Biochemistry of Blood-1. Fundamentals of Acid-base Balance Regulation

Lecture # 28

Lecturer Alexander Koval

Content

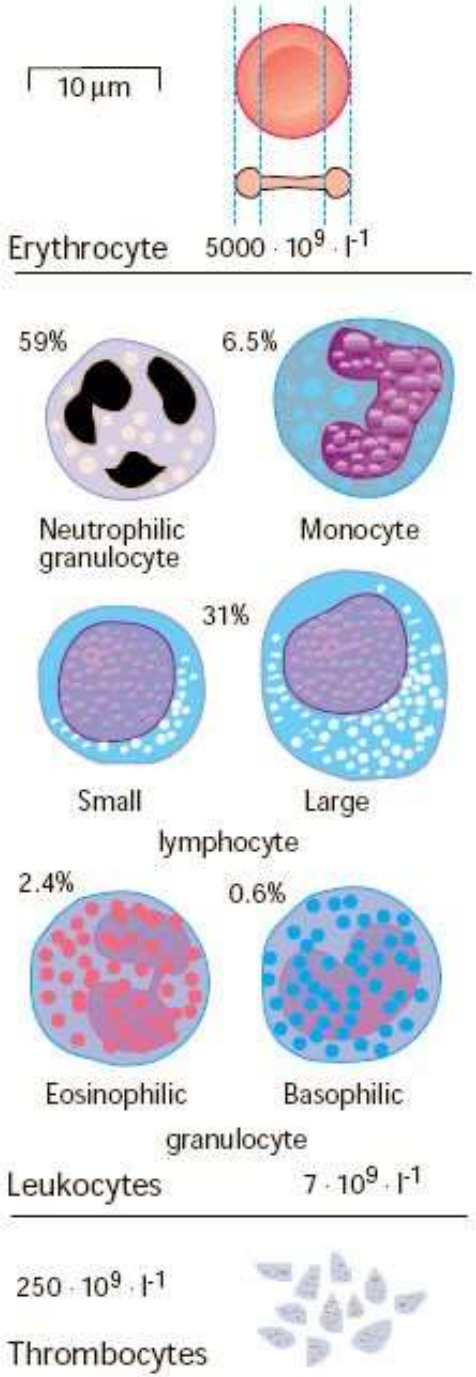
- Functions of blood. Physicochemical constants of blood.
- Blood plasma.
- Proteins of blood plasma. Classification. Changes of protein spectrum at pathology.
- Non-protein components of blood. Rest (nonprotein) nitrogen.
- Concept about acid-base balance (ABB). Base principles of ABB regulation. Mechanisms of ABB regulation:
- Classification of acid-base imbalances:
  - acidoses,
  - alkaloses.
- The basic mechanisms of respiratory, metabolic and secretory acid-base imbalances development.
- Physiological mechanisms of acid-base imbalances correction.
- The ways of evaluation of acid-base imbalances (ABB parameters and electrolytes of blood, urine pH, etc.).

Blood

- **BLOOD** is the river of life that flows through the human body.
  - The heart pumps blood to all our body cells, supplying them with oxygen and food.
  - At the same time, blood carries carbon dioxide and other waste products from the cells.
  - Blood also fights infection, keeps our temperature steady, and carries chemicals that regulate many body functions.
  - Finally, blood even has substances that plug broken blood vessels and so prevent us from bleeding to death.

Plasma & Serum

- **Plasma** is the liquid, straw-colored part of blood.
  - ≈ 50-60 % of the total volume of blood.
  - The formed elements account for the rest.
  - The packed cell volume or **hematocrit** is then about 45 %.
- Plasma consists of about 90 % of water. Hundreds of other substances make up the balance. They include
  - proteins that enable blood to clot and to fight infection;
  - dissolved nutrients (foods);
  - and waste products.
- Plasma also carries hormones, which control growth and certain other body functions.
- The term **serum** is applied to the liquid medium which separates out after the blood clots. Serum does not contain **fibrinogen**.

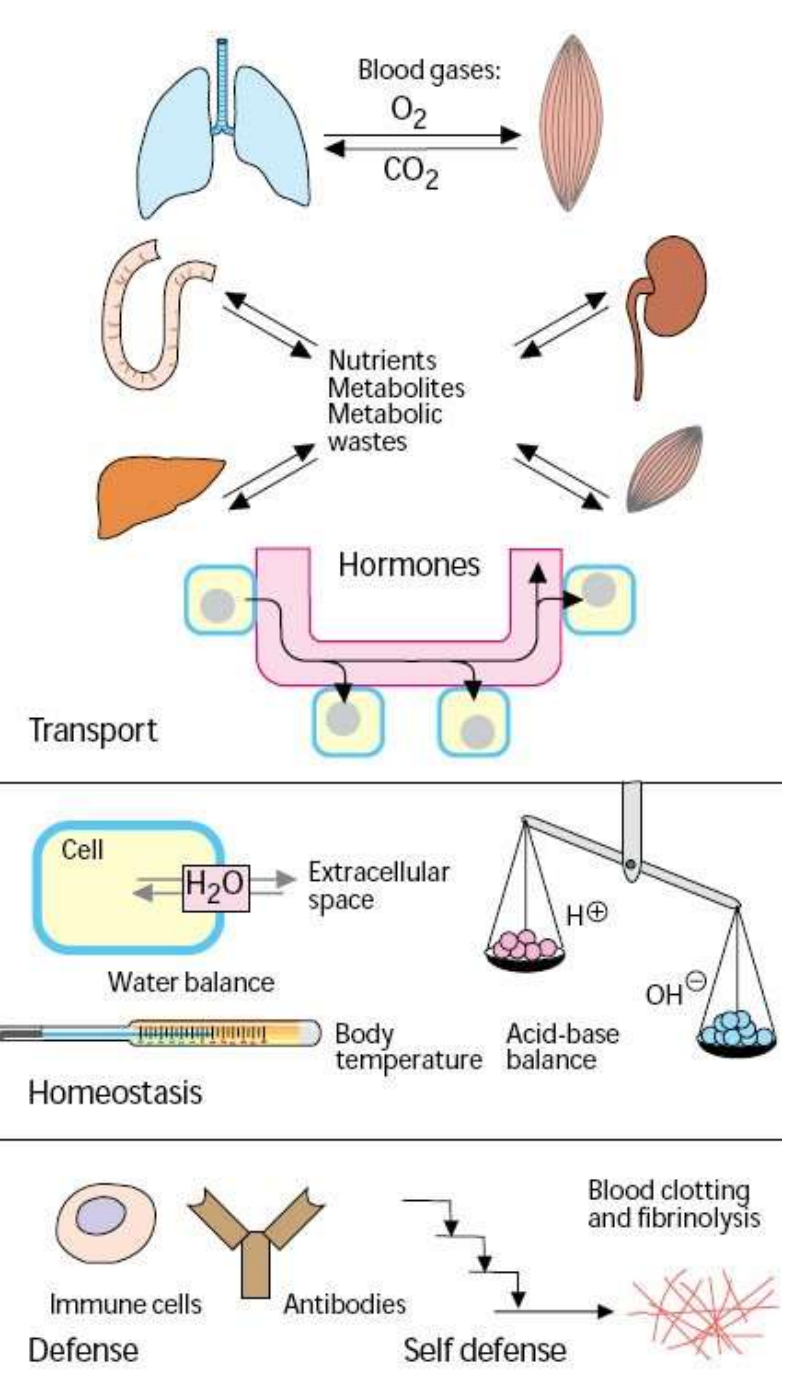


Cellular elements

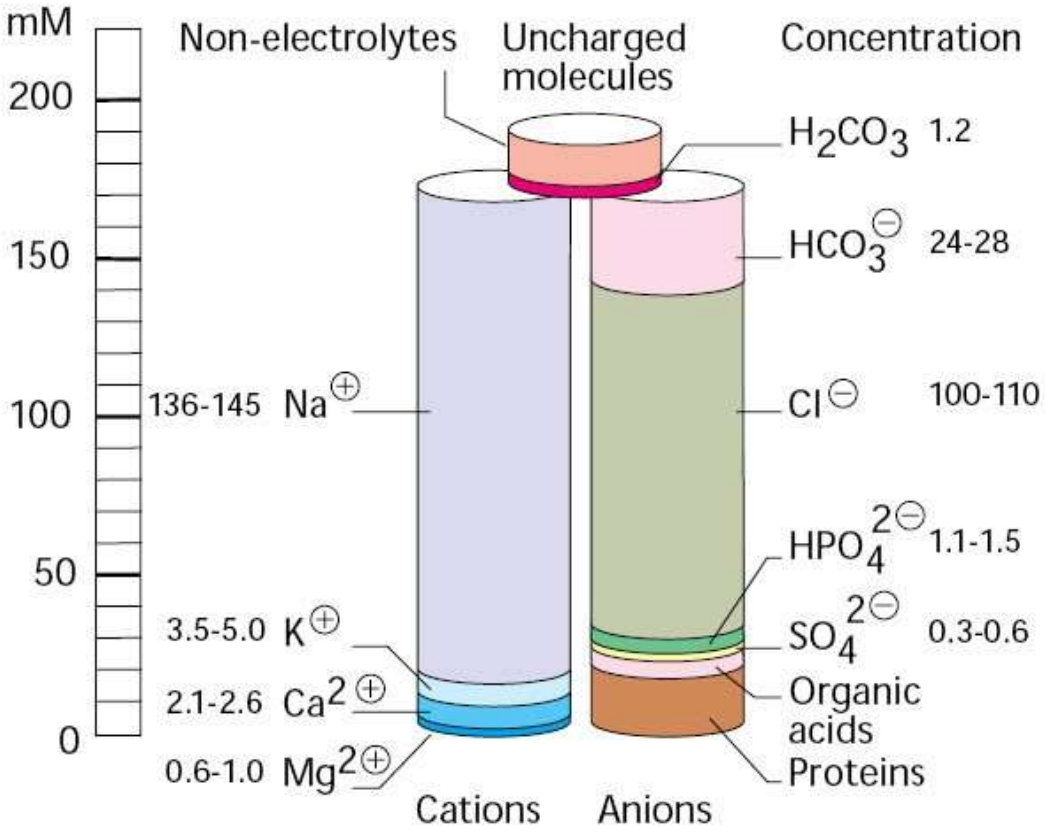
- The **erythrocytes** provide for gas transport in the blood.
- The **leukocytes** include various types of
  - granulocyte,
  - monocyte,
  - lymphocyte.
- immune defense functions.
  - The *neutrophil granulocytes*¸ *monocytes,* and the *macrophages* derived from monocytes are phagocytes.
  - The *lymphocytes* are divided into two groups, B lymphocytes and T lymphocytes. B lymphocytes produce *antibodies,* while T lymphocytes regulate the immune response and destroy virus-infected cells and tumor cells.
  - *Eosinophilic* and *basophilic granulocytes* have special tasks for defense against animal parasites.
- **Thrombocytes** are cell fragments that arise in the bone marrow from large precursor cells, the megakaryocytes. Their task is to promote hemostasis.

Importance of Blood

- Total volume in adult ≈4.5 – 5.0 liters.
- Several functions:
  - Respiration
  - Excretion
  - Acid-base maintenance,
  - Water balance,
  - Transport of metabolites, hormones and drugs,
  - Body defense and coagulation.



Blood plasma: composition



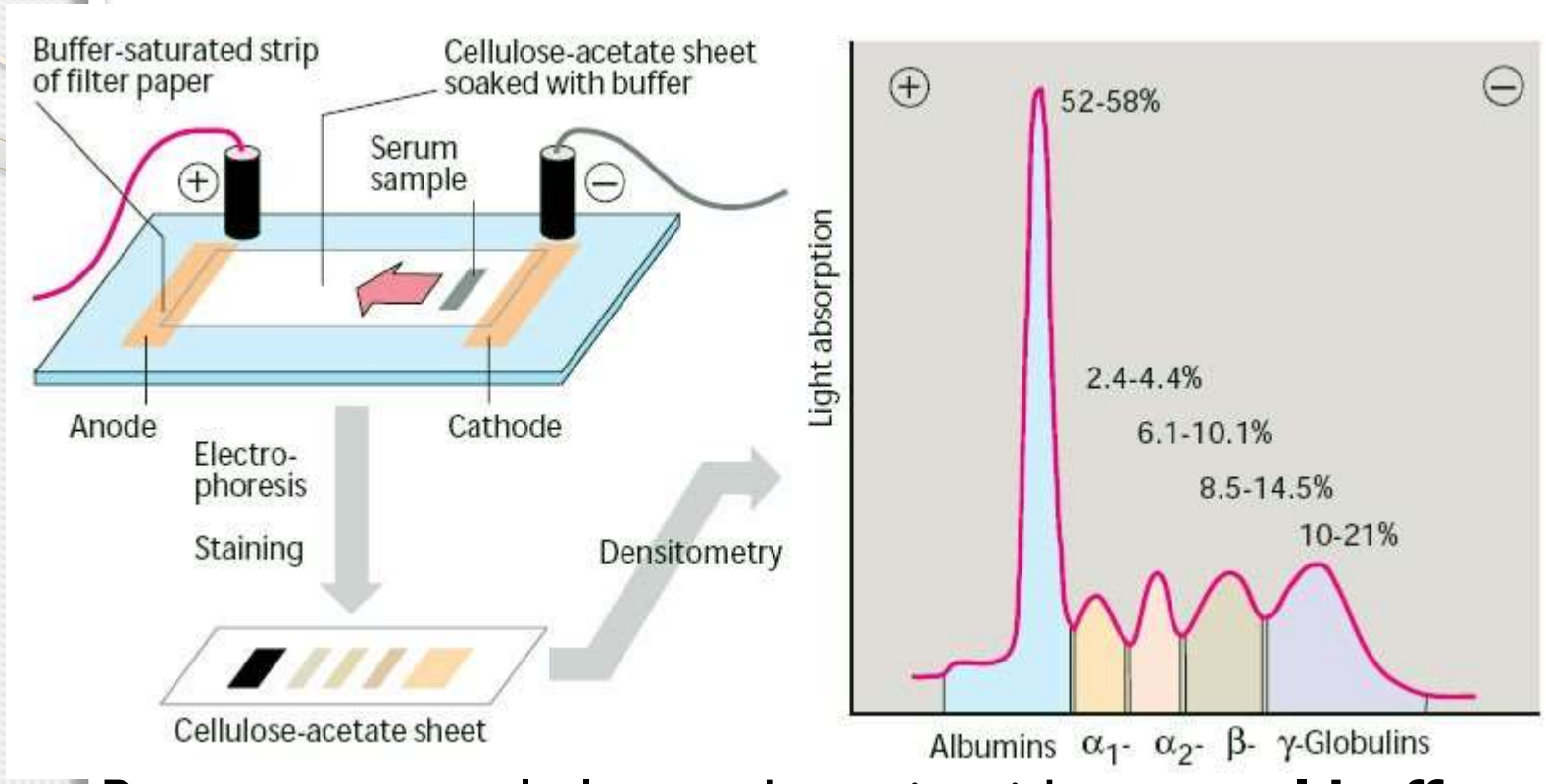
Metabolite	Concentration (mM)
Glucose	3.6 – 6.1
Lactate	0.4 – 1.8
Pyruvate	0.07 – 0.11
Urea	3.5 – 9.0
Uric acid	0.18 – 0.54
Creatinin	0.06 – 0.13
Amino acids	2.3 – 4.0
Ammonia	0.02 – 0.06
Lipids (total)	5.5 – 6.0 $g \cdot l^{-1}$
Triacylglycerols	1.0 – 1.3 $g \cdot l^{-1}$
Cholesterol	1.7 – 2.1 $g \cdot l^{-1}$

Plasma Proteins and Immunoglobulins

Separation of plasma proteins

- The total concentration of plasma protein is about 65-85 g/L.
- Their tasks include transport, regulation of the water balance, hemostasis, and defense against pathogens.
  - **Electrophoresis** is the most commonly employed analytical technique for the separation of plasma (serum) protein.

Electrophoresis



- Paper or agar gel electrophoresis with **veronal buffer** (pH=8.6) separates plasma proteins into 5 distinct bands namely **albumins**, α_1, α_2, β and γ **globulins**.

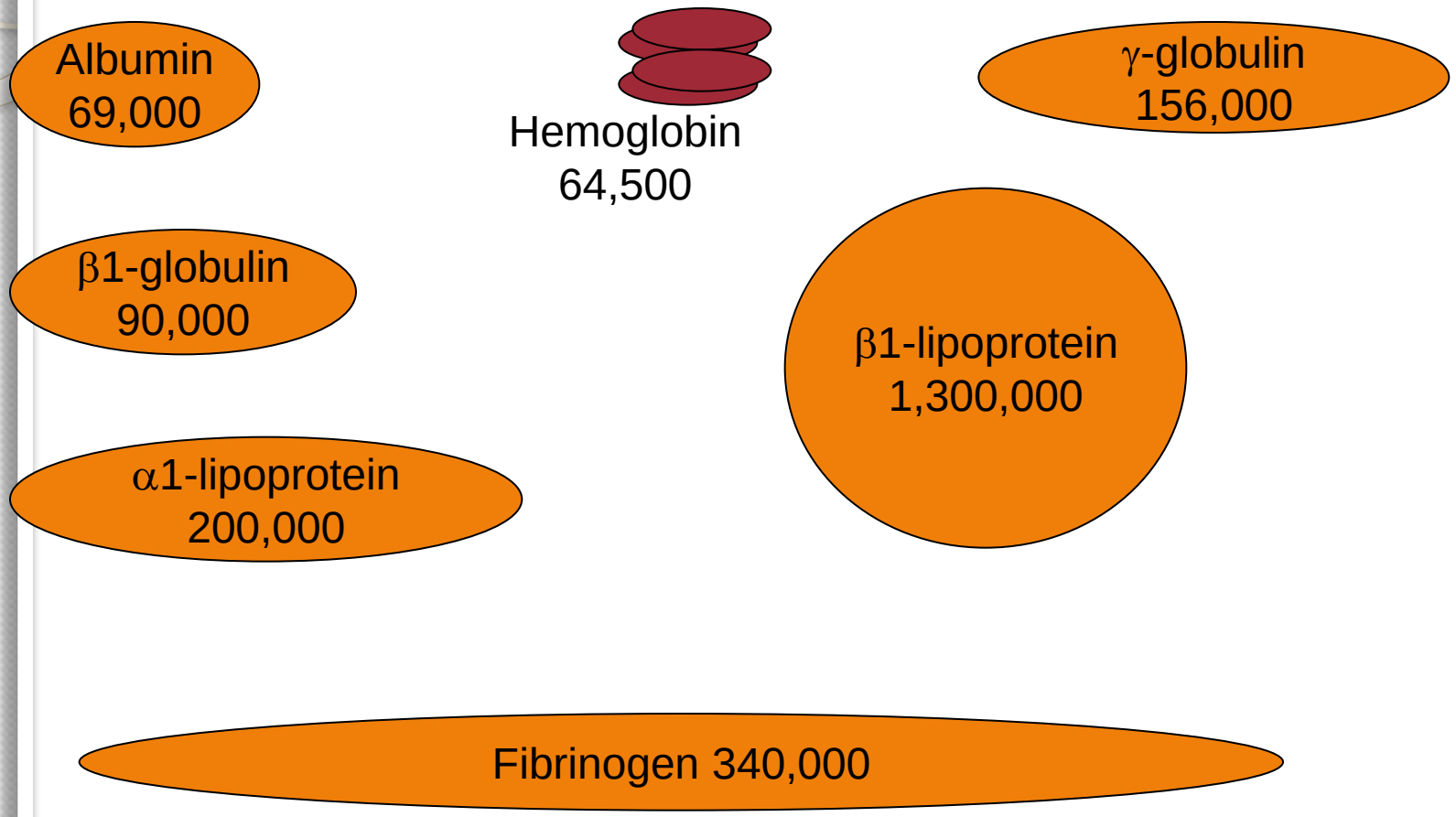
Some functions of plasma proteins

Function	Plasma Protein
Antiproteases	Antichymotrypsin, α_1-Antitrypsin, α_1-Macroglobulin, Antithrombin
Blood clotting	Various coagulation factors, fibrinogen
Enzymes	Function in blood, eg, coagulation factors, cholinesterase Leakage from cells or tissues, eg, aminotransferases
Hormones	Erythropoietin
Immune defense	Immunogobulins, complement proteins, β_2-microglobulin
Involvement in inflammatory responses	Acute phase response proteins (eg, C-reactive protein, α_1-acid glycoprotein [orosomucoid])
Oncofetal	α1-Fetoprotein (AFP)

Some functions of plasma proteins (cont'd)

Function	Plasma Protein
Transport or binding proteins	Albumin (various ligands – bilirubin, FFA, ions [Ca^{2+}], metals [Cu, Zn, etc], metheme, steroids, other hormones, drugs) Ceruloplasmin (contains Cu^{2+}) Corticosteroid-binding globulin (transcortin) – binds cortisol Haptoglobin (binds extracorpuscular hemoglobin) Lipoproteins (chylomicrons, VLDL, LDL, HDL) Hemopexin (binds heme) Retinol-binding protein (binds retinol) Sex hormone-binding globulin (binds testosterone, estradiol) Thyroid-binding globulin (binds T_3, T_4) Transferrin (transport iron) Transthyretin (formely prealbumin) – binds T_4 and forms a complex with retinol-binding protein

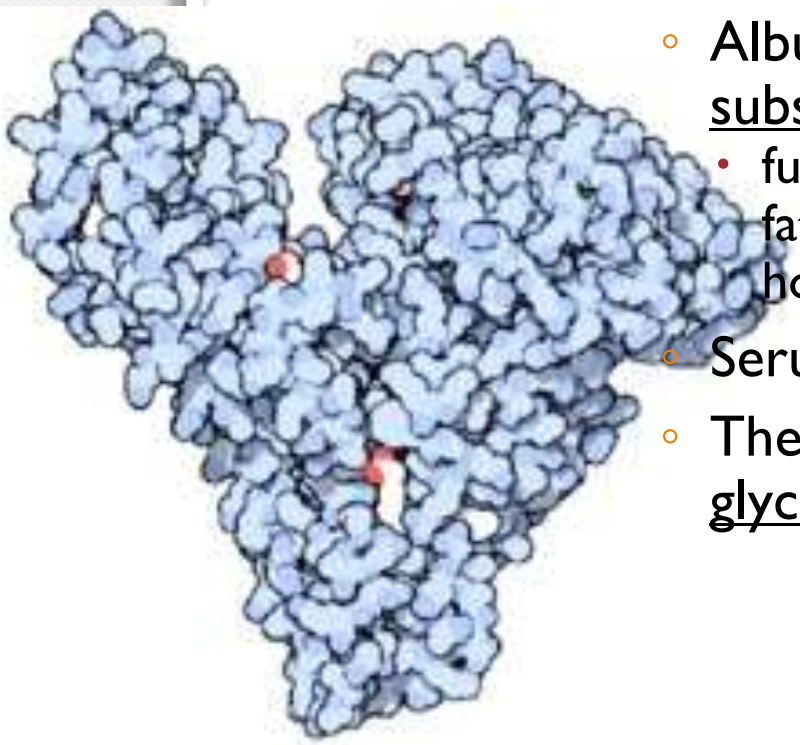
Relative Dimensions and Approximate Molecular Masses of Protein Molecules in the Blood



Albumin

- The major constituent of the total plasma proteins (55-60 % or 35-50 g/L).
- Human albumin has a molecular weigth of 69,000 and consists of a single polypeptide chain of 585 aminoacids, 17 disulfide bonds.
  - The liver produces ~12 g of albumin per day (25 % of total hepatic protein synthesis and ½ of its secreted proteins)
  - Preproalbumin – contains signal peptide to pass through the RER.

Albumin Structure & Functions



- Albumin plays a crucial role in maintaining the blood's colloid osmotic pressure;
  - represents an important amino acid reserve for the body.
  - Albumin has binding sites for apolar substances:
    - functions as a transport protein for long-chain fatty acids, bilirubin, drugs, and some steroid hormones and vitamins.
  - Serum albumin binds Ca^{2+} and Mg^{2+} ions.
  - The only important plasma protein that is not glycosylated.

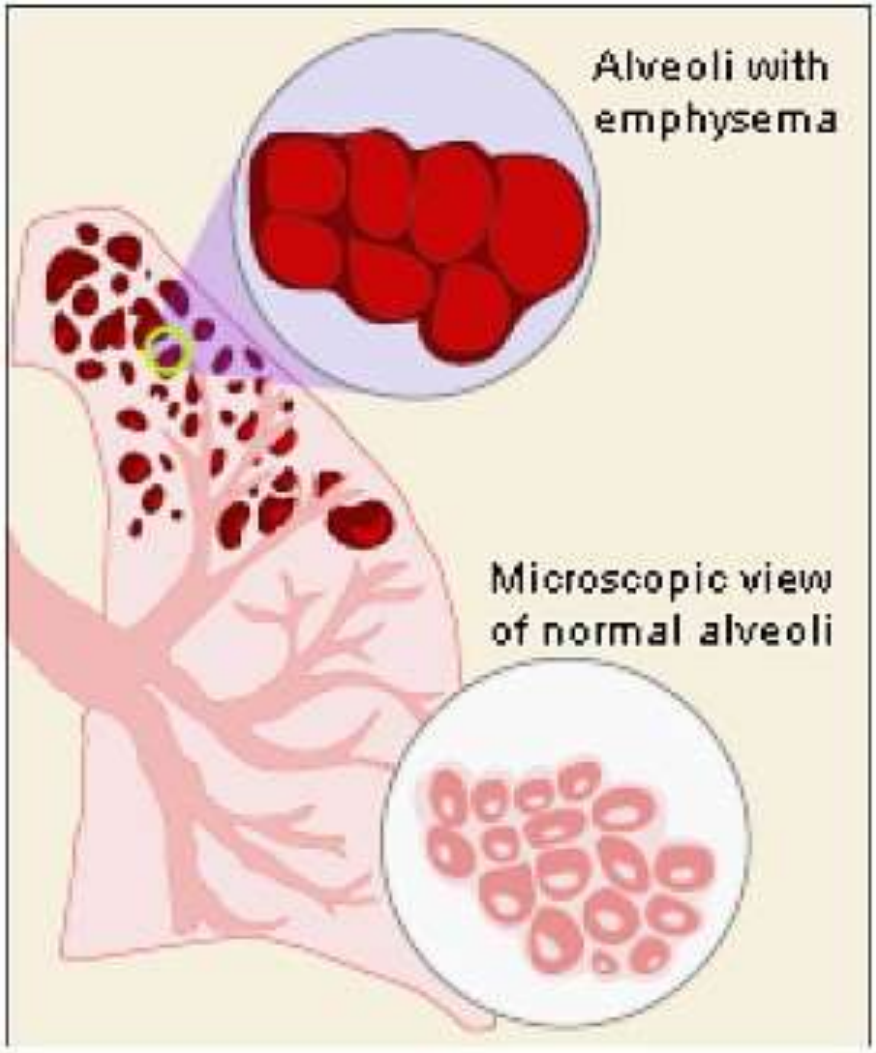
Albumin/Globulin (A/G) Ratio

- The normal A/G – 1.2 – 1.5.
- A/G is lowered in:
  - Decreased synthesis of albumins by liver – liver disease, severe protein malnutrition.
  - Excretion of albumin into urine – kidney damage.
  - High production of globulins – chronic infections, multiple myelomas etc.

α-1-Antitrypsin Deficiency

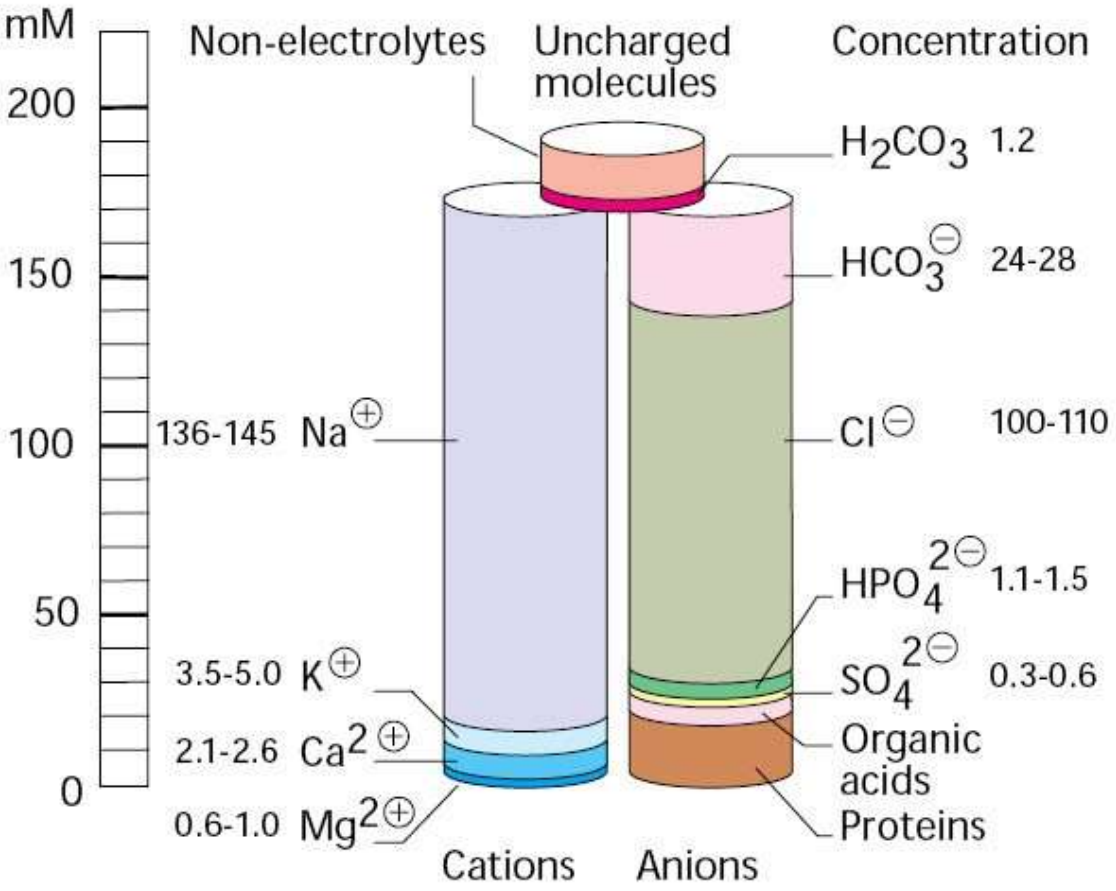
- **α-1-antitrypsin** (AAT) is a protein that protects the body from damage by its immune cells.
- Deficiency of this protein leaves the lung, and occasionally the liver, vulnerable to injury.
- The lung is made of thin outpouchings called *alveoli*. These contain air, and oxygen travels across their walls into the bloodstream.

Emphysema



- White blood cells release **elastase**, a powerful enzyme that can fight infections. But it can also attack normal tissues. If uncontrolled elastase is released around alveoli, it would destroy their walls and surrounding tissue, leaving areas of trapped air.
- This abnormal accumulation of air in the lungs is called emphysema and causes shortness of breath.
- AAT inhibits elastase around normal tissue.

Acid-Base Balance



Acid-base balance (ABB) – is the system of homeostasis for internal medium.

The principles of acid-base balance regulation:

- Isoosmolarity (310 mosm);
- Electroneutrality (155 A^-, 155 K^+);
- Constance of pH (7.40).

Hydrogen Ion Concentration in the Blood Plasma

- The H^+ concentration in the blood and extracellular space is approximately 40 nM (4×10^{-8} mol×L^{-1}).
  - This corresponds to a pH of 7.40 (pH = -lg[H^+]).
  - The body tries to keep this value constant, as large shifts in pH are incompatible with life.
  - Precise mechanism.
- The pH value is kept constant by **buffer systems** that cushion minor disturbances in the *acid–base balance*.
- In the longer term, the decisive aspect is maintaining a balanced equilibrium between H^+ production and uptake and H^+ release.

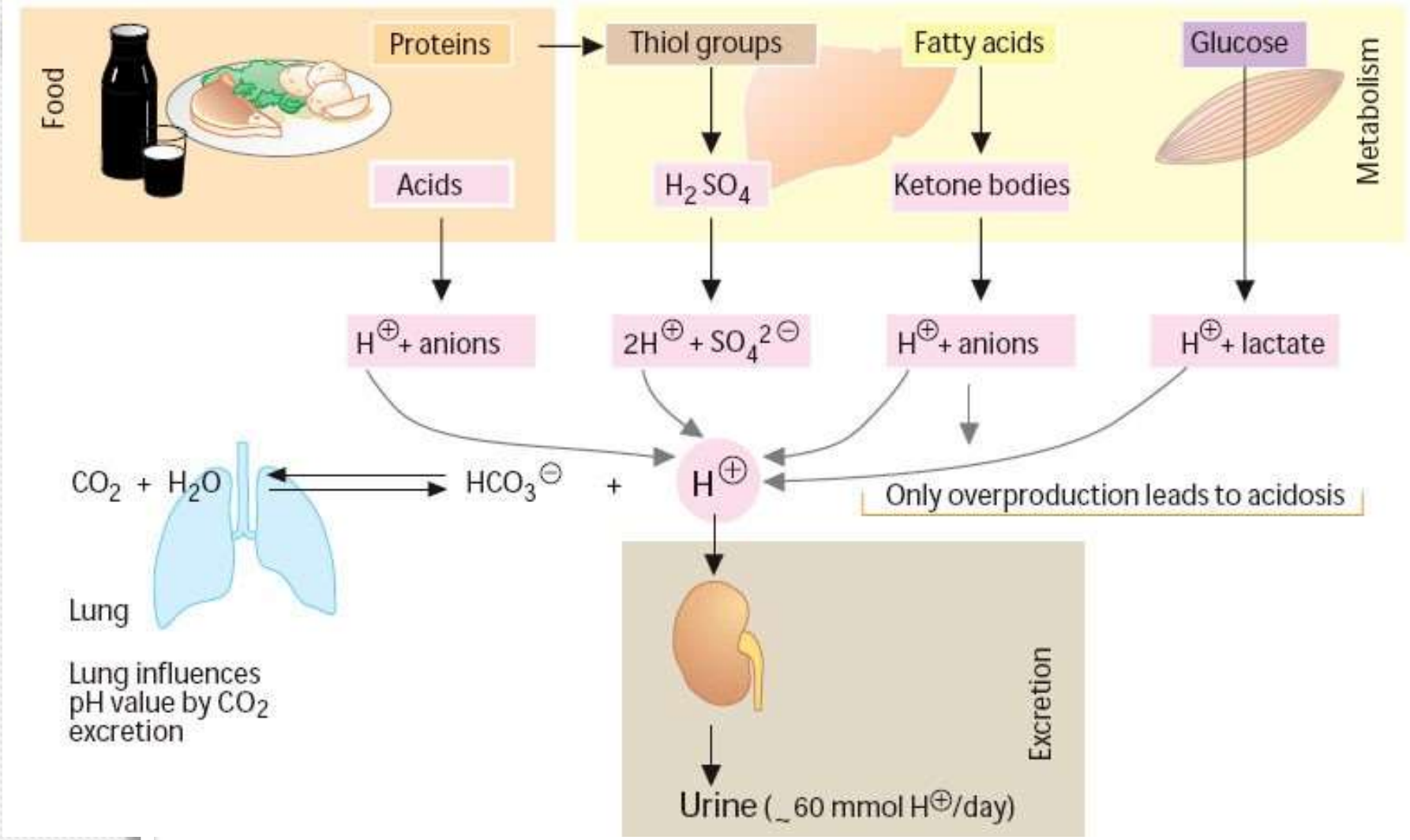
$$\mathrm{pH} = \mathrm{pK}_a + \log_{10}\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

*the **Henderson–Hasselbalch equation** describes the derivation of pH as a measure of acidity*

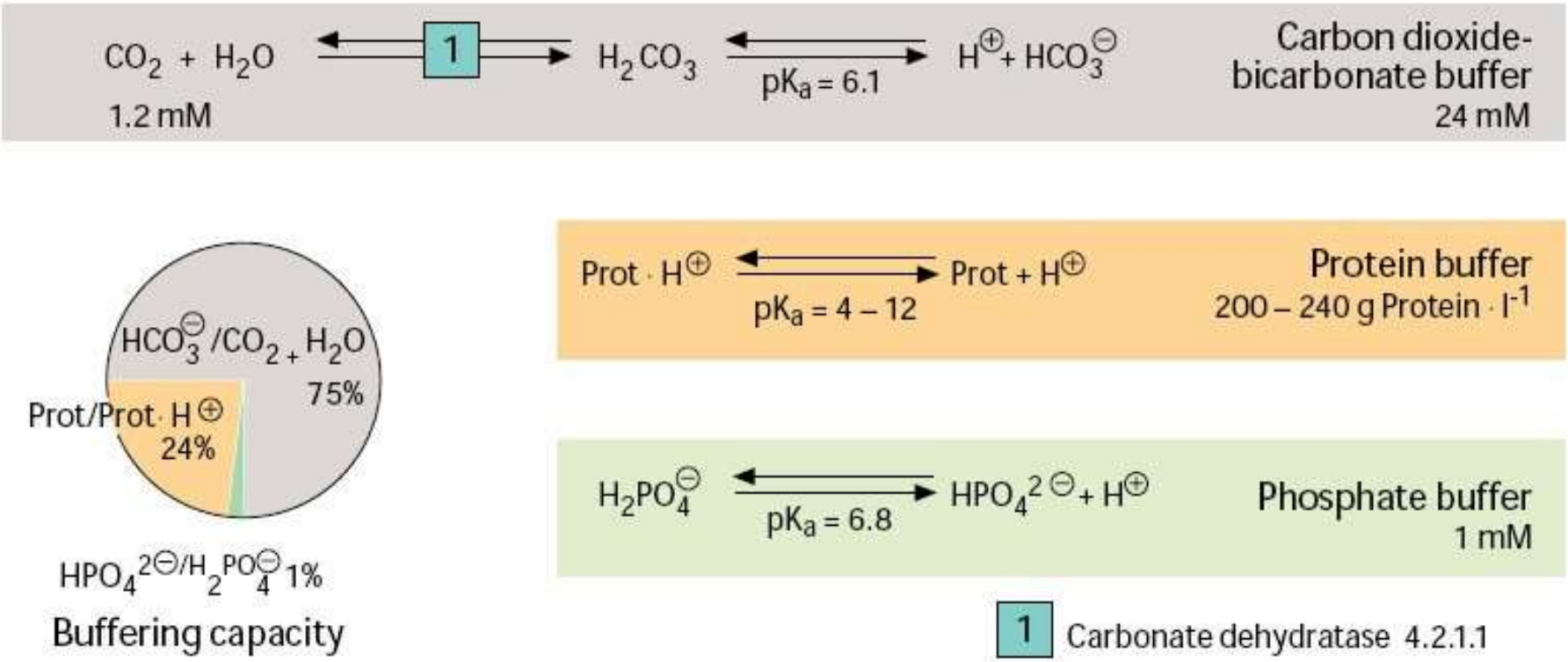
Distribution of Ions in Body Fluids

	ECF* *mmol/L*	ICF
Cations		
Na^+	145	12
K^+	4	150
Anions		
Cl^-	105	5
HCO_3^-	25	12
Inorganic Phosphate	2	100

Acid-base Regulation



Buffer systems in the plasma



Estimating blood pH

- The Henderson-Hasselbalch equation can be applied to relate the pH of blood to constituents of the bicarbonate buffering system:

$$\mathrm{pH} = \mathrm{pK}_{a\ \mathrm{H_2CO_3}} + \log_{10}\left(\frac{[\mathrm{HCO_3^-}]}{[\mathrm{H_2CO_3}]}\right)$$

- where:
  - $\mathrm{pK}_{a\ H2CO3}$ is the cologarithm of the acid dissociation constant of carbonic acid. It is equal to 6.1.
  - $[\mathrm{HCO_3^-}]$ is the concentration of bicarbonate in the blood
  - $[\mathrm{H_2CO_3}]$ is the concentration of carbonic acid in the blood

Mechanisms of ABB Regulation

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$$

- There are many others reactions in the organism accompanied with H^+ formation.
- Mechanisms of ABB regulation:
  - Physico-chemical:
    - Dilution, formation of insoluble compounds.
  - Physiological:
    - Enforced respiration, kidney, liver (GNG), gastro-intestinal tract function.

Acidosis and Alkalosis

$$CO_2\uparrow + H_2O \rightarrow H_2CO_3 \rightarrow H^+\uparrow + HCO_3^-$$

- If the blood's buffering capacity is not suficient, or if the acid-base balance is not in equilibrium – e. g., in kidney disease or during *hypoventilation* or *hyperventilation* – shifts in the plasma pH value can occur.
- A reduction by more than 0.03 units is known as **acidosis,** and an increase is called **alkalosis.**

$$CO_2\downarrow + H_2O \leftarrow H_2CO_3 \leftarrow H^+\downarrow + HCO_3^-$$

Respiratory and Metabolic Compensation in Acid-Base Disorders

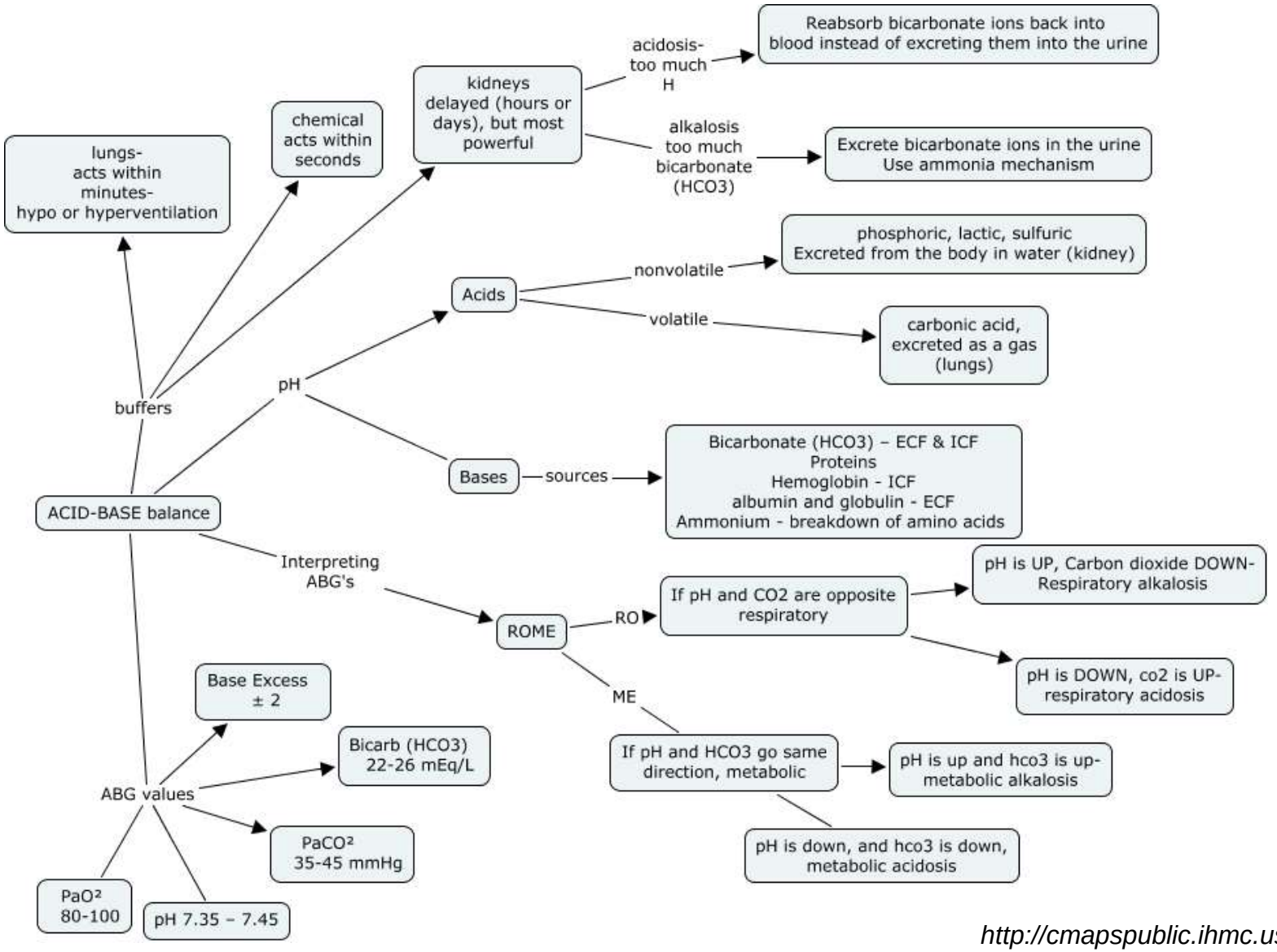
Acid/base disorder	Primary change	Compensatory change	Timescale of compensatory change
metabolic acidosis	decrease in plasma bicarbonate concentration	decrease in pCO_2 (hyperventilation)	minutes/hours
metabolic alkalosis	increase in plasma bicarbonate concentration	increase in pCO_2 (hypoventilation)	minutes/hours
respiratory acidosis	increase in pCO_2	increase in renal bicarbonate reabsorption: increase in plasma bicarbonate concentration	days
respiratory alkalosis	decrease in pCO_2	decrease in renal bicarbonate reabsorption: decrease in plasma bicarbonate concentration	days

Baynes,

Clinical Causes of Acid-base Disorders

Metabolic acidosis	**Respiratory acidosis**	**Metabolic alkalosis**	**Respiratory alkalosis**
diabetes mellitus (ketoacidosis)	chronic obstructive airways disease	vomiting (loss of hydrogen ion)	hyperventilation (anxiety, fever)
lactic acidosis (lactic acid)	severe asthma	nasogastric suction (loss of hydrogen ion)	lung diseases associated with hyperventilation
renal failure (inorganic acids)	cardiac arrest	hypokalemia	anemia
severe diarrhea (loss of bicarbonate)	depression of respiratory center (drugs, e.g. opiates)	intravenous administration of	salicylate poisoning
surgical drainage of intestine (loss of bicarbonate)	weakness of respiratory muscles (e.g. poliomyelitis, multiple sclerosis)	bicarbonate (e.g. after cardiac arrest)	
renal loss of bicarbonate (renal tubular acidosis type 2 - rare)	chest deformities		
impairment of renal H^+ excretion (renal tubular acidosis type 1 - rare)	airway obstruction		

ABB Disturbances Evaluation



http://cmapspublic.ihmc.us

Biochemistry of Blood-2. Hemoglobin Metabolism

Lecture # 29

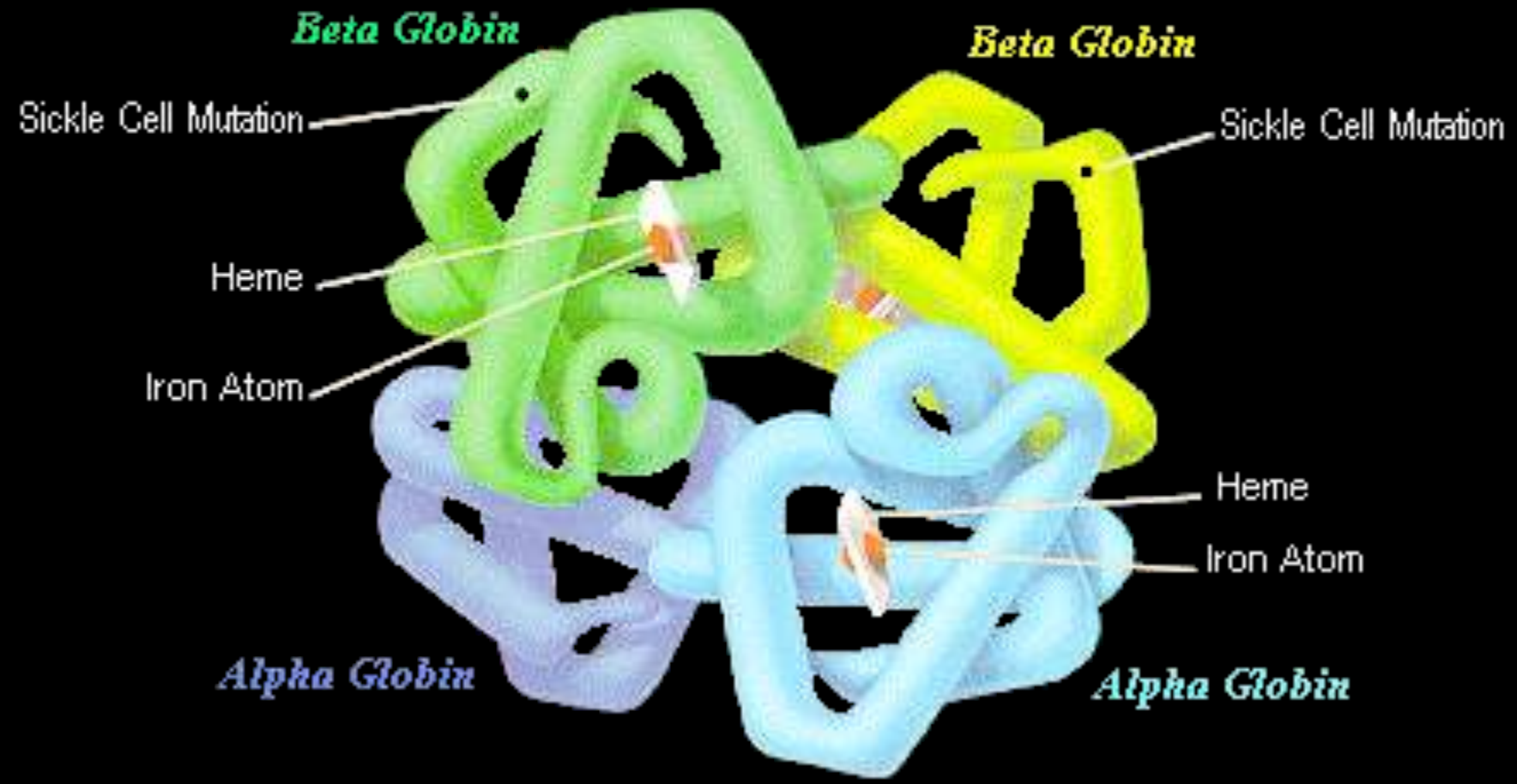
Lecturer Alexander Koval

Content

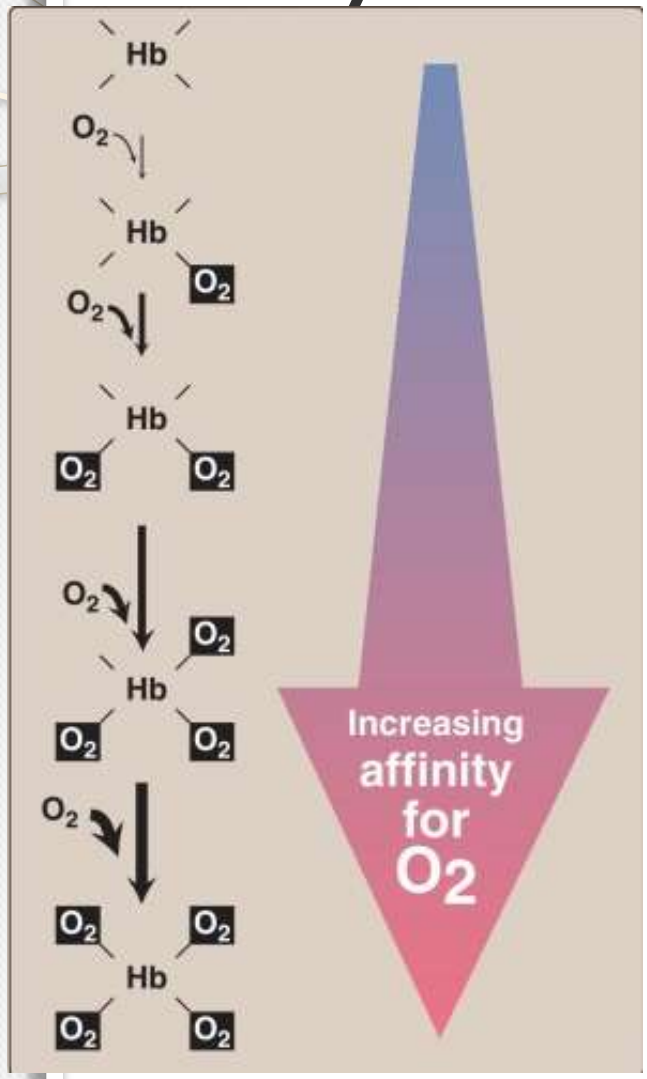
- General characteristic, features of metabolism of erythrocytes.
  - glycolysis, pentose phosphate pathway, isocitrate dehydrogenase, malate dehydrogenase, transaminases, etc.)
  - Na+/K+-ATPase, mineral content of erythrocytes.
  - Glutathione: structure, functions, enzymes of glutathione metabolism.
  - Antioxidant protection.
  - The characteristics of proteins and phospholipids of erythrocyte membranes.
- Hemoglobin (Hb), structure, properties, derivants, types of Hb.
  - Abnormal Hb. Comparative characteristics of Hb and myoglobin.
- Respiratory function of blood, its regulation.
  - Spectrum of blood for hemoglobin and its derivants.
  - Hypoxia, anoxia: types. Metabolic disorders at hypoxia.
  - Regulation of affinity of Hb to oxygen. Role of glycerate-2,3-bisphosphate.
- Chromoproteid metabolism. Digestion and absorption.
  - Hemoglobin metabolism. Biosynthesis of hem.
  - The mechanism of conjugation of bilirubin in liver. Transformation of bilirubin in the intestine.
  - Diagnostic value of definition of bilirubin and its metabolites in blood and urine at various types of jaundice (hemolytic, parenchymatous, obstructive).
- Iron metabolism. Mechanisms of absorption, transport and deposition.
- Characteristics of leukocyte metabolism. Biochemical bases of phagocytosis.
- Features of platelet metabolism.

A Molecule To Breathe With

HEMOGLOBIN



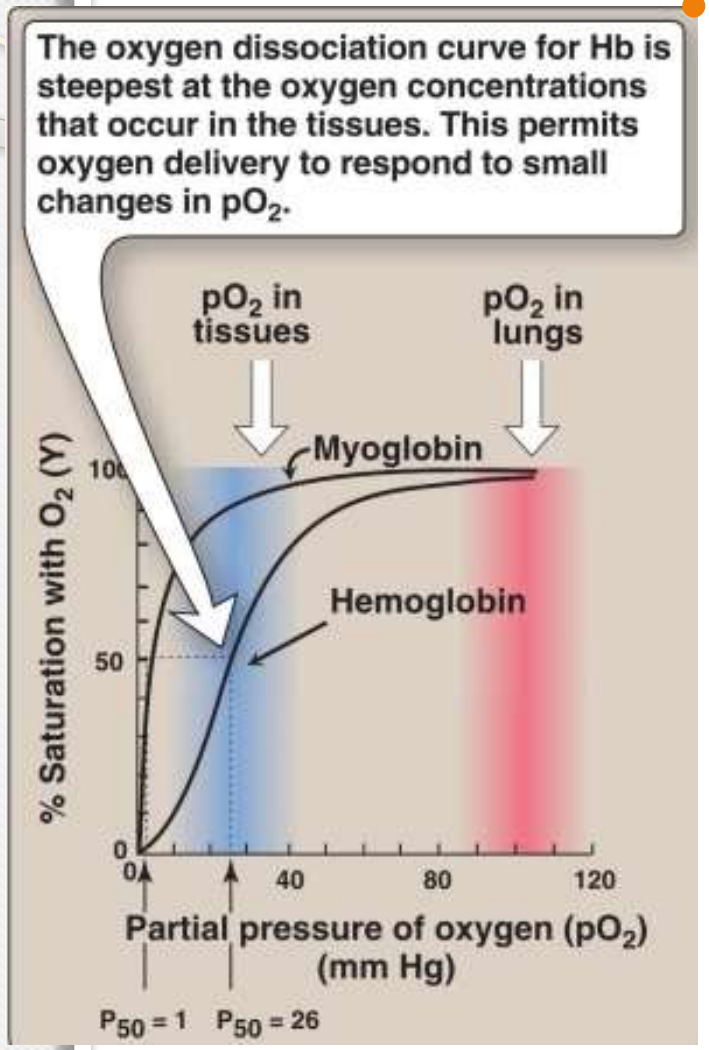
Hb binds oxygen with increasing affinity



- Cooperative binding of oxygen by the four subunits of hemoglobin:
  - the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule.
- This effect is referred to as **heme-heme interaction**.
- The subsequent binding of oxygen occurs with high affinity in the region near 20–30 mm Hg.

Lippincot's Biochemistry, 5th ed.

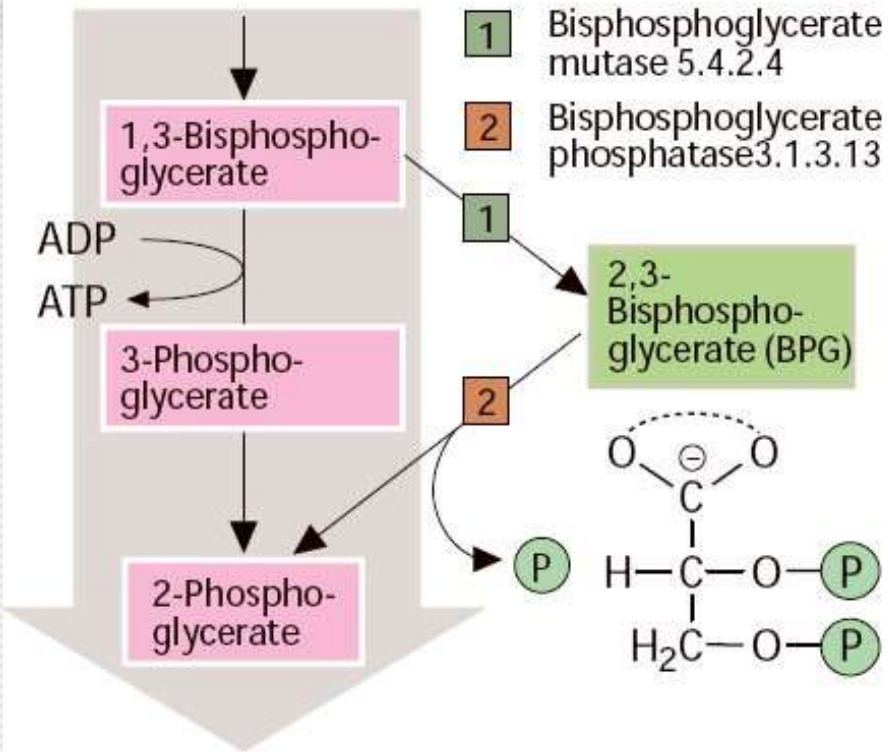
Oxygen dissociation curves for Mb and Hb



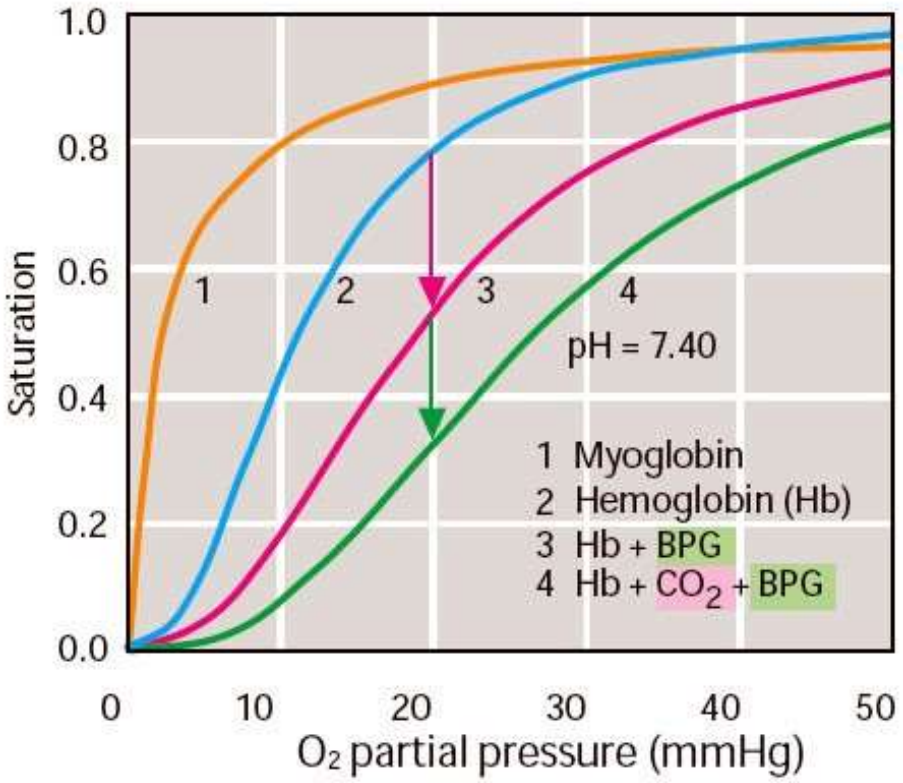
- The curves for myoglobin and hemoglobin show important differences.
  - Myoglobin has a higher oxygen affinity at all pO_2 values than does hemoglobin.
  - The partial pressure of oxygen needed to achieve half-saturation of the binding sites (P_{50}) is approximately 1 mm Hg for myoglobin and 26 mm Hg for hemoglobin.
  - The higher the oxygen affinity (that is, the more tightly oxygen binds), the lower the P_{50}.

Lippincot's Biochemistry, 5th ed.

Regulation of O_2 Transport



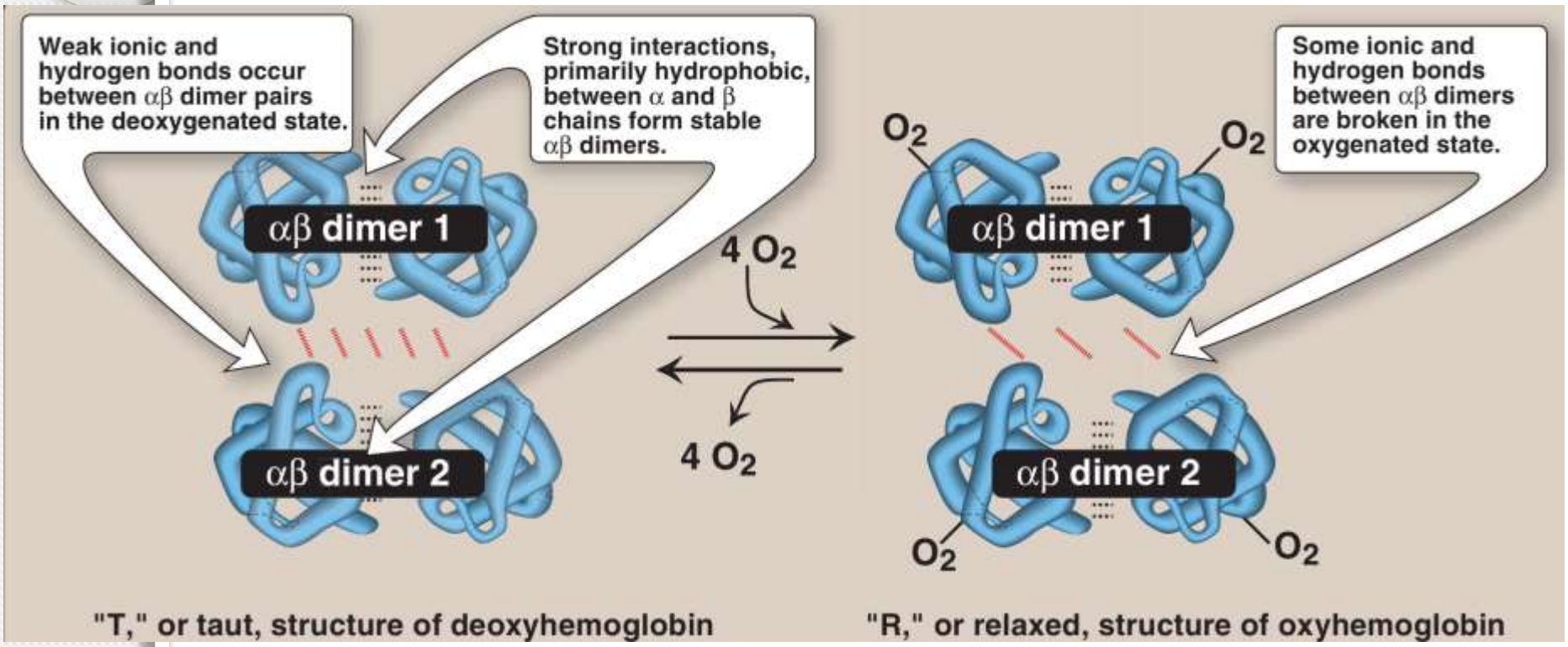
1. BPG metabolism



2. Saturation curves

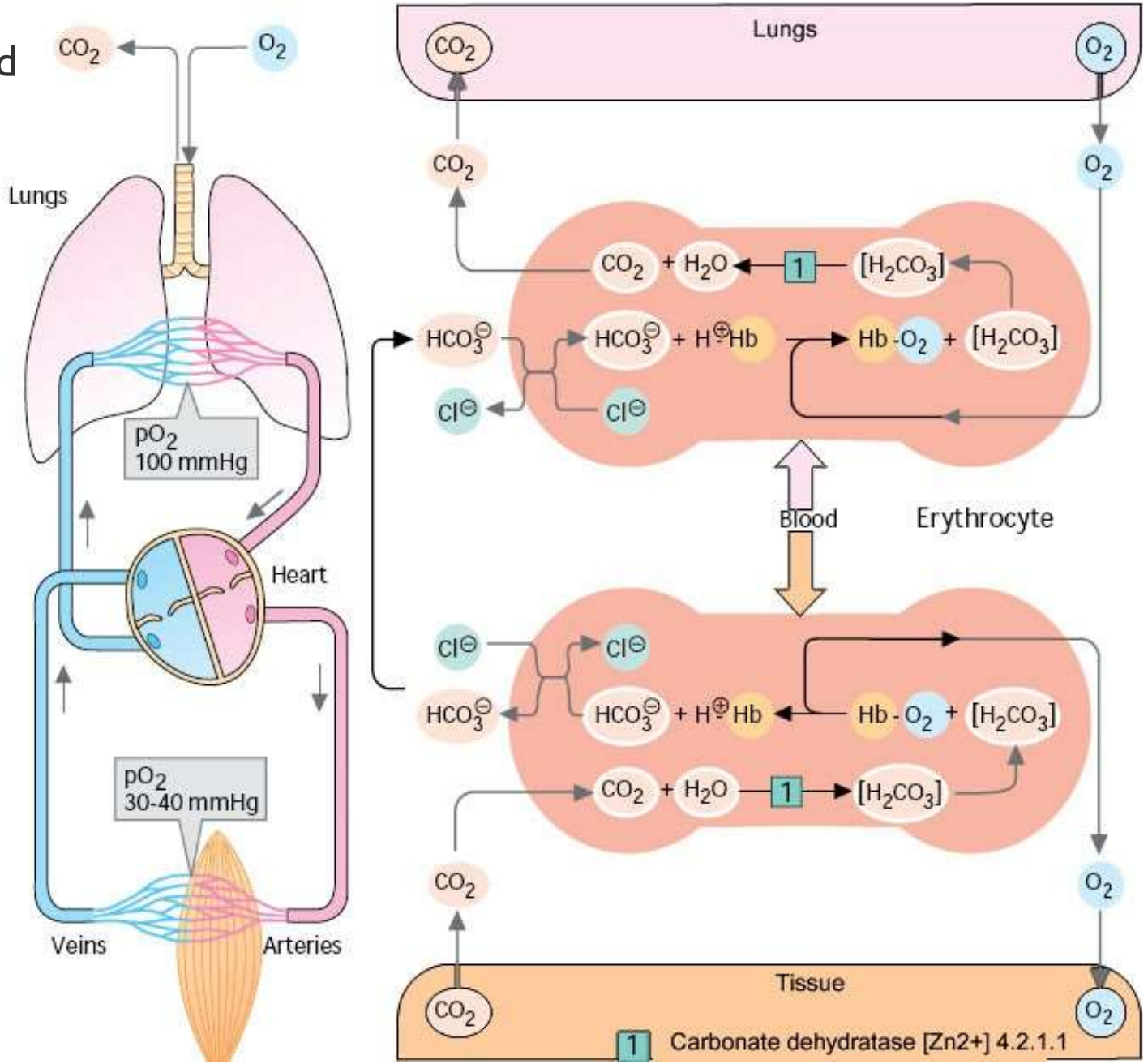
Koolman, 2005

Structural changes in oxygenated and deoxygenated hemoglobin

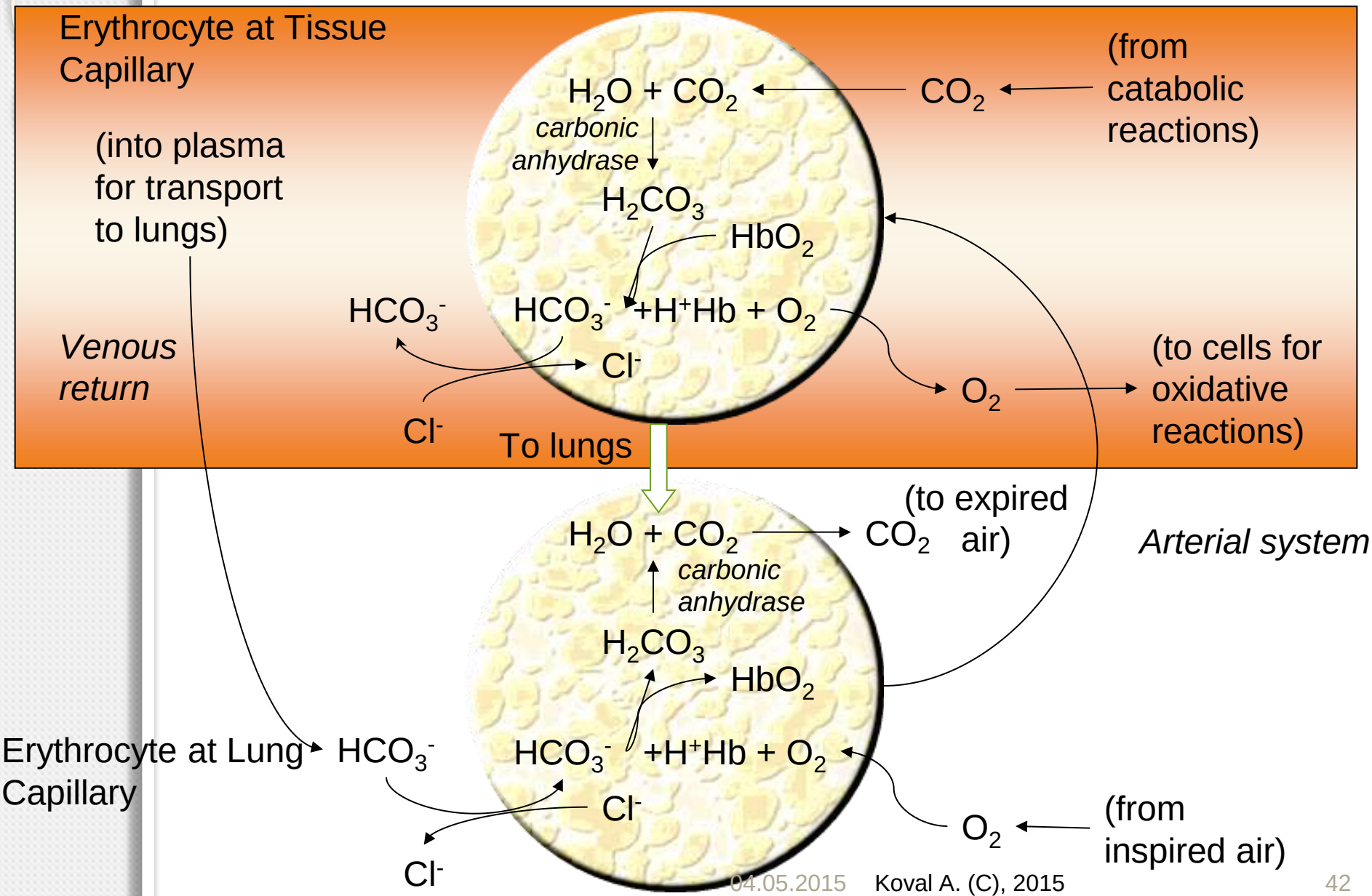


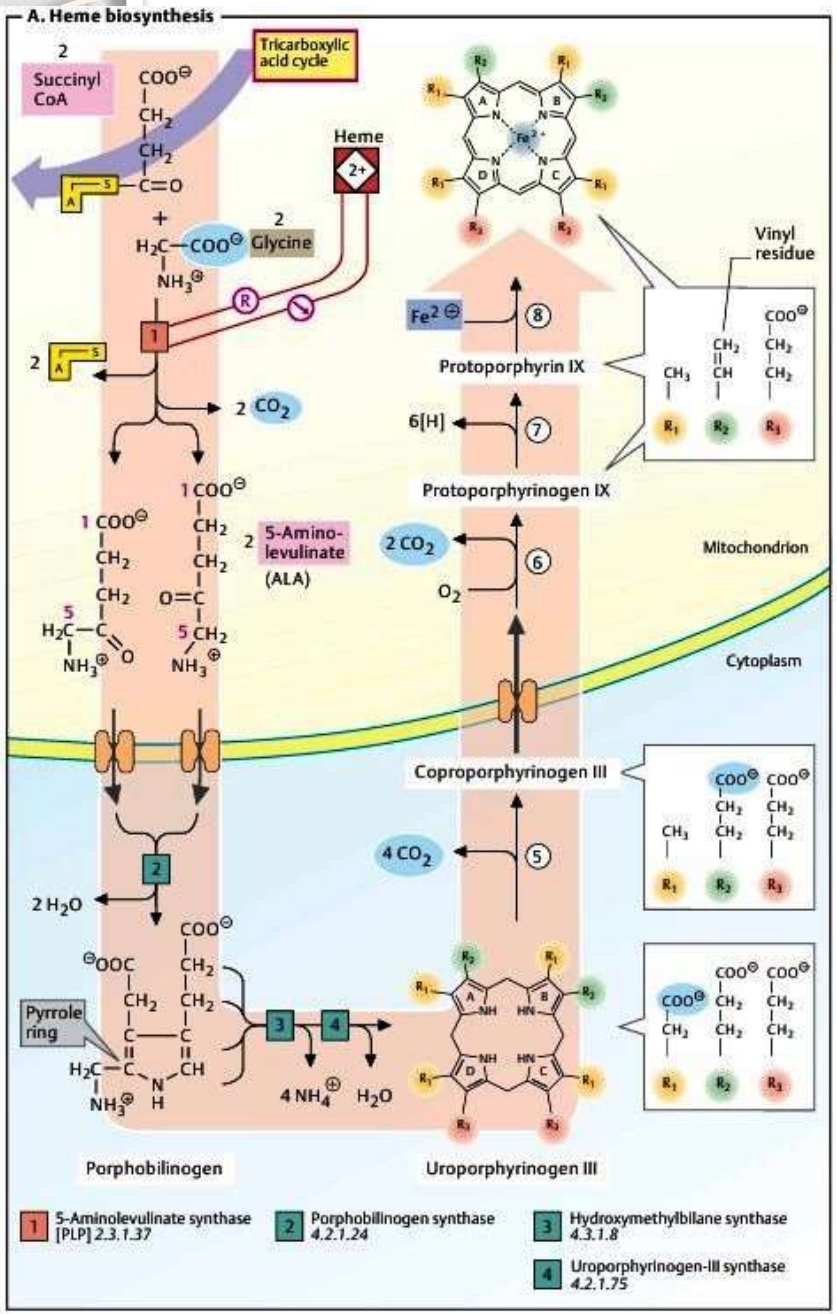
Lippincot's Biochemistry, 5th ed.

Hemoglobin and CO_2 Transport



Reactions of Gases With Erythrocytes

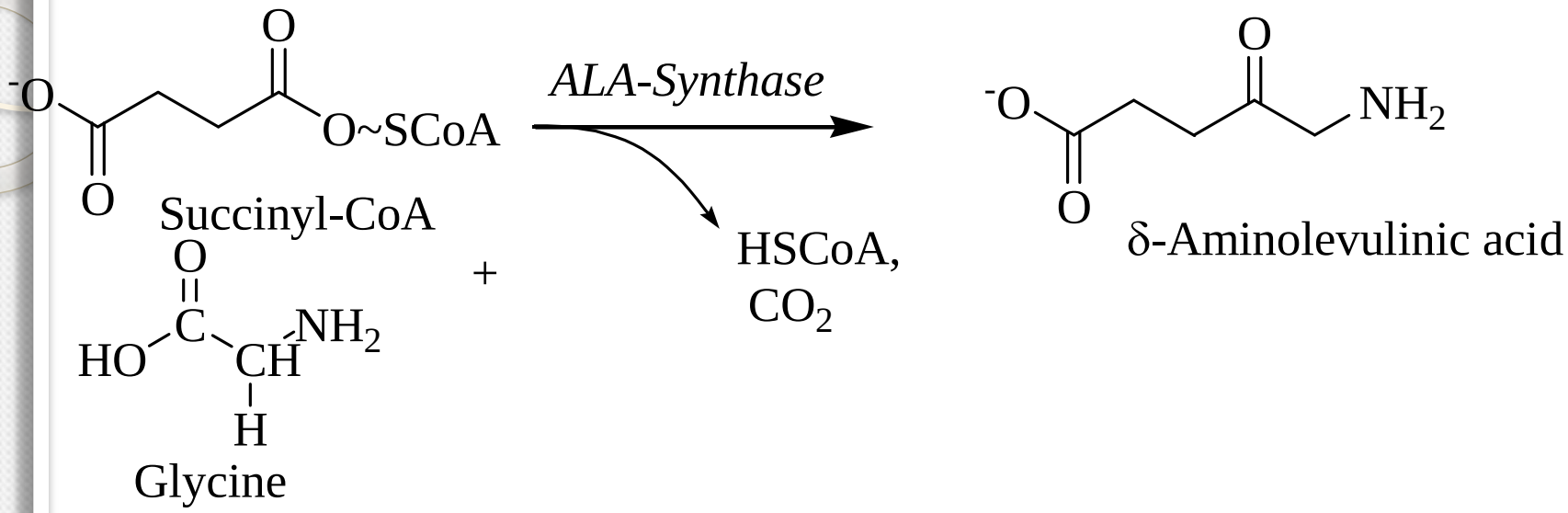




Heme Biosynthesis

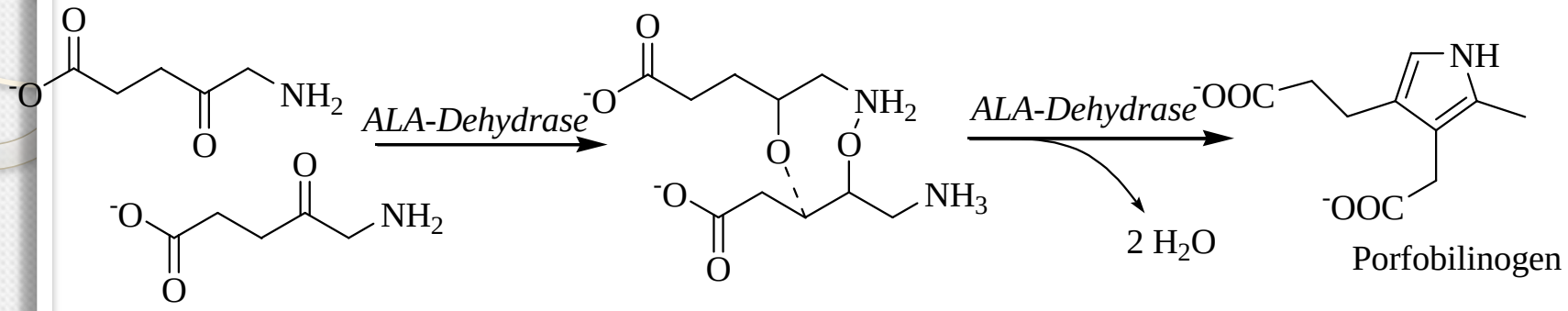
- Synthesis of the tetrapyrrole ring starts in the mitochondria.

Synthesis of Porphobilinogen and Heme: δ-ALA



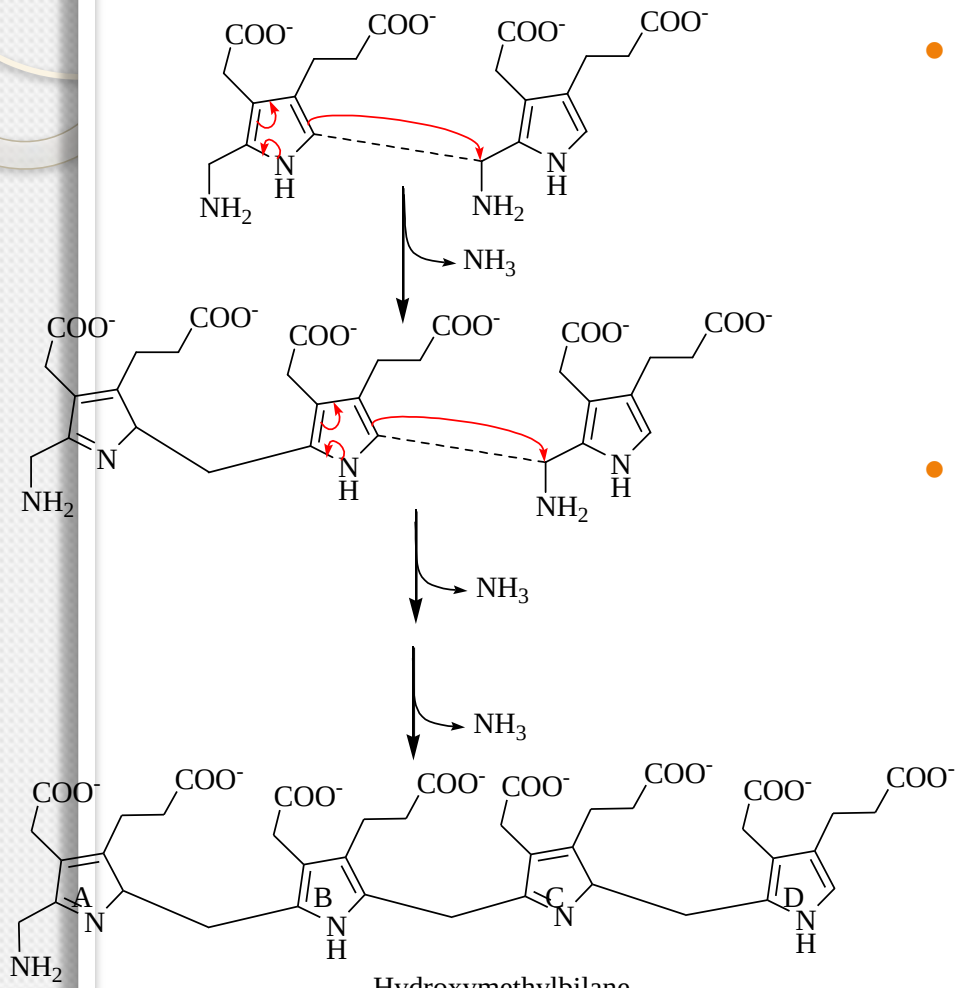
- The first reaction in heme biosynthesis takes place in the mitochondrion and involves the condensation of 1 *glycine* and 1 *succinyl-CoA* by the pyridoxal phosphate-containing enzyme, **δ-aminolevulinic acid synthase** (**ALA synthase**).
- This reaction is both the rate-limiting reaction of heme biosynthesis, and the most highly regulated reaction.

Porphobilinogen



- Mitochondrial δ-aminolevulinic acid (ALA) is transported to the cytosol, where **ALA dehydratase** (also called **porphobilinogen synthase** or **hydroxymethylbilane synthase**) dimerizes 2 molecules of ALA to produce the pyrrole ring compound **porphobilinogen**.

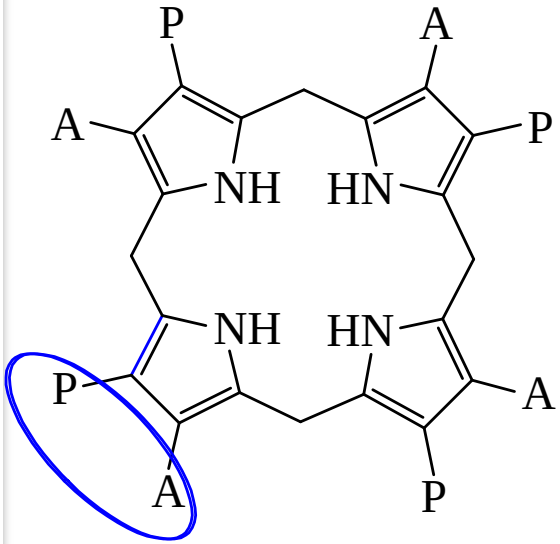
Hydroxymethylbilane



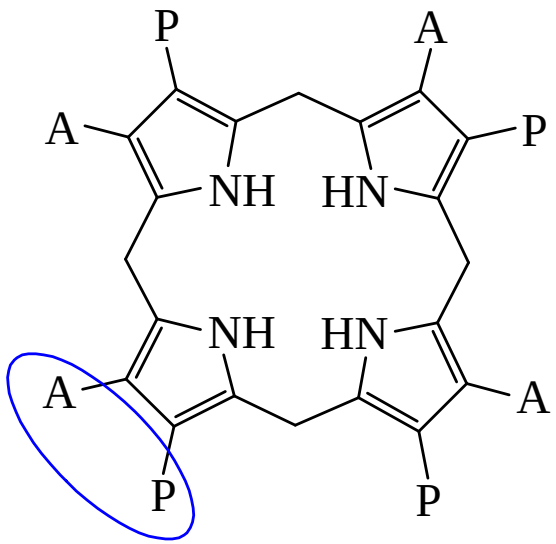
Hydroxymethylbilane

- The next step in the pathway involves the head-to-tail condensation of 4 molecules of porphobilinogen to produce the linear tetrapyrrole intermediate, **hydroxymethylbilane**.
- The enzyme for this condensation is **porphobilinogen deaminase** (**PBG deaminase**).
  - This enzyme is also called **uroporphyrinogen I synthase**.

Uroporphyrinogen III



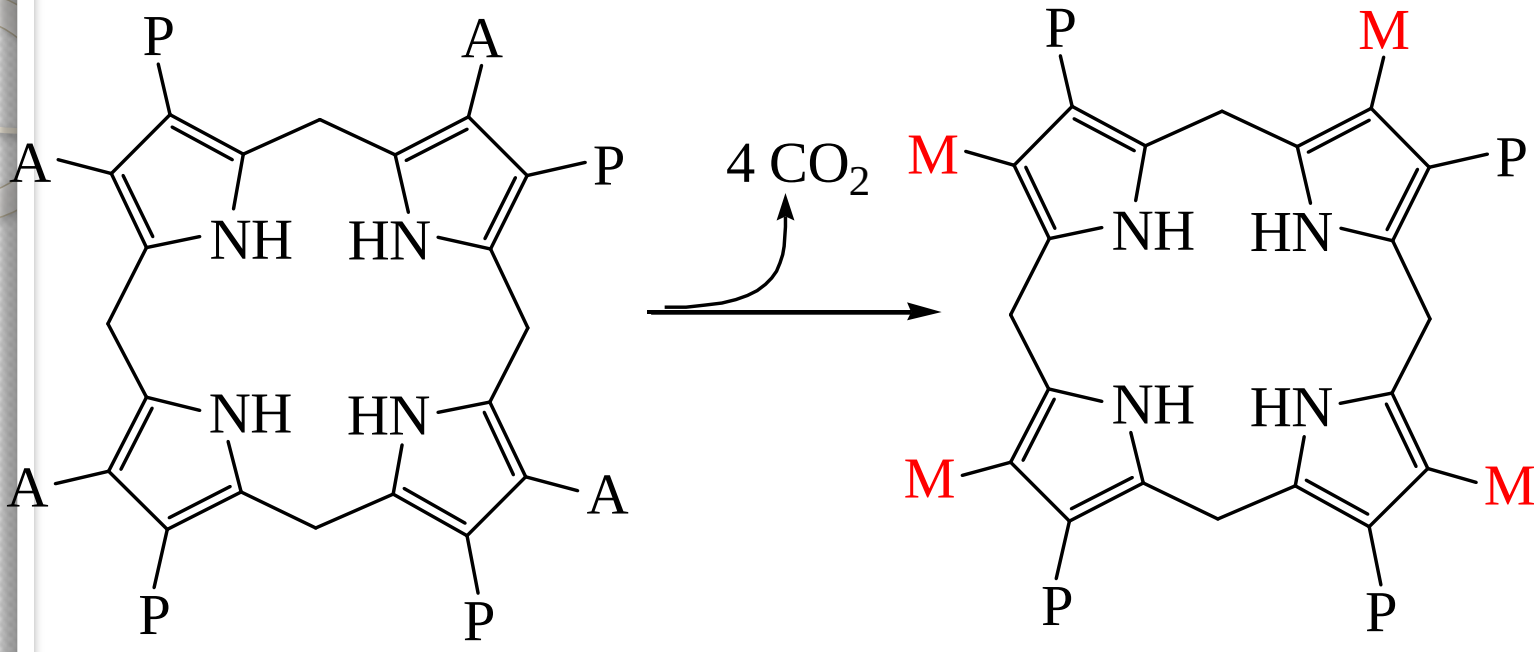
Type I uroporphyrinogen



Type III uroporphyrinogen

- Hydroxymethylbilane then undergoes enzymatic conversion to *uroporphyrinogen III*, the next intermediate on the path to heme.
  - This step is mediated by a holoenzyme comprised of **uroporphyrinogen synthase** plus a protein known as **uroporphyrinogen III cosynthase**.

Intermediates in Heme Synthesis

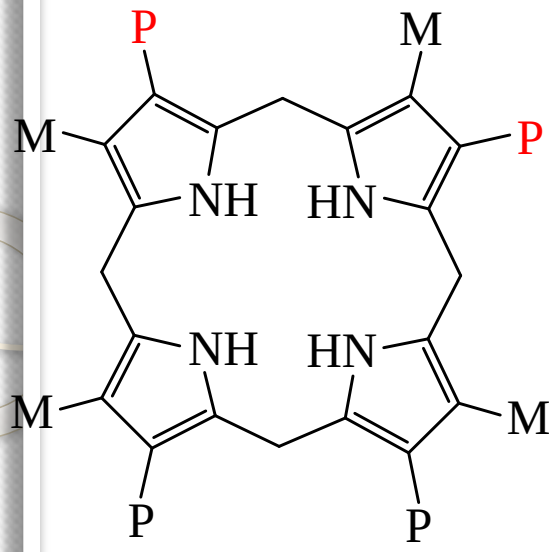


Type III uroporphyrinogen

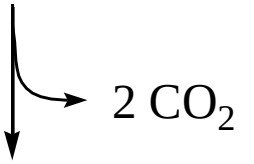
Type III Coproporphyrinogen

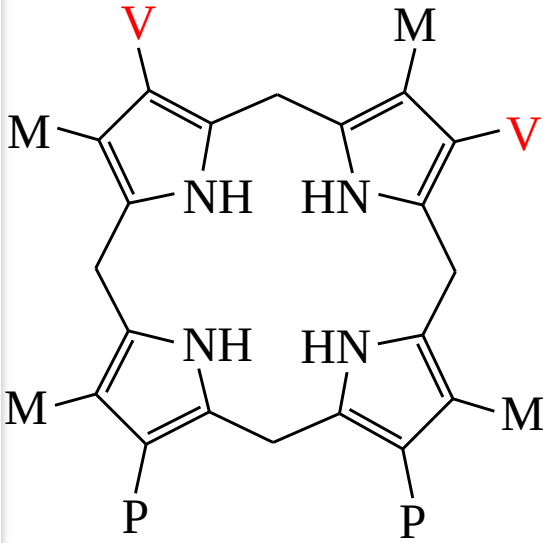
- In the cytosol, the acetate substituents of uroporphyrinogen are all decarboxylated by the enzyme **uroporphyrinogen decarboxylase**.
  - The resultant products have methyl groups in place of acetate and are known as **coproporphyrinogens**, with **coproporphyrinogen III** being the , important, normal intermediate in heme synthesis.

Intermediates in Heme Synthesis (cont'd)



Type III Coproporphyrinogen





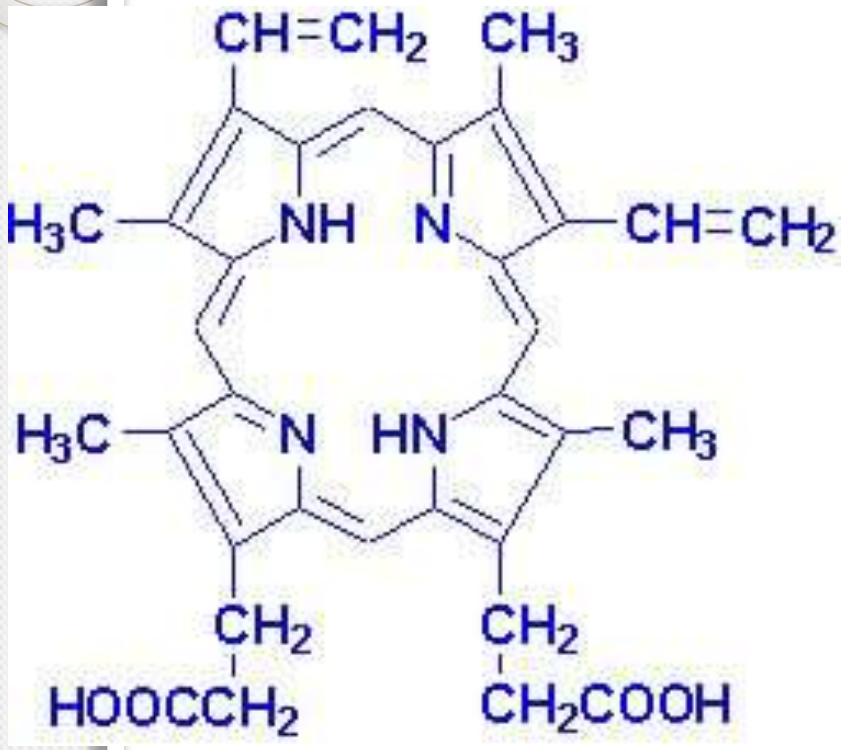
Type IX Protoporphyrinogen

- Coproporphyrinogen III is transported to the interior of the mitochondrion, where 2 **propionate** residues are decarboxylated, yielding **vinyl** substituents on the 2 pyrrole rings.
- The enzyme is **coproporphyrinogen oxidase**
- The colorless product is **protoporphyrinogen IX**.

Final Steps in Heme Synthesis

- In the mitochondrion, protoporphyrinogen IX is converted to **protoporphyrin IX** by **protoporphyrinogen IX oxidase**.
  - The oxidase reaction requires molecular oxygen and results in the loss of 6 protons and 6 electrons, yielding a completely conjugated ring system, which is responsible for the characteristic red color to hemes.
- The final reaction in heme synthesis also takes place in the mitochondrion and involves the insertion of the iron atom into the ring system generating **heme b**.
- The enzyme catalyzing this reaction is known as **ferrochelatase**.

Protoporphyrin IX and Lead Poisoning



Protoporphyrin IX

- The enzymes **ferrochelatase**, **ALA synthase** and **ALA dehydratase** (a sulfhydryl containing enzyme) are highly sensitive to inhibition by heavy metal poisoning.
- Indeed, a characteristic of **lead poisoning** is an increase in ALA in the circulation in the absence of an increase in porphobilinogen.

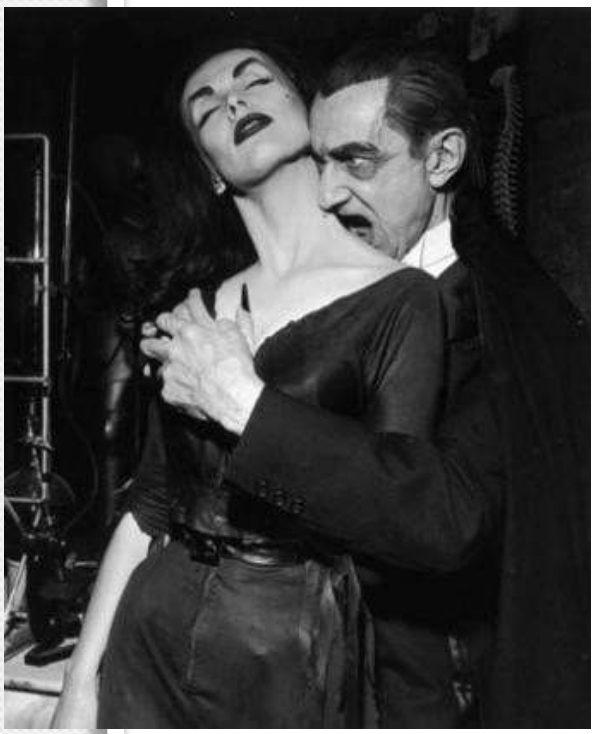
Heme Metabolism Disorders

- Some disorders of heme biosynthesis are more insidious such as the various **porphyrias**.
  - Accumulation of *porphyrins* in the skin can also occur, and exposure to light then causes disfiguring, poorly healing blisters.
  - Neurological disturbances are also common in the porphyrias.

Abnormalities of Heme Synyhesis

- Aside from its importance as the prosthetic group of hemoglobin and a small number of enzymes (e.g., redox cytochromes and the P_{450} class of detoxifying cytochromes), **heme** is important because a number of genetic disease states are associated with deficiencies of the enzymes used in its biosynthesis.
  - Some of these disorders are diagnosed because they cause *δ-aminolevulinic acid*, (ALA) and other colored intermediates to appear in the circulation, the urine, and in other tissues such as teeth and bones.

Vampires…



- It is possible that the medieval legends about human vampires (*"Dracula"*) originated in the behavior of porphyria sufferers:
  - avoidance of light,
  - behavioral disturbances,
  - drinking of blood in order to obtain heme - which markedly improves some forms of porphyria.

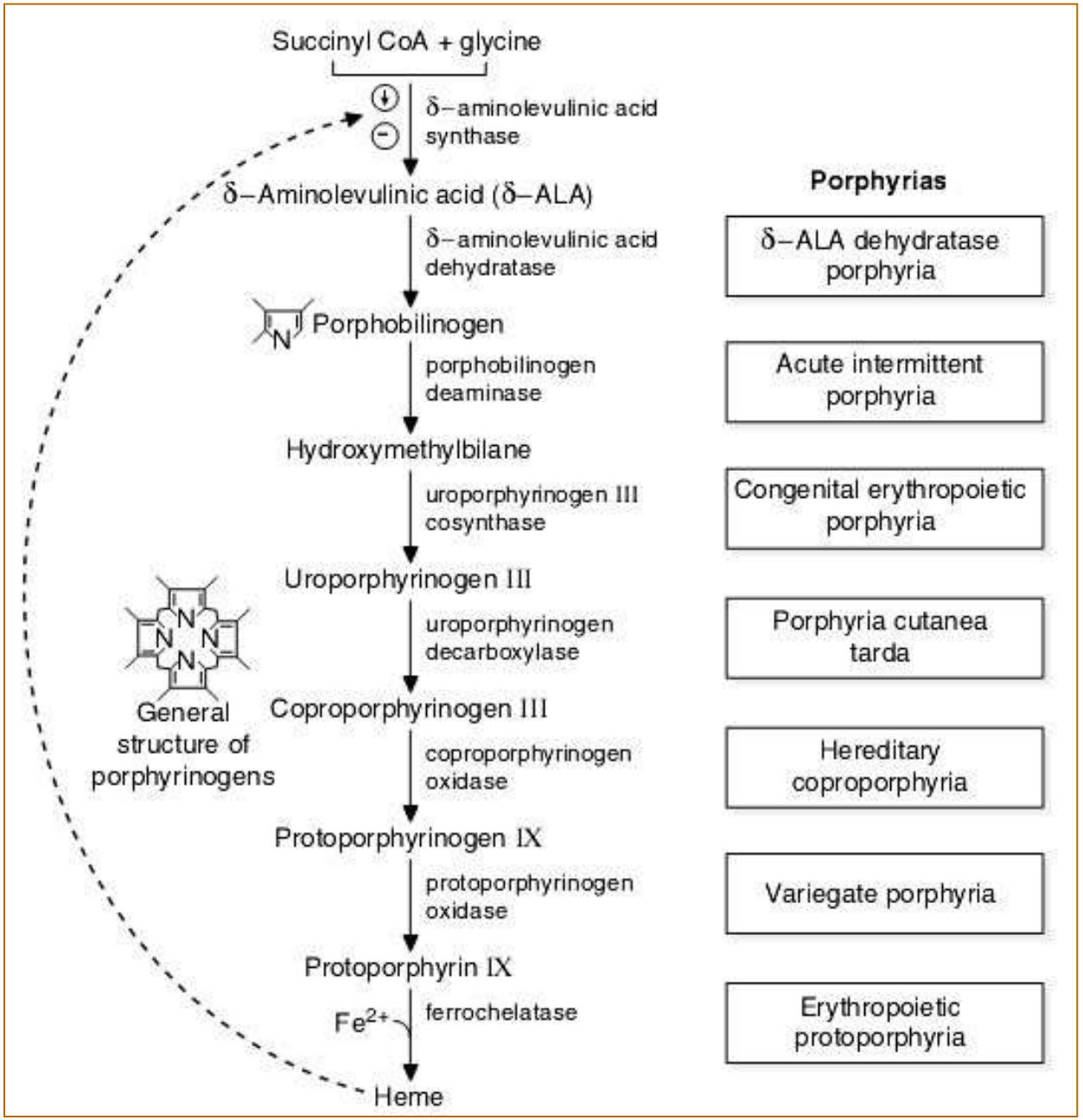
Regulation of Heme Biosynthesis

- The largest repository of heme in the human body is in red blood cells, which have a life span of about 120 days. There is thus a turnover of about 6 g/day of hemoglobin, which presents 2 problems.
  - First, the porphyrin ring is hydrophobic and must be solubilized to be excreted.
  - Second, iron must be conserved for new heme synthesis.

Porphyrias

- The **porphyrias** are both inherited and acquired disorders in heme synthesis. These disorders are classified as either erythroid or hepatic, depending upon the principal site of expression of the enzyme defect.
- Eight different porphyrias have been classified.
- With the exception of the reaction catalyzed by ALA synthase, defects in each of the other enzymes of heme synthesis have been identified.
  - The most commonly occuring porphyria is **acute intermittent porphyria, AIP** which is caused by a defect in **porphobilinogen deaminase**, (PBG deaminase). This enzyme is also called **hydroxymethylbilane synthase** or **uroporphyrinogen synthase**.

Overview of Heme Synthesis



Marks, 2000

Genetic Defects: Intermittent Porphyria

- Genetic defects that cause increased ALA synthase activity or decreased uroporphyrinogen I synthase activity lead to the disease known as acute **intermittent porphyria**, which is diagnosed by the excretion of excess porphobilinogen (a condition that is not obvious from the color of the urine).

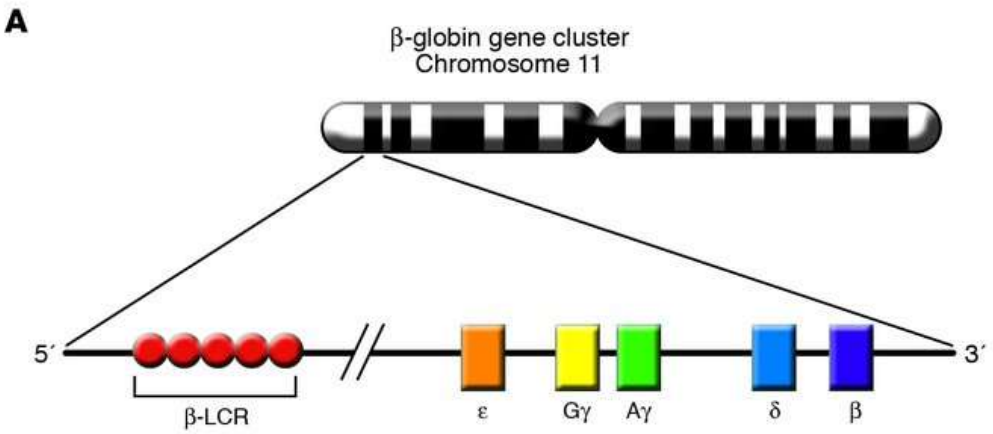
Porphyrias (cont'd)

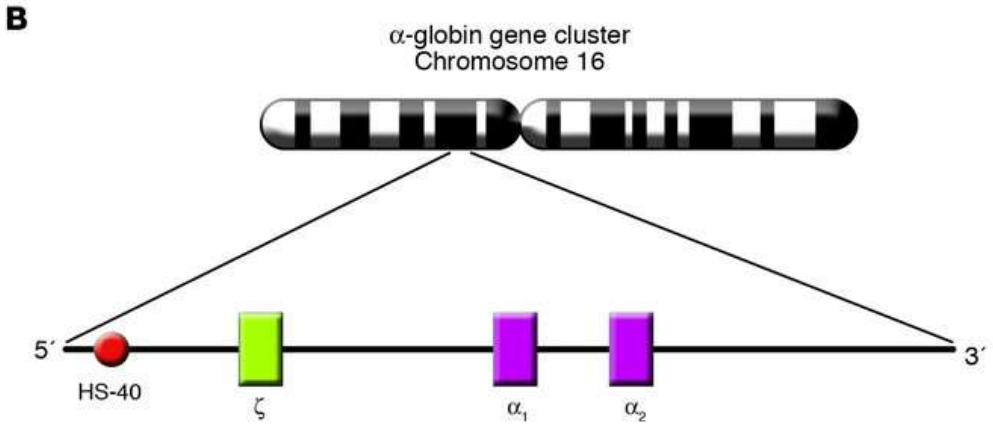
Porphyria	Enzyme Defect	Primary Symptom
Erythropoietic Class		
Congenital erythropoietic porphyria, CEP	Uroporphyrinogen III cosynthase	Photosensitivity
Erythropoietic protoporphyria, EPP	Ferrochelatase	Photosensitivity
Hepatic Class		
ALA dehydratase deficiency porphyria, ADP	ALA dehydratase	Neurovisceral
Acute intermittent porphyria, AIP	PBG deaminase	Neurovisceral
Hereditary coproporphyria, HCP	Coproporphyrinogen oxidase	Neurovisceral, some photosensitivity
Variegate porphyria, VP	Protoporphyrinogen oxidase	Neurovisceral, some photosensitivity
Porphyria cutanea tarda, PCT	Uroporphyrinogen decarboxylase	Photosensitivity
Hepatoerythropoietic porphyria, HEP	Uroporphyrinogen decarboxylase	Photosensitivity, some neurovisceral

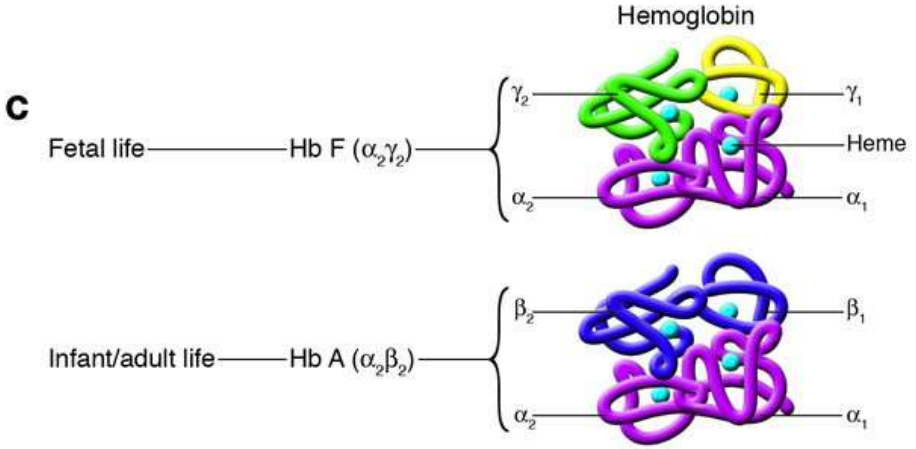
Normal Adult Human Hemoglobins

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	< 2%
HbA_2	$\alpha_2\delta_2$	2-5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3-9%

Globin Gene Clusters

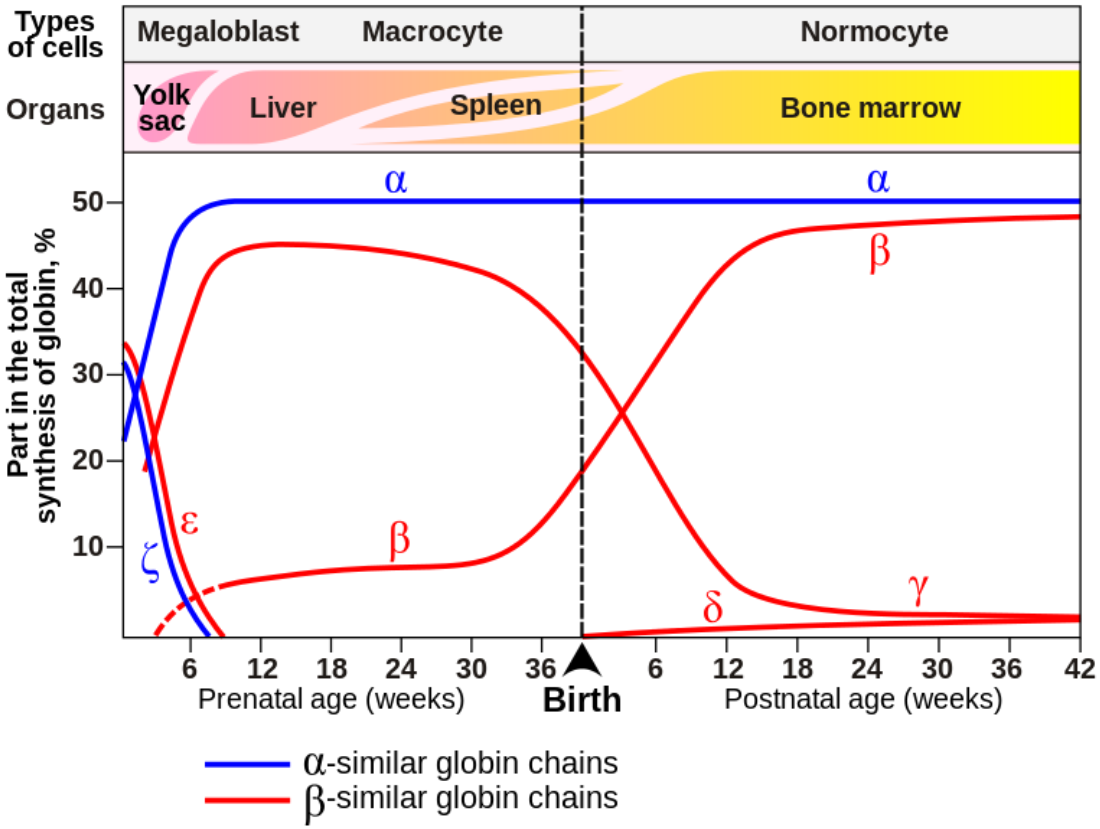






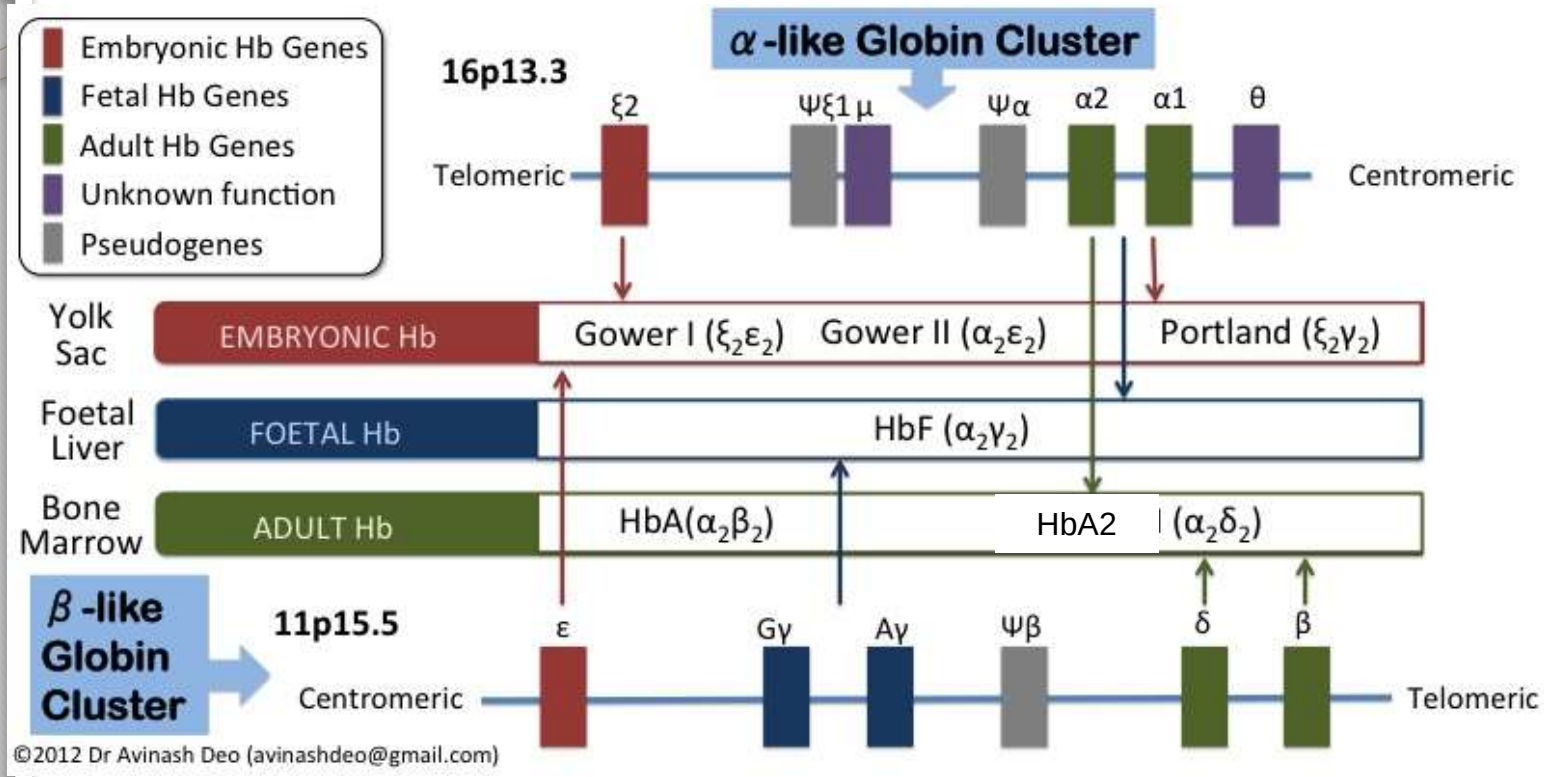
	ζ chain	α chain
ε chain	**HbE Gower I**	**HbE Gower 2**
γ chain	**HbE Portland I**	HbF
β chain	**HbE Portland II**	HbA
δ chain	N/A	HbA_2

Globin Gene Expression in Development

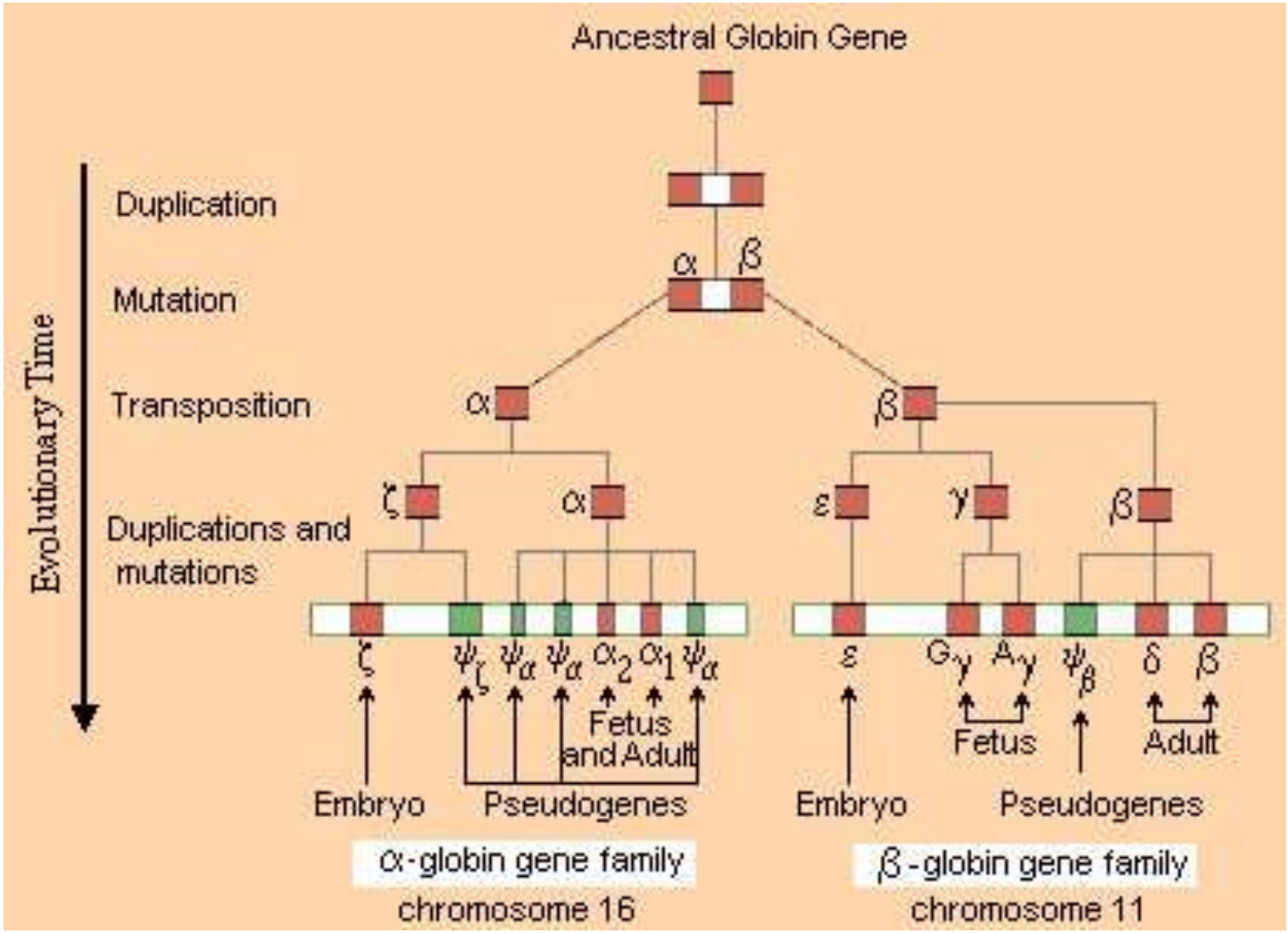


- Multiple **globin** genes are expressed at different times in human development.
  - In the <u>early embryo:</u> chains, ζ and ε.
  - As the <u>fetus</u> develops:
    - replaced by α and γ chains.
    - at about the time of birth: the γ chains are replaced by β chains.
      - after birth a small amount of a δ chain is produced.
  - By age <u>6 months:</u> almost all $\alpha_2\beta_2$ (adult) hemoglobin.

Temporal Regulation of Globin Genes



Ancestry of The Hemoglobin Genes



Clinical aspects of hemoglobin

1. Glycosylation of HbA1 and diabetes mellitus.
   1. HbA_1 reacts with glucose to form a derivative known as hemoglobin A_{1c} (HbA_{1c}).
   2. Normally the concentration of HbA_{1c} in blood is very low, but in patients with diabetes mellitus, in whom blood sugar levels may be high, the concentration of HbA_{1c} may rich 12% or more of the total hemoglobin.
   3. Because the average life of an red blood cells is 120 days, the amount of HbA_{1c} becomes a good indicator of blood glucose levels over a 2–4-month period. For example, determination of the amount of HbA_{1c} can tell a physician if patients have maintained their blood glucose levels over the preceding months or have or have lowered their glucose levels just before their clinical examination.

Methemoglobins

2. Hemoglobin M (HbM).
    1. A number of rare hemoglobinopathies lead to a high percentage of methemoglobins in red blood cells. These usually arise due to mutations in either the proximal or distal histidines of either α or β chains, which bond with the iron in the heme group. These mutations stabilize the iron in the ferric form (Fe^{3+}), which cannot bind oxygen. Only patients who are heterozygous for these mutations have been found. Presumably, homozygosity is lethal.

Mutant Forms of Hemoglobin

- Each of the mutant forms of hemoglobin exists in only a small fraction of the total human population. Many of the mutant forms are deleterious. Others appear to be harmless, and are often referred to as neutral mutations.
- A very few may have advantages. Inheritance of globin genes occurs as a result of standard genetic processes.
- **Pathological Effects** - Deleterious mutations are mostly clustered about the heme pockets and in the vicinity of the $\alpha-\beta$ contact region that is so important in the allosteric transition.

Effect	Residue Changed	Change	Name	Consequences of Mutation	Explanation
Sickling	β6 (A3)	Glu → Val	S	Sickling	Val fits into EF pocket in chain of another hemoglobin molecule.
	β6 (A3)	Glu → Ala	G Makassar	Not significant	Ala probably does not fit the pocket as well.
	β121 (GH4)	Glu → Lys	O Arab, Egypt	Enhances sickling in S/O heterozygote	β121 lies close to residue β6; Lys increases interaction between molecules.
Change in O_2 affinity	α87 (F8)	His → Tyr	M Iwate	Forms methemoglobin, decreased O_2 affinity	The His normally ligated to Fe has been replaced by Tyr.
	α141 (HC3)	Arg → His	Suresnes	Increases O_2 affinity by favoring R state	Replacement eliminates bond between Arg 141 and Asn 126 in deoxy state.
	β74 (E18)	Gly → Asp	Shepherds Bush	Increases O_2 affinity by decrease in BPG binding	The negative charge at this point decreases BPG binding.
	β146 (HC3)	His → Asp	Hiroshima	Increases O_2 affinity, reduced Bohr effect	Disrupts salt bridge in deoxy state and removes a His that binds a Bohr-effect proton.
	β92 (F8)	His → Gln	St. Etienne	Loss of heme	The normal bond from F8 to Fe is lost, and the polar glutamine tends to open the heme pocket.
Heme loss	β42 (CD1)	Phe → Ser	Hammersmith	Unstable, loses heme	Replacement of hydrophobic Phe with Ser attracts water into heme pocket.
Dissociation of tetramer	α95 (G2)	Pro → Arg	St. Lukes	Dissociation	Chain geometry is altered in subunit contact region.
	α136 (H19)	Leu → Pro	Bibba	Dissociation	Pro interrupts helix H.

Amino Acid Composition of Normal Human Chain, and Some Hemoglobins with Abnormal Chains

	Positions on Polypeptide Chain of Hemoglobin						
Hemoglobin	**1 2 3**	**6 7**	**26**	**63**	**67**	**121**	**146**
A (normal)	Val-His-Leu	Glu-Glu	Glu	His	Val	Glu	His
S (sickle cell)		Val					
C		Lys					
$G_{San\ Jose}$		Gly					
E			Lys				
$M_{Saskatoon}$				Tyr			
$M_{Milwaukee}$					Glu		
O_{Arabia}						Lys	

Thalassemias

- Variant Hemoglobins - Hemoglobin variants arise from missense mutations. By contrast, if one or more of the chains of hemoglobin are produced in insufficient amounts, a pathological condition called **thalassemia** arises. Thalassemia can arise in the following ways:
  1. One or more of the genes coding for hemoglobin chains is deleted.
  2. One or more of the genes coding for hemoglobin chains may have undergone a nonsense mutation that produces a shortened chain or a frameshift mutation that produces a nonfunctional chain (see Figure 7.21b and c).
  3. A mutation may have occurred outside the coding regions, leading to a block in transcription or to improper processing of the pre-mRNA, so the protein is not produced or is not functional.
- In case 1 or 2, the gene produces no functional protein. In case 3, limited transcription and translation of the correct polypeptide sequence may occur.

Two Major Classes of Thalassemias

- There are two major classes of thalassemias:
- α-thalassemia
- β-thalassemia.

Two Major Classes of Thalassemias: β-Thalassemia

- β-Thalassemia - In individuals where the β globin gene is lost or cannot be expressed, no β chains are made. These individuals are dependent upon continued production of the fetal γ chains to make a functional hemoglobin, α2γ2. Such individuals may produce chains well into childhood, but they usually die before reaching maturity.
- Much less serious is the heterozygous state, in which one β gene is still functioning. Milder thalassemias (called β_1) are known in which transcription or processing of the β genes are partially inhibited, reducing the amount of β globin synthesized.

Two Major Classes of Thalassemias: α-Thalassemia

- α–Thalassemias involving the α chain are more complicated.
- Two copies of the gene (α1 and α2) are next to each other on human chromosome 16. Their sequences differ by only one amino acid, and one can replace the other in the assembled hemoglobin tetramer.
- An individual can have 4, 3, 2, 1, or 0 copies of an α gene. Only if three or more genes are nonfunctional are serious effects observed.

α-Thalassemia (cont'd)

- Individuals with only one α gene are anemic, because their total hemoglobin production is low. The low level of α hemoglobin is partially compensated for by formation of β4 tetramers (**hemoglobin H**) and γ4 tetramers (**hemoglobin Bart's**).
  - These tetramers can bind and carry oxygen, but they do not exhibit the allosteric transition (they remain always in the R state), nor do they exhibit a Bohr effect. As a result, the unloading of oxygen to tissues is inefficient.
- If all four α gene copies are missing, individuals with this condition are inevitably stillborn. They can form only γ4 hemoglobin and, because the supply of γ chains falls near birth, not enough hemoglobin is available to support the near-term fetus.

Functional Role for Gene Duplication

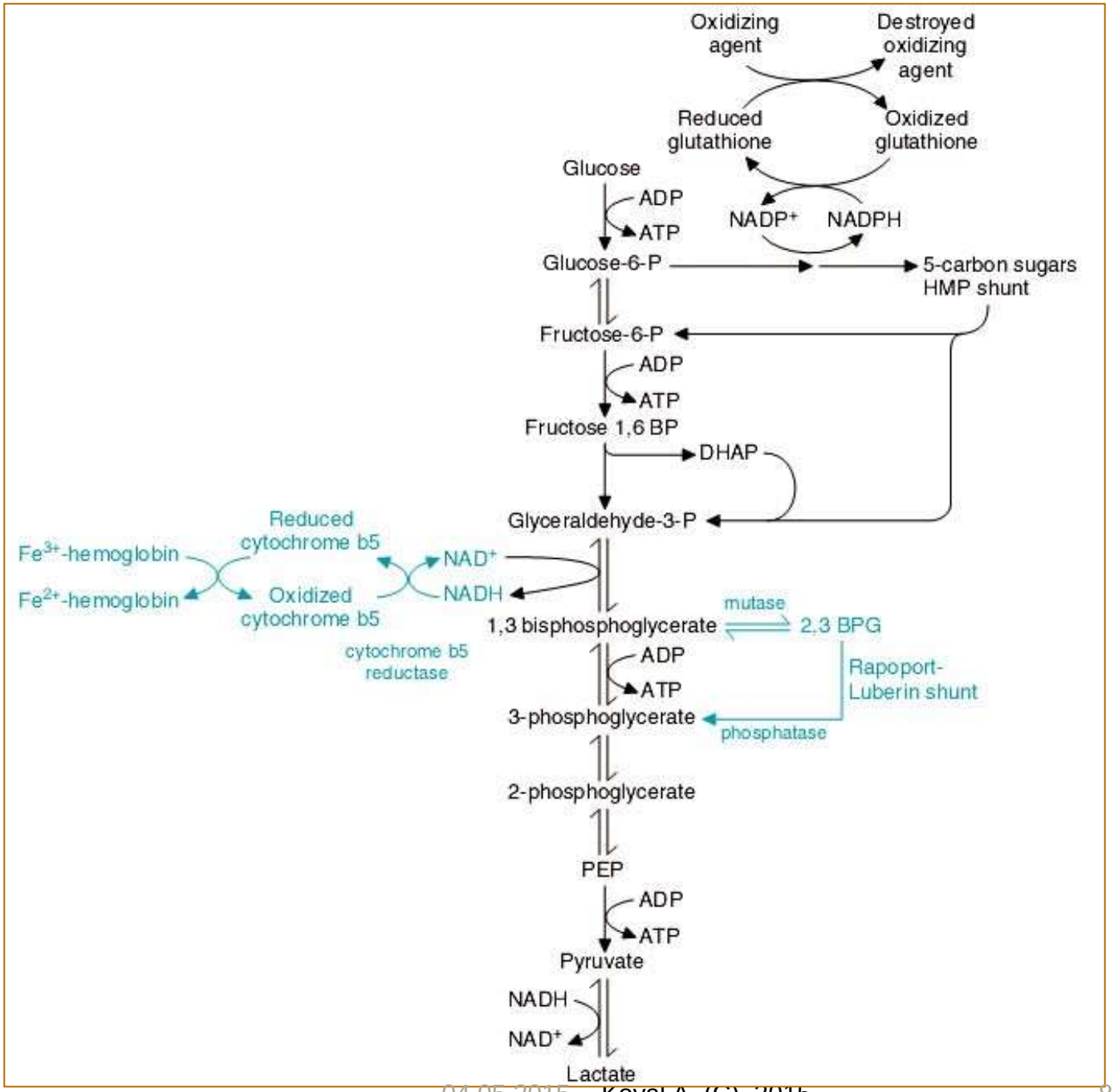
- Because there are two copies of the α gene but only one of the β gene, the most deleterious mutations in mammalian hemoglobins usually occur in the β chains.
- This phenomenon may suggest a functional role for gene duplication. That is, if two or more copies of a gene are present, the species is somewhat protected from the harmful effects of mutations.

Features of Erythrocytes Metabolism

- Two main processes are occurred in the erythrocytes: **glycolysis** and **pentose phosphate pathway**.
- Glycolysis and erythrocyte metabolism

1. Mature erythrocytes contain no mitochondria, so they are totally dependent on glycolysis for ATP production.
2. ATP is required for the activity of the sodium- and potassium-stimulated ATPase-ion transport system, which is necessary to maintain the proper biconcave shape of the erythrocyte membrane.
3. Disorder of glycolysis typically present as disorders of erythrocyte metabolism.

Erythrocyte metabolism

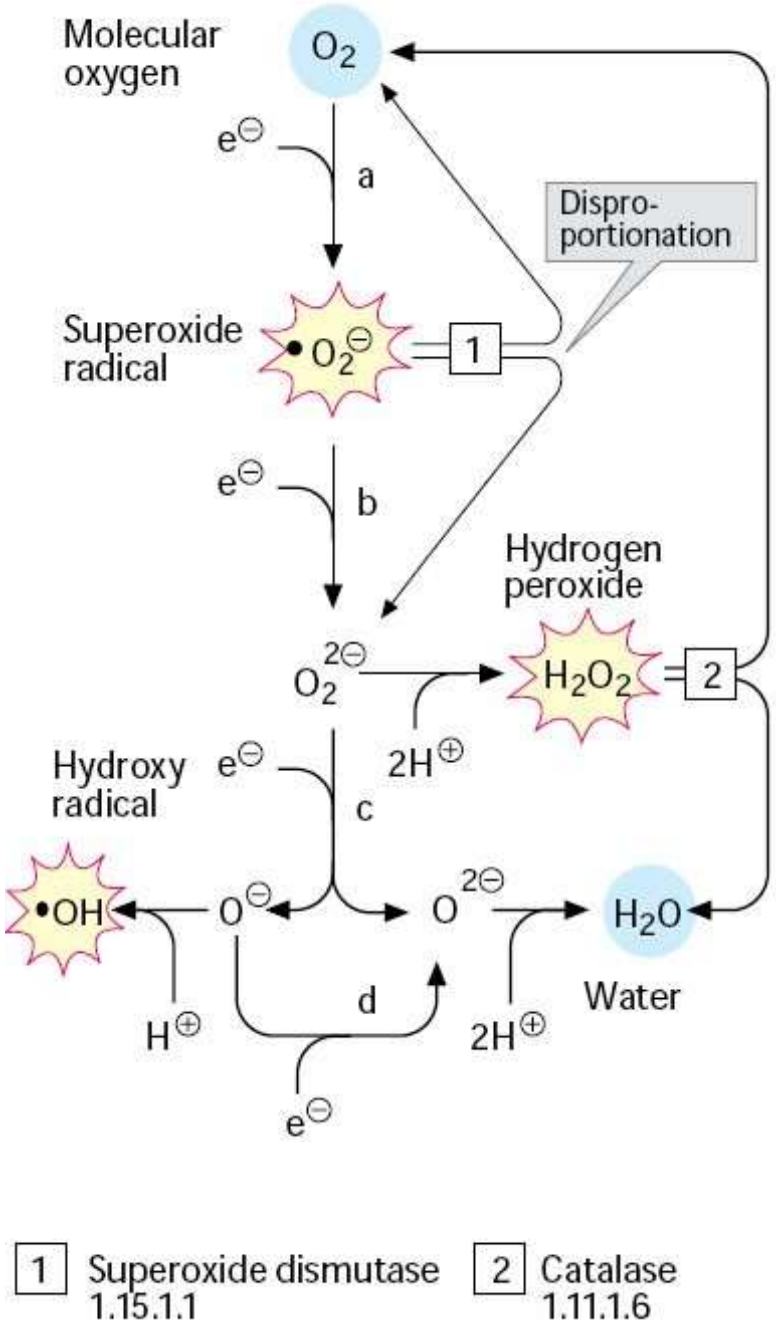


Marks, 2000

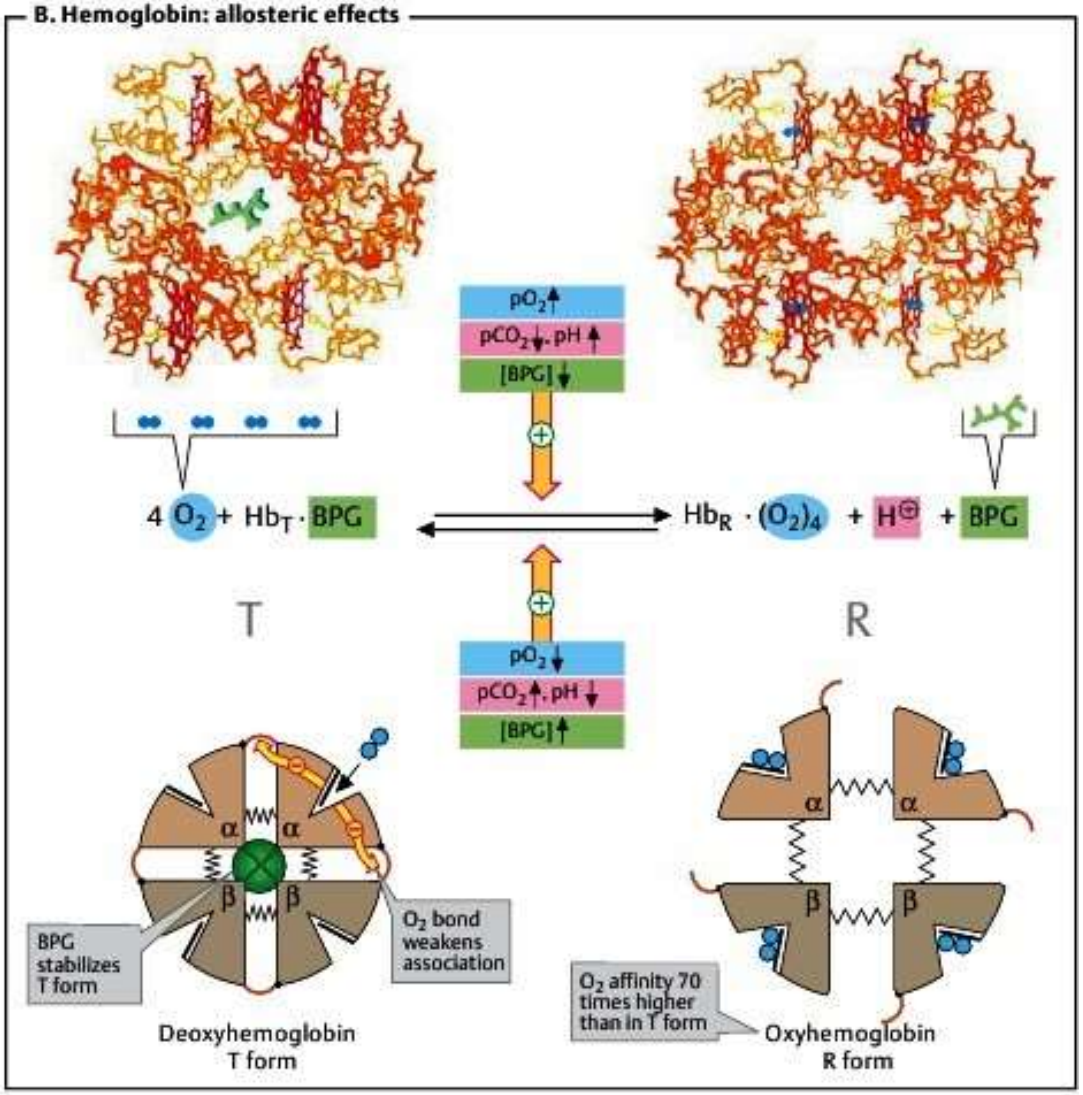
- 2,3-Bisphosphoglycerate (2,3-BPG) is the most abundant organic phosphate in the erythrocytes. Its molar concentration is approximately equivalent to that of hemoglobin. 2,3-Bisphosphglycerate is produced in the erythrocytes from intermediate (1,2-bisphosphoglycerate) of glycolysis. 2,3-BPG regulated the binding of O_2 to hemoglobin. It specially binds to deoxyhemoglobin (and not to oxyhemoglobin) and decreases the, O_2 affinity to Hb.
  - Storage of blood in acid citrate-dextrose medium results in the decreased concentration of 2,3-BPG.
  - 2,3-BPG levels are increased in severe anemia in order to cope up with the oxygen demands of the body. This is an adaptation to supply as much O_2 as possible to the tissue, despite the low hemoglobin levels.

Reactive Oxygen Species

- Due to their role in O_2 transport, the erythrocytes are constantly exposed to high concentrations of O_2 and are therefore particularly at risk from ROS.



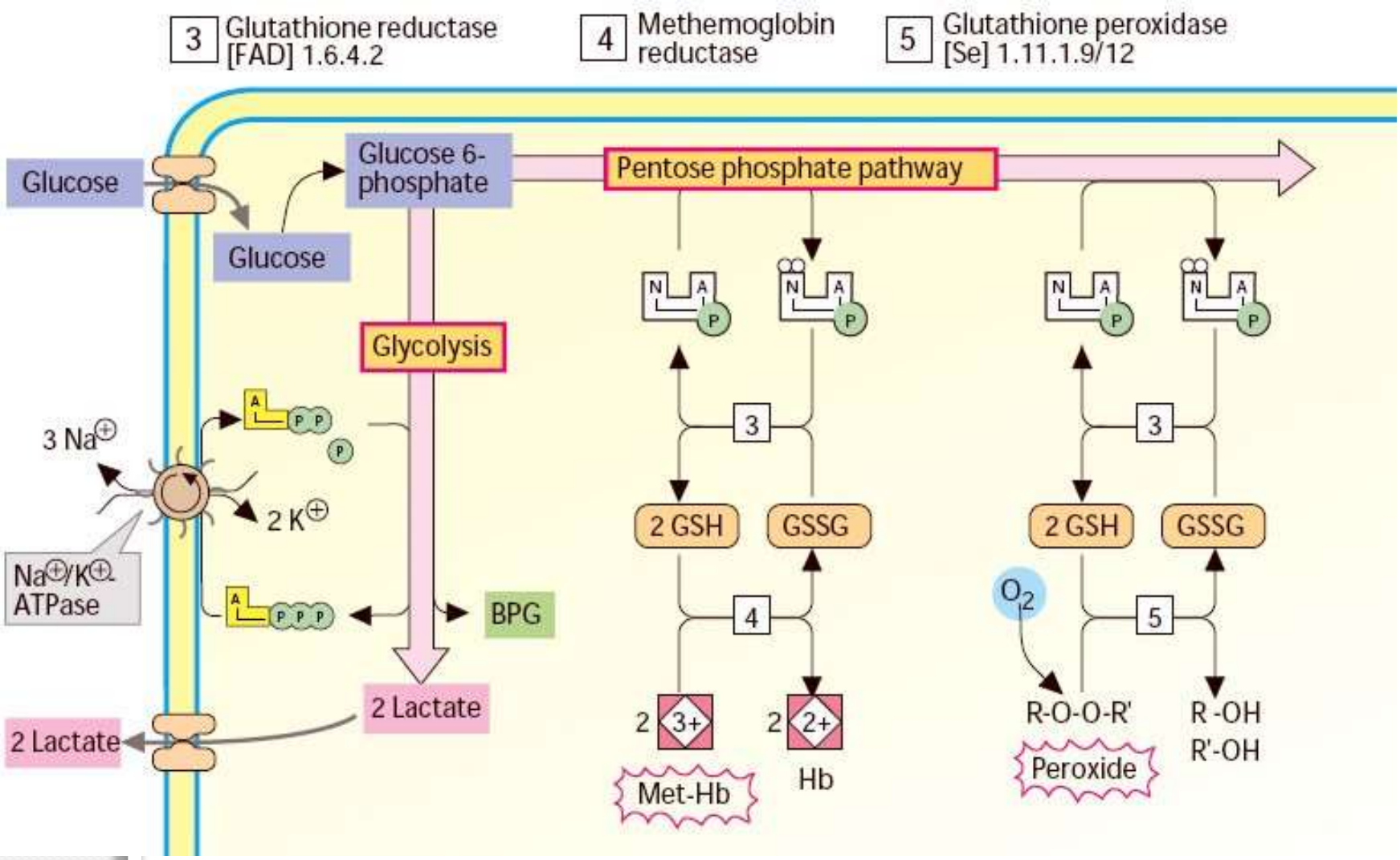
Hemoglobin: allosteric effects



Erythrocytes can inactivate ROS (superoxide dismutase, catalase, GSH)

- Requires products that are supplied by the erythrocytes' **maintenance metabolism**, - from anaerobic glycolysis and the pentose phosphate pathway (PPP).

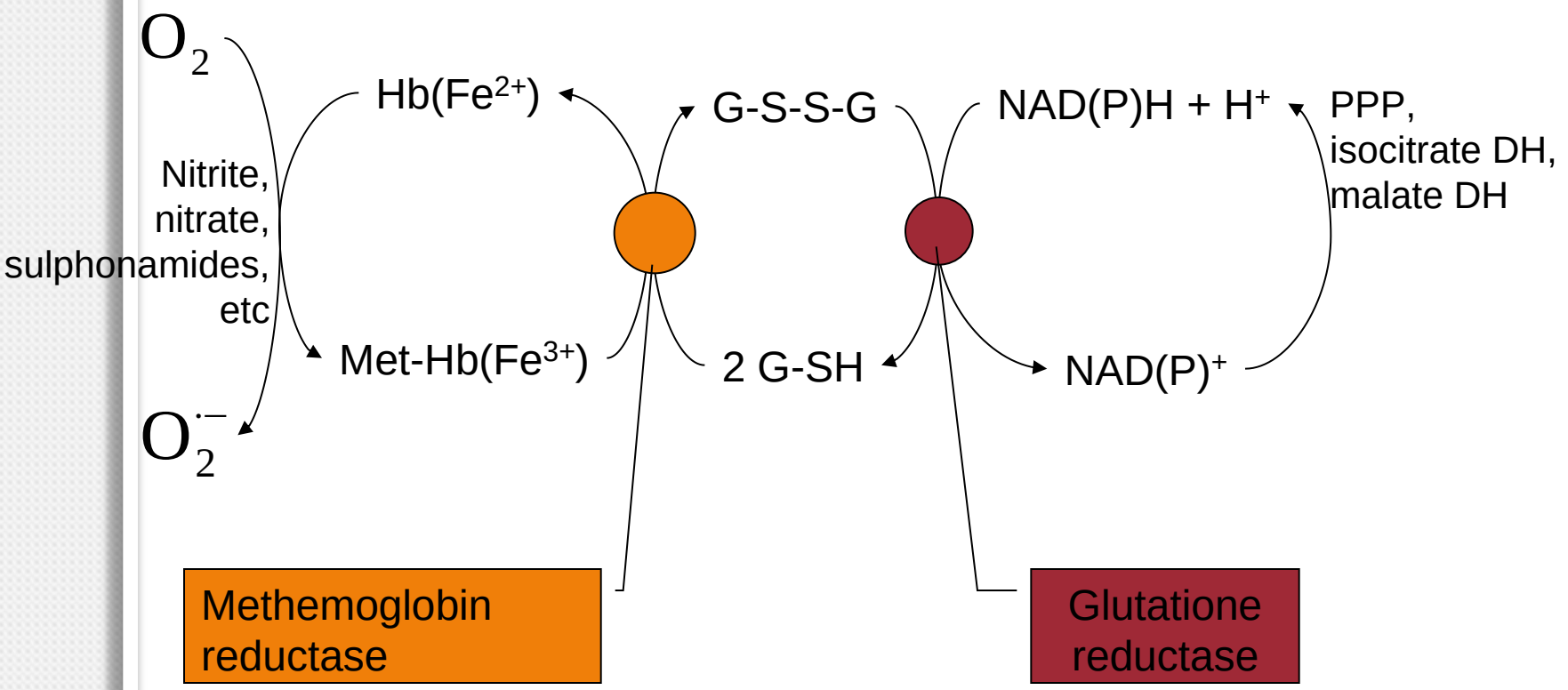
Erythrocyte metabolism



Role of pentose phosphate pathway in erythrocytes

- Role of pentose phosphate pathway NADPH produced in erythrocytes has special functions to perform.
  - It maintains the concentration of reduced glutathione which is essentially required to preserve the integrity of the red blood cell membrane.
  - NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

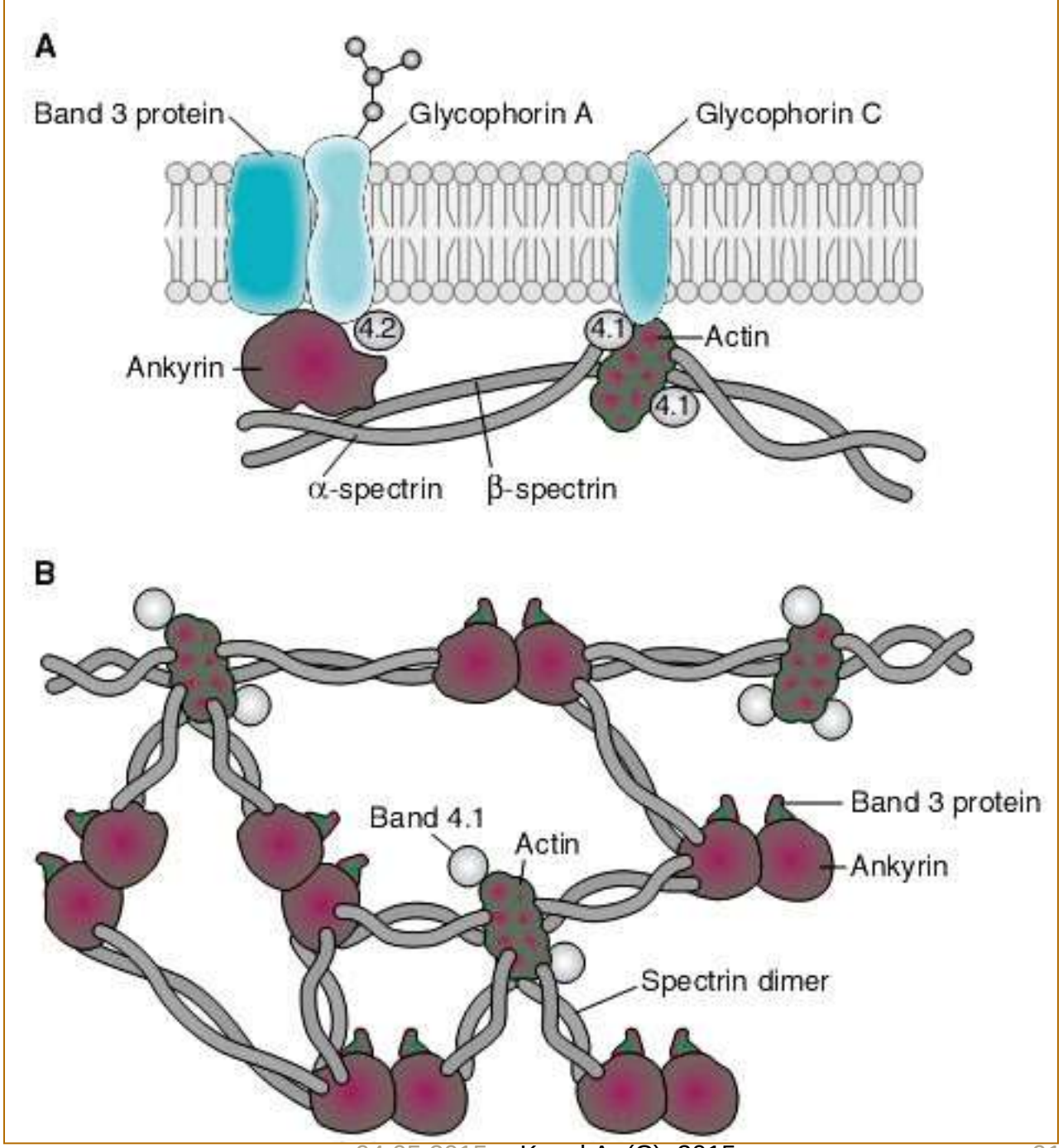
Methaemoglobin reductase system



The Role of ATP and $NADPH^+ H^+$ in RBC

- The **ATP** formed during glycolysis serves mainly
  - to supply *Na^+/K^+-ATPase (*maintains the erythrocytes' membrane potential).
  - The allosteric effector **2,3-BPG** is also derived from glycolysis.
- The PPP supplies **$NADPH^+H^+$**, to regenerate **glutathione** (GSH) from GSSG with the help of *glutathione reductase* [3].
  - GSH, the most important antioxidant in the erythrocytes,
  - coenzyme for *glutathione peroxidase* [5].
  - Contains selenium:
    - enzyme detoxifies H_2O_2 and hydroperoxides, which arise during the reaction of ROS with unsaturated fatty acids in the erythrocyte membrane.
  - The reduction of methemoglobin (Hb Fe^{3+}) to Hb (Hb Fe^{2+}, [4]) is carried out by GSH or ascorbate by a non-enzymatic pathway; however, there are also NAD(P)Hdependent Met-Hb reductases.

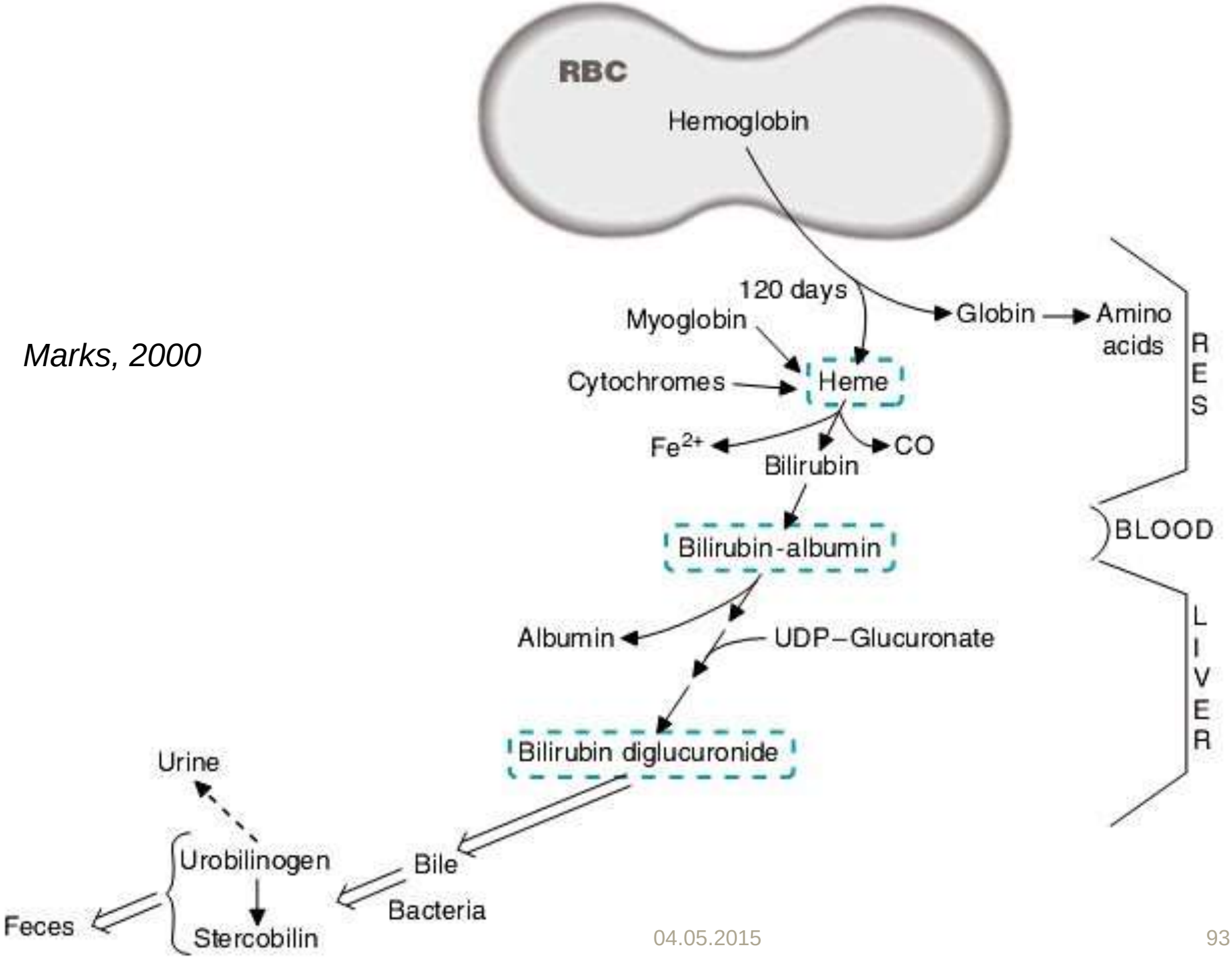
Erythrocyte skeleton



Heme Catabolism

- Normally, senescent red blood cells and heme from other sources are engulfed by cells of the reticuloendothelial system.
- The **globin** is recycled or converted into amino acids, which in turn are recycled or catabolized as required.

Marks, 2000


RBC
Hemoglobin
120 days
Myoglobin
Globin
Amino acids
Cytochromes
Heme
Fe^{2+}
CO
Bilirubin
Bilirubin-albumin
Albumin
UDP–Glucuronate
Bilirubin diglucuronide
Urine
Urobilinogen
Bile
Bacteria
Feces
Stercobilin
RES
BLOOD
LIVER

Summary of RBC Life Cycle

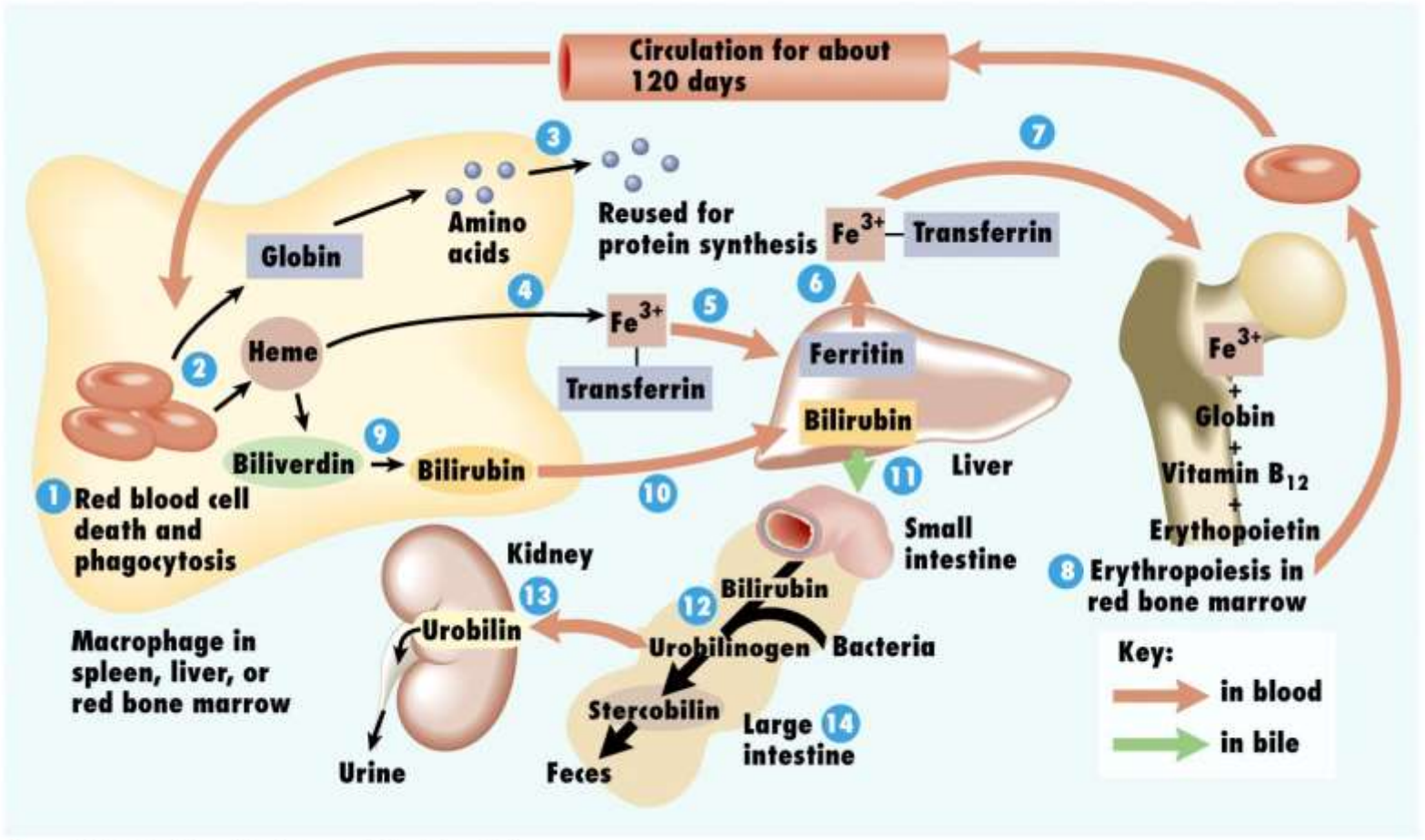
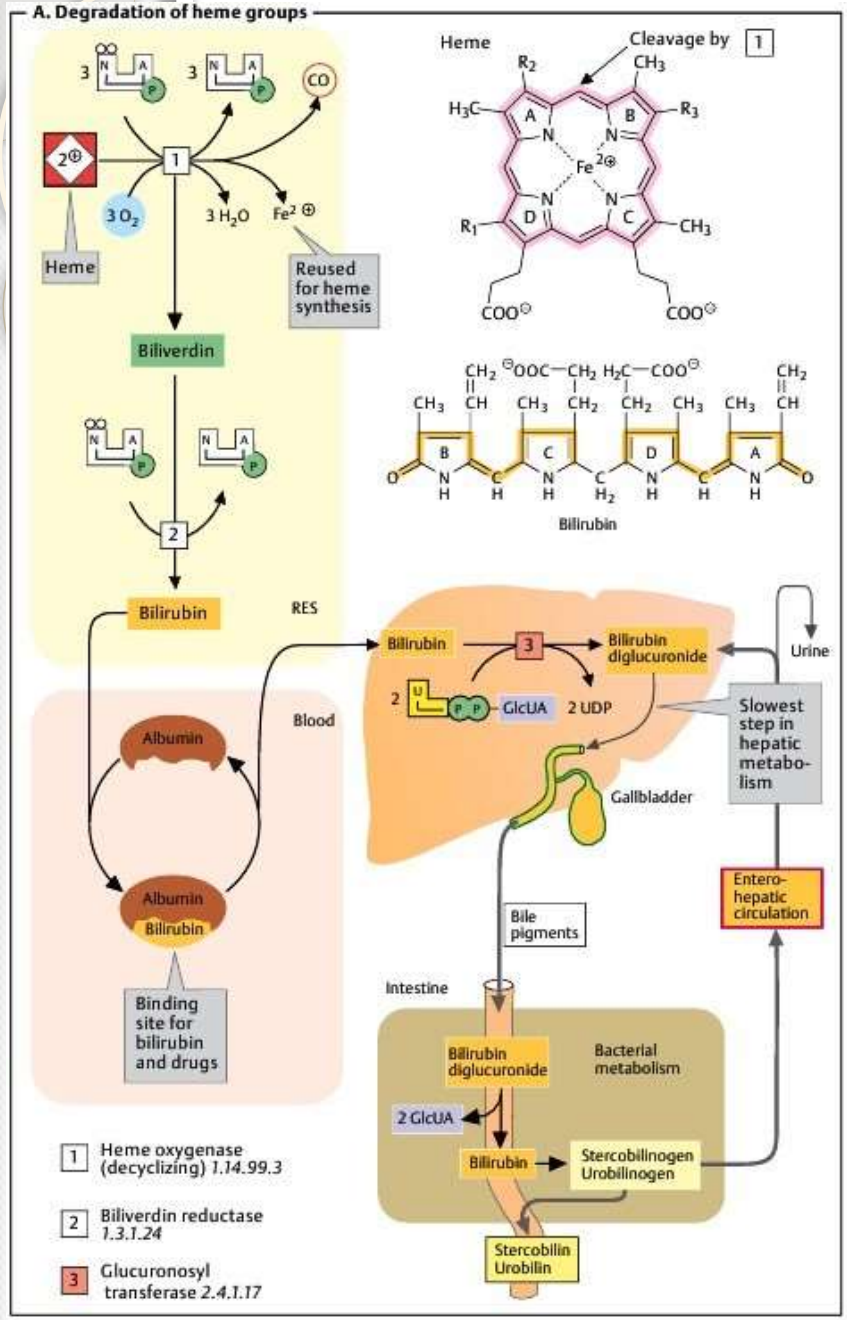
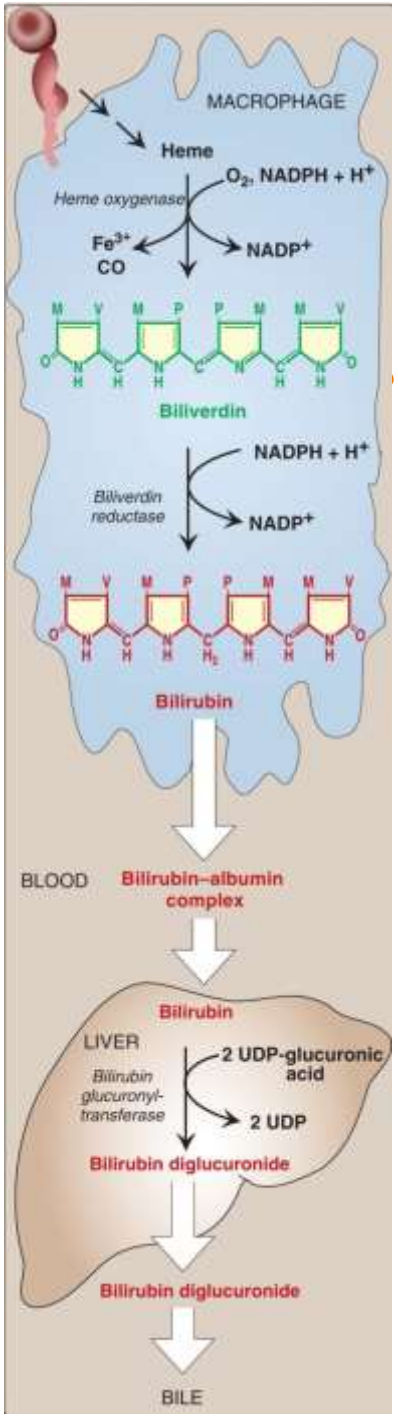


Figure 19-5 Principles of Anatomy and Physiology, 11/e



Heme Catabolism

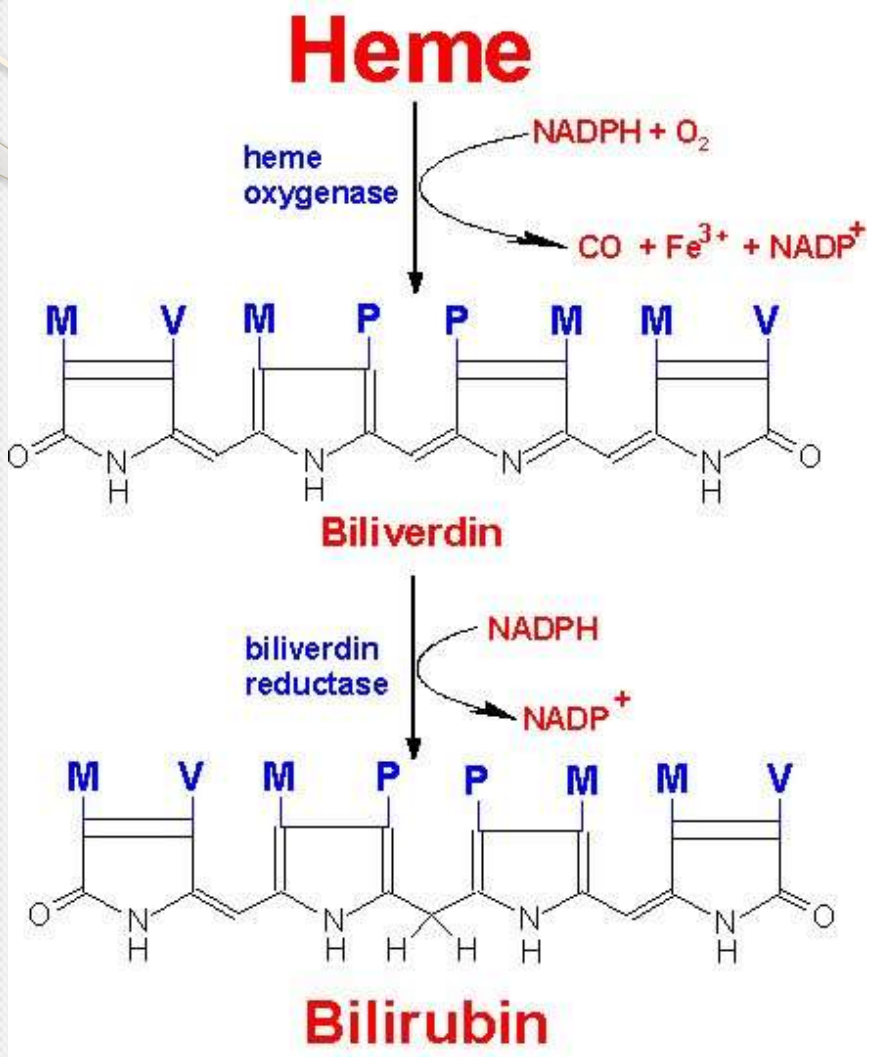
- **Heme** is oxidized, with the heme ring being opened by the endoplasmic reticulum enzyme, *heme oxygenase*.
- The oxidation occurs on a specific carbon producing the linear tetrapyrrole **biliverdin**, ferric iron (Fe^{3+}), and carbon monoxide (CO).
  - This is the only reaction in the body that is known to produce CO.
  - Most of the CO is excreted through the lungs.
  - => CO content of expired air is dependent on heme oxygenase.



Formation of Bilirubin from Heme

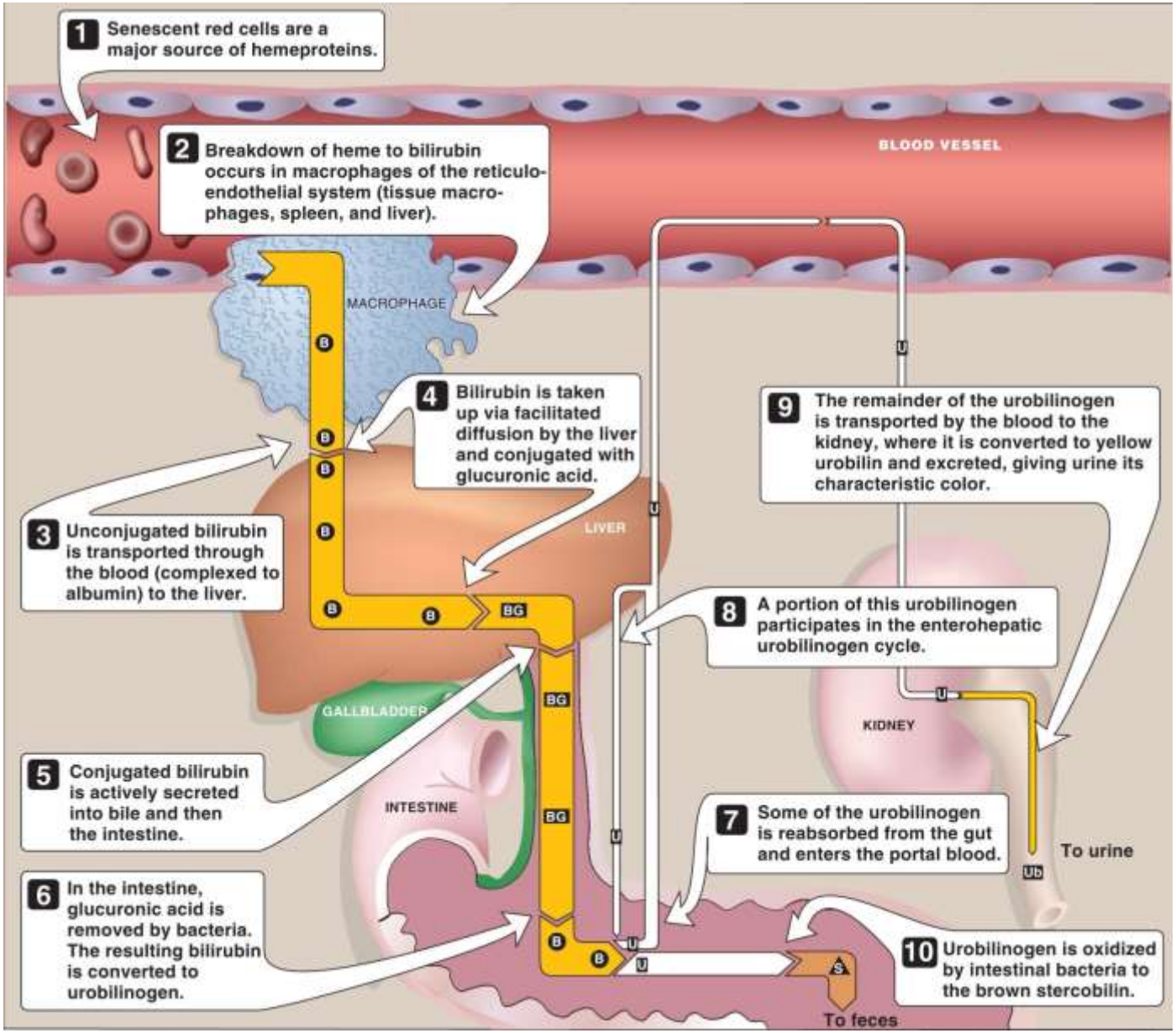
In the next reaction a second bridging methylene (between rings III and IV) is reduced by **biliverdin reductase**, producing **bilirubin**.

- Bilirubin is significantly less extensively conjugated than biliverdin causing a change in the color of the molecule from blue-green (biliverdin) to yellow-red (bilirubin).
- The latter catabolic changes in the structure of tetrapyrroles are responsible for the progressive changes in color of a **hematoma**, or bruise, in which the damaged tissue changes its color from an initial dark blue to a red-yellow and finally to a yellow color before all the pigment is transported out of the affected tissue.
- Peripherally arising bilirubin is transported to the liver in association with albumin, where the remaining catabolic reactions take place.

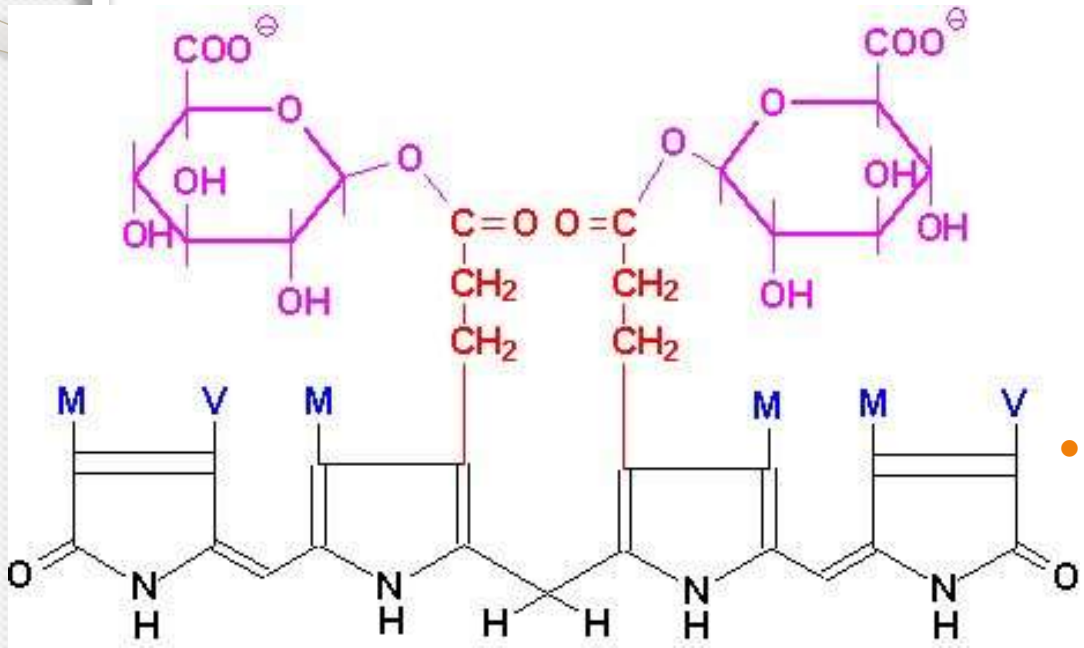


- Pathway for the degradation of heme to bilirubin substituents: **M=methyl, P=propionic, V=vinyl**

Catabolism of Heme: in Order of Events



Bilirubin Diglucuronide



- In hepatocytes, **UDP glucuronyl transferase** adds 2 equivalents of glucuronic acid to bilirubin to produce the more water soluble, **bilirubin diglucuronide** derivative.
- The increased water solubility of the tetrapyrrole facilitates its excretion with the remainder of the bile as the bile pigments.

Jaundice

- In individuals with abnormally high red cell lysis, or liver damage with obstruction of the bile duct, the bilirubin and its precursors accumulate in the circulation; the result is **hyperbilirubinemia**, the cause of the abnormal body pigmentation known as **jaundice**.
  - In normal individuals, intestinal bilirubin is acted on by bacteria to produce the final porphyrin products, **urobilinogens** and **urobilins**, that are found in the feces.
  - Bilirubin and its catabolic products are collectively known as the bile pigments.

Jaundice (cont'd)

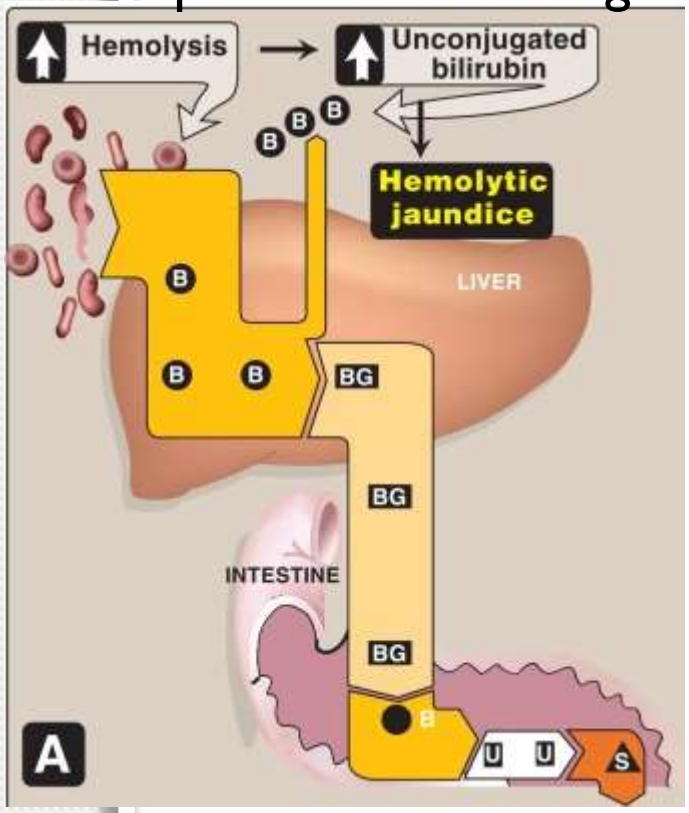
- The normal serum total bilirubin concentration is in the range of 0.2 to 0.8 mg/dl. Of this, about 0.2 - 0.6 mg/dl is uncojugated while 0 to 0.2 mg/dl is conjugated bilirubin.
- **Jaundice** (*French: Jaune — yellow*) is a clinical condition characterized by yellow colour of the white of the eyes (sclerae) and skin.
  - It is cause by the deposition of bilirubin due to its elevated levels in the serum.
  - The term hyperbilirubinemia is often used to represent the increased concentration of serum bilirubin.

Classification of jaundice

- Jaundice (also known as *icterus*) may be more appropriately considered as a symptom rather than a disease.
- It is rather difficult to classify jaundice, since it is frequently caused due to multiple factors.
- For the sake of convenience to understand, jaundice is classified into three major types **- hemolytic**, **hepatic** and **obstructive**.

Hemolytic Jaundice

- This is condition is associated with increased hemolysis of erythrocytes (e.g. incompatible blood transfusion, malaria, sickle cell anemia). This results in the overproduction of bilirubin beyond the ability of the liver to conjugate and excrete the same. It should, however be noted that liver possesses a large capacity to conjugate about 3.0 g of bilirubin per day against the normal bilirubin production of 0.3 g/day.



- In hemolytic jaundice, more bilirubin is excreted into the bile leading to the increased formation of urobilinogen and stercobilinogen. Hemolytic jaundice is characterized by

a. Elevation in the serum unconjugated bilirubin.

b. Increased excretion of urobilinogen in urine.

c. Dark brown colour of feces due to high content of stercobilinogen.

Hepatic (Hepatocellular) Jaundice

- This type of jaundice is cause by dysfunction of the liver due to damage to the parenchymal cells. This may be attributed to viral infection (viral hepatitis), poisons and toxins (chloroform, carbon tetrachloride, phosphorus etc.), cirrhosis of liver, cardiac failure etc. Among these, viral hepatitis is the most common.
- Damage to the liver adversely affects the bilirubin uptake and its conjugation by the liver cells. Hepatic jaundice is characterized by

a. Increased levels of conjugated and unconjugated bilirubin in the serum.

b. Dark coloured urine due to the excessive excretion of bilirubin and urobilinogen.

c. Increased activities of alanine transaminase and aspartate transaminase released intocirculation due to damage to hepatocytes.

d. The patients pass pale, clay coloured stools due to the absence of stercobilinogen.

e. The affected individuals experience nausea and anorexia (loss of appetite).

Obstructive (Regurgitation) Jaundice

- This is due to an obstruction in the bile duct that prevents the passage of bile into the intestine. The obstruction may be caused by gall stones, tumors etc.
- Due to the blockage in bile duct, the conjugated bilirubin from the liver enters the circulation. Obstructive jaundice is characterized by

a. Increased concentration of conjugated bilirubin in serum.

b. Serum alkaline phosphatase is elevated as it is released from the cells of the damaged bile duct.

c. Dark coloured urine due to elevated excretion of bilirubin and clay coloured feces due to absence of stercobilinogen.

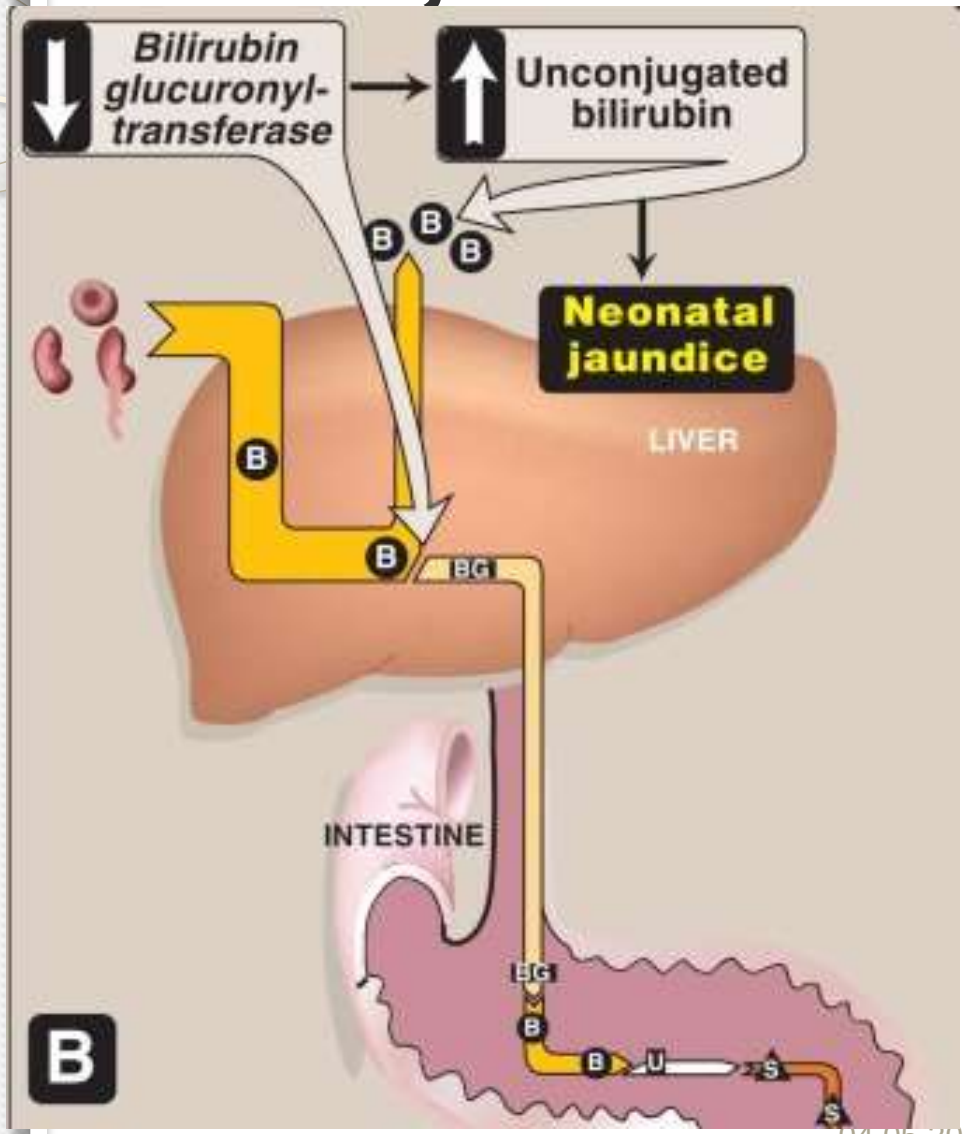
d. Feces contain excess fat indicating impairment in fat digestion and absorption in the absence of bile (specifically bile salts).

e. The patients expenence nausea and gastrointestinal pain.

Laboratory results in patients with jaundice

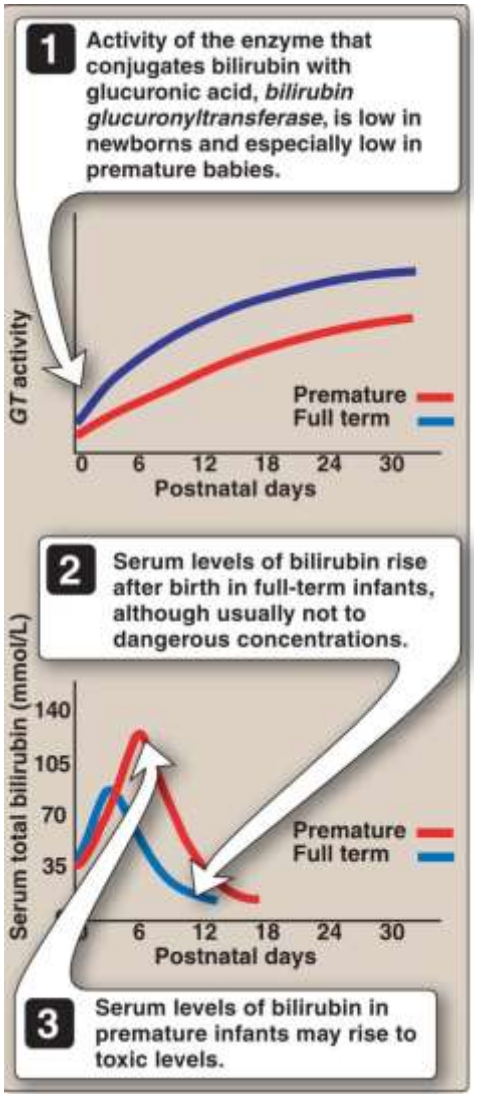
	normal	Hemolytic jaundice	Hepatocellular jaundice	Obstructive jaundice
Serum bilirubin				
total	< 1mg/dl	> 1mg/dl	> 1mg/dl	> 1mg/dl
direct	0~ 0.8mg/dl		↑	↑↑
indirect	< 1	↑↑	↑	
Urine bile pigments				
urobilirubin	–	–	+ +	+ +
urobilinogen	A few	↑	uncertainty	↓
urobilin	A few	↑	uncertainty	↓
Color of feces	normal	dark	Simple or normal	Clay color

Neonatal jaundice



Neonatal-physiologic jaundice. This is not truly a genetic defect. It is caused by increased hemolysis coupled with immature hepatic system for the uptake, conjugation and secretion of bilirubin.

Neonatal jaundice (cont'd)



The activity of the enzyme UDP-glucuronyltransferase is low in the newborn. Further, there is a limitation in the availability of the substrate UDP-glucuronic acid for conjugation.
The net defect is the serum unconjugated bilirubin is highly elevated (may go beyond 25 mg/ml), which can cross the blood-brain barrier and cause damage to the brain leading to mental retardation.

Crigler-Najjar syndrome

Crigler-Najjar syndrome type I. This is also known as congenital nonhemolytic jaundice. It is a rare disorder and is due to a defect in the hepatic enzyme UDP-glucuronyltransferase. Generally, the children die within first two years of life.

Crigler-Najjar syndrome type II. This is again a rare hereditary disorder and is due to a less severe defect in the bilirubin conjugation. It is believed that hepatic UDP-glucuronyltransferase that catalyses the addition of second glucuronyl group is defective. The serum bilirubin level concentration is usually less than 20 mg/dl and this is less dangerous than type I.

Gilbert's disease

This is not a single disease but a combination of disorders. These include

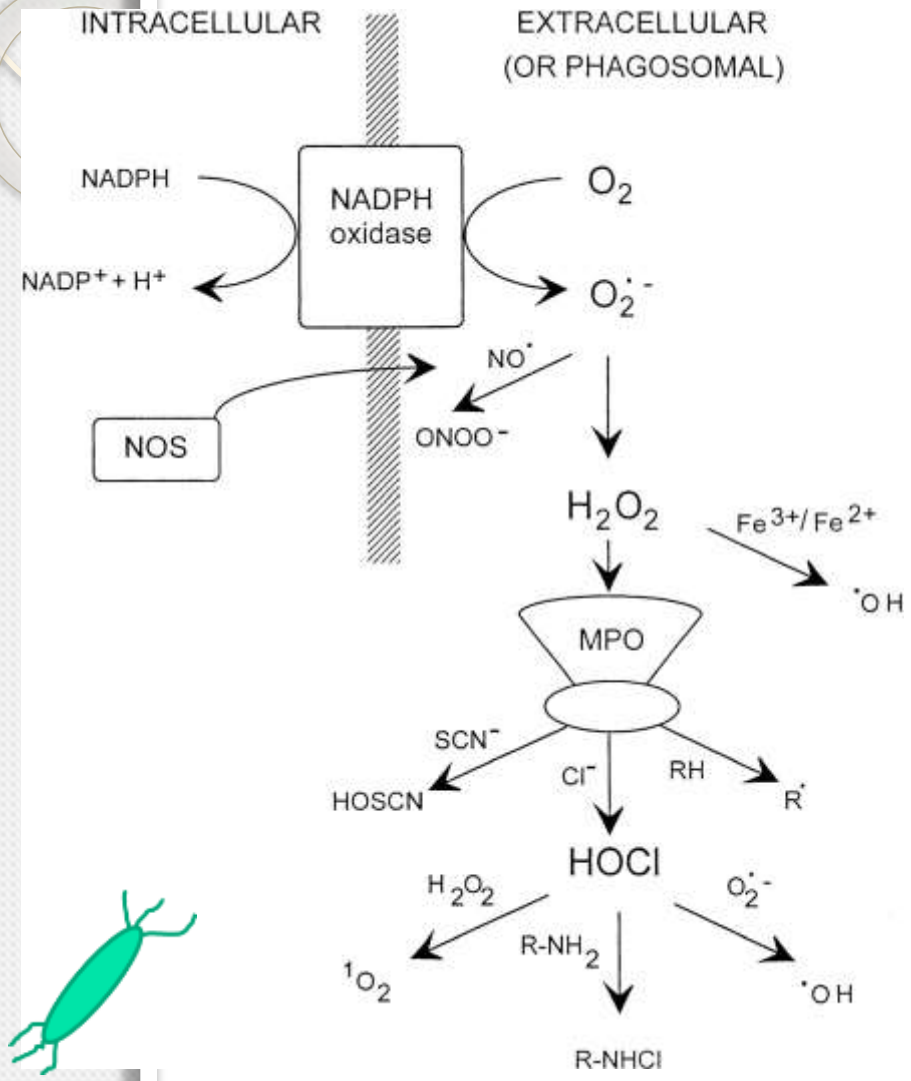
a. A defect in the uptake of bilirubin by liver cells.

b. An impairment in conjugation due to reduced activity of UDP-glucuronyl-transferase.

c. Decreases hepatic clearance of bilirubin.

NEUTROPHIL AND PLATELET METABOLISM

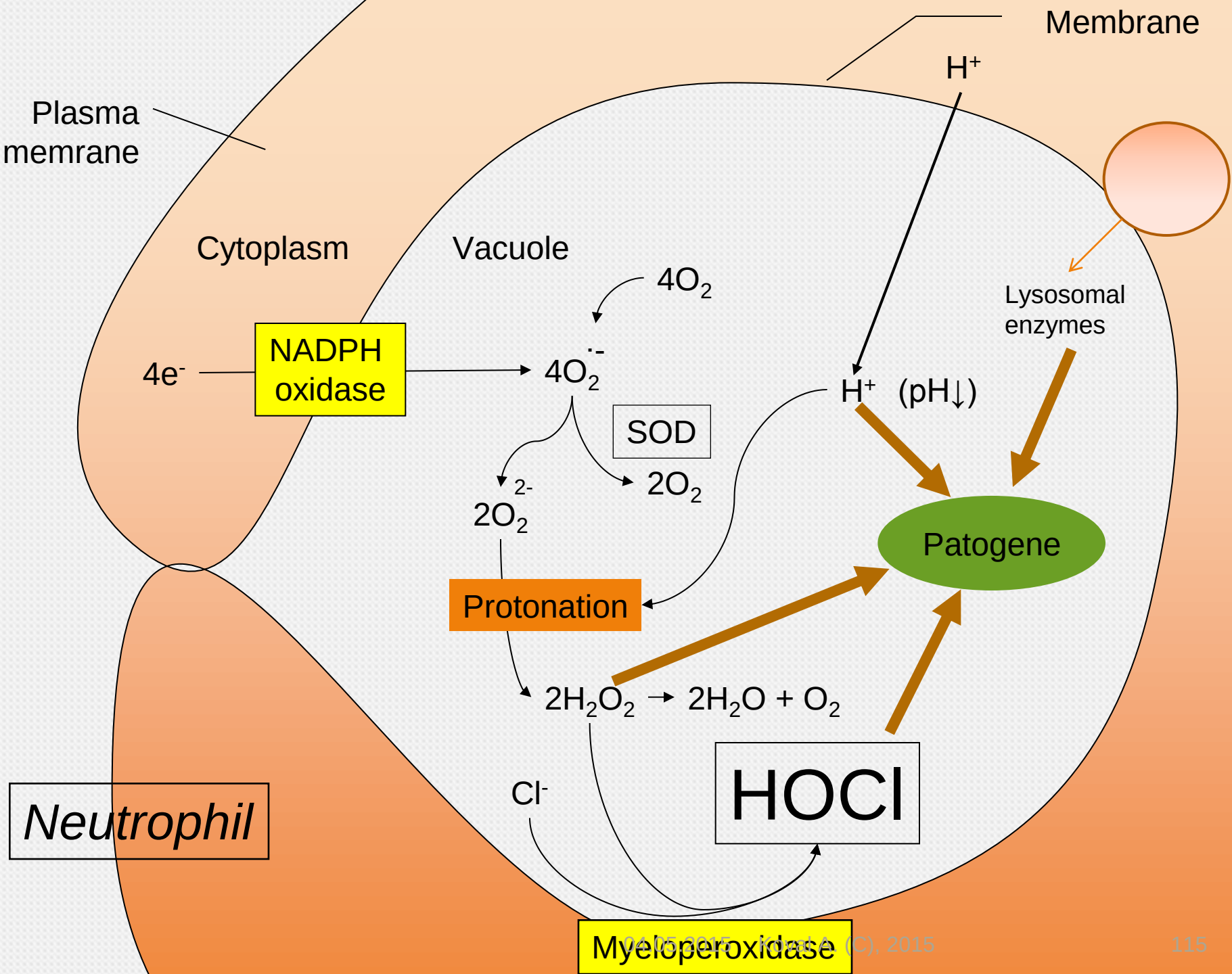
“Oxidative Burst”, and ROS generation



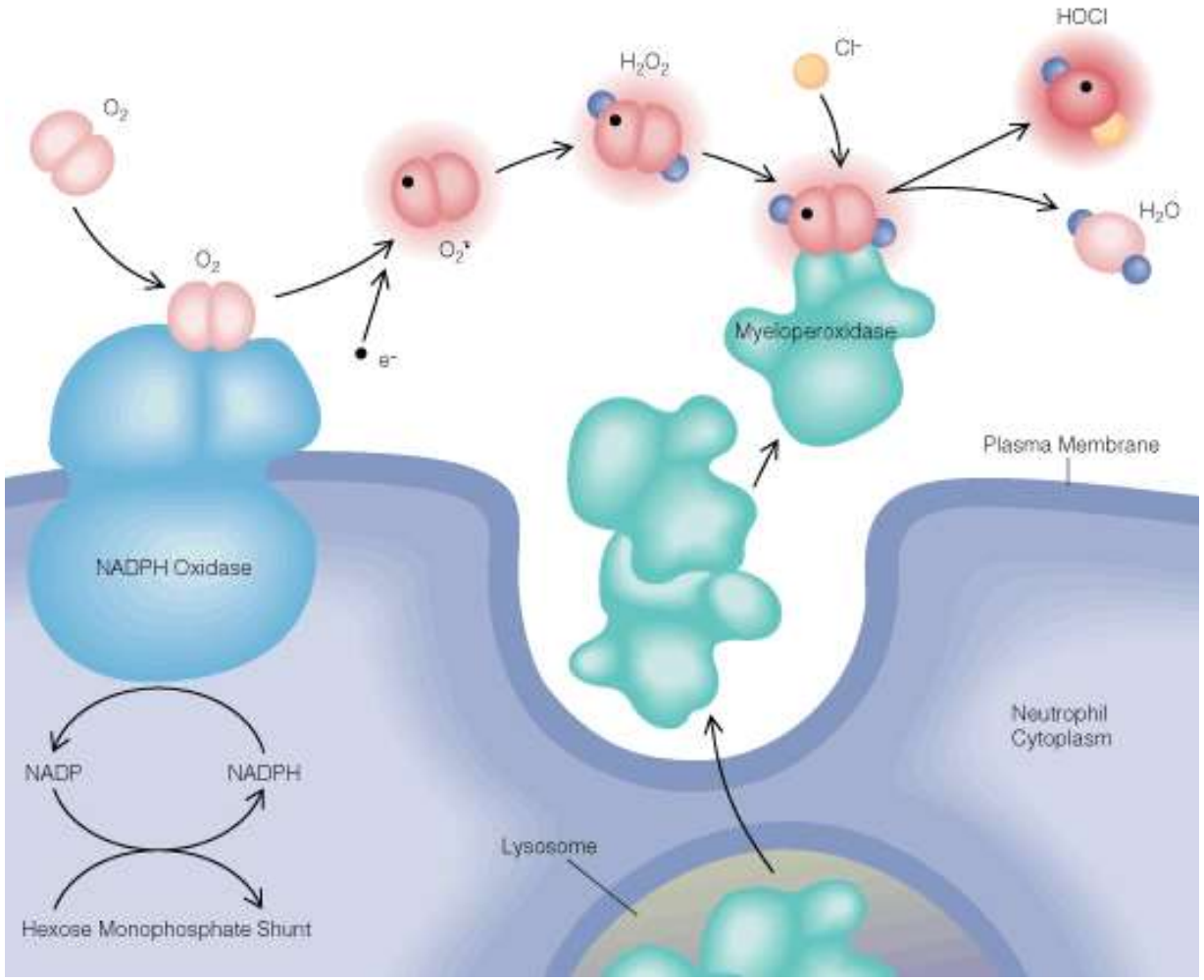
Hampton M B et al. Blood 1998;92:3007-3017

- In a minute after fagocytosis there is sharp increase of O_2 consumption by neutrophil (“oxidative burst”).
- Formed ROS are of bactericide action.

NOS - NO-synthase,
MPO – myeloperoxidase
$ONOO^-$ - peroxynitrite-anion.
HOCl – hypochloric acid

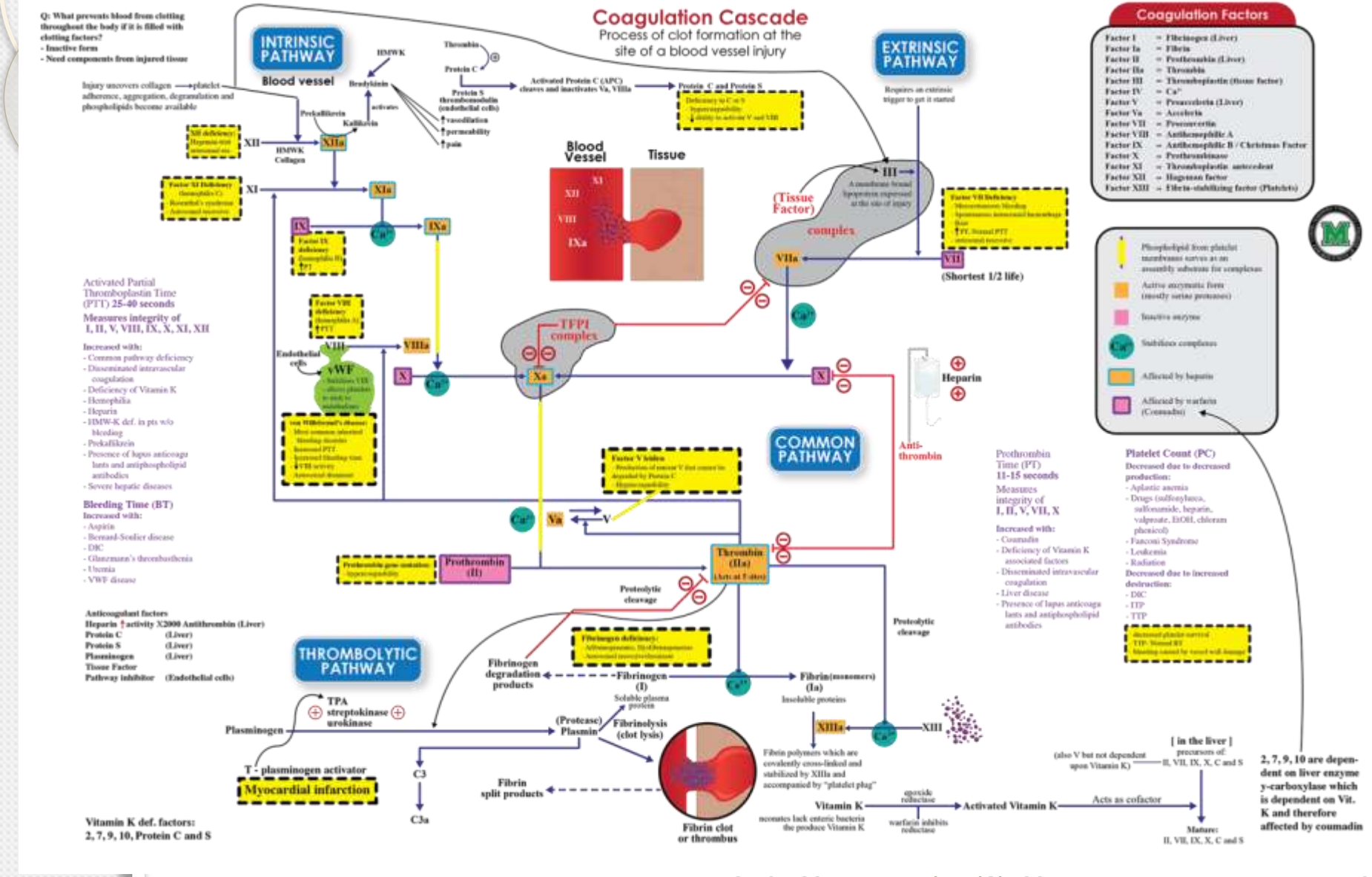

Membrane
H+
Plasma memrane
Cytoplasm
Vacuole
4O2
Lysosomal enzymes
4e-
NADPH oxidase
4O2 ·-
H+ (pH↓)
SOD
2O2 2-
2O2
Patogene
Protonation
2H2O2 → 2H2O + O2
Cl-
HOCl
Neutrophil
Myeloperoxidase

Enzymes of Neutrophils

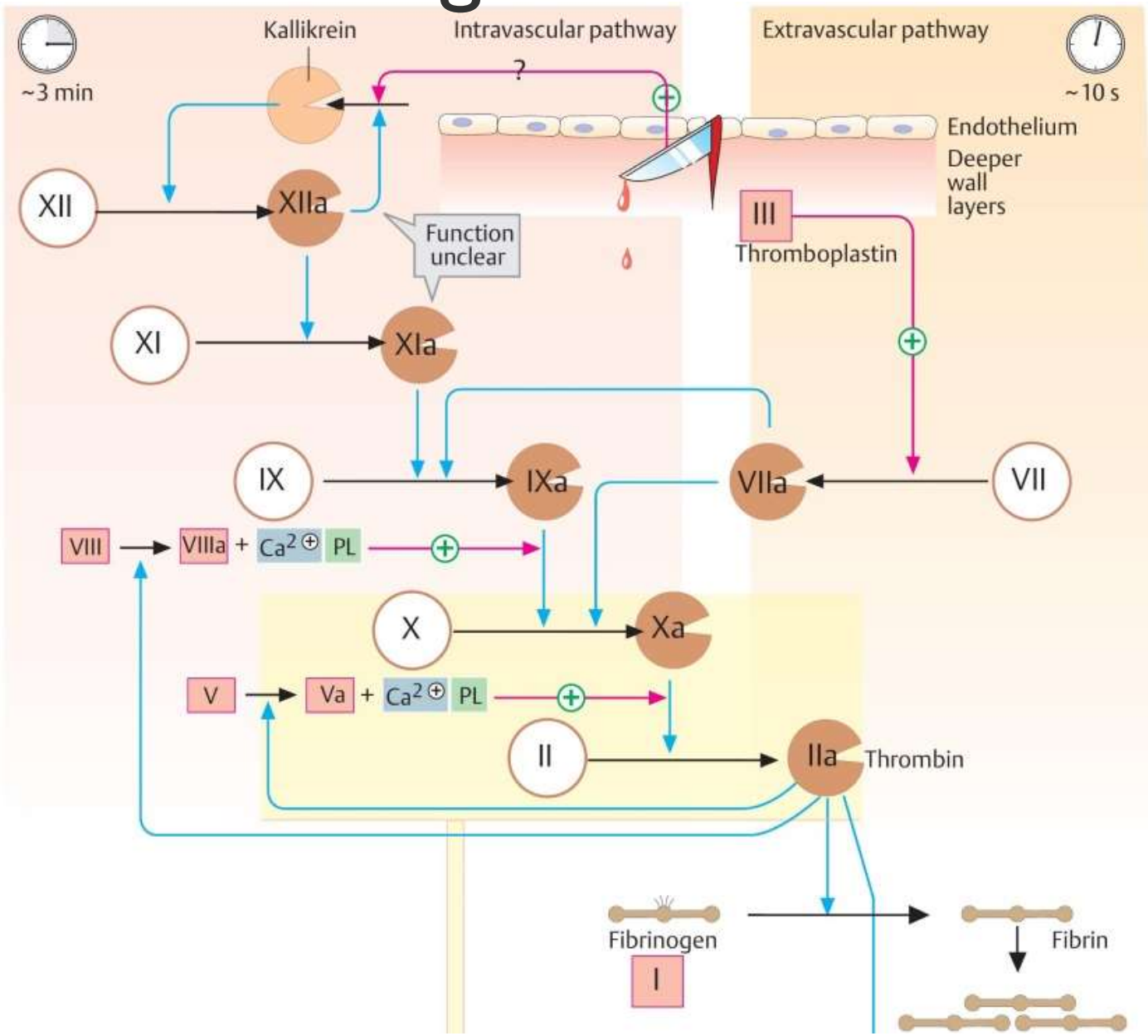


BLOOD CLOTTING

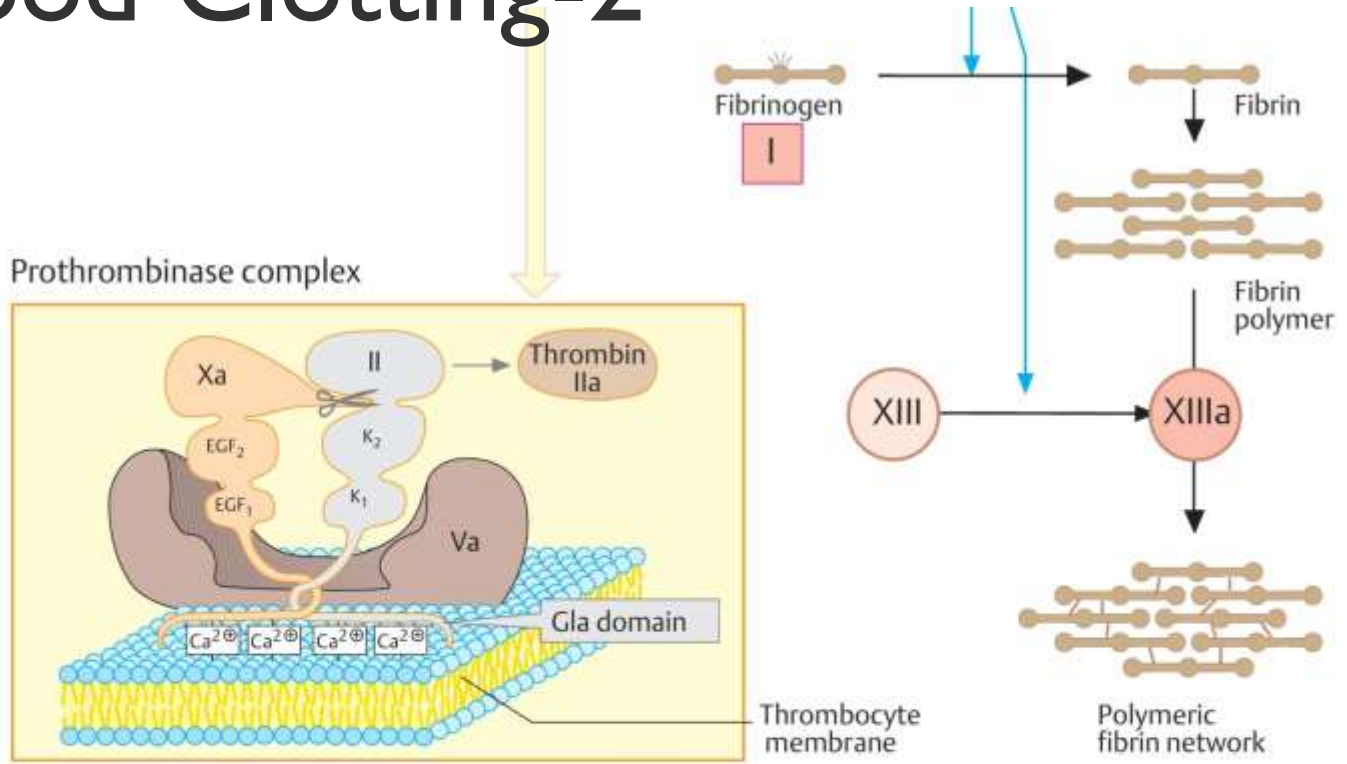
Coagulation Cascade



Blood Clotting-I



Blood Clotting-2



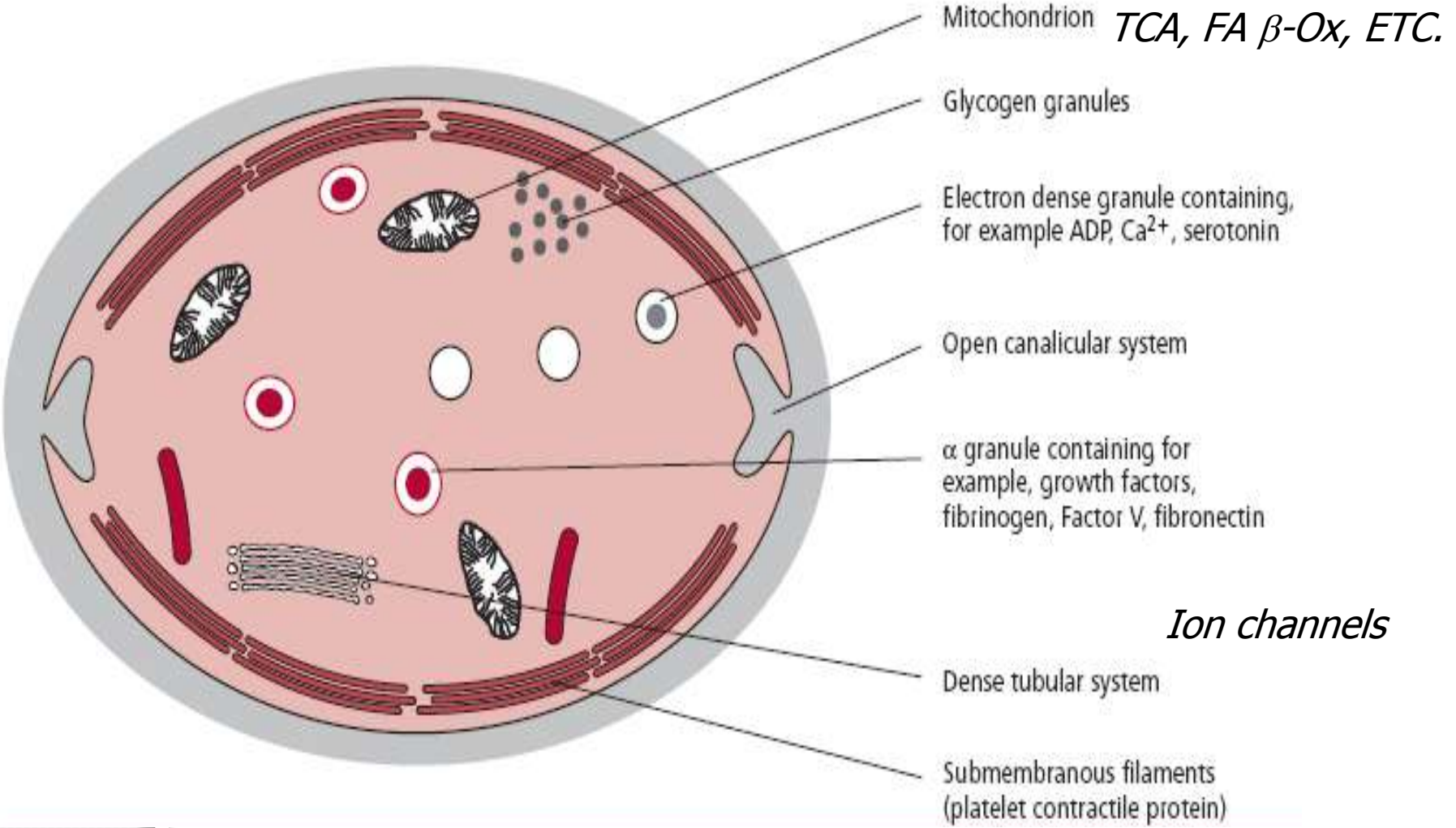
Coagulation factors

- I Fibrinogen
- ◆ II Prothrombin* *3.4.21.5*
- III Tissue factor/thromboplastin
- IV $Ca^{2\oplus}$
- V Proaccelerin
- VI Synonym for Va
- ◆ VII Proconvertin* *3.4.21.21*
- VIII Antihemophilic factor A
- ◆ IX Christmas factor* *3.4.21.22*
- ◆ X Stuart–Prower factor* *3.4.21.6*
- XI Plasma thromboplastin antecedent* (PTA) *3.4.21.27*
- XII Hageman factor* *3.4.21.38*
- XIII Fibrin-stabilizing factor* *2.3.2.13*

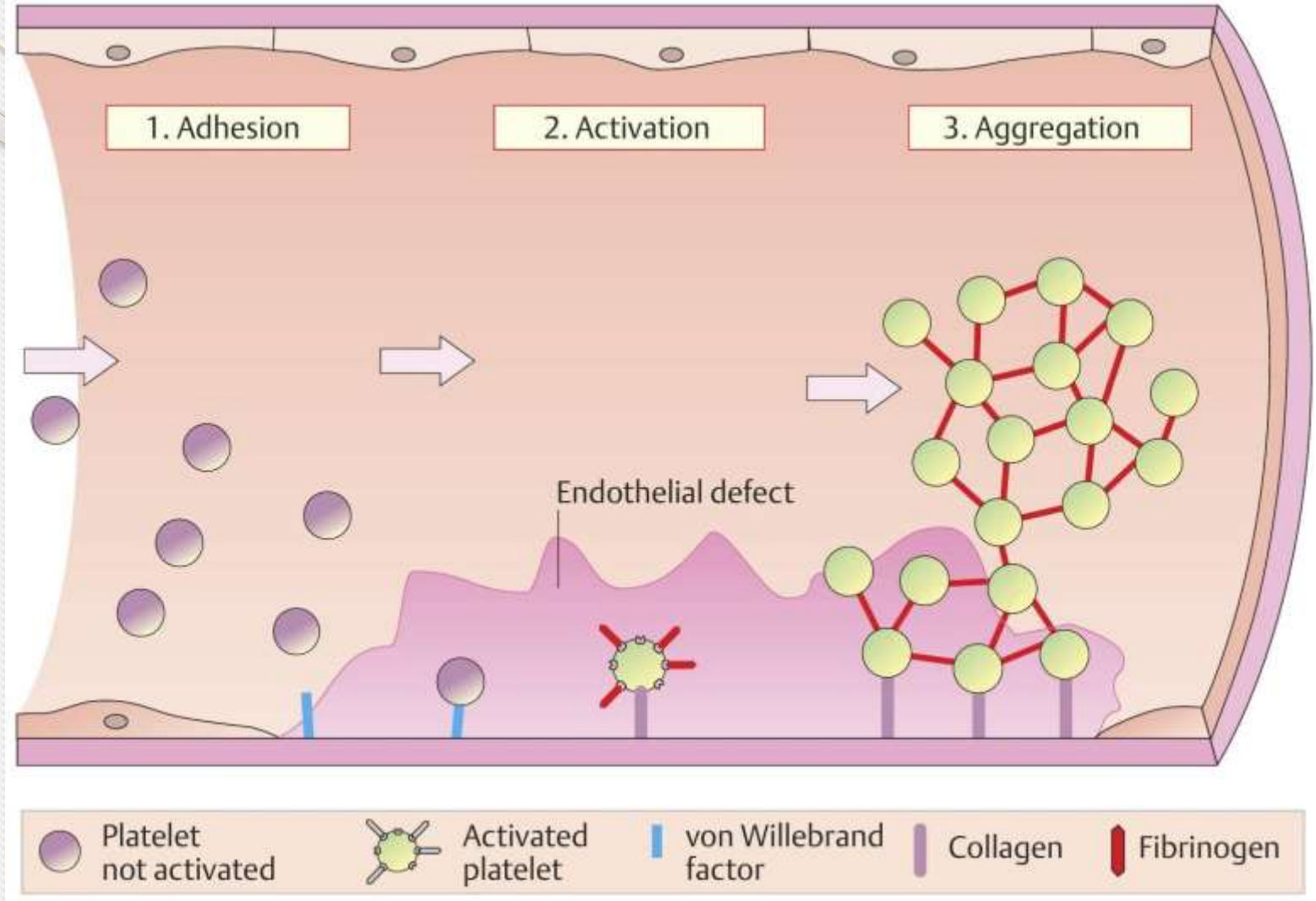
* Proenzyme

◆ Contains γ-carboxyglutamate

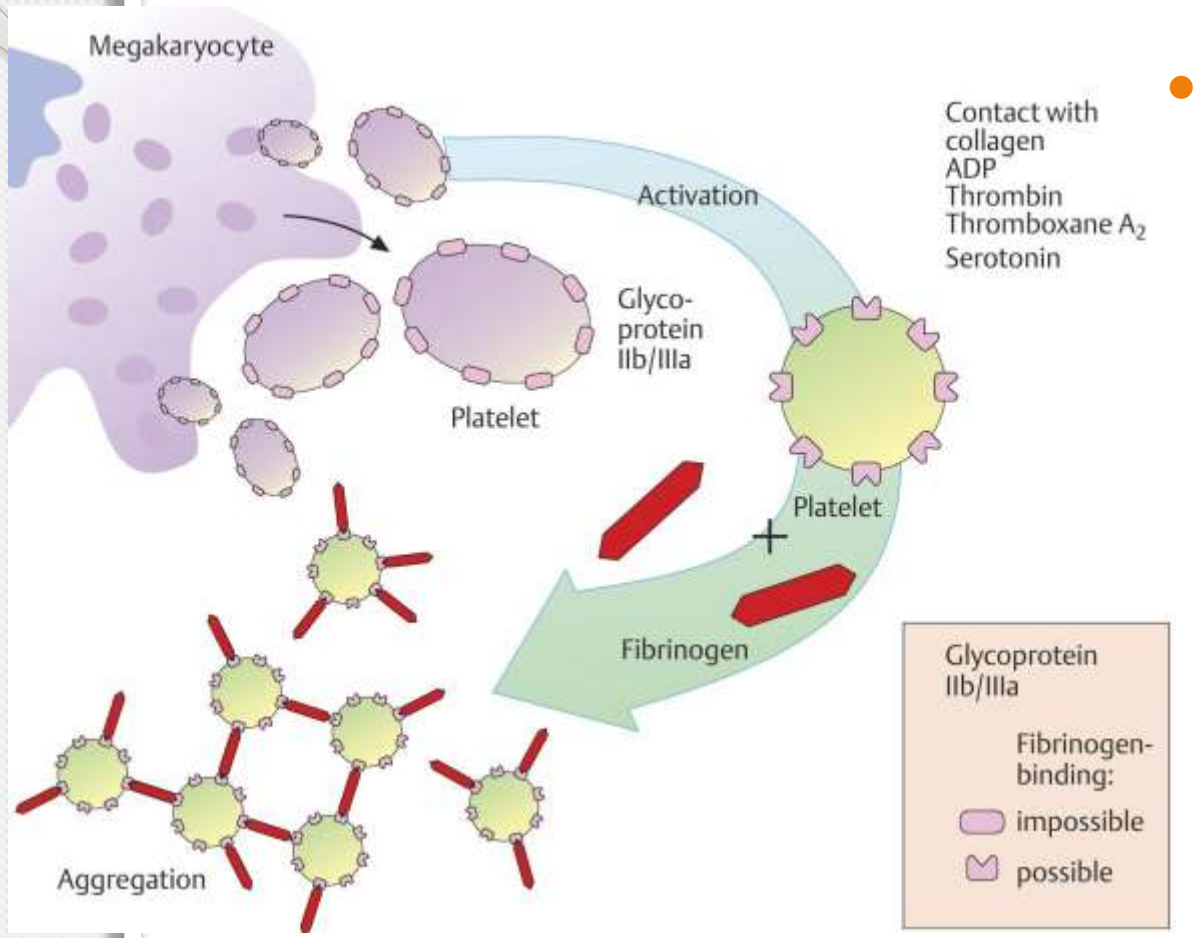
Platelet Structure



Thrombogenesis



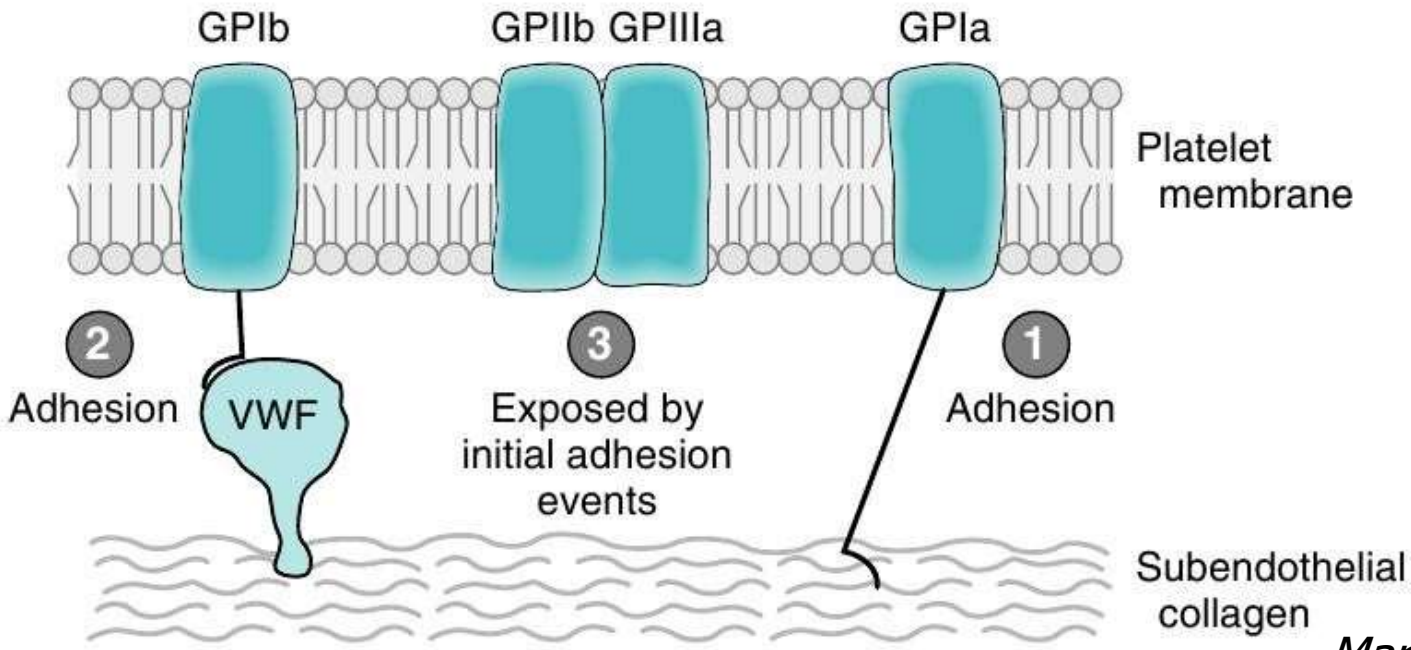
Platelet Aggregation. Role of Integrins GPIIb/IIIa



- Platelet activation – ability to bind fibrinogen. Activators – collagen, ADP, thrombin, throbmoxane A2, serotonin.

Platelet Adhesion. Activation Mechanism

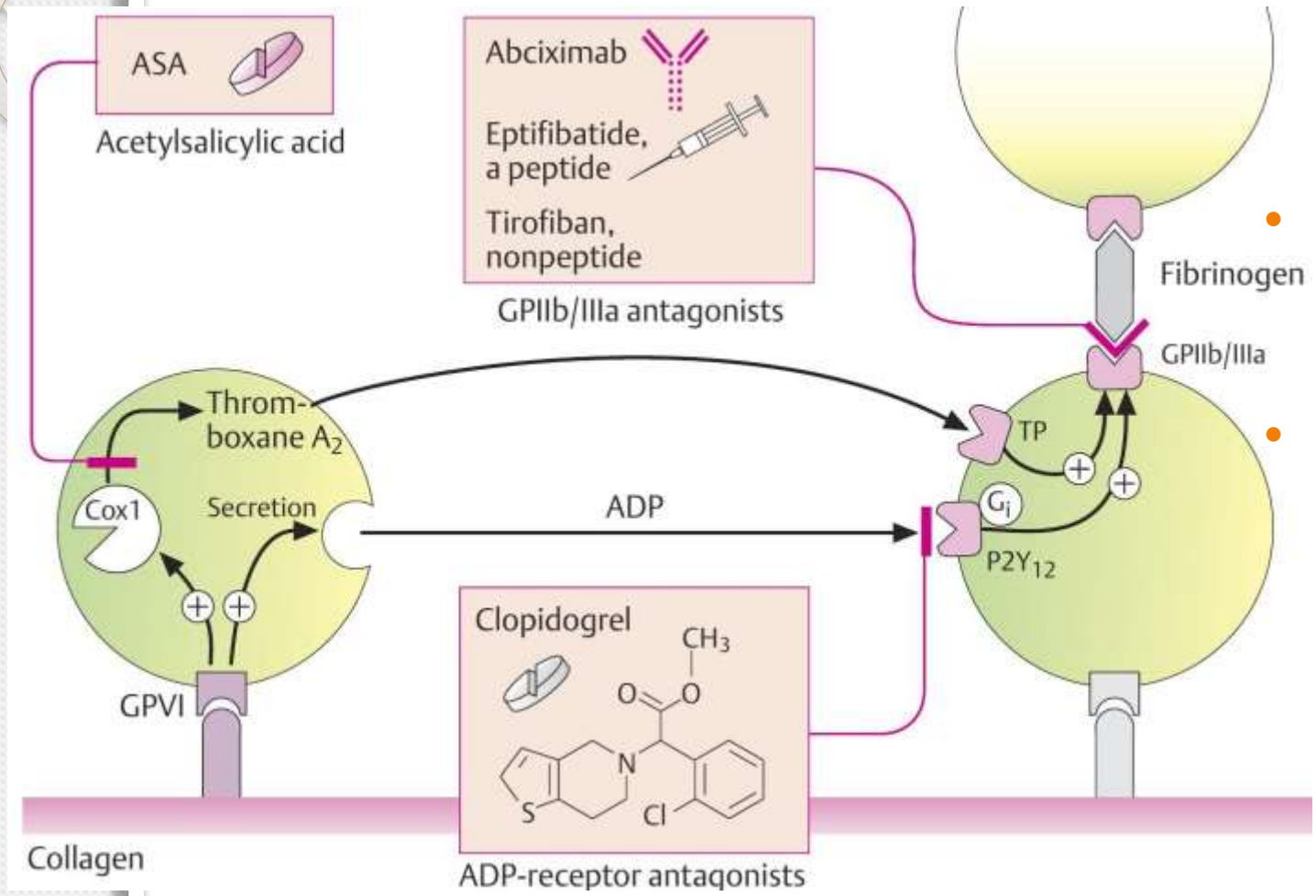
GP – glycoprotein



Marks, 2005

1. Binding of GPIa with subendothelial collagen.
2. Binding of GPIb with vWF (von Willebrand factor).
3. Exposed GPIIb/GPIIIa complex then binds vWF and **fibrinogen**.

Platelet Aggregation Regulation. Inhibitors.

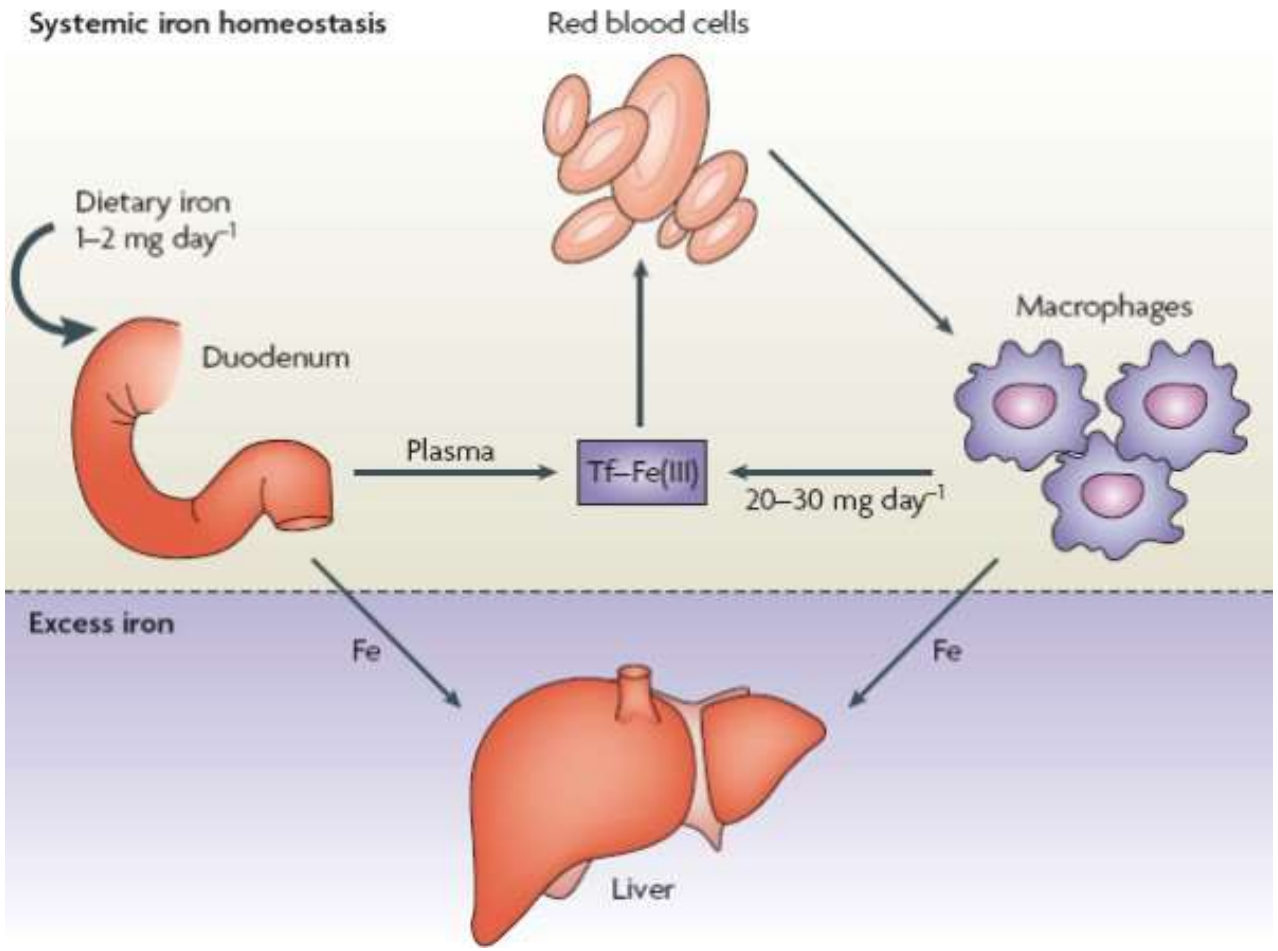


- Platelet activation, TxA_2 and ADP secretion.
- Acetylsalycylic acid and clopidrogel inhibits aggregation.

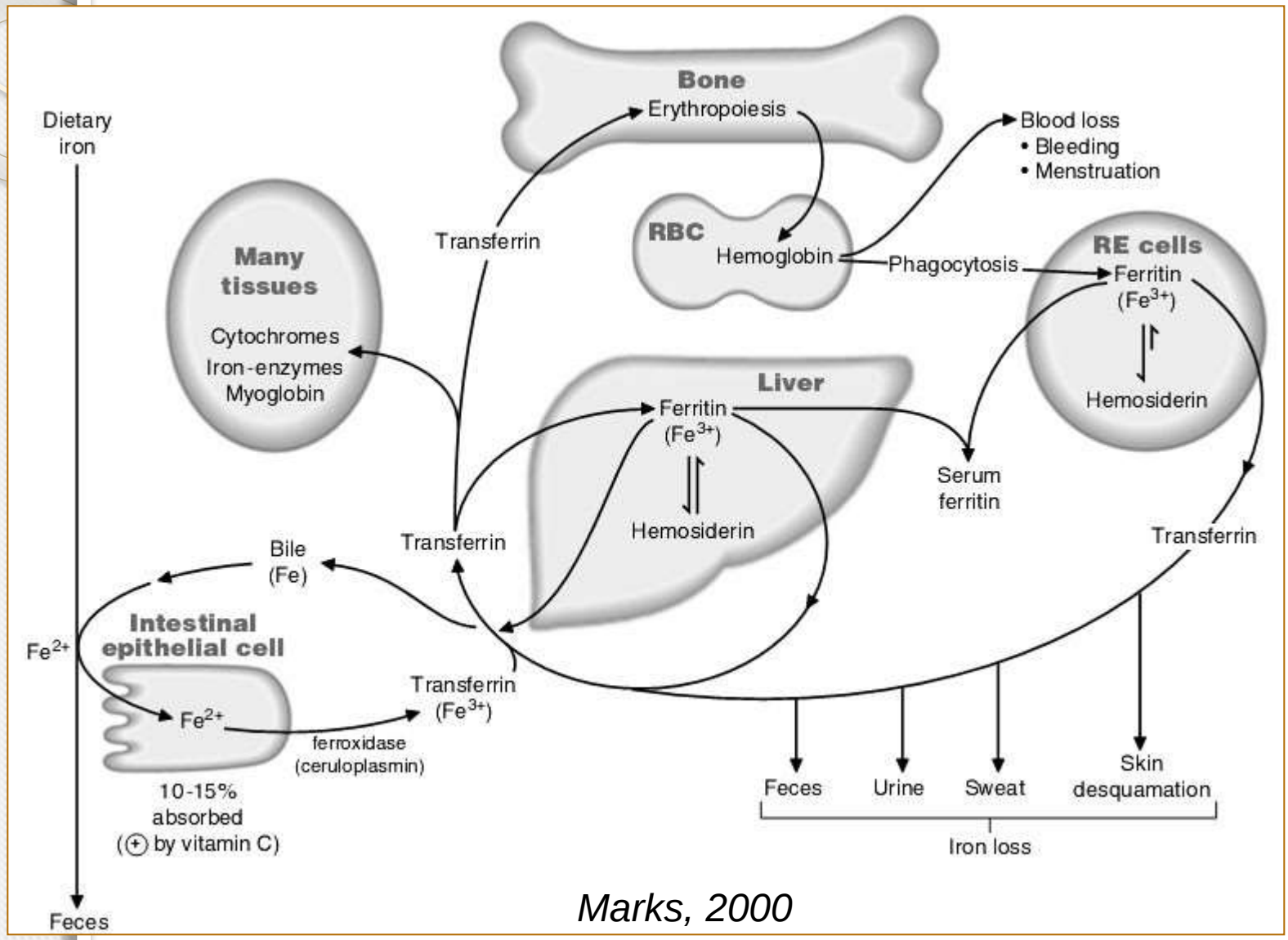
Pathology

- Bernard-Soulier syndrome (GP1b gene mutation)
- Willebrand disease (vWF gene mutation).
- Manifested as hemmoragias.

Systemic Iron Homeostasis

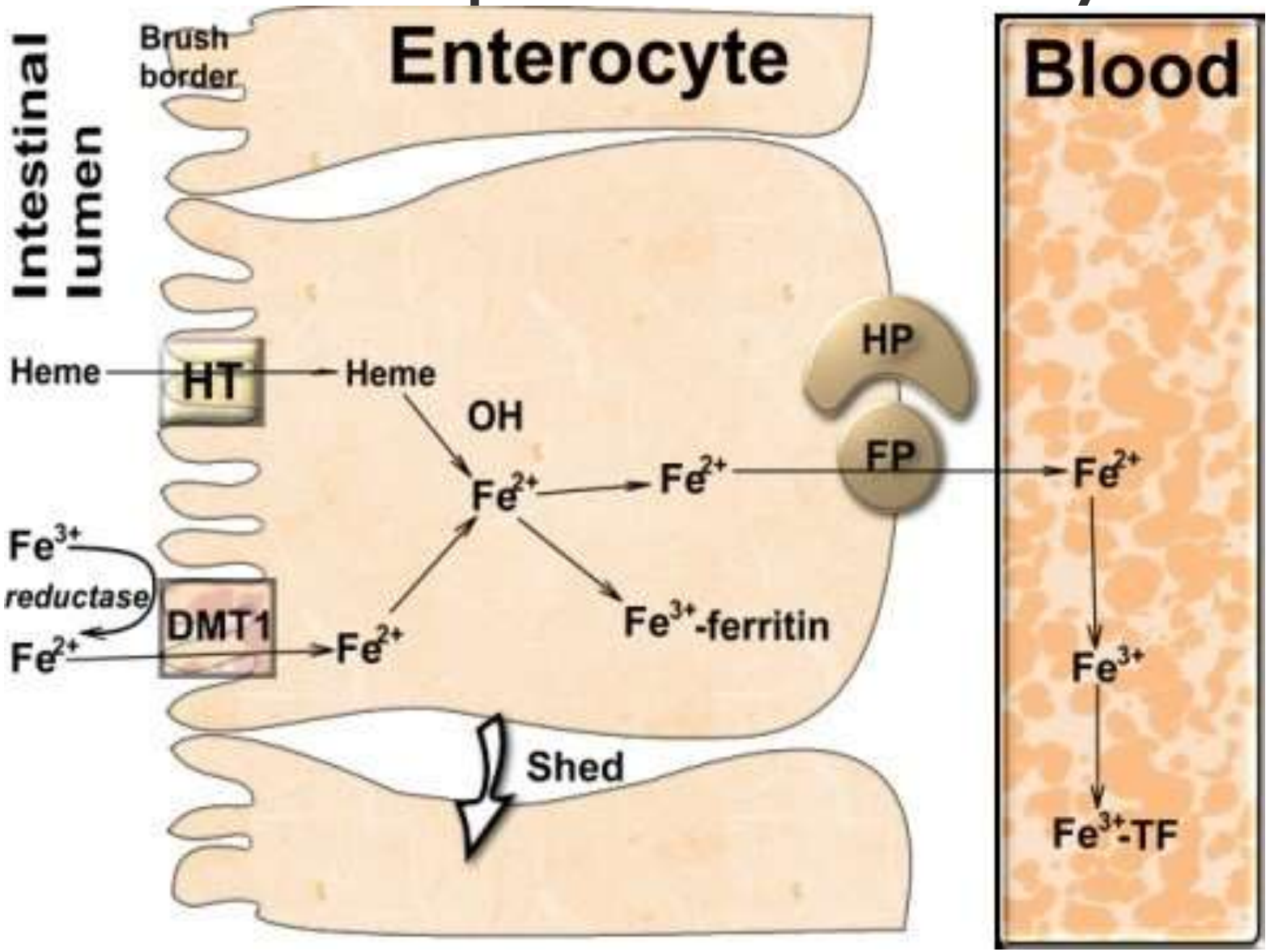


Iron Metabolism



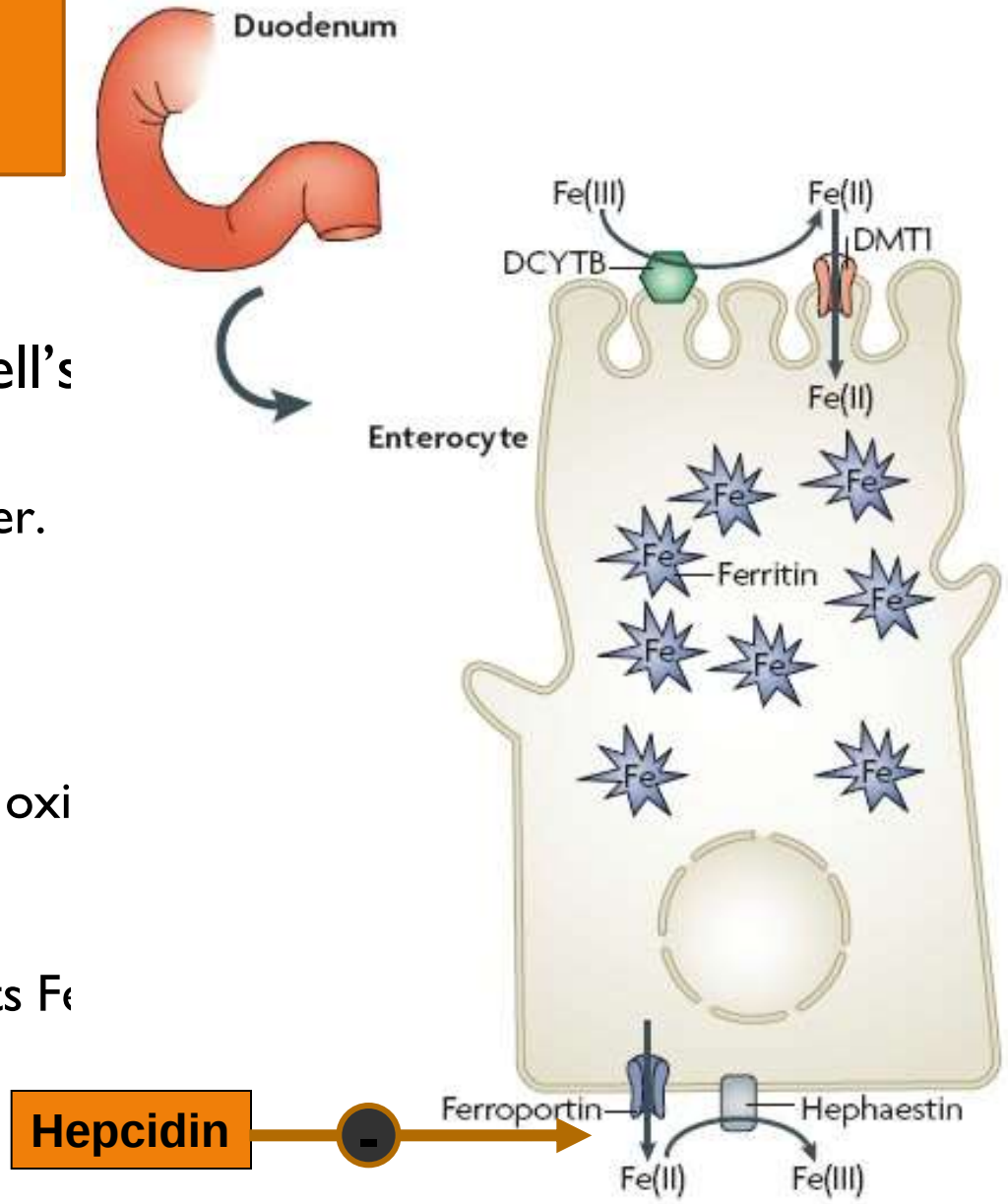
Marks, 2000

Iron Absorption in Enterocytes

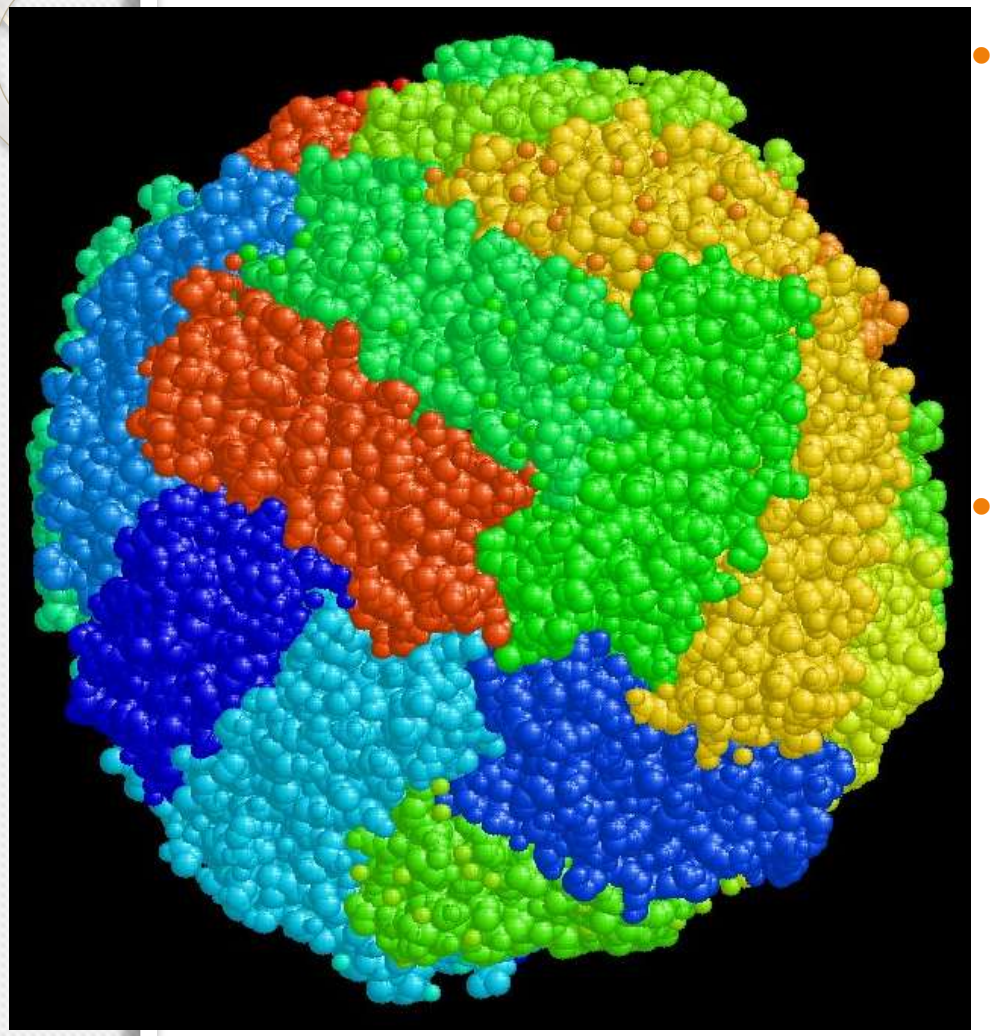


Passing of Iron Through Enterocyte

- DCYTB
  - reduces Fe on the cell's
- DMT1
  - divalent metal transporter.
- Ferroportin
  - iron exporter.
- Hephaestin
  - Cu-containing oxidase – oxi exported Fe^{2+}.
- Hepcidin
  - produces by liver, inhibits Fe overloading.



Ferritin

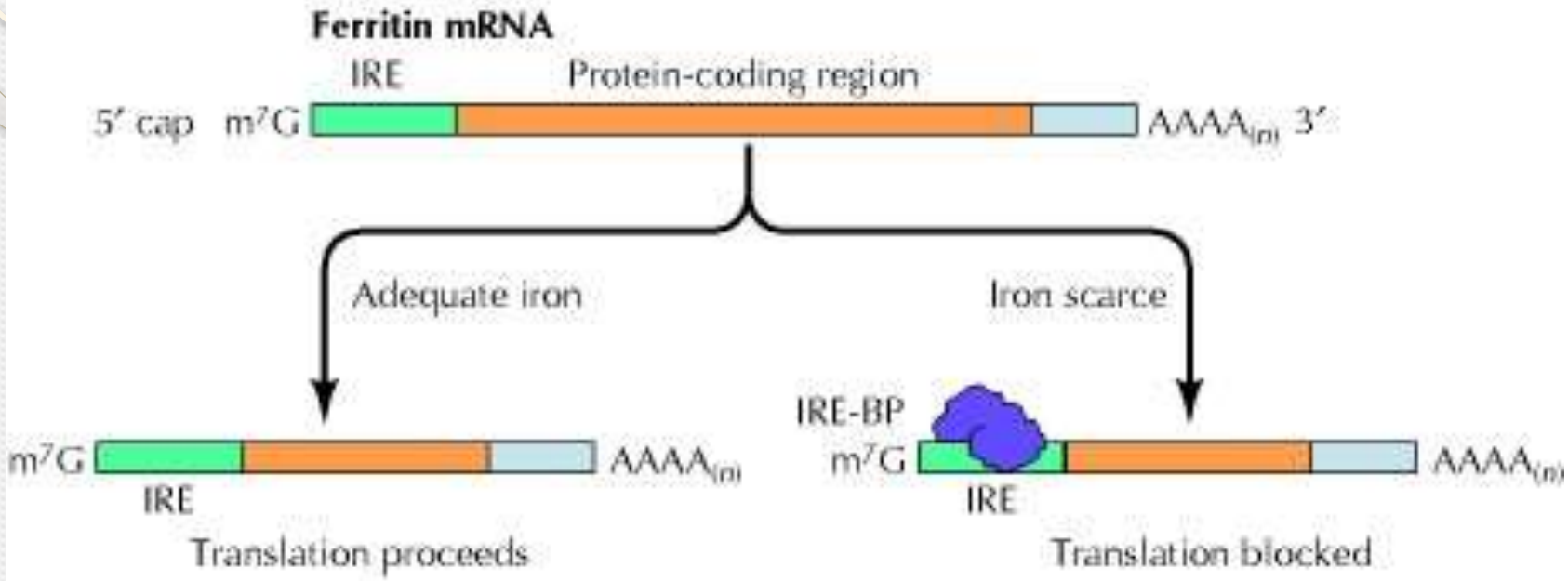


- **Ferritin** is the major intracellular iron storage protein in all organisms.
  - Shape: hollow sphere
  - variable amount of iron is stored as ferric hydroxide phosphate complexes.
- Mammalian liver and spleen ferritin (M_r = 450,000) consists of 24 subunits of 2 species.
  - The heavy subunit (relative mass = 21,000),
  - the light subunit (relative mass = 19,000).

Regulation of the Synthesis of Ferritin

- The best understood example of this mechanism in eukaryotic cells is regulation of the synthesis of **ferritin**, a protein that stores iron within the cell.
- The translation of ferritin mRNA is regulated by the supply of iron:
  - More ferritin is synthesized if iron is abundant. This regulation is mediated by a protein which (in the absence of iron) binds to a sequence (the iron response element, or IRE) in the 5´ untranslated region of ferritin mRNA, blocking its translation.
  - In the presence of iron, the repressor no longer binds to the IRE and ferritin translation is able to proceed.

Translational Regulation of Ferritin

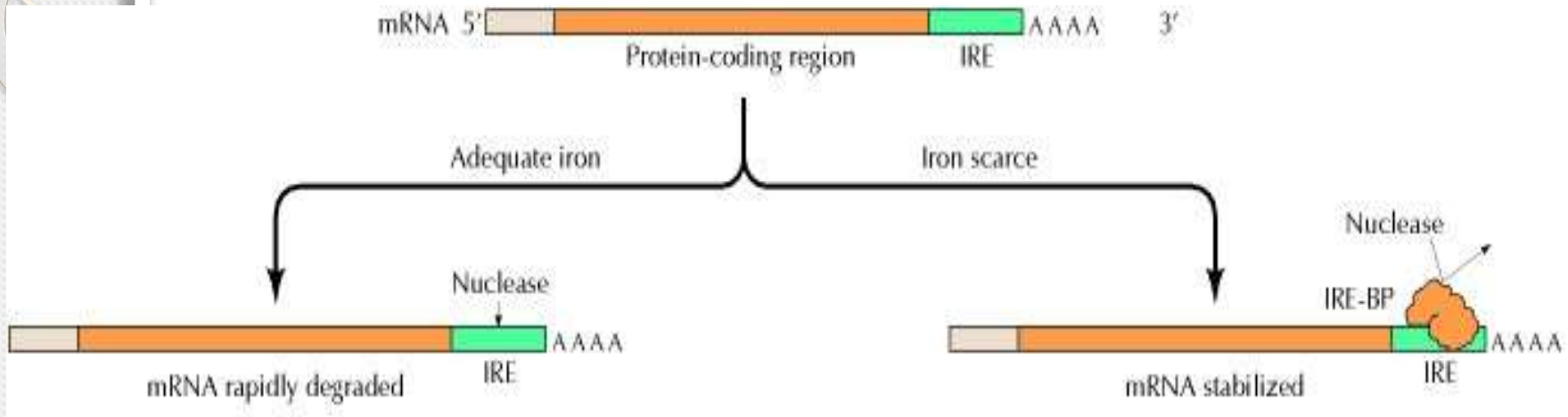


- The mRNA contains an iron response element (IRE) near its 5´ cap. In the presence of adequate supplies of iron, translation of the mRNA proceeds normally.
- If iron is scarce, however, a protein (called the iron response element binding protein, or IRE-BP) binds to the IRE, blocking translation of the mRNA.

Regulation of Transferrin Receptor mRNA Stability

- The stability of transferrin receptor mRNA is regulated by protein binding to an IRE in its 3´ untranslated region.
  - The same protein binds to the IREs of both ferritin and transferrin receptor mRNAs.
  - However, the consequences of protein binding to the two IREs are quite different.
- Protein bound to the transferrin receptor IRE protects the mRNA from degradation rather than inhibiting its translation.

Regulation of Transferrin Receptor mRNA Stability

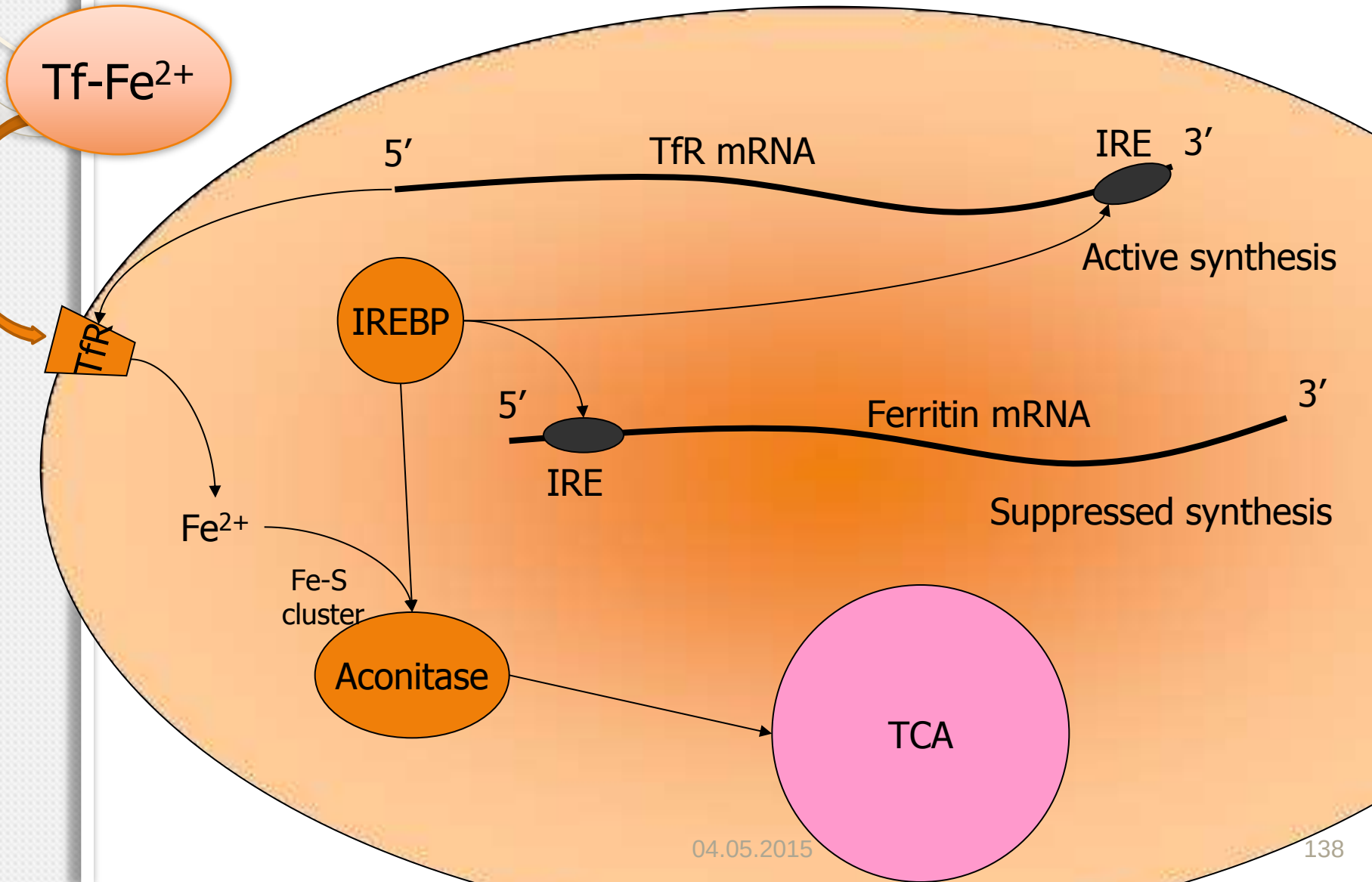


- If the supply of iron is adequate, the mRNA is rapidly degraded as a result of nuclease cleavage near the 3' end.
- If iron is scarce, a regulatory protein (called the iron response element-binding protein, or IRE-BP) binds to a sequence near the 3 end of the mRNA (the iron response element, or IRE), protecting the mRNA from nuclease cleavage.

Exlanation of 2 Different Effects of IRE

- Different locations of the IRE in the two mRNAs.
  - Repressor-binding site: IRE must located within 70 nucleotides of the 5´ cap of ferritin mRNA,
    - protein binding to the IRE blocks translation
  - In the 3´ untranslated region of transferrin receptor mRNA protects the mRNA from nuclease degradation.
    - The protein synthesis is increased.

Aconitase-IREBP




Thank you
for your attention!