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Mechanisms of Sickle Cell Anemia

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Title: Mechanisms of Sickle Cell Anemia

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Institution Affiliation: Boonshoft School of Medicine, Wright State University
Resources Associated: Individual and Group Readiness Assessment Test
Applications Worksheet
Summary Points Review Sheet
Comprehensive Author List: Gary M. Onady MD, PhD
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Description:

This team-based learning program is designed to supplement the first year Molecular Basis of Medicine course (Biochemistry Content) covering quaternary protein structure. This session compares the impact of amino acid substation on HbA, HbS, HbF on quaternary structure and function. Protein solubility, role and rate of oxygenation, hemoglobin concentration and rate of microvascular transient time relative to polymerization reactions are detailed to enhance appreciation as to mechanisms of disease impacted by quaternary structural defects. The team-based learning format includes a readiness assessment test that is taken individually and by teams, an applications worksheet that integrates basic knowledge components into medical decision making tasks, and a summary point review sheet used as a study guide toward formal examination assessments used throughout the course. Students prepare in advance of this session by reading the assigned article: Epstein FH. Pathogenesis and treatment of sickle cell disease. NEJM. 1997;337:762-769, and review of lecture material from “Protein Structure” and “Hemoglobin” course content that introduces protein biochemistry and electrophoresis properties.

Educational Objectives:

1. Demonstrate the importance of Hb role in disease states seen in clinical practice
   A. The type of cellular morphologies that can result in HbS disease
   B. Polymer effects on the erythrocyte membrane
   C. Mechanisms and importance of hydration status of the red blood cell
      i. Understand the ion transport systems that impact on cellular hydration
   D. Mechanisms involving vascular endothelium and vaso-occlusive disease

2. Identify potential of therapeutic interventions from the disease concepts identified above
   A. Know at which point in the mechanism of action these agents intervene
   B. Identify measured benefits for each of these agent
   C. Recognize potential harm that may result with the use of these agents

3. Integrate information previously received from lecture material into the most current and relevant information obtained from the medical literature: Epstein FH. Pathogenesis and treatment of sickle cell disease. NEJM. 1997;337:762-769.

4. Utilize Team-based Learning to integrate basic scientific and clinical information into creative problem solving thereby enhancing learning

Primary Learning Resource: Team-based Learning

Mechanisms of Sickle Cell Anemia Readiness Assessment Test for Both Individual (IRAT) and Group (GRAT) Administration

1. Which hemoglobin S amino acid is substituted for the β-6 glutamic acid in hemoglobin A?

   A. Lysine
   B. Arginine
   C. Valine
   D. Proline
   E. Glycine

2. Which microcirculation process is responsible for the irreversible double stranded hemoglobin polymer-fibrous membrane interaction resulting in distorted red blood cell morphology?

   A. Rapid deoxygenation
   B. Slow deoxygenation
   C. Decreased 2,3 DPG concentrations
   D. Decreased hemoglobin concentrations
   E. Increased concentrations of fetal hemoglobin

3. Which ion enhances Gardos channel opening and subsequent cellular dehydration resulting in the characteristic morphological red blood cell distortion in sickle cell disease?

   A. Cl^-
   B. K^+
   C. Na^+
   D. Mg^{++}
   E. Ca^{++}

4. Which agent has been demonstrated to induce fetal hemoglobin production?

   A. Hydroxyurea
   A. Clotrimazole
   B. Magnesium sulfate
   C. Calcium channel blocker
   D. Amiloride

5. How does the solubility of equal amounts of HbS and HbF compare to HbS alone?

   A. Half as soluble
   B. Equally soluble
   C. Twice as soluble
   D. Infinitely soluble
   E. Totally insoluble

6. HbF inhibits polymerization by which of the following mechanisms?

   A. γ87 glutamine prevents contact between β subunits of neighbor tetramers
   B. γ87 glutamine prevents contact of the double strand with the sickle fiber
   C. β6 valine prevents contact of the double strand with the sickle fiber
   D. β6 valine prevents contact between β subunits of neighbor tetramers
   E. γ87 valine prevents contact of the double strand with the sickle fiber

7. How is the time that elapses between deoxygenation and polymerization effected by HbS concentration where t_d is the elapsed time (delay time)?

   A. $t_d \approx k/[HbS]^2$
   B. $t_d \approx k/[HbS]^5$
   C. $t_d \approx k \times [HbS]^5$
   D. $t_d \approx k/[HbS]^{15}$
   E. $t_d \approx k \times [HbS]^{15}$

8. Which factor is most important factor in minimizing [HbS] density within the RBC?

   A. Decreasing K^+- Cl^- cotransport
   B. Increasing K^+-Cl^- cotransport
   C. Increasing cytosolic $[Ca^{2+}]$
   D. Stimulating the Ca^{2+}-dependent Gardos K+ channel
   E. Decreasing the intracellular [HbF] concentration

9. Which medication decreases potassium loss from the HbS red blood cell?

   A. Hydroxyurea
   B. Butyrate
   C. Acetate
   D. 5-azacytidine
   E. Clotrimazole

10. Delay of HbS microvascular transit time leads to vaso-occlusive crisis through enhanced binding of which intravascular molecular components?

    A. Thrombospondin binding to fibronectin
    B. CD36 binding to integrin complex $\alpha_4\beta_1$
    C. Fibronectin binding to vascular-cell adhesion molecule 1
    D. Activated platelet binding to fibronectin
    E. Inflammatory cytokines binding to CD36

Answers to IRAT Questions

1. The answer is C: The substitution is at the β-6 site where glutamic acid is replaced by valine. The β-6 site is also substituted with lysine but this is known as the HbC variant. Refer to page 762, first column, second paragraph of the Bunn article for this answer.

2. The answer is B: The key is to delay both the deoxyhemoglobin state and the transit time as much as possible during the microcirculation in the tissue beds. A more rapid deoxygenation results in multiple, independent polymers that result in granular deposits that do not alter the disk shape of the RBC. A decreased 2,3DPG concentration actually would favor the oxyhemoglobin proportion therefore decreasing the amount of Hb available for polymerization. Decreased [Hb] leads to decreased rate of polymerization as well. Increased [HbF] will help decrease the interaction between HbS units an further decrease the polymerization reaction. Refer to page 764, first column, third paragraph of the Bunn article for this answer.

3. The answer is E: The Gardos channel is a calcium dependent channel and is triggered by release of calcium in to the cytosol, which is stimulated by membrane distortion. The initial dehydration process is initiated by cell swelling or acidification, which stimulates the potassium-chloride cotransport channels. This begins the polymerization process, distorting the RBC membrane, which then stimulates calcium release from vesicles into the cytosol. Magnesium actually counteracts the effects initiated by the potassium-chloride channels. Refer to page 764, second column, fourth paragraph of the Bunn article for this answer.

4. The answer is A: Hydroxyuria is well known to induce HbF production, at lease initially in the first months of treatment of HbS patients. Clotrimazole sit of action is at the membrane side of the Gardos channel, magnesium countering the effects involving K-Cl cotransport, and amiloride with effects at GTP-dependent sodium channels. Refer to page 767, first column, second paragraph of the Bunn article for this answer.

5. The answer is A: The solubility of equal mixture of HbS and HbF is about twice that of HbS alone. Refer to page 764, first column, second paragraph of the Bunn article for this answer.

6. The answer is C: HbF inhibits polymerization whereby the γ87 residue prevents lateral contact of the double strand from the sickle fiber. Refer to page 764, first column, second paragraph of the Bunn article for this answer.

7. The answer is D: The time elapsing between deoxygenation and polymerization (delay time) is inversely proportional to the intracellular HbS concentration. Refer to page 763, figure and caption of the Bunn article for this answer.

8. The answer is A: Enhanced K^+- Cl^- cotransport has a major role in marked erythrocyte dehydration and when transient increases in cytosolic Ca^{2+} is present, the Ca^{2+}-dependent Gardos K^+ channel is triggered leading to further induced K^+ loss and water leading to additional cellular dehydration. Refer to page 764, second column, third and fourth paragraphs of the Bunn article for this answer.

9. The answer is E: Clotrimazole results in prompt reduction in cellular density and increases in intracellular K+ through inhibition of the Gardos channel. The other agents A-D effect cycling through increasing [HbF]. Refer to page 766, first column, second paragraph of the Bunn article for this answer.

10. The answer is C: The appropriate binding cascade in inflammatory triggered binding is depicted on page 766 Figure 3.

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Sickle Cell Anemia – Applications Worksheet

TEAM NUMBER: _____

Case:

An 18-year-old African-American female with known sickle cell anemia presents to the emergency room the morning after indulgent drinking of Tequila spiked punch, preceded by periods of nausea and vomiting. Her complaints more recently consist of severe pain in her back, chest and extremities. She relates she had a similar crisis two years ago, during an episode of influenza. Physical examination reveals a nauseated, individual writhing in pain with vitals demonstrating tachypnea, mildly elevated blood pressure, and pale nail beds/conjunctiva with diffusely tender extremities on limb palpation.

The following laboratory evaluation returns:

	Patient Value	Normal Value
Hemoglobin	9 g/dl	14-18 g/dl
Hematocrit	27%	37-48%
Sickledex	Positive	Negative

1. Vaso-occlusive ischemic pain experienced by this patient is triggered by which correct pairing of HbS fiber bundle orientation to red cell configuration?

	HbS Fiber Bundle Orientation	RBC Configuration
A.	Long axis orientation	Target cell formation
B.	Long axis orientation	Sickle cell formation
C.	Radial axis orientation	Sickle cell formation
D.	Projection along each axis	Sickle cell formation
E.	Projection along each axis	Holly leaf formation

2. Which clinical factor most likely accelerated this particular pain crisis?

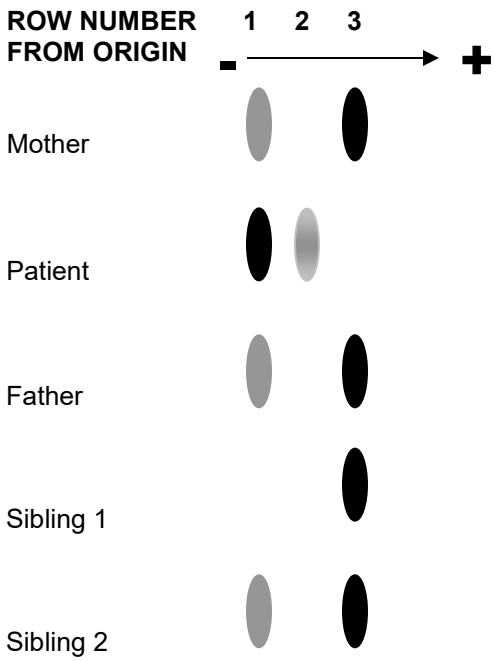
   A. Cellular dehydration resulting from effects of alcohol
   B. Allosteric inhibition of ethanol on O_2 binding at the β-HbS subunits
   C. The underlying anemia at 9 g/dl hemoglobin; 27% hematocrit
   D. Additionally finding that large volumes of water were consumed between Tequila shots
   E. Hormonal changes relating from adolescent maturation

3. What molecular change causes **polymerization** resulting in the **sickle cell** morphology?

   A. Deoxygenation must be rapid for the structural alignment leading to polymerization
   B. Oxygenation exposes β subunit interaction between strands forming polymerization site
   C. Deoxy state exposes β subunit interaction between strands forming polymerization site
   D. FIRST OxyHbS polymerizes THEN Deoxy HbS fibers align
   E. HbS fiber bundles point in direction of each cellular axis projection

A cellulose acetate hemoglobin electrophoresis sequence is run at pH 7.8 on the family of a patient diagnosed with sickle cell anemia results in the pattern indicated in the **FIGURE**:

FIGURE: Cellulose Acetate Hb Electrophoresis



4. Identify **ALL** Sickle Cell carriers (individuals without disease) in this family.

   A. *Mother*
   B. *Father*
   C. Sibling 1
   D. Sibling 2
   E. Patient

5. Identify the band from Row 2 in the **FIGURE**.

   A. HbA
   B. HbS
   C. HbF
   D. HbA/HbS
   E. HbSC

6. What is the therapeutic mechanism of action observed for clotrimazole?

   A. Stimulates the Gardos channel enhancing K^+ transport out of the cell
   B. Inhibits the Gardos channel diminishing K^+ transport out of the cell
   C. Decreases intracellular vesicle concentrations of calcium
   D. Induces Mg^{2+} efflux into red cells diminishing K^+ transport out of the cell
   E. Induces Mg^{2+} efflux into red cells increasing K^+ transport out of the cell

7. What therapeutic mechanism is responsible for the **long-term** benefit from hydroxyurea?

   A. Initially increases the concentration of HbF
   B. Initially decreases the concentration of HbF
   C. Induces a brief high white cell (neutrophillic) count increasing HbS-fibronectin binding
   D. Gradually induces low white cell (neutropenia) count decreasing HbS-fibronectin binding
   E. Increases reticulocyte (immature red cell) numbers preventing adherence interaction with endothelium

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Sickle Cell Anemia – Applications Worksheet

(ANSWER KEY)

TEAM NUMBER: _____

Case:

An 18-year-old African-American female with known sickle cell anemia presents to the emergency room the morning after indulgent drinking of Tequila spiked punch, proceeded by periods of nausea and vomiting. Her complaints more recently consist of severe pain in her back, chest and extremities. She relates she had a similar crisis two years ago, during an episode of influenza. Physical examination reveals a nauseated, individual riving in pain with vitals demonstrating tachypnea, mildly elevated blood pressure, and pale nail beds/conjunctiva with diffusely tender extremities on limb palpation.

The following laboratory evaluation returns:

	Patient Value	Normal Value
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1. Vaso-occlusive ischemic pain experienced by this patient is triggered by which correct paring of HbS fiber bundle orientation-red cell configuration?

	HbS Fiber Bundle Orientation	RBC Configuration
A.	Long axis orientation	Target cell formation
B.	Long axis orientation	Sickle cell formation
C.	Radial axis orientation	Sickle cell formation
D.	Projection along each axis	Sickle cell formation
E.	Projection along each axis	Holy leaf formation

- The polymer orientates along potential exoskeleton structures associated with the RBC leading to the sickle shape (sickle if along longitudinal axis, holly-leaf if each axial orientation).

2. Which clinical factor most likely accelerated this particular pain crisis?

   A. Cellular dehydration resulting from effects of alcohol
   B. Allosteric inhibition of ethanol on O_2 binding at the β-HbS subunits
   C. The underlying anemia at 9 g/dl hemoglobin; 27% hematocrit
   D. Additionally finding of large volumes of water consumed between Tequila punch shots
   E. Hormonal changes relating from adolescent maturation

- Alcohol leads to cellular dehydration; vomiting leads to intravascular dehydration. Refer to pg 764, 2nd col, 2nd para of article. Not C as td = k/C[15] predicts anemia protects against sickling.

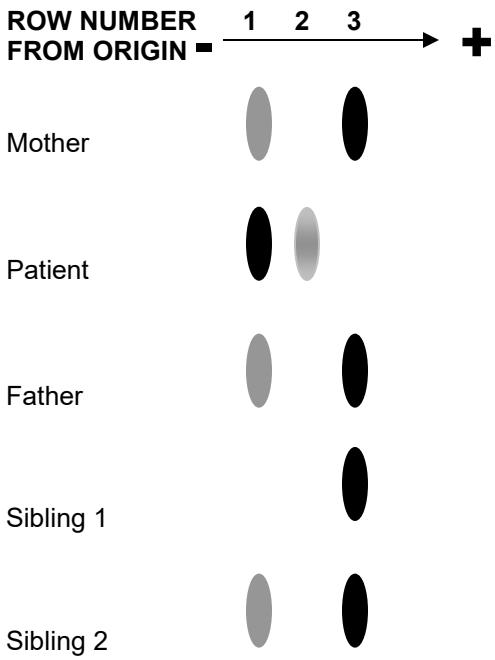
3. Which is true of the HbS structural change that leads to the **sickle** polymerization reaction?

   A. Deoxygenation must be rapid for structural alignment that leads to polymerization
   B. Oxygenation exposes β subunit interaction between strands forming polymerization site
   C. Deoxy state exposes β subunit interaction between strands forming polymerization site
   D. FIRST OxyHbS polymerizes THEN Deoxy HbS fibers align
   E. HbS fiber bundles point in direction of each cellular axis projection

- Oxygenation prevents β-6 interacting between Hb molecules. The deoxy β-6 interaction forms initial polymer nidus. Refer to pg 762, 1st column, 3rd paragraph and Figure 1 of the article. The polymer then orientates along potential exoskeleton structures associated with the RBC leading to the sickle shape (sickle if along longitudinal axis, holly-leaf if each axial orientation).

A cellulose acetate hemoglobin electrophoresis sequence is run at pH 7.8 on the family of a patient diagnosed with sickle cell anemia results in the pattern indicated in the **FIGURE**:

FIGURE: Cellulose Acetate Hb Electrophoresis



4. Identify all Sickle Trait individuals in this family.

   A. Mother
   B. Father
   C. Sibling 1
   D. Sibling 2
   E. Patient

   - Refer to lecture notes and Bunn article which Integrates this detail

5. Identify the band from Row 2 in the **FIGURE**.

   A. HbA
   B. HbS
   C. HbF
   D. HbA/HbS
   E. HbSC

   - HbF is the intermediate band between HbS (Row 1) and HbA (Row 3). HbSC would require that the Mother and father have different single gray bands.

6. Which therapeutic mechanism of action is observed for clotrimazole?

   A. Stimulates the Gardos channel enhancing K^+ transport out of the cell
   B. Inhibits the Gardos channel diminishing K^+ transport out of the cell
   C. Decreases intracellular vesicle concentrations of calcium
   D. Induces Mg^{2+} efflux into red cells diminishing K^+ transport out of the cell
   E. Induces Mg^{2+} efflux into red cells increasing K^+ transport out of the cell

   - Inhibition decreases K^+ channel activation by Ca^{2+} decreasing cell K^+ and the accompanying water loss with cellular dehydration. Refer to pg 766, 1st col, 2nd para, article.

7. What therapeutic mechanism is responsible for the **long-term** benefit from hydroxyurea?

   A. Initially increases the concentration of HbF
   B. Initially decreases the concentration of HbF
   C. Induces a brief high white cell (neutrophillic) count increasing HbS-fibronectin binding
   D. Induces chronic phase neutropenia leading to decreased HbS-fibronectin binding
   E. Increases reticulocyte numbers preventing adherence interaction with endothelium

- Initial increase concentration of HbF dilutes interaction between HbS molecules. Neutropenia induced by hydroxyurea further contributes to drug efficacy. Refer to pg 767, 1st column, 2nd paragraph of the Bunn article for this answer. Answer A is only true of SHORT TERM activity. Discussing this answer would serve bring out the importance of inflammation in sickle cell disease.

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Sickle Cell Anemia Team Learning Curriculum
Summary Review Points

1. Understand the pathogenesis of sickle cell anemia

Sickle Cell (HbS) Anemia is a molecular disease involving a single base substitution (HbA-β6 Glutamic acid replaced by valine in HbS) for the gene encoding the human β-globin subunit.

RBC Hb packaging requires extraordinary protein solubility, whereby oxygenated HbS creates hydrophobic interaction between other Hb molecules that triggers large polymer aggregation. Deoxy-HbS polymerization is the primary event in pathogenesis of SS disease resulting in RBC shape distortion that decreased deformability leading to rigid HbS RBCs, which is responsible for vaso-occlusive process characterizing this disease. Fiber structure elucidated by 3-D High-resolution EM demonstrates twisted rope structure consisting of a 14-strand polymer. Deoxygenated HbS assumes variety of shapes by Transmission EM. One includes the sickle shaped (or banana shaped) cell resulting from fiber bundles oriented along cellular long axis **THIS IS THE SHAPE THAT CAUSES MICROVASCULAR OBSTRUCTION** AND a holly-leaf shaped cell resulting from HbS fiber bundles point in direction of each projection.

2. Understand mechanisms for sickling

RBC in microcirculation releases O_2. The deoxyHbS so formed induces conformational change that results in β subunits move away from each other. The β6 valine hydrophobic patch then binds to hydrophobic β subunit site of **another** HbS tetramer. **Only one** of two β6 valines sites in each HbS tetramer makes this contact.

Kinetic Rate Effects. Delay time elapsing between DeoxyHbS and polymer formation is inversely proportional to [HbS]. The typically microcirculation transit time is short relative to HbS delay time and >80% of cells do not form polymer. Rate of deoxygenation (rapid or slow) is dependent on the three **independent** factors that include 1) Cellular degree of deoxygenation; 2) Intracellular [HbS] whereby dense HbS cells more likely to distort and become rigid (see below); 3) Amount of HbF present. However, rapid deoxygenation results in multiple independent polymerization, which creates granules that **DO NOT** alter RBC shape. Slow deoxygenation allows for single HbS nucleus molecular aggregate forms leading to growth of fiber, fiber alignment, which leads to cell sickling. Continued distortion of cell shape by alignment of HbS fiber projections plays a critical role in perturbing HbS RBC membrane structure and function. DeoxyHbS polymerizes forming fibers which then align distorting RBCs into characteristic shape.

Solubility is effected by: 1) Hb intracellular concentration 2) delay times AND also 3) other Hb types. HbS-HbF is nearly twice as soluble as HbS-HbS. HbFγ87 glutamine residue prevents lateral double strand fiber contact.

Interaction of HbS RBCs and Vascular Endothelium creates the potential for sickle cell initiation of vaso-occlusive event that primarily depends on a rate of polymer formation within range of capillary transit time. Any delay of HbS transit in microcirculation has a critical effect on vaso-occlusion. HbS cells have a sticky surface and attach more readily to endothelial cells. Adherence strongly correlates with severity of disease. Reticulocytes have integrin comples $\alpha_4\beta_1$, which binds fibronectin and vascular-cell adhesion molecule 1. This fibronectin is expressed on endothelial cells after inflammatory cytokine activation (TNF_a). Microvascular endothelial cells and HbS reticulocytes have CD36 which binds to thrombospondin secreted by activated platelets. Thrombospondin binds sulfated glycans on HbS RBCs**.** VHW von Willebrand factor also

contributes to adhesion. Interactions of the above reactants with inflammatory stress ultimately leads to HbS RBC adhesion to endothelial cells. Neutrophil binding to fibronectin may additionally influence vaso-occlusive episodes.

3. Be aware of specific histo/biochemistry of sickling process.

RBC Volume Dysregulation is related to the broad range of density distributions are seen in HbS RBCs. Least dense interactions are due to presence of high number of reticulocytes. Polymerization begins to damage cellular membrane leading to **INTRACELLULAR** dehydration.

K^+-Cl^- co-transport is the most important contribution to dehydration in all RBC types (with **HbA** this cotransport only active in reticulocytes). K^+-Cl^- cotransport rates are much higher in mature HbS (and HbC) RBCs. Dysregulation is then induced by both cell swelling and acidification (particularly in sites of stagnant circulation). This process is **inhibited** by Mg^{2+} ions. This inhibition leads to decreasing intracellular [HbS] concentration resulting in less sickling. With less sickling there will be less hemolysis therefore a higher total hemoglobin concentration.

Ca^{2+}-activated K^+ efflux also plays a role. HbS have **increased** amounts of Ca^{2+} **compartmentalized** in intracellular vesicles. While there is **normal** [Ca^{2+}] **in cytosol** UNTIL the cell sickles leading to transient cytosolic Ca^{2+} increase. This triggers Ca^{2+} dependent K^+ (Gardos) channel which provides a second pathway for sickling induced K^+ loss AND water. This response is **inhibited** by clotrimazole.

Increased cellular density leads to final stage of process – **Irreversible Rigid** cell sickling even if fully oxygenated (lacking polymer) the characteristic sickle shape is maintained.

4. Know therapeutic mechanisms and strategies used in treating sickle cell anemia

Inhibition of HbS Polymerization. No such agent currently exists. Such an agent needs to be readily absorbed through the GI tract circulate unbound to plasma proteins and readily penetrates the erythrocyte membrane that is then specific and binds strongly to HbS that inhibits polymerization.

Reduction of Intracellular Hb Concentration can be achieved by induction of hyponatremia leading to osmotic RBC swelling. This requires meticulous laboratory monitoring making it cumbersome for outpatient use.

Inhibition of K^+ and water loss leading to reduction in [HbS]. Specific inhibition of Gardos channel by the antifungal drug clotrimazole can achieve this. Lower doses than used for anti-fungal activity are effective. Striking reduction in density and irreversible sickled cells is achieved, resulting in increased intracellular K^+, small increases in [Hb] and significant decrease in bilirubin (indicating amelioration of hemolysis). Intracellular divalent cations (Mg^{2+}) can be utilized which has demonstrated 50% K^+-Cl^- cotransport inhibition, which further resulted in 50% inhibition of K^+-Cl^- cotransport that is accompanied by a significant decrease in erythrocyte density which increases [Hb].

HbF Induction can be achieved with 5-azacytidine – Antineoplastic inhibiting DNA methylation that markedly increases HgF; however, hydroxyurea is the only agent currently in use, as it is relatively nontoxic. Any observed myelosuppresive effects are readily reversible and this agent is not known to induce tumors. This agent results in marked increases in HbF and HbF%. Reduction in hemolysis is associated along with a slight increase in [Hb]. Clinically, there is a reduced incidence of acute chest syndrome. Hydroxyurea also reduces need for transfusions and clinical events requiring hospitalizations. There is an inverse correlation of F cells with rate of painful crises; however, this is observed only for 3 month of Rx.

A strong correlation between neutropenia accompanying treatment and pain crisis however is seen in >2 years. It is suspected that there is less SS neutrophil binding to

fibronectin. Reduction in reticulocytes and young, low-density HbS distributes cells less likely to adhere to vascular endothelium.

Erythropoietin has a possible role to stimulate HbF production.

Butyric acid analogues role in HbF regulation are being studied. This agent results in delayed switch of HbF to HbA of infants to diabetic mothers. Enhancement in expression of□ g-globin gene in erythroid progenitors from patients with HbS results.

Bone marrow transplantation as a cure is also being considered.

This **Summary Review Points** section is provided to the students AFTER THE TBL SESSION as a study guide for questions that appear on formal written examinations covering the entire course content.

INSTRUCTOR'S GUIDE:

General Procedures

1. Sessions begin with an Individual Readiness Assessment Test (IRAT), taken by each student, designed to assess background knowledge from a pre-assigned reading that covers session content. This same test is again administered to the entire team as a Group Readiness Assessment Test (GRAT), which serves to bring all students on a team to the same level of preparedness prior to starting the third Applications component of team learning. This session is **CLOSED BOOK**. General format of these components is as follows:
   A. IRATs and GRATs consist of the same 10 questions.
   B. IRAT answers are recorded on scantron sheets and individual grades assigned.
   C. GRAT answers are recorded on IF-AT forms (Scratch and See Answer Sheets, for more information got to www.epsteineducation.com/) Use of IF-AT forms encourages active teaching by student team members through academic discussion then consensus answer choice. Group grades are assigned to each student for this component).
   D. An additional 5 minutes are used for teams to discuss selected GRAT clarifications after the IFAT forms have been collected for grading.

2. The Applications Worksheet consists of multiple choices, best answer questions regarding this case presentation, which serves to integrate **BOTH** Team reading material **WITH** background knowledge obtained from the lecture content. This session is **OPEN BOOK AND LECTURE NOTES**.
   A. Students actively discuss their team reasoning for an answer, which provides insightful student teaching through comparison of opinions of answer options. This session uses a "diving card" – A,B,C,D, E format of simultaneous report. When time is up, each group must indicate their answer by holding up their answer card.
   B. The remaining time (a minimum of 30 minutes) consist of inter-TEAM review of the Applications Worksheet answers. More time can be allotted to this discussion phase with early advancement from the flag system (each group receives a flag; flag up indicates the group is ready to move on).

3. Faculty serves as facilitators (not lectures) in this format and direct the knowledge exchange by choosing the teams that will contribute to the discussion.

4. Same group assignments are maintained throughout this course.

5. Appeals Process
   A. IRAT/GRAT appeals for reconsideration of alternative answers must be submitted within 24 hours of the TBL session.
   B. Applications appeals for reconsideration of alternative answers must be submitted within 1 week of your TBL session.

This guide attempts to optimize a teaching environment for a class of 100 students. The room is divided to accommodate 4 to 5 teams per facilitator to optimize more intimate constructive discussion. Our approach is to run 2 sessions with 2 facilitators in one afternoon as illustrated below. Prior experience from running sessions with 9 teams made it difficult to control peripheral discussion, resulting in students becoming detached and therefore unable to contribute to the main discussion. Facilitators are advised to read pertinent background lecture material and the assigned article in anticipation of integrative questions that may arise from discussions, as the Application constructively integrates lecture and article content. Facilitators are additionally provided with an answer key that anticipates integrative discussion. All facilitator material is distributed 2 weeks prior to the session to provide lead for clarifications.

TBL Operational Structure

Time 0 minutes Begin IRAT
Time 12 minutes End IRAT
At the end of this session:
1) IRAT scantrons **ONLY** are placed into IRAT packet which is then collected.
2) Quiz question sheets are withheld for use in the GRAT (Do not place this in the IRAT folder).

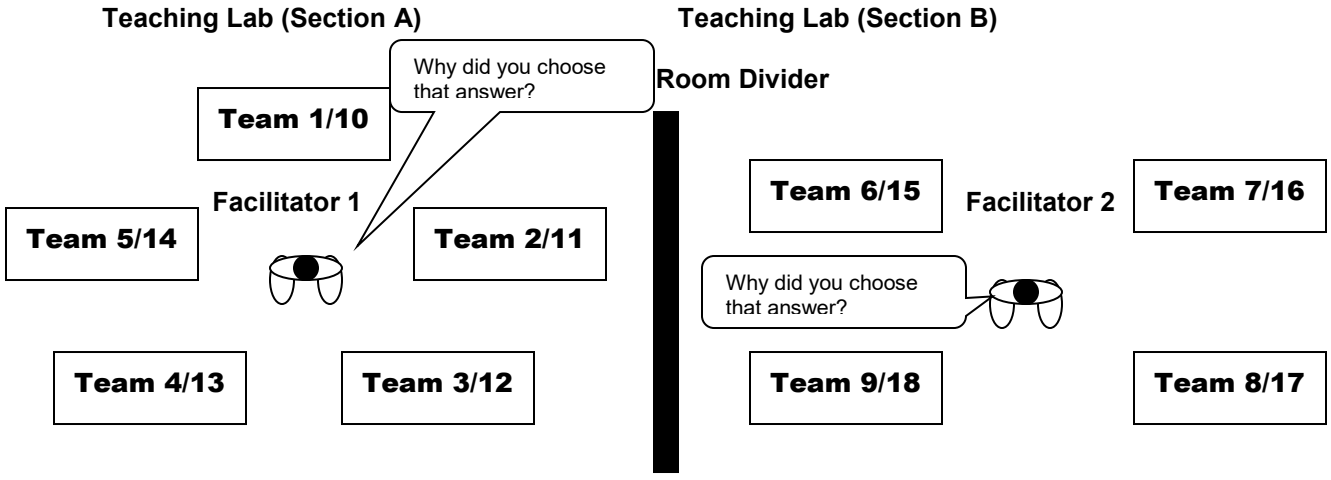
Time 0 minutes Begin GRAT
Time 10 minutes End GRAT
At the end of this session:
1) GRAT IF-AT forms **AND ALL** Quiz Question sheets are placed into the GRAT packet.
2) The GRAT Q/A Session follows.

Time 5 minutes GRAT Q/A Session

Time 0 minutes Begin Applications Worksheet
Time 52 minutes End Applications Worksheet
At the end of this session:
1) Groups Composite Worksheet is placed into the APPLICATIONS packet which is then collected.

Time 0 minutes Begin Discussion Session*
Time 30 minutes End Discussion/TBL Session
At the end of this session:
1) All applications material is collected.
2) Any further changes in team answers are entertained through the appeals process.

Suggested Teaching Lab Set Up



Time/Grade Weight Detailed

The combined grade average for the TBL sessions account for 20% of the entire course grade. The weight of each TBL component is itemized below. Other components contributing toward the course grade (80%) is assessed by 3 comprehensive written exams. Peer evaluation is a method used in holding students accountable for team participation (the IRAT additionally serves this purpose); therefore, students are asked to formally grade each group member at the conclusion of the course. A full discussion of grading and peer evaluation used in TBL can be found at http://www.ou.edu/idp/teamlearning/materials.htm, then choosing the link to peer evaluation.

Component	Grading
IRAT	20% of final score
GRAT (Same questions as IRAT)	20% of final score Correct 1st choice – 1 point Correct 2nd choice – 0.5 point Scratches thereafter – Pointless
Application Worksheets	60% of final score
Diving Card/Discussion Review	Multiple correct answers 1 point each correct answer
Peer Evaluation	Weighing Factor to Final Team Grade

The 0.25 GRAT and 0.75 Applications = Team Grades (TG)
TG/[1 – TG] x Peer Evaluation Weighing Factor = Weighted Team Grade (WTG)
The Final TBL Grade = 0.20 IRAT x 0.80 WTG

Lessons Learned From This TBL Session

Question 1: Some students question why the holly leaf cell deformity does not contribute to the vaso-occlusive process. Discussion should bring out that the holly leaf shape is less rigid and therefore diminishes inflammatory surface contact between the RBC and epithelium that appears to play a major role in the vaso-occlusive crisis. This also provides the chance to try to elicit that rapid deoxygenation only leads to a hemoglobin precipitate (not a polymer) that additionally has little effect at leading to a distorted rigid cellular structure.

Question 3: The facilitator may need to emphasize (from distractor choice C) that the anemia is the effect not the cause of the disease.

Questions 4/5: Students may not appreciate that there is no gene for HbA and therefore no ability to produce HbA. Appreciation for increased production of HbF as a genetic potential to help mediate the effects of HbS interactions may also be missed and therefore some discussion of the electrophoresis band patterns identified in these questions may need to be highlighted during the discussion.

These issues were clarified during discussion and no appeals were submitted from this session.

Keywords:
Quaternary Protein Structure; Molecular Biology Hemoglobins; Anemia, Sickle Cell

ACGME Competencies:

- Patient Care covering appropriate and effective treatment of Sickle Cell disease
- Medical Knowledge that integrates molecular biological mechanisms of disease with rational treatment options that incorporate this theoretical knowledge
- Practice-Based Learning and Improvement that involves appraisal and assimilation of scientific evidence toward improving patient care