HEMATOLOGY LABORATORY AND LECTURE
WEEK 1

HEMATOLOGY

- the study of blood and its components
- clinical, morphologic, and laboratory disorders of the blood and other blood-forming organs.
    - Clinical – understanding symptoms
    - Morphologic – structure and form; observable size and shape
    - Laboratory disorder – laboratory techniques and tests
    - Blood-forming organs – bone marrow, spleen, lymph nodes

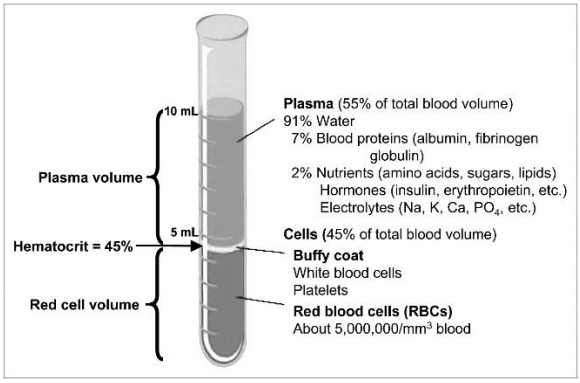
William Hewson

- Father of hematology
- Coagulation factor 1
- Investigated the structure of the lymphatic system and described red blood cells.
- Described that red blood cells are biconcave in shape – and its importance

Total Blood Volume: 5-6 L

BLOOD COMPONENTS

- Packed red blood cells (45%)
- Buffy coat (<1%)
    - White blood cells
        - Neutrophil – presence of bacteria
        - Eosinophil – there's occurrence of allergy
        - Basophil – presence of parasite
        - Monocyte – responsible for phagocytosis
        - Lymphocyte – presence of virus
    - Platelets
- Plasma (55%); Serum - clotted
    - Has fibrinogen
    - Unclotted
    - 90% H2O
    - 10% proteins, CHON, vitamins, hormones, enzyme lipids, and salts.



COMMON LABORATORY TESTS

- Complete Blood Count (CBC)
    - RBC Count
    - WBC Count
    - Platelet Count
    - Hemoglobin
        - Measurement relies on a weak solution of potassium cyanide and potassium ferricyanide, called *Drabkin reagent*.
    - Hematocrit
        - Ratio of the volume of packed RBCs to the volume of whole blood
    - Differential count
        - Which type of WBCs are present
    - RBC Indices
        - Hemoglobin and hematocrit ratio
- Reticulocyte count – precursor of RBC; to access those with anemia or if the bone marrow is functional
- Bleeding time and clotting time
- PT (Prothrombin Time) and APTT (Activated Partial Thromboplastin Time
    - PT: monitoring warfarin, liver function
    - APTT: monitoring heparin, clotting factor deficiency
- Toxic granules – seen in the granulated WBC (Neutro, Eo, Baso); shows a larger and darker granules
- D-dimer test – protein fragments; for blood clotting process
- ESR (Erythrocytes Sedimentation Rate) - monitor the course of inflammatory conditions such as rheumatoid arthritis, infections, or certain malignancies.
- DVT (Deep Vein Thrombosis) /DIC (Disseminated Intravascular Coagulation) – symptoms of blood clotting

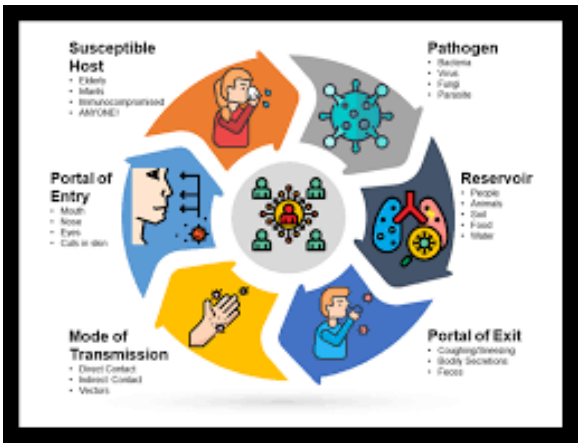
CHARACTERISTICS

- Color
    - Arterial Blood
        - Bright scarlet red
        - Oxygenated, high oxygen hemoglobin
    - Venous Blood:
        - Dark red
        - Deoxygenated, low oxygen content
- Viscosity
    - Resistance to flow
    - Thick and sticky fluid that normally flow with difficulty
- Specific Gravity
    - 1.055 – 1.065
- pH
    - 7.35 – 7.45
    - Problems id pH goes off:
        - Impaired enzymes
        - Oxygen exchange problems
    - <7.35 – Acidosis
    - >7.45 – Alkalosis

HEMATOLOGY LABORATORY AND LECTURE
WEEK 1

SAFETY IN HEMATOLOGY LABORATORY

- Chain of infection is the spread of infection from person to person.



- Disrupting one link of the chain will stop the cycle
- Handwashing
  - Targets the 4th link
  - Dirty materials are eliminated
  - Soap action inactivates microorganisms
  - Requires running water
- Hand hygiene
  - Handwashing
  - Alcohol based antiseptic hand cleanse
- Routine hand washing
  - Uses plain soap and water
- Hand antisepsis
  - Uses antimicrobial soap to remove transient microorganisms

When to do handwashing?

- Before and after:
  - Removing gloves
  - Patient contact
  - Contact with contaminated things

LABORATORY PPE

- Protects from splashes of blood and specimen
- Must be removed after leaving the room of the patient

DONNING	DOFFING
1. Gown	5. Gloves
2. Mask	6. Goggles
3. Goggles	7. Gown
4. Gloves	8. Mask

BASIC METHODS

- Phlebotomy – blood collection

2 General sources of blood

- Peripheral Blood/ Capillary
  - Small quantities needed
  - Mixture of venous blood, arterial blood, and tissue juices
  - Micro chemical techniques
  - Patient: babies, burned patients or amputees
  - Point-of-care testing
  - Site of puncture:
    - Adult – 3rd or 4th non dominant finger
  - Infants – plantar surface of heel or large toe
  - Ivy's method of bleeding time, puncture site is in the arm
- Venous Blood
- Arterial Blood
  - For Blood Gases Determination (ABG)
  - Requires extensive skills (only doctors and RMTs that undergone seminar is permitted)
  - Radial Arteries (thumb side of wrist)
- Umbilical cord Blood – rarely used
  - Extraction for newborn babies
  - Collect and preserve stem cells from the tissues of umbilical cord

SKIN PUNCTURE DEVICE

- Stainless steel blood lancet
- Plastic lancet with pinching device
- Automatic lancet

CONTAINERS:

- Capillary tube
  - Blue – no anti-coagulant
  - Red – heparinized tube
    - NOTE: coagulant + coagulant = dilute
- Microcontainer or bullet tubes

PROCEDURE:

- Perpendicular to the lines of patient's finger
- Must not be deeper than 2mm; deeper may be at risk or bone injury

HEMATOLOGY LABORATORY AND LECTURE
WEEK 1

- Wipe first drop of blood – due to presence of tissue juices
- Small amount of pressure – since hemolysis might occur

VENOUS BLOOD

- Tests requiring large quantities of blood
- Patients with visible vein
- Use of tube

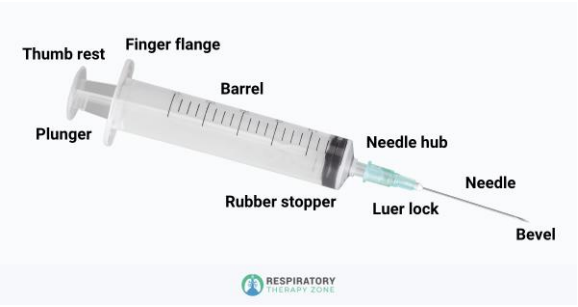
SITES OF PUNCTURE: Antecubital fossa

- Median cephalic/ cubital
- Cephalic
- Basilic – near median and bronchial artery

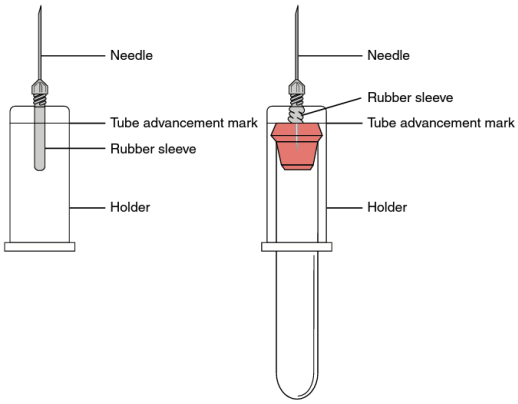
SYRINGE METHOD

- Adult: 21g (1-1.5")
- Pediatric: 23-25g
- Blood donation 16-18g

PARTS OF THE SYRINGE:



EVACUATED TUBE SYSTEM (ETS)



- Rubber sleeve/ sheath – to prevent leakage of blood when changing tube

VENIPUNCTURE DEVICES

- S-Monovette System by Sarstedt (tube and syringe in one
- Torniquet
  - Length: 18-20" inches
  - Width: 1 inch
  - Place: 3-4 inches away from the puncture site (7.5 – 10cm) – improper placing of torniquet results to collapsing of vein
  - Not to exceed 1 minute – exceeding will result to hematocrit
  - BP cuff as torniquet: 40 – 60 mmHg – recommended for bleeding time
  - Seraket – seatbelt torniquet; for allergic patients
- Winged infusion sets/ butterfly
  - Used for delinquent veins (elderly and very young)
- Evacuated tubes

Closure Color	Collection Tube	Mix by Inverting
BD Vacutainer® Blood Collection Tubes (glass or plastic)		
	• Blood Cultures - SPS	8 to 10 times
	• Citrate Tube*	3 to 4 times
or	• BD Vacutainer® SST™ Gel Separator Tube	5 times
	• Serum Tube (glass or plastic)	5 times (plastic) none (glass)
	• Heparin Tube	8 to 10 times
or	• BD Vacutainer® PST™ Gel Separator Tube With Heparin	8 to 10 times
or	• EDTA Tube	8 to 10 times
	• Fluoride (glucose) Tube	8 to 10 times

HEMATOLOGY LABORATORY AND LECTURE
WEEK 1

- Antiglycolytic Agent
  - Inhibits the use of glucose of blood cells
  - Inhibition may be necessary if testing of glucose is delayed
  - Sodium fluoride and lithium iodoacetate
- Anticoagulant
  MECHANISM OF ACTION:
  - EDTA (Ethylenediaminetetraacetic acid)
    - Chelate/ binds calcium
  - Sodium citrate
    - Chelates/ binds calcium
  - Lithium heparin
    - Inhibits thrombin (III) – clotting factor
- Clot activator
  - Silica clot activator
    - Platelet activation and clotting factor thrombin
    - Increase surface area
  - Helps initiate/ enhance the clotting mechanism
- Separator gel
  - Serve as a separation barrier between serum and cells or between plasma and cells
  - Cannot be used for blood banking since it might interfere with test (especially cross matching)
    - BB – use plain red tube

SPECIMEN CONSIDERATION IN HEMATOLOGY

Psychologic Factors affecting:

- Posture
  - Due to shifting of body water from blood vessels to interstitial spaces
  - Elevated: lipid, enzymes, and proteins
- Diurnal Rhythm
  - Daily body fluctuations that occur
  - Affected: cortisol, ACTH, Fe, and eosinophil
  - Sleep-wake cycle
  - Cortisol: has inhibitory effect in eosinophil; normally elevated in the morning
- Exercise
  - Affected: creatinine, proteins, creatinine kinase, aspartate, transaminase, and lactate dehydrogenase
  - Muscle breakdown
  - Metabolic release
- Stress
- Diet
  - 10-12 hrs. prior to blood draw
  - Glucose: 6-8 hrs.
- Smoking
  - Increased: WBC count, cortisol level
  - Cortisol: due to nicotine, release by angina glands
  - Initiates inflammation

COMPLICATIONS

- Hematoma
  - Petechiae: < 3mm
  - Purpura: 3-10mm
  - Ecchymoses: > 10mm
- Syncope/ Fainting – if occurred, continue the blood draw
- Failure to draw blood
- Edema – due to improper needle placement, vein damage, prolonged pressure
- Obesity
- Hemoconcentration – due to prolonged torniquet
- Hemolysis
- Vomiting and choking
- Allergies – ask patient

10 COMMANDMENTS OF PHLEBOTOMY

THOU SHALL...

1. Protect thyself from injury
2. Identify the patients correctly
3. Puncture the skin at about 15 deg angle
4. Glorify the median cubital vein
5. Invert tube with additives immediately after collection
6. Collect specimens only from an acceptable site
7. Label specimens at the bed side
8. Stretch the skin at the puncture site
9. Know when to quit (2 attempts)
10. Treat all patients as if they were family

HEMATOLOGY LABORATORY AND LECTURE
WEEK 1

11.

ADDITIONALS FOR HEMATOLOGY LABORATORY

- Ideal handwashing:
    - Surgical: 2 mins
    - Routine (WHO) 1 min
- Handwashing song: Happy Birthday

SPECIMEN

- Blood
- Total blood volume
    - Male – 5-7L
    - Female – 4-6L
- Blood components
    - Fluid (55%)
        - Plasma
        - Serum
    - Formed components (45%)
        - RBCs
        - WBCs
        - Platelets
    - Gaseous portion
        - O
        - CO2
        - CO – biproduct of hemolysis

ORDER OF DRAW

- For Syringe and ETS:
    - Yellow – Sterile tube for blood culture; Sodium polyanethole sulfonate
    - Light Blue – Sodium citrate
    - Red – No anti-coagulant
    - Green - Heparin
    - Purple/ Lavander - EDTA
    - Gray – Sodium fluoride

    - Black = Sodium citrate

SKIN PUNCTURE

1. Blood gases
2. EDTA micro-collection tubes
3. Other micro-collection tubes with anti-coagulant
    - Gray
    - Green
4. Serum micro-collection tubes

- Optimum concentration of EDTA
- 1.5mg/mL
- EDTA:
    - Choice of hematology
    - Maintains the morphology of cells (particularly RBCs)
    - Duration: 2 hrs.
    - Liquid EDTA: sequestrene

STEP-BY-STEP (VENIPUNCTURE) PROCEDURE:

1. Handwash
2. Prepare materials
3. Explain procedure to the patient
4. Introduce yourself and know your patient
5. Palpate the site – antecubital fossa
    - Alternatives: back of hand, wrist, ankle
    - Options: median, cephalic, and basilic
6. Disinfect the puncture site – use 70% isopropyl alcohol

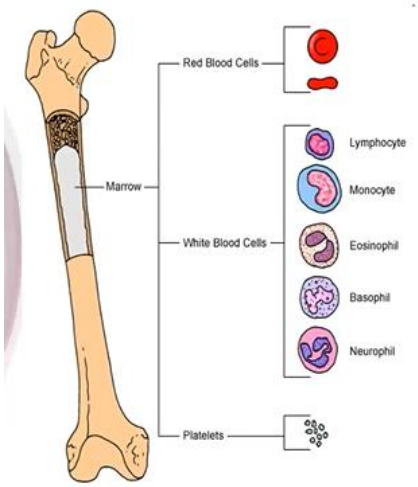
- Angle: 15-30 deg

SKIN PUNCTURE

- 42 deg
- Sites:
    - Ear lobe – less pain, less tissue juices
    - Adult: 3rd and 4th non dominant hand
    - Pediatric: plantar surface of heel or large toe
- To facilitate the free flow of blood
- To remove epidermal cells
- To remove the excess tissue fluids

HEMATOLOGY LECTURE
WEEK3 - MED225

HEMATOPOIESIS/ CELL KINETICS

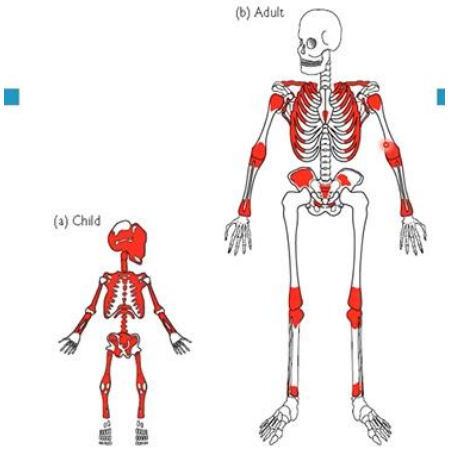


Hematopoiesis

- The process through which the body produces blood cells
- It is a highly regulated and a dynamic process that ensures the continuous replenishment of a various cellular component
- Occurs in the bone marrow, where **hematopoietic stem cells** reside
    - Multipotent
    - Has the ability to transform into various types of blood cells: RBCs, WBC, and platelets

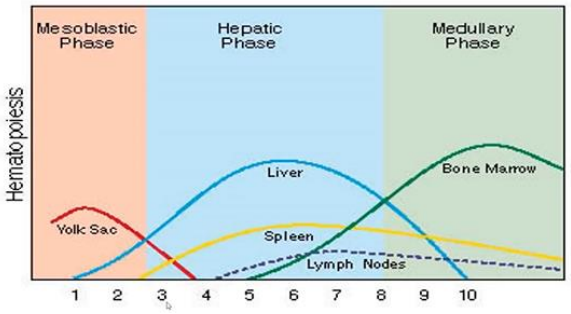
HEMATOPOIETIC TISSUES

- Organs and tissue
- The site of blood cell regulation
- Adults: restricted to the bone marrow within the cortical bones of the body
- Fetus: different organs
    - Mesoblastic phase: Yol sac
    - Hepatic phase: Liver (spleen, thymus, lymph nodes)
    - Medullary phase: Bone Marrow



- Child
    - Total marrow space: 1600 ml
    - Active red marrow:1000 – 1400 g
- Adult
    - Total marrow space: 2600 – 4000 ml
    - Active red marrow: 1200 -1500 g
- As the person ages, red marrow is replaced by **yellow marrow**
    - Stores fats

STAGES OF HEMATOPOIESIS



- Mesoblastic Phase
    - Yolk sac
        - Responsible for blood cell production
    - 1-3 week
    - Primitive blood cells are produced
- Hepatic Phase
    - Liver with contribution from spleen, lymph nodes
    - 3 – 8 weeks
- Medullary Phase
    - Bone Marrow
    - Week 10 – adulthood

MESOBLASTIC/ YOLK SAC PHASE

- As early as 19th day of gestation in the blood islands of the yolk sac of the human embryo
- Confined to erythropoiesis (PRIMITIVE HEMATOPOIESIS) producing primitive cells containing embryonic hemoglobin:
    - **Portland**: 2 zeta + 2 gamma
        - 1st hemoglobin that is produces during early embryogenesis
    - **Gower I**: 2 zeta + 2 epsilon
        - Plays the role in oxygen transport during the embryonic stage
    - **Gower II**: 2 alpha + epsilon
        - Reflecting the shift toward the alpha globulin genes that will later produce fetal and adult hemoglobin
- Portland, Gower I and II are specific to early embryonic period and are not found after birth
- As development progresses, these primitive cells are replaced by fetal hemoglobin:
    - Hemoglobin F
    - Hemoglobin A – adult hgb

HEMATOLOGY LECTURE
WEEK3 - MED225

HEPATIC PHASE

- In the 3rd month, yolk sac discontinues its role, fetal liver becomes active (erythrocytes and granulocytes in production)
- By the end of 4th month, primitive cells are disappearing, with an increase in the more definitive erythroblast, granulocytes and megakaryocytes
- Also active are the:
    - **Spleen and Lymph nodes**
        - Contributes to the overall immune response and blood storage
    - **Thymus**
        - Primarily involved in the maturation of **T lymphocytes**
            - Essential part of the adaptive immune system
- Start of definitive hematopoiesis producing definitive erythroblasts containing fetal hemoglobin:
    - **Hgb F**: 2 alpha + 2 gamma
        - Predominant hemoglobin during fetal life
        - Has higher affinity for oxygen compared to adult hemoglobin – efficient for crucial hemoglobin transfer of mother to the fetus
    - **Hgb A1**: 2 alpha + 2 beta
        - Major form
        - Becomes the dominant type after birth
        - Gamma into beta
    - **Hgb A2**: 2 alpha + 2 delta
        - Minor hgb
        - 2 – 3 % of adult hgb
        - Present in small amount
        - **A1 and A2**: adult hgb

MEDULLARY/ MYELOID PHASE

- Between 5th and 6th month gestation, the bone marrow becomes the primary site of hematopoiesis
- At birth, BM becomes the primary source of cell production
- Hematopoiesis occurs in the most bones but primarily in the **flat bones of the sternum**, **ribs**, **vertebrae**, **skull**, **pelvis**
    - Provides supportive environment to produce blood cells
- In adult, the principal source of production is the **sternum** and **other flat bones**.
    - Due to the ability of flat bones to maintain **active marrow** – essential for continuous blood cell production throughout life

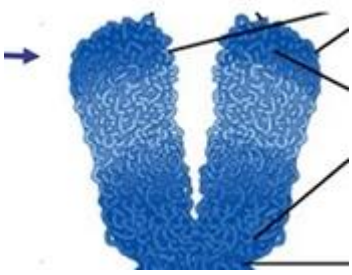
NORMAL CELL MATURATION

- Nuclear Maturation
    - Loss of nucleoli
    - Decrease in diameter o nucleus
    - Condensation of nuclear chromatin
    - Possible change in shape of nucleus
    - Possible loss of the nucleus

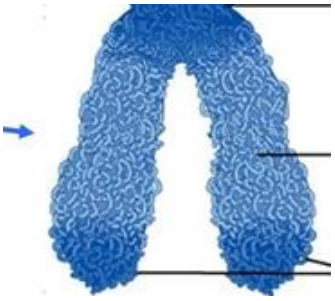
Most reliable indicator of maturity: chromatin pattern

- Cytoplasmic maturation
    - Decrease in **basophilia**
        - Tendency of cells to stain with basic dyes
        - Indicates in reduction of RNA content
    - Increase in the proportion of cytoplasm
    - Appearance of cytoplasmic granules

CHROMOSOME PARTS



- Heterochromatin
    - More condensed
    - Silenced genes (methylated)
        - Methylation – a chemical modification that suppresses gene expression
    - Gene poor (high AT content)
        - Fewer active genes; contains adenine and thymine
    - Stains darker



- Euchromatin
    - Less condensed
    - Gene expressing
    - Gene rich (higher GC content)
    - Stains lighter

ERYTHROPOIESIS – RED BLOOD CELLS

- Process by which **erythroid precursor cells** differentiate to become mature RBC
    - Immature cells in the bone marrow
- ERYTHROPOIETIN: primary regulator of erythropoiesis
    - Plays a crucial role in promoting in the survival in the maturity of erythroid precursor cells
    - Produces in the kidney
    - HYPOXIA: primary stimulus

HEMATOLOGY LECTURE
WEEK3 - MED225

- Lack of sufficient oxygen in the tissue
- When oxygen is lacked, the kidneys produce erythropoietin which helps carry more oxygen

- Retics: 1-2 days in bone marrow + 1 day in peripheral blood
- The blood can maintain an adequate supply of RBCs to meet its oxygen carrying needs.

BLASTS

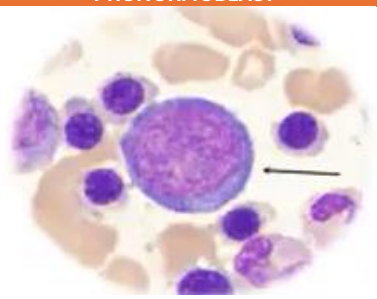
- characteristics of blast cells which is an immature precursor cells found in the bone marrow

- Large size
- Basophilic cytoplasm
- Absence of granules
- Large nucleus
- High N/C (nucleus/cytoplasm) ratio
- Presence of nucleoli
- **Fine chromatin**
  - DNA materials in the nucleus
  - Not yet fully condensed
  - These loose structures allow for a high level of transcriptional activity needed for the cell to grow and differentiate.

RBC MATURATION SERIES

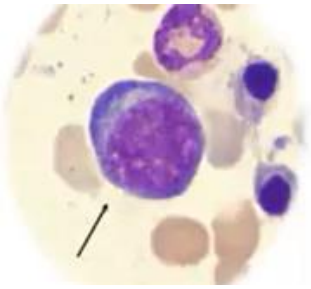
Rubriblast	Pronormoblast	Proerythroblast
Prorubricyte	Basophilic Normoblast	Basophilic erythroblast
Rubricyte	Polychromatophilic Normoblast	Polychromatic erytroblast
Metarubricyte	Orthochromic Normoblast	Orthochromic erythroblast
Reticulocyte	Polychromatophilic Erythrocyte	Diffusely basophilic erythrocyte
Mature Erythrocyte	Mature Erythrocyte	

PRONORMOBLAST



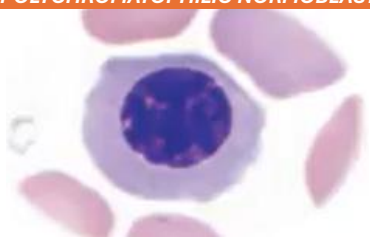
- Rubriblast/ Proerythroblast
- Diamete = 14-20 um
- Deeply basophilic cytoplasm (BLUE)
- Non-granular with fine chromatin
- N/C ration = 8:1
- With 1-2 nucleoli

BASOPHILIC NORMOBLAST



- Basophilic Erythroblast/ Prorubricyte
- Diameter = 12-17 um
- Intensely basophilic cytoplasm
- Chromatin slightly coarse
- N/C = 6:1
- Nucleoli not usually visible

POLYCHROMATOPHILIC NORMOBLAST



- Polychromic Erythroblast/ Rubricytes
- Last stage capable of mitosis
- Hemoglobin synthesis begins
- Murky blue-gray to pink-gray cytoplasm
- Diameter = 10-15 um
- N/C ration = 4:1

ORTHOCHROMIC NORMOBLAST

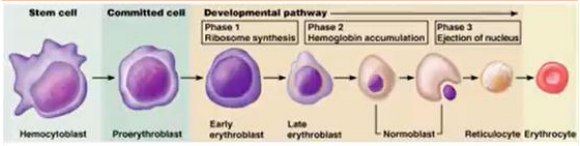
- Orthochromic Erythroblast/ Metarubricyte
- Diameter = 7-12 um
- Pink cytoplasm w/ **small pyknotic nucleus**
  - Nucleus when it's fully condensed
- N/C ratio = 1:2
- Last nucleated stage

RETICULOCYTE

HEMATOLOGY LECTURE
WEEK3 - MED225

- Polychromatophilic Erythrocyte
  - Its presence suggests active erythropoiesis
  - Last stage of RBC development where the protein responsible for oxygen transport is synthesized
- Index of bone marrow activity
- Last stage with hemoglobin synthesis
  - After, the cells mature into fully erythrose form of RBC and no longer produce hgb.
- Diameter = 7-10 um
- Pink to slightly pinkish gray cytoplasm
- Contains fine basophilic reticulum of RNA which is only visible with supravital stain
  - Brilliant Cresyl Blue
  - New Methylene Blue

MATURE ERYTHROCYTE



- Red Blood Cells/ Discocyte
- Diameter = 6-8 um
- Non-nucleated, round and biconcave with a **central pallor** 1/3 of its size
  - Surface area of gas exchange and allows RBCs to deform easy as they pass through the narrow capillaries
- Pink in color (Salmon-pink)
  - Color comes from the presence of hgb
- Not capable of hgb synthesis
- Highest in the morning
  - Due to body fluid distributions and hormonal variations
- Life span: 120 days
  - Old/ damage red blood cells are removed from the circulation and put in the spleen

MUST KNOW!

- Up to 16 orthochromic normoblasts are produced from a single pronormoblast
- Last stage capable of mitosis: **Polychromatic Normoblast**
- Last nucleated stage: **Orothochromic Normoblast**
- Index of bone marrow activity: **Reticulocytes**
- Hemoglobin synthesis:
  - Start: **Polychromatic Normoblast**
  - End: **Polychromatic Erythroblast**

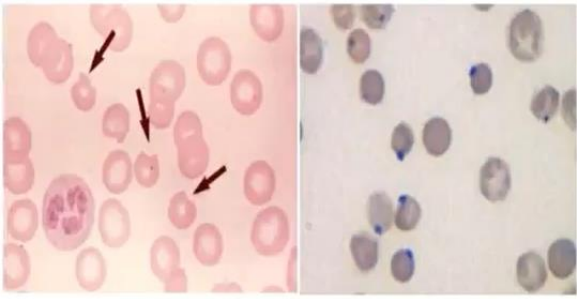
PHYSIOLOGIC VARIATIONS

(^) high; (v) low

- Males: ^RBC ^Hct (Hematocrit) ^Hgb
  - Due to female menstruation and male testosterone
- Older males: vHgb
  - Older males have higher chances of having GI bleeding and colon cancer than older women
- RBC, Hct, HGB:
  - ^ upon standing from a recumbent position
    - Due to the redistribution of blood volume
  - ^ in the morning
    - Due to circadian rhythms and due to fluid shifts during the night
  - ^ with high altitude
    - As the body produce more RBCs to confiscate for lower hgb level in the air
  - ^ in smokers
    - Due to body compensation for the lower oxygen carrying capacity of the blood due to the presence of carbon monoxide during smoking.

MUST KNOW!

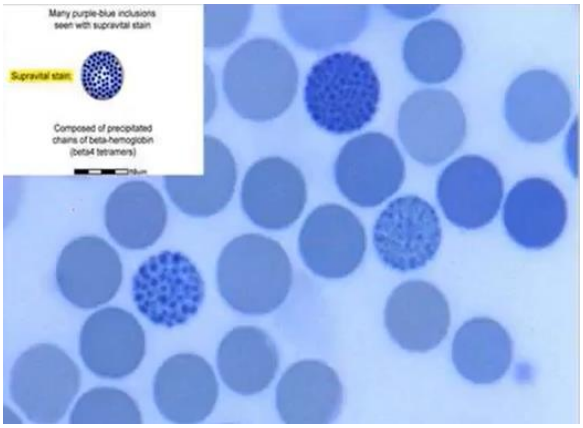
- HLA (Human Leukocytes Antigen) on RBC: BENNET-GOODSPEED
- Specific HLA markers that can be present on the surface of a red blood cell:
  - Bg^a: HLA-B7
  - Bg^b: HLA-B17
  - Bg^c: HLA-A28
- Visible with SUPRAVITAL STAIN (RHH)
  - Reticulum of reticulocytes
    - Refers to the network of RNA and mature RBC which can be stained and seen under the microscope with SVS
  - Heinz bodies
    - Inclusion within RBCs composed of denatured hgb which can be detected using SVS
  - HbH (Hemoglobin H)
    - Refers to an abnormal hgb that can also be detected with SVS



Bite cells | Heinz bodies

- Bite cells are a type of RBC that has a small bite taken out of it.
- This appearance is caused by the removal of denature hgb (Heinz body) by macrophage in the spleen.

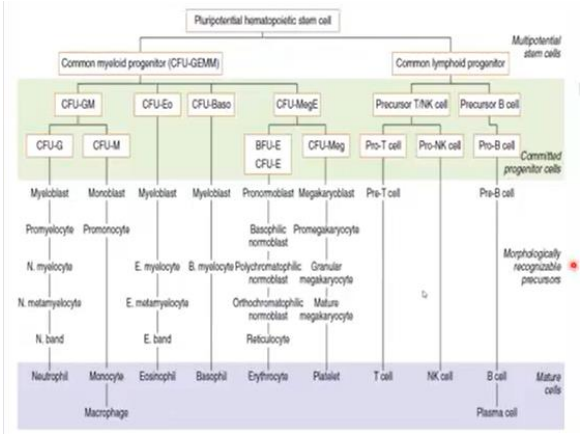
- Often associated with hemolytic anemia, particularly those caused by oxydative damage to the RBC
- Conditions:
    - **G6PD deficiency (Glucose-6-phosphate dehydrogenase deficiency)** – genetic disorder that leads to deficiencies in the enzymes of glucose 6 phosphate dehydrogenase making RBCs susceptible to oxidative damage
    - **Heinz body anemia** – RBCs are damaged by oxidizing agents leading to the formation of Heinz bodies



- Characteristics of HbH – composed of beta 4-tetramers
    - Abnormal forms of hgb
- Conditions:
    - HbH disease – result of alpha thalassemia where there is a deficiency in the alpha globulin chain leading to the transformation of beta 4-tetramer
    - Hemolytic anemia – due to the instability and abnormal functions of RBCs

LEUKOPOIESIS – WHITE BLOOD CELLS

- The process of producing WBC (leukocytes) in the bone marrow
- Crucial components of the immune system



- All blood cells originate in the hematopoietic stem cells in the bone marrow
- **Pluripotential hematopoietic stem cell**
    - Mother of all cells

LEUKOCYTE CLASSIFICATION

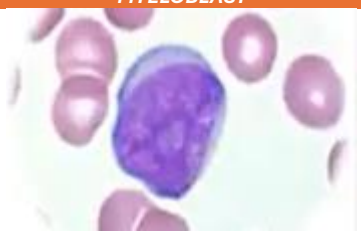
GRANULOCYTES AND POLYMORPHOMUCLEAR - **Neutrophil** - **Eosinophil** - **Basophil**	**NONGRANULOCYTE AND MONONUCLEAR** - Monocyte - Lymphocyte
PHAGOCYTES - **Neutrophil** - **Eosinophil** - **Monocyte**	**IMMUNOCYTE** - Lymphocyte

GRANULOPOIESIS - GRANULOCYTES

- This maturation refers to the stages through which a WBC develops and matures from stem cells into final functional form
- Production of mature granulocytes
- Takes about 14 days

	IMMATURE CELLS	MATURE CELLS
CELL	Large	Smaller
NUCLEOLI	With nucleoli	Without nucleoli
CHROMATIN	Fine & delicate	Coarse & clumped
NUCLEUS	Round	Round. Lobulated or segmented
CYTOPLASM	Dark blue	Light blue
N/C RATIO	High	Low

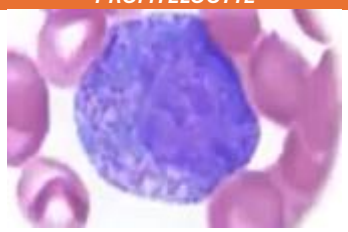
MYELOBLAST



- Diameter = 15-20 um
- Basophilic cytoplasm
- Earliest recognizable granulocytic precursor using light microscope
- Only nongranulated WBC precursor
- N/C ratio = 4:1

- Round or slightly oval nucleus
- With fine chromatin
- 2-5 nucleoli

PROMYELOCYTE

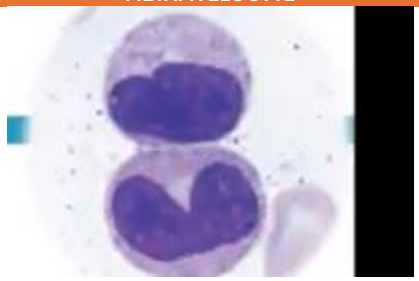


- Diameter = 15-21 um
- Larger than its immediate precursor
- Basophilic cytoplasm
    - Continues to stain dark blue or purple
- Contains **primary/ nonspecific granules**
    - Essential for future role in the immune response
- N/C ratio = 2-3:1
- With slightly coarser chromatin
- 2-3 nucleoli

MYELOCYTE

- Diameter = 12-18 um
- N/C ratio = 1:1
- No nucleoli
- Contains **secondary/ specific granules**
- With coarse chromatin
- **Last stage capable of mitosis**
    - Can increase the number of cells
- Youngest stage wherein specific granulocyte can be identified through color
    - Neutrophil myelocyte – **rose-pink granules**
    - Eosinophil myelocyte – **orange red granules**
    - Basophil myelocyte – **dark purple/ blue-black**

METAMYELOCYTE



- Critical stage in maturation of neutrophils marking the transition from proliferative to non-proliferative cells
- **Juvenile leukocyte**
- Diameter = 10-15 um
- N/C ratio = decreased
- Contains **tertiary/ <u>gelatinase granules</u>**
    - Allows the ability to move through tissues and response to infections
- **Youngest stage not capable of mitosis**
- **Predominant cell in the adult bone marrow**
- **Indented or kidney-shaped nucleus**
    - Less than ½ of the diameter of the nucleus
- With coarse and clumped chromatin

BAND FORM

- Stab/ Staff
- Diameter = 9-15 um
- Youngest WBC to appear in the PB
- Elongated/ Band-shaped/ sausage-shaped nucleus
    - More than ½ of the diameter of the nucleus
- With coarse and clumped chromatin
- LEFT SHIFT: an increase in the number of immature cell types among the blood cells in a sample of blood

MATURE NEUTROPHIL

- Segmented neutrophil/ Segmenters
- Diameter = 9-15 um
  + this size allows their body to effectively move to blood vessels and tissues to perform their roles
- Pink to Rose Violet granules
    - granules contain ACP (acid phosphatase – deals with a role of degradation and cellular), acid hydrolase (breakdown of substrate within the cells), muramidase, lysozymes (destroys bacteria sa walls), collagenase (breaks down collagen, plasminogen activator (convert from plasminogen to plastic which is important for fibrinolysis , aminopeptidase (invoke liquidation by removing enter ominous of peptide) and lactoferrin (binds iron that in which are essential for phagocytosis)
- Nucleus has 2-5 lodes (<2: Pelger-Huet / >5: hyper lobed)
- With coarse and lumped chromatin
- Phagocytic (segmenters highly phagocytic)
- Phagocytosis – they can invoke and digest microorganisms and other particles, crucial in innate immune response
- Highest in the AFTERNOON (peaks and these variations can attribute to a various physiological and hormonal factors affecting neutrophil production

NEUTROPHILS

- Primary defense against infection
- Most common and most abundant of WBCs
    - Pseudoneutrophilia
        - Caused by a shift of marginated cells to the circulatory pool due to exercise, extreme temperatures, nausea,

vomiting, pregnancy, labor, rage, panic and stress
- Neutrophilia (increase in the number of neutrophilia)
  - Bacterial infection
  - Inflammation/ injury
  - Leukemia and other bm disorders
  - response to medications (chemotherapy)
- Neutropenia
  - Chemical toxicity (benzene)
  - BM replacement
  - Nutritional deficiencies
  - Cytotoxic drugs
- Agranulocytosis
  - WBC count: <0.5 x 10^9/L
  - Associated with drugs: Amidopyrine and Cephalosporin

MATURE EOSINOPHIL

- Diameter = 9-15 um
- **Reddish-orange granules**
  - **Larger granules:** MBP, acid hydrolases (enzymes that helps to breakdown substrates) , peroxidase (breakdown oxygen), phospholipase (helps breakdown phospholipids which can affect inflammatory senses), cathepsin (protein degradation), EOSINOPHILIC CATIONIC PROTEIN (ECP) (has a toxic effect on parasite, also contribute to inflammatory response), EDN (Eosinophil derived neurotoxin- a neurotoxin with antiviral properties and involves in modulating inflammation) and eosinophil protein X (a protein involved in the inflammatory process and tissue damage during a legit reactions)
  - **smaller granules**: arylsulfatase (glycosaminoglycans) , peroxidase (contribute in antimicrobial activity) & ACP)contribute with aedes cells)
  -
- Usually has a bilobed nucleus
- With coarse and clumped chromatin

EOSINOPHILS

- Act as phagocytes and **modulate inflammatory responses**
- Plays a role in **allergic disorders and parasitic infections**
- **Dampen hypersensitivity** and inflammatory reactions
  - **Eosinophilia**
    - Allergic disorders (Hay fever, asthma)
    - Parasitic infections
    - Scarlet fever
    - Skin inflammation (Psoriasis, eczema)
    - Eosinophilic leukemia and other BM disorders
  - **Eosinopenia** – ACTH administration
  - When intense prolonged eosinophilic inflammatory actions occur, there is often a formation of Charcot -Leyden crystals (hexagonal bipyramidal crystals are composed of lysophospholipase localized in the cytoplasm of eosinophil)

MATURE BASOPHIL

- Diameter = 10-16 um
- **Dark purple/ Bluish-black granules**
  - Water-soluble granules
  - Granules contain **histamine ad heparin**, peroxidase, eosinophilic chemotactic factor A and chondroitin sulfates
- Unsegmented of bilobed nucleus
- Rarely has 3-4 lobes

BASOPHILS

- Responds to adrenal corticosteroids in similar fashion to eosinophils
- Involved in **immediate hypersensitivity reactions** such as allergic reactions, basophils release histamines and other mediators to regulate symptoms like itching, swilling, and renest)
- IgE – antibody triggering histamine and other inflammatory mediators
- Also involved in some **delayed hypersensitivity reactions, or cutaneous basophil hypersensitivity**, such as contact allergies
- Assess in modulating immune response and sustaining inflammation through their granulations and release of various chemical mediators

  - Basophilia
    - Leukemia
    - Chronic inflammation
    - Hypersensitivity to food
    - Radiation therapy
  - Basopenia (theoretical)
    - Acute infections
    - Stress
    - Hyperthyroidism
    - Increased level of glucocorticoids
    - Chronic urticaria

MAST CELLS

- Widely distributed throughout the body including the bone marrow, thymus, and spleen but they do not normally appear in blood
- Crucial component of the immune system, involves in inflammatory responses and allergic reactions
- Tissue mask cells: NOT A WBC
- Usually larger than basophils
- Low N/C ratio
- Round or oval reticular nucleus that is usually obscured by abundant red-purple granules
- Contains heparin, bradykinin and histamine

HEMATOLOGY LECTURE
WEEK3 - MED225

- **Bradykinin:** peptides in regulations of blood pressure and promotion of inflammation such as induced vasodilation, widening blood vessels and increase vascular permeability contributing to the symptoms of inflammations and allergic reactions such as weakness

PHYSIOLOGIC VARIATIONS

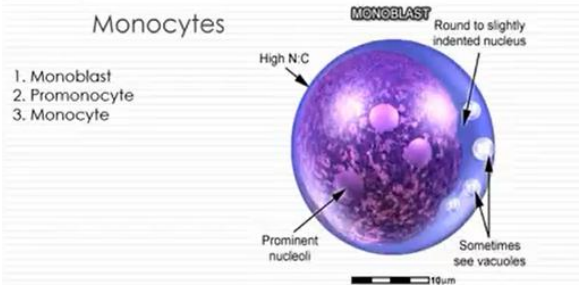
- Specific: nonspecific granule Ration = 2—3:1
- WBC
  - ^ in the afternoon
  - ^ after exercise
  - ^ in smokers
  - low in blacks (people)

PRIMARY	SECONDARY	TERTIARY
(APESBAL) ACP Peroxidase Esterase Sulfated mucosubstance B-galactosidase Arylsulfatase Lysozyme (MACDEPO) Myeloperoxidase Acid B-glycerophosphatase Cathepsins Defensins Elastase Proteinase-3 Cationic bacterial proteins	(CLAML) Collagenase Lysozyme Aminopeptidase Muramidase Lactoferrin Gelatinase Plasminogenactivator B_2-microglobulin Neutrophil gelatinase-associated lipocalin	(GCLAB) Gelatinase Collagenase Lysozyme Acetyltransferase B_2-microglobulin

Granules

MONOPOIESIS

Maturation of monocyte



- Monoblast
- Promoblast
- Monocyte

MONOBLAST

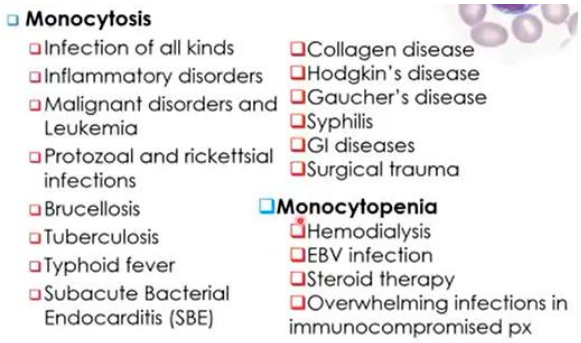
- Diameter = 12-20 um
- Basophilic cytoplasm
- Non granular
- N/C ratio = 3-4:1
- With 1-2 nucleoli

PROMONOCYTE

- Earliest recognizable cell in this series (first stage in which it can be recognized in a specific lineage)
- Diameter = 14-18 um
- Blue-gray cytoplasm cytoplasm (typically immature cells that synthesize proteins) (fine chromatin layer)
- N/C ratio = 2-3:1
- With fine chromatin pattern
- 1-5 nucleoli (involves ribosome production – active protein synthesis)

MONOCYTE

- **Type of a white blood cells that can be seen in a peripheral blood**
- **LARGEST CELL IN THE PB**
- Diameter = 14-20 um
- **Blue-gray** cytoplasm
- Many fine **azurophilic granules** (lysosomes containing enzymes used for phagocytosis)
- giving a **ground-glass/ frosted-glass** appearance of the cytoplasm
- **Round/ kidney-shaped/ horseshoe-shaped nucleus**
  - May show slight lobulation
  - May be folded over on top if itself showing **brainlike** convolutions
- highly active in response to inflammation, infection, and foreign bodies
- Responds to inflammation, infection and foreign bodies via phagocytosis
- PRIMARY ROLE: **Phagocytosis** (they engulf and digest pathogens, dead cells and other debris)
-



MACROPHAGES

- **TISSUE COMPONENT OF THE MONOCYTE SYSTEM**
- **Can be fixed or wandering**
- arise from emigrated blood monocytes
- Diameter = 15-80 um
- have irregular cell membranes, often with **blebs and pseudopodia: MOTILE**
- N/C ratio is high with an oblong and/ or indented nucleus
- located in virtually all tissues of the body
  - the greatest numbers are found in the bowel, liver, bone marrow, and spleen

MONOCYTE/MACROPHAGE

- In promonocytes, monocytes, and macrophages, the granules contain acid hydrolase, arylsulfatase, nonspecific esterase, and peroxidase.

HEMATOLOGY LECTURE
WEEK3 - MED225

- Monocyte/macrophage functions
    - **Phagocytosis** - bacteria, cellular debris, senescent cells
    - **Antigen processing**
    - **Cell-mediated immunity** - antibody dependent cellular cytotoxicity
    - **Synthesis of bioactive molecules**

LYMPHOPOIESIS

LYMPHOBLAST

- Diameter = 10-18 um
- Scanty cytoplasm
- N/C ratio is 4:1
- Loose chromatin
- 1 to 2 distinct nucleoli

PROLYMPHOCYTE

- Size may be the same as the lymphoblast or smaller
- Moderate to dark blue cytoplasm
- Round, oval or slightly indented nucleus
- 1 to 2 nucleoli
- Chromatin pattern is more clumped

LYMPHOCYTE

- **Scanty** cytoplasm
- Bluish cytoplasm described as **"Robin Egg Blue"**
- Small Lymphocyte = 8-10 um diameter
    - as large as RBC
- Medium Lymphocyte =10 -12 um diameter
- Large Lymphocyte = (12 to 16 um diameter
    - if granular (LGL) = **Natural Killer Cells**
    - If nongranular = **Hematopoietic Stem Cells**
- **Lymphocytosis**
    - Viral infections
    - Infectious mononucleosis
    - Infectious lymphocytosis
    - CMV infections
    - Acute Viral Hepatitis
    - Bordetella pertussis infection
- **Lymphocytopenia/ Lymphopenia**
    - HIV infection/ AIDS (T lymphocytopenia)

FUNCTIONAL GROUPS OF LYMPHOCYTES

- **Tlymphocytes (T cells):** cell-mediated immunity
    - 60-80%
    - long-lived (4-10 years)
    - **CD3:** T-cell receptor
    - **CD4+:** T-helper
    - **CD8+:** T-cytotoxic
    - NOTE: CD4:CD8 ratio 2:1
- **B lymphocytes (B cells):** precursor cell in Ab production
    - 0-20%
    - short-lived (3-4 days)
    - with surface immunoglobulins (1. **IgM** 2. **IgD**)
- **Null lymphocyte/Natural Killer cells (NK cells/LGL)**
    - 10%; 3rd POPULATION LYMPHOCYTE
- **Virgin/ Naive lymphocytes/ 4th Population lymphocyte**
    - Never been exposed to antigens

PLASMA CELLS MATURATION

1. **Plasmablast**
    - eccentric nucleus with halo
2. **Proplasmacyte**
    - with hof/perinuclear halo
3. **Plasmacyte/Plasma cell: activated B cell**
    - 8 to 20 um in diameter
    - Deeply basophilic cytoplasm
    - **LARGE WELL-DEFINED HOF** next to nucleus (light staining area in the cytoplasm near the nucleus/ **Golgi Zone)**
    - Eccentrically located nucleus
    - Chromatin pattern is condensed and coarse with **CARTWHEEL pattern**
    - No nucleoli are visible
    - Commonly mistaken as **OSTEOBLAST**
        - Osteoblast: specialized cells involved in synthesis of new bone matrix

MEGAKARYOBLAST

- **EARLIEST RECOGNIZABLE PRECURSOR CELL OF PLATELETS**
- Diameter = 20-50 um
- Blue cytoplasm
- N/C ratio = 10:1
- Has multiple nucleoli
- With fine chromatin

PROMEGAKARYOCYTE

- Diameter = 20-60 um
- N/C ratio = 4-7:1
- Irregularly shaped nucleus, may even show slight lobulation
- Chromatin becomes more coarse
- Multiple nucleoli

GRANULAR MEGAKARYOCYTE

- Diameter = 30-90 um
- Very fine and diffusely granular cytoplasm
- N/C ratio = 1-2:1
- Multiple nuclei may be visible or the nucleus may show multi-lobulation
- No nucleoli are visible

MATURE MEGAKARYOCYTE

- Platelets are produced directly from **megakaryocyte's CYTOPLASM**

HEMATOLOGY LECTURE
WEEK3 - MED225

- Cytoplasm contains coarse clumps of granules aggregating into little bundles, which bud off from the periphery to become platelets
- Diameter = 40-120 um
- Multiple nuclei are present
- N/C ratio < 1:1
- **LARGEST CELL IN THE BM**
- Commonly mistaken as **OSTEOCLAST**

THROMBOCYTE/PLATELETS

- Diameter = 1-4 um
- Light blue to purple, very granular
    - **Chromomere**: CENTRAL GRANULAR part
    - **Hyalomere**: PERIPHERAL NONGRANULAR part; clear to light blue in color
- Composed of 6**0% protein, 30% lipid, 8% carbohydrate**, various minerals, water and nucleotides
- Divided anatomically into 4 areas:
    - Peripheral zone
    - Sol-gel zone
    - Organelle zone
    - Membranous system

PLATELETS

- **Thrombocytosis**
    - Primary thrombocythemia
        - Essential thrombocythemia: faulty stem cells produce too many nonfunctional platelets
- **Secondary thrombocytosis: platelets are normal**
    - Cancers
    - IDA
    - Hemolytic anemia
    - Splenectomy
    - Inflammatory or infectious diseases
    - Reaction to certain drugs
    - Thrombocytopenia that lasts for a short time:
        - Recovery from serious blood loss
        - Recovery from thrombocytopenia
        - Acute infection or inflammation
        - Response to physical activity
- **Thrombocytopenia**
    - Occurs because the BM does not make enough platelets or BM makes enough platelets but the body destroys them or the spleen holds on to too many platelets

- Cancers
- Aplastic anemia
- Exposure to toxic chemicals & Alcohol consumption
- Drugs such as diuretics, chloramphenicol aspirin and ibuprofen
- Viruses
- Genetic conditions: Wiskott-Aldrich and May-Hegglin syndromes
- Autoimmune diseases: ITP
- Surgery & pregnancy
- TTP and DIC
- Splenomegaly

MUST KNOW!

- **THROMBOPOIETIN**: primary regulator of plt production
    - produced by the liver, kidney, marrow stroma, and other tissues
- **ENDOMITOSIS**: nuclear division w/o cytoplasmic division
- 1 megakaryocyte = **1000-5000 platelets**
- Platelet maturation: **5 days**
- Platelet lifespan: **8-11 days**
- Circulating platelets: **2/3 of plt pop'n**
- Platelets in spleen: **1/3 of plt pop'n**
    - **Splenomegaly: decrease plt count**
    - **Splenectomy: increase plt count**
- Average platelet per OIO: 7-25 platelets/8-20 platelets
- Platelet estimate = number of platelets per OIO × 20,000

PLATELET STRUCTURE

1. **PERIPHERAL ZONE**
    - responsible for **platelet adhesion and aggregation**
        - **Glycocalyx:** outer surface important in platelet reactions
2. **SOL-GEL/STRUCTURAL ZONE**
    - comprise the platelet **cytoskeleton**
        - **Microfilaments:** actin and myosin (actomyosin/thrombostenin); responsible for CLOT RETRACTION
        - **Microtubules:** contain tubulin; maintains PLATELET SHAPE
3. **ORGANELLE ZONE**
    - **Alpha granules**: platelet factor, platelet derived growth factor (PDGF), fibrinogen, Factor V, Fibrinogen, vWF, b-thromboglobulin, thrombospondin, albumin and fibronectin
    - **Dense granules**: calcium, ADP, pyrophosphate, ATP, serotonin (CAPAS)
    - **Mitochondria**
4. **MEMBRANOUS SYSTEM**
    - **Dense tubular system**
        - **control center** for platelet activation
        - site of **arachidonic acid** metabolism
    - **Open canalicular system/ surface connecting canalicular system**
        - cannal for release of platelet granules

HEMATOLOGY - LECTURE
WEEK 4 | Christina D. Pal | MED225 | Instructor: Dra. Racquel Villanueva

Mature Erythrocyte
- No nucleus
- Biconcave
  - Allows RBC to enter the narrowest capillaries
  - Provides flexibility
- Size:
  - 7-8 um diameter
  - 1.5-2.5 um thickness
- Color:
  - Salmon pink (Stained film)
  - Due to hemoglobin production
- Central pallor
  - ⅓ of the diameter
  - Key indicator of erythrocyte's health and functionalities
- Life span
  - 120 days
  - After 120 days, RBC will be removed and destroy by macrophages and put in the spleen
  - In the spleen, RBC breaks down

Mature Erythrocyte Cellular Activity:
- Delivers 02 to tissues throughout the body which requires a membrane:
  - Flexible
  - Deformable
- Contains **Hemoglobin**
  - 02 carrying component

Red Blood Cell Production
- Hypoxia
  - Stimulus for RBC production
- Oxygen-Sensing system (peritubular fibroblasts of kidney)
  - Mechanism for sensing tissues whether there is adequate 02 being carried to the tissues
- Erythropoietin
  - Thermostable glycoprotein hormone produce by kidney and acts as on bone marrow
- Hypoxia
  - hypoxia → 02 - sensing system in the kidney →
  - Erythropoietin (EPO) > Erythrocyte precursors

Note: Hypoxia occurs when there is deficiencies amount of oxygen that reaches the tissues
  - If low oxygen, kidney and peritubular recognizes the deficiencies and trigger the release of EPO
  - EPO will travel to BM and act pre erythrocyte precursors
  - Then will stimulate new production of red blood cells

RBC Destruction
- *As the RBC ages, there will be:*
  - Decrease enzyme
  - Decrease ATP
  - Decrease size
  - Increase viscosity
- *Culling*
  - Function of spleen where aged RBCs are filtered and destroyed by splenic macrophage
  - Ensures that only healthy RBC will circulate
  - Prevent potential problems and maintaining the efficient of the body's oxygen relieve resistance

Types of RBC Destruction:
- Extravascular Hemolysis
  - Macrophage-mediated
  - 90%
  - Occurs in liver and spleen
  - Macrophages identify aged or damaged red blood cells and breakdown within their ribosomes
  - These process allows the body to recycle variable movement such as Iron from hemoglobin
- Intravascular Hemolysis
  - Mechanical-mediated
  - Fragmentation
  - 10%

Extravascular Hemolysis

Decrease enzyme, decrease ATP, Decrease size, Increase viscosity → loss of membrane deformability, spherical shape → trapped in splenic sieve → ingest by splenic macrophage

Intravascular Hemolysis
- RBC ruptures within the lumen of blood vessels Mechanical factors results in fragmentation and release of cell content in the blood
- Turbulence on heart chamber
- Small breaks on blood vessel
- Conditions:
  - Disseminated Intravascular Hemolysis (DIC)
  - Severe hypertension
- More problematic because it releases hemoglobin
  - Renal damage
  - Jaundice
  - Severe hemolytic

  Example: hemoglobinuria, hemoglobinemia, increase lactate dehydrogenase

Red Blood Cell Membrane
- Deformability

→ depends on *Geometry, Viscosity, Elasticity*

Main function of RBC Membrane:
- Maintain cell and deformability
- Maintain osmotic balance between plasma and cell cytoplasm
- Act as supporting skeletal system for surface antigens and receptors
- Aid in the transportation of essential ions and gasses

RBC Deformability
- Geometry
  - Biconcave
  - 7-8 um
  - MCV:

→ 901L
  - Average SA:

→ 120 um
  - 40% excess area for stretch undamage upto 2.5x their resting diameter

→ To pass through narrow capillaries
- Viscosity
  - Cytoplasmic Viscosity
    → Hemoglobin
  - Mean Cell Hemoglobin Concentration (MCHC)
    → Normal value of 32-36%
  - Increase MCHC = Increase viscosity
    → Loss of deformability
    → Short life span
    → Destroyed by splenic macrophage

HEMATOLOGY - LECTURE
WEEK 4 | Christina D. Pal | MED225 | Instructor: Dra. Racquel Villanueva

• Elasticity
- RBC Membrane:
- 8% carbohydrates
- 52% proteins
- 40% lipids

Protein	Lipid	Cholesterol
Transmembrane Proteins - Glycophorin A - Glycophorin B - Glycophorin C - Band 3 - Aquaporin - RhAg Cytoskeleton Proteins - Spectrin - Ankyrin - Actin - Myosin	Phospholipid Cholesterol	Glycocalyx

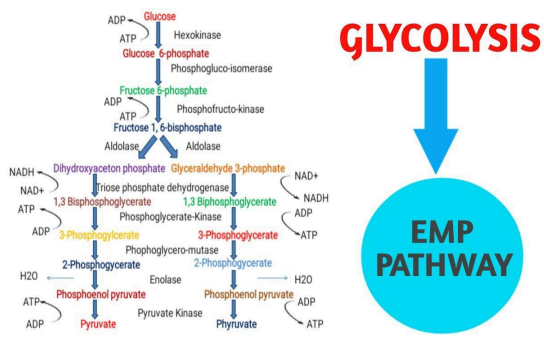
Osmotic Balance and Permeability

- Impermeable: Na, K and Ca
- Na-K ATPase pump
  + Regulate concentration of Na and K
- Ca ATPase pump
  + Expels Ca from cell
- Lack ATP/Pump damage:
  + RBC swelling due to influx of Ca and Na, followed by water
- Permeable: HCO3, Cl and water
- Aquaporin 1
  + Channel that creates inward flow of water
- Hereditary Spherocytosis:
  + Lacks Aquaporin 1

RBC energy production

- RBC lacks mitochondria
- Anaerobic glycolysis / Embden meyerhof
- Pathway: Major energy provider
- Anaerobic Glycolysis / EMP
- Required RBC to produce ATP by relying on glucose from plasma
- How does Glucose from plasma enter the RBC?
- **Glut-1**

→ **Insert EMP Pathway**



Glycolysis Diversion Pathways

1. Hexose Monophosphate Shunt
2. Methemoglobin Reductase Pathway
3. Rapo-port Luebering Pathway

Hexose Monophosphate Shunt

- Also known as Pentose phosphate shunt
- Detoxifies Peroxide from 02
- Reduction of Nicotinamide Adenine Dinucleotide
- Phosphate (NADP) to MADPH is subsequently needed to reduce Glutathione
+ Glucose 6 Phosphate Dehydrogenase (G6PD)
  > Needed to generate NADPH for glutathione reduction
  >. Lack of G6PD
  • RBC vulnerable to oxidative damage

Glucose 6 Phosphate Dehydrogenase Deficiency

- Most common inherited enzyme deficiency associated with heinz body

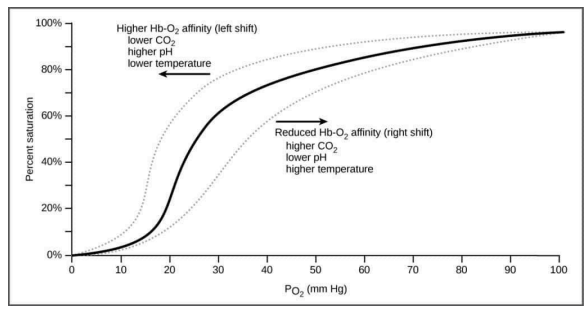
Methemoglobin Reductase Pathway

- Exposure to peroxide oxidizes heme iron from ferrous to ferric state
- Heme in Ferrie state: **Methemoglobin**

Rapoport Luebering Pathway

• Increase 2, 3 DPG
+ Decrease affinity to 02
• Decrease 2, 3 DPG
Increase affinity to 02

→ insert Oxygen Dissociation Curve



RBC Anomalies:

- Anemia
- Decrease in the oxygen carrying capacity of the blood
+ Insufficient hemoglobin
+ Hemoglobin has impaired function

Classic symptoms:

- *Pallor*
- *Fatigue*
- *Shortness of breath*

Mechanisms of Anemia:

Ineffective erythropoiesis

That are defective production of erythroid precursor cells

Insufficient erythropoiesis

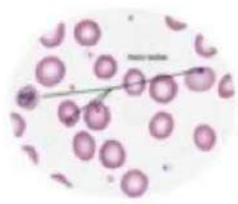
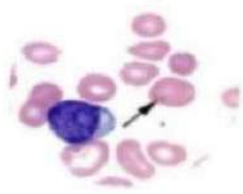
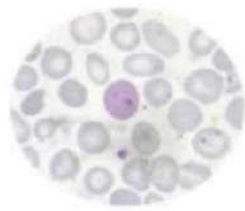
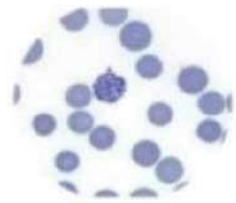
Decrease in the number of erythroid precursors

Acute/Chronic blood loss

+ *Trauma*
+ *Intermittent bleeding*
+ *Hemolysis*

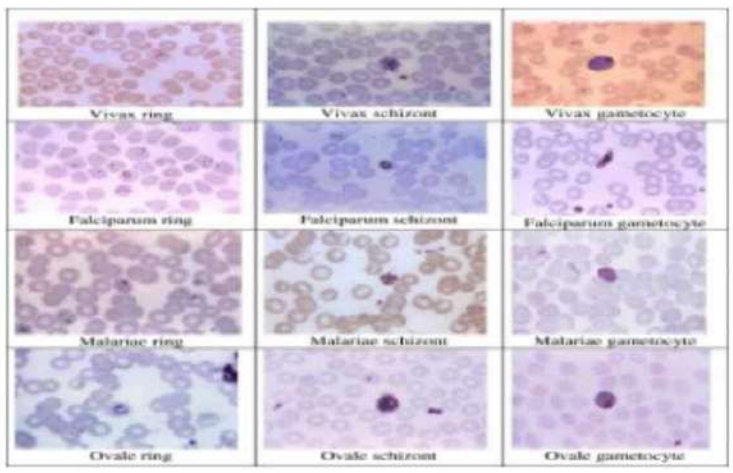
Laboratory Diagnosis:

- CBC (RBC indices)
- Reticulocyte count
- Peripheral blood smear

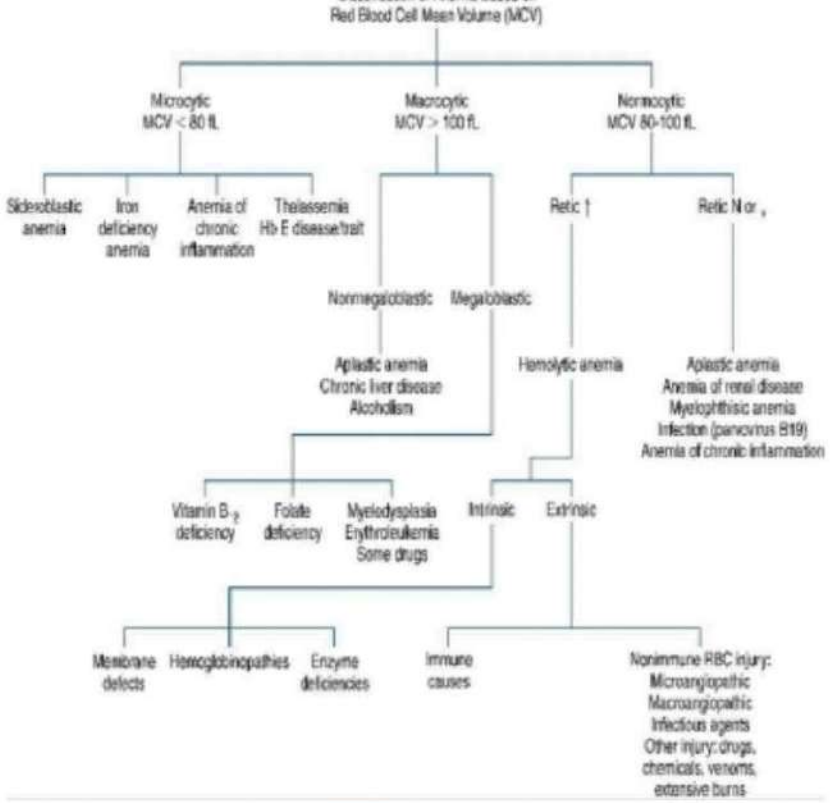
RBC Inclusions		
 **Basophilic Stippling**	 **Howell Jelly Bodies**	 **Heinz Body**
Dark purple-blue fine granules (Precipitated RNA) Distributed throughout cytoplasm - Lead poisoning - Hemoglobinopathy	Single or multiple small round granules (DNA fragment) Near the periphery - Post splenectomy - Megaloblastic anemia	Dark blue-purple granule (Denatured Hemoglobin) Attached to inner cell membrane - Glucose 6 phosphate deficiency
 **Pappenheimer Bodies**	 **Cabot Ring**	 **Hemoglobin H**
Irregular clusters of small light-dark purple granule (Iron) Often near periphery of cell - Sideroblastic anemia - Post splenectomy - Hemoglobinopathy	Rings or figure of 8 (Mitotic spindle remnant) - Megaloblastic anemia - Myelodysplastic syndromes	Fine evenly disperse dark blue granules (Precipitated B-globin chains) *Golf ball* appearance - Hb H disease
 **Hemoglobin SC Crystal**	 **Hemoglobin C Crystal**	 **Babesia Microti**
Finger-like / Quartz-like crystal (Dense hemoglobin) Formed within RBC membrane - Hb SC disease	Hexagonal crystal protruding from RBC membrane - Hb SC disease	Trophozoite from: Pyriform Vacuolated Tetrads Forming a Maltese-cross pattern - Babesiosis

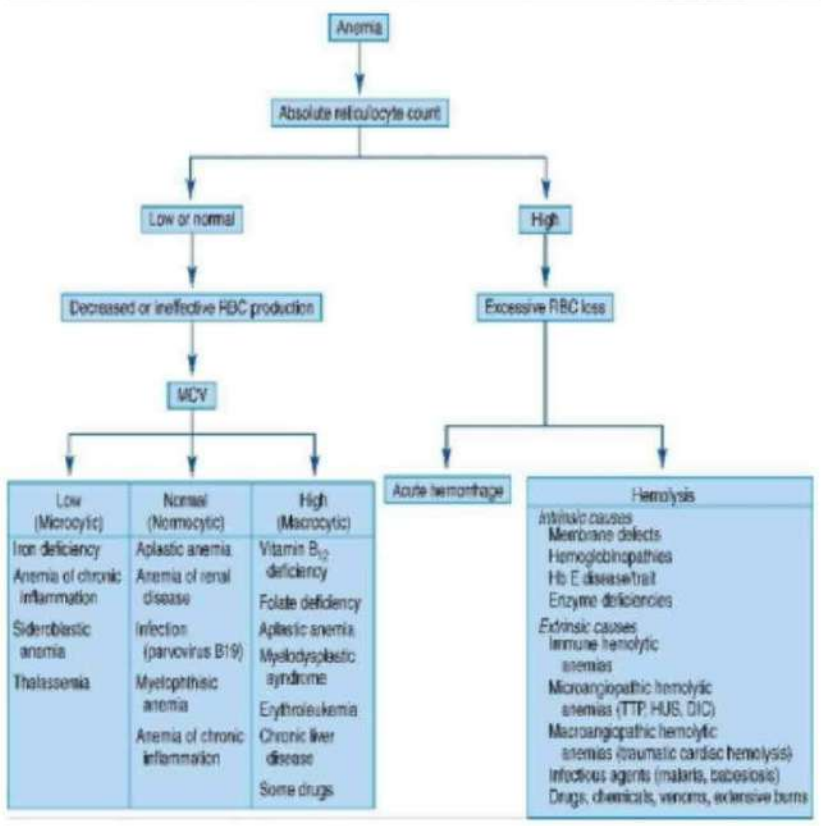
Malaria Infection

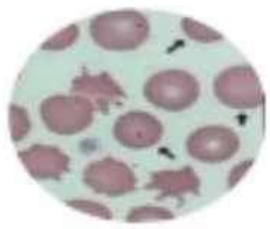
 **Microcyte**	 **Anisocytosis**	 **Poikilocytosis**
Small RBC <6 um MCV: <90 fL - Hemolytic anemia - IDA	Variation in size - Megaloblastic anemia - IDA - Hemolytic anemia	Variation in shape - Severe anemia
 **Elliptocyte**	 **Stomatocyte**	 **Codocyte**
Oval RBC Hemoglobin at end of RBC - Hereditary elliptocytosis	Slit-like area of central pallor - Hereditary stomatocytosis - Alcoholism - Rh null - Cirrhosis	Also known as *Target Cell* Hemoglobin concentrated at center and periphery of RBC - Thalassemia - Hemoglobinopathy - Post-splenectomy
 **Schistocyte**	 **Keratocyte**	 **Dacrocyte**
Fragmented RBC due to rupture in the peripheral circulation - Microangiopathic hemolytic anemia - Extensive burns	Also known as *Helmet cell* Contains 2 or more horn-like spicules - Microangiopathic hemolytic anemia - DIC - Severe burns	Also known as *Teardrop cell* RBC with single point extension Pear shape - Myelophthisic anemia - Primary myelofibrosis - Thalassemia
 **Echinocyte**	 **Depranocyte**	 **Acanthocyte**
Also known as *Burr cell* RBC with blunt, short projections evenly throughout the surface of cell - Uremia - Pyruvate kinase deficiency	Also known as *Sickle cell* Elongated RBC with pointed end - Sickle cell anemia	Also known as *Spurr cell* RBC with few irregular projections of varying length - Severe liver disease - Abetalipoproteinemia



Classification of Anemia Based on Red Blood Cell Mean Volume (MCV)





RBC Morphology		
 **Hypochromic RBC**	 **Hyperchromic RBC**	 **Macrocyte**
Larger area of central pallor - Iron deficiency anemia - Malignancy	Central pallor smaller than normal - Hereditary Spherocytosis	Large oval RBC >8 um MCV: 100 fL - Megaloblastic anemia

HEMATOLOGY LECTURE
WEEK 5 (MED225)

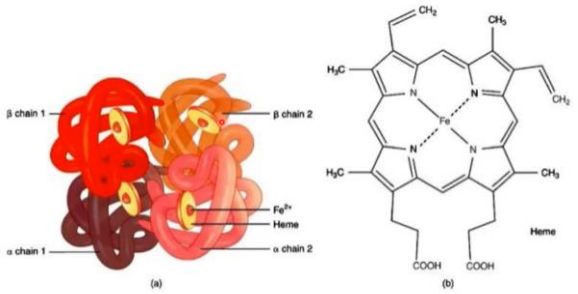
HEMOGLOBIN

HEMOGLOBIN STRUCTURE

- **Primary function of RBC**: transport oxygen to the tissues to the lungs which are carried out by hemoglobin
- Hemoglobin molecule: composed of four subunits, each containing heme (molecule that contains **iron** – binds to oxygen) and globin (surrounds and protect the heme)
    - **1 hemoglobin** = 4 heme + 4 globin chains
    - 1 heme can carry 1 mole of O2
    - 1 gram of Hgb carried **1.34mL O2** and **3.47 mg Fe**
- Heme – crucial; site where oxygen binds
- Globulins – gives hgb flexibility – allows change

Why is HGB important?

- Because it allows oxygen to travel in the lungs and tissues
- Hgb structure allows picking up of O in the lungs, hold into it while travelling in the blood stream, and release where it is needed



3D STRUCTURE

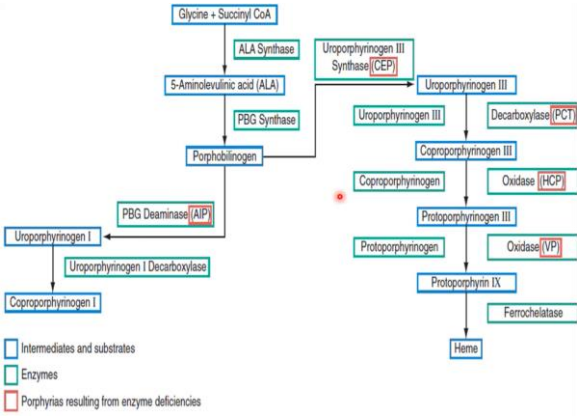
- 2 subunits: 2 alpha, 2 beta chain – each are folded
- Yellow part – heme
- Ferrous – essential for binding oxygen

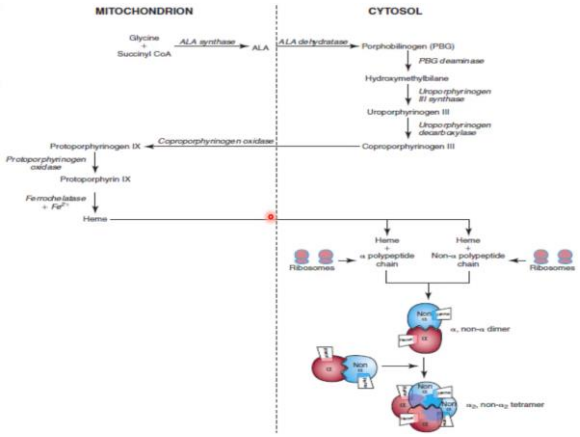
CHEMICAL STRUCTURE

- Porphyrin ring – ring-like structure that surrounds the central ring; has several C, H, N atoms forming a stable environment for the forming Fe

HEMOGLOBIN SYNTHESIS

- The synthesis of heme begins in the **mitochondria** with the formation of **D-ALA** from **glycine** and **succinyl coenzyme**
    - D-ALA: delta aminolevulinic acid
    - Glycine – amino acid
    - Succinyl coenzyme – molecule that has a role in the citric acid cycle
- **MITOCHONDRIA** – early and late heme synthesis
- **CYTOPLASM** – heme assembly (middle step) – fluid outside the mitochondria





- Synthesis of heme
- Incorporation of heme to hgb
- Assembly of molecules

HEMOGLOBIN

- Is composed of 4 heme and 4 globin chains
- Genetic coding for globin chains
    - **Chromosome 16**: alpha and zeta
    - **Chromosome 11**: beta, delta, epsilon, gamma
- 1 gram of Hgb carries **1.34 mL O2** and **3.47 mg Fe**
- **FERRITIN** – storage form of iron (4000 ferric)
- **Hemosiderin** – partially degraded ferritin
- **FRANSFERRIN** – transport protein of iron (ferric)

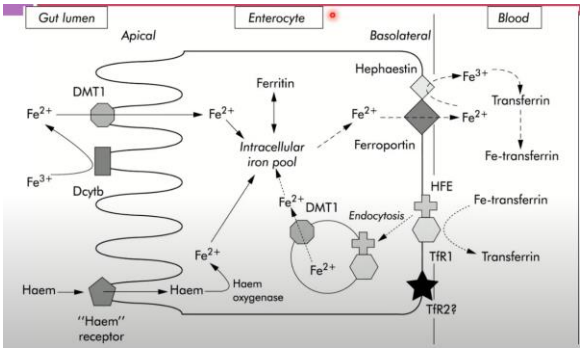
IRON KINETICS

- Iron is absorbed in **FERROUS** FORM
    - Fe 2+ → Fe 3+
- **Duodenal cytochrome b** – converts into ferrous in the GIT lumen to facilitate absorption

HEMATOLOGY LECTURE
WEEK 5 (MED225)

- **DMT1** – iron transporter in the GIT lumen – facilitates uptake of ferrous iron into the **enterocytes** (cell lining the intestine)
- **Ferroportin** – iron transporter in the basolateral membrane
- **Hepcidin** – a regulatory hormone produced by the LIVER; inhibits ferroportin
    - Increased hepcidin causes degration of ferroportin
- **Hephaestin** – converts ferrous into ferric for apotransferrin receptor found on most cells
    - For transport around the body
- **TfR1** – transferrin receptor found on most cells
- **Iron is absorbed in the ferrous form, requires conversion from ferric form by duodenal cytochrome b (Dcytb)**

IRON ABSOPTION



2 TYPES OF IRON:

- Ferrous iron – form that can be directly absorbed
- Ferric iron – less absolvable form
- Dcytb – Ferric iron to ferrous iron
- Ferrous enters the cell → DMT1 → inside the iron pool – cut into smaller pieces
- Iron leaving intestine → blood → ferroportin → ferric iron → Fe-transferrin → transferrin

LABORATORY ASSESSMENT

- Serum iron (SI)
- Total iron-binding capacity (TIBC)
- Percent transferrin saturation
- Prussian blue staining of tissues
- Ferritin assay
- Soluble transferrin receptor (sTfR)
- Hemoglobin content of reticulocytes
- Zinc protoporphyrin

SERUM IRON (SI)

- Release of iron from apotransferrin (transport protein) by acid
- Color reagent (ferrozine) reacts with freed iron forming a colored complex detected **spectrophotometrically** – for concentrate iron determination
- NOTE: diurnal variation, increased after meal and supplementation (iron)
- Preferred specimen = early morning and fasting (8-12 hrs)

TOTAL IRON-BINDING CAPACITY (TIBC)

- Test that measures blood capacity to bind iron with a protein - transferrin
- Apotransferrin is first saturated with ferric iron
- Amount of iron detected presents all the **binding sites available in transferrin** – the total iron-binding capacity (TIBC)
- Indirect measure of transferrin
- Increased in Iron Deficiency anemia (IDA) – body produces more transferrin

PERCENT TRANSFERRIN SATURATION

- Measures the available sites on apotransferrin

Serum iron/TIBC X 100% = % transferrin saturation

- Low transferrin saturation percentage may indicate iron deficiency
- High transferrin – iron overload

ZINC PROTOPORPHYRIN

- Accumulates in RBCs when iron is not incorporated into heme and zinc binds instead to **protoporphyrin IX** – precursor molecules for heme
- Molecules responsible for oxygen transport into blood
- Value of this test is greatest when the activity of the **ferrochelatase** is impaired, as in lead poisoning
    - Responsible for inserting iron into protoporphyrin IX into heme
    - If not working properly, zinc will bind to protoporphyrin IX leading to high levels of protoporphyrin in RBCs – measuring zinc protoporphyrin can help in the diagnosis where iron incorporating into heme is disrupted (IDA, lead poisoning)

HEMATOLOGY LECTURE
WEEK 5 (MED225)

GLOBIN CHAINS IN HEMOGLOBIN

GREEK DESIGNATION	GREEK NAME	No. of AMINO ACIDS	Other Info
α	Alpha	141	
β	Beta	146	
δ	Delta	146	Differs from beta chain by 10 amino acids
γ	Gamma	146	Differs from beta chain by 39 amino acids
ε	Epsilon	unknown	Embryonic only
ζ	Zeta	141	Enbryonic only

- Alpha and beta – main components of adult hemoglobin
- Gamma – important for fetal hemoglobin; has a higher affinity of oxygen than adult hemoglobin – allowing efficient oxygen transfer from the mother to the fetus
- Delta – part of adult hemoglobin
- Epsilon and zeta – seen only in the earliest stage of development – embryonic hemoglobin

NORMAL HUMAN HEMOGLOBINS

HEMOGLOBIN	MOLECULAR STRUCTURE	STAGE OF LIFE
Gower 1	2 zeta, 2 epsilon	Embryonic
Portland	2 zeta, 2 gamma	Embryonic
Gower 2	2 alpha, 2 epsilon	Embryonic
Fetal (F)	2 alpha, 2 gamma	Newborn and adult
A1	2 alpha, 2 beta	Newborn and adult
A2	2 alpha, 2 delta	Newborn and adult

- Gower 1, 2, and Portland – present in the earliest stage of development
- Fetal – predominant in the fetal; gradually replaced by adult forms after birth (after 6 months)
- A1 and A1 – main types of hgb seen in adults – healthy individual
- A1 – most common form of hgb in adult

OXYHEMOGLOBIN DISSOCIATION CURVE

- Shows how readily hgb in the blood picks up and release oxygen under certain conditions
- NOTE:
    - **Curve/shift to the RIGHT – hgb RELEASES oxygen more easily**
    - **Curve/shift to the LEFT – hgb HOLDS on to oxygen more tightly**

Effects of:

- pH
    - Acidic – curve to the right – hgb releases oxygen more easily (BOHr EFFECT)
    - Alkaline – shift curve to the left – hgb holds into oxygen more tightly
- 2,3-DPG
    - Molecules that binds to hgb which reduces its affinity for oxygen
    - Increased levels – curve to the right
    - Low levels – curve to the left
- Body temperature
    - Increased BT – shift to the right – useful during fever or exercising
    - Decreased BT – shirt to the left
- Carbon dioxide
    - Increased CO2 – shift to the right – CO2 combines with water forming carbonic acid – lowers pH
    - Lower CO2 – curve to the left
- Hb F admixture
    - Higher – curve to the left – allow efficient transfer from mother to fetus
- Abnormal hemoglobins
    - Ex: sickle cell or thalassemia

HEMOGLOBIN DERIVATIVES

- Abnormal hgb **DO NOT transport O2**

1. **CARBOXYHEMOGLOBIN (HbCO)**
    - Carbon monoxide – **210 times** greater affinity to heme than oxygen
        - Carbon monoxide binds more to hemoglobin than oxygen
    - CO – **binds hgb** even if its concentration in the air is extremely low
    - High concentrations of HbCO shift the Hb-oxygen dissociation curve increasingly to the **left**, thus adding to **anoxia** – lack of oxygen
    - Light sensitive with typical, brilliant, **CHERRY RED COLOR OF SKIN** – carbon monoxide poisoning
    - Chief sources – gas line motors, illuminating gas, gas heaters, defective stoves, smoking of tobacco, etc.
    - **Reversible** – through high concentrations of oxygen

2. **METHEMOGLOBIN/ HEMIGLOBIN (HI)**
    - Derivative of hgb in which the **ferrous iron is oxidized to the ferric state**, resulting in the instability of Hi to combine reversibly with O2
    - Causes **CHOCOLATE BROWN** discoloration of blood, cyanosis and functional anemia if present in high enough concentration
    - Reversible
    - Quantitated by spectrophotometry

- An abnormal Hb (Hb M) may also be responsible for methemoglobinemia noted at birth or in the first few months of life

3. SULFHEMOGLOBIN

- Is a mixture of oxidized**, partially denatured form of hgb** that form during oxidative hemolysis
- During oxidation of hgb, **sulfur** from some source, which may vary is incorporated into heme rings of hgb, resulting in **green hemochrome**
- Blood is **MAUVE-LAVANDER** in sulfhemoglobinemia
- Reported in px receiving tx with sulfonamides or aromatic amine drugs (phenacetin, acetanilid) as well as in px with severe constipation, in cases of bacteremia due to **C. perfringes** and in condition known as enterogenous cyanosis
- Cannot transport oxygen; can combine with CO to from **carboxydulfhemoglobin**
- CANNOT be reduced back to hgb and remains in the cells until they break down; **IRREVERSIBLE**