Biochemistry of Kidney and Liver

Lecture # 30-31

Lecturer Alexander Koval

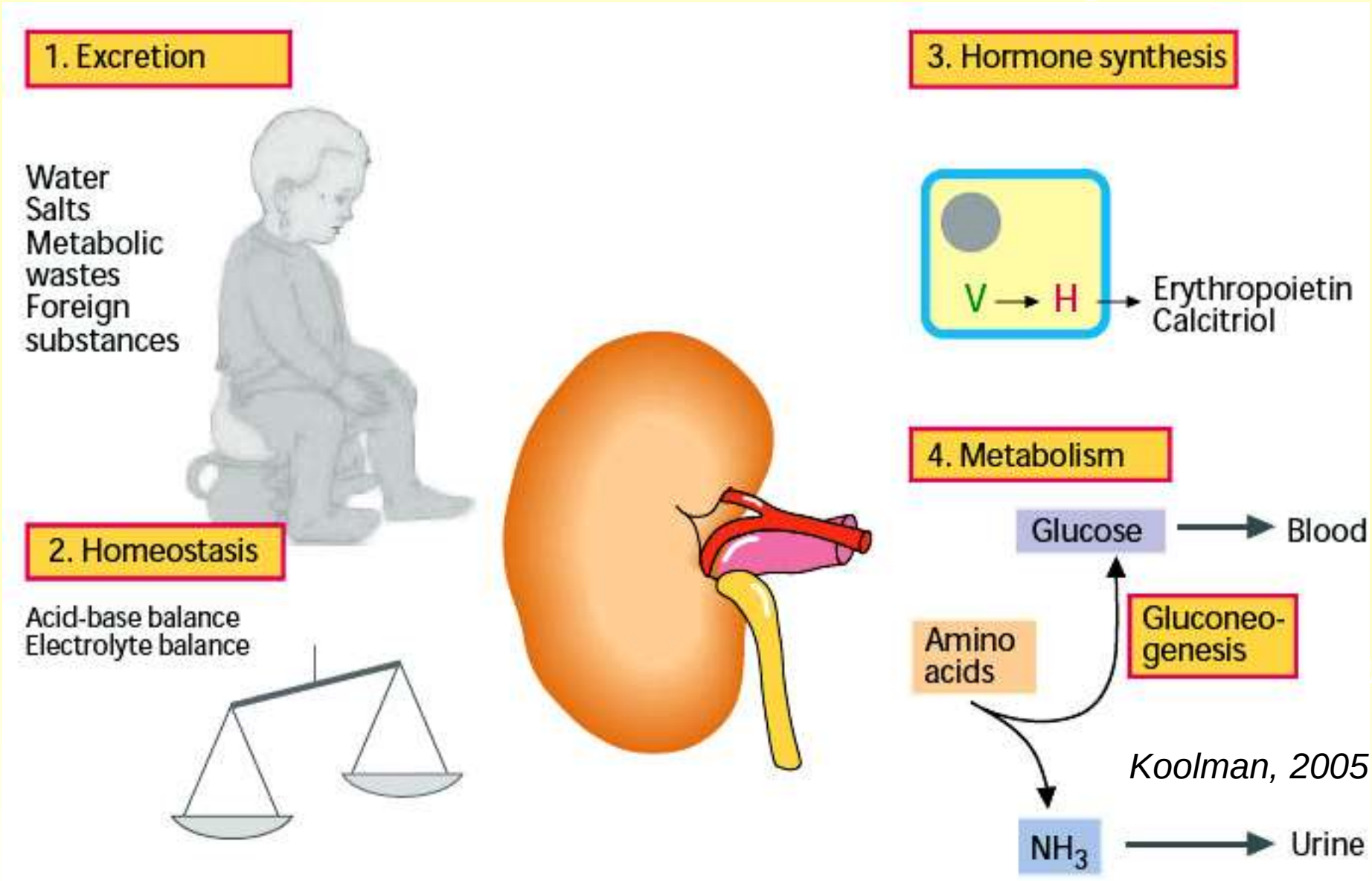
Biochemistry of Kidney

Lecture # 30

There are 2 Main Homeostatic Functions of Kidneys

- Excretory
  - the most important
  - the production of **urine**.
    - carries various waste materials out of the body.
- Non-excretory
  - Regulation of water, electrolyte and acid-base balance
  - Regulation of arterial pressure
    - Renin
  - Regulation of RBC
    - Erithropoietin
  - Formation of active form of vitamin D (calcitriol).

Functions of the Kidneys



Excretion of Compounds in the Urine

Component	g/24 hr	Nitrogen (mmol)
H_2O	1000	-
SO_4^{2-}	2-5	-
HPO_4^{2-}	2-5	-
K^+	1-2	-
Urea	12-20	400-650
Creatinine	1-1.8	25-50
Uric acid	0.2-0.8	4-16
NH_4^+	0.2-1 (up to 10 in acidosis)	11-55 (up to 550 in acidosis)

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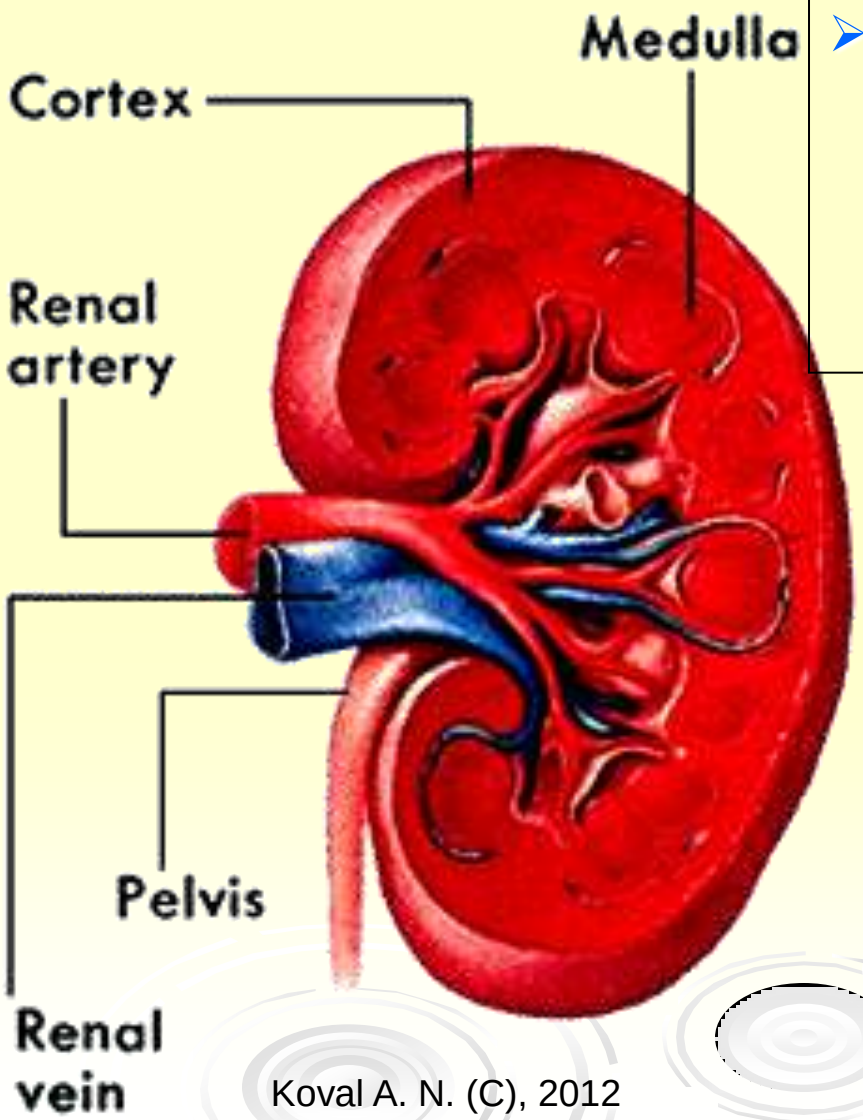
Metabolism of Kidney

- In the **cortex**
  - Aerobic metabolism
    - Aerobic glycolysis



- In the **medulla**
  - Anaerobic metabolism
    - Anaerobic glycolysis

Concentrating urine and transporting it in the kidneys are of very high energy demands

- In the proximal tubule, the ATP is obtained from oxidation of *fatty acids, ketone bodies,* and several *amino acids.*
  - Less: lactate, glycerol, and citric acid.
- In the distal tubule and Henle's loop, *glucose* is the main energy substrate.
- The endothelial cells in the proximal tubule are also capable of *gluconeogenesis.*
  - The substrates: amino acids.
  - $-NH_2 \rightarrow NH_3$ (buffering urine).
- Enzymes for peptide degradation and the amino acid metabolism occur in the kidneys at high levels of activity (e. g., amino acid oxidases, amine oxidases, glutaminase).

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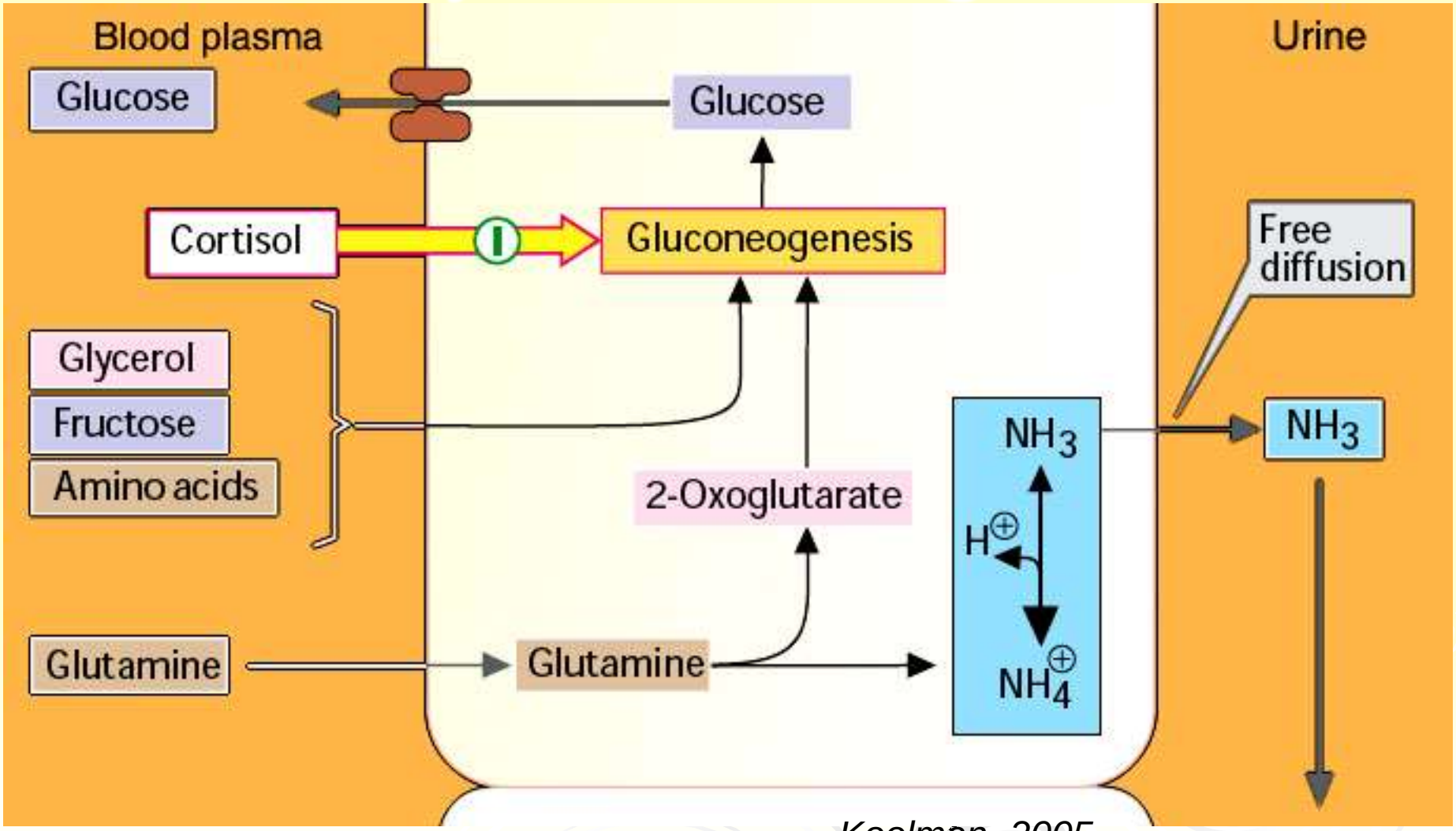
Carbohydrate Metabolism in Kidney

- Regulation of blood glucose level
  - Renal threshold for glucose is 9.99 mmol/l
- Pentose phosphate pathway
  - NADPH:
    - detoxication,
    - synthesis of fatty acids, cholesterol, amino acids, pentoses.
- Gluconeogenesis
  - 50% of GNG are in kidneys (50% in liver)
  - Activated in acidosis
  - Less sensitive to hormones, comparing to liver
  - Predominant substrates are lactate, pyruvate, citrate.

Kidney: Gluconeogenesis



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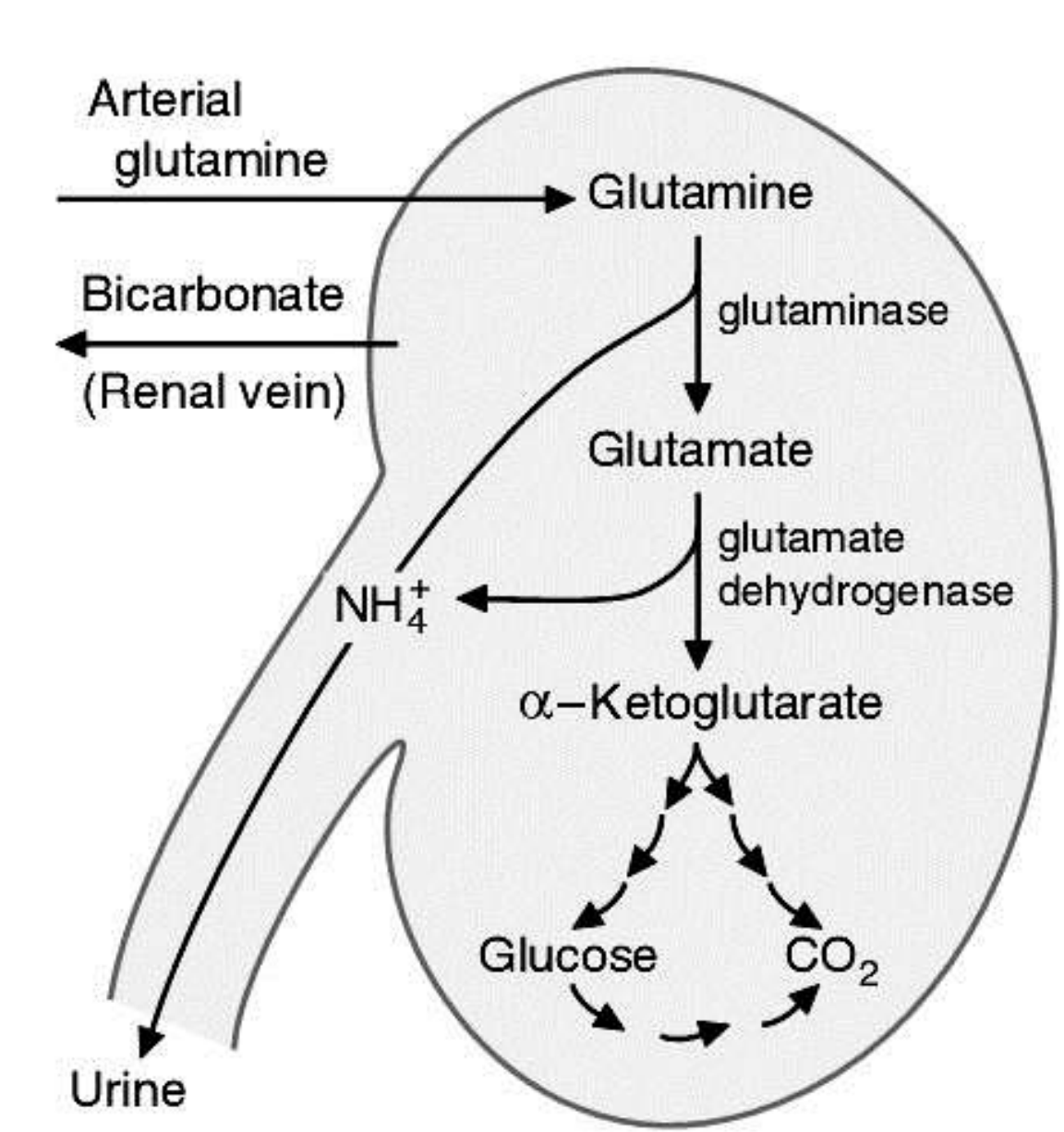
Protein Metabolism in Kidney

- Most proteins (M > 60 000 D) are filtrated and reabsorbed (endocytosed) in the tubules.
- All amino acids are reabsorbed by several transporting mechanisms
  - Negatively charged AA (glu, asp)
  - Basic AA (arg, lys, orn)
  - Neutral AA (ala, leu, etc.)
  - Smallest AA (gly).
- 40% of insulin are degraded in the kidney.

Renal Glutamine Metabolism



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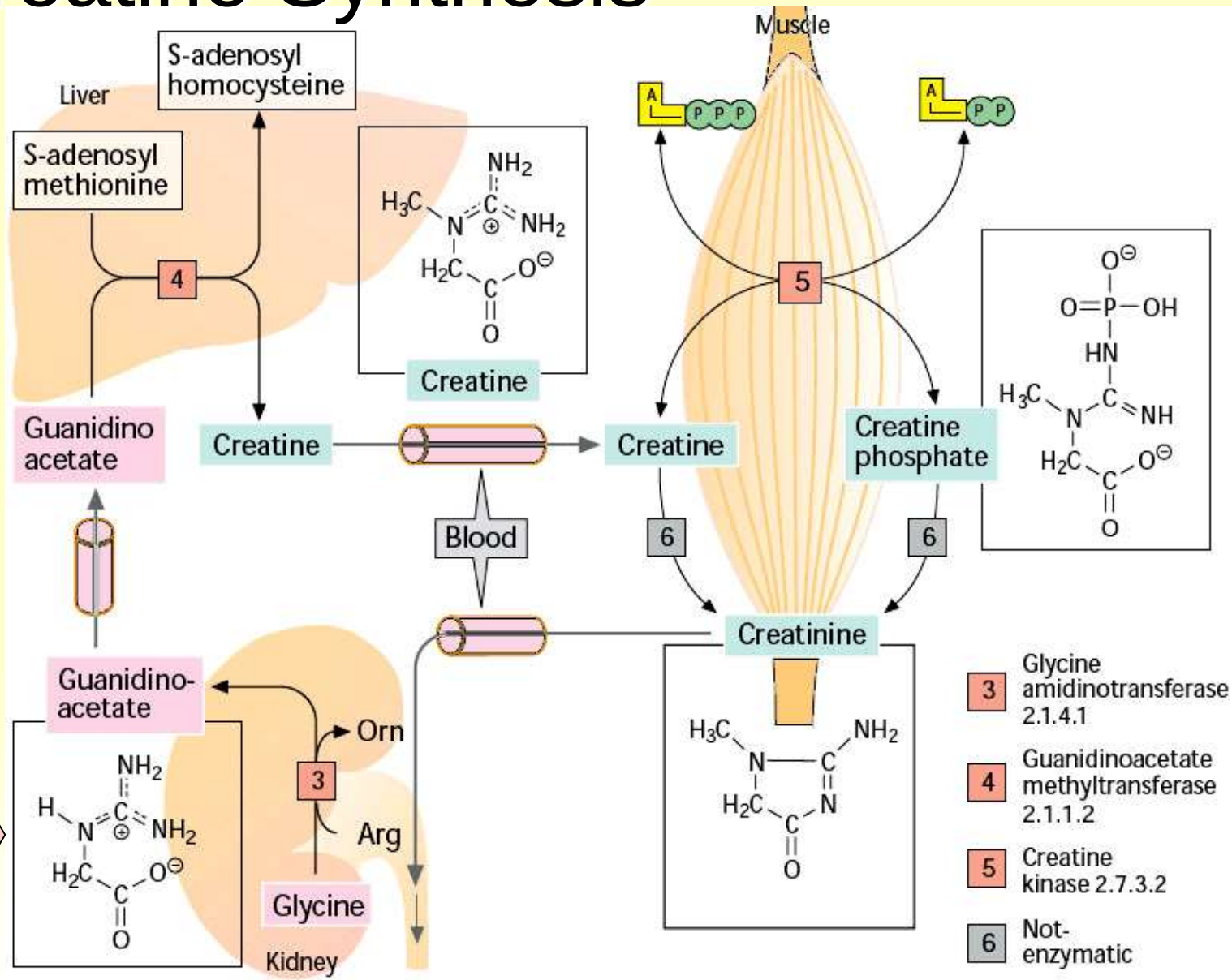
Creatine Synthesis

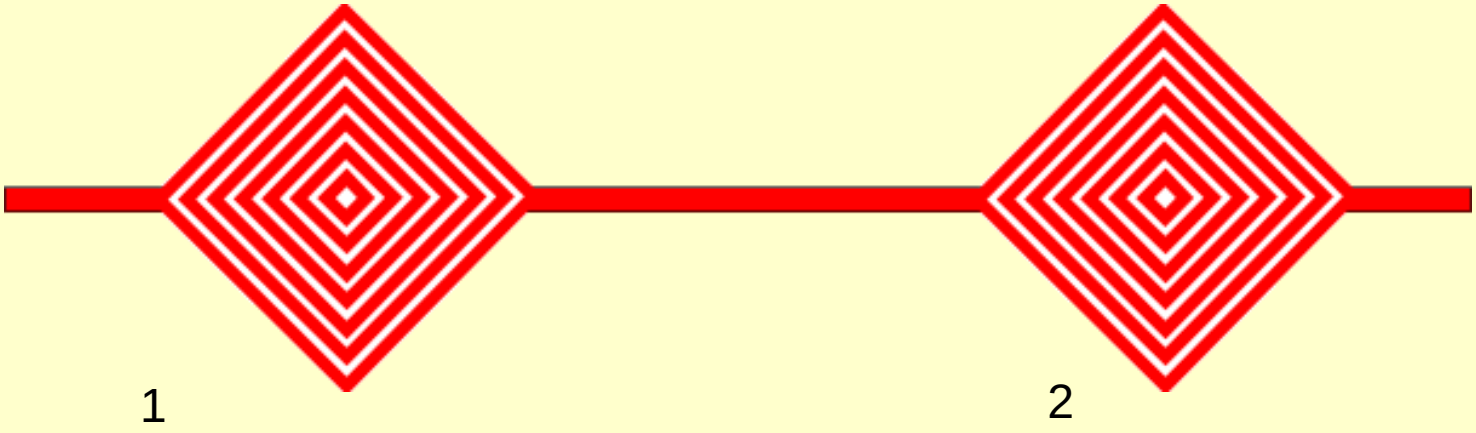
1st step of creatine synthesis occurs in kidney.



Lipid Metabolism in Kidney

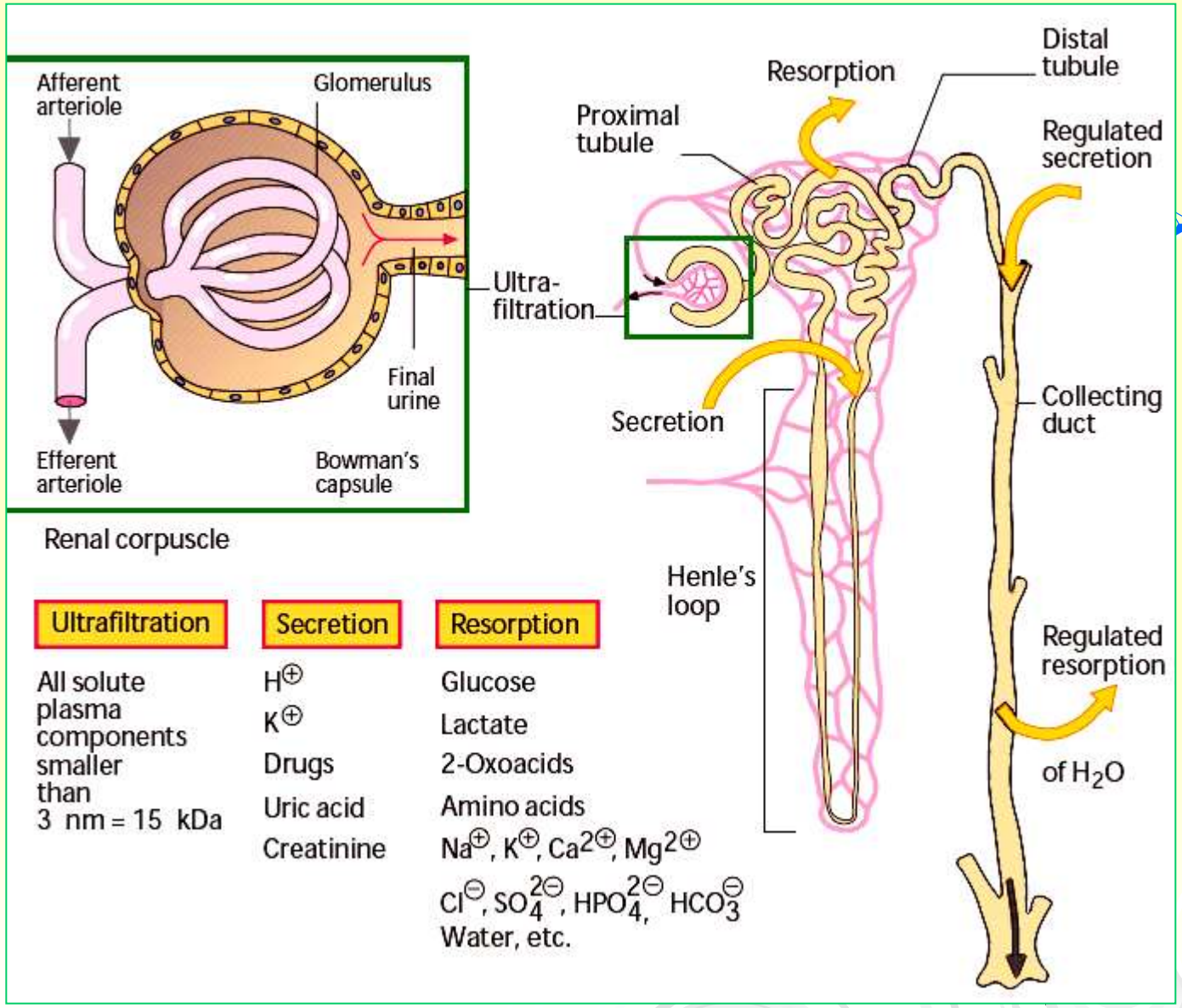
- VLDL, LDL, HDL are metabolized in kidney.
- Kidneys active synthesize cholesterol.
- Kidneys can use ketone bodies as fuels.
- Also vitamin D belongs to lipids. It is metabolized to its active form.

Renal Blood Circulation



1. Glomerular capillari
2. Tubular capillari

Urine formation



- The functional unit of the kidney is the nephron
  - Ultrafiltration
  - Resorption
  - Secretion
  - Clearance

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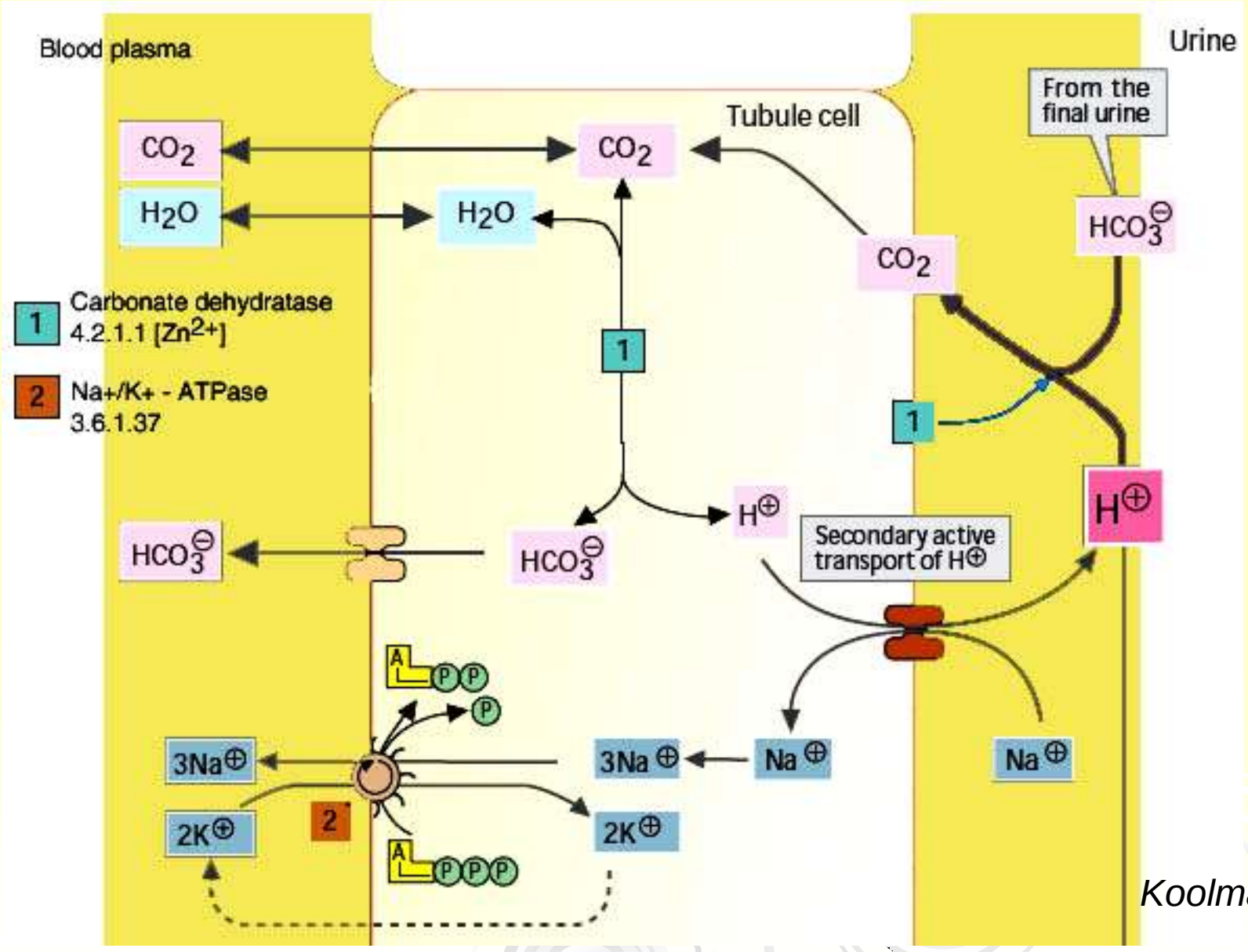
Proton Secretion



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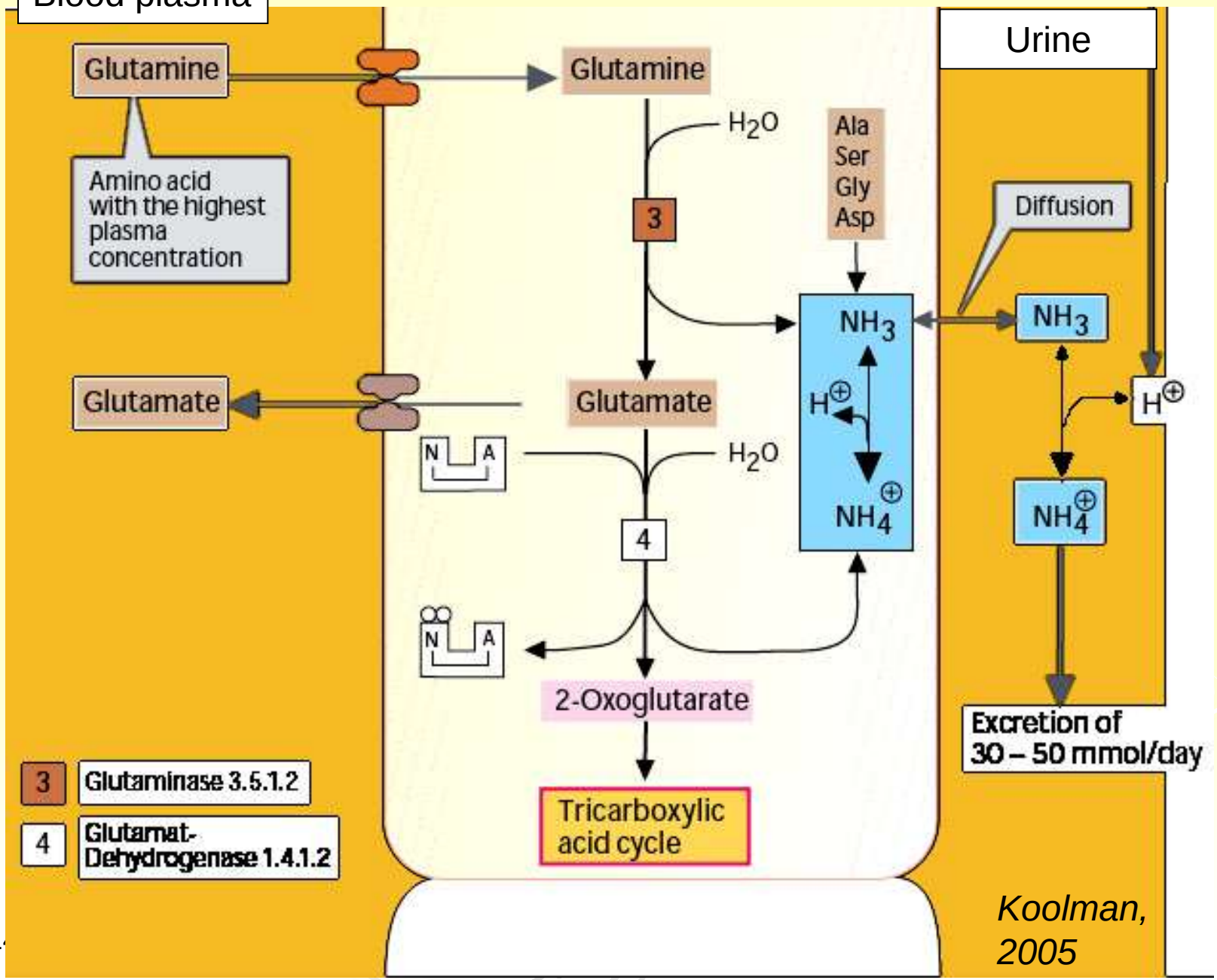
Ammonia excretion



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Ammonia excretion by the kidney

- The protons in the tubule are bufered by phosphate, by bicarbonate and by NH_3.
- The ammonia is able to diffuse through the membrane into the urine.
- As it combines with a proton in the urine, it forms NH_4^+, which cannot be transported back into the cells.

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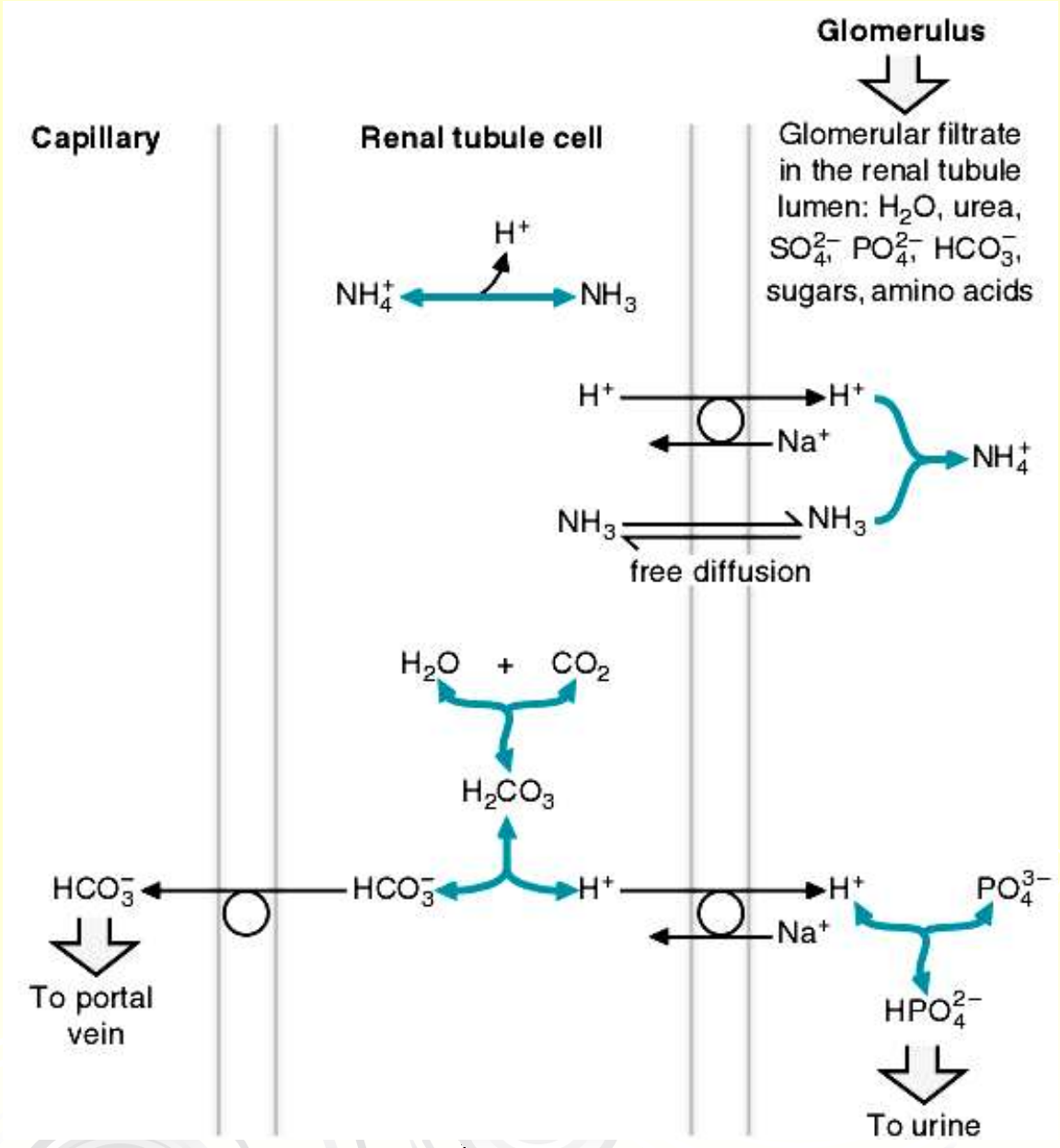
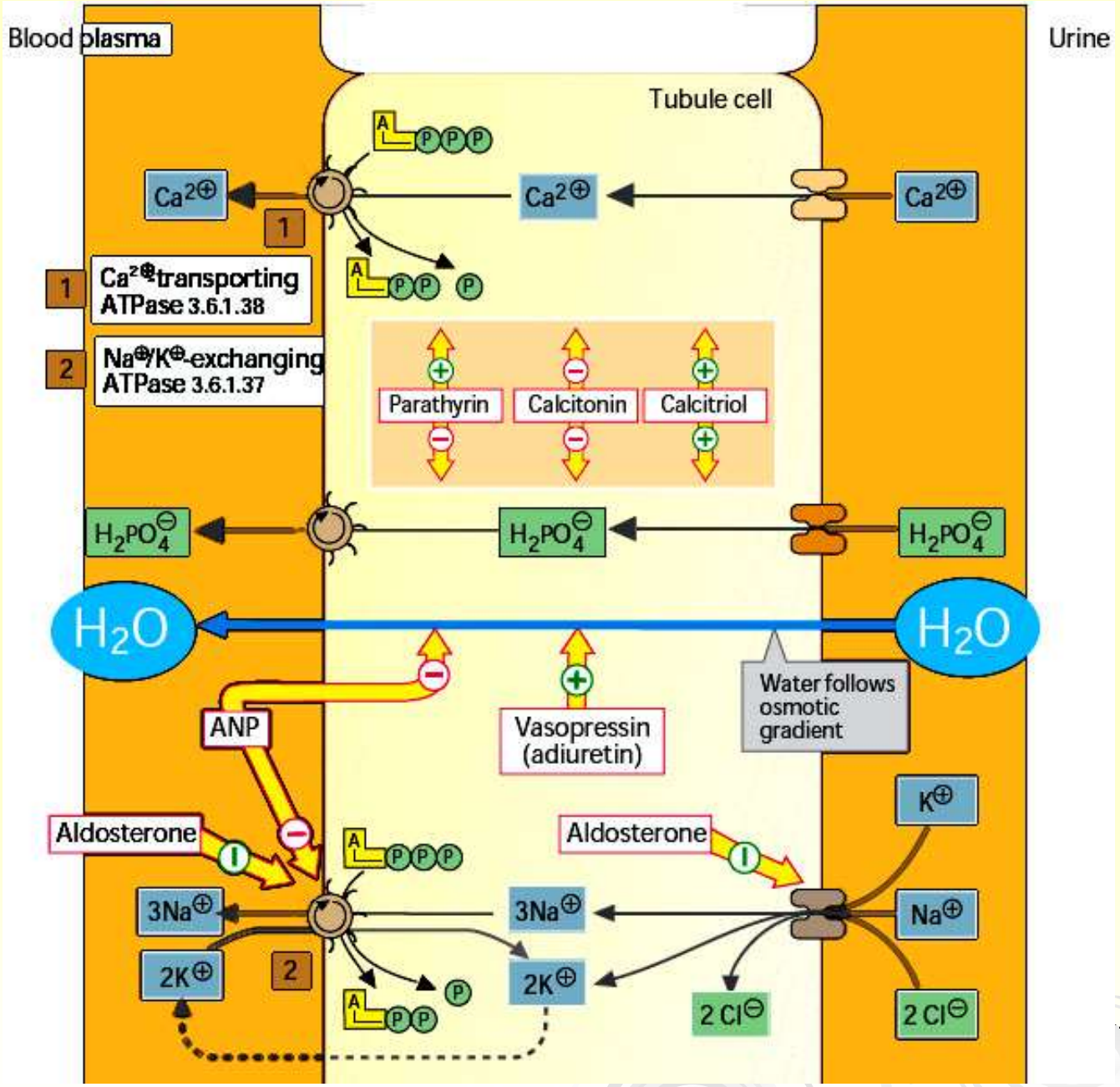


Kidney: Electrolite and Water Recycling



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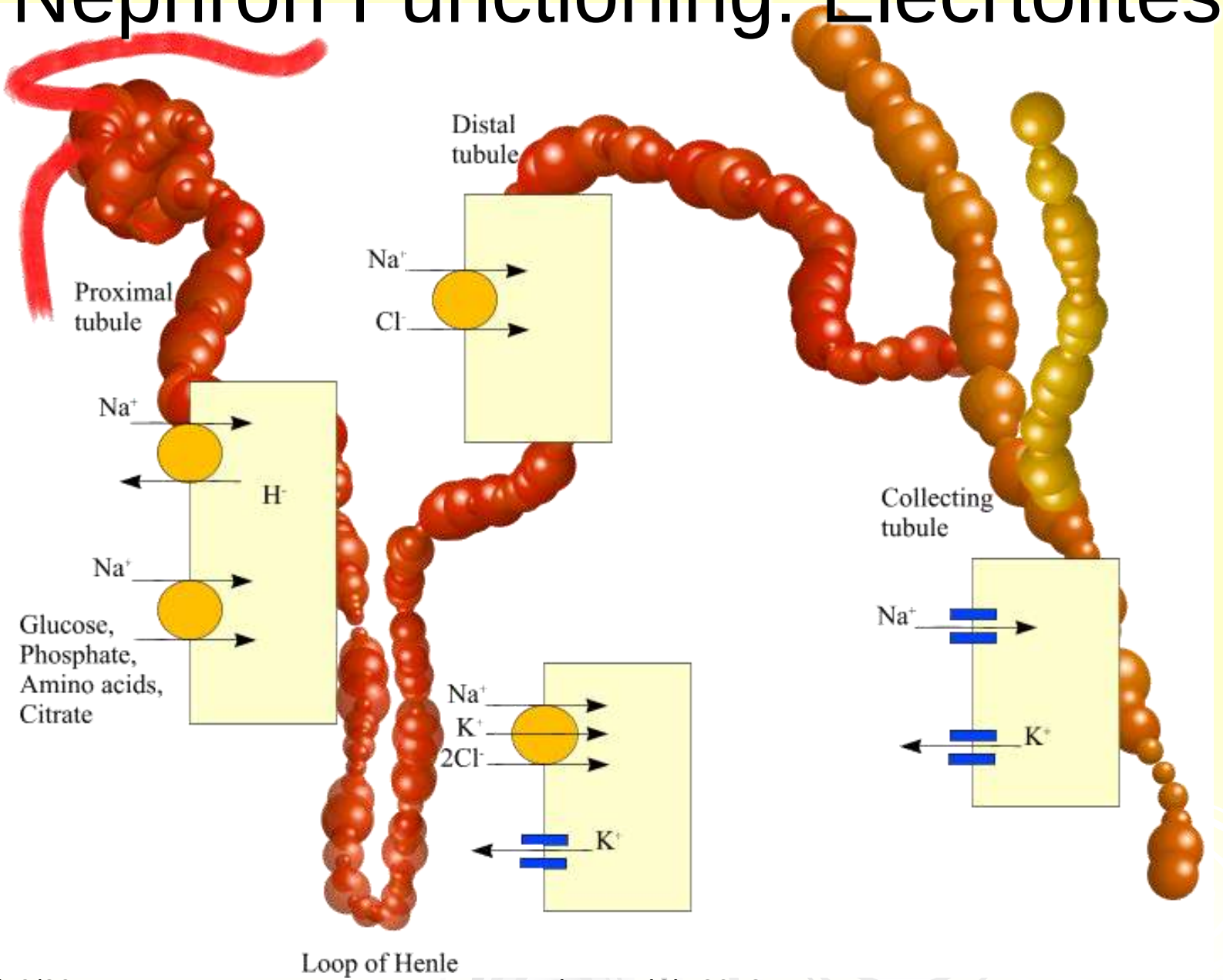
Nephron Functioning: Elecrtolites

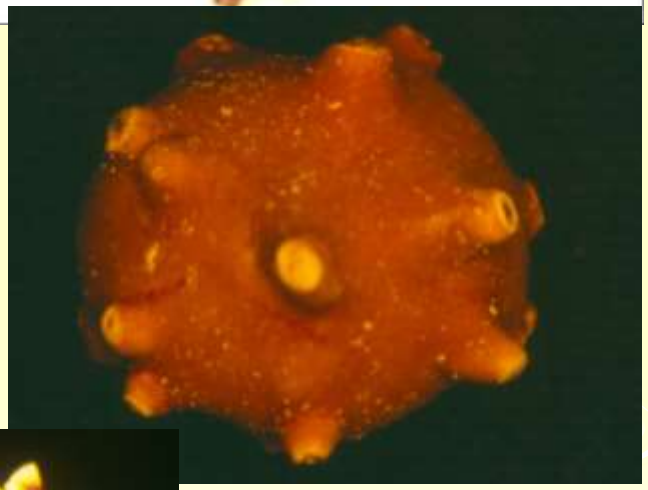


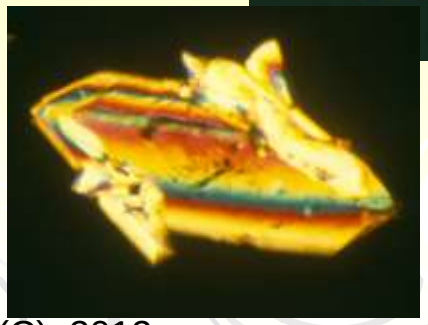
Diuretic Action

Site of Action and Transporter Inhibited	Drug	Percent of Filtered Na^+ Excreted	Effects on Other Ions
Proximal tubule Na^+-H^+-exchanger	Acetazolamide	Limited effect	Increased HCO_3^- and K^+ excretion
Loop of Henle Na^+-K^+-$2Cl^-$ transporter	Furosemide, Bumetanide, Ethacrynic acid	≤ 25	Increased K^+ and H^+ excretion
Distal tubule Na^+-Cl^- cotransporter	Thiazides, Chlorthalidone, Metolazone	≤ 3-5	Increased K^+ and H^+ excretion
Collecting tubules Na^+ channel	Amiloride, Spironolactone, Triamterene	1-2	Decreased K^+ and H^+ excretion

Kidney Stones

- **Kidney stones**, or **Renal calculi**, are solid concretions (crystal aggregations) of dissolved minerals in urine; calculi typically form inside the kidneys or ureters.
- Nephrolithiasis - presence of calculi in the kidneys.
- Urolithiasis - presence of calculi in the urinary tract.
- Renal calculi can vary in size.
- Kidney stones leave the body by passage in the urine stream. 2-3 millimeters stones can cause obstruction of the ureter.
- Renal colic can be associated with nausea and vomiting.





From Wikipedia.org

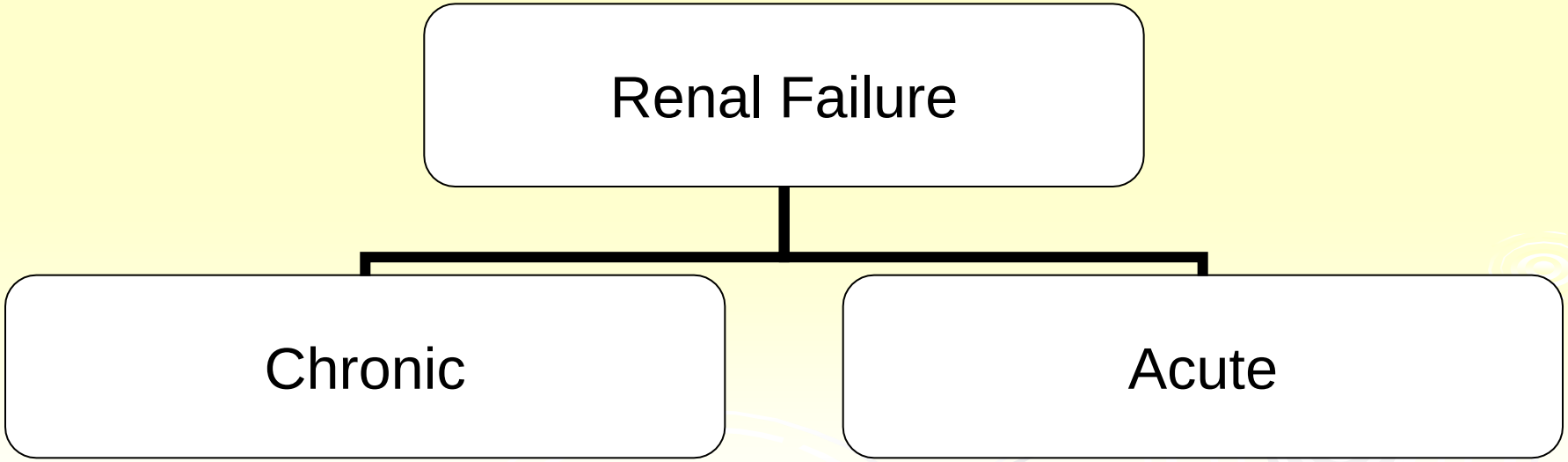
Etiology of Kidney Stones

- Most commonly composed of calcium oxalate crystals.
- In the urine, oxalate is a very strong promoter of calcium oxalate precipitation.
- Other components of kidney stones:
  - struvite (magnesium, ammonium and phosphate) - associated with the presence of urea-splitting bacteria, most commonly *Proteus mirabilis.*
  - uric acid - associated with conditions that cause high blood uric acid levels, such as gout, leukemias/lymphomas treated by chemotherapy (secondary gout from the death of leukemic cells), and acid/base metabolism disorders.
  - calcium phosphate - is associated with conditions such as hyperparathyroidism and renal tubular acidosis.
  - cystine - found only in high urinary concentrations in people suffering from cystinuria).
- Renal calculi also are in renal tubular acidosis, Dent's disease and medullary sponge kidney.

From Wikipedia.org

Renal Failure

- **Renal failure** or **kidney failure** is the condition in which the kidneys fail to function properly.
  - Physiologically, renal failure is described as a decrease in the glomerular filtration rate.
  - Clinically, this manifests in an elevated serum creatinine.
    - However, serum creatinine levels are also affected by the patient's existing muscle mass.



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Renal Failure: Chronic

- Chronic renal failure (CRF) develops slowly and shows few symptoms initially.
- It can be the complication of a large number of kidney diseases, such as IgA nephritis, glomerulonephritis, chronic pyelonephritis and urinary retention.
  - End-stage renal failure (ESRF) is the ultimate consequence, in which case dialysis is generally required until a donor for a renal transplant is found.
  - Treatment modalities for ESRF include peritoneal dialysis, hemodialysis, and transplantion from either a living or cadaveric donor.
- Of these, renal transplantation affords the best quality and quantity of life to the patient, provided they are an appropriate candidate.

From Wikipedia.org

Renal Failure: Acute

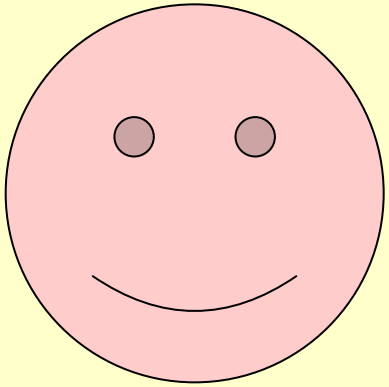
- Acute renal failure (ARF) is, as the name implies, a rapidly progressive loss of renal function, generally characterised by
  - **oliguria** (decreased urine production, quantified as less than 400 mL per day in adults, less than 0.5 mL/kg/h in children or less than 1 mL/kg/h in infants);
  - **body water** and body fluids disturbances;
  - **electrolyte** derangement.
- An underlying cause must be identified to arrest the progress, and dialysis may be necessary to bridge the time gap required for treating these fundamental causes.

From Wikipedia.org

Conclusion

- Kidney fulfill a number of important functions:
  - excretory, ABB regulation, blood pressure regulation, glucose level regulation, RBC synthesis.
- The excretory function is of high energy demand:
  - ATP is necessary for osmotic work – resorption of substances and electrolytes.
- All the groups of organic substances are metabolized in kidney:
  - Carbohydrates: aerobic glycolysis, PPP, GNG.
  - Lipids: Cholesterol biosynthesis,
  - Proteins an amino acids: Reabsorption of AA, biosynthesis or guanidineacetate (precursor of creatine). Very important metabolism of Gln.
- Diuretics inhibits most of electrolyte carriers in the nefron.
- Renal failure can be chronic and acute.

Antimilitary Function of Urine

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Metabolism of Liver. Metabolism of Xenobiotics

Lecture # 31

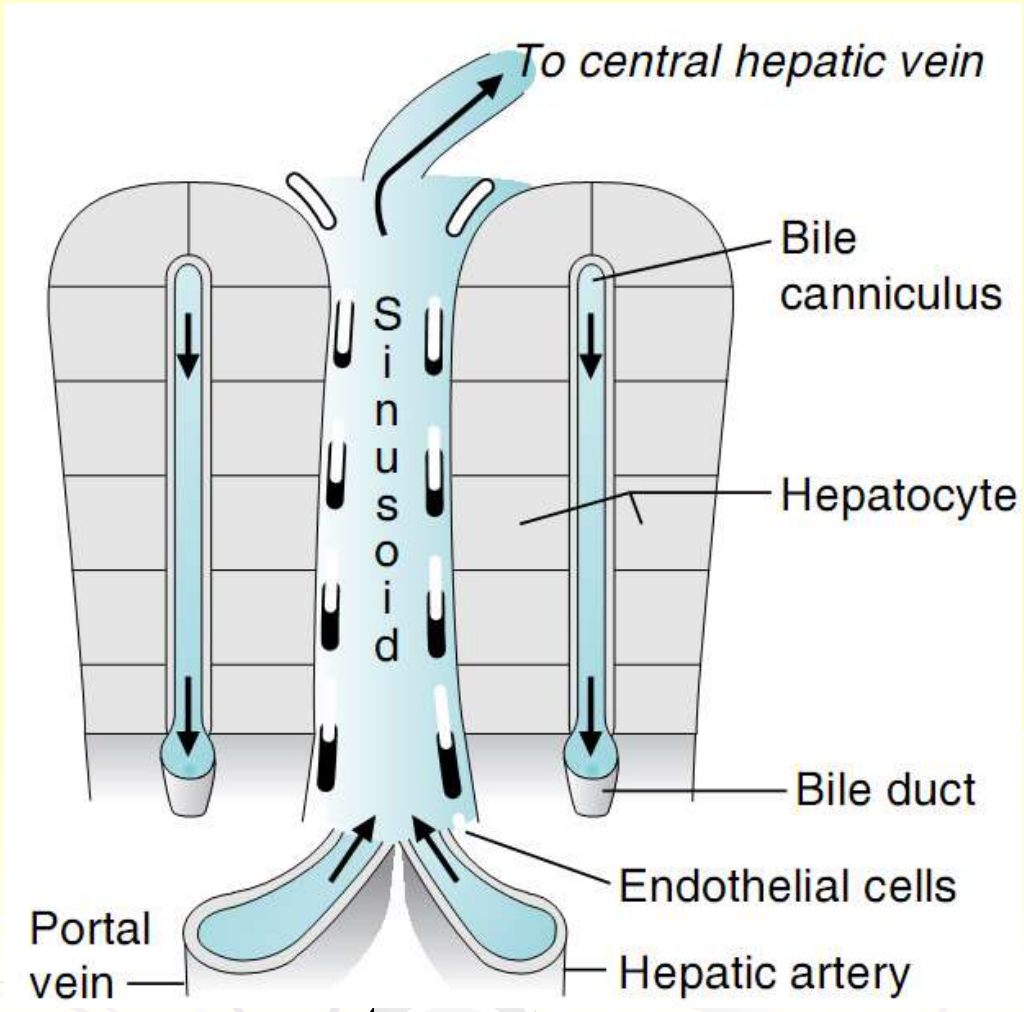
Lecturer Alexander Koval

Metabolism of Liver

- Some facts
  - Liver weight is 1.5 kg – 2-3 % of body weight
  - Oxygen consumption – 20 – 30 % of total O_2
- Liver is metabolically active organ.

Liver Lobule

- Blood from the portal vein and hepatic artery empty into a common conduit – the liver sinusoids.
  - They are “leaky”: the contents of plasma have free access to the hepatocytes.
  - The hepatocytes secrete bile into the bile canniculi, which empty into the bile ducts.



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Liver Cells

- Hepatocytes:
  - 80% (volume);
  - 60% of the total number.
- Nonparenchymal cells:
  - 40% of the total number.
  - Lining cells of the walls of the sinusoids.
    - Endothelial cells,
    - Kupffer cells,
    - Hepatic stellate cells (aka perisinusoidal or Ito cells).
  - Intrahepatic lymphocytes
    - Pit cells (liver-specific natural killer cells).

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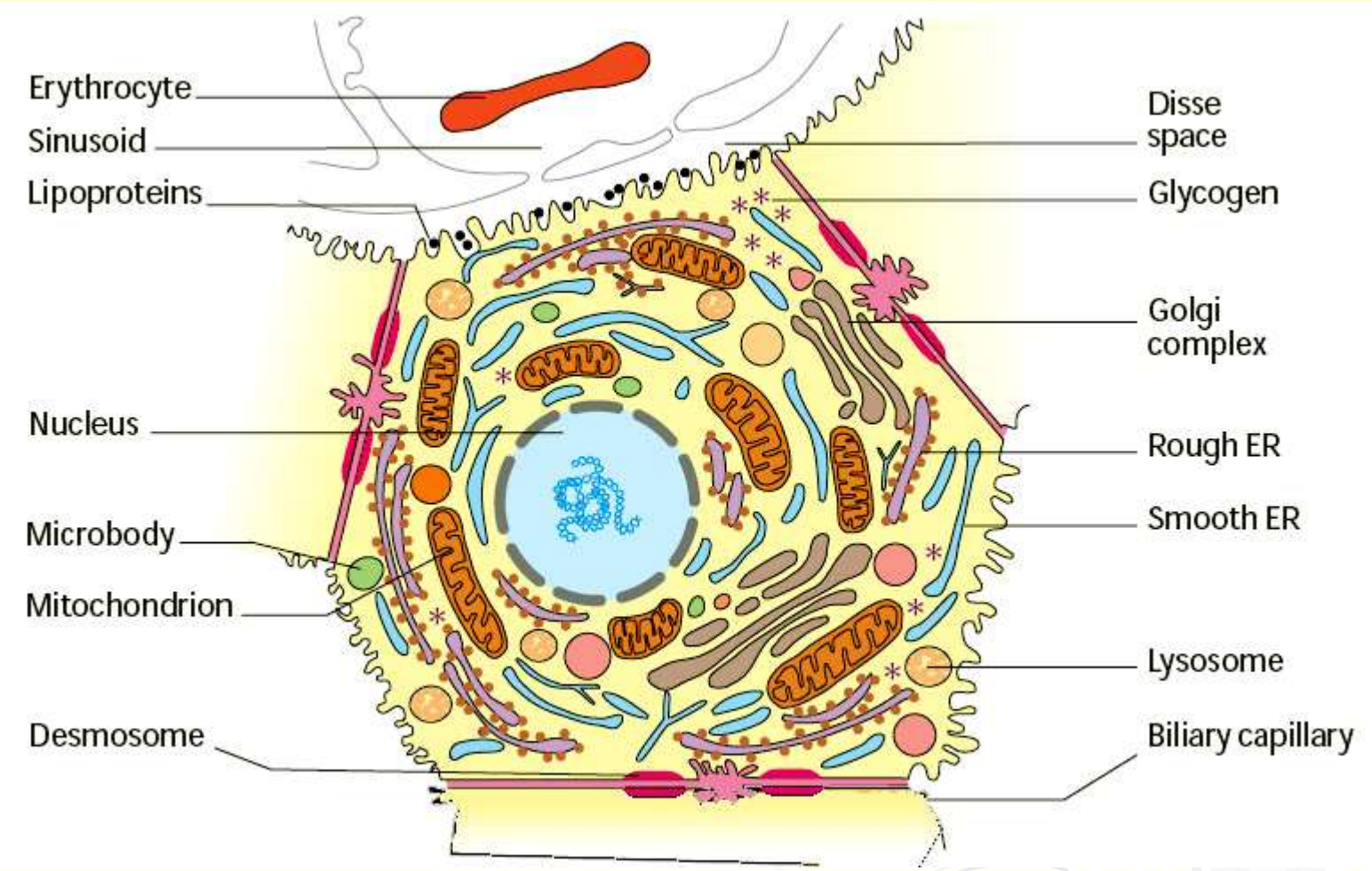
Hepatocyte structure



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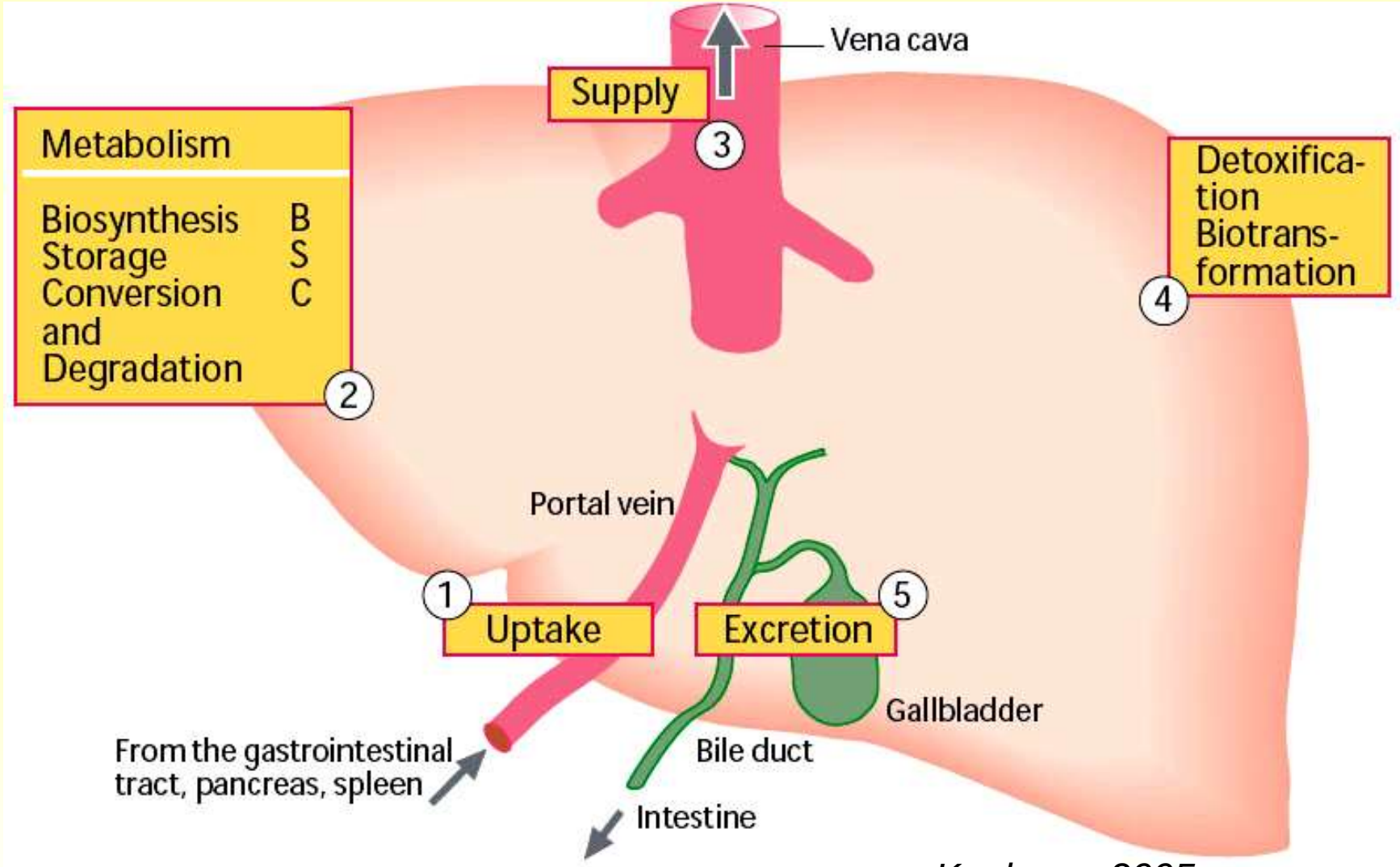
Functions of the Liver



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Liver metabolism

Carbohydrates	Lipids	Amino acids	Biotrans-formation
Glucose (BSC), Galactose (C), Fructose (C), Mannose (C), Pentoses (BC), Lactate (C), Glycerin (BC), Glycogen (BSC)	Fatty acids (BC), Fats (BC), Ketone bodies (B), Cholesterol (BSC), Bile acids (BE), Vitamins (SC).	Amino acids (BC), Urea (B) **Plasma proteins** Lipoproteins (BC), Albumines (BC), Coagulation factors (BC), Hormones (BC), Enzymes (BC)	Steroids (CE), Bile pigments (CE), Ethanol (C), Drugs (CE)

Designation of functions:
B – Biosynthesis, S – Storing, C – Conversion, E – Excretion

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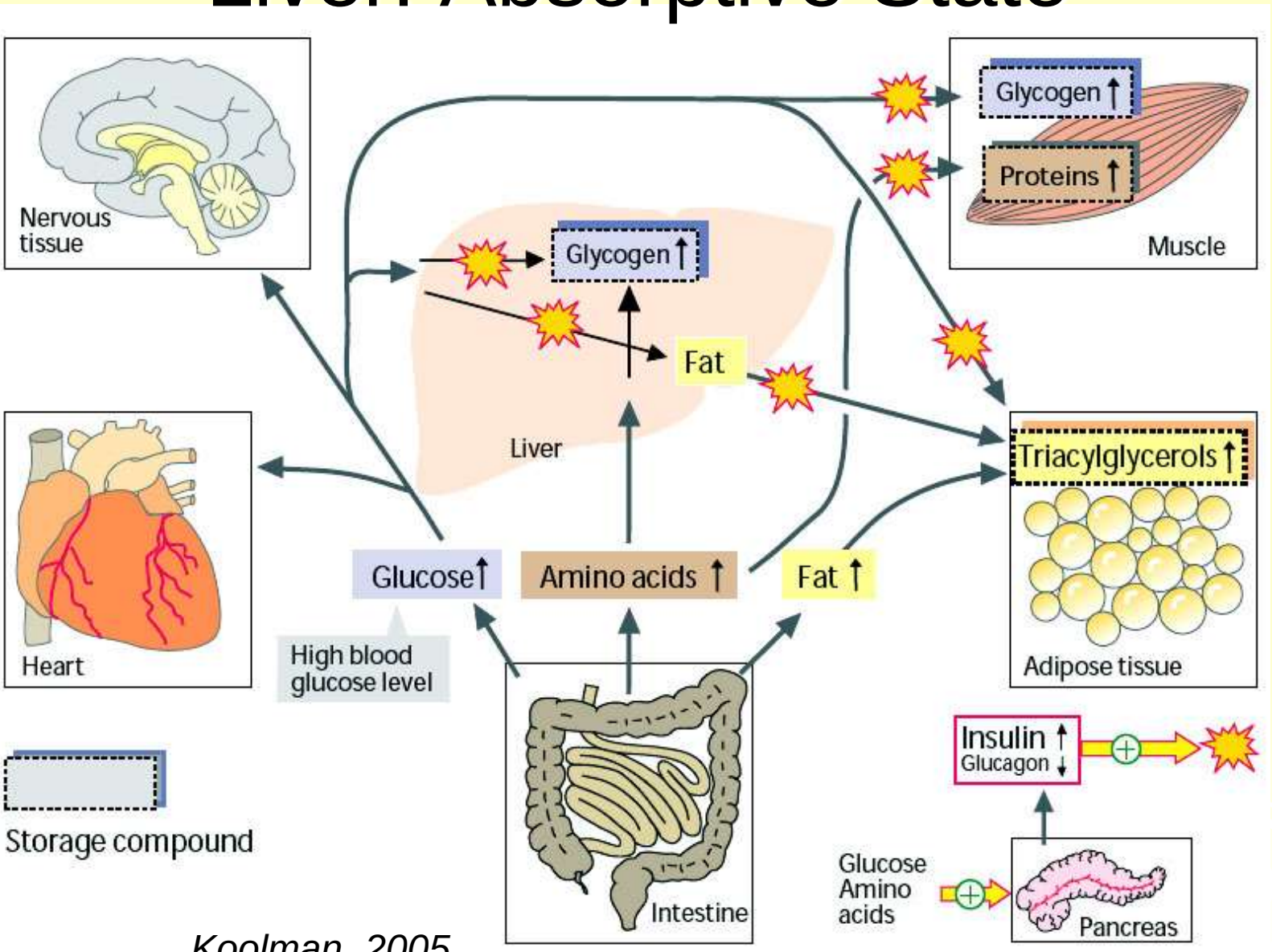
Liver: Absorptive State



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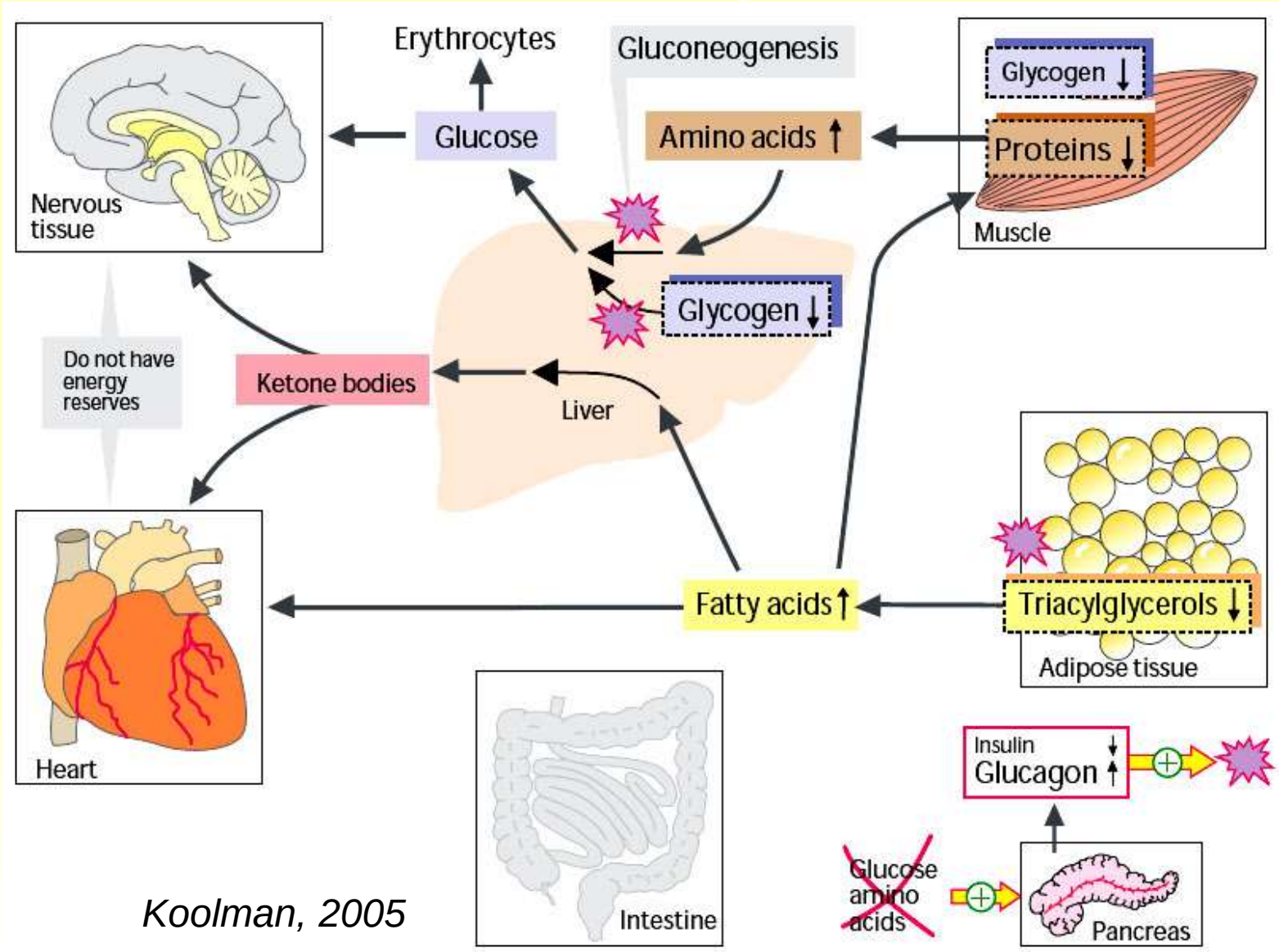
Liver: Postabsorptive State



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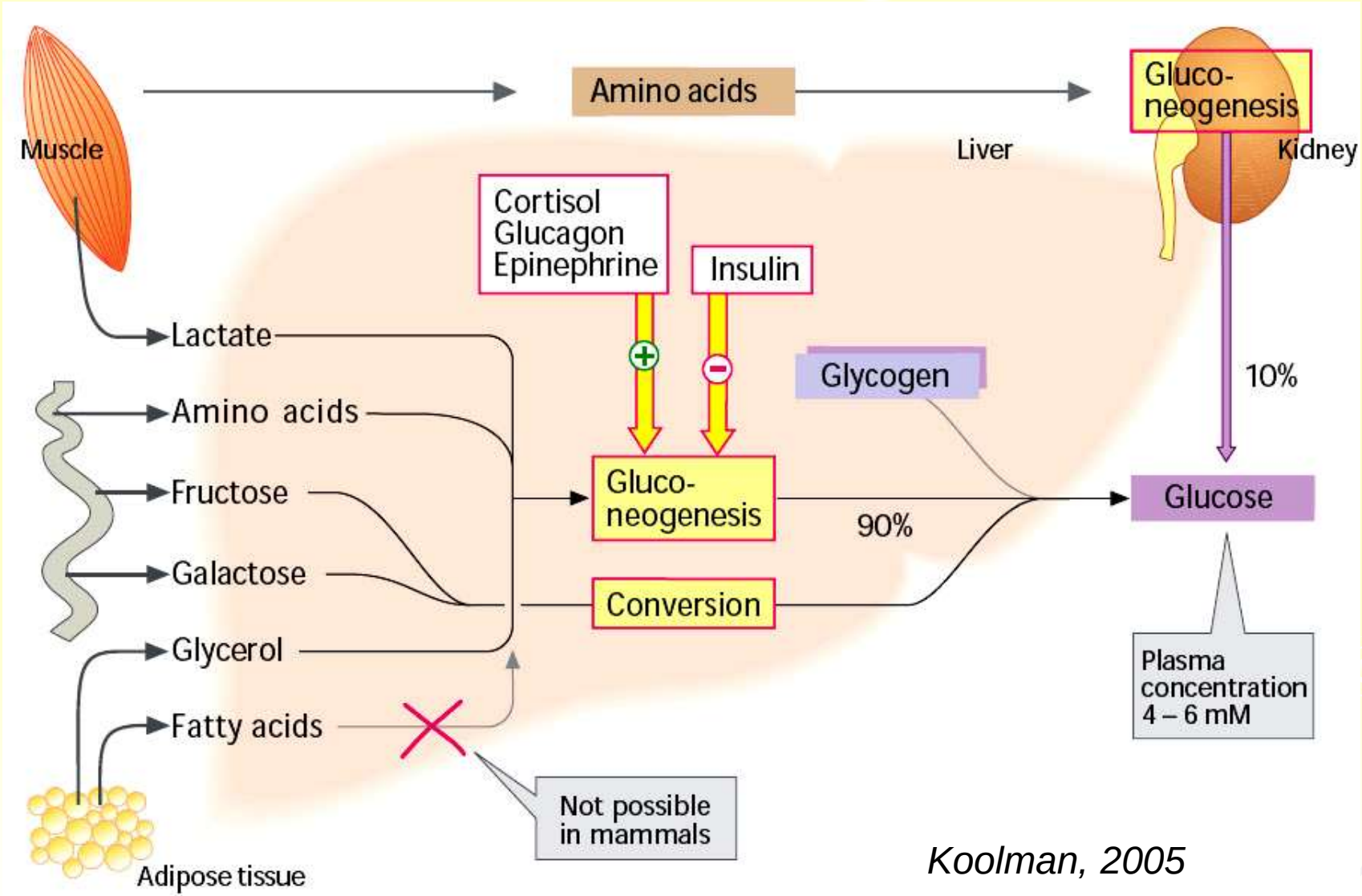
Liver: Gluconeogenesis



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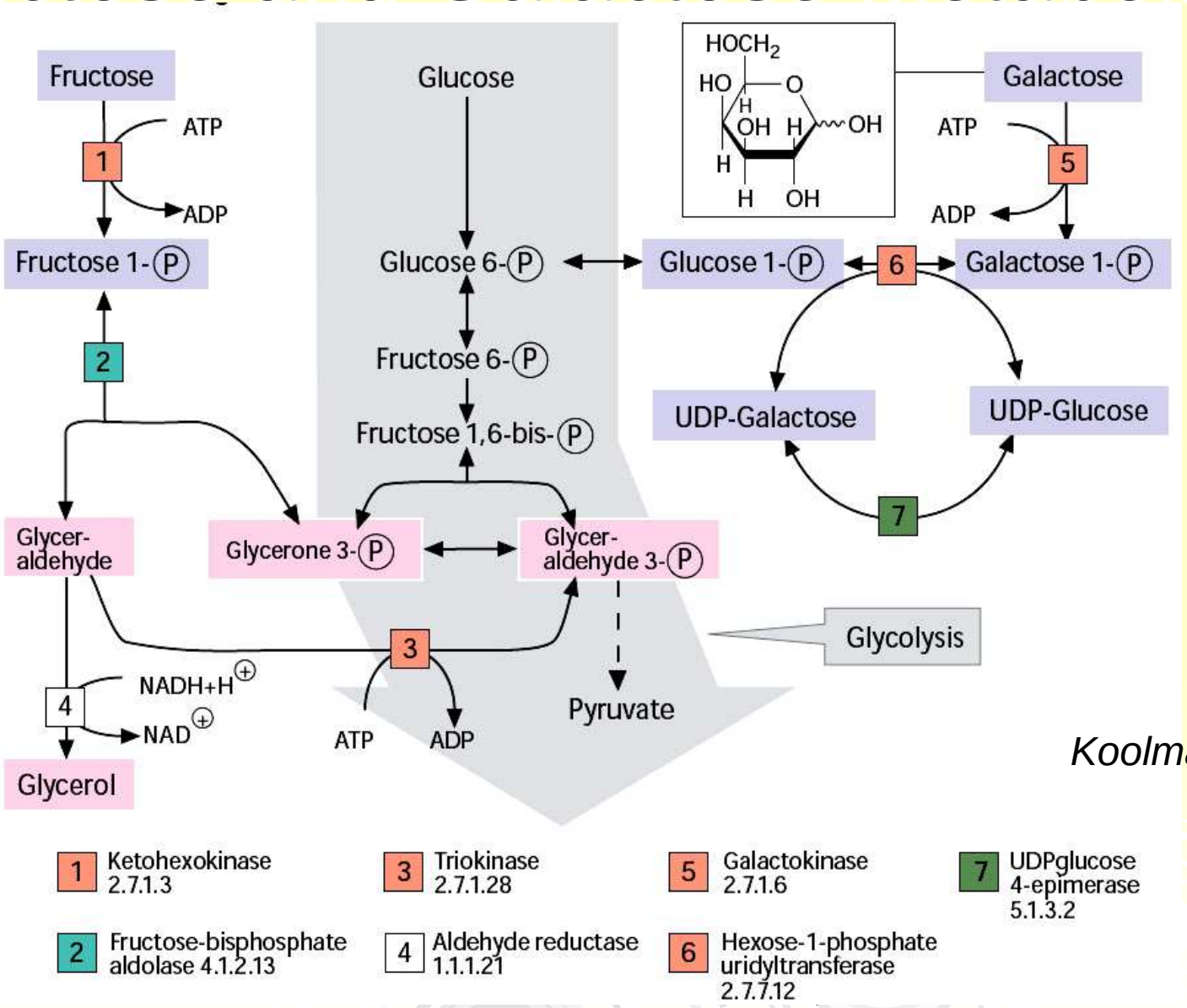
Fructose and Galactose Metabolism



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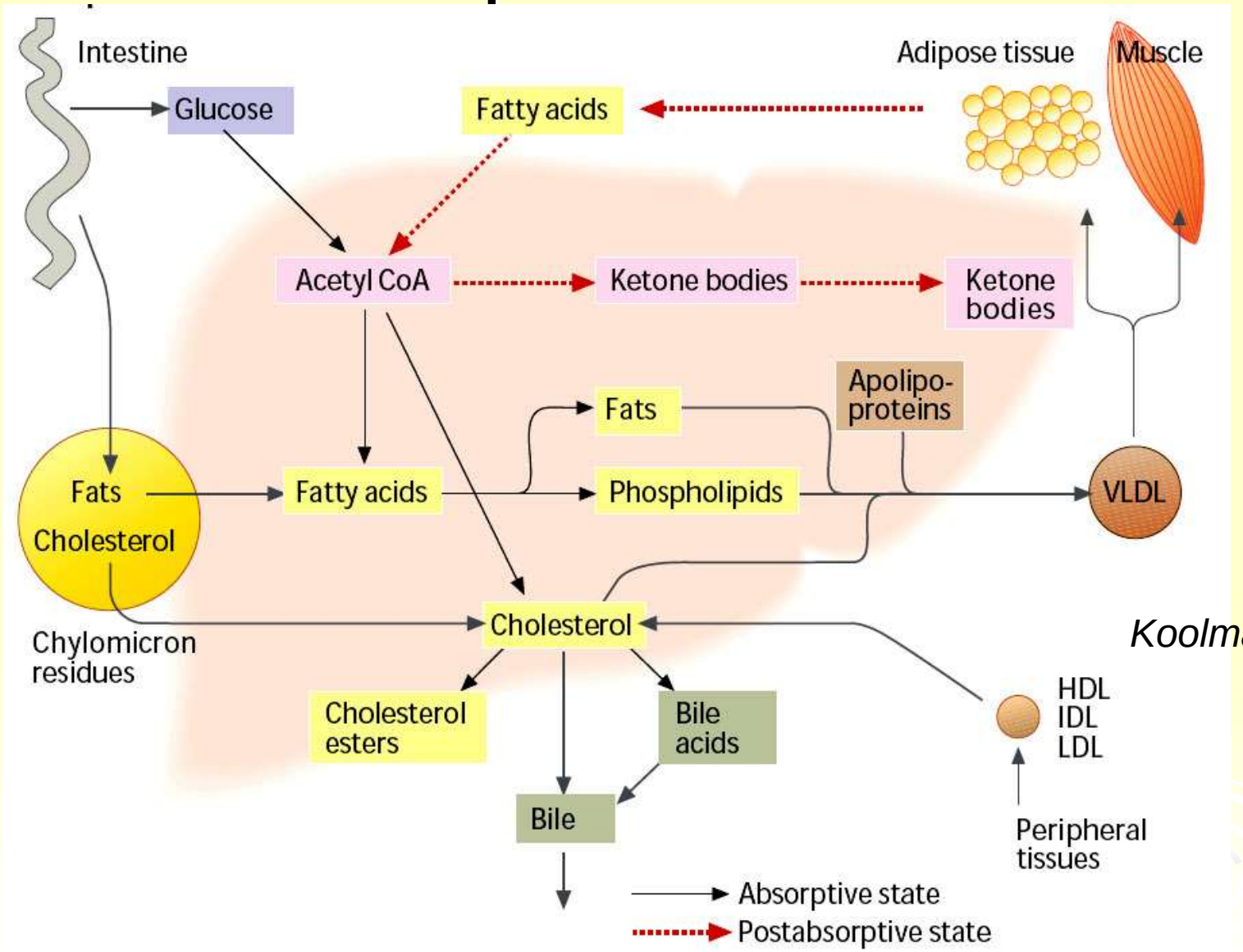
Liver: Lipid Metabolism



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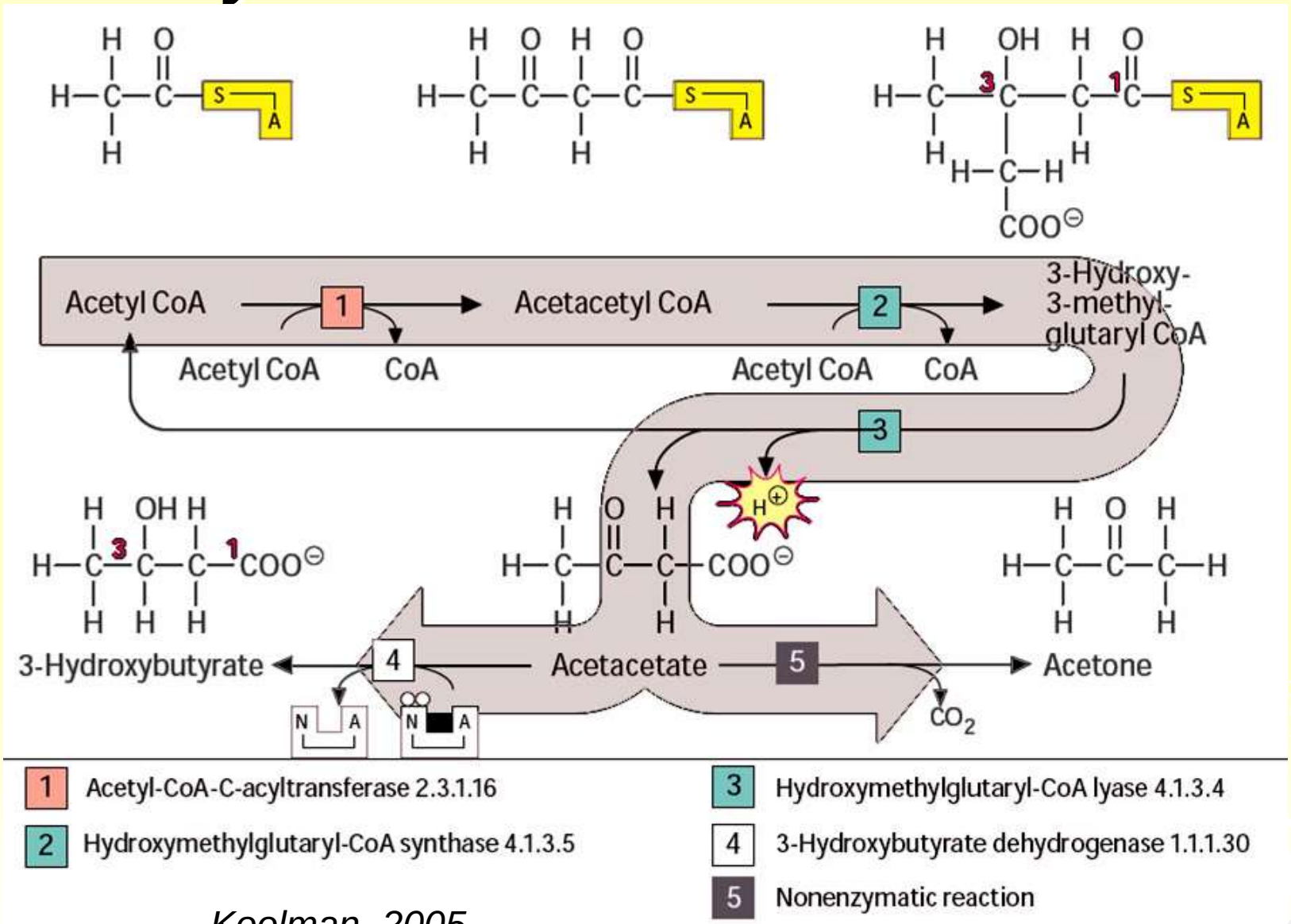
Biosynthesis of Ketone Bodies



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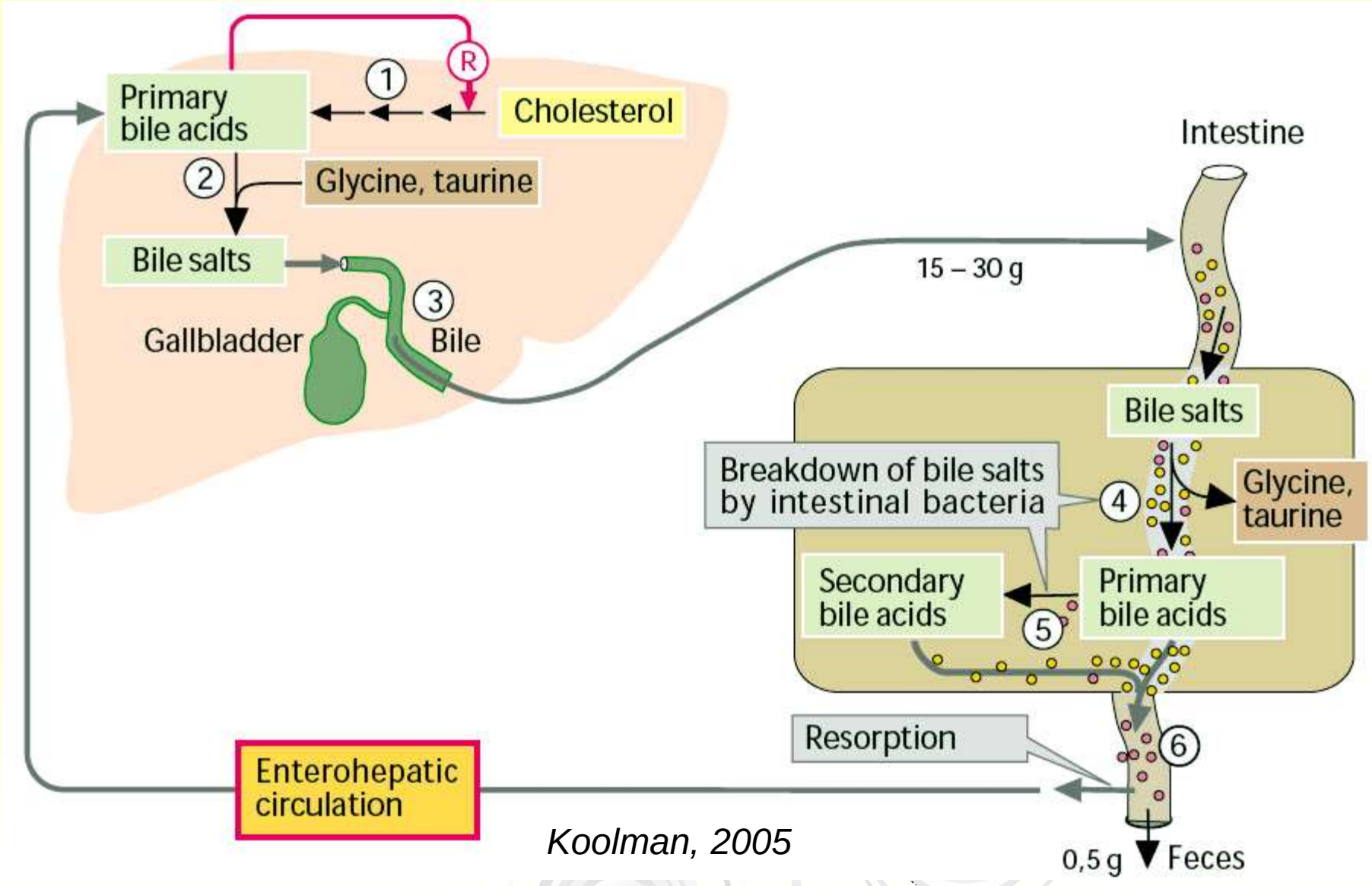
Metabolism of Bile Salts



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Five different glucose transporters have been identified – expressed in different tissues

Name	Tissue	K_m
GLUT-1	Erythrocytes, brain	5-7 mM
GLUT-2	Liver, kidney, pancreatic B cell	7-20 mM
GLUT-3	Brain	1.6 mM
GLUT-4 (insulin-sensitive)	Muscle, adipose tissue	5 mM
GLUT-5	Duodenum	5 mM (fructose)

Blood circulation in liver

Special blood supply

1. Hepatic artery – 20% of blood supply
2. Hepatic portal vein (HPV) – 80% of blood supply
   - carries blood directly from intestinal tract and from endocrine pancreas
   - uniquely placed to pick up nutrients that enter the body

Liver and carbohydrate metabolism

GLUT-2 (high Km transporter)
Allows glucose concentration in the hepatocyte to equilibrate with that in the blood

Glucokinase
phosphorylates glucose to generate Glc 6-P

much higher Km than hexokinase – not overwhelmed as [Glc] increases

unlike hexokinase is not inhibited by product (Glc 6-P)

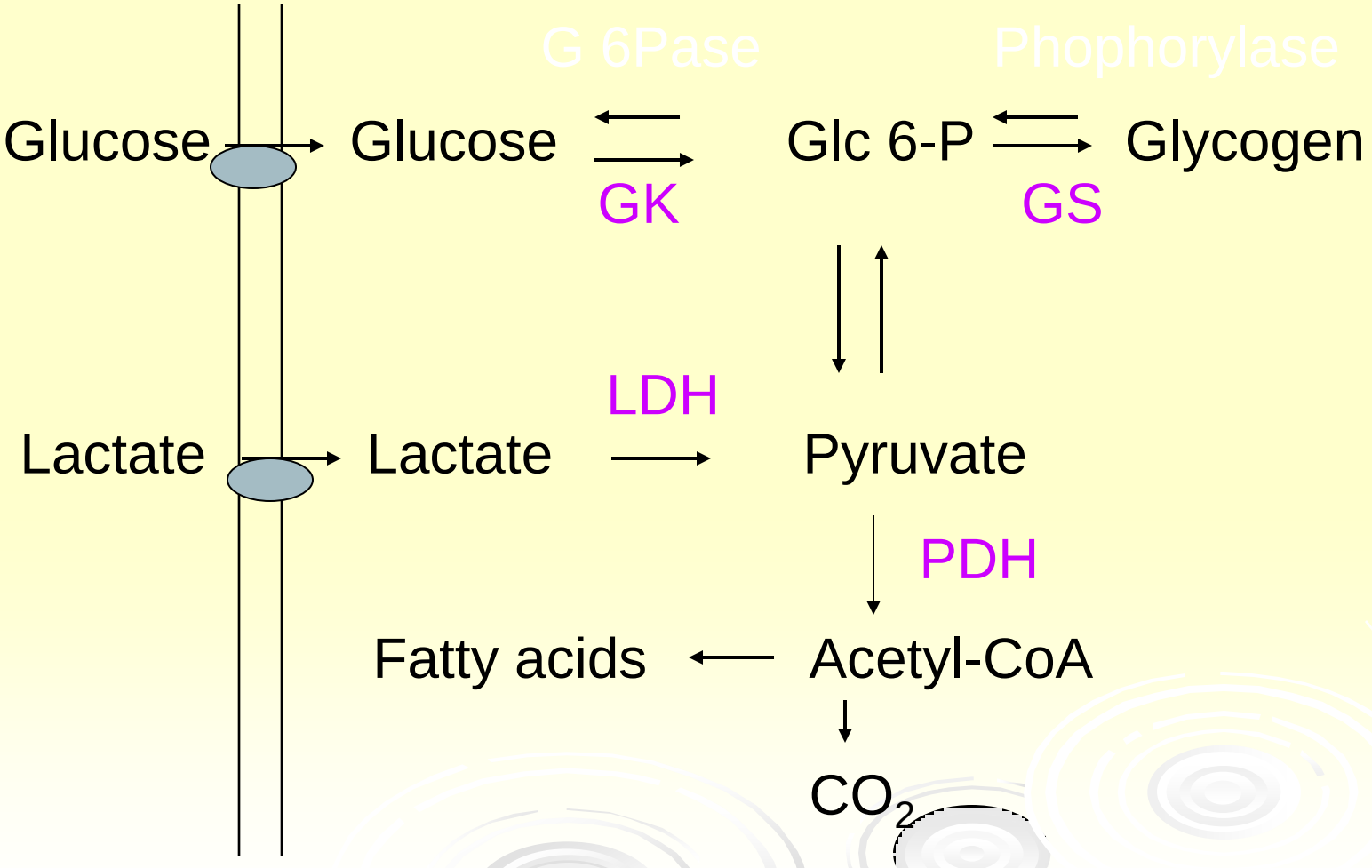
Liver Glc 6-P can be:

- Converted to glycogen (glycogen synthase)
- Dephosphorylated by G-6P-ase to yield glucose
- Metabolised to pyruvate by glycolysis
  - small amount enters TCA cycle for energy production
  - some is released as lactate
  - most of acetyl CoA generated is used in fatty acid synthesis
- Used in pentose phosphate pathway to generate NADPH and ribose 5-P

Liver and carbohydrate metabolism

- Liver effectively acts as a “sink” for glucose – takes it up from blood and stores it after meal and releases it as required.
  - Note: most of energy required by the liver comes from fatty acid (FA) and amino acid breakdown.

Liver and carbohydrate metabolism (2)



How carbohydrate metabolism in the liver is regulated (1)

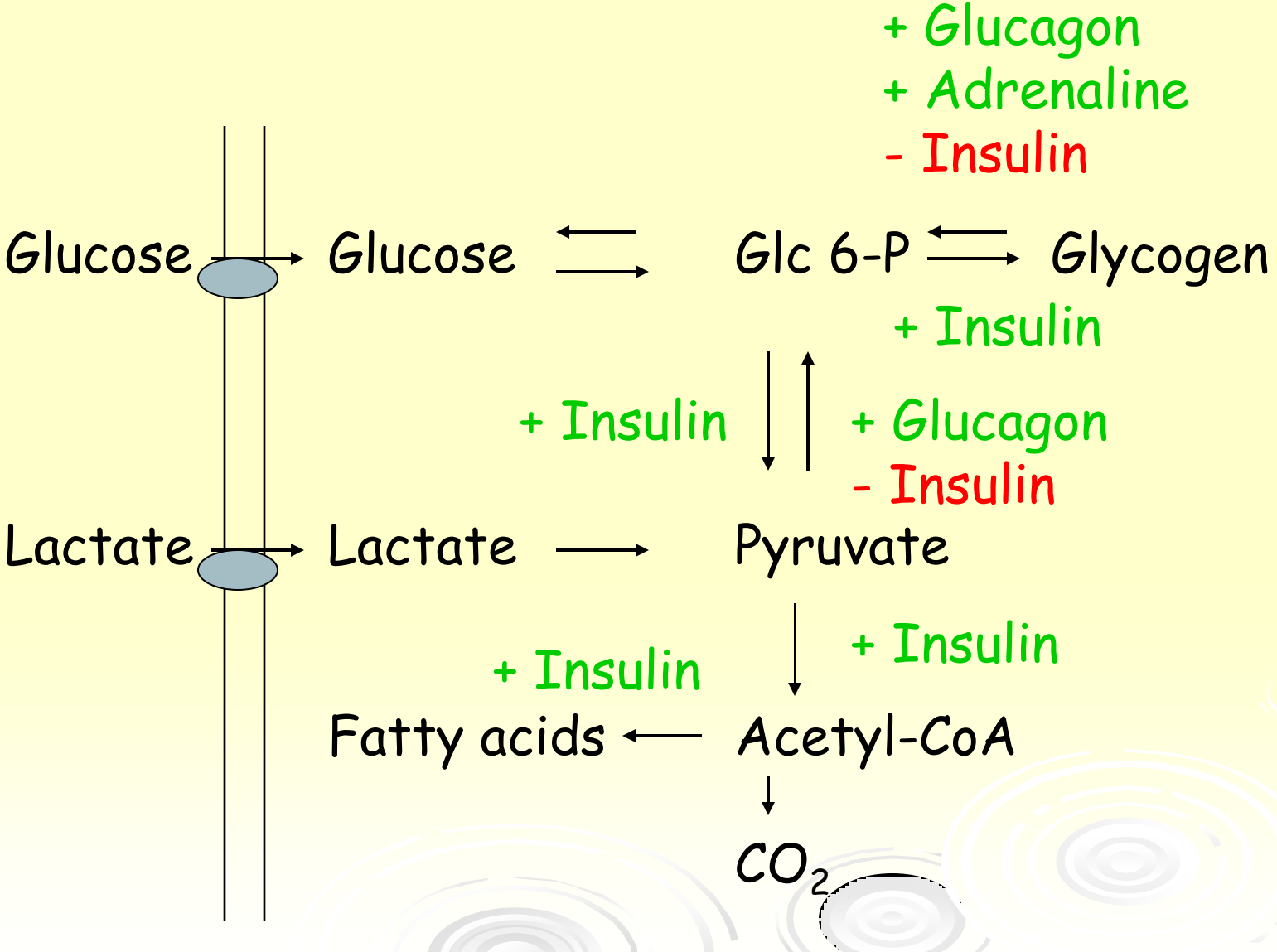
1. In fed state high [Glc] in blood stimulates insulin secretion

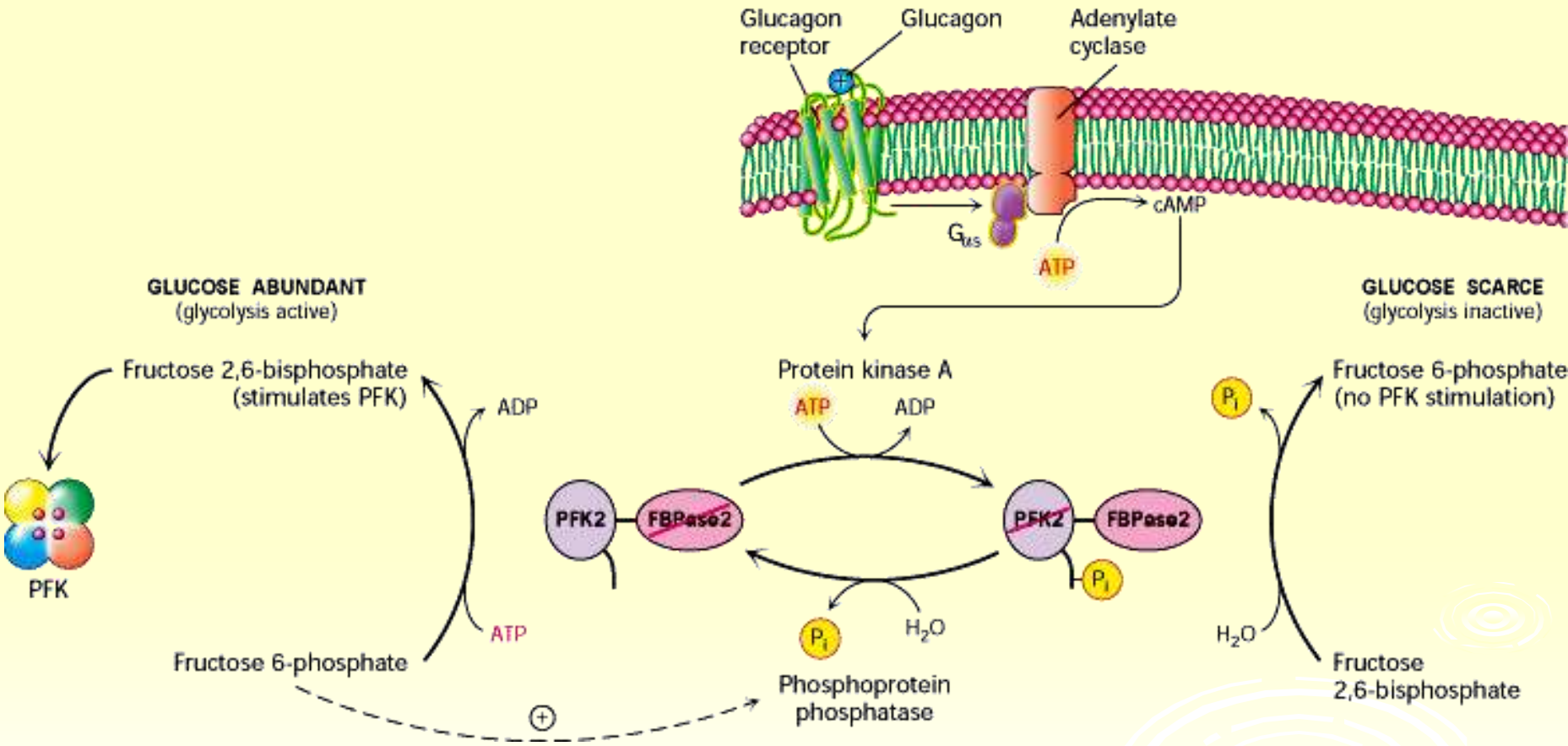
2. Insulin stimulates Glc 6-P to glycogen via activation of glycogen synthase.
Insulin also inhibits the breakdown of glycogen.

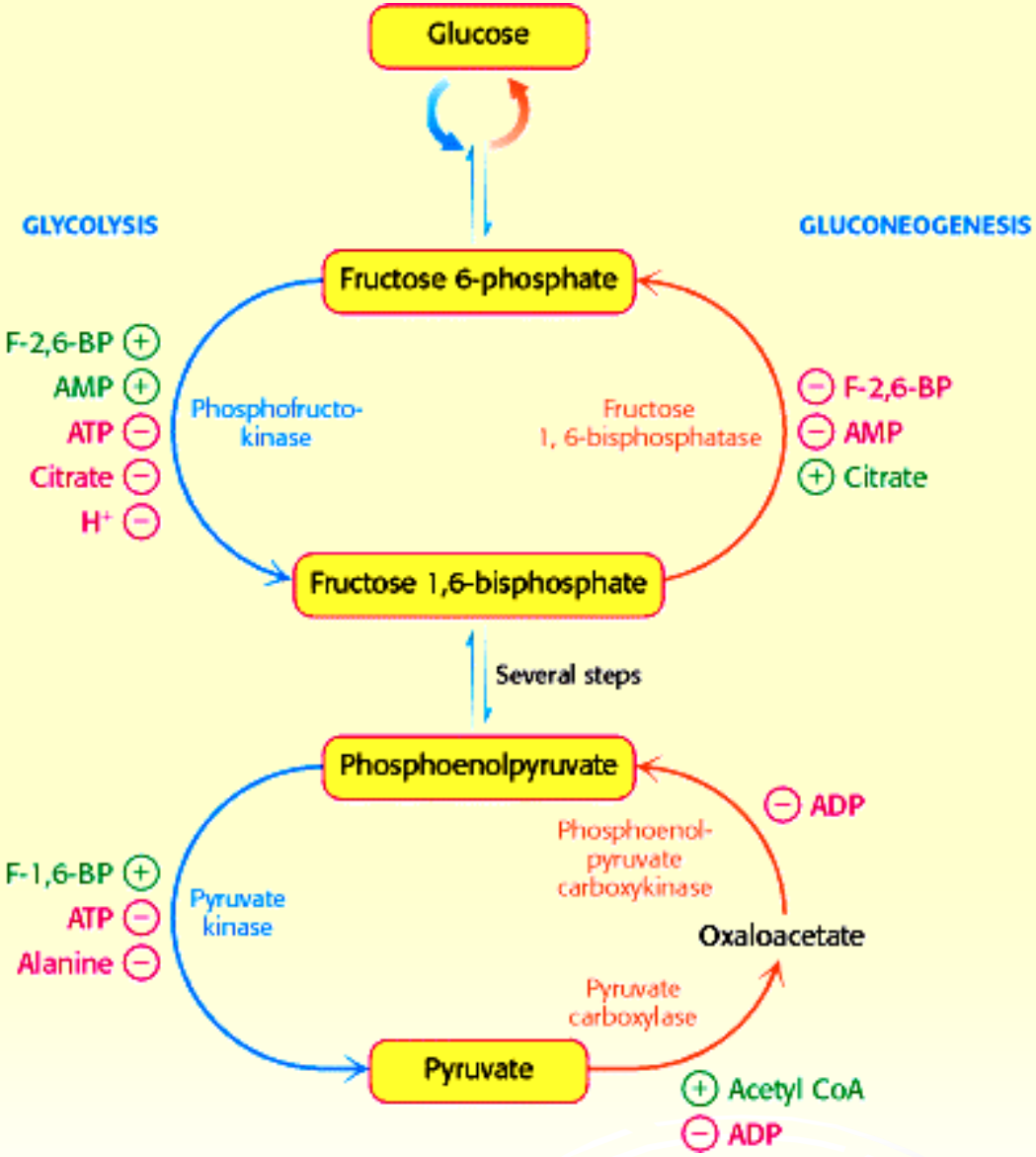
How carbohydrate metabolism in the liver is regulated (2)

3. When blood glucose levels drop glucagon and adrenaline are released
– stimulate glycogen breakdown to generate Glc 1-P which is converted to Glc 6-P

4. Glucose 6 phosphatase can convert Glc 6-P to glucose which is released back into blood as required.


+ Glucagon
+ Adrenaline
- Insulin
Glucose
Glucose
Glc 6-P
Glycogen
+ Insulin
+ Insulin
+ Glucagon
- Insulin
Lactate
Lactate
Pyruvate
+ Insulin
+ Insulin
Fatty acids
Acetyl-CoA
CO_2


Glucagon receptor
Glucagon
Adenylate cyclase
cAMP
$G_{\alpha s}$
ATP
GLUCOSE ABUNDANT
(glycolysis active)
GLUCOSE SCARCE
(glycolysis inactive)
Fructose 2,6-bisphosphate
(stimulates PFK)
PFK
ADP
ATP
Fructose 6-phosphate
Protein kinase A
ATP
ADP
PFK2
FBPase2
PFK2
FBPase2
P_i
P_i
H_2O
Phosphoprotein phosphatase
P_i
Fructose 6-phosphate
(no PFK stimulation)
H_2O
Fructose 2,6-bisphosphate


Glucose
GLYCOLYSIS
GLUCONEOGENESIS
Fructose 6-phosphate
F-2,6-BP
AMP
ATP
Citrate
H+
Phosphofructo-
kinase
Fructose
1, 6-bisphosphatase
F-2,6-BP
AMP
Citrate
Fructose 1,6-bisphosphate
Several steps
Phosphoenolpyruvate
ADP
Phosphoenol-
pyruvate
carboxykinase
F-1,6-BP
ATP
Alanine
Pyruvate
kinase
Oxaloacetate
Pyruvate
carboxylase
Pyruvate
Acetyl CoA
ADP

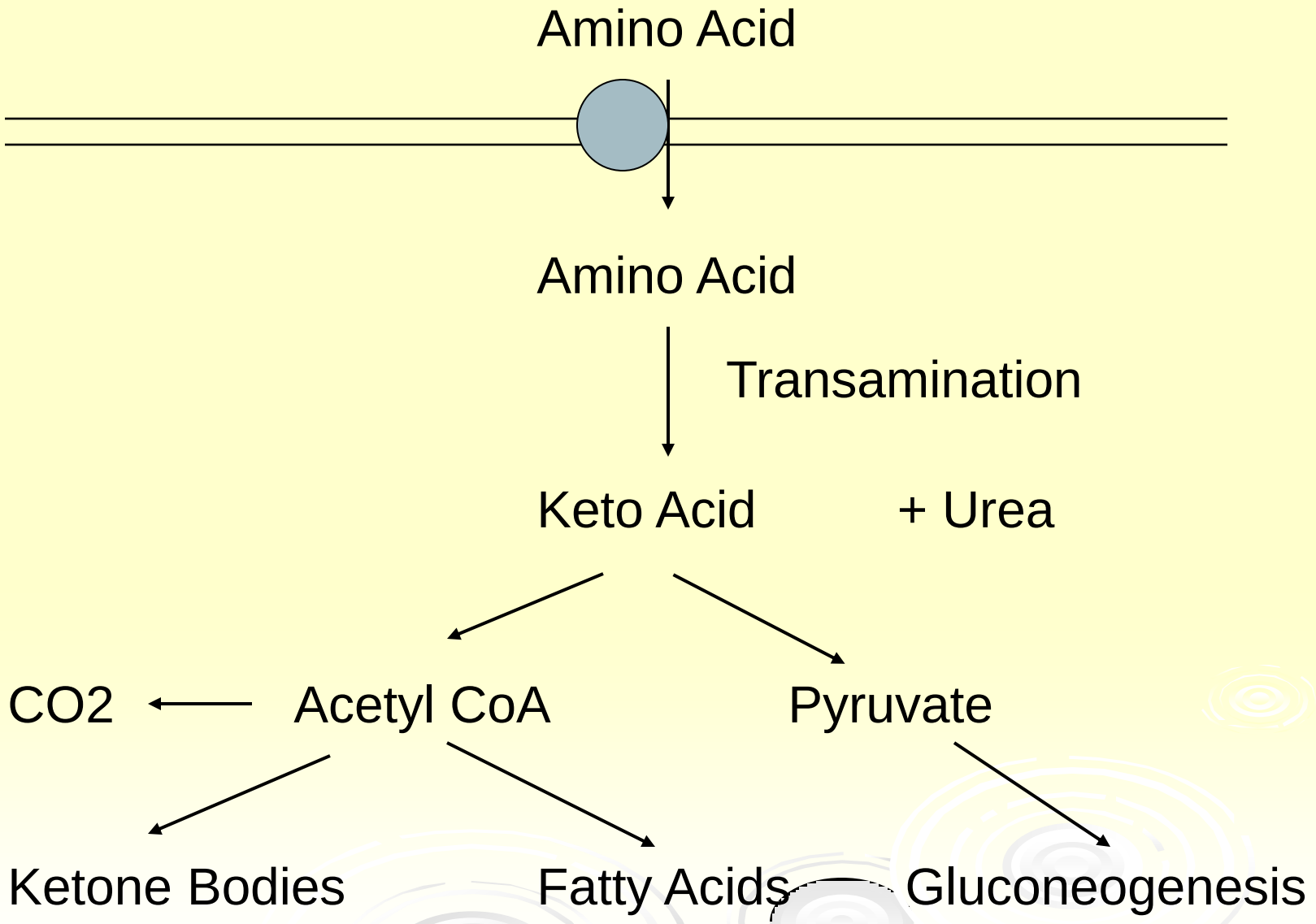
Liver and amino acid (protein) metabolism

1. Takes up amino acids from the hepatic portal vein. Some amino acids arrive intermittently from other tissues. Glucose-alanine cycle operates normally between meals.

2. Amino acid catabolism provides about 50% of liver's energy requirement.

Liver and amino acid (protein) metabolism (2)

3. Substrates for synthesis of glucose, fatty acids and ketone bodies.

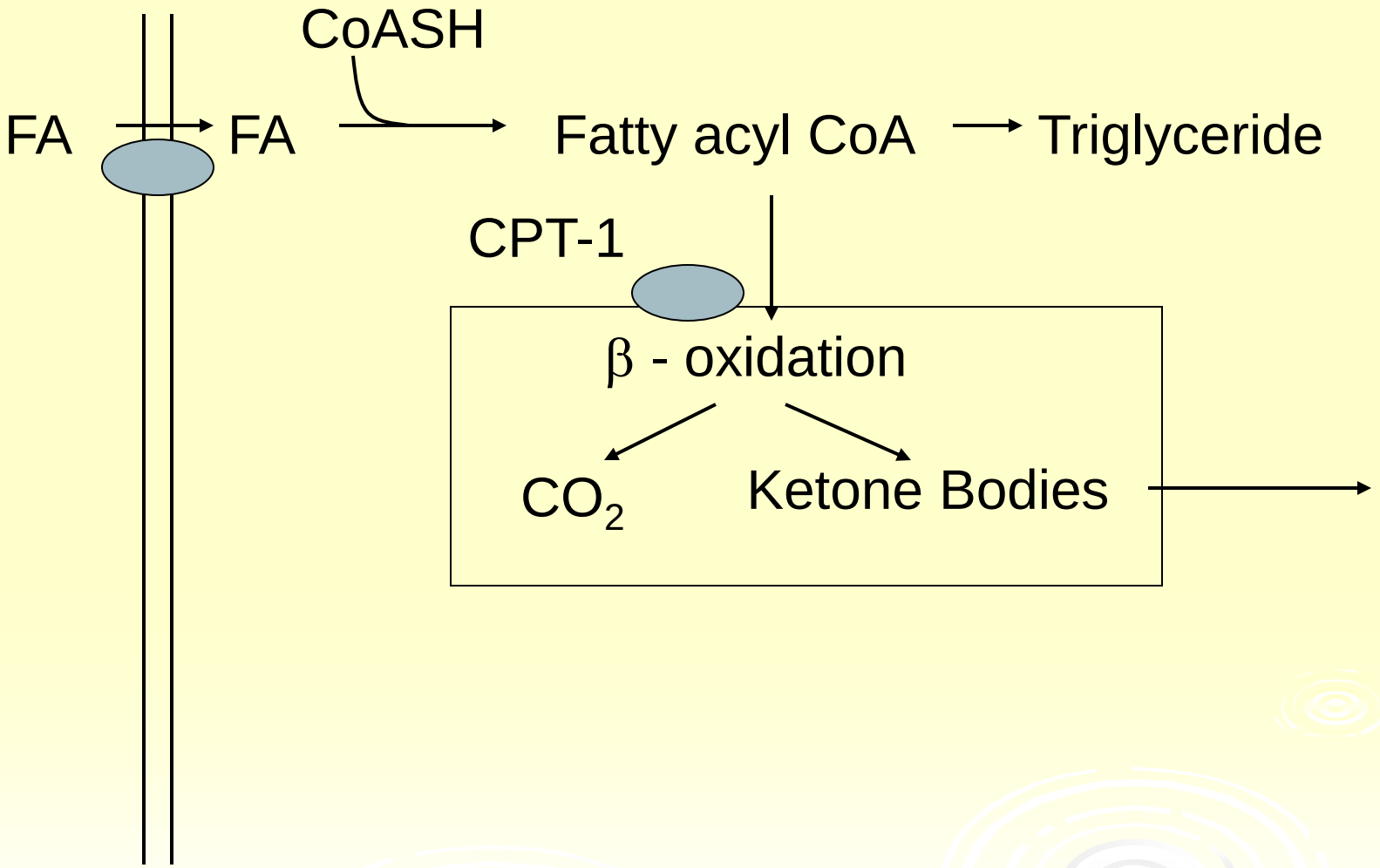

Amino Acid
Amino Acid
Transamination
Keto Acid
+ Urea
CO2
Acetyl CoA
Pyruvate
Ketone Bodies
Fatty Acids
Gluconeogenesis

Liver and Fatty Acid Metabolism

Liver takes up non-esterified fatty acids (NEFAs) from plasma. Two major fates:

A.) oxidation (energy source for liver – production of ketone bodies);

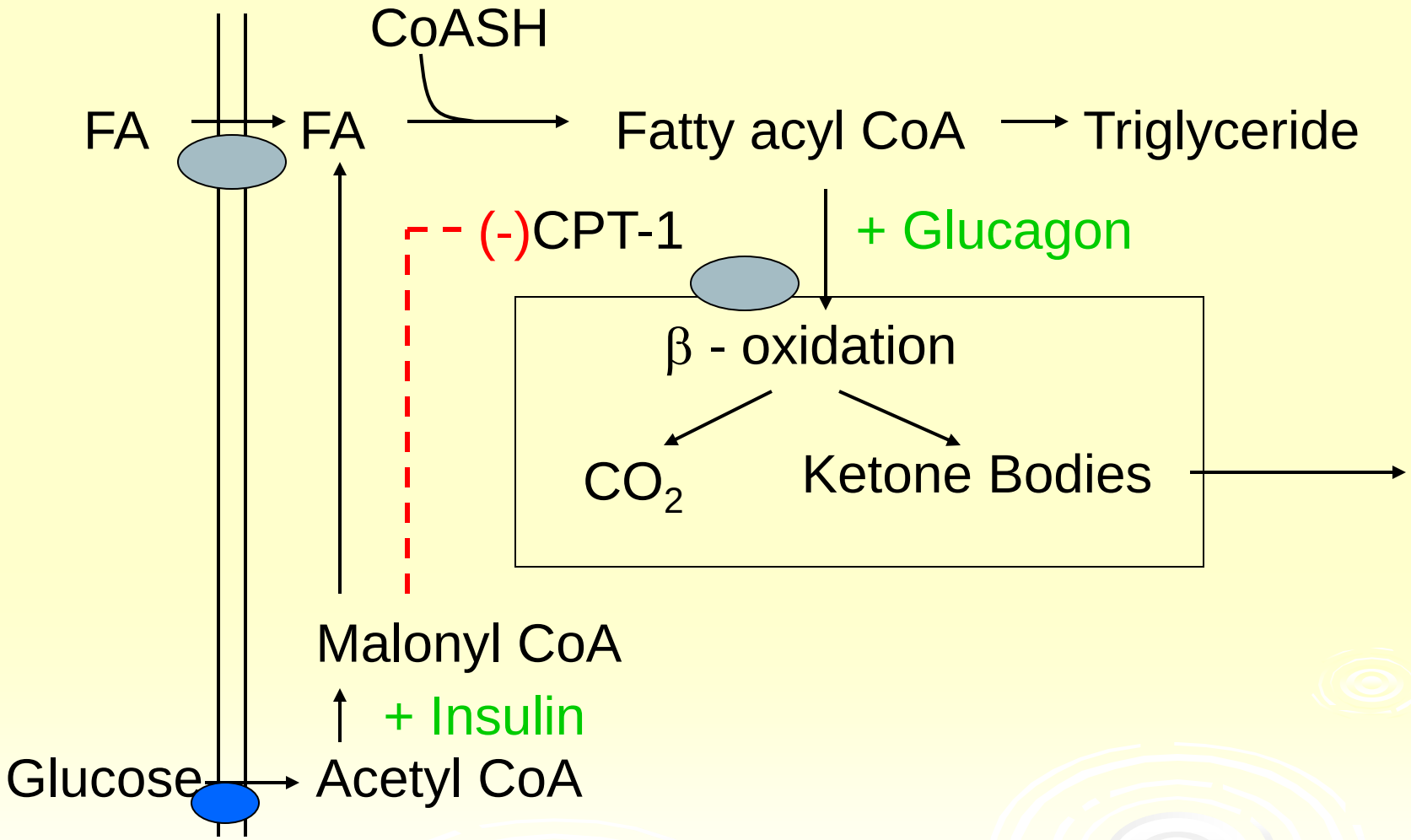
B.) triacylglycerol formation (local store for liver energy needs and distributed to other tissues as VLDL).


CoASH
FA
FA
Fatty acyl CoA
Triglyceride
CPT-1
β - oxidation
CO_2
Ketone Bodies

Regulated by insulin and glucagon

Fed state
– insulin is elevated
– malonyl CoA is high and CPT-1 is inhibited
– fatty acids esterified to TGs.

In fasting state
- glucagon elevated
- stimulates CPT-1
- FA oxidation is favoured.


CoASH
FA
FA
Fatty acyl CoA
Triglyceride
(-)CPT-1
+ Glucagon
β - oxidation
CO_2
Ketone Bodies
Malonyl CoA
+ Insulin
Glucose
Acetyl CoA

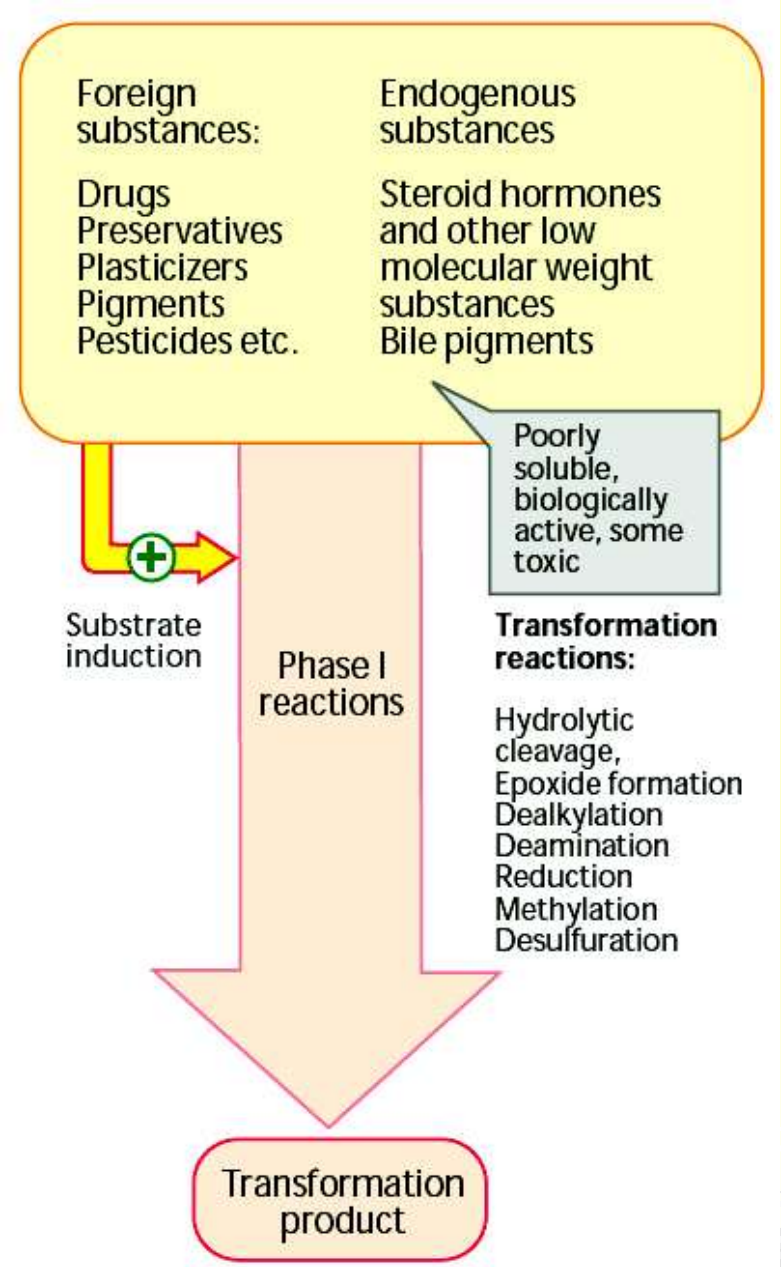
Xenobiotics

- **Xenobiotics** (ξενοσ – gk.) – various foreign chemicals.
  - *"As crude a weapon as the cave man's club, the chemical barrage has been hurled against the fabric of life"*

    Rachel Carson
- Biomedical importance:
  - Pharmacology,
  - therapeutics,
  - pharmacy,
  - toxicology,
  - management of cancer,
  - drug addiction.

Detoxification

- 2 phases:
  - **Phase 1:** oxidation, reduction, hydrolysis.
  - **Phase 2:** conjugation reactions (glucuronate, glycine, amino acids, sulfate, acetate, and methyl groups)
- About 30 different reactions are involved in xenobiotic metabolism.



Phase 1 reactions

- **Oxidation** occurs with:
  - Alcohols, aldehydes, amines, aromatic hydrocarbons, and sulfur compounds.
- In general: aliphatic compounds are more easily oxidized than aromatic.

Koolman, 2005

Cytochrome P450

- Other name – monooxygenase. Mixed function oxidase.
- Associated with microsomes.
- Absorption peak (CO-derivative) at 450 nm.

$$RH + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$$

- Also: deamination, dehalogenation, deslulfiration, epoxidation, peroxygenation, and reduction.

Substrates for Cytochrome P450

- Exogenous:
  - Drugs
  - Carcinogens
  - Pesticides
  - Pertoleim products
  - Pollutants
- Endogenous:
  - Cetrtain steroids
  - Eicosanoids
  - Fatty acids
  - Retinoids

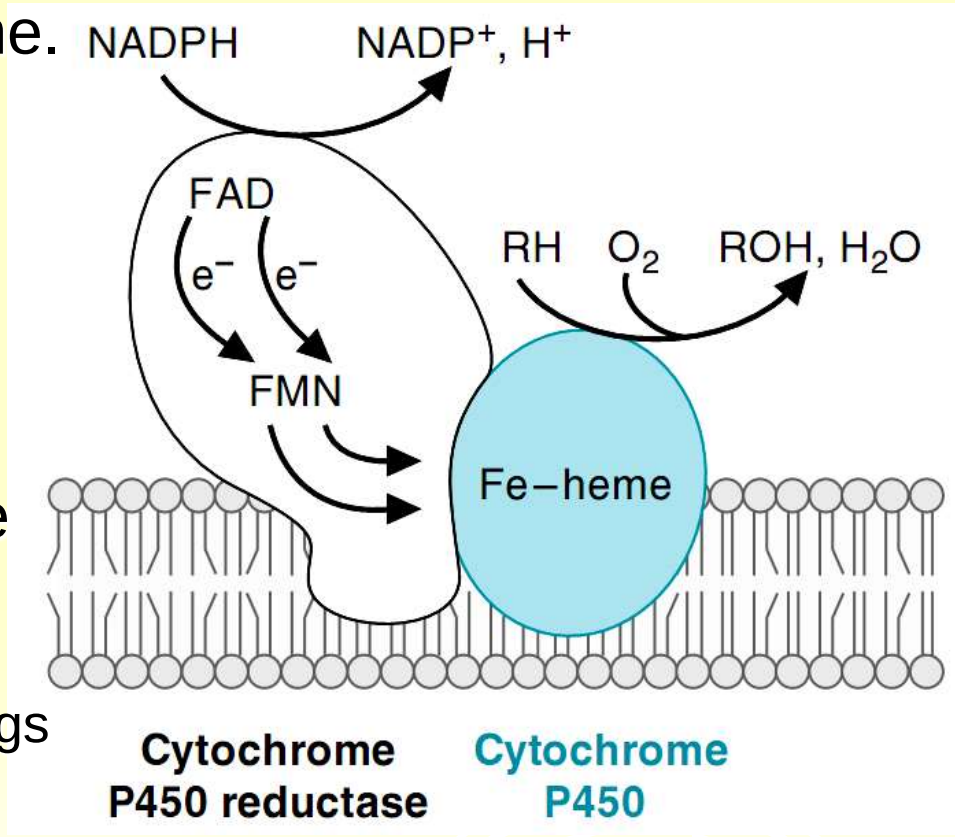
- The substrates are **lipophilic** and are rendered more **hydrophilic** by hydroxylation
- ~50% of the drugs humans ingest are metabolized by isoforms of cytochrome P450

Important Points of Cytochrome P450

1. Multiple forms: 35 – 60 (to 200), 14 families
   - Systematics:
     - CYP1A1 – cytochrom P450, member of family 1, subfamily A, and is the first individual member of that subfamily.
     - Italized *(CYP1A1)* – gene encoding CYP1A1.
2. Hemoprotein
3. Abundant in liver (microsomes of SER), small intestine, and adrenal (mitochondria and ER)
   - Mitochondrial cytochrome P450 uses **adrenodoxin reductase** and **adrenodoxin.** Restricted substrate specificity.

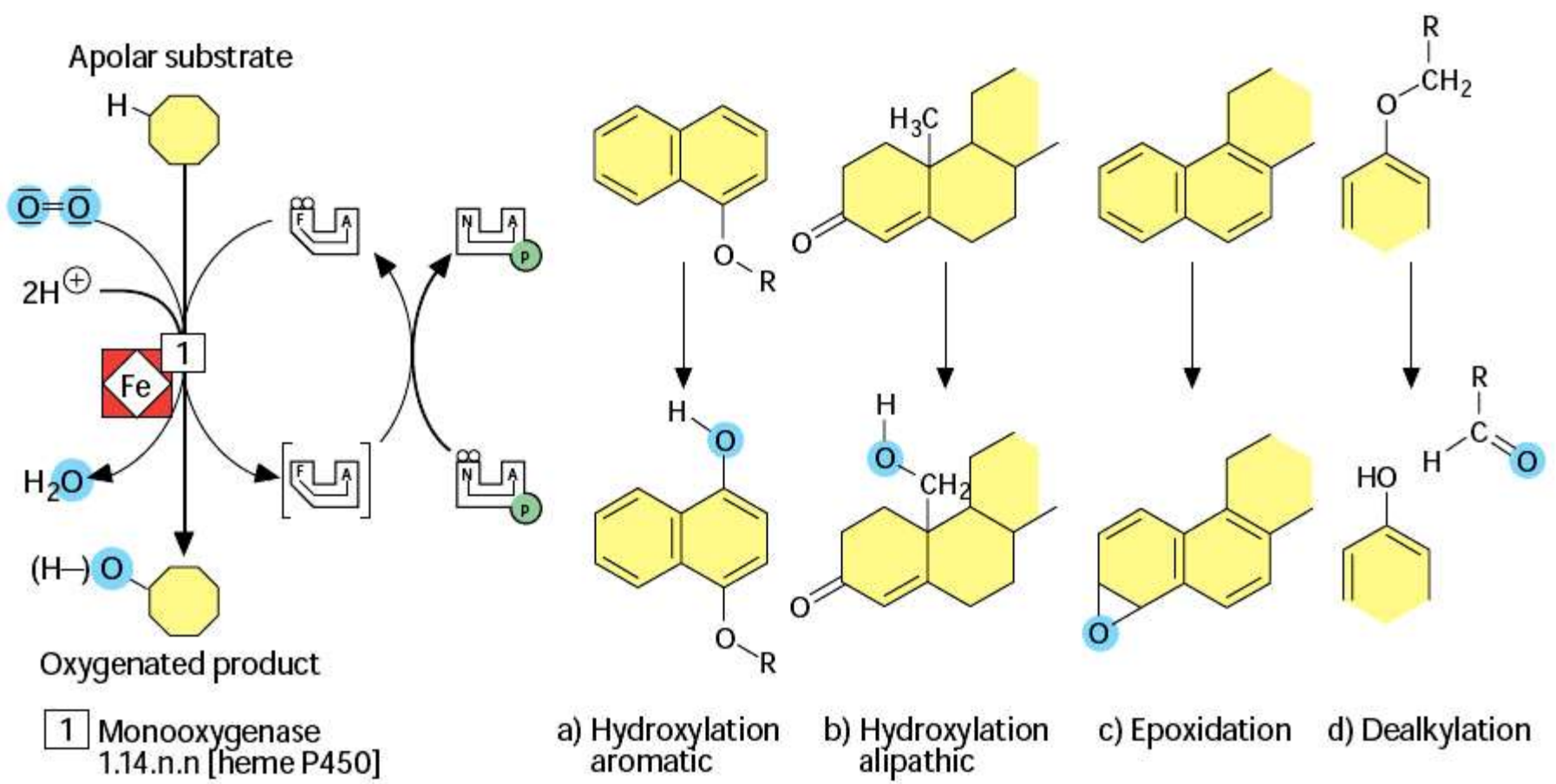
Important Points of Cytochrome P450 (cont'd)

4. NADPH-dependent enzyme.
   - NADPH-cytochrome reductase.
   - Reductive activation of molecular oxygen.
   - Cytochrom b5 – as electron donor.
5. Phosphatidylcholine is a consistuent of Cytochrome P450
6. Inducible enzyme.
   - Phenobarbital and other drugs – 3-4-fold increase in Cytochrome P450 amount within 4-5 days.



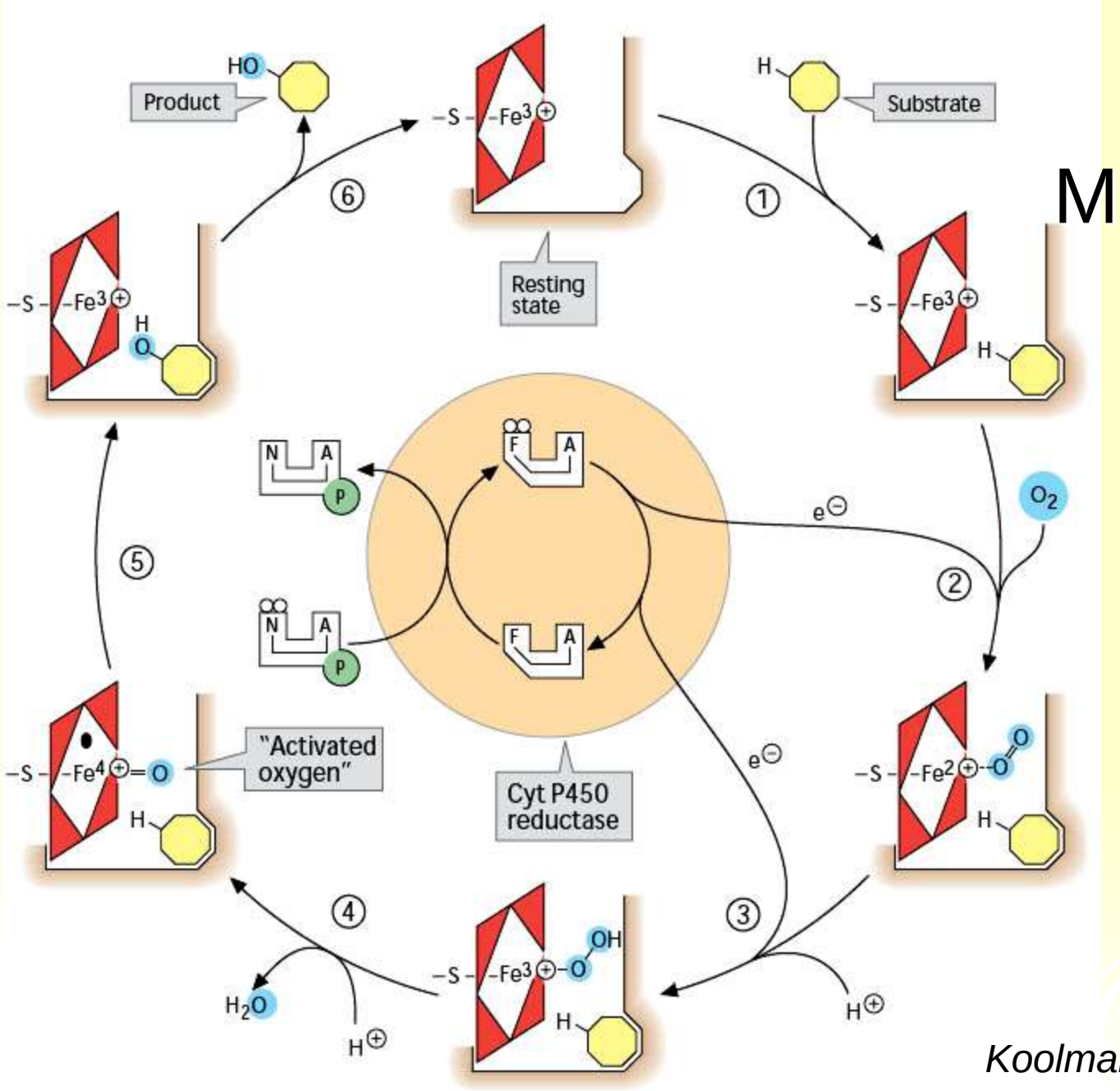
Marks, 2000

P450 Reactions

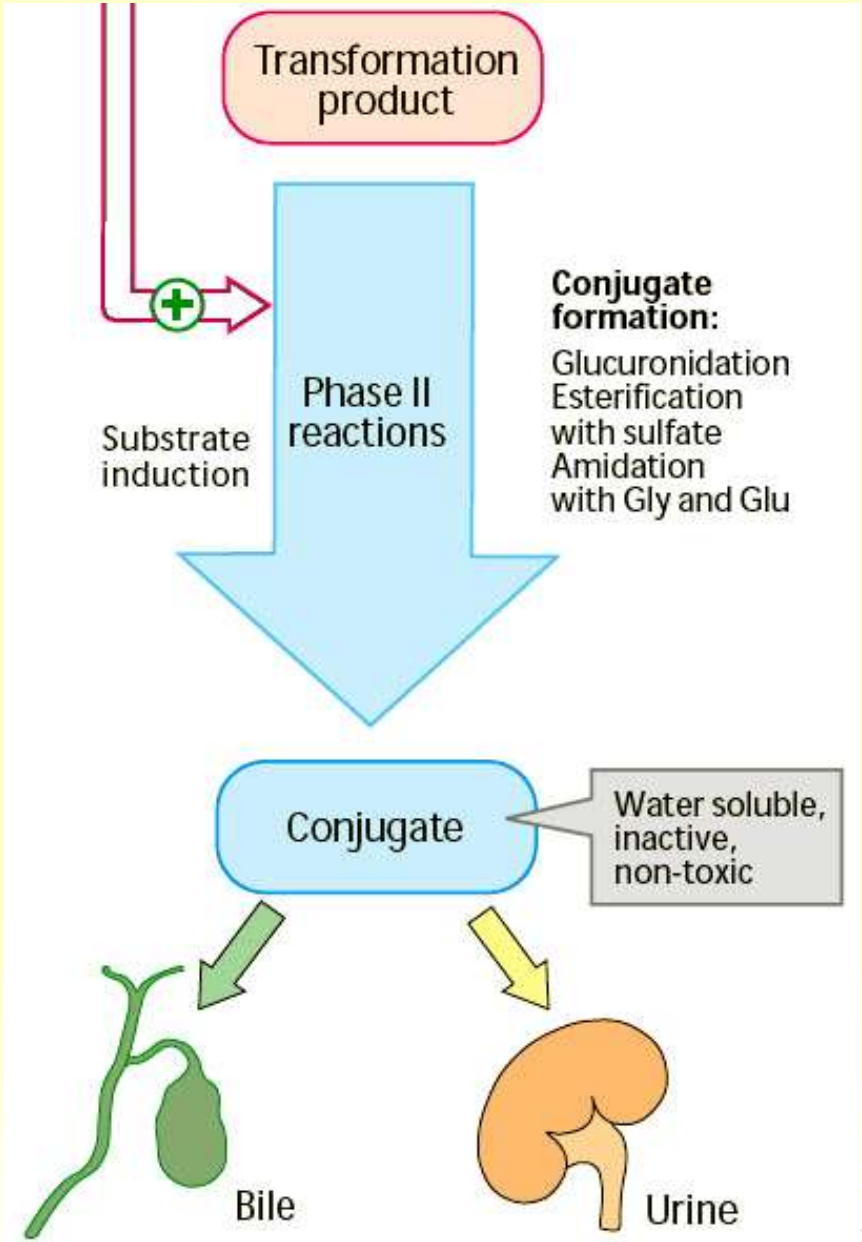


Koolman, 2005

P450 Mechanism



Koolman, 2005



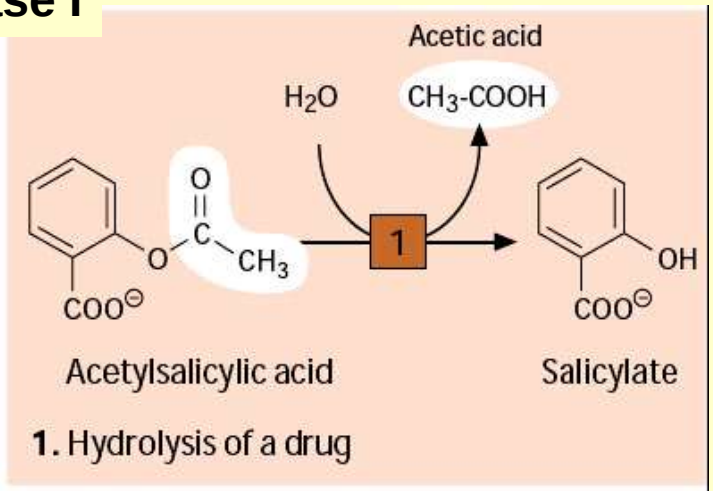
Biotransformations - Phase 2 reactions

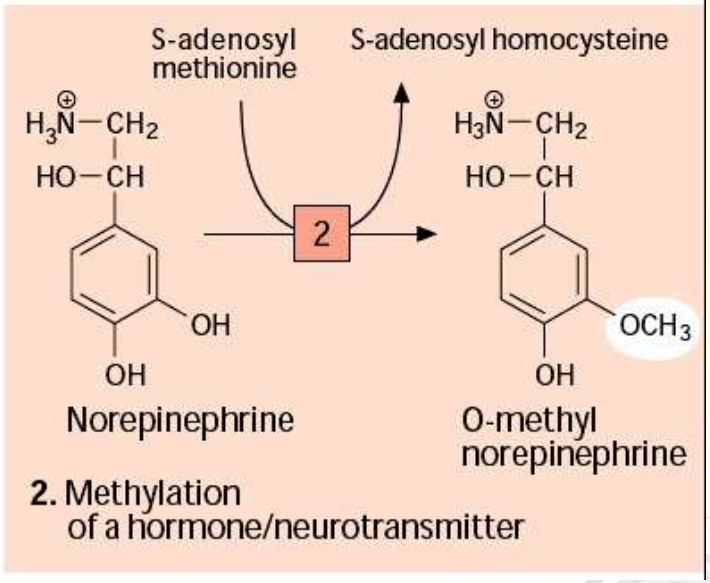
- Conjugation reactions, 5 types:
  - Glucuronidation
  - Sulfation
  - Conjugation with glutathione
  - Acetylation
  - Methylation

Koolman, 2005

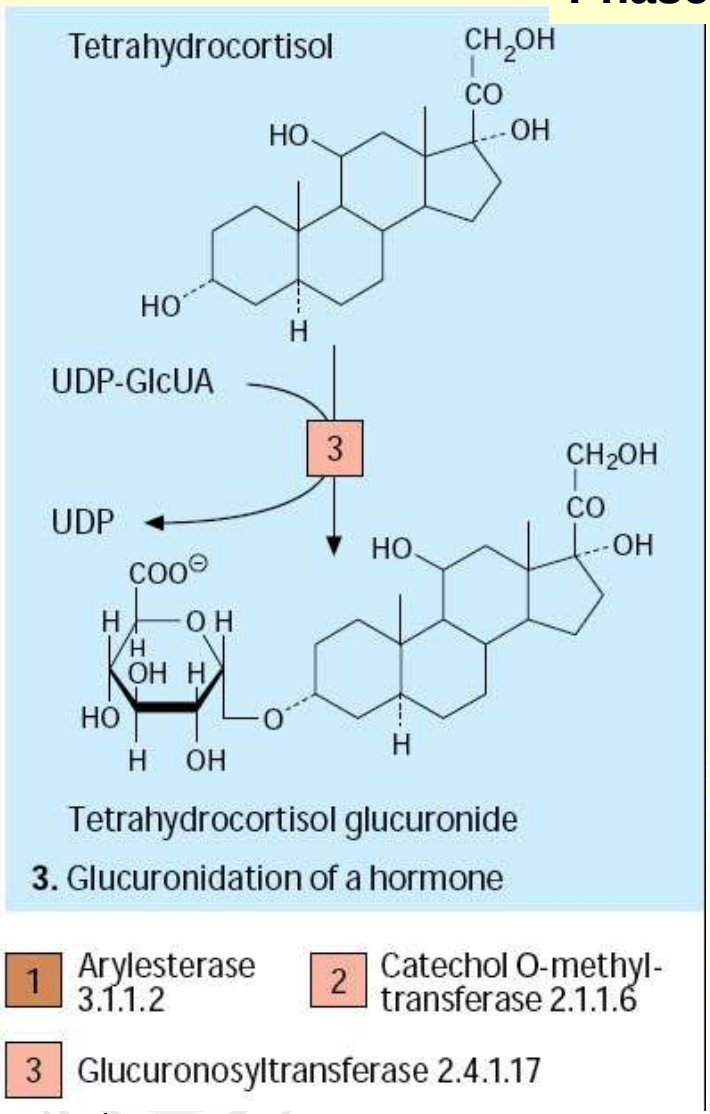
Examples of Reactions Phase 1 and 2

Phase I



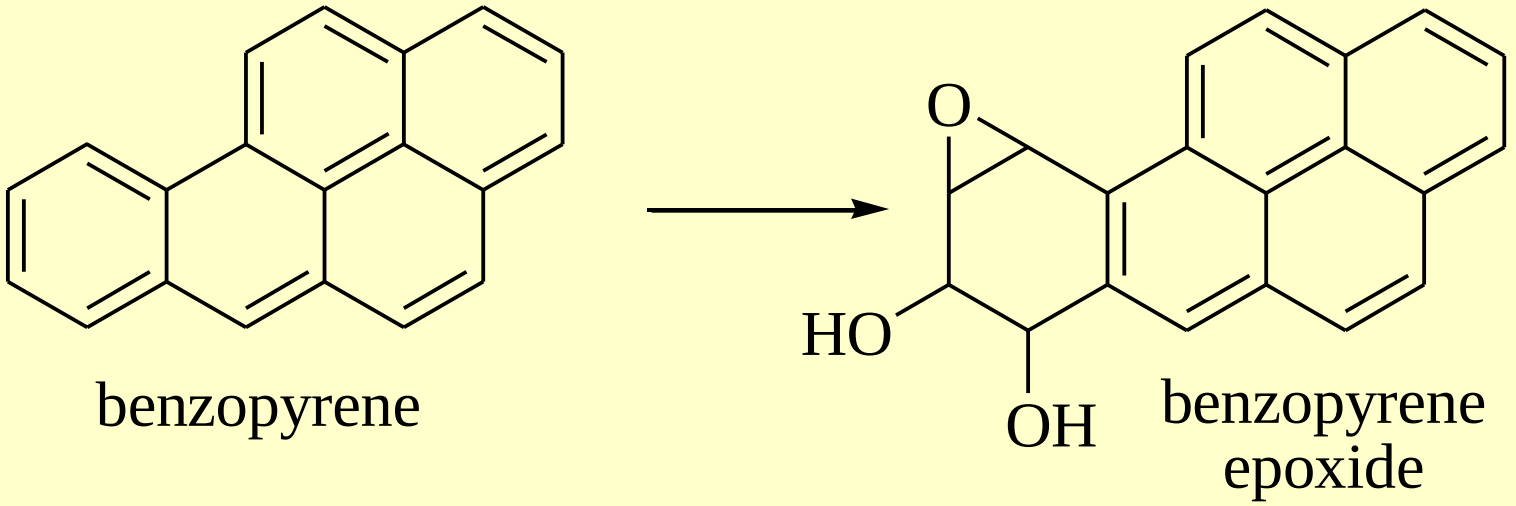


Phase II



Koolman, 2005

Lethal Synthesis

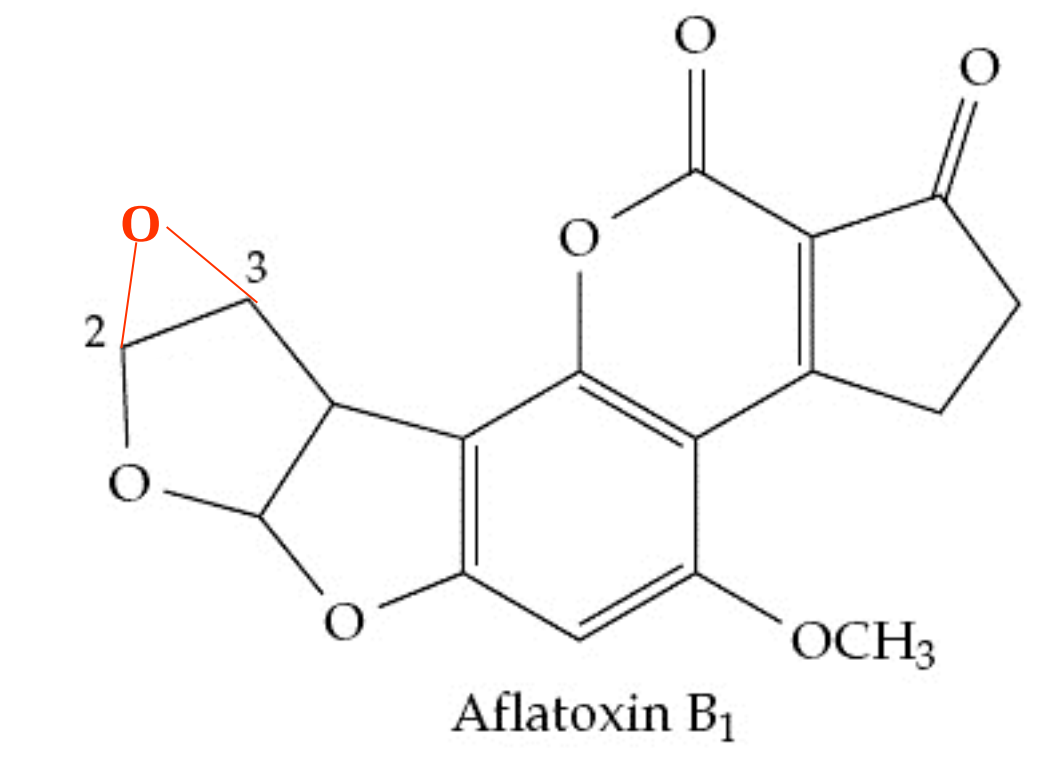


benzopyrene

benzopyrene epoxide

- In some cases P450 system produce even more toxic product than xenobiotics.
  - Examples:
    - benzopyrene from tobacco smoke, and aflatoxine B from Aspergillus flavus
    - are metabolized into the benzopyrene epoxide and aflatoxine epoxide – extremely carcinogenic substances.

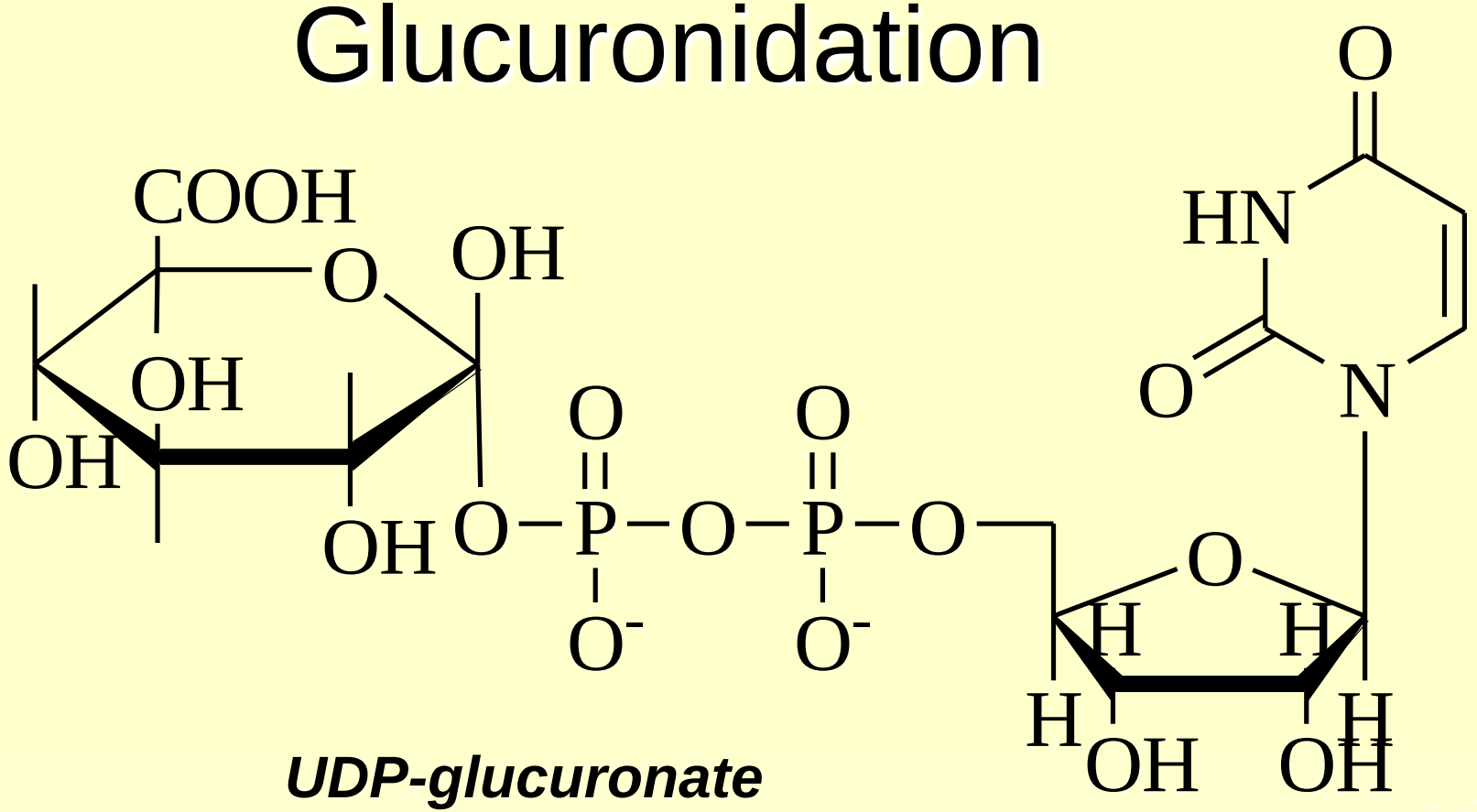
Lethal Synthesis (cont'd)



Aflatoxin B_1

- Aflatoxin B_1 is converted into 2,3-epoxide.

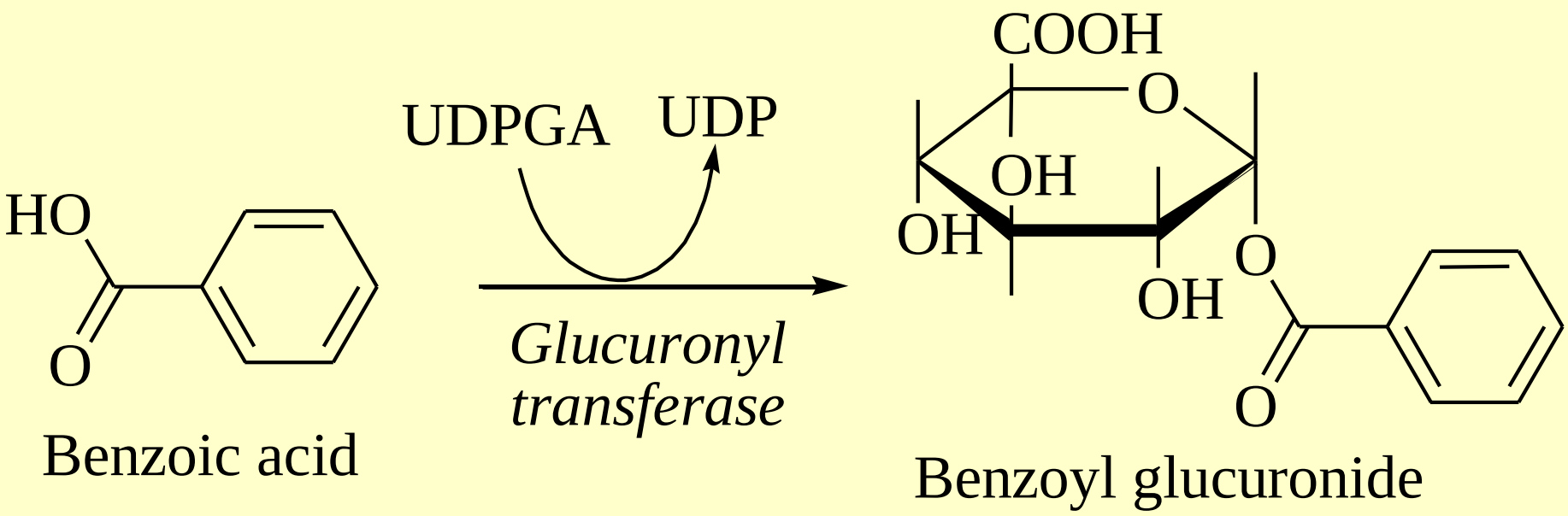
Glucuronidation



UDP-glucuronate

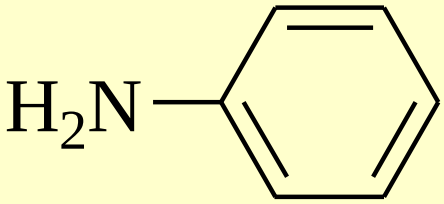
- UDP-glucuronic acid is glucuronyl donor in a variety of reactions.

Glucuronidation (cont'd)

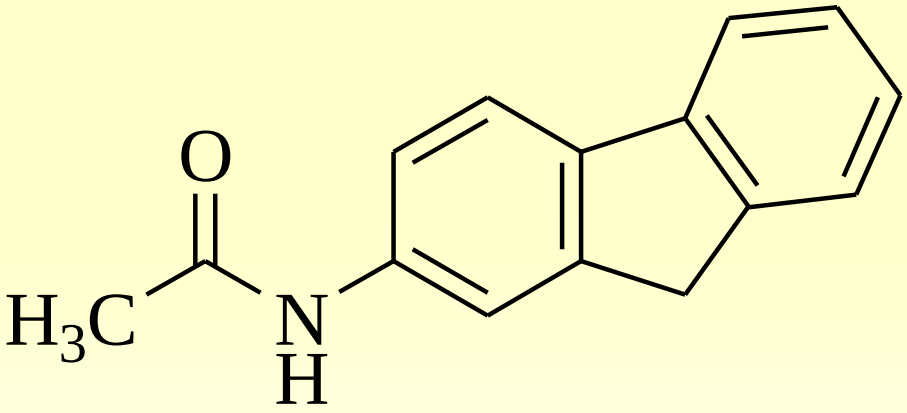


- Glucuronidation of benzoic acid.
  - Here the glucuronic acid is attached to oxygen, but might also to nitrogen or sulfur atoms of the substrate.

Glucuronidation (cont'd)



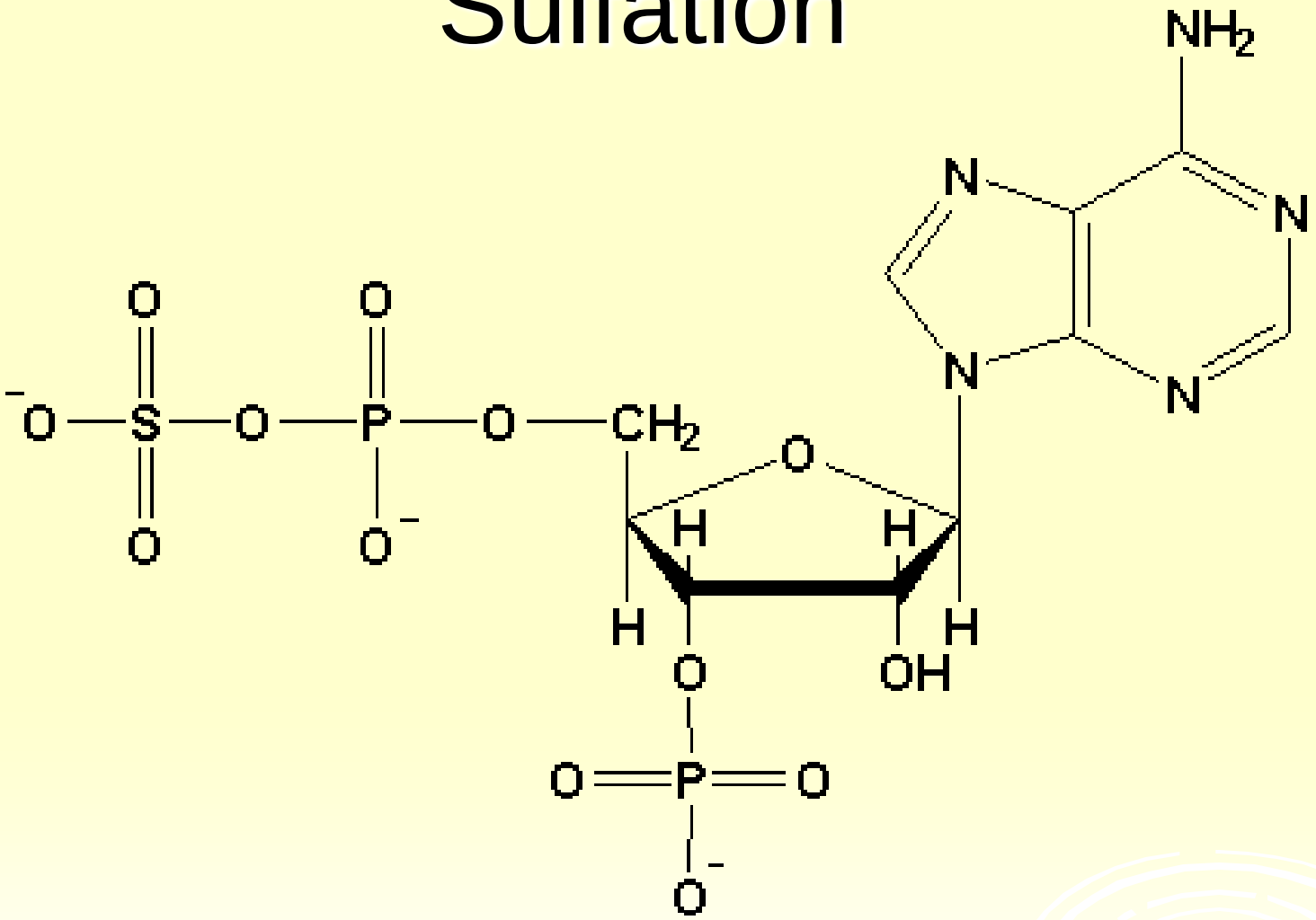
aniline



2-acetylaminofluoren

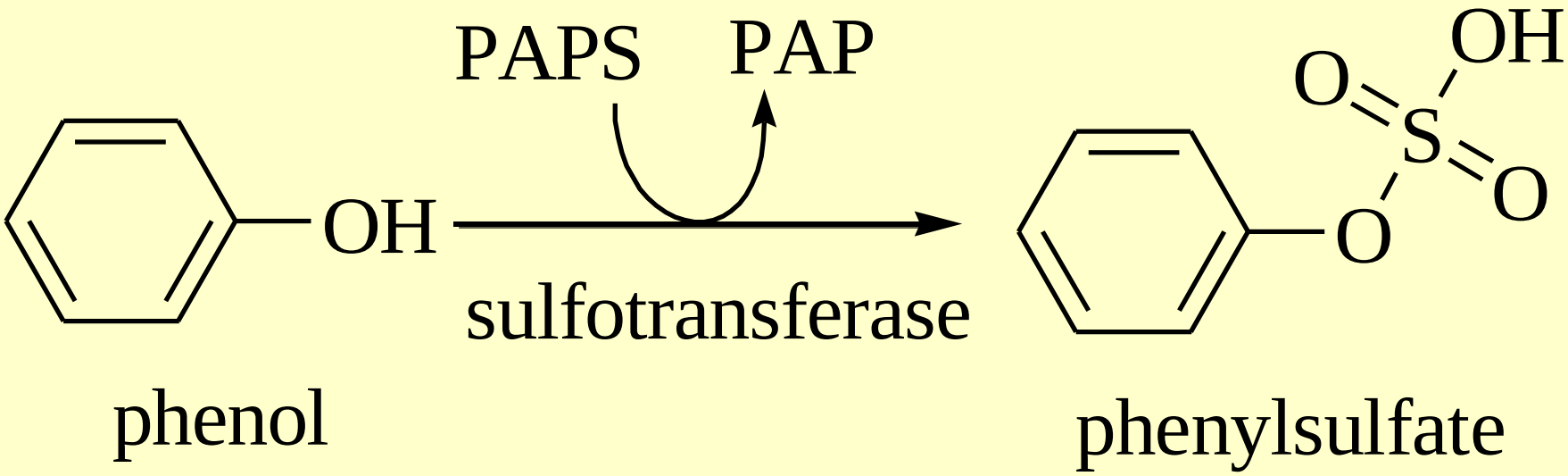
- Molecules such as 2-acetylaminofluoren, aniline, meprobamate, phenol and many steroids are excreted as glucuronides.

Sulfation



3'-Phosphoadenosine-5'-phosphosulfate

Sulfation (cont'd)

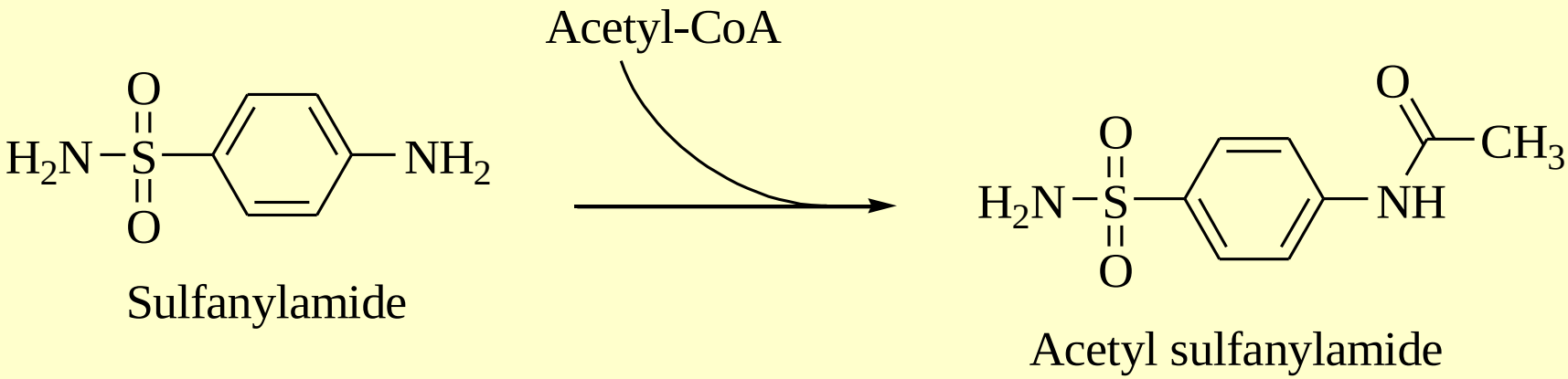


- Several aliphatic and aromatic compound undergo sulfation

Conjugation with glutathione

- Glutathione (GSH) is γ-glu-cys-gly.
  - R + GSH → R-S-G
  - Enzymes – various glutathione S-transferases
  - GSH – is an important defence mechanism
    - Participate in the decomposition of potentially toxic substances (H_2O_2).
    - Maintain essential –SH groups in their reduced state
    - Transport of certain amino acids across the membranes in kidney.
    - AA + GSH → γ-glu-AA + cys-gly
    - Enzyme γ-glutamyltransferase (GGT)

Acetylation



- Common reaction
  - X + Acetyl-CoA → Acetyl-X + CoA
    - X – xenobiotic.

Methylation

- A few xenobiotics are subject to metylation be methyltransferases, employing **S-adenosylmethionine** as the methyl donor.

Conclusion

- Liver is metabolically active organ.
- There are many functions of liver:
  - Synthesis, detoxication, barrier, storing.
- There are many reactions in hepatocyte.
  - Synthesis, hydroxilation, conjugation.


Thank you
for your attention!

Come in WITH NO NOICE!!!