylalaninemia type I (phenylketonuria)	1	↑ Phe, phenylpyruvate, phenylacetate, phenyllactate phenylacetylglutamine,	Mental retardation, seizures, psychoses, mousy odour
Hypertyrosinemia type II	2	↑ Tyr	
Alkaptonuria	3	↑ Homogentisate	Black urine
Hypertyrosinemia type I	4		Liver failure, early death
Albinism	5		Absence of skin and eye pigments

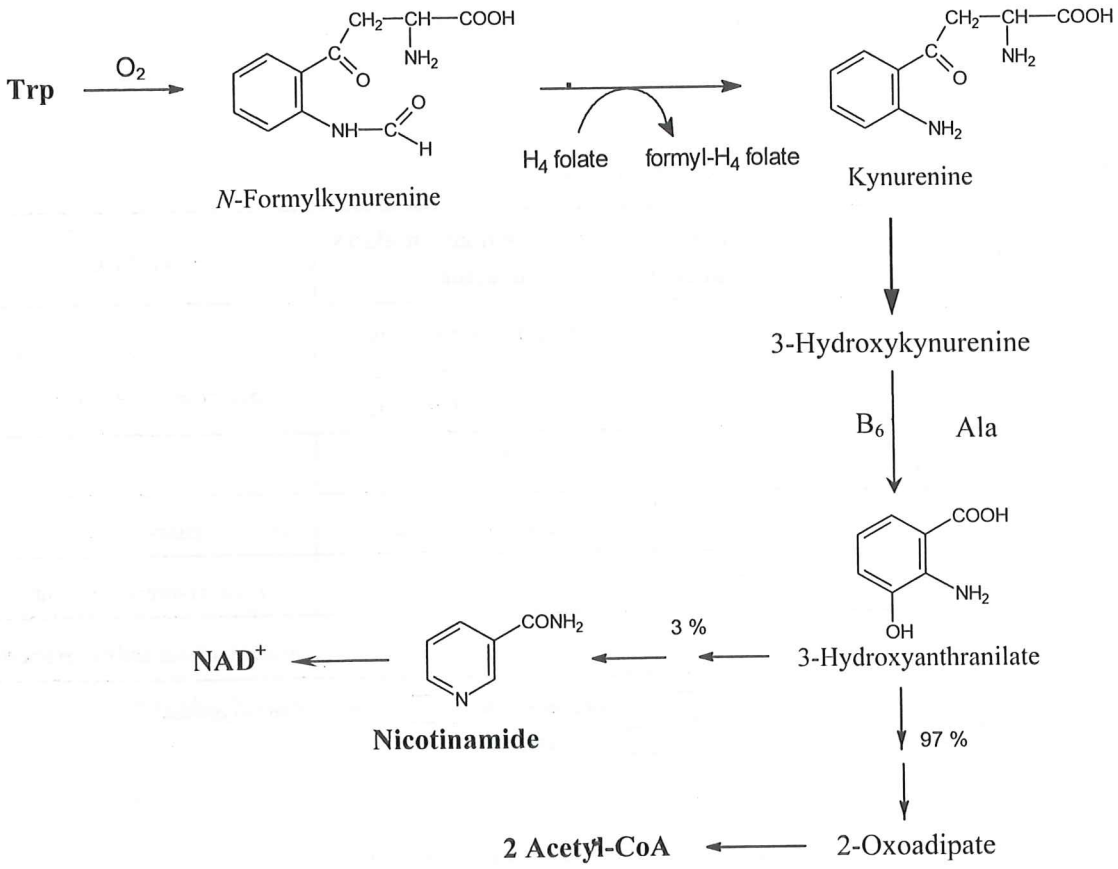
Enzyme defects: [1] Phenylalanine hydroxylase, [2] Tyroxine transaminase, [3] Homogentisate oxygenase
[4] Fumaroyl acetoacetate hydrolase, [5] Tyroxine hydroxylase

31. Explain the formation of phenylalanine catabolites at phenylketonuria.

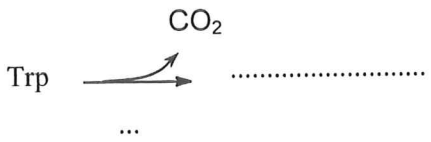
32. The symptom of alkaptonuria is black urea. Try to explain, what the cause of the dark colour is.

Tryptophan

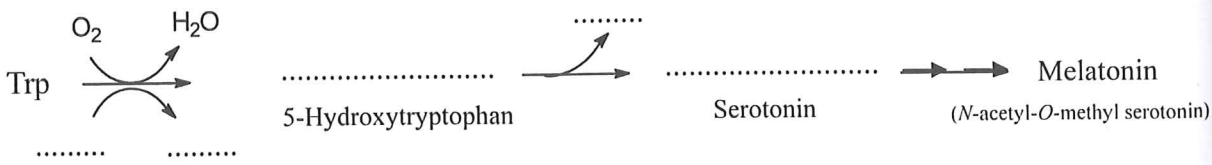
A. Catabolism and nicotinamide formation



B. Decarboxylation



C. Conversion to melatonin



33. The clinical syndrome resulting from nicotinic acid deficiency is called pellagra. Explain, why this deficiency is widespread within maize-eating areas.