

BIOCHEMISTRY AND MOLECULAR BIOLOGY

Problem Unit Five 1999/2000

Blood and Hemoglobin

**Copyright 1999, E.C. Niederhoffer. All Rights Reserved.
All trademarks and copyrights are the property of their respective owners.**

- Module 1:** Blood Clotting and Heme Metabolism
- Module 2:** Hemoglobins and Red Cells
- Module 3:** O₂ Transport and O₂-Hemoglobin Affinity
- Module 4:** Modes of CO₂ Transport and CO₂-O₂ Interrelationship in Transport

Faculty: Dr. Ramesh Gupta
Biochemistry & Molecular Biology
Office: 210 Neckers Bldg.
email: rgupta@som.siu.edu
Telephone: 453-6466

Estimated Work Time: 40 hours.

Learning Resources:

A. This study guide is provided in two forms: printed and electronic (produced by Dr. E.C. Niederhoffer, Biochemistry and Molecular Biology). ***It is best viewed in electronic form as a pdf file which can be read on your computer using Adobe Acrobat Reader.*** See [Appendix I](#) for an introduction on how to view a pdf file. The pdf file can be downloaded from the biochemistry server (<http://www.siu.edu/departments/biochem>) and Acrobat Reader can be downloaded free from Adobe's web page (<http://www.adobe.com/acrobat>). They should also be installed on the student computers. There are a number of advantages to using the electronic version including color, a hypertext index, and hypertext links within the text. Hypertext links in the text body are in blue underlined characters ([such as this](#)). Clicking on these will lead to a jump to the linked material for further details. The destination material is indicated by red underlined characters ([such as this](#)). (Clicking on the black double arrows in the menu bar will allow you to "hyper-jump" back and forth.)

The study guide for heme metabolism is condensed and one of the better resources for this subject area. Also note that there is an appendix for Module 2.

This and other study guides are provided to help you focus on the topics that are important in the biochemistry curriculum. These are designed to guide your studying and provide information that may not be readily available in other resources. They are not designed to replace textbooks, and are not intended to be complete. They are guides for starting your reading and reviewing the material at a later date. Some of the terms in Nomenclature and Vocabulary, and Keywords are linked to their reference in the Study Guide.

B. Textbooks:

1. Champ & Harvey, Lippincott's Illustrated Reviews: Biochemistry, 2nd ed. ('94), Lippincott. Efficient presentation of basic principles.
2. Murray et al., Harper's Biochemistry, (24th ed.) ('96),

Appleton & Lange. An excellent review text for examinations.

3. Devlin, Textbook of Biochemistry with Clinical Correlations, 4th ed. ('97), Wiley-Liss. Core text for Biochemistry & Molecular Biology.
4. Marks, Marks, and Smith, Basic Medical Biochemistry: A Clinical Approach, ('96), Williams & Wilkins. Good basic presentation with clinical relevance.

Most texts of biochemistry have sections on blood and hemoglobin. The content of the subject is much the same from text to text; the differences are basically in style and rigor. The Study Guide, Pretest, and Post Test in the Problem Unit will set the level of rigor expected of you. Read the sections on blood and hemoglobin in several texts. What differences there will be between these texts and the Study Guide will be helpful to you in gaining perspective on the subject. Additional material can be found on the web at the National Institutes of Health (<http://www.nih.gov>), the National Library of Medicine (<http://www.nlm.nih.gov>), and the free MEDLINE PubMed Search system at the National Library of Medicine (<http://www3.ncbi.nlm.nih.gov/PubMed/>).

You may find worthwhile reading in some of the more popular journals and review series (see also the searchable [SIU-SOM database](#)). These resources typically contain specific articles involving blood and hemoglobin. Suggestions for journals include *American Family Physician*, *Journal of Biological Chemistry*, *Nature*, *Science*, and *Scientific American* (and SA's *Science and Medicine*). Excellent reviews may be found in the *Annual Review of Biochemistry*, *Cell and Developmental Biology*, *Genetics*, *Medicine*, and *Microbiology*.

C. Practice Exams.

Practice exams for Module 1 and Module 4 are included.

D. Lecture/Discussions

All of the major points with emphasis on the more difficult concepts will be presented in lectures.

Evaluation Criteria and Testing Information:

These modules will be examined as part of Problem Unit 5. Answers to questions, discussions, solving of problems, and definitions will be judged against the learning resources. A written secure examination covering the objectives in Problem Unit 5 will be scheduled. The pass level is 70%. The examination will be confidential and will not be returned to the students. Students can make arrangements for

biliverdin

urobilinogens

bilirubin

4. Answer questions about the breakdown of hemoglobin such as:
 - a. In what organ are old RBC's removed from the blood and phagocytized?
 - b. How is free hemoglobin transported to the liver?
 - c. What are the substrates and products of heme oxygenase?
 - d. If heme should appear in plasma, how would it be transported to the liver?
 - e. What is the purpose of the conversion of bilirubin into the diglycoside of D-glucuronic acid?
 - f. What is meant by "indirect" bilirubin and "direct" bilirubin?
 - g. How is bilirubin related to jaundice?
 - h. Why is unconjugated bilirubin considered dangerous?
5. Describe the three mechanisms by which hemostasis is achieved in normal individuals.
6. Response time in blood clotting is an important aspect of this mechanism of defense. Explain how the activation of a series of specific proteolytic enzymes of an enzymatic cascade amplifies a small initial signal to achieve the rapid and timely formation of a clot.
7. Describe the molecular events involved in the conversion of fibrinogen to fibrin. When you have accomplished this goal, you should be able to answer questions such as:
 - a. What is the subunit organization of fibrinogen? of fibrin?
 - b. How does the structure of fibrin monomers differ from fibrinogen?
 - c. Why is fibrinogen soluble and fibrin insoluble?
 - d. What is a fibrin polymer clot?
 - e. Explain how such agents as oxalate, EDTA and citrate inhibit clot formation.
 - f. What keeps fibrinogen from spontaneously aggregating in the blood?
8. A primary event in clotting is the conversion of prothrombin to the active proteolytic enzyme, thrombin. What is the composition of the complex which serves to catalyze this conversion and what roles do each of the components serve in the process.

9. How is the fibrin polymer clot stabilized? What enzyme is involved in this process? Which amino acids are involved in the cross-linking reaction and which subunits of fibrin are cross-linked?
10. Describe the role of vitamin K in blood clotting.
 - a. What clotting factors require vitamin K?
 - b. How do dicoumarol, coumarin, and the rat poison, warfarin, prevent clotting?
 - c. Draw the structure of γ -carboxyglutamic acid.
 - d. Why is the presence of this posttranslationally modified amino acid important?
11. What roles do Ca^{2+} and negatively charged phospholipids play in clotting?
12. Antithrombin III is a protein inhibitor of thrombin.
 - a. Explain why it is necessary to have such an inhibitor.
 - b. What is heparin and how is it involved with antithrombin III to regulate blood clotting?
 - c. How is it possible for clotting to occur in the presence of antithrombin III?
 - d. Under what conditions does a decreased level or absence of antithrombin III occur and what is the medical consequence of the decrease or absence of antithrombin III?
13. Describe the mechanism of clot dissolution.
14. Describe the mechanism of platelet activation in bloodclotting.
15. Explain why blood coagulation uses a complex cascade of enzymes to achieve red thrombi formation.
16. How does aspirin affect clotting?
17. Be able to define, and use correctly each of the terms in the [NOMENCLATURE and VOCABULARY](#) and [Key Word](#) list.
18. After reading a passage from a medical journal or textbook on porphyrin metabolism or blood clotting (which may be either a clinical investigation or a biochemical description) answer questions about the passage (which may involve the drawing of inferences or conclusions) or use the information given to solve a problem.

Nomenclature and Vocabulary:

<u>α1-Antitrypsin</u>	<u>Albumin</u>
<u>Anti-trypsin</u>	<u>Antithrombin III</u>
<u>Barbiturates</u>	<u>Bilirubin diglucuronide</u>
<u>Bilirubin</u>	<u>Biliverdin</u>
<u>Carbon monoxide</u>	<u>Ceruloplasmin (ferroxidase I)</u>
<u>Collagen</u>	<u>Common pathway</u>
<u>Coproporphyrinogen I and III</u>	<u>Cyclooxygenase</u>
<u>δ-Aminolevulinate</u>	<u>Dicoumarol</u>
<u>Enzymatic cascade</u>	<u>Extrinsic pathway</u>
<u>Ferritin</u>	<u>Ferrochelatae</u>
<u>Fibrin</u>	<u>Fibrinogen</u>
<u>γ-Carboxyglutamate</u>	<u>Haptoglobin</u>
<u>Heme oxidase</u>	<u>Hematin</u>
<u>Heme</u>	<u>Hemopexin</u>
<u>Hemophilia</u>	<u>Hemostasis</u>
<u>Heparin</u>	<u>Intrinsic pathway</u>
<u>Jaundice</u>	<u>Lead poisoning</u>
<u>Plasma</u>	<u>Plasmin</u>
<u>Plasminogen</u>	<u>Platelet plug</u>
<u>Porphobilinogen</u>	<u>Porphyria</u>
<u>Proenzyme</u>	<u>Prothrombin</u>
<u>Protoporphyrin III (IX)</u>	<u>Red thrombus</u>
<u>Thrombin</u>	<u>Thromboplastin (lipoprotein tissue factor)</u>
<u>Thrombus</u>	<u>Transferrin</u>
<u>Transglutaminase</u>	<u>UDP-glucuronic acid</u>
<u>Urobilin</u>	<u>Urobilinogens</u>
<u>Urokinase</u>	<u>Uroporphyrinogen I and III</u>
<u>vWF</u>	<u>Van den Bergh reaction</u>
<u>von Willebrand's disease</u>	<u>Vitamin K</u>
<u>Warfarin</u>	<u>White thrombus</u>
<u>Zymogen</u>	

Key Words:

Antithrombus	Bilirubin
Biochemistry	Blood
Blood coagulation	Blood proteins
Erythrocytes	Fibrin
Fibrinogen	Heme
Hemoglobins	Hemostasis
Jaundice	Proteins
Thrombin	Vitamin K

STUDY GUIDE-1

Blood Clotting

I. **Hemostasis:** The cessation of bleeding.

Primary hemostasis: **Platelet plug (white thrombi)** formation at sites of damage. Initiation of white thrombi formation occurs with platelet adhesion to a site of damage. Platelet binding takes place between components of the vascular subendothelium and platelet surface receptors; **collagen** and glycoprotein (GpIb) receptors on the platelets. Platelet binding is enhanced by glycoprotein **von Willebrand factor, (vWF)** binding to GpIb. Aggregation of additional platelets is mediated by fibrinogen binding to platelet glycoproteins receptors IIb and IIIa.

Platelet activation: External signals (collagen, epinephrine, thrombin) bind to platelet surface receptors. Signal binding triggers transmembrane signaling and a signal transduction pathway. Arachidonic acid is released from membrane phospholipids and converted to thromboxane A₂ signal path continues ultimately stimulating platelet granular content release. Originally disc shaped, bound platelets upon activation change shape swelling into spiky spheres. Platelet granule contents are secreted into the **plasma**. α -granules release von Willebrand factor, fibronectin, calcium ions (factor IV) and thrombospondin. Dense granules release calcium, serotonin, and ADP. Lysosomes release a heparin-cleaving enzyme and endoglycosidases. Serotonin promotes vasoconstriction, adenosine diphosphate (ADP) signal modifies platelet surface promoting platelet aggregation. Released calcium is crucial for propagation of clotting signal in secondary hemostasis. White thrombi plugs are effective for the initiation of coagulation even in arteries where blood flow is rapid. White thrombi are very important in stopping blood loss in capillaries and small vessels.

II. **Secondary homeostasis:** The plasma coagulation system resulting in **fibrin** formation. Fibrin traps formed cells including red blood cells hence **red thrombi**. Red thrombi can form at site of platelet plug or where blood flow has slowed (even without damage). Clots in arteries; areas of high flow rate generally are white thrombi as little fibrin and few red cells are trapped. Formation of fibrin requires a cascade of **proteolytic** reactions involving nearly 20 substances, many of which are liver-synthesized plasma glycoproteins. All, but two of the factors have a

common name and a roman numeral designation for historical reasons.

- Seven are **zymogens** (inactive forms) of **serine proteases**.
- **Accessory factors** or **cofactors** which are activated by the serine protease's enhance the rate of activation of some of the zymogens.
- For both factors and accessory factors the active form is usually designated by the subscript a.

Conversion of **fibrinogen** to fibrin is catalyzed by **thrombin**. A series of four reactions involving two pathways that merge into a final **common pathway** result in the activation of thrombin from **prothrombin** (Figure 1).

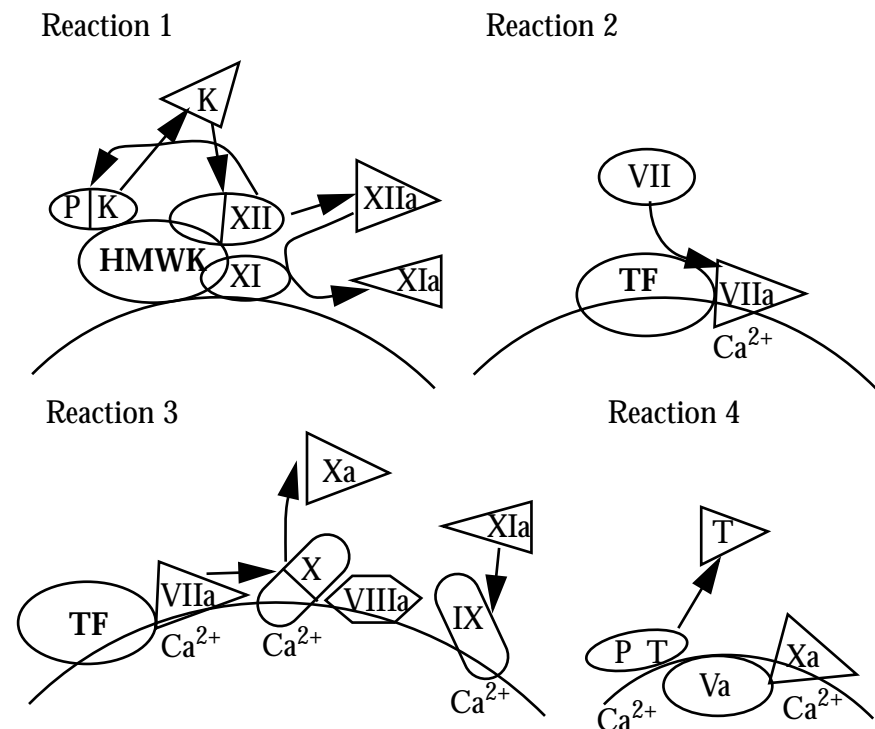


Figure 1. The four coagulation pathway reactions prior to the final common pathway; see text for details and Figure 2. Abbreviations are HMWK, high-molecular-weight kininogen; PK, prekallikrein; K, kallikrein; TF, tissue factor; PT, prothrombin; T, thrombin.

(1) Reactions initiating the **intrinsic pathway**; Factor XII (Hageman factor), prekallikrein (PK), and high-molecular weight kininogen (HMWK) complex on collagen exposed in damaged or abnormal vessels. In the presence of HMWK factor XII is slowly activated. Factor XIIa converts PK to kallikrein and plasma thromboplastin ante-

cedent (PTA or factor XI) to factor XIa. Kallikrein catalyzes the formation of more XIIa.

(2) Reaction initiating the **extrinsic pathway** dependent on **tissue-factor**. Tissue-factor forms an activation complex with factor VII in appropriate calcium phospholipid environment.

(3) From the intrinsic pathway factor XIa catalyzes the proteolytic activation of factor IX (Christmas factor) and factor IXa slowly activates factor X (Stuart factor) in the presence of calcium and phospholipids by cleaving the same Arg-Ile peptide bond as does factor VIIa of the extrinsic pathway. The activation of factor X can be accelerated 500-fold by factor VIIIa (Antihemophilic factor).

(4) Factor Xa represents the merger of the extrinsic and intrinsic coagulation pathways in the final common pathway. Factor Xa by itself is slow to cleave prothrombin. Factor Va (proaccelerin) accelerates factor Xa activity (20,000-fold).

Factors II (prothrombin), IX, X, and VII (and protein C) contain **Gla residues**; **γ -carboxyglutamate residues**. These residues are glutamate residues **posttranslationally** modified to contain an extra carboxyl group by a carboxylation reaction (that indirectly requires **vitamin K**) that occurs in the liver the site of synthesis of all of the coagulation factors. The Gla residues concentrate negative charges that promotes association with the calcium/phospholipid environment in white thrombi. Compounds that chelate divalent cations such as Ca^{2+} inhibit clot formation by blocking binding of Gla containing factors to the forming clot; chelators include **EDTA, citrate and oxalate**.

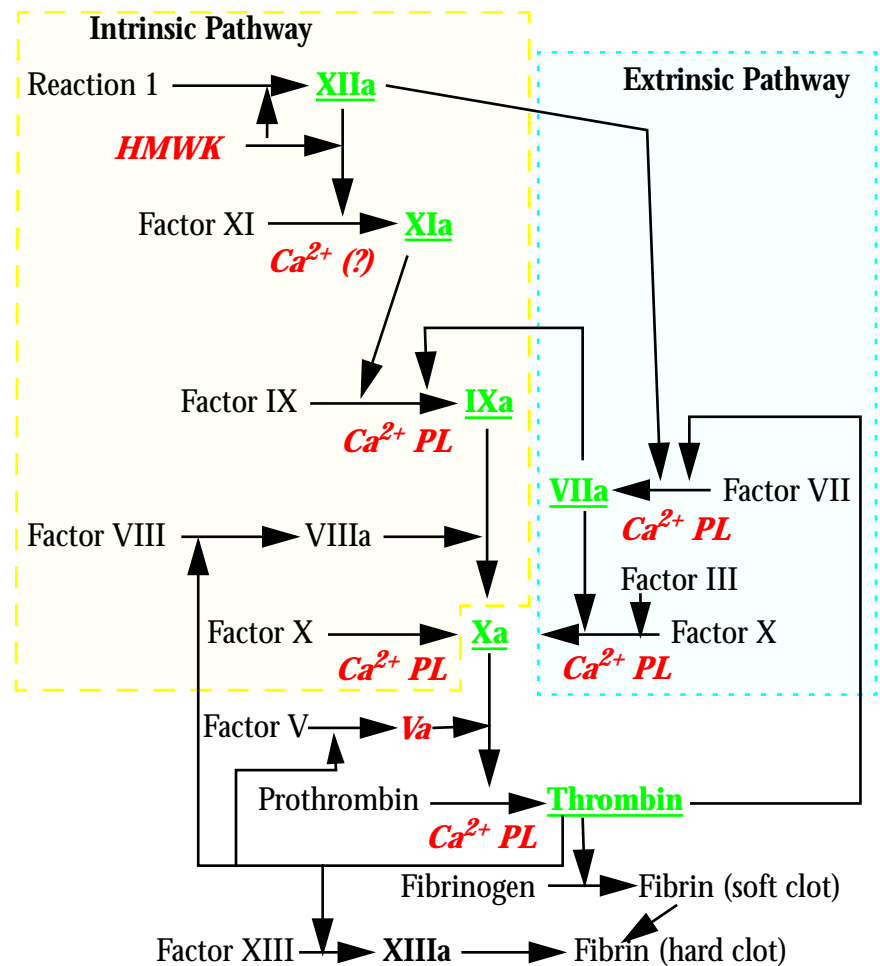


Figure 2. The blood clotting cascade in humans. The active factors which are serine proteases, are underlined in bold green text. Active accessory factors, including calcium and membrane phospholipids (PL), are indicated in bold italic red text. Inactive factors are in plain text. Initiation of the intrinsic pathway (Reaction 1) is shown in detail in Figure 1. Abbreviations are HMWK, high-molecular-weight kininogen; PL, phospholipid.

Thrombin's **serine protease** activity cleaves Arginine-Glycine (Arg-Gly) peptide bonds in a number of proteins. Thrombin plays a key role in clot formation and in clot dissolution.

- Thrombin cleaves four Arg-Gly peptide bonds in human fibrinogen. This results in the loss of about 2% of the original molecule the fibrinopeptides A and B (FPA and FPB) that contain most of the negatively charged residues in the central region of the molecule. Thus, these cleavages result in a net positive charge at the central region of **fibrin** that can associate with the negatively charged globular ends of

other fibrin molecules forming a regularly (half) staggered array that traps formed cells (red cells, platelets, and leukocytes).

- Thrombin activates factor XIII to XIII_a (fibrin-stabilizing factor, FSF). XIII_a is a plasma **transaminase (transglutaminase)** that catalyzes formation of a covalent bond between the γ-carboxyl group of Gln residues and the ε-amino group of Lys residues. The arrangement of the fibrin subunits is such that XIII_a activity forms bonds between α subunits of two monomers and between γ subunits of two monomers. As the fibrin monomers are covalently linked to each other the clot strengthens.

- Thrombin cleaves factor V to yield Va which accelerates Xa activity potentially leading to the formation of more thrombin and more Va.

III. Limitations to coagulation

- Plasma contains thrombin inhibitors whose presence helps prevent clots from spreading from injury sites. **Antithrombin III** binds and inactivates thrombin and other serine protease factors in the clotting pathway; thrombin, IXa, Xa, XIa and XIIa (not VIIa). **α₂-macroglobin** accounts for most of the remaining antithrombin activity.

- **Heparin**, a negatively charged polysaccharide, found on the surface of endothelial cells accelerates Antithrombin III activity.

- Thrombomodulin integral membrane glycoprotein projects from vascular endothelium, binds thrombin. Thrombomodulin bound thrombin has altered substrate specificity changing its activity from a procoagulant protease to an anticoagulant protease. As a cofactor thrombomodulin induces thrombin to be more than 1000 fold more active on **protein C zymogen**.

- Activated protein C (a **Gla** containing protease) cleaves (inactivates) the two plasma cofactors VIIIa and Va to reduce the rate of two critical coagulation reactions; activation of factor X and thrombin respectively. Protein C protease activity is enhanced by association with Protein S.

IV. Fibrinolysis:

Dissolution of clots. **Plasmin** is a plasma serine protease that inactivates fibrinogen, fibrin, prothrombin, factors V_a, VIII_a and XII. Normally present in blood in its zymogen form, **plasminogen** is activated by **tPA, tissue plasminogen activator** (vascular tissues), or **urokinase** (kidney/bladder). Plasminogen, through association with fibrinogen and fibrin, is adsorbed into clots thus, the three components required for plasmin formation, plasminogen, tPA and fibrin (see below) are typically found only in clots - where plasmin activity

is needed.

V. Anti-coagulation drugs:

- **Tissue plasminogen activator, tPA**, is a serine protease that is inactive until exposed to fibrin.
- **Streptokinase** binds to plasminogen as a 1:1 complex that can activate other plasminogen molecules to plasmin.
- Aspirin inhibits platelet aggregation; **acetylates** a **cyclooxygenase** essential for prostaglandin biosynthesis including Thromboxane A₂ which stimulates platelet aggregation. Other nonsteroidal anti-inflammatory drugs are competitive inhibitors of the cyclooxygenase.

VI. Coagulation disorders

- **von Willebrand's disease** common bleeding disorder an autosomal dominant affecting 1 in 800 to 1000 individuals. Von Willebrand factor important for platelet aggregation and is the plasma carrier for factor VIII.
- **Bernard-Soulier Syndrome** rare autosomal recessive defective in platelet GpIb (for vWF binding).
- **Glanzmann's disease** rare autosomal recessive defective in platelet GpIIb-IIIa complex (essential for fibrinogen mediated association of platelets).
- **Hemophilia A** A defect in factor VIII encoded by an X-linked gene, permanent tendency for hemorrhages.
- **Hemophilia B** defect in Christmas factor, IX also an X-linked gene.

Other coagulation factor defects are inherited as autosomal recessives are rare occurring in factors, II, V, VII, and X, and components of intrinsic pathway, i.e., factors IX, and XII, prekallikrein, and high molecular weight kininogen. Deficiencies in vitamin K can deplete Gla residue containing factors (factors present, but contain unmodified Glu residues). Liver damage or disorders can lead to bleeding disorders due to impaired vitamin K metabolism and/or synthesis of the various coagulation factors. Protein C pathway defects can cause venous thrombosis.

Heme Metabolism

I. Heme Synthesis

Porphyryns are colored fluorescent compounds that have a basic structure of four **pyrrole rings** joined by methenyl bridges, as shown

below.

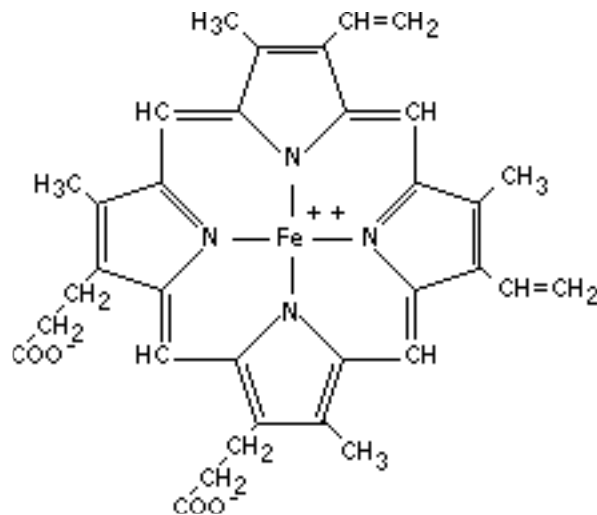


Figure 1. Heme

The various porphyrins differ according to the nature of the side chains.

Uroporphyrins - each pyrrole group has an **acetate** and a **propionate** side chain.

Coproporphyrins - each pyrrole group has a **methyl** and a **propionate** group.

Protoporphyrins - each of two pyrrole groups has a **methyl** and a **propionate** side chain; each of the other two has a **methyl** and **vinyl** side chain.

Porphyrinogens are colorless precursors of **porphyrins**. Their structures contain four pyrrole rings joined by methylene bridges. **Porphyrinogens** are readily oxidized, especially in the presence of light, by non-enzymatic means, to their stable **porphyrin** products.

Porphyrins are associated as **prosthetic groups** with a wide variety of proteins. The best known porphyrin, the **heme** moiety of hemoglobin, is the primary concern of this study guide.

The biosynthesis of heme occurs in all cells for use in cytochromes. The two major sites of synthesis are the liver (15%) and in reticulocytes, erythrocyte precursor cells. Regulation of heme biosynthesis differs at these two sites. Heme biosynthesis in reticulocytes is turned on massively during red blood cell development to provide heme for hemoglobin. In the liver heme biosynthesis is induced to provide

prosthetic groups for the P₄₅₀ group of cytochromes that are important in detoxification reactions. In this organ heme synthesis is repressed until demand relieves repression. Hence defects in heme biosynthesis (porphyrias, see below) are usually manifested in the liver as the inability to supply sufficient heme further derepresses the heme biosynthetic pathway leading to the accumulation of large amounts of specific intermediates in the pathway. The sequence of reactions is as follows (regulation is for the hepatic system; see your textbook for structures).

- (1) The precursors, **glycine** and **succinyl-CoA**, condense to form **δ -aminolevulinic acid (ALA)** in a reaction catalyzed by **ALA synthase**. This reaction occurs in mitochondria, is rate-limiting, and is subject to feedback inhibition by heme. (Also, the synthesis of the enzyme is repressed by hemin, see below).
- (2) Two molecules of ALA condense to form **porphobilinogen**. This reaction occurs in the cytosol and is catalyzed by ALA dehydrase.
- (3) Four molecules of porphobilinogen condense to form a linear tetrapyrrole, which then cyclizes to yield **uroporphyrinogen III**. This occurs in the cytosol and requires two enzymes. **Uroporphyrinogen I synthase** alone will catalyze the formation of the symmetric molecule **uroporphyrinogen I** whose structure is given below.

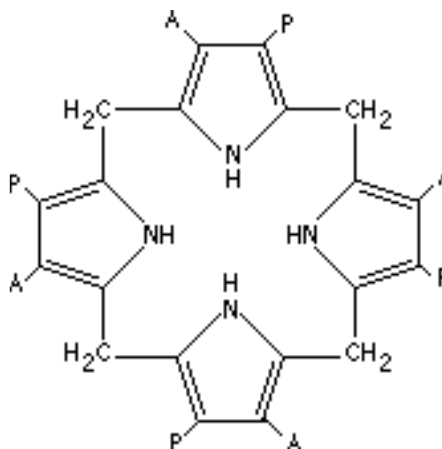


Figure 2. Uroporphyrinogen I

A represents acetic acid groups and P, propionic acid groups

Uroporphyrinogen III cosynthase is essential for the conversion of the above species to the asymmetric isomer, **uroporphyrinogen III**.

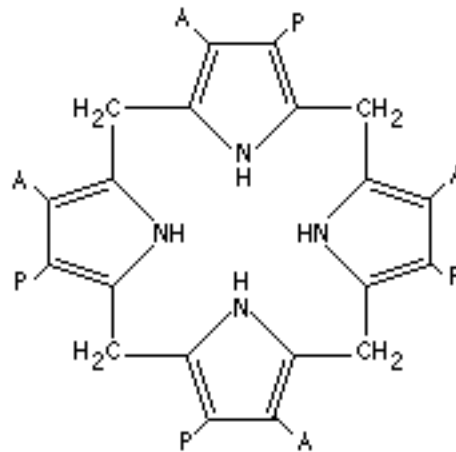


Figure 3. Uroporphyrinogen III

- (4) Uroporphyrinogen III is next converted to **coproporphyrinogen III** by decarboxylation of the acetic acid side chains by uroporphyrinogen decarboxylase, leaving methyl groups. This reaction also occurs in the cytosol.
- (5) **Protoporphyrin IX** is synthesized by converting two propionic acid side chains of coproporphyrinogen III to vinyl ($-\text{CH}=\text{CH}_2$) groups by coproporphyrinogen oxidase and the by protoporphyrinogen oxidase oxidizing three methylene bridges to methyldi-ene bridges.

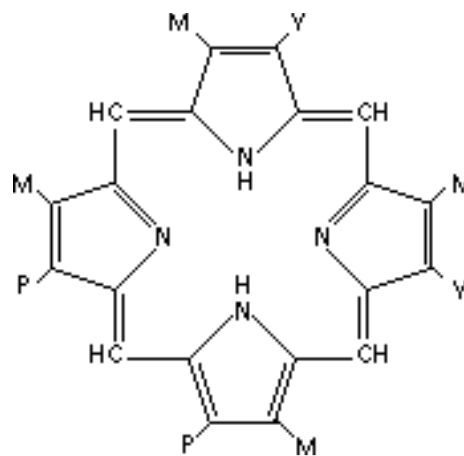


Figure 4. Protoporphyrin IX

These enzymes are associated with the outer mitochondrial membrane.

- (6) The ferrous form of iron is then inserted into **protoporphyrin**

IX. This reaction is catalyzed by **ferrochelatase** in mitochondria. Iron is transported in the plasma by **transferrin**, a protein that binds two ferric ions, and is stored in the tissues (liver) inside molecules of **ferritin**. The large internal cavity of ferritin can hold as many as 4500 ferric ions.

(7) Heme is attached to the protein globin in the cytoplasm.

Porphyrin synthesis is regulated mainly by *controlling the activity of δ -aminolevulinic acid synthase*. In addition, heme feedback inhibits δ -aminolevulinic acid dehydratase and ferrochelatase. **Hemin** (which is made by the spontaneous oxidation of the iron in heme from Fe^{2+} to Fe^{3+} when heme is in its free state) represses the formation of δ -aminolevulinic acid synthase and is also a negative effector of this enzyme. Moreover, in erythrocytes, hemin activates the synthesis of globin polypeptide chains and effects transport of ALA to the mitochondria from the cytoplasm.

Several aspects of this pathway are of clinical significance. For example, **lead poisoning** results from general enzyme inhibition caused by the binding of lead ions to sulfhydryl (thiol, -SH) groups. Ferrochelatase, uroporphyrinogen I synthase, and δ -aminolevulinic acid dehydratase are all sensitive to inhibition by lead.

Hence, lead poisoning leads to an accumulation of heme biosynthetic intermediates, particularly ALA and protoporphyrin IX. It has been suggested that the protoporphyrin IX content of red blood cells would be a more accurate measure of lead toxicity than plasma lead concentration, because the former reflects the effect of the dose while the latter is only a gross measure of lead ingestion.

Porphyrias are disorders in the synthesis of heme. "Over the last century, clinicians have been interested in the porphyrias to a far greater extent than would seem justified by their relatively rare occurrence. This undoubtedly was due in part to the complexity and unusually wide range of their clinical manifestations which extend all the way from mild and inconsequential photosensitivity to rapidly developing attacks of central or peripheral neuropathy, terminating at times fatally. In addition, the intense red fluorescence of free porphyrins permits their easy and often dramatic detection in microgram quantities in excreta and tissues." (Rudi Schmid, Hepatic Porphyrias, Current Hepatology, No. 4, Dr. Falk & Co.)

Porphyrias are generally divided into groups, hepatic and erythropoietic, or erythrohepatic (both), depending on the site of porphyrin accumulation. About 15% of heme synthesis occurs in the liver and

nearly all of the remainder in erythropoietic tissues of spleen and bone marrow. Most porphyrias are of genetic origin though lead and other heavy metals, hexachlorobenzene, and some drugs (griseofulvin and apronalide) can cause toxic acquired porphyria.

Laboratory tests used in analysis of porphyria include urinary porphobilinogen or uroporphyrin levels; fecal coporphyrin or protoporphyrin levels and red cell protoporphyrin levels. Porphyria victims must avoid drugs (including alcohol) that cause induction of cytochrome P₄₅₀ and anesthetics. Treatments include a carbohydrate rich diet (glucose loading) and administration of **hematin** (the hydroxide form of heme) that may suppress ALA synthase reducing heme precursor levels. Porphyrias expressing photosensitivity can be treated with use of sunscreens; the free radical quencher β -carotene may also help.

(1) **Protoporphyrin** has been traced to decreased activity of **ferrochelatase** in all **erythropoietic** tissues. This leads to an increase in protoporphyrin IX titer. This disease is characterized clinically by photo-sensitivity of the skin with painful itching and edema.

(2) A rarer, but more severe, disease is **congenital erythropoietic uroporphyrin** (often referred to simply as congenital erythropoietic porphyria). This autosomal recessive disease is due to reduced activity of **uroporphyrinogen III cosynthase**. Hence, uroporphyrinogen I accumulates in the red blood cell, along with related symmetric porphyrins. This leads to extreme photosensitivity (scarring and mutilation will eventually occur). Also, because these compounds are colored and fluorescent, the urine is pink to red in color, and the teeth may even glow, due to deposits of the pigment. It has been suggested that individuals with this disease might have been the origin of Werewolf legends. (Note: Harper's incorrectly summarizes this defect as not leading to photosensitivity - Table 34-1.)

(3) **Acute intermittent porphyria**, is characterized by neurotic or even psychotic behavior. One in 1000 Laplanders is afflicted. It is an autosomal dominant disease which results from inherited partial deficiency of **uroporphyrinogen I synthase**. in both liver and erythropoietic cells. This leads to decreased synthesis of heme which, in turn, results in less feedback inhibition of ALA synthase. Hence, the disease is associated with increased activity of **ALA synthase**. Patients with this disorder excrete massive quantities of porphobilinogen and δ -aminolevulinic acid. The accumulation of biosynthetic intermediates leads to periodic attacks of abdominal pain and erratic behavior. Administration of hemin (usually hematin, the hydroxide form) appears to suppress ALA synthase activity and relieves the condition in some.

(4) **Porphyria cutanea tarda** (hepatic) is characterized by excess uroporphyrins in the urine and photosensitivity. This autosomal dominant disease is due to reduced **uroporphyrinogen decarboxylase** activity.

(5) **Hereditary coproporphyria** (hepatic; **coproporphyrinogen oxidase**) and **Variegate porphyria** (hepatic; **protoporphyrinogen oxidase**) are characterized by abdominal pain, photosensitivity and neuropsychiatric problems. Each condition is result the result of an autosomal dominant defect. Afflicted individuals accumulate urinary prophobilinogen and uroporphyrin. Hereditary coproporphyria patients also accumulate fecal coproporphyrin while variegate porphyria patients also accumulate fecal protoporphyrin.

II. Heme Catabolism

Degradation of hemoglobin (about 6 g daily for a 70-kg human) is initiated with the breakdown of erythrocytes at the end of their approximately 120-day lifetime. The red blood cells are removed from the bloodstream by **reticuloendothelial** cells, of the liver, bone marrow and spleen. Any free hemoglobin that has escaped into the bloodstream from the rupture of RBCs is captured as methemoglobin dimers by **haptoglobin** (which prevents hemoglobin and hence iron loss in the urine) and is transported to the liver, while free heme, the oxidation product of heme, is transported bound to the plasma protein, **hemopexin**.

In the liver, and in other reticuloendothelial cells to a lesser degree, heme is transformed into **bile pigments** and related compounds. The sequence is as follows. (Also see your textbook.)

1. **Globin** is denatured and separated from heme.
2. Heme is cleaved by heme oxygenase to form the green compound **biliverdin** and Fe and **CO** are released. Biliverdin and related compounds are responsible for the color of bruises.
3. Biliverdin is reduced to **bilirubin** (also referred to as bilirubin IX-A alpha), a yellow pigment, by adding hydrogen to the central methylene group. Because bilirubin is *water insoluble*, this bile pigment is transported to the liver bound to **albumin**. Each molecule of albumin appears to have one high-affinity and one low-affinity site for bilirubin. In 100 ml of plasma, approximately 25 mg of bilirubin can be tightly bound to albumin at its high-affinity site. Bilirubin in excess is bound less tightly, and can be released to diffuse into tissues.
4. Bilirubin is conjugated with **glucuronic acid** to form **bilirubin**

diglucuronide, which is *water-soluble*. UDP-glucuronate is the co-substrate for UDP-glucuronyltransferase which catalyzes this reaction. (Conjugation occurs at the two bilirubin propionic acid groups.) The water-soluble product can be excreted into the intestine via the bile duct.

5. In the duodenum, conjugated bilirubin is deglycosylated (the glucuronic acid groups are removed) and reduced by bacteria to **mesobilirubinogen** and then to the colorless product **urobilinogen** (also known as stercobilinogen). Urobilinogen is found in feces (from 50 to 280 mg per day and excreted in urine (up to 4 mg per day).
6. A small fraction of urobilinogen is reabsorbed from the intestine, eventually returning to the liver. Most, however, is lost in the feces, as mentioned above. In feces (or urine, since some urobilinogen is excreted), urobilinogen is air-oxidized to dark brown **urobilin** (which is also called stercobilin). These compounds are largely responsible for the brown hue of feces and dark urine. Obstruction of the bile ducts leads to light-colored feces and dark urine. The skin is tinted yellow by the bilirubin that normally is converted to urobilin in the feces.

The most significant clinical aspect of the heme catabolic pathway is its relation to **jaundice** or **icterus**. **Jaundice** is caused by a higher than normal concentration of heme derivatives in the blood. There are several types of jaundice. The following classification is often used.

1. **Hemolytic jaundice**--excessive lysis of red blood cells results in an increased bilirubin concentration which exceeds the conjugating capacity of the liver. Hence, free (unconjugated) bilirubin is found in the plasma.
2. **Obstructive jaundice**--blockage of the bile ducts prevents excretion of conjugated bilirubin into the intestine. This leads to an increase in the concentration of conjugated bilirubin in the plasma.
3. **Hepatocellular jaundice**--damage to the liver by poisons, infection, etc., impairs its ability to conjugate and/or excrete bilirubin leading to an increase in either free or conjugated bilirubin concentrations (or both).

It is suggested in Harrison's Principles of Internal Medicine that jaundice be classified on the basis of clinical chemistry rather than pathology (as done above). To this end, the following classifications

are suggested.

- I. Predominantly unconjugated hyperbilirubinemia.
 - A. Overproduction (hemolysis or ineffective erythropoiesis).
 - B. Impaired hepatic uptake (drugs, fasting).
 - C. Impaired conjugation (hepatic disease such as hepatitis or cirrhosis; hereditary deficiency of the conjugating enzyme glucuronyl transferase).
- II. Predominantly conjugated hyperbilirubinemia.
 - A. Impaired excretion (viral or drug-induced hepatitis, hereditary disorders).
 - B. Extrahepatic biliary obstruction (stones, stricture, tumor of bile duct).

One means of distinguishing between these two broad categories is to assay for bilirubin in the urine. Normally unconjugated bilirubin (in contrast to conjugated) is tightly bound to albumin while in the bloodstream. Jaundice occurs when plasma bilirubin levels exceed 2-2.5 mg/dL. Bilirubin is insoluble and cannot be filtered by the glomerulus. High urine bilirubin concentrations are indicative of conjugated hyperbilirubinemia. Only conjugated bilirubin is water soluble and can pass from the liver into the blood and is passed out in the urine. Insoluble bilirubin can diffuse across membranes out of the plasma into tissue leading to the yellow coloration typical of jaundice. Bilirubin also can cross the blood-brain barrier affecting basal ganglia. Encephalopathy due to hyperbilirubinemia is known as **ker-nicterus** and results only from unconjugated bilirubin.

Bilirubin concentrations are usually assayed by the **Van den Bergh reaction**, in which bilirubin is diazotized with sulfanilic acid and then measured spectrophotometrically. This reaction can distinguish between conjugated and unconjugated bilirubin. In aqueous solution (the direct Van den Bergh reaction) only water-soluble conjugated bilirubin (**direct bilirubin**) will react. On the other hand, if the assay is conducted in methanol, both conjugated and unconjugated bilirubin are soluble and will react. Thus, total bilirubin is measured. The difference between the two assays gives the unconjugated bilirubin concentration, often referred to as "**indirect bilirubin**".

- I. Common types of unconjugated hyperbilirubinemia.
 1. Neonatal or physiological jaundice: The most common cause of unconjugated hyperbilirubinemia. Neonatal jaun-

dice can result from accelerated hemolysis (infant red cell levels adjust for normal respiration; there is some thought that excess iron supplements to the expectant mother can contribute to neonatal jaundice as the high iron levels lead to excess red cell formation in the neonate) and the fact that liver functions may not be fully developed. The immature hepatic system often expresses reduced capacity for bilirubin uptake, conjugation (low UDP-glucuronyltransferase activity and reduced synthesis of **UDP-glucuronic acid**) and secretion. Treatment includes administration of phenobarbital to induce hepatic function and phototherapy with visible light. Light can promote conversion of bilirubin deposits in the skin to derivatives that are excreted in the bile.

2. Crigler-Najjar Syndrome, Type I: A rare autosomal recessive mutation results in an absence of bilirubin UDP-glucuronyltransferase activity. Victims usually die within a year of birth although phototherapy may prolong life.
3. Crigler-Najjar Syndrome, Type II: A rare autosomal recessive UDP-glucuronase activity is present but, may be defective in adding the second glucuronyl group to bilirubin monoglucuronide. Afflicted individuals are responsive to phenobarbital treatment.
4. Gilbert's Disease: A heterogeneous group of disorders. Generally several aspects of bilirubin metabolism are affected. Excess hemolysis may be present along with deficient uptake of bilirubin by the liver and bilirubin UDP-glucuronyltransferase activity is often reduced.
5. Toxic Hyperbilirubinemia: Acquired disorders due to hepatic parenchymal cell damage impairing conjugation. Cell damage can also obstruct the liver biliary tree resulting in some conjugated hyperbilirubinemia in addition to **unconjugated hyperbilirubinemia**. Liver toxins include chloroform, cirrhosis, Amanita mushroom poisoning, carbon tetrachloride, and hepatitis viral infection.

II. Common types of conjugated hyperbilirubinemia.

1. Chronic Idiopathic Jaundice or Dubin-Johnson Syndrome: An autosomal recessive mutation results in a defect in hepatic secretion of conjugated bilirubin into the bile. The defect blocks entry into the bile of other compounds that utilize the same secretion system including conjugated estrogens.

2. Obstructive Jaundice: Blockage of the hepatic or common bile ducts can lead to conjugated hyperbilirubinemia. Common causes are liver damage or bile stones.

PRACTICE EXAM

Blood Clotting and Heme Metabolism

(NOTE: This exam was given years ago. The current exam format is multiple choice questions.)

1. Explain why fibrinogen is soluble whereas fibrin spontaneously polymerizes. Include a brief, concise description of the subunit organization of these proteins.
2.
 - a. How is a soft clot converted to a hard clot?
 - b. Name the enzyme(s) that is/are directly involved in this step of clotting.
 - c. If you looked in your blood where would you find the precursor(s) to this/these enzyme activity(s)?
3. Considering the molecular complex that catalyzes the conversion of prothrombin to thrombin: Name each component of the complex and describe the structural and functional roles they play within the complex.
4. Describe the factors in the intrinsic pathway that are directly responsible for the conversion of Stuart's factor (X) to its active form (Xa).
5.
 - a. Diagram, using structures or names, the pathway for heme biosynthesis.
 - b. How does heme regulate the activity of the heme biosynthetic pathway?
 - c. Which enzyme(s) in this pathway is/are inhibited by lead ions?
6. Would the test for direct or indirect bilirubin give a higher than normal value for each of the following? Briefly explain why.
 - a. A newborn Rh-positive child born to an Rh-negative mother?
 - b. Carbon tetrachloride poisoning.
 - c. A person carrying a defective glucose 6-phosphate dehydrogenase following treatment for malaria with primaquin.
 - d. Biliary tree obstruction.

Module 2: Hemoglobins and Red Cells

Basic Concepts:

Pluripotent hematicopoietic stem cells (HSCs) give rise to mature blood cells red, white and lymphoid. Differentiation of red cells from stem cells includes hemoglobinization and extrusion of the nucleus. This module is concerned with the molecular biology of red blood cell (RBC) production and hemoglobin synthesis.

Molecular disease arises as a consequence of a mutational change which alters the structure of a biological molecule and impairs the normal behavior of the macromolecule *in vivo*. This module is also concerned with molecular diseases that affect RBC synthesis/structure and hemoglobin structure and the biological implications of the altered structures. Genetic alterations of other proteins in RBCs lead to changes in functional behavior of oxygen transport.

Objectives:

1. The vast majority of hemoglobinopathies originate from a variety of mutations in the genes coding for the α and β chains of hemoglobin. For the following types of mutations give an example of a hemoglobinopathy resulting from the mutation and indicate how the resulting gene product differs from normal hemoglobin (Hb A).
 - a. point or missense mutation
 - b. frameshift mutation
 - c. deletion of gene segment
 - d. mRNA splicing defect
2. Explain in some detail how Hb Lepore and Anti-lepore-type variants come about. Which of the two types of variants would have the least problems physiologically? Why?
3. It is presumed that Hb variants (HbS, HbC, and α and β Thalassaemia) were genetically selected for during the course of evolution yet homozygous individuals with these traits are not healthy (or do not exist).
 - a. State the general geographical locations where these traits originate.
 - b. What is presumed to be the selective advantage offered by these traits (in terms of cellular processes)?

4. Compare the molecular structure and/or function of each of the following to adult hemoglobin (HbA).
 - a. Fetal Hemoglobin (HbF)
 - b. HbM
 - c. Familial methemoglobinemia
 - d. Carbonmonoxyhemoglobin (carboxyhemoglobin)
 - e. HbH or β_4
 - f. HbA_{1C}
5. Name the three different mechanisms by which methemoglobinemia can arise.
6. Explain how the amino acid replacement at the β -6 position in HbS ultimately leads to sickle-shaped cells.
7. Be able to describe the general process by which stem cells commit to a specific lineage such as red cells.
8. Explain how a pyruvate kinase deficiency results in a hemolytic anemia with markedly elevated P₅₀ whereas a hexokinase deficiency would result in a diminished value for P₅₀.
9. G6P dehydrogenase deficiency results in defective erythrocyte structure. How does the deficiency result in hemolysis? What agents initiate hemolysis? What is the basis for primaquine sensitivity?
10. Be able to define and use correctly the terms and concepts in the [Nomenclature and Vocabulary](#) and [Key Word](#) list.
11. Diagram the gene clusters for human globin genes. Include regulatory elements and other DNA elements found in these regions. With respect to hemoglobin synthesis detail changes in globin synthesis over human development. Describe the general features of hemoglobinization of RBCs (that is that takes place at any stage of human development).

Nomenclature and Vocabulary:[G6P Dehydrogenase deficiency](#)[Glutathione](#)[Heinze bodies](#)[Hexokinase deficiency](#)[Methemoglobinemia](#)[Pyruvate kinase deficiency](#)[Sickle cell trait](#)[Thalassemia](#)**Key Words:**

Anemia

Biochemistry

Blood
Hemoglobins
Sickle cell trait

Erythrocytes
Hemoglobins, abnormal

STUDY GUIDE-2

I. Transcription factors, growth factors and hematopoietic development:

Pluripotent hematopoietic stem cells (HSCs) give rise to mature blood cells red, white and lymphoid. HSCs must be able to maintain a non-cycling state that is both self-renewal and production of progenitor cells with more limited developmental potential. Mammalian embryo hematopoiesis occurs in yolk sac blood islands (primitive hematopoiesis-as product is large nucleated "primitive" red cells). Red cell synthesis shifts to the fetal liver and then to the bone marrow (definitive hematopoiesis). Growth factors trigger cellular proliferation and differentiation by modulating the activity of nuclear regulators (transcription factors) that regulate expression of lineage specific genes.

II. Erythrocyte lineage:

A major regulatory molecule in human erythropoiesis is the glycoprotein **erythropoietin**. Pluripotent stem cells are influenced to develop by a number of growth factors. The first class of progenitor cells specific to erythropoiesis are known as burst-forming unit-erythroid (BFU-E). Erythropoietin acts on BFU-E cells to promote both cell proliferation and differentiation to colony-forming unit-erythroid (CFU-E) cells. Erythropoietin acts on CFU-E cells again promoting cell proliferation and differentiation. Other factors including interleukin-3 and granulocyte stimulating factor contribute to this developmental/proliferative pathway. CFU-E progenitor cells differentiate to proerythroblasts (the earliest red cell precursor that can be recognized on examination of the bone marrow) which respond to erythropoietin by differentiating into erythrocytes. This differentiation is marked by loss of the nucleus. The nucleus has dwindled in size during the three to four cell divisions that typify proerythroblast proliferation/differentiation and this 4-day period is marked by increased hemoglobin synthesis. Loss of nuclei results in reticulocytes that remain in the bone marrow for several days prior to release into the plasma. The first day in the plasma is marked by the loss of mitochondria and ribosomes and assumption of the structure of a mature red cell.

In the fetus erythropoietin is synthesized mainly in the liver. After birth most erythropoietin synthesis occurs in the kidney. Liver or kidney hypoxia leads to increased synthesis of erythropoietin. This negative feedback loop increases the oxygen-carrying capacity of the blood: normally serum erythropoietin levels are inversely proportional to hemoglobin concentration. The normal marrow can increase red cell production 3 to 5 times its normal rate within a week

or two of stimulation. Erythropoietin and other growth factors (which are also glycoproteins) bind to receptors on the surface of target cells eliciting a second messenger response and ultimately influencing the level of expression of specific genes through the activity of transcription factors.

III. Hemoglobinization:

Proerythroblasts are stimulated to massively increase transcription of appropriate globin gene mRNA. This change in regulation of gene expression coupled with organelle loss results in 98 percent of red cell protein consisting of hemoglobin. Normally similar amounts of α -like and β -like globins are synthesized as well as the heme cofactor essential to complete hemoglobin synthesis.

IV. Human globin gene organization:

Figure 1 shows the organization of the human globin gene clusters (the coding strand for the globin genes is shown). The presence of repetitive DNA sequence elements such as the *Alu* and *Kpn* sequences shown have implications for deletions and rearrangements of DNA in these clusters which can give rise to thalassemias. As mentioned above the site of globin gene expression changes during human development. There are three functional (expressed) α -like genes and five functional β -like genes.

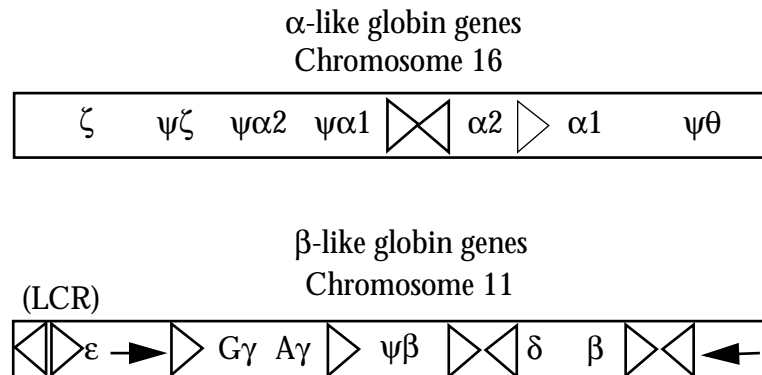


Figure 1. Human globin gene clusters. Symbols indicate genes that are expressed at specific stages of human development except for ψ , indicates a pseudogene; LCR indicates the locus of control region typically found 6 to 20 kb upstream of the ϵ gene. Cartoon not to scale. Arrowheads represent *Alu* sequences in their relative orientations. Arrows indicate *Kpn* sequences in their relative orientations.

Expression of these sets of genes changes during development resulting in the synthesis of a number of distinct forms of hemoglobin as detailed below. Figure 2 shows the general organization of α -like and

β -like genes. The eight genes share a good deal of similarity at the nucleotide level in their exons. The globin gene products are also similar especially with respect to structure. The presence of introns which vary greatly with respect to nucleotide sequence and to a lesser extent with respect to size may help to reduce the frequency of recombination events between globin genes. Each globin gene cluster also contains pseudogenes; a nucleotide sequence that mimics much of the exon/intron structure of a globin gene, but that is not expressed. Not shown in detail in figure 2 are regulatory sequences with the exception of the promoter region.

Promoter	Exon1	Intron1	Exon2	Intron2	Exon3	Poly(A) site
Exon length: 30-31			67-73		42	

Figure 2. Cartoon of the general organization of α -like and β -like genes. Exon length is given in codons. Intron length often varies from gene to gene.

We will use the **Locus Control Region (LCR)** found upstream of the human β -globin gene cluster as an example of transcriptional regulation of gene expression. The LCR is found 6 to 20 kb upstream of the human ϵ gene. The LCR represents a series of DNase I hypersensitive sites that are specific to erythroid lineage cells. Formation of these sensitive sites is required for β -globin gene expression. Positioning of genes near the LCR confers high level expression and allows nonerythroid gene expression in erythroid lineage cells. LCR deletions inactivate downstream β -globin gene and result in a β thalassemia phenotype. Two modes of regulation: autonomous; though LCR is active in definitive cells genes that are not expressed escape LCR effect suggesting mediation by negative mechanisms such as a silencer in the 5' flanking region of the ϵ globin gene. LCR can override the normal developmental regulation of say γ or β when directly linked, but not when proper gene order in the cluster is conserved. Suggests γ and β compete for LCR initially then silencer(s) shuts down γ expression later in development.

During the course of a normal human development 5 different hemoglobin subunit chains are elaborated to form hemoglobin molecules of the following composition: $\zeta_2\epsilon_2$ (Hb Gower); $\alpha_2\gamma_2$ (HbF); $\alpha_2\beta_2$ (HbA); $\alpha_2\delta_2$ (HbA₂). Note all hemoglobins are heterotetramers with two α -like globin subunits and two β -like globin subunits. Over the course of human development two α -like and four β -like polypeptides are synthesized. The first type of hemoglobin ($\zeta_2\epsilon_2$)

appears and disappears during embryonic development. $\alpha_2\gamma_2$ is known as fetal hemoglobin (HbF) and comprises 50 to 90 percent of total hemoglobin in the newborn. This percentage normally decreases to 15%, 5%, and 1% at the ages of 1, 2, and 4 years, respectively, while adult hemoglobin (HbA), $\alpha_2\beta_2$, increases proportionally. $\alpha_2\delta_2$ is a minor hemoglobin (a few percent) also found in adults. Normally, synthesis of α -like and β -like chains is regulated to yield approximately equal amounts of these two types of polypeptides.

Hemoglobin interacts with blood glucose that enters erythrocytes; glucose nonenzymatically glucosylates hemoglobin at Lys residues or at N-termini forming HbA_{1C}. Can look at fraction of total HbA by ion exchange chromatography or electrophoresis; normally about 5% is HbA_{1C} levels are proportionate to blood glucose concentration. Half-life of erythrocytes is 120 days so this data provides a window on last 6-8 weeks of average blood glucose levels. Useful in diagnostics of diabetics.

Problems arise with O₂ transport upon alteration of hemoglobin. Such alterations can occur by inheritance or they can be induced by chemical alteration of the structure. The inherited abnormal hemoglobins are of several types (see Devlin pp. 732-733).

1. **Altered exterior**--amino acid substitution on the surface of the hemoglobin molecule. Sickle-cell hemoglobin (HbS) is a striking example.
2. **Altered active site**--structural change near the heme directly affects oxygen binding. Examples include certain methemoglobinemia cases (HbM).
3. **Altered tertiary structure**--abnormal folding of the polypeptide chain results from certain amino acid substitutions.
4. **Altered quaternary Structure**--Mutations at subunit interfaces result in loss of allosteric properties.
5. **Loss of or diminution in ability to synthesize α or β chains**--This characterizes the **thalassemia** cases.

In addition to defects in globin genes other inheritable cases with defects in genes encoding certain enzymes can also result in O₂ transport problems. Familial **methemoglobinemia** results from deficient activity of methemoglobin reductase and is a good example of this type of problem. In this module, we intend to discuss many of these abnormal and/or reagent induced hemoglobin alterations from a

structural and functional viewpoint.

Practice Questions:

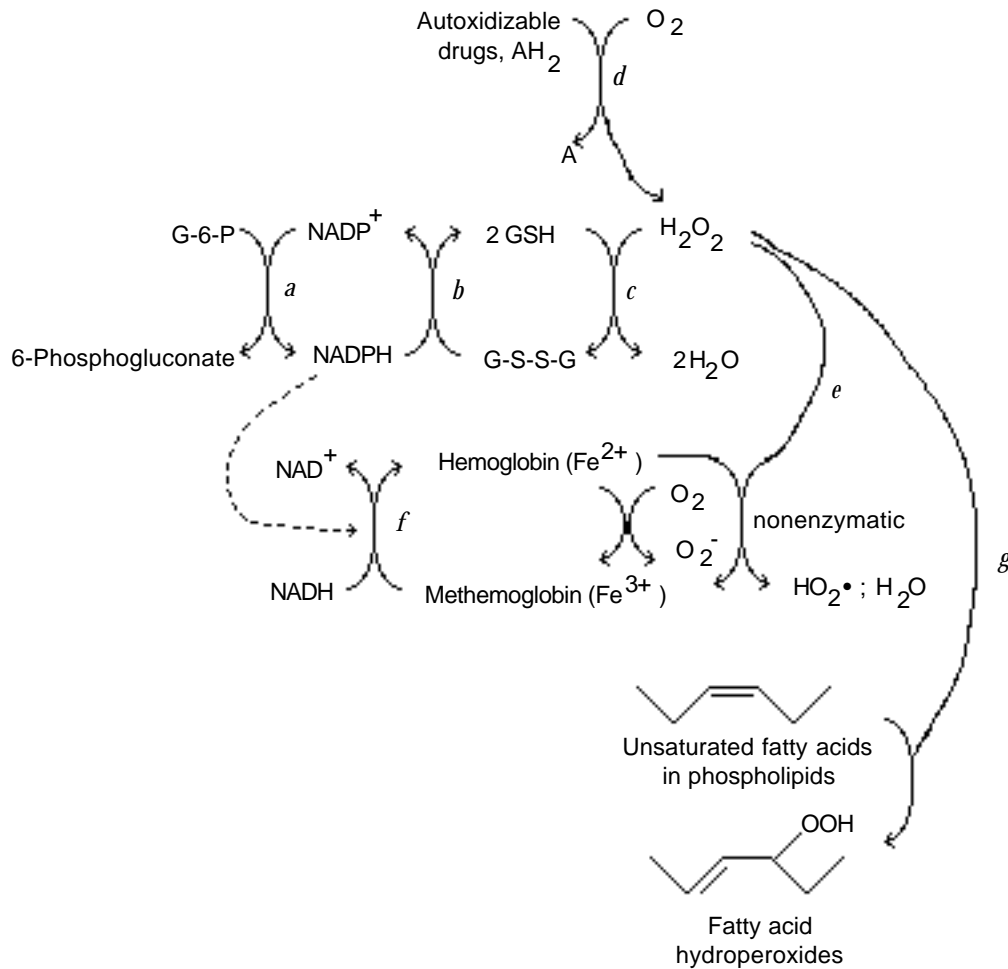
1. Why is the sickle cell trait called an anemia?
2. How does the oxygen dissociation curve of purified HbF compare to purified HbA?
3. How does the O₂ dissociation curve for fetal erythrocytes compare to that for normal adult erythrocytes? Why? What is the physiological significance of this?
4. What are the molecular and physiological consequences of Thalassemia α and β ?
5. Compare the O₂ dissociation curves for HbH and HbA.
6. HbF normally disappears after the first few months following birth. Under what circumstances might HbF persist in the blood long after birth?
7. Explain in molecular terms how "sickling" comes about.
8. What is meant by the term "molecular disease"?
9. What is meant by "hemolytic anemia"?
10. Give examples of reagents capable of causing methemoglobinemia.
11. What is Pamaquine sensitivity?

Appendix I: GLUTATHIONE PEROXIDASE AND ABNOR- MALITIES OF RED BLOOD CELLS

Excerpted From Biochemistry, David E. Metzler, Academic Press, 1977, p. 564

The processes by which hemoglobin is kept in the Fe(II) state and functioning normally within intact erythrocytes is vital to our health. Numerous hereditary defects leading to a tendency toward anemia have helped to unravel the biochemistry indicated in the accompanying scheme.

About 90% of the glucose utilized by erythrocytes is converted by glycolysis to lactate but ca. 10% is oxidized (via glucose 6-phosphate) to 6-phosphogluconate. The oxidation (reaction a) is catalyzed by glucose-6-phosphate dehydrogenase using NADP⁺. This is the principal reaction providing the red cell with NADPH for reduction of **glutathione** according to reaction b. Despite the important function of glucose-6-P dehydrogenase, over 100 million persons, principally in tropical and Mediterranean areas, have a hereditary deficiency of this enzyme. Furthermore, genetic variations are numerous, at least 22 types having been identified. The lack of the enzyme is truly detrimental and leads to excessive destruction of red cells and anemia during some sicknesses and in response to administration of certain drugs. The survival of the defective genes, like that for sickle cell hemoglobin is thought to result from increased resistance to malaria parasites.



Other erythrocyte defects that lead to drug sensitivity include a deficiency of glutathione (resulting from a decrease in its synthesis) and a deficiency of glutathione reductase (reaction b). The effects of drugs have been traced to the production of H₂O₂ from oxygen (reaction d). Current thinking is that the function of glutathione and of the enzymes catalyzing reactions a, b, and c is to destroy hydrogen peroxide arising naturally or from autoxidation of drugs. The selenium-containing peroxidase is the principal enzyme destroying H₂O₂ (reaction c) in red blood cells; catalase is thought to function in a similar way and both enzymes are probably necessary for optimal health. Oxidizing agents can appear in the blood from dietary sources (e.g. nitrates and nitrites) and the administration of certain drugs (such as sulfonamides, pamaquine, etc.). The oxidative insult

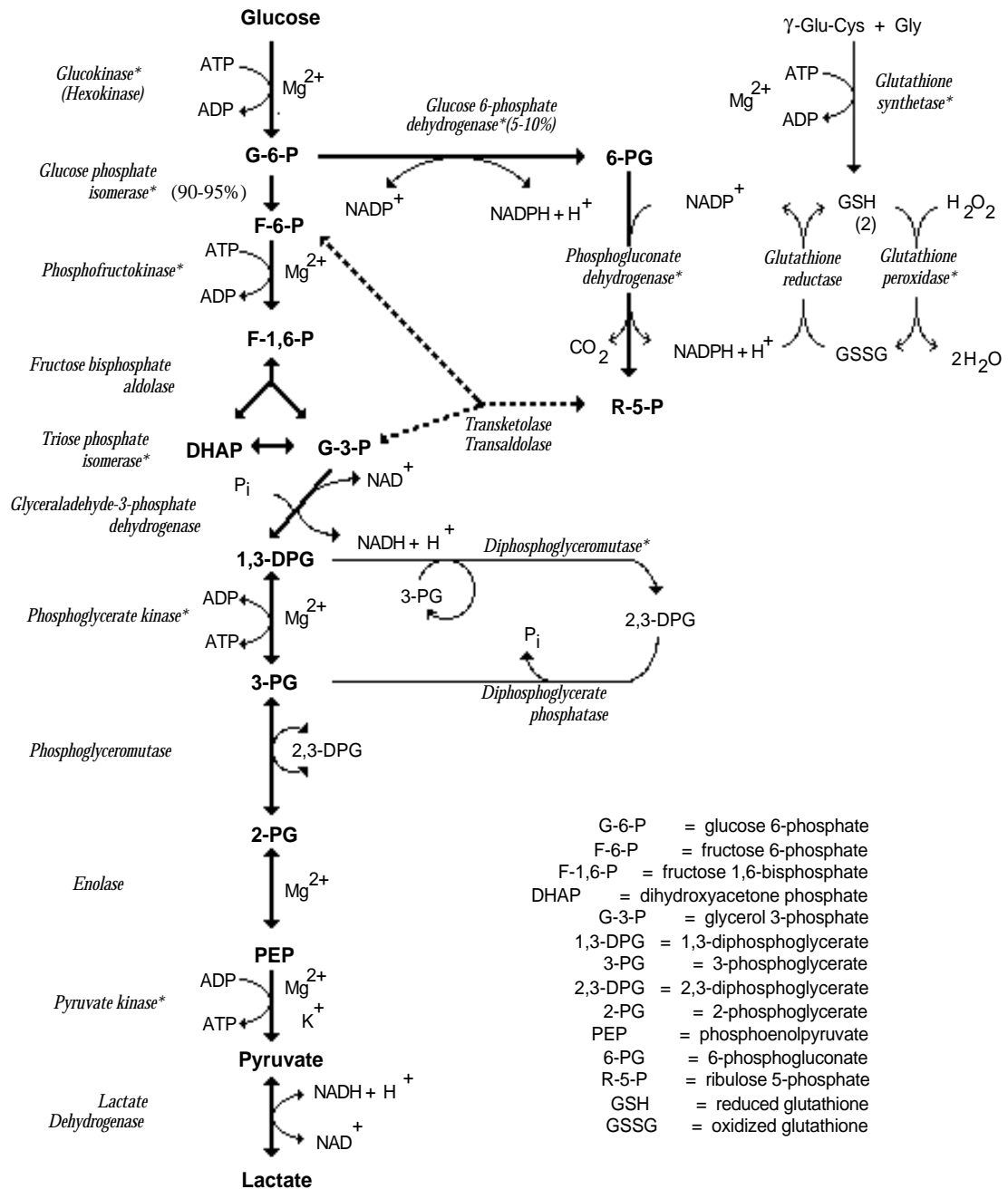
provided by these agents may lead to reagent induced methemoglobinemia as well as an increase in H_2O_2 .

An excess of H_2O_2 can damage erythrocytes in two ways. One is to cause excessive oxidation of functioning hemoglobin to the Fe(III)-containing methemoglobin. (Methemoglobin is also formed spontaneously during the course of the oxygen-carrying function of hemoglobin. It is estimated that normally as much as 3% of the hemoglobin may be oxidized to methemoglobin daily.) The methemoglobin formed is reduced back to hemoglobin through the action of NADH-Methemoglobin reductase (reaction f). A smaller fraction of the methemoglobin is reduced by a similar enzyme requiring NADPH (as indicated by the dashed arrow). A hereditary lack of the NADH-methemoglobin reductase (familial methemoglobinemia) is also known resulting in a high level of methemoglobin in these individuals.

A second destructive function of H_2O_2 is attack on double bonds of unsaturated fatty acids of the phospholipids in cell membranes. The resulting fatty acid hydroperoxides can react further with C—C chain cleavage and disruption of the membrane. This is thought to be the principal cause of the hemolytic anemia induced by drugs in susceptible individuals. Glutathione peroxidase is thought to decompose these fatty acid hydroperoxides. Vitamin E, acting as an antioxidant, is also needed for good health of erythrocytes.

Some cases of granulomatous disease are accompanied by a decreased glutathione peroxidase activity and a decreased microbicidal activity of phagocytes. It has been suggested that hydroperoxides of fatty acids interfere with normal phagocytosis by inhibiting required enzymes.

Hemolytic anemia can also arise from hereditary changes in other enzymes in red cells. The accompanying figure illustrates some of the known anemias resulting from deficiencies of certain glycolytic and other enzymes in the red cell. **Pyruvate kinase deficiency**, inherited as an autosomal recessive trait, results in a rather severe anemia while phosphofructokinase deficiency is transmitted as a sex-linked recessive with males being severely affected, exhibiting neurological disease and behavioral disturbances.



Glycolysis and Glutathione Metabolism in the Human Erythrocyte

*Known enzymic steps in which the deficiencies are associated with hereditary hemolytic anemia.

In all of these enzyme deficiencies the red cell is shorter lived presumably because there is less energy (as ATP) available from glycolysis. Energy is needed to maintain the integrity of the cell membrane so less ATP production should shorten cell life. Depending upon where the deficiency occurs in the pathway, the red cell will have either an excess or a deficiency of 2,3-BPG. That is, if the deficiency occurs for reactions along the pathway between glucose and 1,3-BPG, a deficiency of 2,3-BPG will be observed. On the other hand, a deficiency of an enzyme catalyzing any reaction from 1,3-BPG to pyruvate will result in a buildup (excess) of 2,3-BPG. Since 2,3-BPG is a negative effector of oxygen binding to hemoglobin, the concentration of 2,3-BPG in the red cell will affect the shape of the oxygen saturation curve for those erythrocytes.

Module 3: O₂- Transport and O₂- Hemoglobin Affinity

Objectives:

1. Give a word description of characteristics of hemoglobin (Hb) and myoglobin (Mb) in terms of:
 - a. their 3-D character (quaternary structure with Hb)
 - b. their secondary structural characteristics.
 - c. their prosthetic groups.
2. Both Mb and Hb function by binding oxygen. Diagram or graph and clearly describe the functional differences on O₂ binding for Mb and Hb. Illustrate by the use of saturation curves how hydrogen ion, CO₂, and organic phosphates affect oxygen binding to myoglobin and hemoglobin.
3. Describe in terms of binding constants why Hb has a sigmoid binding curve. (Does it mean at 50% saturation that all hemoglobin molecules have two molecules of O₂ bound? Why?)
4. What is meant by the term Heme-Heme interaction?
5. What is the functional significance of a sigmoid dissociation curve? (Hint) Contrast hyperbolic and sigmoid curves with the same P₅₀ in terms of their ability to transport oxygen.
6. In general terms, what quaternary changes take place upon oxygenation of hemoglobin?
7. Describe the Bohr effect in terms of oxygen saturation curve. Write an equation which expresses the relationship of hemoglobin to [H⁺] for the oxygenation-deoxygenation process and explain in some detail where the [H⁺] come from when one mole of O₂ combines with deoxyhemoglobin. What is the physiological significance of the Bohr effect at the tissue and lung?
8. Describe, in general terms, where and how 2,3-bisphosphoglycerate (BPG) binds to deoxyhemoglobin. Explain the significance of the effect of BPG on oxygen transport at the physiological level.
9. What is the role of globin in oxygen transport and storage in

myoglobin and hemoglobin?

10. What is the significance of the Hill equation, Hill coefficient, and Hill Plot? What is the meaning of the term "cooperativity" with respect to Hb-O₂ binding and why is the Hill coefficient a measure of cooperativity?
11. Describe iron metabolism with respect to O₂ transport and the role of iron in O₂ transport.
12. Given a passage from a medical journal or text that describes a clinical investigation or description of a problem involving hemoglobin or O₂ transport, be able to answer questions about the passage (which may involve drawing inferences or conclusions) or use the information given for solving a problem.
13. Given an excerpt from a text, journal, or clinical case concerned with oxygen carriage, be able to discuss the abnormality in terms of alterations in the oxygen dissociation curve and the molecular characteristics of the oxygen carrier molecule.
14. Be able to define and use correctly the terms and concepts in the [Nomenclature and Vocabulary](#) and [Key Word](#) list.

Nomenclature and Vocabulary:

[2,3-Bisphosphoglycerate](#)
[Bohr effect](#)
[Heme-heme interaction](#)
[Hill Coefficient](#)

[Allosteric](#)
[Cooperativity](#)
[Hemoglobin](#)
[Myoglobin](#)

Key Words:

Biochemistry
Erythrocytes
Myoglobin
Oxyhemoglobins

Blood
Hemoglobins
Oxygen

STUDY GUIDE-3

Practice Questions:

1. What noncovalent forces are present at the interfaces of α and β subunits, i.e., α contacts as well as β contacts?
2. Define prosthetic group.
3. Define P_{50} .
4. What does the term allosteric mean?
5. Construct a graph of Mb binding.
6. What is meant by the term cooperativity, as it applies to Hb- O_2 binding?
7. What are the quantitative relationships of O_2 binding for Hb, Mb, and cytochrome oxidase?
8. When BPG binds to deoxyhemoglobin, what is the effect on oxygen affinity and why? How does CO_2 effect oxygen affinity to hemoglobin?
9. Does heme-heme interaction mean that the protoporphyrin ring systems physically bond in some fashion?
10. People living at high altitudes have more 2,3-BPG in their red cells than those living at sea level. Explain how this is advantageous for those people living at high altitudes.

Module 4: Modes of CO₂ Transport and CO₂-O₂ Interrelationship in Transport

Objectives:

1. Write equations which describe the chemistry which occurs when CO₂ enters an erythrocyte.
2. Explain the importance of carbonic anhydrase in terms of its necessity in respiration as well as where it is physically located to aid respiration.
3. Carbon dioxide exists in several states in whole blood. What are the primary ways CO₂ is transported in blood?
4. Explain the phenomenon of isohydric shift and its importance in the biochemistry of respiration. What are the principal buffers in the circulatory system?
5. Describe the reaction of carbon dioxide with Hemoglobin in terms of: (a) an equation (b) the name of the compound formed.
6. Describe the events taking place upon "chloride shift" and explain why they take place.
7. The HCO₃⁻/CO₂ (dissolved) buffer pair (pK=6.1) is unusual in that it can buffer at physiological pH (7.4) even though this pH is more than 1 pH unit past the pK for the buffer. Discuss how this unusual characteristic is accounted for.
8. What is hematocrit and why does it differ for venous and arterial blood?
9. Give a complete schematic representation of processes which occur when O₂ and CO₂ are transported during respiration. It will be necessary to give separate schematics for the events which take place when arterial blood reaches peripheral tissue as well as for return of venous blood to the lung (alveoli). In your discussion be certain to indicate: (1) the direction of transport, (2) the results of the isohydric shift, chloride shift, Bohr effect and (3)

the names of proteins and enzymes involved in the processes.

10. Explain how each of the following affect oxygen transport and/or utilization. CO, CN⁻, dinitrophenol, azide, rotenone, oxidizing agents.
11. What causes O₂ to be released by oxyhemoglobin and taken up by tissue cells e.g., skeletal muscle? Upon exercising, what causes the O₂ transport system to work more efficiently at the tissue level?
12. Define metabolic respiratory quotient and explain how it is that more equivalents of O₂ are inspired than equivalents of CO₂ are expired when different food stuffs are compared.
13. How do Hb, Mb, and cytochrome oxidase operate together in the transport and utilization of O₂ in living systems?
14. Be able to define and use correctly the terms and concepts in the [Key Word](#) list.

Key Words:

Acid-base equilibrium
Blood
Carbonic anhydrase
Erythrocytes
Hemoglobins

Biochemistry
Carbon dioxide
Carboxyhemoglobin
Hematocrit
Respiration

STUDY GUIDE-4

I. Basic Concepts:

CO₂ is produced in tissues as the final product of complete oxidation of food stuffs. Since as much as 13 to 15 equivalents of acid are produced per day from the hydration of carbon dioxide to carbonic acid, it is essential to eliminate CO₂ rapidly and efficiently so as not to create a pH imbalance. The mechanisms for CO₂ transport and the resulting effects on other ions and molecules are considered here.

II. What is the fate of oxygen in respiration?

While much emphasis has been given to oxygen transport by hemoglobin, the real chemistry of respiration actually occurs at the tissue level. The overall reaction of importance is the generation of energy by oxidation of fatty acids, glucose, and other food stuffs. (i.e., C₆H₁₂O₆ + 6O₂/6CO₂ + 6H₂O + energy) As is well known, this overall reaction is the consequence of the summation of individual reactions of the glycolytic pathway and the citric acid cycle along with oxidation of requisite coenzymes performed by the electron transport system of the mitochondrion. Thus, the site at which oxygen becomes reduced is the terminal event in the electron transport system which is catalyzed by cytochrome oxidase. This enzyme, then, is the ultimate acceptor of molecular oxygen in respiration and its characteristics play an important role in understanding respiration and some of the pathology associated with respiration.

III. What is the driving force for O₂ to get to cytochrome oxidase?

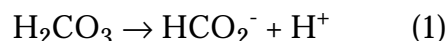
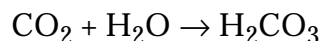
Clearly, since O₂ is being used in the peripheral tissue its concentration is lower at the tissue than it is in the arterial blood arriving at the peripheral tissue capillary bed. Thus O₂ is driven by the concentration gradient to the tissue cells. Certain muscle tissues have myoglobin present (deep diving animals have very high concentrations of myoglobin) and since myoglobin has a lower p50 than hemoglobin, the myoglobin will bind the O₂, further promoting a steep O₂ concentration gradient and promoting the unloading of oxygen within the capillary bed. Cytochrome oxidase has a lower p50 than myoglobin, so the O₂ prevailing in the tissue is further sequestered by cytochrome oxidase. The rank order of p50 values for hemoglobin, myoglobin, and cytochrome oxidase helps to ensure that the O₂ gets to its ultimate site of utilization.

A number of respiratory inhibitors and poisons act at the level of the electron transport chain. CO, CN⁻, dinitrophenol, azide, rotenone, and oxidizing agents all act directly at the mitochondrion level and

some are also inhibitors at other steps in respiration.

IV. CO₂ is the second half of the story in gas exchange.

Along with water, CO₂ is a product of complete combustion of carbohydrates and fats in tissues. Consequently CO₂ concentration in peripheral tissues is high while that in the newly arrived arterial blood at the capillary bed is quite low. This concentration gradient serves as a driving force for CO₂ to enter the blood and, therefore, the red cell. CO₂ readily diffuses through membranes but on passage into the red cell, it is acted upon by an enzyme, carbonic anhydrase, associated with the inner membrane of the red cell. Carbonic anhydrase readily hydrates CO₂ to carbonic acid with the reaction reaching equilibrium in approximately one second and the carbonic acid spontaneously dissociates into bicarbonate and hydrogen ion.



These reactions are extremely important since production of bicarbonate and the law of mass action permits additional CO₂ to enter the red cell, thereby increasing the capacity of the blood for CO₂. CO₂ can hydrate without being enzyme catalyzed but the noncatalyzed reaction takes about 100 seconds to reach equilibrium. Since blood completes its cycle from lung to tissue and back on the order of 60 seconds and remains in the capillary bed for only about one second, it is clear that carbonic anhydrase plays an essential role in CO₂ transport back to the lung.

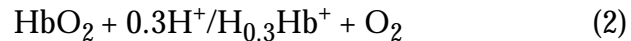
V. How does the blood in the capillary bed accommodate the hydrogen ion produced by hydration of CO₂?

Increased acidity inside the red cell sets forth a host of chemical events that are reversed when the venous blood reaches the capillary bed of the lung.

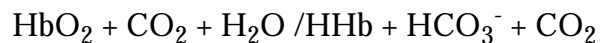
First, let us discuss how the hydrogen ion is taken care of by considering simple buffering which will occur in the presence of ionizable species with pK_as in the neutral pH range. The buffers which occur in blood include organic phosphates such as 2,3-DPG (also denoted 2,3-BPG) which occur at a concentration of 4-5 mM in red cells, proteins which contain imidazole groups (pK_a=6.0) (Hb contains 38 imidazoles per tetramer) and alpha amino groups (pK_a=7.8), and the CO₂/H₂CO₃ pair. Buffering by these buffering systems accommodates about 60% of the acid created by CO₂ hydration.

VI. Much of the remaining acidity is accommodated by an important reaction involving hemoglobin called the Bohr effect

This reaction takes place as oxyhemoglobin unloads or dissociates its oxygen and is illustrated by the following equation.



pK_a s of certain groups on hemoglobin are perturbed by the conformational change brought about by the oxy/deoxy conversion which makes deoxyHb a weaker acid than oxyHb. A deceptively simple but illustrative composite reaction which may be written to illustrate CO_2 hydration, carbonic acid dissociation, followed by the Bohr effect involving hemoglobin is:



Note that hydrogen ion does not appear in the overall (composite) reaction.

Hemoglobin's ability to accommodate hydrogen ion produced from the influx of CO_2 into the red cell is referred to as the *isohydric carriage* of CO_2 . But in reality the Bohr effect only accommodates 30% or so of the H^+ produced from the CO_2 influx and the remainder is taken care of by simple buffering by phosphates and imidazole groups on the surface of hemoglobin. Even then not all of the acidity is accommodated since the pH of venous blood (pH=7.35) is a little more acidic than that of arterial blood (pH=7.4). The bottom line, however, is that the red cell through simple buffering and isohydric carriage accommodates a significant amount of acidity with only a very modest change in pH.

VII. CO_2 also reacts in a direct chemical reaction with hemoglobin.

This reaction occurs with an alpha amino group on one of the subunits of hemoglobin to form a compound called carbamino hemoglobin.

VIII. The increase in bicarbonate equivalents and positive charge on hemoglobin results in swelling of the red cell and the chloride shift.

Note that when CO_2 enters the red cell, bicarbonate ions increase in concentration as the red cell changes from high oxygen tension conditions of the arterial state to high CO_2 conditions of venous blood. This increase in equivalents of species inside the red cell naturally results in an increase in the osmotic pressure and water diffuses in to reduce the osmotic effect. This results in swelling of the red cell and this swelling effect is evident from the fact that the hematocrit (the fraction of volume occupied by the red cells in whole blood) of venous blood is greater than that of arterial blood. The increase in bicarbonate ions causes it to want to diffuse to the plasma but since movements the major counterions (K^+ , Na^+) are under strict control, it turns out that bicarbonate ions in the red cell and chloride ions from the plasma exchange positions in accord with their concentration gradients. This effect is known as the chloride shift and it allows the $\text{CO}_2/\text{H}_2\text{CO}_3$ pair in the plasma to readjust to the new pH prevailing in venous blood.

The reverse of these reactions occurring at the tissue level occurs on return of venous blood from tissue to the alveoli capillary bed.

IX. During exercise the physiological response is to increase oxygen delivery to muscle tissue.

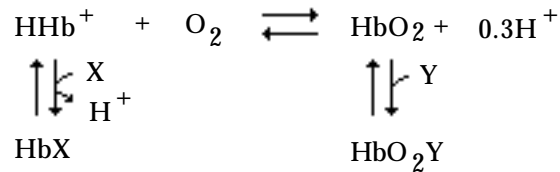
The mechanics of the physiological response occurs through application of the principles of CO_2/O_2 exchange which we have just discussed. In vigorous exercise, the muscles are respiring so concentration of CO_2 is very high while that of O_2 is very low. The arterial blood reaching the capillary bed of the respiring tissue will respond in proportion to these concentration gradients which exist by rapid diffusion of oxygen out of the red cells and into the tissues and vice versa for the CO_2 . The concentration gradients are major driving forces in the physiological response. The increased CO_2 entering the red cell has two effects, first by the law of mass action (see equation 2 above), the increase in excess acidity on hydration of the additional CO_2 will promote the dissociation (unloading) of oxyhemoglobin. And secondly, since CO_2 preferentially binds to deoxyhemoglobin to form carbaminoHb, the increase in red cell CO_2 will promote deoxygenation of oxyhemoglobin, thereby promoting the unloading of oxygen. In addition, hydrogen ion will also diffuse into the blood from excess lactic acid produced through anaerobic metabolism further promoting the unloading of oxygen via the Bohr effect. And finally, the increased heat generated in the muscles tends to shift the p_{50} of oxyHb to the right, thus, promoting the unloading of oxygen at the tissue level.

Practice Exam

O₂/CO₂

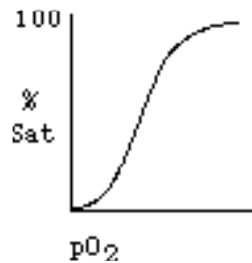
(Note: This exam was given years ago. The current exam format is multiple choice questions.)

Given: The following equilibria describe many of the observations concerning O₂ transport and hemoglobin function.

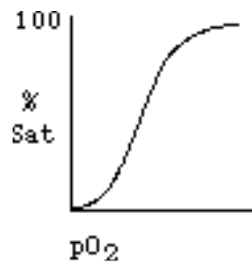


1. What two different physiological compounds can act as described by compound X in the above system? (a) _____ (b) _____.
2. Addition of O₂ to the above system in which X is not present would increase/decrease the pH? (circle the correct answer)
3. Carbon dioxide reacts with _____ groups on α and β subunits to form _____ compounds.
4. Much of the 0.3 [H⁺] taken up by oxyhemoglobin occurs as a result of shifts in the _____ values of amino acids in the protein.
5. Upon oxygen binding to hemoglobin certain _____ bonds are broken between α and β subunit. These changes result in the _____ observed in the oxygen dissociation curve.
6. What is the relationship between the P₅₀ values for myoglobin, hemoglobin and cytochrome oxidase?
7. Cooperativity exhibited in the oxygen saturation curve for hemoglobin is often referred to as _____
8. What advantage does an oxygen carrier system with a sigmoid dissociation curve have over an oxygen carrier system with a hyperbolic dissociation curve?

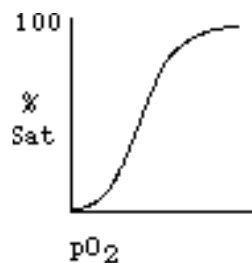
9. Since the heme group itself is the O_2 binding site in hemoglobin, what is the function of the globin part of the molecule?
10. Why would erythrocytes as packages of oxygen carriers be more effective in delivering oxygen in capillaries than having oxygen carriers circulate free in solution through the capillaries?
11. Each of the following represent the oxygen saturation curve for normal adult hemoglobin within mature erythrocytes. Sketch the curve one would expect for each of the following conditions listed relative to the "normal" curve given for adult red cells.



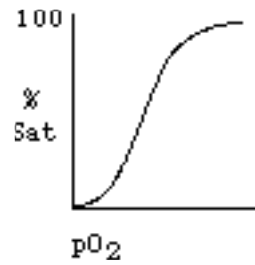
- a. Fetal erythrocytes



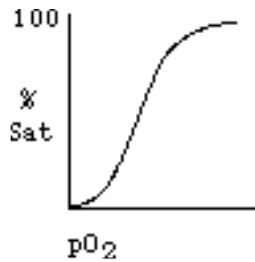
- b. CO_2 increased in the normal erythrocyte



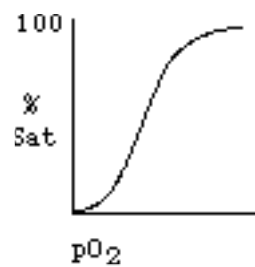
- c. A hypothetical compound X which preferentially binds to deoxyhemoglobin in normal adult erythrocytes



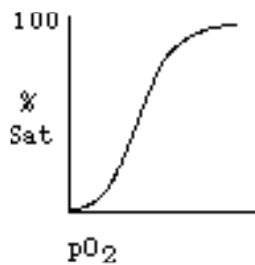
d. Comparison with cytochrome oxidase O₂ affinity



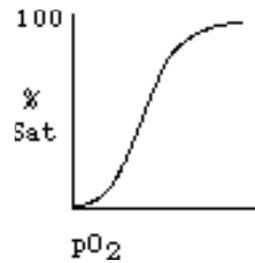
e. Increased 2,3-DPG in the adult RBC



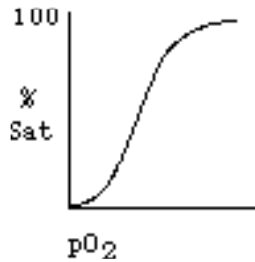
f. Affect of lactic acid produced in muscle on O₂ dissociation curve in adult RBC



g. Effect of increase in temperature on dissociation curve



h. Effect of pyruvate kinase deficiency



i. Comparison with myoglobin O₂ affinity

12. Biochemistry: A Case Oriented Approach, Montgomery et. al. 4th edition, p. 87.

Sickledex test: The Sickledex test is the proprietary name for a test that is rapidly gaining popularity as a quick, simple, and convenient test for HbS. It appears to depend on the lysing of the erythrocyte and the insolubility of reduced HbS. However, serious errors in this test are possible unless one has a clear understanding of its molecular basis.

There are always a few sickled cells in the blood of patients with the disease, but they are not present in the blood of those with the trait. The cells from the carrier can be made to sickle, however, as is done in the Sickledex test. The reagents used for this test consist of a phosphate buffer, saponin, which is used to lyse the erythrocytes, the dithionite, which is used to reduce the hemoglobin so that it will form visible aggregates if any HbS is present.

As one might suspect, the test will pick up the trait as well as the disease. It also responds positively to several other abnormal hemoglobins, to elevated amounts of plasma proteins, and to the use of too much blood in relation to the amount of reagents.

Screening programs have improved considerably in recent years. It is now even possible to diagnose the disease prenatally by analyzing for the defective gene itself, using DNA restriction

- enzymes and other modern DNA methods.
- a. The term "reduced" is used here to mean deoxygenate. Why does the test call for deoxygenation of the sample?
 - b. The statement is made: "As one might expect, the test will pick up the trait as well as the disease".
 - i. What is meant by the "trait" as opposed to the "disease" in the context?
 - ii. What assures that the test will pick up the trait as well as the disease?
13. At the lung, the chloride shift involves the transport of (ion)_____ into the red cell concomitant with the transport of (ion) _____ to the plasma.
 14. In the Haldane effect, _____ triggers the release of_____.
 15. a. What catalyzed reaction occurs in the whole blood which makes carbon dioxide carriage possible? (Write equation)
b. Which enzyme catalyzes the reaction?
 16. If the red cell intracellular pH is 7.25 and the dissolved CO₂ concentration is 0.9 meq/L, what is the intracellular bicarbonate concentration? $pK_a = 6.1$

Log Table	
(Abbreviated)	
<u>N</u>	<u>log N</u>
1.0	0
2.0	0.3
3.0	0.48
4.0	0.60
6.0	0.78
7.0	0.85
8.0	0.90
9.0	1.0

17. Why is there a coupled movement of anions across the red cell

membrane when the chloride shift takes place?

18. CO₂ reacts chemically with deoxyhemoglobin, illustrate (by equation) that chemical reaction and name the compound formed.
19. How does CO affect oxygen transport and/or utilization?
20. Explain how the isohydric shift is important in the physiological sense.
21. Circle the correct answer or fill in the blank as indicated.
 - a. The pH of the red cell interior is high/ low/the same in comparison with the pH of the plasma.
 - b. The concentration of a given diffusible anion in the red cell is less/more/unchanged in comparison with its concentration in the plasma. This relationship is due to the .
 - c. An individual with a diet of fats will have a higher/lower/ unchanged respiratory quotient in comparison with one who has a diet high in starch.
22. Biochemistry: A Case Oriented Approach, Montgomery et. al. 4th edition, p. 268.

A 23-year old woman from a nearby Amish community was seen with complaints of weakness and abdominal pain. Physical examination revealed moderate jaundice and distinct splenomegaly. She denied taking any drugs. Her hemoglobin was 1.6 mmol/L (10.9 g/dL), and her hematocrit was 27%. Her reticulocyte count was 4.5% of the erythrocyte count. She was therefore referred to a hematologist, who sought the cause of her problems through three sets of experiments described here.

Blood samples were collected from the patient and from a normal volunteer laboratory worker. Equal volumes of each were dispensed into a series of sterile tubes containing a citrate-oxalate anticoagulant mixture. Additions to each tube were made as shown in Table 5.9. All the tubes were gently shaken at 37°C for 48 hrs; then they were gently centrifuged so that any hemolysis could be observed in the clear supernatant. Results of these experiments are shown in Table 5.9, where presence of hemolysis is indicated on a scale of 1+ to 4+.

In the second set of experiments, fresh samples were collected from the patient and the volunteer. Each was analyzed for lactate before and after incubation for 48 hrs. and the change in lactate concentration was calculated for each. Incubation increased the lactate concentration of the patient's blood by 9.5% but in

the volunteered sample the difference in lactate concentration was 36%.

In the third set of experiments, blood cells from the patient and the volunteer were packed by gentle centrifugation, hemolyzed by addition of water, and samples of the hemolysates were separated by electrophoresis. The electropherograms were then stained by means appropriate to locate pyruvate kinase activity. The pyruvate kinase (PK) activity in the patient's blood was found to have a lower electrophoretic mobility than the activity from the control samples.

Based on the total evidence, a diagnosis of pyruvate kinase deficiency was made. From your review of this report respond to the following questions.

Tube Number	Additions to blood samples	Extent of hemolysis after incubation for 48 hours at 37°C	
		Patient	Volunteer
1	0	+++	0
2	Glucose, 1 mol/L	+++	0
3	ATP, 5 mol/L	0	0

- Hemolysis occurs when RBCs are unable to maintain cell integrity. In general, red cells need to produce two classes of metabolites to be used in maintaining cell integrity and preventing hemolysis. What are these two kinds of metabolites?
- Explain what the tests in Table 5.9 reveal. Leave no doubt that you understand the basic metabolic result which underlies the test.
- What is the significance of the second set of experiments in terms of the etiology?
- Define hematocrit and state the significance of the hematocrit of this patient in this case.
- How would you expect the 2,3-DPG content of the red cells of the patient to compare with that of the volunteer? Why?

APPENDIX I: Using Acrobat Reader with pdf Files

Portable Document Format (PDF) files can be read by Acrobat Reader, a free program which can be downloaded from the Adobe Web site (<http://www.adobe.com/acrobat>). If Acrobat Reader is installed on your system, it will automatically open simply by double-clicking on the pdf file that you wish to read.

Acrobat Window

The document will be displayed in the center of your window and an index will appear at the left side of the screen. Each entry in the index is a hypertext link to the associated topic in the text.

Using hypertext links in a pdf document is exactly like that in a web page or html document. When you place the cursor over a hypertext link, it changes to a hand with the index finger pointing to the underlying text. Clicking the mouse causes the text window to jump to that location. The index does not change. Magnification may need to be adjusted using the menu option in the lower part of the screen to optimize the view and readability. The best magnification is usually around 125%.

Subheadings in the index can be viewed by clicking on the open diamonds to the left of appropriate entries to cause them to point downwards. Clicking again will close the subheadings lists.

Hypertext links

Hypertext links in the text (not in the index) are indicated by blue underlined text. The cursor should change to a hand with the index finger pointing to this text when it passes over it. Clicking will cause the text page to move to the associated or linked text which will be highlighted in red underlined text. Red underlined text is not a hyperlink, only a destination.

How to back up to a previous window:

If you wish to return to a previous text window after following a hypertext link, use the black double solid arrow key at the top of the Acrobat window (or use the key equivalent "command - "). Acrobat keeps a record of your last 20 or so windows so that multiple steps back can be made by repeating the command.

Links to web sites

A number of url links to web sites are located in the pdf file and appear in blue underlined type starting with http:// (e.g. <http://>

www.som.siu.edu). Clicking on these should open a web browser such as Netscape and take you to those web sites. You may need to resize the Acrobat Window to view the web browser window displayed underneath it.

COMMENTS

I hope that you find this pdf file useful. Comments on how to make it better would be greatly appreciated. Please notify me in person or by email (enieder@som.siu.edu) of any errors so that they can be removed. The online version on the Biochem server can be easily updated.