NUCLEOTIDE BIOSYNTHESIS

JIŘÍ JONÁK

An ample supply of nucleotides is essential for many life processes.

- activated precursors of nucleic acids – nucleoside 5' triphosphates
- ATP is a universal currency of energy
  GTP is an energy source for a more select group of biol. processes (protein synthesis)
- UDP-glucose participates in biosynthetic processes such as the formation of glycogen, gangliosides
  CTP – formation of phosphoglycerols (Phosphatidyl-choline, -inositol, Cardiolipin) (PIP_2)
- essential components of signal transduction pathways
  – cyclic AMP, cyclic GMP = second messengers: transmit signals both within and between cells

Nucleotide biosynthetic pathways are tremendously important as intervention points for therapeutic agents.

Many of the most widely used drugs in the treatment of cancer block steps in nucleotide biosynthesis, particularly steps in the synthesis of DNA precursors.

Drugs against certain viruses.

NUCLEOTIDE BIOSYNTHESIS

(i) **de novo pathway** : the nucleotide bases are assembled from simpler compounds

[Activated ribose (PRPP)] + amino acids (glycine, aspartate)
+ ATP
+ NH_4^+ (glutamine) ⟶
+ CO_2 ....
n-nucleoside mono P =
⟶ n - NUCLEOTIDE (PRPP)

a) PYRIMIDINE: base is assembled first and then attached to ribose
b) PURINE: base is synthesized directly onto a ribose-based structure (PRPP)

(ii) **salvage pathway** : preformed bases are recovered and reconnected to a ribose unit

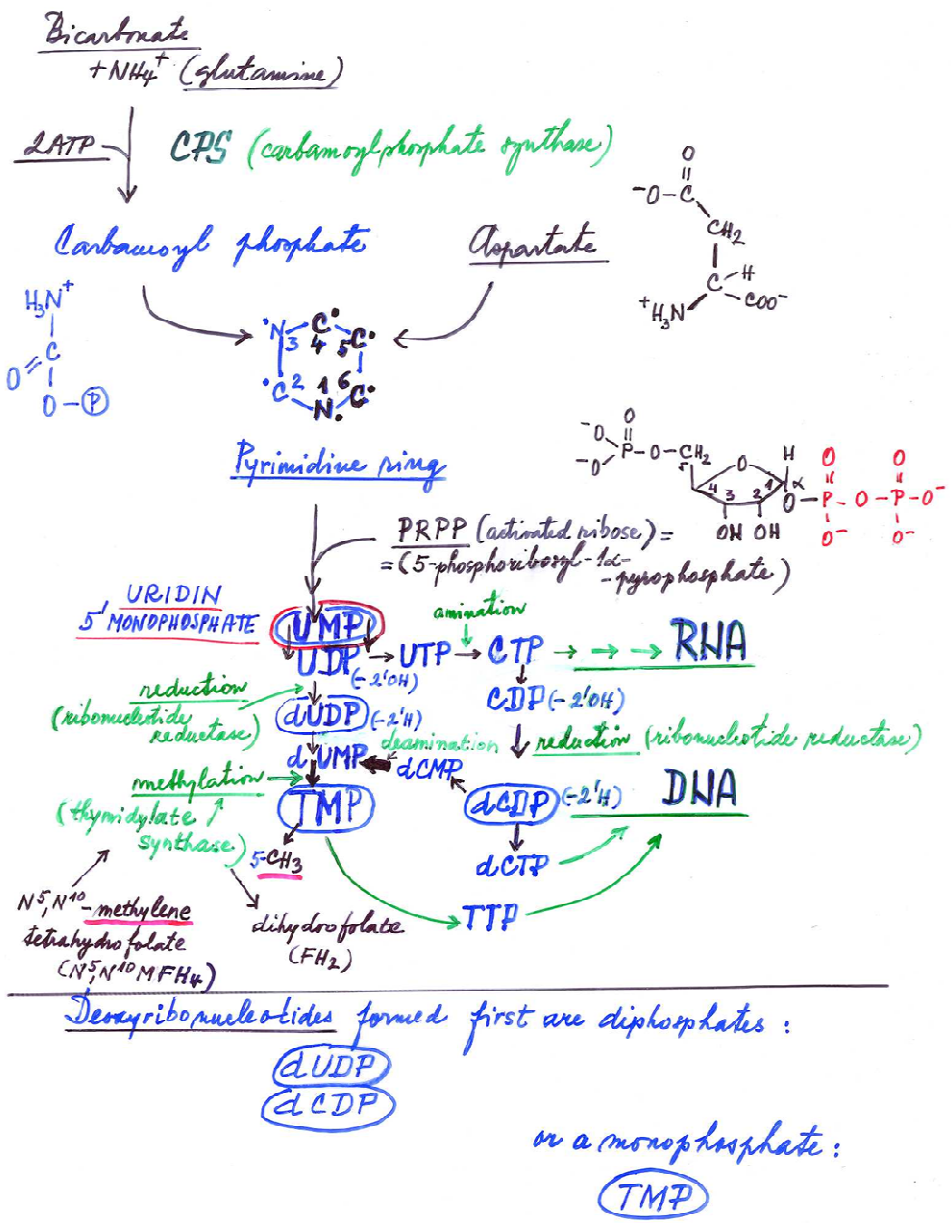
Preformed base + activated ribose (PRPP) ⟶ n-nucleoside mono P = n - NUCLEOTIDE

RNA preceded DNA in the course of evolution

(a) all deoxyribonucleotides are synthesized from the corresponding ribonucleotides. The deoxyribose sugar is generated by the reduction of ribose within a fully formed n-nucleotide - not earlier

(b) the methyl ($-CH_3$) group that distinguishes the thymine of DNA from the uracil of RNA is added at the last step in the pathway

De novo pathway for pyrimidine nucleotide synthesis (simplified)



Deoxyribonucleotides formed first are diphosphates:

dUDP

dCDP

or a monophosphate:

TMP

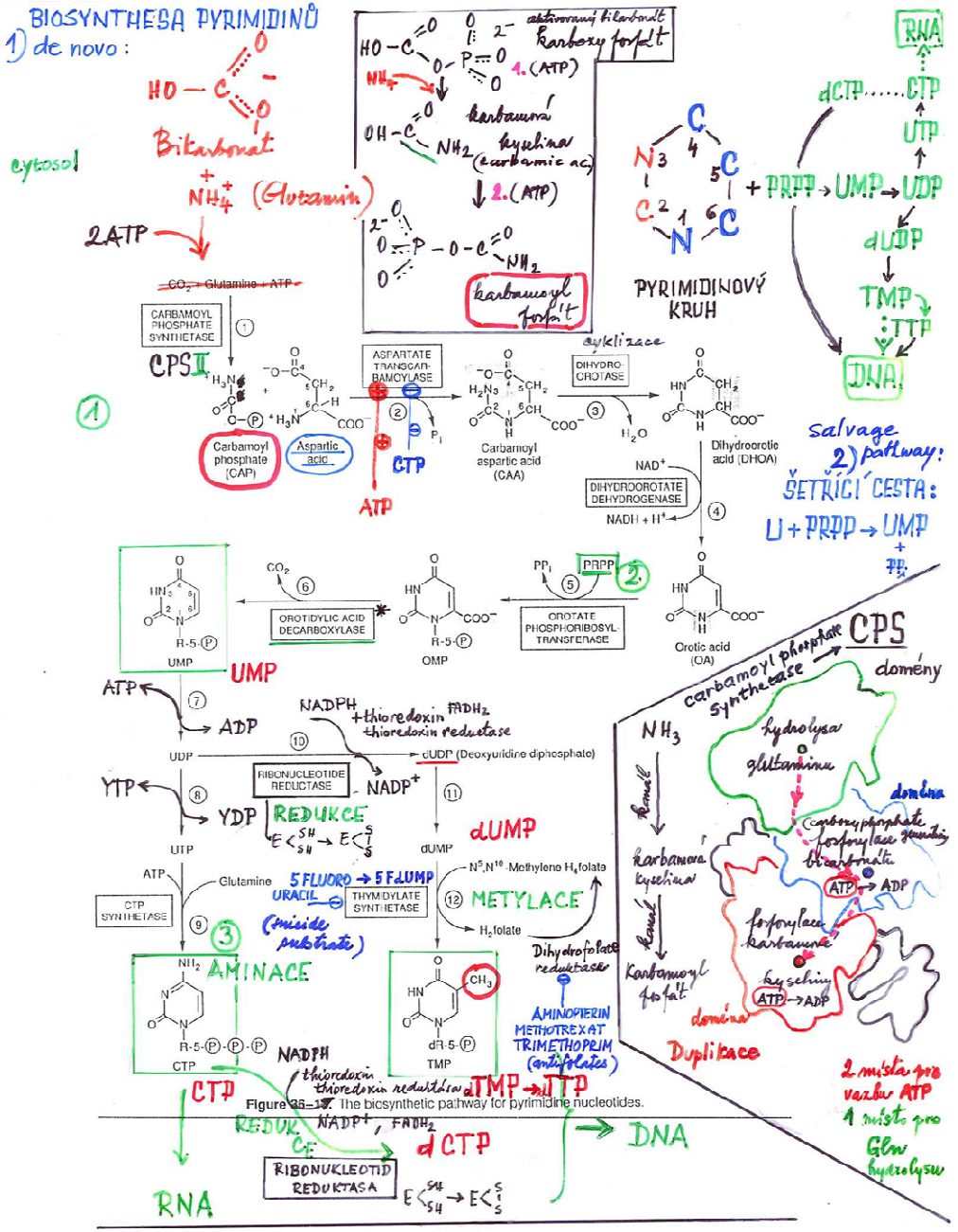


Figure 25–17 The biosynthetic pathway for pyrimidine nucleotides.

* orotidylát dekarboxylasa : jeden z nejúčinnějších známých enzymů (1/s); bez něj by došlo k dekarboxylaci 1x/78 milionů let zrychlení reakce enzymem : 10^{17} násobné

The *pyrAb* Gene Coding for the Large Subunit of Carbamoylphosphate Synthetase from *Bacillus stearothermophilus*: Molecular Cloning and Functional Characterization

(carbamoylphosphate synthetase / pyrimidine biosynthesis pathway / *pyr* operon / *pyrAb*)

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Two binding sites for ATP binding in the molecule of karbamoyl phosphate synthetase

start : ATP 1 ATP 2

```
  -64                                                                aatt
  -60 cgctgtttgacgagt ttttggcgctcatcc gccagttcaacaaga aaggagaaagtcatcc
start pyrAb  stop pyrAa
    1 atgcctaaacgccgc gacattgaaacgatt ttagtcatcggctcg gggccgatcgtcatc
   61 ggccagggcggcgag ttcgcctacgcaggg acgcaagcgtgcttta gccctaaaagaagaa
  121 ggatacaaagtcatt ctggtgaactcaaac ccggcgacgatcatg accgatacggaaatc
  181 gccgataaagtctat atggaagccgctgacg ctcgatttctgccgcc cgcatcatccgcaaa
  241 gagcggcccggacgcg attttgccgacgctt ggcgggccaaacgggc cttaacttggcggtg
  301 gaacttggccaaagcc ggagtgctcgaggag tgcggcgtcgaaatt atcggcgacgaagctt
  361 gaagcaatcgaaaaa gcggaagacccgcaa cagttccgcgccctc atgaacgagcttggc
  421 gagccggtgccggaa agcgcgattattcac agctttggaagaagcg tacgccctttgtcgaa
  481 caaatcggctatccg gtcatcgtccgtccg gcgttcacgctcggc ggcacggccgcgggc
  541 atttgcacgaacgaa gaagaacttgttgag attgtgtccaaccggc ctgaagctgaqtccg
  601 gtgcacccaatgcttg cttgaacggagcatc gccggctacaaccaa atcgaatatgaagtg
  661 atgcgcgacgccaac gacaacgcgatcgtc gtctgcaacatggaa aacatcgatccggtc
  721 ggcattcatacgggt gattcgatcgtcgtc gccccgagccagacg ttaagcgacccgcgag
  781 tatcagctgctccgc aacgcgtcgcttaag atcatccgcgcgctt ggcatcgaaggcggc
  841 tgcaacgtccagctg gcgcttgacccggac agcttccgctattac gtcattgaagtgaac
  901 ccgcgcgtcagccgt tcgtcggcgctggcg tccaaagcaacgggg tatccgatcgcgaaa
  961 ctggcggcgaaaatc gcggtcgggttgacg cttgatgaaatgatc aacccggtcacccggg
 1021 aaaacgtacgcctgc ttcgagccgqcgctc gactatgtggtgacg aaaattccgcgcttt
 1081 ccgtttgccaaattt gaatcggcgaaccgc cgccttggcacgcaa atgaaagcgaccggc
 1141 gaagtgatgtcgatc ggccggacgttcgaa gaatcactgttaaaa gccgttcgttcgctc
 1201 gagatcgggggtgcat catctggaattgaac gaggcgaaaacggcc gccgacgacgtgatg
 1261 qaaaaacggatccgc aaggcgggcgatgaa cggctcttttacatc gccgaggcgctccgc
 1321 cgcgggggtgacagtg gagacgctgcatgaa tggagccaaatcgac cgcttttcctgcat
 1381 aagattcaaaaacatt attgagatggaaacg gtcctgaaaacccac ccgggcgaccttgac
 1441 gtgttaaaaaaagcg aaggggctcggcttc tccgatgcggcgata gcggcattatggaac
 1501 aaaacgagcgcgcgc gttcacgcgctgcgc cgccaggaaggcatc gtgccggtgtacaaa
 1561 atggtcgatacgtgc gcggccgagttcacg tcggaaacgcccgtac tactacagcacgtac
 1621 gaggaaagaaaatgaa tcgatcgtgacggaa aaaccgagtgtcatc gtgcttggttccggc
 1681 ccgatccgcatccgc caagggattgagctt gacrracgcgaccgtc cattgcgtcttggcg
 1741 atcaaacaagccggc tacgaggcgatcatc atcaacaacaacccg gaaacggtctcgacc
 1801 gacttcagcacatcg gacaaactgtatttc gaaacgttgacggct gaagatgtgatgcat
 1861 gtcatcgaccttcgaa cagccggtcggcgtc atcgtccaattccgc ggtcagacggccatc
 1921 aacttggcggcgaa ctcgaagcgcgcggc gtccgcttgcttggg acgactcttgaagat
 1981 ttggaccgtgccgaa gaccgcgacaaattt gaacaggcgctctca gagcttggcattccg
 2041 aaaccggccggcaaa accgctgtttcggtc gaagaagcggttgcc atcgcggaagaaatc
 2101 cgttatccggtgctc gtccgcccatcgtac gtgctcggcggcggg gcgatggaaattgtc
 2161 tacaaccgcgaagag ctgcttcattacatg gaacatccccgtcgc gtcaatccgcagcac
 2221 ccggtgctccgttgac cggtacatcacccgc aaggaagtcgaagtc gacgccatcgccgac
 2281 ggcgagacggtcgtc attccggggatcatg gaacatattcgaacgg gccggcgtccattcc
 2341 ggcgactcgatcgcc gtcatatccgccgcaa acgttaagcgatgac gtcatcgcaaaaatt
 2401 accgactatacggtg aaactggcgcgcggg ctgcatattgtcggg ctgttgaacatccag
 2461 ttcgtcgtcgcaggc agcgacgtctacgtc ttggaagtgaacccg cgctcaagccgcacg
 2521 gtgccgttttaagt aaaatcaccggcgtg ccgatggcgaatctc gccacgaaagccatt
 2581 ttaggggcgaagctc gccgacatgggttat gagacgggcgtctgc ccggtgccgcccggc
 2641 gtgtacgtgaaagtg ccggtgttctcgttt gccaaattgcgcaac gtcgacatttcgctc
 2701 ggcccggaaatgaaa tcgacggcgaagtg atcggcaaagacgtg acgtttgaaaaagcg
 2761 ctctataagggccc gtcgcctccggcatc cagatccagccgcat gggcggtgccgttg
 2821 acggtggccgacaaa gacaaagaagaagcc gtcgagctggcgcgc cgcttgccgacatc
 2881 ggctaccagctgttg gcgacgaacggcacg gcggaaacgttgaaa gcggccggcattccg
 2941 gtgacggtcgtcaat aaaatccactcggcg tcaccgaacattttg gatgtcatccgccaa
 3001 ggaaggggcgaagtc gtcatcaacacgctg acgaaagggaagcag ccggaaagcgacggc
 3061 ttccgcatccgccgc gaagtcgaaaacggc atcccatgcttgacg tcgcttgataaggca
 3121 agggcgatgctgcaa gtgctcgaatcgatg acgttttcaacgaca gcgatgaccgaagag
 3181 ccggtgcgctcatga tcggccgtgaacgaa tgacggtcgcaagcc agc
                 stop pyrAb
          start pyrD  stop
```

Fig. 2. Nucleotide sequence of the *Bacillus stearothermophilus pyrAb* gene and its 5' and 3' flanking regions. Putative Shine-Dalgarno sequences for *pyrAb* and *pyrD* are depicted in underlined italics. The start codons of *pyrAb* and *pyrD* as well as the stop codons of *pyrAa* and *pyrAb* are highlighted by underlined bold letters.

malá podjednotka CPS

dihydroorotát dehydrogenáza

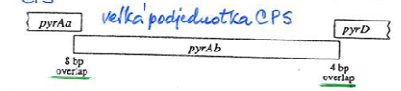


Fig. 3. Schematic representation of the *pyrAb* gene of *Bacillus stearothermophilus* within the pyrimidine biosynthetic operon.

(pyr operon) ~ 10 genů

DNA sekvence genu pyrAb = 3195 nt dlouhý ORF - kodoje polypeptidický řetězec dlouhý 1064 aminokyselin

BIOSYNTHESA PURINŮ – PURINE BIOSYNTHESIS

(u savců hlavně v játrech)

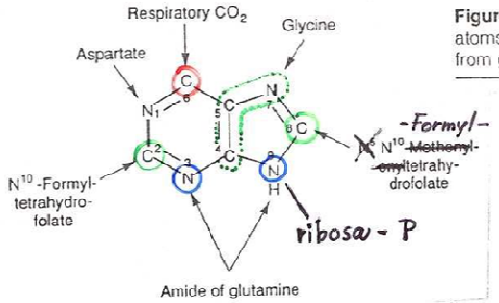


Figure 36–2. The sources of the nitrogen and carbon atoms of the purine ring. Atoms 4, 5, and 7 (shaded) derive from glycine.

1) de novo:

IMP

ATP GTP ....> RNA

dATP dGTP...> DNA

SALVAGE PATHWAY :

2) „ZACHRANNÁ" CESTA ŠETŘÍCÍ :

Adenin + PRPP ⟶ AMP + PP_i

Guanin + PRPP ⟶ GMP + PP_i

Hypoxanthin + PRPP ⟶ IMP + PP_i

HGPRT

HGPRT = hypoxanthin – guanin fosforibosyl transferase

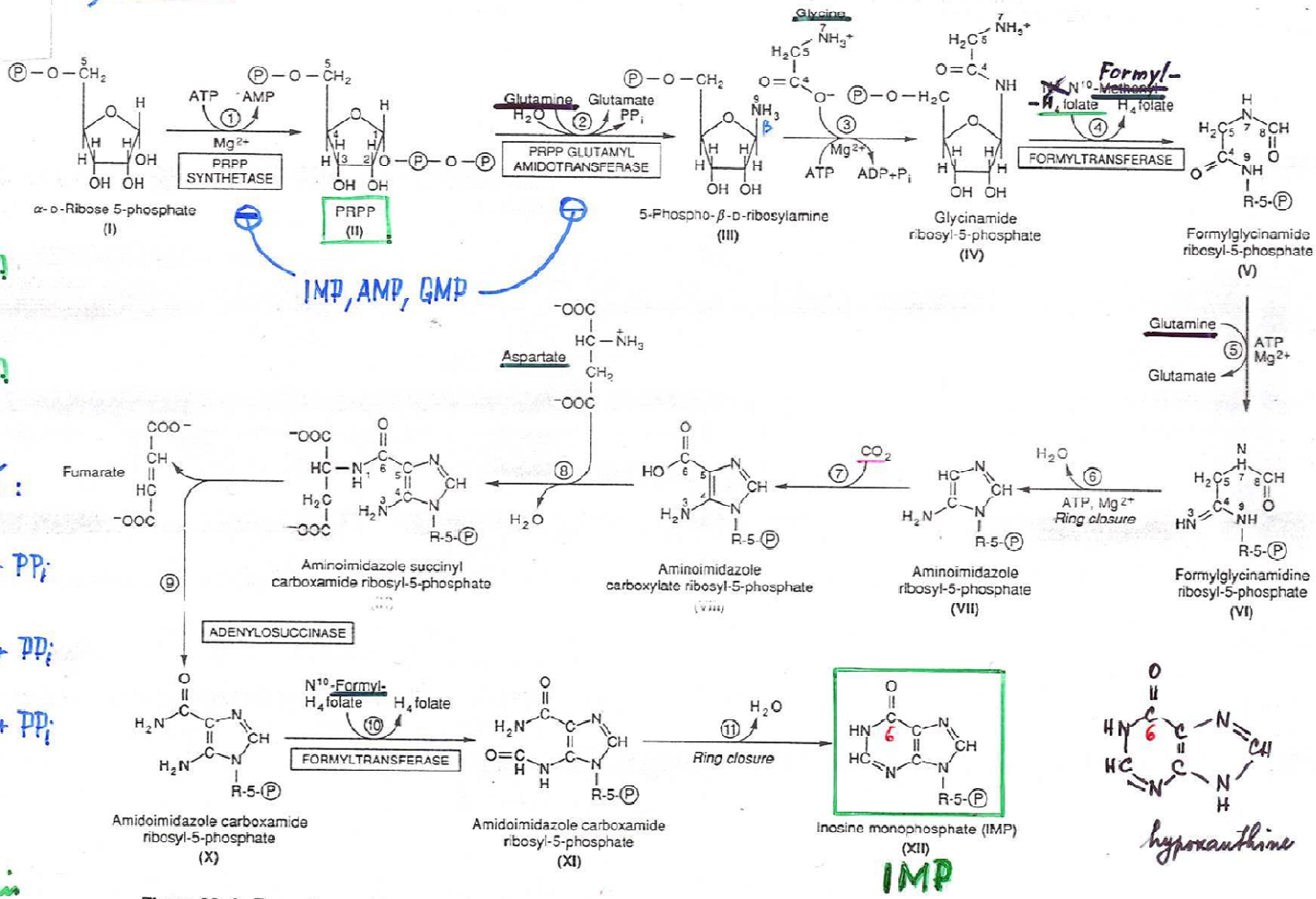


Figure 36–3. The pathway of de novo purine biosynthesis from ribose 5-phosphate and ATP. (See text for explanation.) Ⓟ, PO_3^{2-} or PO_2^-.

CONVERSION OF IMP TO AMP AND GMP

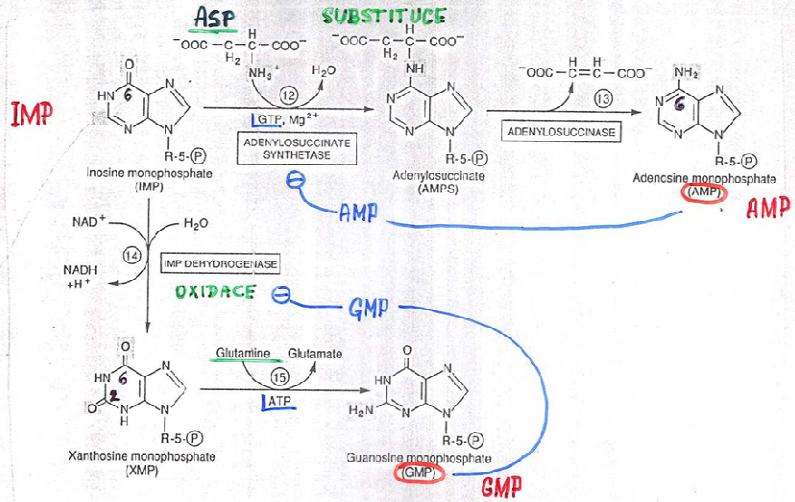


Figure 36–4. Conversion of IMP to AMP and GMP.

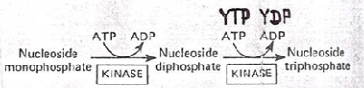


Figure 36–5. Conversion of nucleoside monophosphates to nucleoside diphosphates and nucleoside triphosphates.

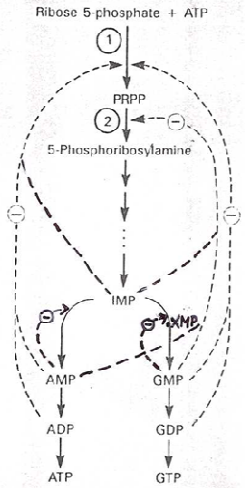


Figure 36–8. Control of the rate of de novo purine nucleotide synthesis. Solid lines represent chemical flow, and broken lines represent feedback inhibition (⊖) by end products of the pathway. Reactions ① and ② are catalyzed by PRPP synthetase and by PRPP glutamyltransferase (see Fig 35–3), respectively.

amido

FORMATION OF DEOXYRIBONUCLEOTIDES

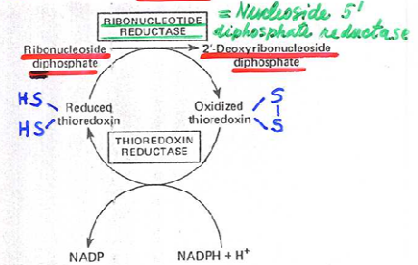
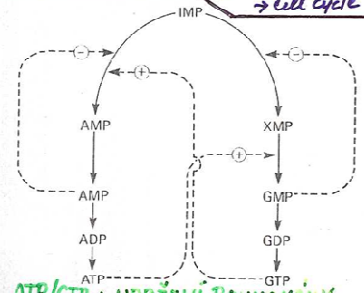


Figure 36–10. Reduction of ribonucleoside diphosphates to 2'-deoxyribonucleoside diphosphates.

Inhibitors of ribonucleotide reductase are potent inhibitors of DNA synthesis → → cell cycle



ATP/GTP : UDRŽENÍ ROVNOVÁHY

Figure 36–9. Regulation of the interconversion of IMP to adenosine nucleotides and guanosine nucleotides. Solid lines represent chemical flow, and broken lines represent both positive (⊕) and negative (⊖) feedback regulation.

PURINE DEGRADATION:
DEGRADACE PURINŮ

DNA, RNA → (DNases, RNases, Endonucleases; hydrolysis) → NUKLEOTIDY (5'mono?) → (nucleotidases; hydrolysis) → NUKLEOSIDY → (nucleosidases (nucleoside phosphorylases); phosphorolysis) → BASE + ribosa-1-P → ribosa-5-P → PRPP (šetřící cesta (salvage))

BASE → DEGRADACE

d-ribosa-1-P

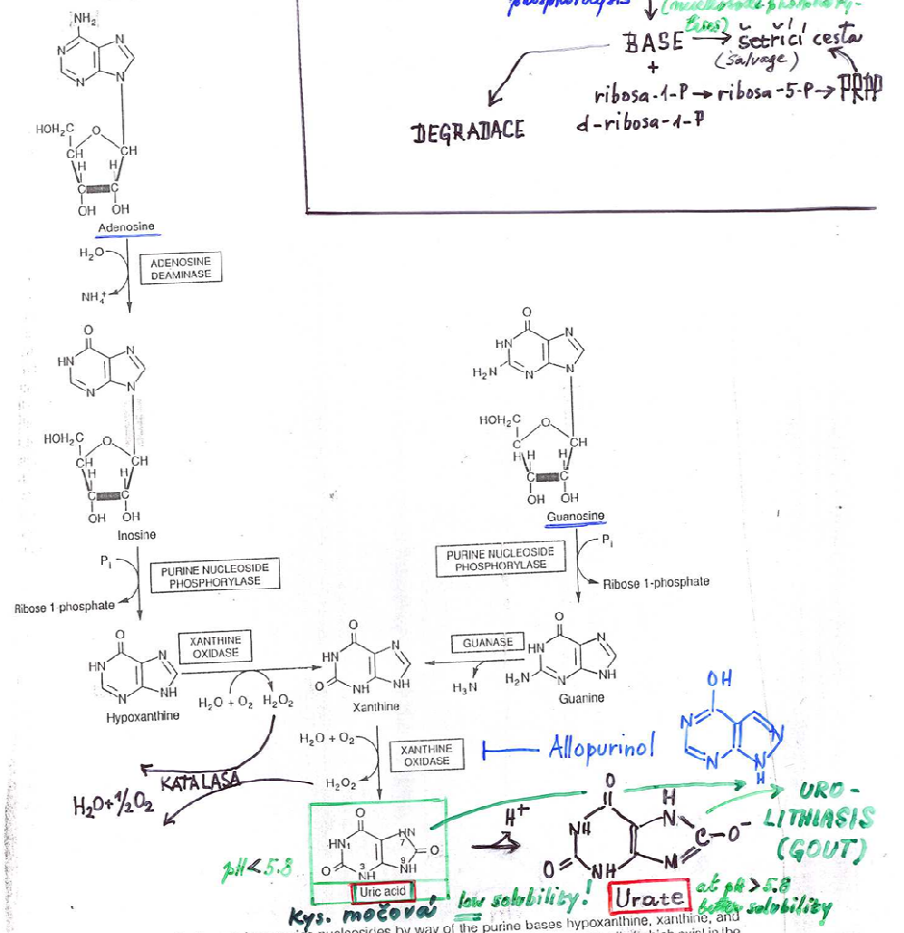


Figure 36–1. Formation of uric acid from purine nucleosides by way of the purine bases hypoxanthine, xanthine, and guanine. Purine deoxyribonucleosides are degraded by the same catabolic pathway and enzymes, all of which exist in the mucosa of the mammalian gastrointestinal tract.

URO-LITHIASIS (GOUT)

in mammals but not in primates
savci, ale ne vyšší primáti

URICASE

Allantoin (soluble)

VÝHODA: ÚČINNÝ VYCHYTÁVAČ ROS, tj. ANTIOXIDANT → ? PRODLOUŽENÍ ŽIVOTA

DEGRADACE NUKLEOTIDŮ = PYRIMIDINY
PYRIMIDINE DEGRADATION

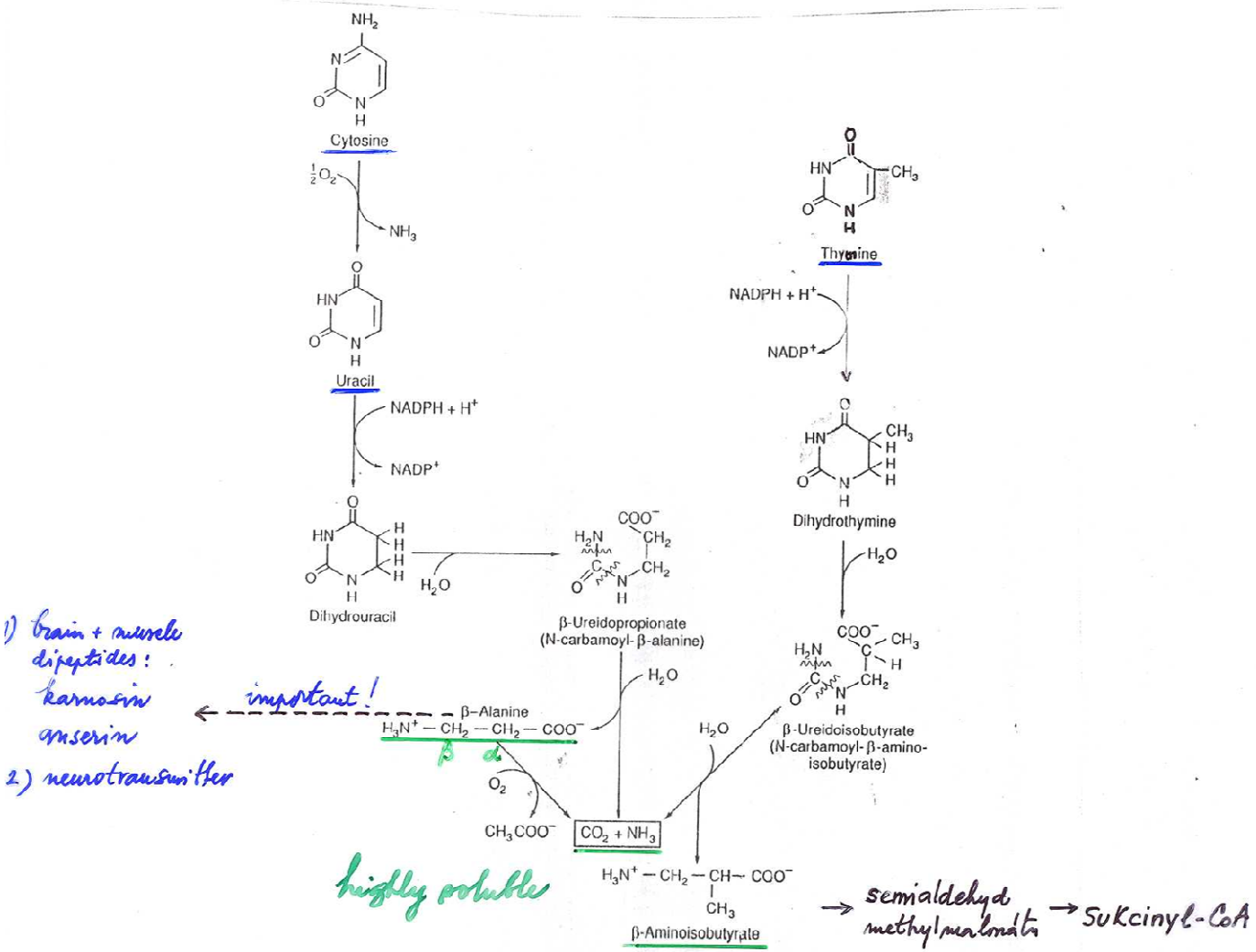


Figure 36–15. Catabolism of pyrimidines.

Table 35–1. Inherited disorders of purine metabolism and their associated enzyme abnormalities.

Clinical Disorder	Defective Enzyme	Nature of the Defect	Characteristics of Clinical Disorder	Inheritance Pattern
Gout	PRPP synthetase	Superactive (increased V_{max})	Purine overproduction and overexcretion	X-linked recessive
Gout	PRPP synthetase	Resistance to feedback inhibition	Purine overproduction and overexcretion	X-linked recessive
Gout	PRPP synthetase	Low K_m for ribose 5-phosphate	Purine overproduction and overexcretion	Probably X-linked recessive
Gout	HGPRTase*	Partial deficiency	Purine overproduction and overexcretion	X-linked recessive
Lesch-Nyhan syndrome	HGPRTase* (SEŘÍCÍ CESTA)	Complete deficiency →↑↑ PRPP	Purine overproduction and overexcretion; cerebral palsy and self-mutilation. →↑↑ urát	X-linked recessive + NEPŘÁTELSKÉ CHOVÁNÍ
Immune deficiency	Adenosine deaminase	Severe deficiency SCID *	Combined (T cell and B cell) immunodeficiency, deoxyadenosinuria	Autosomal recessive
Immune deficiency	Purine nucleoside phosphorylase	Severe deficiency	T cell deficiency, inosinuria, deoxyinosinuria, guanosinuria, deoxyguanosinuria, hypouricemia	Autosomal recessive
Renal lithiasis	Adenine phosphoribosyltransferase	Complete deficiency	2,8-Dihydroxyadenine renal lithiasis	Autosomal recessive
Xanthinuria	Xanthine oxidase	Complete deficiency	Xanthine renal lithiasis, hypouricemia	Autosomal recessive

*HGPRTase = hypoxanthine-guanine phosphoribosyltransferase.

* léčení: génová terapie (10/11 dětí vyléčeno × leukemie 2x)
(génová transplantace pomocí retrovirového konstruktu)
* SCID: severe combined immunodeficiency
* nadbytek purinů, ne nukleotidů!

NENORMÁLNÍ CHOVÁNÍ
až
SEBEMRZAČENÍ
způsobeno chybějícím JEDINÉHO ENZYMU!

* HGPRT = hypoxanthin-guanin fosforibosyl transferáza ("sběrná" cesta)

Table 36–3. Inherited disorders of pyrimidine metabolism and their associated enzyme abnormalities.

Clinical Disorder	Defective Enzyme	Characteristics of Clinical Disorder	Inheritance Pattern
β-Aminoisobutyric aciduria	Transaminase	No symptoms; frequent in Orientals.	Autosomal recessive
Orotic aciduria, type I	Orotate phosphoribosyltransferase and orotidylate decarboxylase	Orotic acid crystalluria, failure to thrive, and megaloblastic anemia. Immune deficiency. Remission with oral uridine.	Autosomal recessive
Orotic aciduria, type II	Orotidylate decarboxylase	Oroticinuria and orotic aciduria, megaloblastic anemia. Remission with oral uridine.	Autosomal recessive
Ornithine transcarbamoylase deficiency	Ornithine transcarbamoylase	Protein intolerance, hepatic encephalopathy, and mild orotic aciduria.	X-linked recessive.

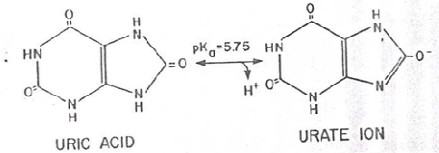


FIG. 49-10 Ionization of uric acid. The weakly acidic nature of uric acid is due to ionization of hydrogen atoms. Ionization at position 9 (pK_a = 5.75) is shown above. The ionized forms of uric acid readily form salts. In extracellular fluids in which sodium is the principal cation, about 98 percent of uric acid is in the form of the monosodium salt at pH 7.4. The crystals, which form in the synovial fluid or the tophi of gouty patients when solubility limits are exceeded, are composed of monosodium urate monohydrate.

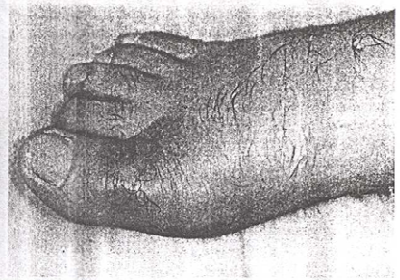


FIG. 49-1 Acute gouty arthritis of the first metatarsophalangeal joint (podagra). Intense swelling and discoloration (redness) spreading well beyond the confines of the joint (periarticular inflammation) are typical of acute gout.

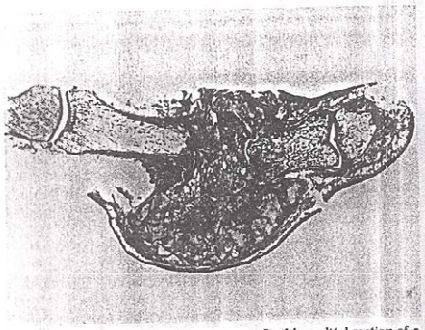


FIG. 49-2 Chronic tophaceous gout. In this sagittal section of a surgical specimen, complete destruction of the first metatarsophalangeal joint is evident. Light microscopy with polarization confirmed replacement of articular and adjacent bony structures as well as the subcutaneous layers by fibrous tissue containing relatively few chronic inflammatory cells but masses of monosodium urate crystals. The tarsometatarsal and interphalangeal joints remain intact. *(Courtesy of M. A. Simon, University of Chicago.)*

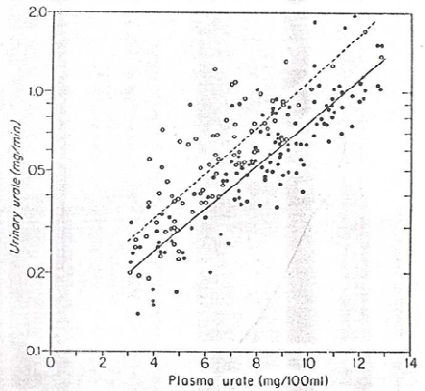
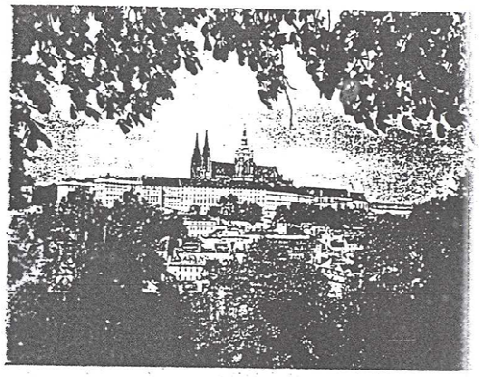


FIG. 49-14 Urate excretion at varying concentrations of plasma urate. Urinary urate is expressed in mg/(min ● 100 ml of glomerular filtrate). The slopes of the normal (○—○) and gout (●—●) regressions are not significantly different from each other. The average gouty individual must have a serum urate 1.7 mg/dl higher than normal in order to equal the normal rate of urate excretion. *(From P. A. Simkin.[141] Used by permission.)*



Prof. Dr. Jan Horbaczewski
(1854 – 1942)

1. chemická syntéza
KYSELINY MOČOVÉ
1st chemical synthesis
of URIC ACID ~ 1882

Aus dem LXXXVI. Bande der Sitzb. der k. Akad. der Wissensch. II. Abth. Nov.-Heft. Jahrg. 1882.

Synthese der Harnsäure.

Von Dr. Johann Horbaczewski,
Assistenten am Laboratorium für angewandte medicinische Chemie in Wien.

(Aus dem Laboratorium des Professors E. Ludwig.)

(Vorgelegt in der Sitzung am 2. November 1882.)

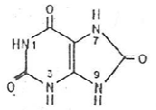
Monatshefte für Chemie 10: 624 – 641 (1889)

Untersuchungen über die Entstehung der Harnsäure im Säugethierorganismus

von

J. Horbaczewski.

(Vorgelegt in der Sitzung am 4. Juli 1889.)



Uric acid

Monatshefte für Chemie 12: 221 – 275 (1891)

Beiträge zur Kenntniss der Bildung der Harnsäure und der Xanthinbasen, sowie der Entstehung der Leucocytosen im Säugethierorganismus.

(Nach Versuchen, die zum Theile von den Herren Sadowenj, Mrazek und Formanek ausgeführt wurden.)

Von

J. Horbaczewski.

(Vorgelegt in der Sitzung am 16. April 1891.)