Amino acid metabolism II
Fates of amino acid carbon skeleton – degradation to common intermediates – pyruvate, intermediates of citric acid cycle, acetyl-CoA

Glucogenic AA
– precursors of glucose
- degradation to pyruvate or citric acid cycle intermediates – can be converted to oxaloacetate – key intermediate of gluconeogenesis
- most AA

Ketogenic AA
– precursors of ketone bodies
- degradation to acetoacetate (a ketone body) or to acetyl-CoA (a substrate of ketogenesis)
- Lys, Leu, Ile, Phe, Tyr, Trp

both glucogenic and ketogenic AA
Conversion of amino acids to glucose (examples)

Alanine → Pyruvate
Aspartate → Oxaloacetate

Pyruvate → Phosphoenolpyruvate

Phosphoenolpyruvate → 2-Phosphoglycerate

2-Phosphoglycerate → 3-Phosphoglycerate

3-Phosphoglycerate → 1,3-Bisphosphoglycerate

1,3-Bisphosphoglycerate → glyceraldehyde 3-phosphate → Dihydroxyacetone phosphate

Dihydroxyacetone phosphate → Fructose 1,6-bisphosphate

Fructose 1,6-bisphosphate → Fructose 6-phosphate

Fructose 6-phosphate → Glucose 6-phosphate → Glucose
Metabolism of individual aminoacids

Each AA
- specific pathway of degradation to common intermediates (pyruvate, citric acid cycle intermediates, acetyl-CoA)
- specific transformation to specialized nitrogenous substances (catecholamines, nucleotides, porphyrins, creatine…)
- numerous enzyme defects in AA metabolism – genetic diseases
  ~ 120 genetic disorders of AA transport and metabolism
  ~ 25 disorders associated with mental retardation
Tetrahydrofolate (THF) – important cofactor in AA metabolism

- derived from vitamin - folic acid

- the cofactor function – mobilization, interconversion and utilization of single-carbon functional groups = one-carbon units

  methyl
  methylene( hydroxymethyl)
  formyl
  formimino

sources: serine, glycine, histidine

-one-carbon units attached to N⁵ and/or N¹⁰ of the THF molecule

- THF-one-carbon unit - involved in biosynthesis of purine nucleotides, pyrimidine nucleotides (donor of thymine methyl group), methionine synthesis from homocysteine
S-Adenosylmethionine (SAM) – methyl group donor

- Methyl group transferred to an acceptor (dopamine, noradrenaline, ethanolamine…)
- Resulting S-adenosylhomocysteine hydrolyzed to homocysteine
- Folate/THF deficiency → hyperhomocysteinemia - risk factor of cardiovascular diseases
Metabolism of aromatic amino acids

Proteins $\xrightarrow{1}$ Phenylalanine $\xrightarrow{2, 3 \text{ liver}}$ Tyrosine $\xrightarrow{4 \text{ melanocytes}}$ Melanin $\xrightarrow{\text{adrenal medula, CNS}}$ Catecholamines $\xrightarrow{\text{CO}_2 + \text{H}_2\text{O} + \text{urea}}$ Proteins $\xrightarrow{\text{Thyroxine}}$

Metabolic defects (enzyme deficiencies) $\longrightarrow$ genetic diseases:
1 - Hyperphenylalaninemia - phenylketonuria
   $1 : 10,000$ (world)
   $1 : 5 - 8,000$ (CR)
2 - Tyrosinemia (I, II, III)
3 - Alcaptonuria
4 - Albinism
Degradation of phenylalanine and tyrosine

Phe, Tyr – both glucogenic and ketogenic AA
Phenylketonuria (PKU)

- Deficiency of *phenylalanine hydroxylase*, tetrahydrobiopterin (THBP) or dihydropteridine reductase
- Accumulation of phenylalamine in blood and tissues
- Alternative metabolism – transamination of Phe and transformations of resulting phenylpyruvate
- Mental retardation
- Treatment:
  - *phenylalanine hydroxylase deficiency* – diet low in phenylalanine, high in tyrosine throughout first decade or for life
  - *THBP or dihydropteridine reductase deficiency* – phenylalanine-low diet, supplying THBP and Dopa, 5-OH-tryptophane – precursors of neuro-transmitters

Detection in urine with FeCl₃

1-2 g/day
**Alcaptonuria** – deficiency of *homogentisate oxygenase* – homogentisic acid eliminated in urine – darkening of urine owing to oxidation of homogentisate – „dark urine disease“

**Tyrosinemias** – increased level of tyrosine in blood, tyrosinuria; inflamations (from intracellular crystalization of tyrosine), mental Retardation

**Albinism** - lack of melanin (brown pigment of skin, hair, eyes) production – deficiency of *tyrosinase*

Tyrosine $\xrightarrow{\text{tyrosinase}}$ Dihydroxyphenylalanine $\xrightarrow{\text{tyrosinase}}$ Dopa quinone $\xrightarrow{}$ Melanin
Catecholamines
= dopamine, norepinephrine, epinephrine
(noradrenalin) (adrenalin)
neurotransmitters hormone
synthetized in: brain, adrenal medulla

Structural basis:
catechol → pyrocatechol = o-dihydroxybenzen

Synthesis: from tyrosine via dihydroxyphenylalanine (= Dopa)
hydroxylation, decarboxylation – essential reactions

Essential role of tetrahydrobiopterine in hydroxylation reactions
Disorders in catecholamine biosynthesis

**Parkinson’s disease** – deficiency of dopamine synthesis – affect nerve transmission in the substantia nigra of the upper brain stem → involuntary tremor, decreased motor power and control, postural instability, muscular rigidity

Treatment: **dopamine cannot cross blood-brain barier** - thus administration of its precursor = **Dopa** (crosses blood-brain barrier) together with **Dopa-decarboxylase inhibitors**

↓

prevent decarboxylation of Dopa to dopamine in liver

**Increased dopamine** production – associated with **schizophrenia, drug abuse**

**Pheocytochroma** – tumor of the adrenal medulla – overproduction of norepinephrine, epinephrine → permanent hypertension, hyperglycemia, glucosuria
Inactivation of catecholamines

- Rapid inactivation – half-life ~ 20 seconds
- Enzymes for inactivation present in most tissues, particularly in liver

1. **MAO – monoamine oxidase** (flavoprotein – FAD)
2. **COMT - catechol-O-methyltransferase** *(S-adenosylmethionine = SAM)*

\[
\begin{align*}
1. \quad & R - CH_2 - NH_2 \xrightarrow{\text{FAD, FADH}_2} R - CH = NH \xrightarrow{\text{H}_2\text{O}} R - C = O + NH_3 \\
& \text{Amine} \quad \text{Aldehyde}
\end{align*}
\]

2. S-adenosylmethionine

Detection in urine, *marker of catecholamine overproduction* – neuroblastoma, pheochromocytoma

**MAO, COMT, oxidation**
Biosynthesis of other neurotransmitters

Decarboxylation of AA – essential reaction

Hydroxylation in serotonin synthesis – THBP-dependent
Biosynthesis of amino acids – transamination of α-keto acids (= metabolic intermediates)

10 amino acids (nonessential)

Precursors: α-ketoglutarate → glutamate
pyruvate → alanine
oxaloacetate → aspartate → asparagine
3-phosphoglycerate → serine
intermediate of glycolysis
transamination

Disposal of amino acids

Synthesis of amino acids

3-Phosphoglycerate
## Essential amino acids

required to be supplied by the diet – precursors of the synthesis (keto acids) does not exist: histidine, tryptophane, phenylalanine, leucine, isoleucine, valine, lysine, threonine, methionine

<table>
<thead>
<tr>
<th>Category</th>
<th>Amino Acids</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Essential</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Totally essential</td>
<td>Lys, Thr</td>
<td>Human can synthesize neither carbon chain, nor introduce $-\text{NH}_2$ group</td>
</tr>
<tr>
<td>b) With essential carbon chain</td>
<td>Ile, Leu, Val, Met, Phe, Trp, His</td>
<td>Human cannot synthesize carbon chain, however can synthesize these AA from the supplied oxo acids</td>
</tr>
<tr>
<td>2. Conditionally essential</td>
<td>Tyr, Cys, Tau, Orn</td>
<td>Synthesized in the organism from non-essential AA: Phe → Tyr, Met → Cys → Tau, Arg → Orn</td>
</tr>
<tr>
<td>3. Essential in overloaded organism</td>
<td>Cys, Tyr, Arg, Orn, Cit, Tau</td>
<td>Long-term parenteral nutrition, elevated protein catabolism</td>
</tr>
<tr>
<td>4. Nonessential</td>
<td>Ala, Asp, Asn, Glu, Gln, Gly, Pro, Ser</td>
<td>Synthetized de novo in humans</td>
</tr>
</tbody>
</table>
Methylmalonate acidemia/aciduria

- disorder of metabolism of amino acids degraded to propionyl-CoA (Ile, Val, Thr Met)
- genetic defect of methylmalonyl-CoA-mutase = the enzyme essential for conversion of propionyl-CoA to succinyl-CoA; or deficiency of vitamin B12 = cofactor of the enzyme
- elevated level of methylmalonate in the blood, excretion of methylmalonate into the urine
- metabolic acidosis, developmental problems, death

Scientific Sleuths Solve a Murder Mystery

Truth can sometimes be stranger than fiction—or at least as strange as a made-for-TV movie. Take, for example, the case of Patricia Stallings. Convicted of the murder of her infant son, she was sentenced to life in prison—but was later found innocent, thanks to the medical sleuthing of three persistent researchers.

The story began in the summer of 1989 when Stallings brought her three-month-old son, Ryan, to the emergency room of Cardinal Glennon Children’s Hospital in St. Louis. The child had labored breathing, uncontrollable vomiting, and gastric distress. According to the attending physician, a toxicologist, the child’s symptoms indicated that he had been poisoned with ethylene glycol, an ingredient of antifreeze, a conclusion apparently confirmed by analysis at a commercial lab.

After he recovered, the child was placed in a foster home, and Stallings and her husband, David, were allowed to see him in supervised visits. But when the infant became ill, and subsequently died, after a visit in which Stallings had been briefly left alone with him, she was charged with first-degree murder and held without bail. At the time, the evidence seemed compelling as both the commercial lab and the hospital lab found large amounts of ethylene glycol in the boy’s blood and traces of it in a bottle of milk Stallings had fed her son during the visit.

But without knowing it, Stallings had performed a brilliant experiment. While in custody, she learned she was pregnant; she subsequently gave birth to another son. David Stallings Jr., in February 1990. He was placed immediately in a foster home, but within two weeks he started having symptoms similar to Ryan’s. A blood sample was tested with a rare metabolic disorder called methylmalonic acidemia (MMA). A recessive genetic disorder of amino acid metabolism, MMA affects about 1 in 48,000 newborns and presents symptoms almost identical with those caused by ethylene glycol poisoning.

Stallings couldn’t possibly have poisoned her second son, but the Missouri state prosecutor’s office was not impressed by the new developments and pressed forward with her trial anyway. The court wouldn’t allow the MMA diagnosis of the second child to be introduced as evidence, and in January 1991 Patricia Stallings was convicted of assault with a deadly weapon and sentenced to life in prison.

Fortunately for Stallings, however, William Sly, chairman of the Department of Biochemistry and Molecular Biology at St. Louis University, and James Shoemaker, head of a metabolic screening lab at the university, got interested in her case when they heard about it from a television broadcast. Shoemaker performed his own analysis of Ryan’s blood and didn’t detect ethylene glycol. He and Sly then contacted Piero Casanovas, a metabolic disease expert at Yale University School of Medicine whose lab is equipped to diagnose MMA from blood samples.

When Rinaldo analyzed Ryan’s blood serum, he found high concentrations of methylmalonic acid, a breakdown product of the branched-chain amino acids isoleucine and valine, which accumulates in MMA patients because the enzyme that should convert it to the next product in the metabolic pathway is defective. And particularly telling, he says, the child’s blood and urine contained massive amounts of ketones, another metabolic consequence of the disease. Like Shoemaker, he did not find any ethylene glycol in a sample of the baby’s bodily fluids. The bottle couldn’t be tested, since it had mysteriously disappeared. Rinaldo’s analyses convinced him that Ryan had died from MMA, but how to account for the results from two labs, indicating that the boy had ethylene glycol in his blood? Could they both be wrong?

When Rinaldo obtained the lab reports, what he saw was, he says, “scary.” One lab said that Ryan Stallings’ blood contained ethylene glycol, even though the blood sample analysis did not match the lab’s own profile for a known sample containing ethylene glycol. “This was not just a matter of questionable interpretation. The quality of their analysis was unacceptable,” Rinaldo says. And the second laboratory? According to Rinaldo, that lab detected an abnormal component in Ryan’s blood, “a rare metabolic disorder called methylmalonic acidemia (MMA). A recessive genetic disorder of amino acid metabolism, MMA affects about 1 in 48,000 newborns and presents symptoms almost identical with those caused by ethylene glycol poisoning.

Rinaldo presented his findings to the case’s prosecutor, George McElroy, who called a press conference the very next day. “I no longer believe the laboratory data,” he told reporters. Having concluded that Ryan Stallings had died of MMA after all, McElroy dismissed all charges against Patricia Stallings on September 20, 1991.

Amino acids converted to propionyl-CoA $\rightarrow$ succinyl-CoA
Degradation of isoleucine
– example of branched-chain amino acids breakdown

1. Transamination
   - \( \text{NH}_4^+ \)
   - \( \text{L-
   Isoleucine} \)
   - \( \alpha\text{-Keto acid} \)
   - \( \alpha\text{-Amino acid} \)

2. Oxidative decarboxylation
   - \( \alpha\text{-Keto-\beta\text{-methyvlvalerate}} \)
   - \( \text{CoASH} \)
   - \( \text{CO}_2 \)

3. \( \beta\text{-oxidation} \)
   - \( \alpha\text{-Methylbutyryl-CoA} \)
   - \( [\text{H}] \)

\( \beta\text{-oxidation} \)
- \( \text{Tiglyl-CoA} \)
- \( \text{α-Methyl-β-hydroxybutyryl-CoA} \)
- \( \text{α-Methylacetoacetyl-CoA} \)
- \( \text{Acetyl-CoA} \)
- \( \text{Propionyl-CoA} \)