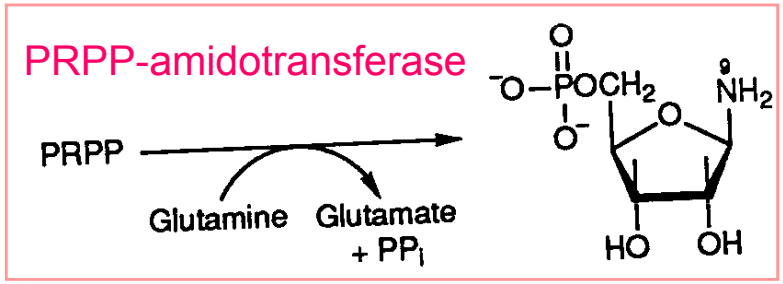
Purine and uric acid metabolism disorders

Clinical biochemistry 2008 (J.S.)

Synthesis of purine *de novo* :



Rate-limiting step

Allosteric inhibition by the end-products - AMP, GMP

Glycine

Methenyl-H_4folate

C_1

FH_4

Glutamine

$-NH_2$

ADP

ATP

P_i

CO_2

Aspartate

$-NH_2$

Formyl-H_4folate

C_1

H_2O

Inosine-5´-phosphate

AMP

GMP

Purine salvage pathways (salvage reactions, reutilization of purines)**:**

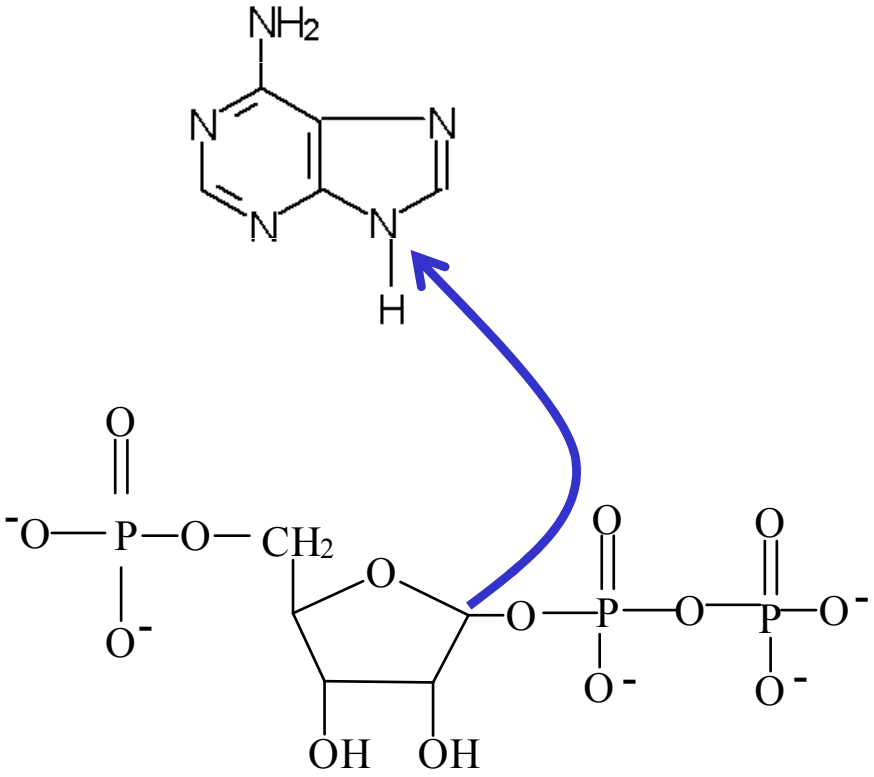
Adenosine + ATP → adenosine monophosphate + ADP

Adenosine kinase

Purine (hypoxanthine, guanine, adenine) + PRPP →

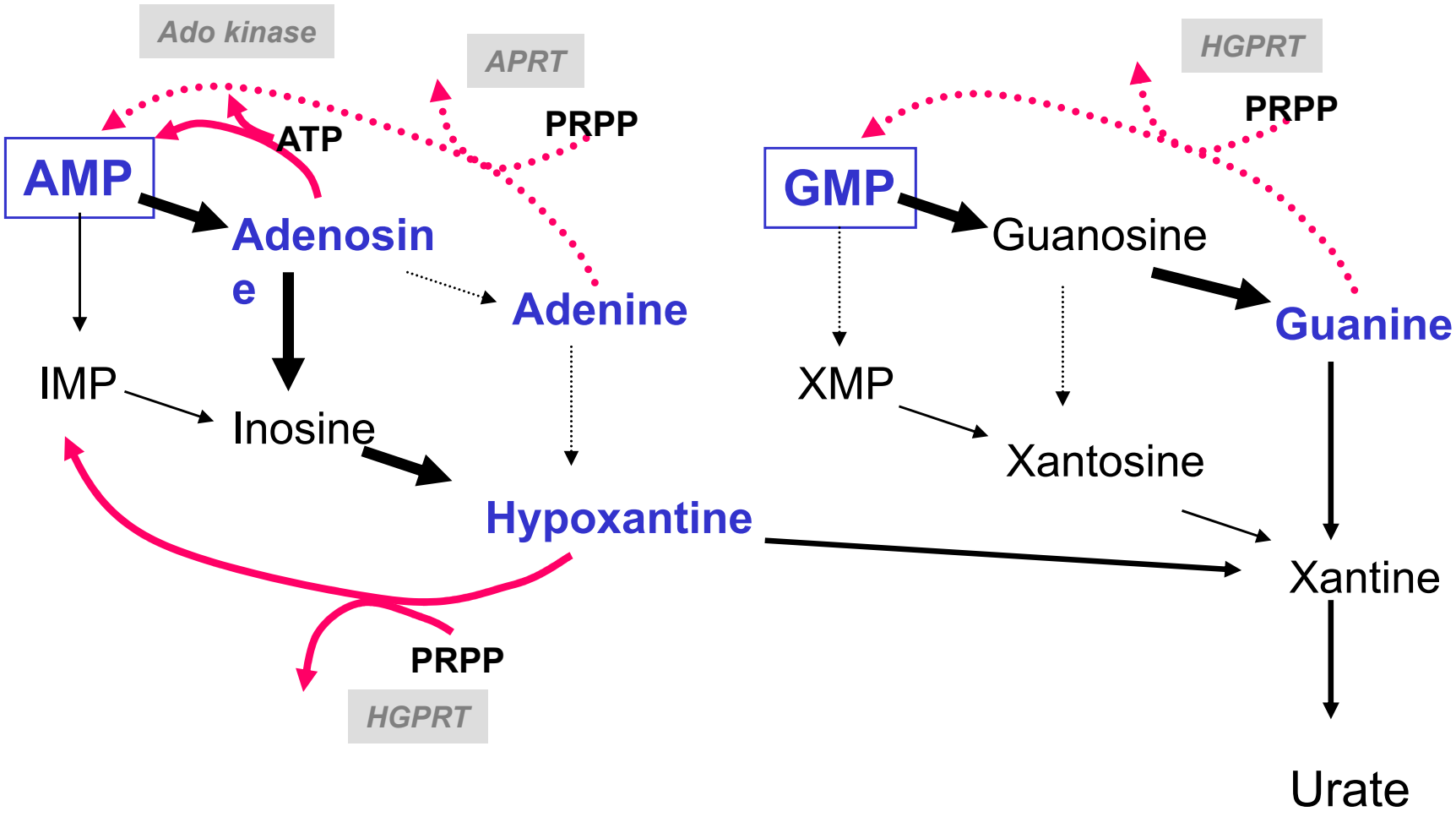
→ purine nucleotide monophosphate + PP_i

Phosphoribosyl transferases **HGPRT** a **APRT**

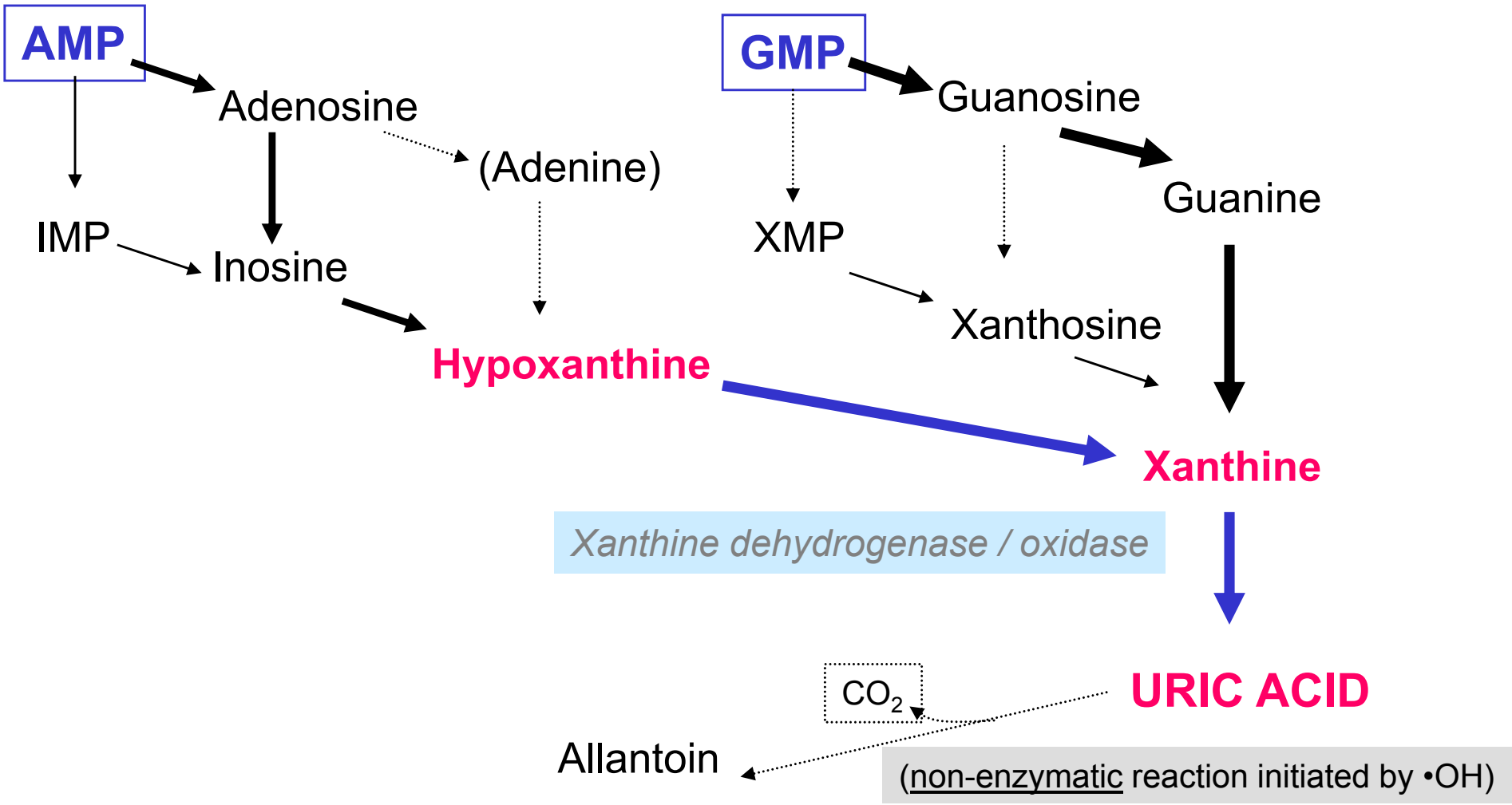


Salvage pathways occur in considerable extent in tissues with low synthesis of purines *de novo*, namely in **CNS, bone marrow, and leukocytes**. At low concentration of purine nucleotide catabolism end-products, reutilization of purine bases through salvage reactions in preferred over the purine degradation..

Purine salvage pathways (salvage reactions, reutilization of purines)**:**

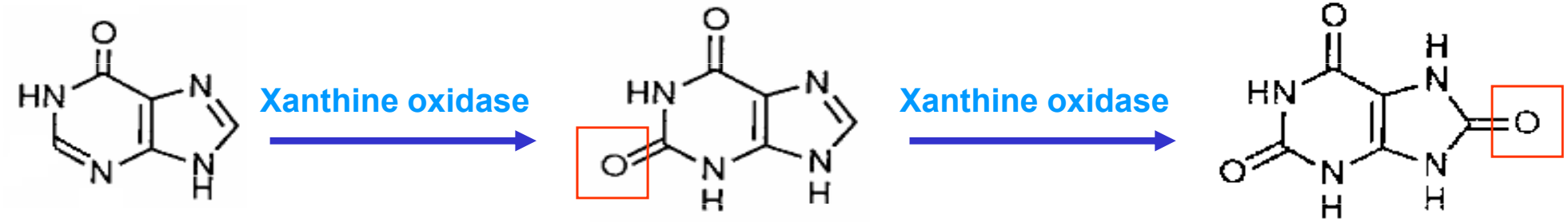


Purine nucleotide degradation:



In humans and uricotelic animals *urate oxidase* that catalyzes the oxidative splitting of urate to allantoin is absent !

Xanthine oxidase oxidizes hypoxanthine to xanthine and this same enzyme oxidizes xanthine to uric acid. The lactam forms of those compounds are shown:



Hypoxanthine
(6-hydroxypurine)

Xanthine
(2,6-dihydroxypurine)

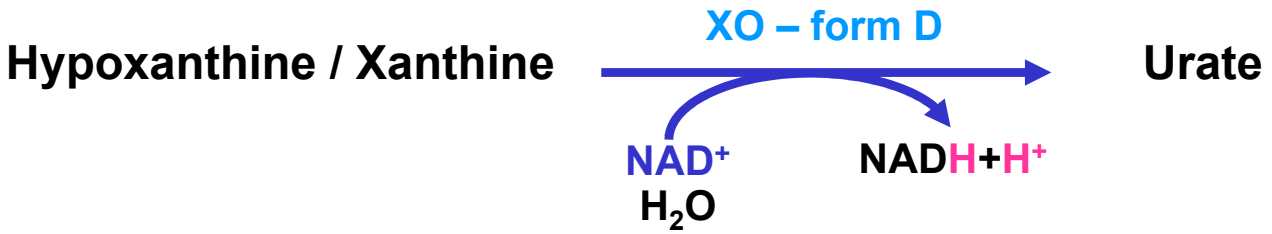
Uric acid
(2,6,8-trihydroxypurine)

Uric acid is the **final metabolic product of purine catabolism in humans** and other primates (also in uricotelic animals - birds and land-dwelling reptiles), and is excreted in the urine.

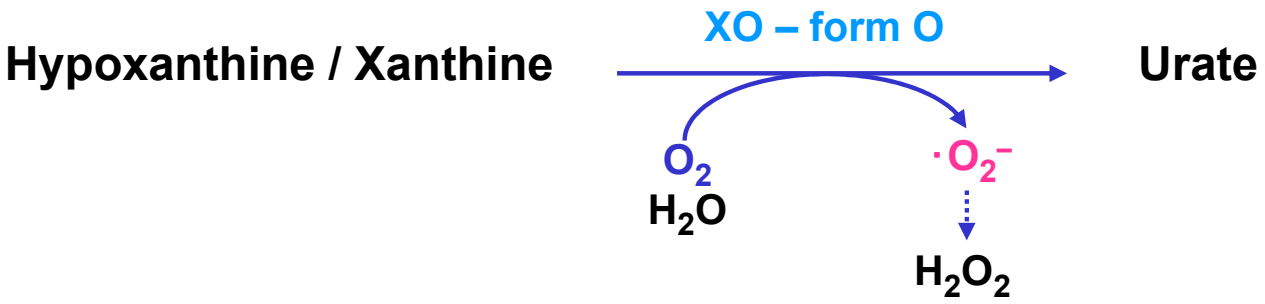
Other organisms, incl. most mammals, metabolize urate to allantoin or other simpler forms.

Xanthine oxidase ((XO) is a molybdenum- and iron-containing flavoprotein, which may exist in two forms – D-form and O-form.

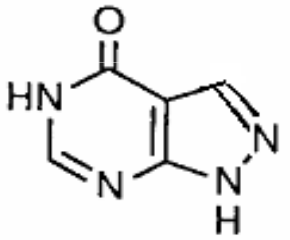
Under physiological conditions, the **D-form** catalyses the oxidation (hydroxylation) as a **dehydrogenase**, the acceptor of electrons is NAD^+.



In tissue hypoxia, the D-form is transformed into the **O-form** by the proteolytic splitting of 20 amino acyl residues. The O-form is a **oxygenase**, the acceptor of electrons is dioxygen. Then the reaction produces superoxide anion-radicals which dismutase into hydrogen peroxide by the action of SOD (the cause of so-called "**reperfusion injury**" after the restoration of a sufficient oxygen supply to the ischaemic tissue).



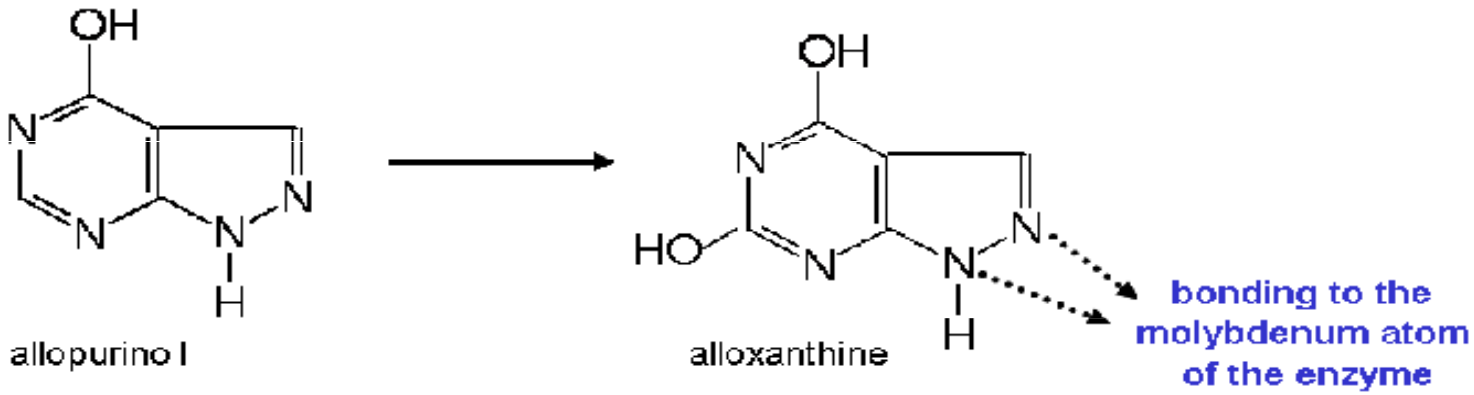
Allopurinol inhibition of xanthine oxidase



Allopurinol

Hyperuricacidaemias and gouty syndromes are usually treated with **allopurinol**, a "suicide" **inhibitor of xanthine oxidase**.

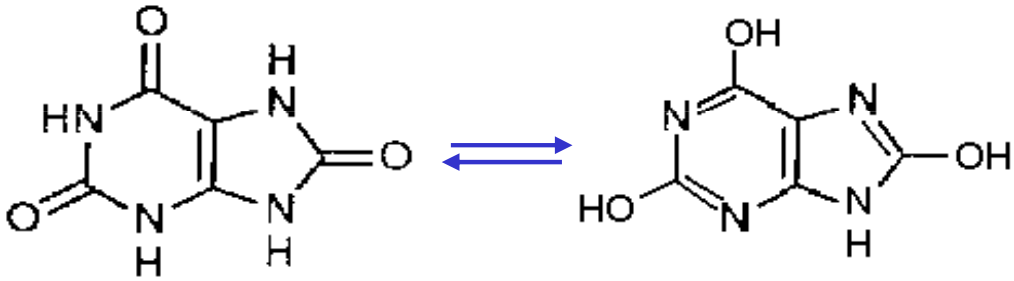
Instead of urate, the final products of purine catabolism are then hypoxanthine and xanthine, which are more soluble and thus more easily excreted into the urine. Allopurinol is oxidized by xanthine oxidase to 2-hydroxyallopurinol (alloxanthine) that remains bound to the molybdenum atom of the enzyme, thereby inactivating it.



Alloxanthine is released from the enzyme with the half-life of about 5 h. Allopurinol thus acts as a **uricostatic** (Milurit, Urosin, etc.). After allopurinol, oxidation to uric acid is inhibited.

Uric acid (2,6,8-trihydroxypurine)

$M_r = 168$



Lactam form Lactim form

is a very weak diprotic acid. The pK_{a1} equals 5,75, therefore the predominant form of uric acid in body fluids is the **monovalent hydrogen urate anion**.

Unfortunately, uric acid and its urate salts have a **low solubility** in water. The average serum concentrations in humans (the upper limit ~ 400 μmol/l) is close to the solubility limit, above which the precipitation of needle-shaped monosodium urate crystals may begin. Urate crystals then occur frequently as deposites in the soft tissues, particularly in interstitium of the kidney and in joints, or in renal stones.

On the other hand, an increase in urate concentration in primates has a markedly beneficial action. Urate is a **highly effective antioxidant** - a scavenger of reactive oxygen species which is about as effective as ascorbate taken in the diet.

In the reaction with oxygen radicals, urate is changed into the radical that is non-enzymatically (spontaneously) transformed into allantoin. Primates thus excrete small amounts of allantoin proportionally to their exposure to oxidative stress, although they lack the urate oxidase.

Elimination of urate

The total amount of uric acid in a normal man is approximately 7 mmol (1.2 g), about 2 mmol being produced each day and eliminated.

• About 20 % of the eliminated amount is excreted **in the bile** and is partially degraded in the GIT by intestinal microflora to CO_2 and NH_3.

• Approximately **80 % of urate is excreted in the urine**:

- **filtrated** freely by the glomerulus,
- over 90 % of filtrated urate **reabsorbed in proximal tubules**,
- **tubular secretion**, the mechanism is the same as that for other organic acids (and so the urate excretion diminished competitively at high secretion of lactate, acetoacetate, 3-hydroxybutyrate, and during therapy with thiazide diuretics or acetylsalicylate),
- **postsecretion reabsorption** in the distal parts of nephron that is inhibited (urate excretion increased) by **uricosurics** as, e.g. probenecid (Benemid) or NSAD with exception to salicylates.

Plasma clearance of urate is rather low, about 0,1 - 0,2 ml / s**,** i.e. the **excretion fraction F/E** of urate is only **10 %** on the average.

Investigation of purine metabolism

Basal:

Serum uric acid – reference range **200 - 420 μmol/l** in men,
140 - 360 μmol/l in women.

Urinary excretion without purine restriction 1.5 – 4.4 mmol/d (250 - 750 mg/d)
low-purine diet < 2.5 mmol/d (< 420 mg/d)

Kaufman's index - urine ratio urate/creatinine 0,2 - 0,4 (mmol/mmol)

In hyperuricaemia:

Serum uric acid after a purine load

Plasma urate clearance and excretion fraction (to exclude a renal disorder)

Search for dyslipidaemia, decreased glucose tolerance, ischaemic heart disease, test for the liver functions.

Special tests:

Urinary excretion of xanthine, blood activities of HGPRT and adenine deaminase.

Hyperuricaemia

Mild latent hyperuricaemia without clinical symptoms occurs in approximately 4 - 10 % of healthy individuals. (Is it the sign of good adaptation to an increased production of reactive oxygen species ?)

Defects in purine metabolism

Inborn gouty syndromes

Gout (primary gout) is a metabolic disease caused by accumulation of excess urate in body fluids due to the miscontrol of endogenous purine *de novo* synthesis supported by high intake of purines in the diet. Gout predominantly affects males, the peak incidence between the fourth and sixth decades. It is rare in adolescents, extremely rare in women.

The crystals of monosodium urate are deposited in joints (painful arthritis), around them, or in other tissues, particularly in kidneys (may result in urate **urolithiasis** or **renal failure**).

Asymptomatic stage – characteristic acute gout attacks (big toe is the typical site of the first attack) – progression to chronic gout (joint swelling and destruction, tophi in the ears and around joints).

Lesch-Nyhan syndrome is a rare nearly complete deficit in hypoxanthine-guanine phosphoribosyl transferase (HGPRT, see scavenger pathways of nucleotide synthesis), that stimulates an overproduction of purines.
Children with this disease exhibit mental retardation, spasticity, compulsive self-destructive behaviour (biting their fingers and lips) and aggression toward others.

An incomplete deficit in HGPRT leads in adults only to the formation of renal stones followed by the gouty arthritis years later (**Kelley-Seegmiller syndrome**).

Familial hyperuricaemic nephropathy of juveniles is a serious defect of urate renal excretion of young persons that leads progressively to the renal failure.

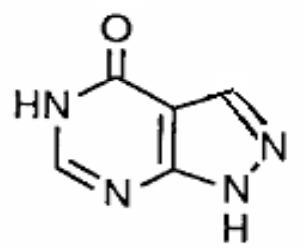
Immunodeficiency syndromes

The autosomal recessive form of severe combined immunodeficiency syndrome (**SCIDS**) has its cause in approximately 50 % of patients in a genetic deficiency of adenosine deaminase, which is oft associated with defects of 5´-nucleotidase and nucleoside phosphorylase, too. All these enzymes take part in the purine salvage pathway.

"Secondary" hyperuricaemia and/or gout

may have its cause in

- an **intensified desintegration of cells**, e.g. in myelo- and lymphoproliferative diseases, during the cytostatic cancer chemotherapy, in **hypercatabolic states** (prolonged starvation, chronic alcoholism, high doses of corticosteroids),
- a **decreased renal excretion of urate** in renal diseases or due to the side-effect of some kind of diuretics (thiazides, also furosemide).



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Hypouricaemia

- decrease in the purine synthesis – e.g. in acute liver disorders,
- decrease in the activity of xanthine dehydrogenase – xanthinuria,
  – allopurinol therapy
- decreased tubular urate reabsorption – some tubular defects,
  – inherited renal hypouricaemia,
  – uricosurics - probenecid, ketazon and other NSAD

Purine content in foodstuffs

Purines in mg per 100 g of edible fraction

Meat, poultry		**Fish**		**Cereals and pastry**	
calves thymus	**400**	(pickled) herring	**790**	oat flakes	30
spleen	**104**	salted anchovy	**540**	wholemeal bread	14
liver	**95**	sardine	**120**	white bread, rolls	8
kidneys	**80**	carp, trout	55	flour	0
horse meat	**80**	pike	48	**Vegetables**	
tongue	55	salmon	22	potatoes	6
veal, pork	48	**Milk, eggs**		peasecod	**80**
beef	40	egg-yolk	5	lentils	**70**
ham	24	white of an egg	1	peas, beans	45
chicken	40	eggs	2	spinach	23
little pigeons	**80**	milk	1	cauliflower, celery	10