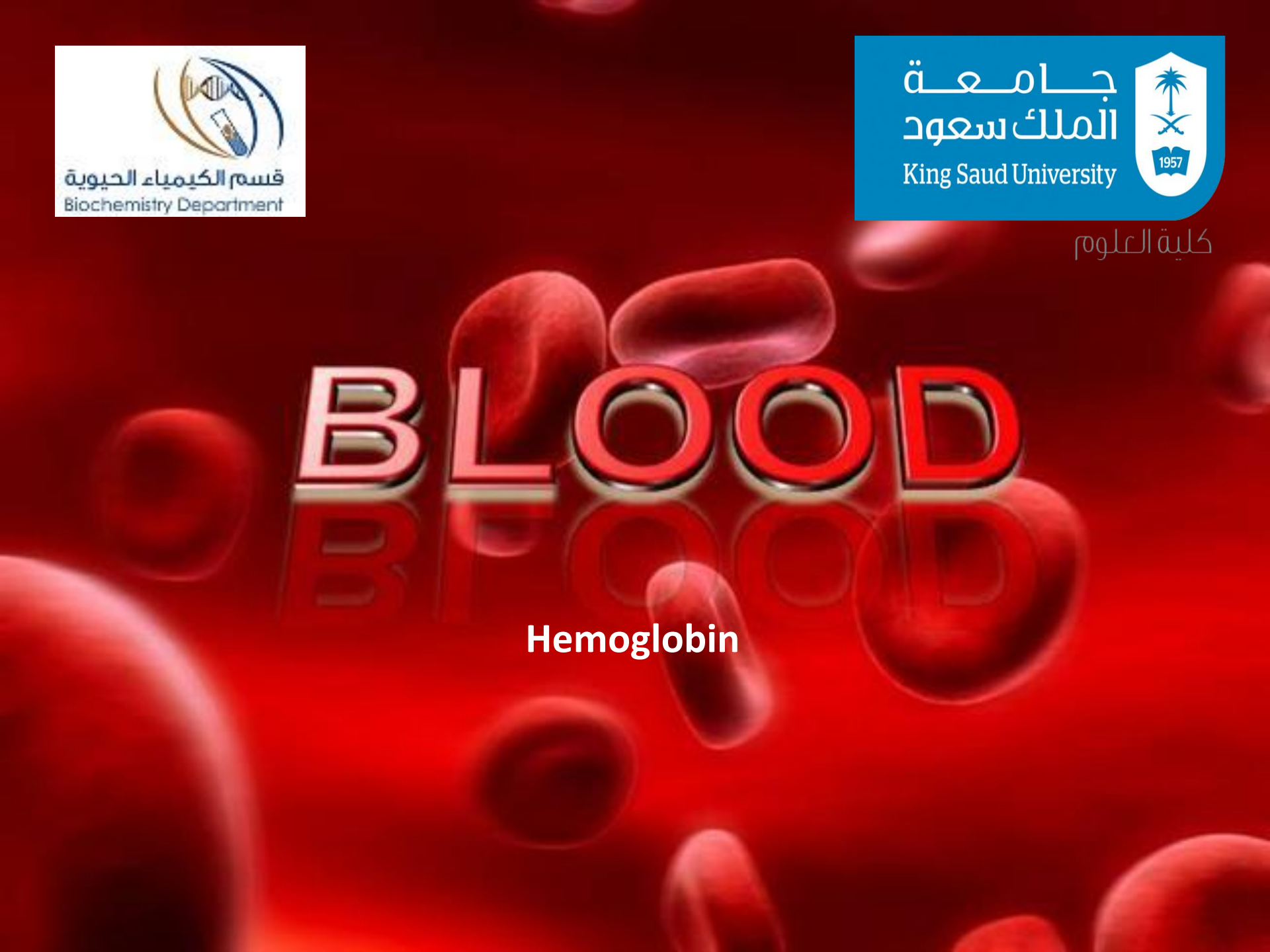
قسم الكيمياء الحيوية
Biochemistry Department

جامعة الملك سعود
King Saud University
1957
كلية العلوم

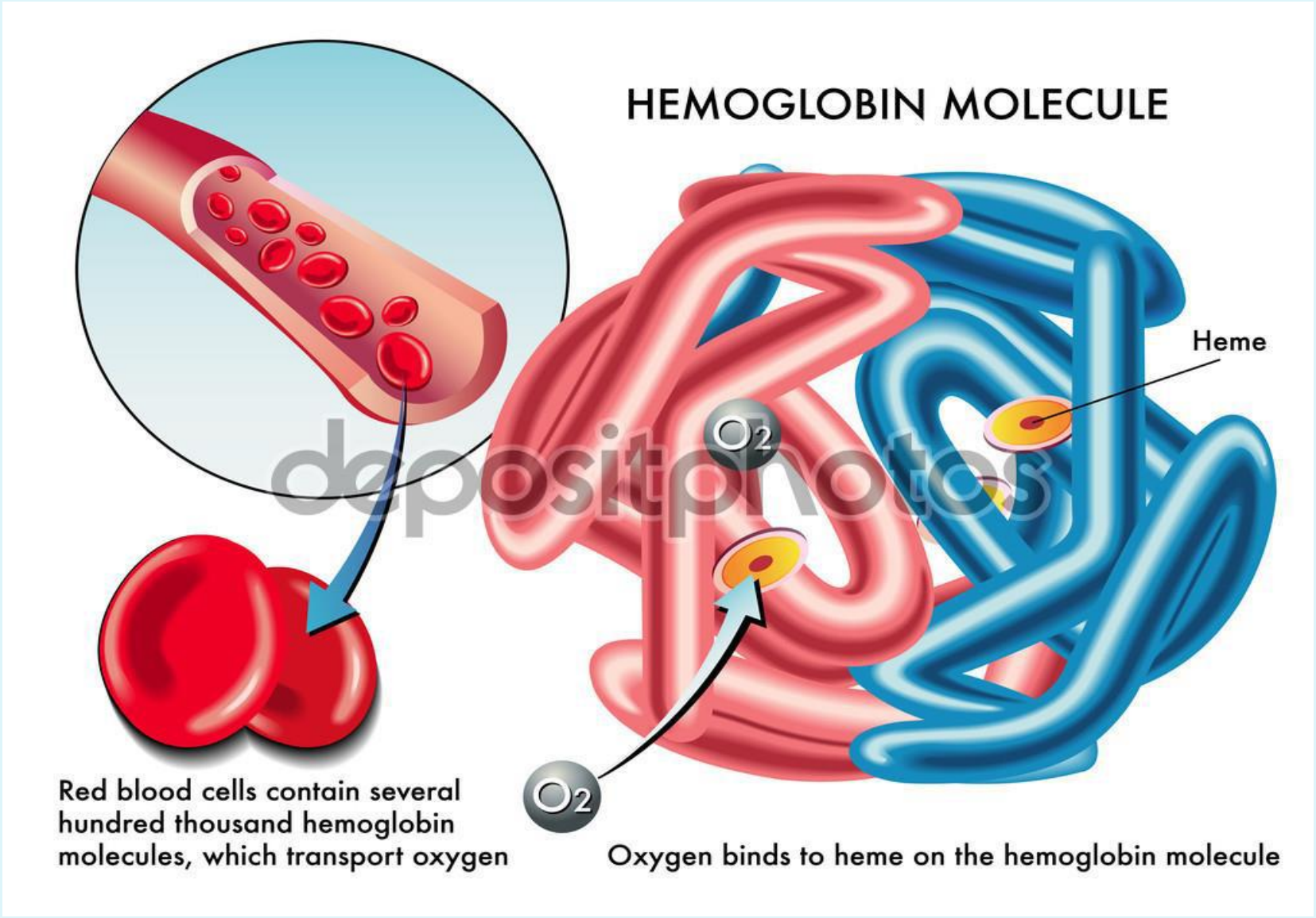


Hemoglobin

Hemoglobin

- **The most important function of the red blood cells is totransport (O2) from the lungs into the tissues, and carbon dioxide (CO2) from the tissues back into the lungs.**
- **O2 is poorly soluble in water (3.2 mL soluble in 1L blood plasma).**
- **The hemoglobin (Hb) can bind a maximum of 220 mL O2 per liter.**
- **The Hb content of blood in men, (140–180 g/L).**

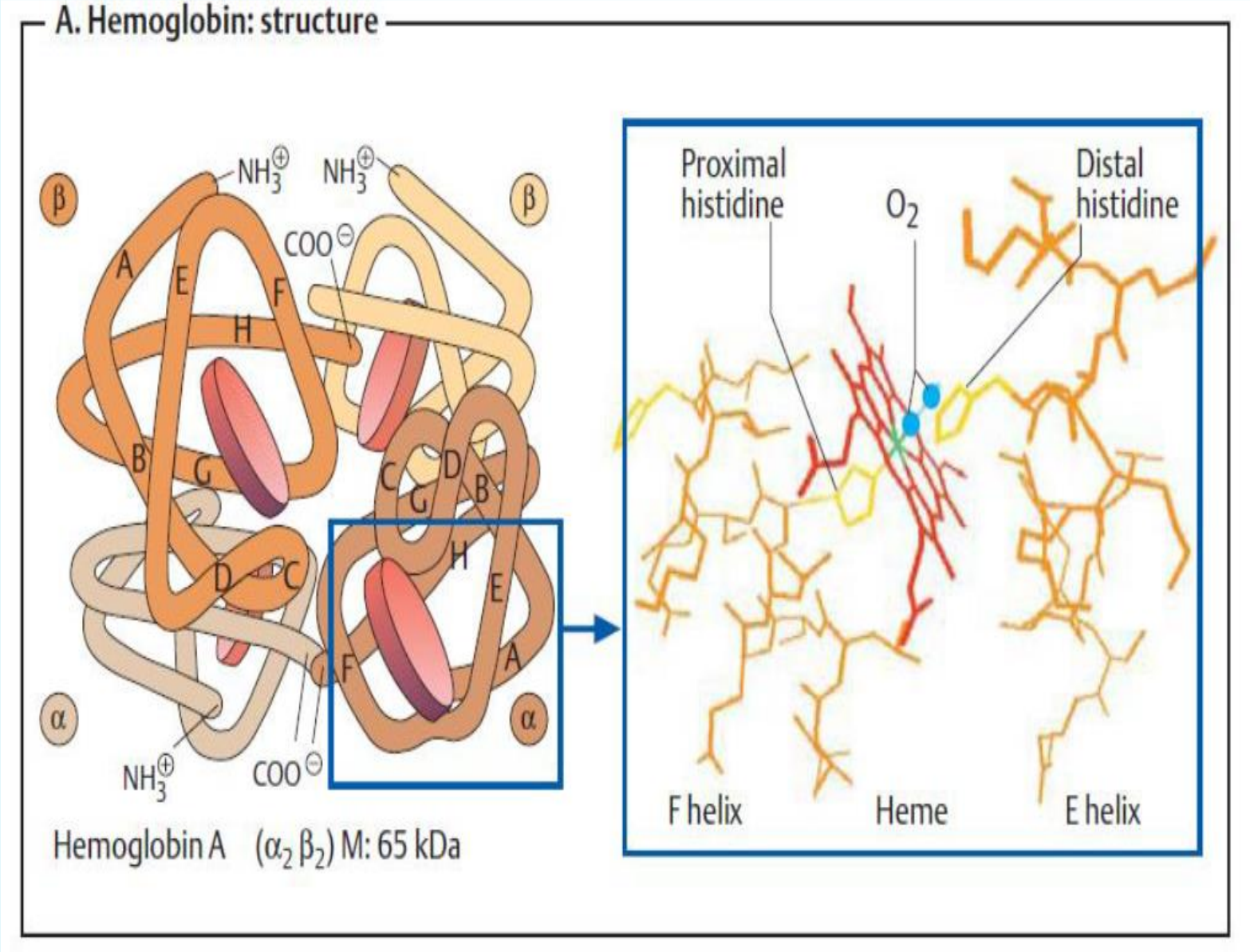
in women (120–160 g/L). Twice as high as that of the plasma proteins (50–80 g/L). Hb is therefore also responsible for the majority of the blood proteins'pH buffer capacity.

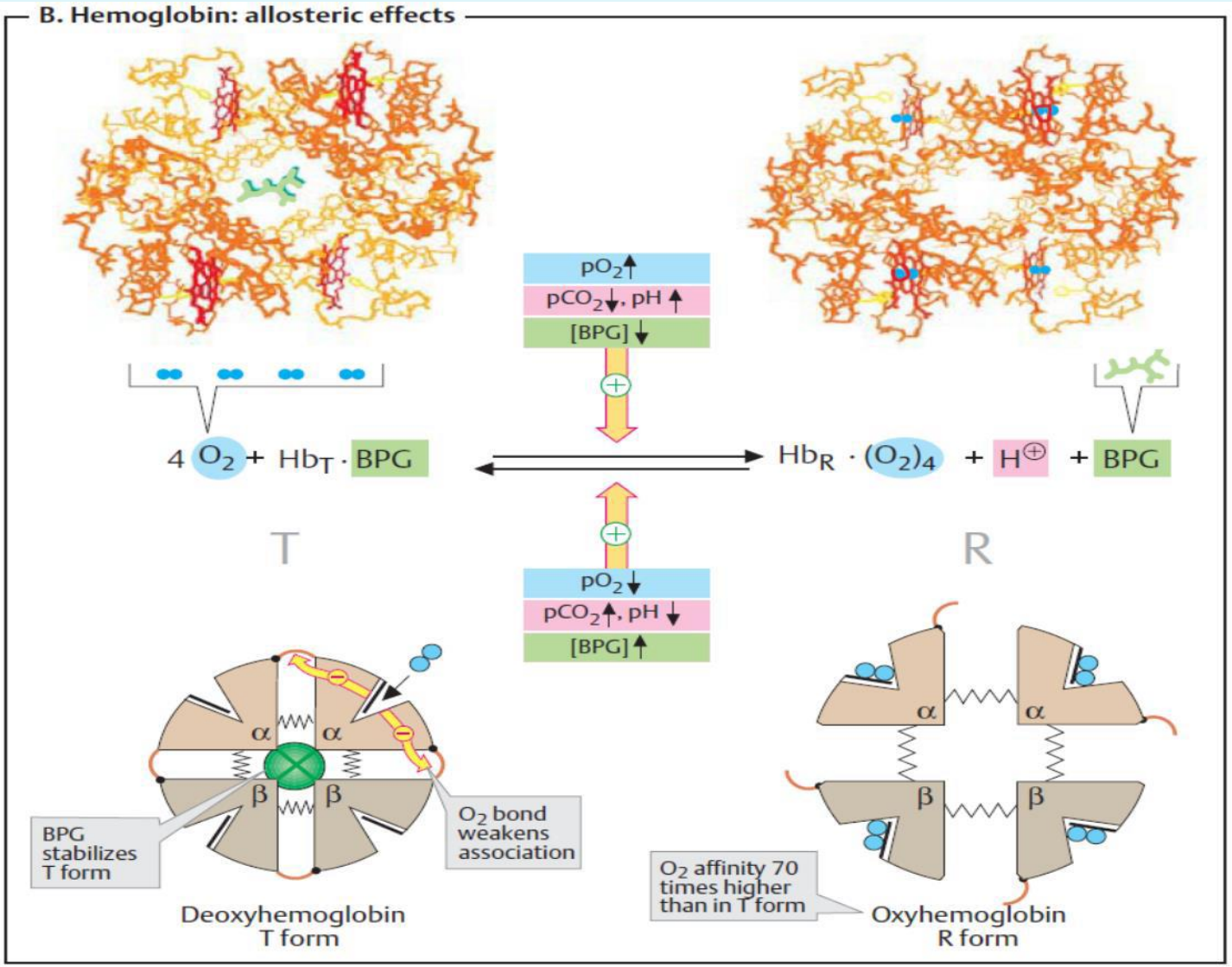

HEMOGLOBIN MOLECULE
Heme
O2
O2
Red blood cells contain several hundred thousand hemoglobin molecules, which transport oxygen
Oxygen binds to heme on the hemoglobin molecule

Hemoglobin: structure

- In adults, hemoglobin (HbA) is a heterotetramer consisting of two α-chains and two β-chains, each with masses of 16 kDa.
- The α- and β-chains have different sequences, but are similarly folded. Some 80% of the amino acid residues form α-helices, which are identified using the letters A–H.
- Each subunit carries a heme group, with a central bivalent iron ion. When O2 binds to the heme iron (Oxygenation of Hb) and when O2 is released (Deoxygenation), the oxidation stage of the iron does not change.

- **Oxidation of Fe2+ to Fe3+ only occurs occasionally. The oxidized form, methemoglobin, is then no longer able to bind O2. The proportion of Met-Hb is kept low by reduction and usually amounts to only 1–2%.**
- **Four of the six coordination sites of the iron in hemoglobin are occupied by the nitrogen atoms of the pyrrol rings, and another is occupied by a histidine residue of the globin (the proximal histidine). The iron's sixth site is coordinated with oxygen in oxyhemoglobin and with H_2O in deoxyhemoglobin.**

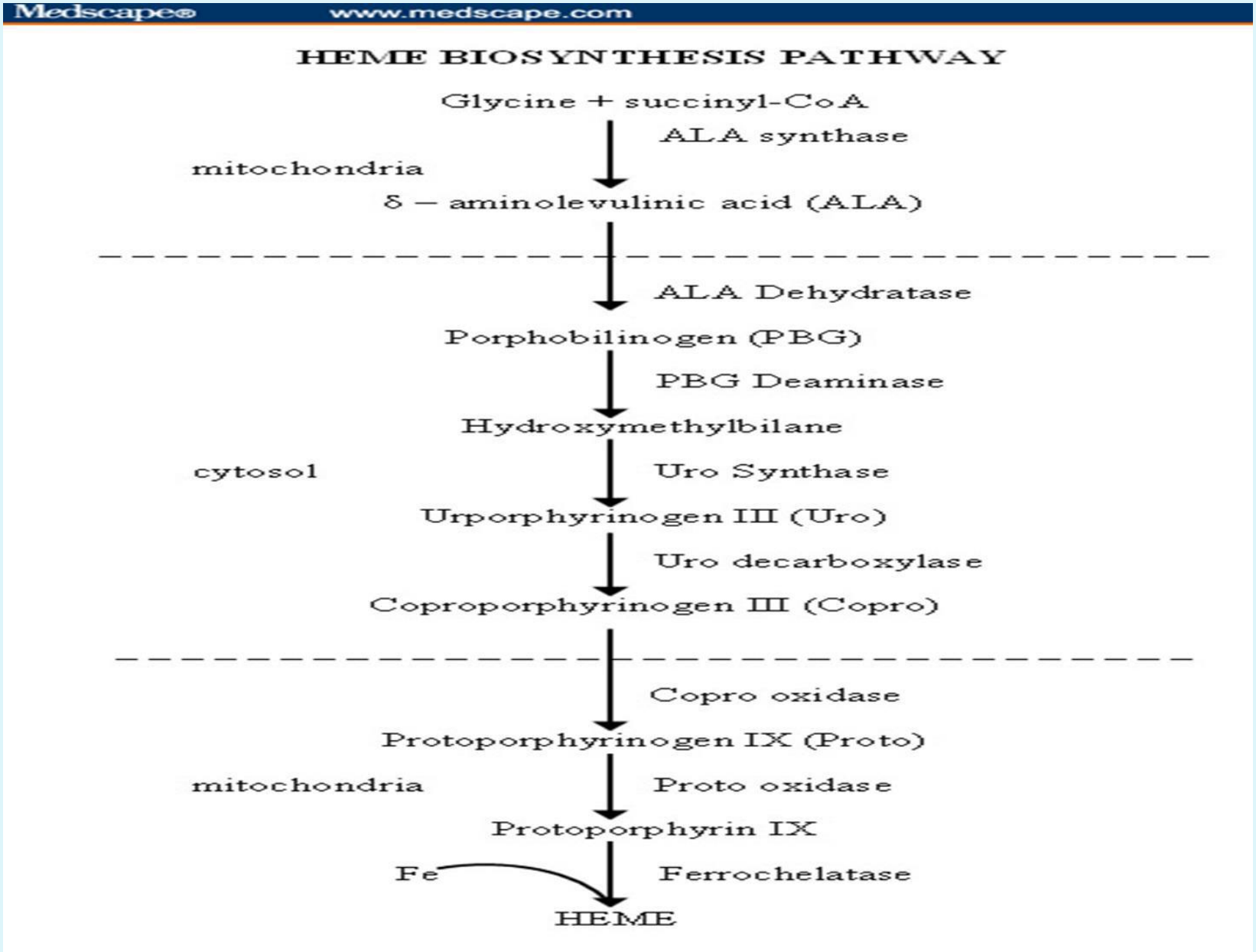

A. Hemoglobin: structure
β
β
α
α
NH3
NH3
COO
NH3
COO
A
E
F
H
B
G
C
D
C
D
B
G
H
E
F
A
Hemoglobin A (α2 β2) M: 65 kDa
Proximal histidine
O2
Distal histidine
F helix
Heme
E helix


B. Hemoglobin: allosteric effects
pO2↑
pCO2↓, pH↑
[BPG]↓
4 O2 + HbT · BPG
HbR · (O2)4 + H⊕ + BPG
T
R
pO2↓
pCO2↑, pH↓
[BPG]↑
α
α
β
β
BPG stabilizes T form
O2 bond weakens association
Deoxyhemoglobin T form
O2 affinity 70 times higher than in T form
Oxyhemoglobin R form

- **hemoglobin in adults consists of two α- and two β-chains.**
- **In addition to this main form (HbA1, α2β2), adult blood also contains small amounts of a second Form with a higher O2 affinity in which the β- chains are replaced by δ-chains (HbA2, α2δ2).**
- **Two other forms occur during embryonic and fetal development. In the first three months, embryonic hemoglobins are formed, with the structure ζ2ε2 and α2ε2.**

- **Up to the time of birth, fetal hemoglobin then predominates (HbF, $\alpha_2\gamma_2$), and it is gradually replaced by HbA during the first few months of life. Embryonic and fetal hemoglobins have higher O_2 affinities than HbA, as they have to take up oxygen from the maternal circulation.**

Hemoglobin	Structure	Stage of Life	% in Adult	% in Newborn
Gower I	$\zeta_2\varepsilon_2$	0-5 weeks embryo	None	Up to 40
Gower II	$\alpha_2\varepsilon_2$	4-13 weeks embryo	None	Up to 35
Portland	$\zeta_2\gamma_2$	4-13 weeks embryo	None	Up to 35
Fetal (F)	$\alpha_2\gamma_2$	Newborn & adult	< 1.0	80
A1	$\alpha_2\beta_2$	Newborn & adult	97	20
A2	$\alpha_2\delta_2$	Newborn & adult	2.5	< 0.5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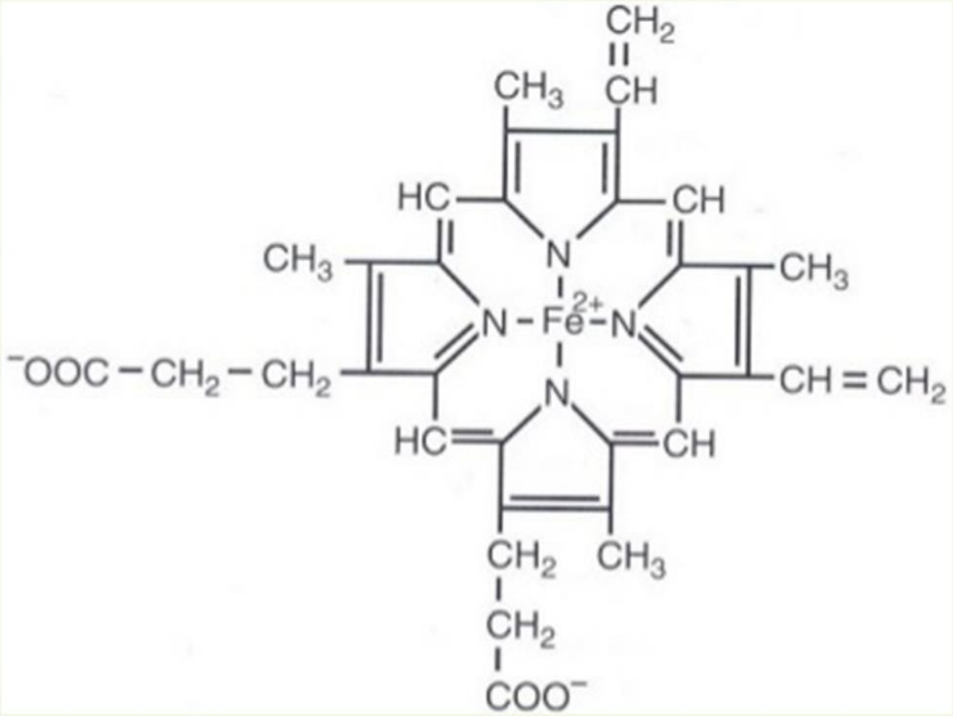
Glycine + succinyl-CoA
ALA synthase
mitochondria
δ – aminolevulinic acid (ALA)
ALA Dehydratase
Porphobilinogen (PBG)
PBG Deaminase
Hydroxymethylbilane
cytosol
Uro Synthase
Urporphyrinogen III (Uro)
Uro decarboxylase
Coproporphyrinogen III (Copro)
Copro oxidase
Protoporphyrinogen IX (Proto)
mitochondria
Proto oxidase
Protoporphyrin IX
Fe
Ferrochelatase
HEME

STRUCTURE OF HEME



Ferrous iron (Fe2+)

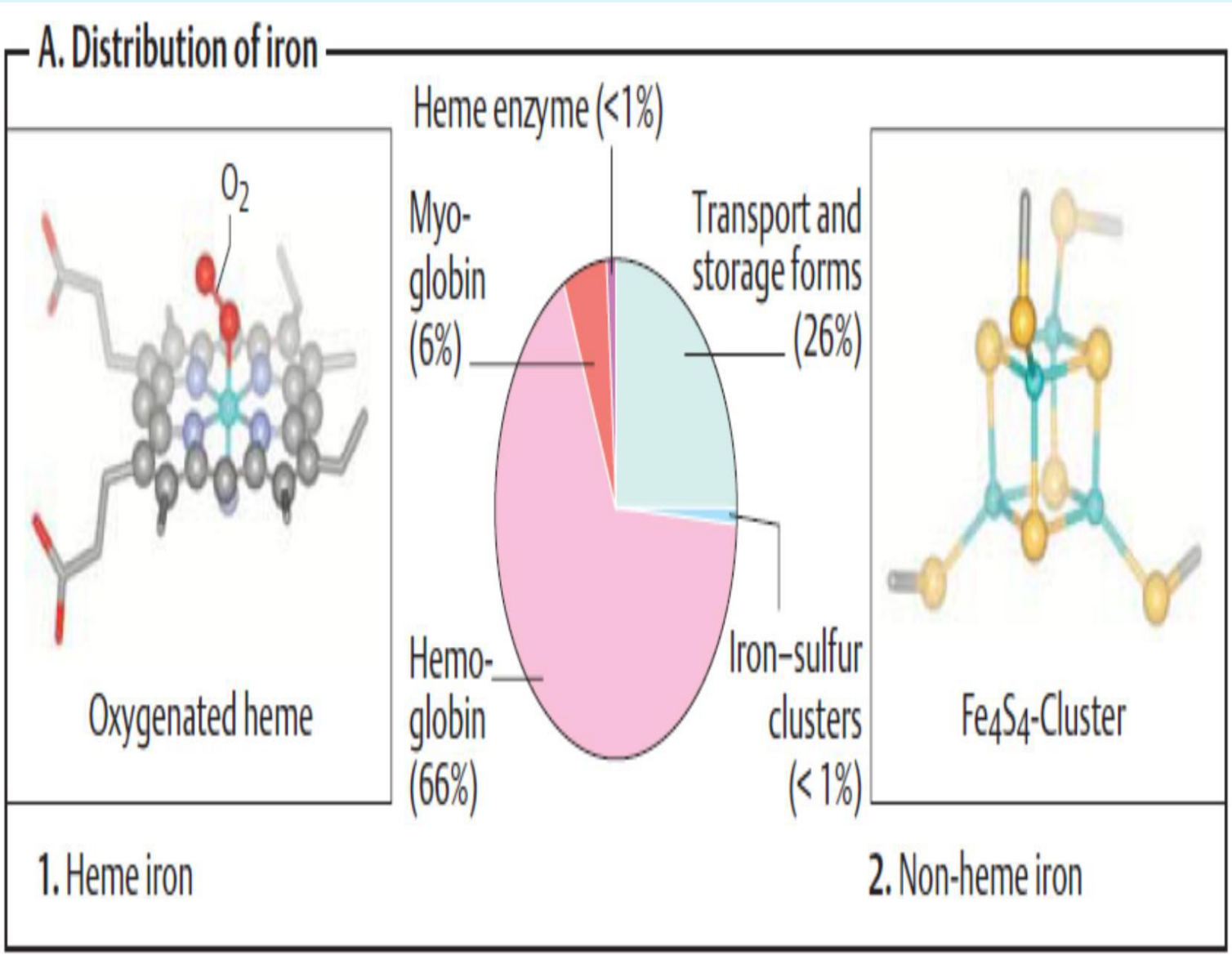
Protoporphyrin IX: contains 4 pyrrole rings linked together by methenyl bridges

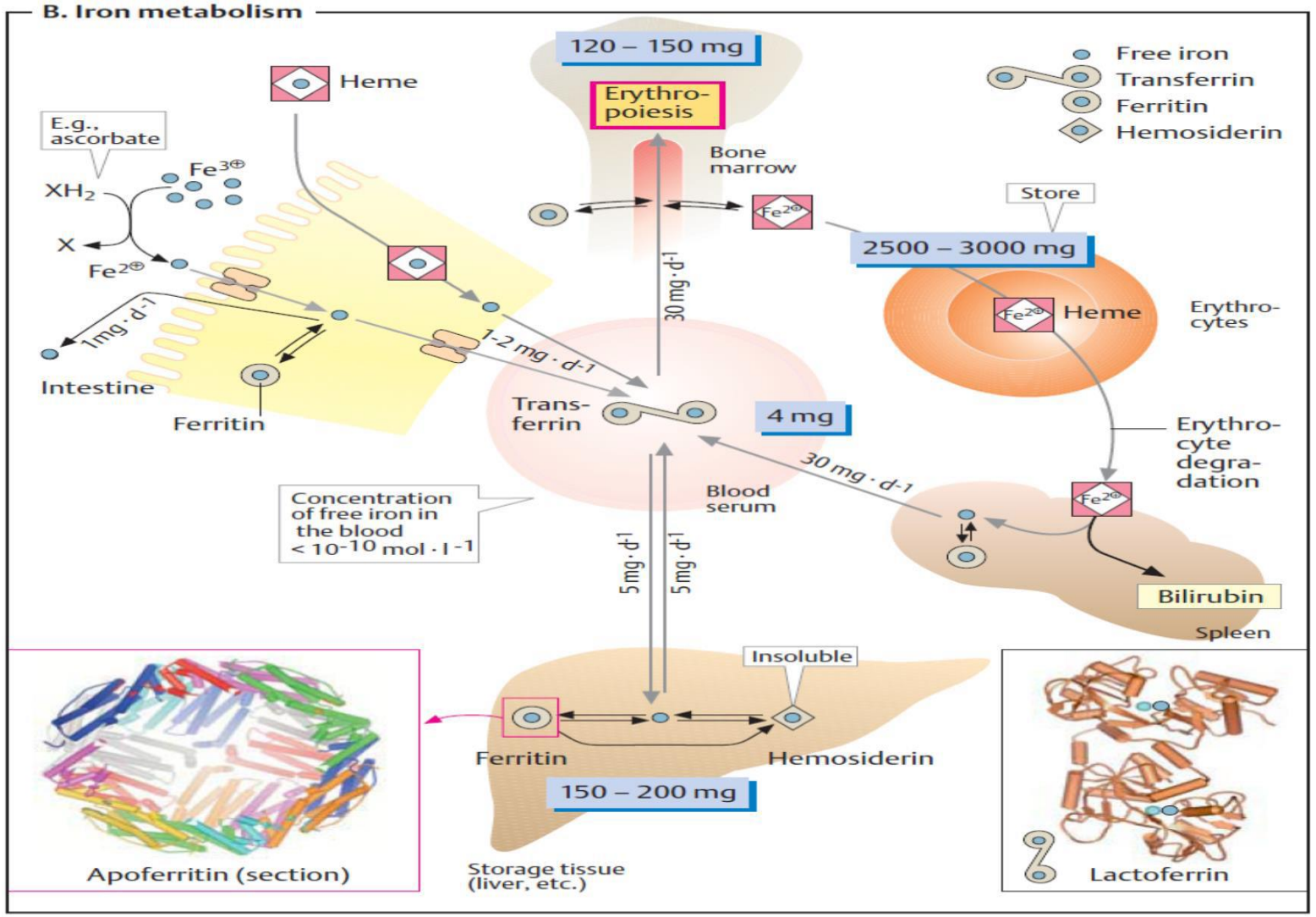
Globin moiety

Globin moiety is formed from amino acids from the body pool in amounts of about 8 g/day in the normal adult 14% of the amino acids from an average daily protein intake are used for globin formation.

Iron

The human body Contains 4–5 g iron, three-quarters of the total amount is found in heme proteins, mainly hemoglobin and myoglobin.


A. Distribution of iron
Heme enzyme (<1%)
Myo-globin (6%)
Transport and storage forms (26%)
Hemo-globin (66%)
Iron–sulfur clusters (< 1%)
O_2
Oxygenated heme
Fe_4S_4-Cluster
1. Heme iron
2. Non-heme iron


B. Iron metabolism
120 – 150 mg
Erythro-poiesis
Heme
E.g., ascorbate
XH_2
X
$Fe^{3\oplus}$
$Fe^{2\oplus}$
1 mg · d-1
Intestine
Ferritin
1-2 mg · d-1
Bone marrow
30 mg · d-1
Trans-ferrin
4 mg
Concentration of free iron in the blood < 10^{-10} mol · l -1
Blood serum
5 mg · d-1
5 mg · d-1
Free iron
Transferrin
Ferritin
Hemosiderin
Store
2500 – 3000 mg
Heme
Erythro-cytes
Erythro-cyte degra-dation
30 mg · d-1
Bilirubin
Spleen
Insoluble
Ferritin
Hemosiderin
150 – 200 mg
Storage tissue (liver, etc.)
Apoferritin (section)
Lactoferrin

- **Iron absorbed by the bowel in Fe^{2+} form. For this reason, reducing agents in food such as ascorbate (vitamin C) promote iron uptake.**
- **Fe^{2+} enters the blood, where it is bound by transferrin. Part of the iron that is taken up is stored in the bowel in the form of ferritin.**
- **Heme groups also absorbed by the small intestine.**
- **Most of the iron serves for the formation of red blood cells in the bone marrow (erythropoiesis).**
- **it is only in the final step of hem biosynthesis that Fe^{2+} is incorporated by ferrochelatase into the previously prepared tetrapyrrol framework.**

In the blood, 2.5–3.0 g of hemoglobin iron circulates as a component of the erythrocytes.

Over the course of several months, the flexibility of the red blood cells declines due to damage to the membrane and cytoskeleton.

Old erythrocytes are taken up by macrophages in the spleen and other organs and broken down. The organic part of the heme is oxidized into bilirubin, while the iron returns to the plasma pool. The quantity of heme iron recycled per day is much larger than the amount absorbed by the intestines.

Transferrin: **A β-globulin (80 kDa), serves to transport iron in the blood. consists of two similar domains, each of which binds an Fe2+ ion very tightly. Similar iron transport proteins are found in secretions such as saliva, tears, and milk; these are known as lactoferrins. Transferrin and the lactoferrins maintain the concentration of free iron in body fluids at values below 10–10 mol /L. This low level prevents bacteria that require free iron as an essential growth factor from proliferating in the body. Transferrin and the lactoferrins are taken up into cells by receptor-mediated endocytosis.**

Ferritin:

Excess iron stored in the liver and other organs. Consists of 24 subunits and has the shape of a hollow sphere. It takes up Fe^{2+} ions, which in the process are oxidized to Fe^{3+} and then deposited in the interior of the sphere as ferri-hydrate. Each ferritin molecule is capable of storing several thousand iron ions in this way. In addition to ferritin, there is another storage form, hemosiderin.

Disturbances of the iron metabolism:

Iron deficiency is usually due to blood loss, or inadequate iron uptake. During pregnancy, increased demand can also cause iron deficiency states. In severe cases reduced hemoglobin synthesis can lead to anemia ("iron deficiency anemia"). In these patients, the erythrocytes are smaller and have less hemoglobin. As their membrane is also altered, they are prematurely eliminated in the spleen.

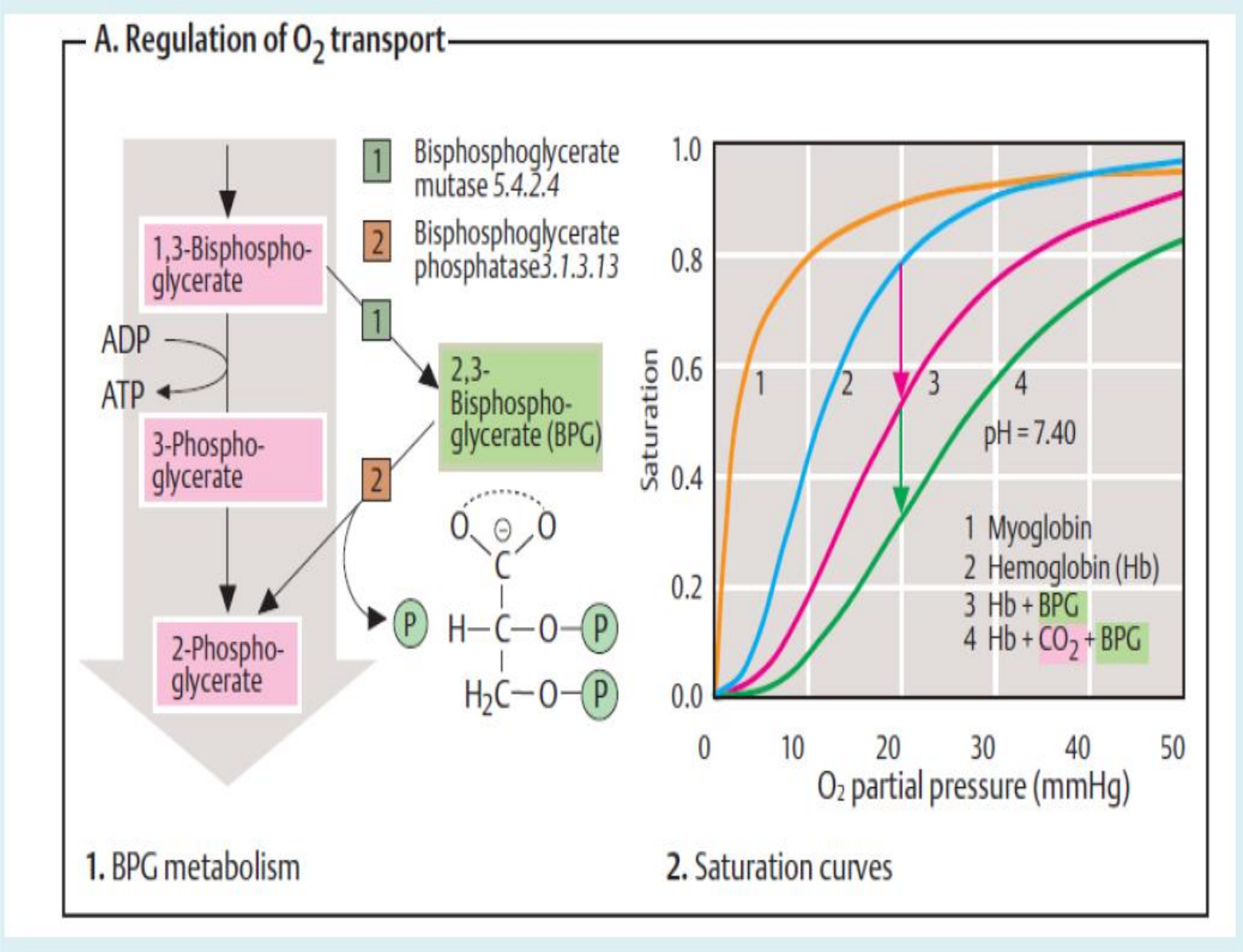
Disturbances resulting from raised iron concentrations are less frequent. Known as hemochromatoses, these conditions can have genetic causes, or may be due to repeated administration of blood transfusions. As the body has practically no means of excreting iron, more and more stored iron is deposited in the organs leading to severe disturbances of organ function.

Gas transport

Most tissues are constantly dependent on a supply of molecular oxygen (O_2) to maintain their oxidative metabolism.

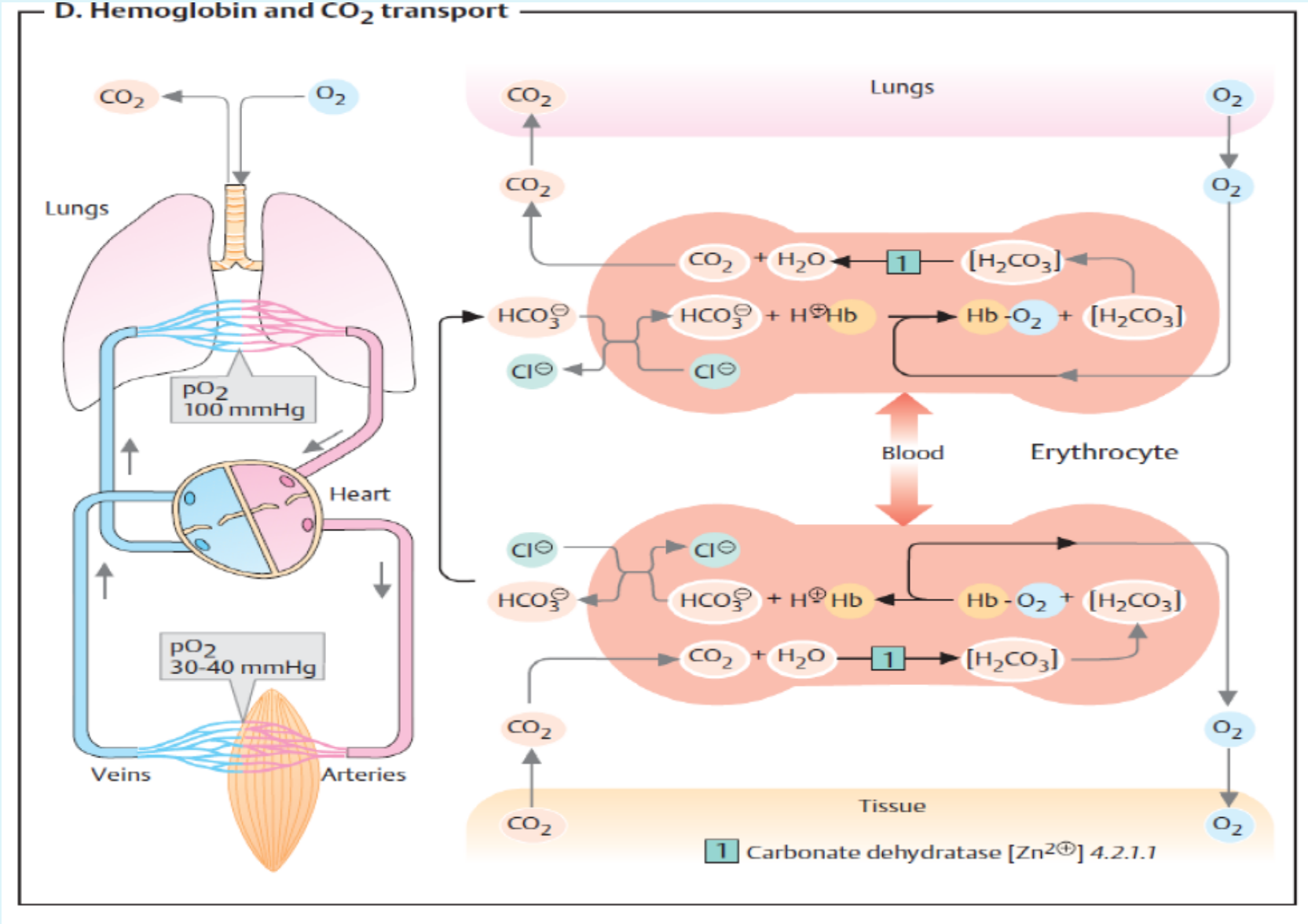
Regulation of O_2 transport

The effectors of Hb include oxygen, which as a positive homotropic effector promotes its own binding. The O_2 saturation curve of hemoglobin is therefore markedly sigmoidal in shape. The non-sigmoidal saturation curve of the muscular protein myoglobin.


A. Regulation of O_2 transport
1 Bisphosphoglycerate mutase 5.4.2.4
2 Bisphosphoglycerate phosphatase 3.1.3.13
1,3-Bisphospho-glycerate
ADP
ATP
3-Phospho-glycerate
2-Phospho-glycerate
2,3-Bisphospho-glycerate (BPG)
1. BPG metabolism
Saturation
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
O_2 partial pressure (mmHg)
pH = 7.40
1 Myoglobin
2 Hemoglobin (Hb)
3 Hb + BPG
4 Hb + CO_2 + BPG
2. Saturation curves

CO_2, H^+, and 2,3 bisphosphoglycerate (BPG) act as heterotropic effectors of hemoglobin. BPG is synthesized from 1,3–bisphosphoglycerate, an intermediate of, and it can be returned to glycolysis again by break down into 2–phosphoglycerate, with loss of an ATP. BPG binds selectively to deoxy Hb, thereby increasing its amount of equilibrium. The result is increased O_2 release at constant pO_2. In the diagram, this corresponds to a right shift of the saturation curve. CO_2 and H+ act in the same direction as BPG.

Their influence on the position of the curve has long been known as the Bohr effect. The effects of CO_2 and BPG are additive. In the presence of both effectors, the saturation curve of isolated Hb is similar to that of whole blood.


D. Hemoglobin and CO_2 transport
CO_2
O_2
Lungs
pO_2 100 mmHg
Heart
pO_2 30-40 mmHg
Veins
Arteries
Lungs
CO_2 + H_2O
1
$[H_2CO_3]$
HCO_3^-
HCO_3^- + H^+Hb
Hb - O_2 + $[H_2CO_3]$
Cl^-
Blood
Erythrocyte
Tissue
1 Carbonate dehydratase [Zn^{2+}] 4.2.1.1

Hemoglobin and CO_2 transport

- **Some 5% of the CO_2 arising in the tissues is covalently bound to the N terminus of hemoglobin and transported as carbaminohemoglobin.**
- **About 90% of the CO_2 is first converted in the periphery into hydrogen carbonate (HCO_3^-), which is more soluble. In the lungs, CO_2 is regenerated again from HCO_3^- and can then be exhaled.**
- **These two processes are coupled to the oxygenation and deoxygenation of Hb. Deoxy Hb is a stronger base than oxy Hb.**

- **It therefore binds additional protons (about 0.7 H^+ per tetramer), which promotes the formation of HCO_3 from CO_2 in the peripheral tissues. The resulting HCO_3 is released into the plasma via an antiporter in the erythrocyte membrane in exchange for Cl^-, and passes from the plasma to the lungs.**

- **In the lungs, deoxy Hb is oxygenated and releases protons. The protons shift the HCO_3/CO_2 equilibrium to the left and thereby promote CO_2 release. O_2 binding to Hb is regulated by H^+ ions (i.e., by the pH value) via the same mechanism.**
- **High concentrations of CO_2 such as those in tissues with intensive metabolism locally increase the H^+ concentration and thereby reduce hemoglobin's O_2 affinity (Bohr effect). This leads to increased O_2 release and thus to an improved oxygen supply.**

- **The adjustment of the equilibrium between CO_2 and HCO_3 is relatively slow in the uncatalyzed state.**
- **It is therefore accelerated in the erythrocytes by carbonate dehydratase (carbonic anhydrase), an enzyme that occurs in high concentrations in the erythrocytes.**