Case 1: "Do I need a CBC?"

Block 4 Module 1

BLOOD

- Blood is a tissue composed of red blood cells (erythrocytes), white blood cells (leukocytes) and platelets suspended in a fluid blood plasma which also contains an immense number of ions, inorganic molecules, and organic molecules including plasma proteins
- Average human possesses: **5 liters** of blood

HEMATOPOIESIS

DEFINITION

- Hematopoiesis is defined as the production, development, differentiation, and maturation of all blood cells

DIFFERENTIATION AS TO SITE OF PRODUCTION BEFORE AND AFTER BIRTH

- The bone marrow is extremely versatile and serves as the body well by supplying life-giving cells with a multiplicity of functions. Various organs serve a role in hemopoiesis, and these organs differ from fetal to adult development.
- The **yolk sac, liver,** and **spleen** are the focal organs in fetal development
  - From 2 weeks until 2 months in the fetal life, most erythropoiesis takes place in the fetal yolk sac (primitive erythroblasts).
  - During the hepatic period (2 through 7 months of fetal life), the **liver** and **spleen** take over the hematopoietic role
    - **Liver** – serves as an erythroid-producing organ primarily but also give rise to fetal hemoglobin (alpha and gamma chains)
    - **Spleen, Thymus, and Lymph Nodes** – also become haematopoietically active during this stage, producing red cells and lymphocytes
  - From 7 months until birth, the bone marrow assumes the primary role in hematopoiesis, a role that continues into adult life.

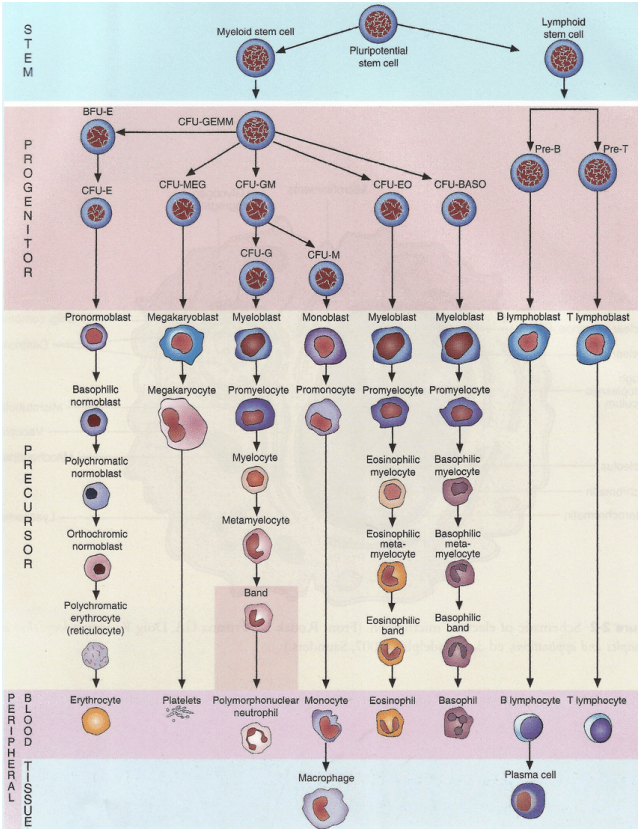
DIFFERENTIATION BETWEEN INTRAMEDULLARY AND EXTRAMEDULLARY HEMATOPOIESIS

- Intramedullary Hematopoiesis - hematopoiesis within the bone marrow
- Extramedullary Hematopoiesis - hematopoiesis outside the bone marrow environment, primarily in the liver and spleen.
- Because these organs play major roles in early fetal hematopoiesis, they retain their hematopoietic memory and capability.
- The liver and spleen can function as organs of hematopoiesis if needed in adult life such as in bone marrow infiltration by leukemic cells.
- If extramedullary hematopoiesis develops, the liver and spleen become enlarged, a condition termed as "hepatomegaly"

FORMED ELEMENTS OF THE BLOOD

DEVELOPMENT

- Formed elements of the blood:
  - Erythrocytes
  - Granulocytes
  - Monocytes
  - Lymphocytes
  - Platelets
- The formed elements of the blood have a common origin in the pluripotent hematopoietic stem cell (found in the red-cell producing bone marrow).
  - This common precursor then gives rise to the lymphoid stem cells which are committed to produce lymphocytes and the trilineage myeloid stem cells which are committed to produce myeloid cells.
- The lymphoid stem cells give rise to precursors of T cells and B cells and possible natural killer (NK) cells.
- From the multipotent myeloid stem cell arise at least three types of "committed stem cells" capable of differentiating along the erythroid/megakaryocytic, eosinophil, and granulocyte – macrophage pathways.
- From the various committed stem cells are derived intermediate stages and ultimately the morphologically recognizable precursors of the differentiated cell lines, that is, proerythroblasts, myeloblast, megalokaryoblasts, monoblasts, and eosinophiloblasts. These turn in give rise to mature progeny.



MECHANISM OF PRODUCTION AND RELEASE

- In the red cell-producing bone marrow are cells called pluripotential hematopoietic stem cells, from which all the cells in the circulating blood are derived.
- Growth and reproduction of the different stem cells are controlled by multiple proteins called growth inducers.
- Four major growth inducers have been described.
  - One of these, **interleukin-3**, promotes growth and reproduction of virtually all the different types of stem cells, whereas the others induce growth of only specific types of committed stem cells.
  - The **growth inducers** promote growth but not differentiation of the cells. This is the function of still another set of proteins called differentiation inducers.
  - Each of these causes one type of stem cell to differentiate one or more steps toward a final type of adult blood cell.
- Formation of the growth inducers and differentiation inducers is itself controlled by factors outside the bone marrow.
  - For instance, in the case of red blood cells, exposure of the body to low oxygen for a long-time results in growth induction, differentiation, and production of greatly increased numbers of erythrocytes.
    - Example: When a person's hemoglobin level is below normal, the oxygen content of the blood drops and the oxygen tension in the kidneys is reduced (hypoxia). This stimulates the kidneys to increase their production of erythropoietin, a low - molecular weight hormone which is dedicated to red cell regeneration. Erythropoietin makes its way through the circulation

and locks onto a receptor on the pronormoblast, the youngest red cell precursor, stimulating the production of 16 mature red cells from every pronormoblast precursor cell.

- The total mass of red blood cells in the circulatory system is regulated within narrow limits. What we know about the control mechanism is diagrammed below:

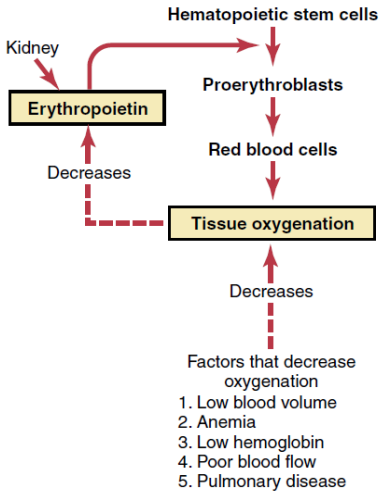


Figure 33-4. Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

ERYTHROCYTIC (RED CELL) SERIES

GENERAL DESCRIPTION

- The mature red blood cell is a magnificently designed instrument for hemoglobin delivery. As a hemoglobin - filled sac, the red cell travels more than 300 miles through the peripheral circulation, submitting itself to the swift waters of the circulatory system, squeezing itself through the threadlike splenic sinuses and bathing itself in the plasma microenvironment.
- Average size is 6 - 8 µm.
- Cellular and environmental factors contribute to red cell survival. In order for the red cell to survive for its 120 - day life cycle, these conditions are necessary:
  - The red cells membrane must be deformable
  - Hemoglobin structure and function must be adequate
  - The red cell must maintain osmotic balance and permeability

FUNCTIONS

- The major function of erythrocytes is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues.
- Erythrocytes also contain a large quantity of carbonic anhydrase, which catalyzes the reversible reaction between carbon dioxide and water, increasing the rate of this reaction several thousand-fold.
  - The rapidity of this reaction makes it possible for the water of the blood to transport enormous quantities of carbon dioxide from the tissues to the lungs in the form of the bicarbonate ion.
- Also, the hemoglobin in the cells is an excellent acid-base buffer, so that the red blood cells are responsible for most of the acid-base buffering power of whole blood.

STAGES OF DEVELOPMENT

Pronormoblast (Rubriblast)

- This is the earliest recognizable erythroid precursor and is the largest of all the erythroid precursors.
- Cell size is variable, nucleus usually occupies more than 80% of the cell and is round to slightly oval with dispersed fine clumps of chromatic and nucleoli.
- Cytoplasm is intensely basophilic.
- This undergoes mitosis and forms two (2) basophilic normoblasts (prorubricyte)

Basophilic Normoblast (Prorubricyte)

- This is slightly smaller than the rubriblast with nucleus usually occupying 75% of the cell.
- The nucleus is round with coarser and more clumped chromatin, parachromatin (the nonstaining area of the nucleus) is slightly visible between the clumps of chromatin, usually nuclei are no longer visible. The cytoplasm is deeply basophilic due to the abundance of RNA.
- After mitosis, evidence of continuing hemoglobin production becomes visible in the cytoplasm of the two daughter cells the polychromatophilic normoblast (rubricyte).

Polychromatophilic Normoblast (Rubricyte)

- Usually smaller than the proubricyte, its round nucleus may be eccentric, nuclear chromatin is very coarse and condensed, and distinct areas of parachomatin are visible.
- The cytoplasm has a varied spectrum of the red- staining of hemoglobin with the of RNA in varying shades. of gray (polychromasia or polychromatophilia)
- This undergoes one or two mitotic divisions. After the last mitosis, the orthochromatophilic normoblast (metarubricyte) stage is reached.

Orthochromaphilic Normoblast (Metarubricyte)

- This is the last nucleated erythrocyte stage and its size is the same as or smaller than a rubricyte.
- Nucleus is pyknotic. The cytoplasm contains more abundant hemoglobin and fewer polyribosomes and remains slightly polychromatophilic (paler blue - gray violet to pink).
- Finally, accompanied by cytoplasmic contractions and undulations, the nucleus and a small rim of cytoplasm are ejected from the orthochromatic normoblast forming the reticulocyte.

Reticulocyte

- This is slightly larger than the erythrocyte and may have irregular cytoplasmic borders.
- The reticulocyte is polychromatophilic as a result of retention of RNA. (pink and blue staining of cytoplasm)

Mature Erythrocyte

- A biconcave disc with a central pale area which gradually fades into a reddish - pink cytoplasm.

LEUKOCYTIC (WHITE CELL) SERIES

GENERAL DESCRIPTION

- The white cell series encompasses those cells that are distinguished by their granules and those that are agranular. In all, there are five maturation stages for neutrophils, four for eosinophils and basophils, and three each for monocytes and lymphocytes. Key features in distinguishing immature and mature stages of any of these cells are: cell size, nucleus - to - cytoplasm (N:C) ratio, chromatin pattern, cytoplasmic quality, and presence of granules.

GRANULOCYTES (NEUTROPHILS, EOSINOPHILS, BASOPHILS)

- In general, as granulocytes mature, the nuclear chromatin becomes more condensed, nucleoli disappear, and abundant basophilic cytoplasm with non-specific granulation progresses to more scant cytoplasm containing granulation specific for the eosinophil, basophil or neutrophil. The nucleus indents and the overall cell size decreases.

Neutrophils

- Stages of maturation of the neutrophilic series from least mature to most mature:
  Myeloblast → promyelocyte (progranulocyte) → myelocyte (granulocyte) → metamyelocyte → band (stab) → segmented neutrophil.
- Morphology:
  - **Myeloblasts**
    - dark blue to blue cytoplasm without visible granules; nucleus is made up of smooth, delicate, uniformly distributed chromatin patter, 2 or more distinct nucleoli, the nucleus occupies most of the cell leaving only a rim of cytoplasm.

- **Promyelocyte**
  - the cytoplasm remains blue; nucleus still with uniform, evenly distributed chromatin pattern; there are large, prominent reddish - purple granules in the cytoplasm, nucleoli may or may not be visible; the nucleus: cytoplasmic ratio decreases
- **Myelocyte**
  - the cytoplasm loses its blue color and shows & pinkish-tan color; specific granules are formed; nucleus is still round, becomes more condensed and the chromatin pattern clumped; nucleoli usually absent.
- **Metamyelocyte**
  - the shape of the nucleus is the chief identification criterion - begins to pattern on one side and begins to constrict or indent (indentation is less than half the width of the nucleus kidney - bean or peanut - shaped); the cytoplasm is uniformly pink with pinkish purple secondary granules evenly distributed.
- **Band (stab)**
  - elongated, curved or sausage - shaped nucleus with no threadlike filament; the cytoplasm stains light pink with numerous specific granules that give it a grainy appearance
- **Segmented neutrophil**
  - the nucleus is segmented into 2 to 5 lobes connected by a threadlike filament, nuclear chromatin is dark purple with densely stained clumps; cytoplasm is like that of a band.

- **General Description**
  - The primary (nonspecific) and secondary (specific) granules of the neutrophils are packaged and released from the Golgi apparatus.
  - **Primary Granules**
    - are membrane bound lysosomes and contain acid phosphatase, peroxidase, esterase, sulfated mucosubstance, β-galactosidase, arylsulfatase, lysozyme and other basic proteins.
  - **Secondary Granules**
    - contain aminopeptidase, collagenase, muramidase, lactoferrin, lysozyme and a number of basic proteins.
- **Functions**
  - Neutrophils are metabolically active. They are capable of both aerobic and anaerobic glycolysis for their source of energy.
  - Their major function is to stop or retard the action of foreign material or infectious agents by means of:
    - moving into the area of inflammation or infection
    - phagocytosis of the foreign material
    - killing and digestion of the offending material

Eosinophils

- **General Description**
  - The eosinophil is primarily a tissue cell. Once it is released into the peripheral blood from the bone marrow, it will be randomly removed from the blood independently of its age. Its half - life in the blood is about 8 hours, there is a diurnal variation in eosinophil counts, with the highest occurring at night. From the blood, it moves into the tissues where it localizes in areas exposed to the external environment, most notably in the skin, nasal membranes, lungs and gastrointestinal tract. For each eosinophil in the peripheral blood, there are 300 - 500 eosinophils in the tissues where their life span is probably several days. Once they migrate to the tissues they are still able to return to the peripheral circulation.
  - The mature eosinophils contain 2 types of granules:
    1. The larger granules are the more numerous, contain a very dense elliptical crystalloid core, which primarily consists of major basic protein (MBP) which is toxic to helminth parasites and may also become toxic to the body's own tissues. Production of these granules stops when the eosinophil becomes mature.
    2. The second type of granule is smaller than the first and may not appear in the cell until after the myelocytic stage.
- The eosinophilic granules contain peroxidase, B - glucorinidase, acid ß - glycerophosphatase, arylsulfatase (in the small granules) phospholipase, acid phosphatase, ribonuclease and cathepsin. The peroxidase is a different form than that found in the neutrophil. Also, the eosinophil granules differ from these in the neutrophil, in that they lack lysozyme, phagocytic, and neutrophil bactericidal cationic proteins.
- **Morphology**
  - The eosinophil usually has a bilobed nucleus with moderately large, refractile cytoplasmic granules that stain deep red or orange.
- **Functions**
  1. Act as phagocytes
  2. Destroy helminths (by generating potent oxidants and releasing cationic proteins from the granules which then damage and degrade the larval wall of the parasitic invaders.)
  3. To dampen hypersensitivity and inflammatory reactions
  - Eosinophils modulate reactions that occur when tissue mast cells and basophils degranulate. Eosinophils express the chemokine receptor CCR3.
  - Among the chemotactic factors that attract eosinophils, eosinophil chemotactic factor of anaphylaxis (ECF - A) is present in basophils and mast cells.
  - Eosinophils also contain substances that inactivate factors released by mast cell and basophils such as histamines, slow - reacting substances of anaphylaxis and platelet - activating factor (PAF).

Basophils

- **General Description**
  - Basophils have a life span similar to eosinophils. Basophils circulate in the blood and are not normally found in tissues, in contrast to mast cells which can spend 9 - 18 months in connective tissue.
  - The granules of mature basophils are metachromatic (red - purple) because of acid mucopolysaccharide (heparin) content. Those granules contain histamine, heparin, peroxidase, eosinophilic chemotactic factor - A, smaller quantities of bradykinin and serotonin.
  - Other cellular constituents are: slow – reacting substance of anaphylaxis and platelet - activating factor.
- **Morphology**
  - Shows large, coarse cytoplasmic granules that stain deep blue) the granules may be seen within the cytoplasm or overlying the irregular nucleus.
- **Functions**
  1. immediate hypersensitivity reactions
  2. some delayed hypersensitivity reactions
  - Basophils as well as mast cells appear to be involved in immediate hypersensitivity reactions such as allergic asthma
  - IgE binds readily to basophil and mast cell membranes. When a specific antigen reacts with the membrane - bound IgE, degranulation occurs with the release of mediators of immediate hypersensitivity (e.g, histamine SRS - A), PAF, heparin and ECF - A). The latter leads to the accumulation of eosinophils, which contain substances that tend to counteract these mediators.
  - Basophils are also involved in some delayed hypersensitivity reactions, "cutaneous basophil hypersensitivity" such as contact allergies, in which they appear to undergo a different type of degranulation response.

AGRANULOCYTES (MONOCYTES, MACROPHAGES, LYMPHOCYTES)

Monocytes and Macrophages

- **General Descriptions**
  - Blood monocytes are distributed in a circulating monocyte pool and marginating monocyte pool, in a ratio of 1:3.5. Once monocytes enter the blood, they leave randomly with a half - time of 8.4 hours.
    - This time period is shortened in splenomegaly or acute infection and may be prolonged in monocytosis. After monocytes leave the blood they spend several months as macrophages.
  - Granules and other constituents: acid hyrolase, arylsulfatase, nonspecific esterase, peroxidase, acid phosphatase.
- **Morphology**
  - In the normal marrow, it is not possible morphologically to distinguish the "monoblast" from the myeloblast.
  - The earliest recognizable cell in the series is the promonocyte, somewhat larger than the myeloblast. The N/C ratio is moderate, and the nucleus may be oval or indented with a fine uniform or slightly streaked chromatin and two to five nucleoli. The cytoplasm is basophilic with a ground - glass appearance and a variable number of five azurophilic granules.
  - The **monocyte** which is present in both blood and marrow, is only slightly smaller; it has a moderate to low N/C ratio and or indented or lobe nucleus with a fine - streaked, only slightly condensed, delicate chromatin pattern. Nucleoli are indistinct or obscured. The cytoplasm is opaque, more gray than blue and contains an abundance of fine azurophilic granules.
  - **Macrophages** are the tissue component of the monocyte system and arise from emigrated blood monocytes. Macrophages are larger than monocytes. They have irregular cell membranes, often with blebs and pseudopods. The N/C ratio is high with an oblong and/or indented nucleus.
- **Functions**
  1. phagocytosis - bacteria, cellular debris, senescent cells
  2. antigen processing
  3. cell - mediated immunity - antibody - dependent cytotoxicity
  4. synthesis of bioactive molecules

MEGAKARYOCYTIC (THROMBOCYTIC) SERIES

GENERAL DESCRIPTION

- **General description**
  - Platelets originate from polypoid megakaryocytes, the largest of all hematopoietic cells which number less than 1% of the total nucleated marrow cells. The fragments of these megakaryocytes are platelets that are released into the bloodstream. The circulating platelets make up about two — third of the platelets that are released from the bone marrow. The other one - third is stored (sequestered) in the spleen.
  - Platelets survive 8 - 12 days in the circulation. Some platelets are utilized in maintaining vascular integrity and in plugging small vascular injuries.
- **Morphology**
  - Megakaryocytes
    - is a giant cell with round multiple nuclei (up to 32) that usually remain connected, and abundant cytoplasm containing numerous small, rather uniformly distributed granules with a reddish - blue hue. The chromatin pattern is linear and coarse with distinct spaces between the strands.
  - Platelets
    - dense blue to purple particles with granules
- **Functions of Platelets**:
  1. maintain the integrity of blood vessels
  2. form hemostatic plugs to stop blood loss from injured vessels and in the process promote coagulation of plasma factors.

COMPLETE BLOOD COUNT

DEFINITION

- The complete blood count (CBC) is one of the most frequently ordered and most time - honored laboratory tests in the hematology laboratory.
- This evaluation consists of several components and offers the clinician a variety of hematological data to interpret and review that directly relate to the health of the bone marrow represented by the numbers and types of cells in the peripheral circulation. It measures the concentration of white blood cells, red blood cells and platelets in the blood and aids in the diagnosis of conditions and diseases

COMPONENTS

- **White Blood Cell Count (WBC)**
  - is the number of white blood cells in a volume of blood.
- **White Blood Cell (WBC) Differential Count**
  - composed of several different types that are differentiated, or distinguished, neutrophils, lymphocytes, monocytes, eosinophils, basophils and Based on their size and shape. The cells in a differential count are neutrophils, lymphocytes, monocytes eosinophils, basophils, and bands
- **Red Cell Count (RBC)**
  - signifies the number of red blood cells in a volume of blood.
- **Hemoglobin (Hb)**
  - this is the amount of hemoglobin in a volume of blood. Hemoglobin is the protein molecule within red blood cells that carries oxygen and gives blood its red color.
- **Hematocrit (Hct)**
  - this is the ratio of the volume of red cells to the volume of whole blood. This is usually measured by spinning down a sample of blood in a test tube which causes the red blood cells to pack at the bottom of the tube.
- **Mean Corpuscular Volume (MCU)**
  - is the average volume of a red blood cell. This is a calculate value derived from the hematocrit and red cell count.
- **Mean Corpuscular Hemoglobin**
  - the average amount of hemoglobin in the average red cell.
- **Mean Corpuscular Hemoglobin Concentration (MCHC)**
  - is the average concentration of hemoglobin in a given volume of red cells.
- **Red Cell Distribution Width (RDW)**
  - is a measurement of the variability of red cell size and shape. Higher numbers indicate greater variation in size.
- **Platelet Count**
  - the number of platelets in a specified volume of blood.

TABLE 31.2
Typical Blood Cell Values in a Normal Population of Young Adults

	Men	Women
White cell count ($\times 10^9$/L blood)	7.8 (4.4–11.3)	
Red cell count ($\times 10^{12}$/L blood)	5.21 (4.52–5.90)	4.60 (4.10–5.10)
Hemoglobin (g/dL blood)	15.7 (14.0–17.5)1	13.8 (12.3–15.3)
Hematocrit (%)	46 (41.5–50.4)	40.2 (35.9–44.6)
Mean cell volume (fL/ red cell)	88.0 (80.0–96.1)	
Mean cell hemoglobin (pg/red cell)	30.4 (27.5–33.2)	
Mean cell hemoglobin concentration (g/dL RBC)	34.4 (33.4–35.5)	
Red cell distribution width (CV, %)	13.1 (11.6–14.6)	
Platelet count ($\times 10^9$/L blood)	311 (172–450)	

The mean and reference intervals (normal range) are given. Because the distribution curves may be nongaussian, the reference interval is the nonparametric central 95% confidence interval. Results are based on 426 normal adult men and 212 normal adult women. Studies were performed on the Coulter model S-Plus IV. From Morris MW, Skrodzki Z, Nelson DA: Zeta sedimentation ratio (ZSR), a replacement for the erythrocyte sedimentation rate (ESR), *Am J Clin Pathol* 164:254–256, 1975.
CV, Cell values; *RBC,* red blood cell.

TABLE 31.3
Normal Leukocyte Count, Differential Count, and Hemoglobin Concentration at Various Ages

Age	LEUKOCYTES* Total Leukocytes	Total Neutrophils	Band Neutrophils	Segmented Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Hemoglobin (g/dL Blood)
12 months	11.4 (6.0–17.5)	3.5 (1.5–8.5) *31*	0.35 *3.1*	3.2 (1.0–8.5) *28*	0.30 (0.05–0.70) *2.6*	0.05 (0–0.20) *0.4*	7.0 (4.0–10.5) *61*	0.55 (0.05–1.1) *4.8*	12.6 (11.1–14.1)
4 years	9.1 (5.5–15.5)	3.8 (1.5–8.5) *42*	0.27 (0–1.0) *3.0*	3.5 (1.5–7.5) *39*	0.25 (0.02–0.65) *2.8*	0.05 (0–0.2) *0.6*	4.5 (2.0–8.0) *50*	0.45 (0–0.8) *5.0*	12.7 (11.2–14.3)
6 years	8.5 (5.0–14.5)	4.3 (1.5–8.0) *51*	0.25 (0–1.0) *3.0*	4.0 (1.5–7.0) *48*	0.23 (0–0.65) *2.7*	0.05 (0–0.2) *0.6*	3.5 (1.50–7.0) *42*	0.40 (0–0.8) *4.7*	13.0 (11.4–14.5)
10 years	8.1 (4.5–13.5)	4.4 (1.8–8.0) *54*	0.24 (0–1.0) *3.0*	4.2 (1.8–7.0) *51*	0.20 (0–0.60) *2.4*	0.04 (0–0.2) *0.5*	3.1 (1.5–6.5) *38*	0.35 (0–0.8) *4.3*	13.4 (11.8–15.0)
21 years	7.4 (4.5–11.0)	4.4 (1.8–7.7) *59*	0.22 (0–0.7) *3.0*	4.2 (1.8–7.0) *56*	0.20 (0–0.45) *2.7*	0.04 (0–0.2) *0.5*	2.5 (1.0–4.8) *34*	0.30 (0–0.8) *4.0*	15.5 (13.5–17.5) 13.8 (12.0–15.6)

From Altman PL, Dittmer DS, editors: *Blood and other body fluids,* Washington, DC, 1961, Federation of American Societies for Experimental Biology (for leukocyte and differential count); Dalman PR: Developmental changes in red blood cell production and function. In: Rudolph AM, Hoffman JIE, editors: *Pediatrics,* ed 18, Norwalk, CT, 1987, Appleton & Lange, pp 1011–1012 (for hemoglobin concentrations).
*Values are expressed as mean (95% reference) values. For leukocytes and differential count cell types, the units are cells × 10^9/μL; the numbers in italics are mean percentages.

SIGNIFICANCE OF ABNORMAL VALUES

- Hct/ Hgb/ RBC
  - Low – Anemia
  - High – Polycythemia, physiologic variation

White Blood Cell Counts

- White cell counts that are reported on the CBC are directly counted from an automated instrument or by manual method.
- The age of the patient directly influences whether this number within or outside of the reference range. Pediatric reference ranges show more variability than do ranges for adults.

White Blood Cell Differential Count

- The WBC differential is an evaluation of the types of mature white cells in the peripheral circulation. Although only a snapshot of the white cell concentration at a particular moment in time, the differential offers valuable information as to the hematological status of an individual and their response to any circumstances which may alter hematological status. In general terms, the differential is performed on a well - stained, well distributed peripheral smear.
- Because white cells have such a short time span in the peripheral circulation, alterations either in the quantity of or the quality of a particular cell can be quite dramatic. Any increase or decrease in a particular type of cell signals the body's unique response "assaults" of any kind. Infection, inflammation, chronic disease, parasitic infestations, etc., each represents an unexpected occurrence, an opportunity for white cells to mobilize. As white cells respond to infection or other stimuli, changes are seen in the number of and types of a particular cell line.
- If a cell line is increased, the suffix used to designate an increase is "osis" or "philia", such as "eosinophilia" and "leukocytosis." If a cell line is decreased, the suffix used to designate a decrease is "penia" such as "neutropenia." Changes are observed in the CBC as well as in the peripheral blood smear.
- Total WBC (white blood cell) count:
  - Low - leukopenia (infection, conditions with pancytopenia)
  - High - leukocytosis (infection, metabolic toxic states)
- In the bone marrow, there is a 4:1 (ME ratio) - the M:E ratio indicating that four myeloid or white cells are produced for one erythroid cell.
- Daily production of white cells is 1.5 billion. Transit from the bone marrow to the peripheral circulation takes place only after white cells have been held in the maturation storage pool of the bone marrow. Once released into the circulation, most white cells are short - lived before they migrate into tissues. The white cells that are observed in the peripheral circulation are only a snapshot of white cells that are located in three distinct cell compartments" the bone marrow, the circulation, and the tissues.
- White blood cells (WBCs) are referred to as leukocytes. For clarity, the word "leukocytic" applies to the white cells of all stages. White cells are a remarkably versatile group of cells whose primary purpose is to defend against bacteria, viruses, fungi or other foreign substances. To this end, most white cells are granulated and these granules contain enzymes used for digestion and destruction of the invading organisms.
- The granular leukocytes are: neutrophils, eosinophils, basophils
- The agranular leukocytes are: monocytes, lymphocytes
- The term "granulocytic" applies only to granulated white cells. The term "myelocytic" is used in describing a particular white cell condition. The two terms may be used interchangeably but only in reference to the leukemias.
- **Relative vs. Absolute Values**
  - Relative and absolute counts are terms referring to the white cell differential. The "absolute" count refers to the count derived from the total white count multiplied by the percentage of any particular white cell. The "relative" count refers to the percentage of a particular cell counted from the 100 WBC differential. Example of how to calculate and interpret the relative and absolute count:

    WBC: 5.0 × 10^9/L

Differential:		Ref. Range:
Segmental neutrophils:	40%	40% - 70%
Bands	3%	0 - 5%
Lymphocytes	55%	20 - 40%
Monocyte	2%	2 - 8%

    Then: Absolute count of lymphocytes would be
    5000 x 0.55 = 2,500
    (from the total WBC) (lymphocytes)

  - Reference range for absolute lymphocyte count: 1000 - 4,800
  - Therefore: In this patient there is relative lymphocytosis but not an absolute lymphocytosis.
- **The Neutrophils**
  - **Neutrophilia**:
    - Neutrophilic leukocytosis or neutrophilia refers to an absolute concentration of neutrophils above normal for age. Key causes are:
      - Acute inflammatory - collagen vascular, vasculitis
      - Acute infectious - bacterial, some viral, fungal, parasitic
      - Drugs, toxins, metabolic - corticosteroids, growth factors, uremia, ketoacidosis
      - Tissue necrosis - burns, trauma, myocardial infarction, red blood cell
      - hemolysis
      - Physiologic - stress, exercise, smoking, pregnancy
      - Neoplastic - carcinomas, sarcomas, myeloproliferative disorders
    - **Mechanisms**: The primary factors influencing the neutrophil count are:
      1. The rate of inflow of cells from the bone marrow (mitosis/proliferation, maturation/storage and release)
      2. The proportion of neutrophils in marginal (cells adhering to vessel! walls) granulocyte pool (MGP) and the circulating (non-adhering cells) granulocyte pool (CGP) of the blood.
      3. The rate of outflow of neutrophils from the blood (migration from and through vessels into tissue, both randomly and at sites of inflammation, infection, etc.)
  - **Neutropenia**:
    - This is a reduction of the absolute neutrophil count (ANC). The term "agranulocytosis" has been used for severe neutropenia. This can also be associated with depletion of eosinophils and basophils. Causes of neutropenia:
      - Drugs - cancer chemotherapy, chloramphenicol, sulfas/other antibiotics, phenothiazines, benzodiazepine, antithyroids, anticonvulants, quinine, quinidine, indomethacine, procainamide, thiazide
      - Radiation

- Toxins - alcohol, benzene compounds
- Intrinsic defects - Fanconi's Kostmann's, cyclic neutropenia, Chediak - Higashi
- Immune - mediated - collagen vascular disorders, rheumatoid arthritis, AIDS
- Hematologic - megaloblastic anemia, myelodysplasia, marrow failure, marrow replacement
- Infectious - any overwhelming infection
- Others — starvation, hypersplenism

■ **Mechanisms** by which neutropenia occur include:

1. Decreased flow of neutrophils from marrow into blood as a result of either lack of production or ineffective production
2. Increased removal of neutrophils from the blood (survival defect)
3. Altered distribution between CGP and MGP
4. Combinations of the above mechanisms

○ **Eosinophilia**

■ This is typically associated with allergic processes and parasitic infections.

■ Causes of eosinophilia:

- Allergic – urticaria, hay fever, asthma
- Parasitic – trichinosis, filariasis, ascariasis, Schistosomiasis
- Nonparasitic infection - systemic fungal, scarlet fever, chlamydial pneumonia of infancy
- Respiratory - pulmonary eosinophilia syndrome (Loeffler's, tropical pulmonary eosinophilia). Churg - Strauss syndrome
- Neoplastic - chronic myelogenous leukemia, Hodgkin's lymphoma, T cell lymphomas

○ **Basophilia**

■ Causes:

- Myeloproliferative disease
- Allergic - food, drugs, foreign proteins
- Infections - variola, varicella
- Chronic hemolytic anemia - especially post – splenectomy
- Inflammatory - collagen vascular disease, ulcerative colitis

○ **Monocytosis**

■ Causes:

- Infections - tuberculosis, subacute bacterial endocarditis, syphilis, protozoan, rickettsial
- Recovery from neutropenia
- Hematologic – leukemia, myeloproliferative disorders, lymphomas, multiple myeloma
- Inflammatory - collagen vascular diseases, chronic ulcerative colitis, sprue, myositis, polyarteritis, temporal arteritis

○ **Lymphocytosis**

■ Causes:

- Infections - many viral, pertussis, tuberculosis, rickettsial
- Chronic inflammatory - ulcerative colitis, Crohn's
- Immune - mediated - drug sensitivity vasculitis, graft rejection, Grave's disease, Sjogren's syndrome
- Hematologic - acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma
- Stress - acute, transient

○ **Lymphopenia**

■ Causes:

- Destructive – radiation, chemotherapy, steroide
- Debilitative - starvation, aplastic anemia, terminal cancer, collagen vascular disease, renal failure
- Infections – viral hepatitis, influenza, typhoid fever, tuberculosis
- AIDS associated HIV cytopathic effect, nutritional imbalance, drug effect
- Congenital immunodeficiency: Wiskott-Aldrich

Platelets

- The number of platelets in the blood is referred to as the platelet count and is normally between 150,000 to 450,000/ µL/ (one-millionth of a litter).
- Platelet counts less than 150,000 are termed thrombocytopenia.
- Platelet counts greater than 450,000 are called thrombocytosis.

HEMOGLOBIN

GENERAL STRUCTURE AND ROLE IN THE TRANSPORT PROCESS OF GAS

- Synthesis of hemoglobin begins in the proerythroblasts and continues even into the reticulocyte stage because when the reticulocytes leave the bone the blood stream, they continue to form minute marrow and pass into quantities of hemoglobin for another day of so. Figure 32-5 shows the basic chemical steps in the formation of hemoglobin. The most important feature of the hemoglobin molecule is its ability to combine loosely and reversibly with oxygen.
- Its primary function in the body is to combine with oxygen in the lungs and then to release this oxygen ready in the tissue capillaries where the gaseous tension of oxygen is much lower than in the lungs.

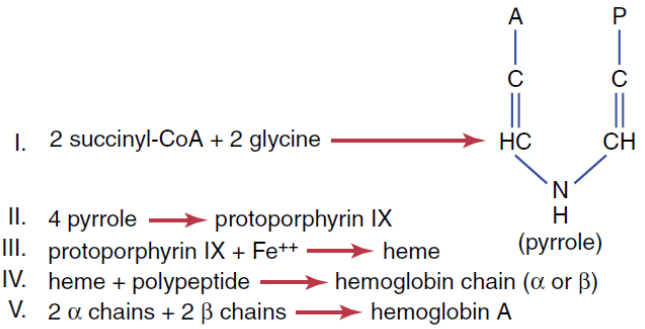


Figure 33-5. Formation of hemoglobin.

PORPHYRIN

- Porphyrins are cyclic compounds formed by the linkage of four pyrole rings through methenyl bridges. A characteristic property of the porphyrins is the formation of complexes with metal ions bound to the nitrogen atom of the pyrrole rings.
  - Examples are the iron porphyrins such as heme of hemoglobin and the magnesium-containing porphyrin chlorophyll, the photosynthetic pigment of plants.
- Proteins that contain heme (hemoproteins) aro widely distributed in nature. The porphyrins found in nature are compounds in which various side chains are substituted for the eight hydrogen atoms numbered in the porphyrin nucleus. As a simple means or showing these substitutions, Fischer proposed a shorthand formula in which the methenyl bridges are omitted and each pyrrole ring is shown as a bracket with the eight substituent positions.
- The arrangement of the acetate and propionate substituents in the uroporphyrin shown in Figure 34-2 is asymmetric. A porphyrin with this type of asymmetric substitution is classified as a type III porphyrin. A porphyrin with a completely symmetric arrangement of the substituents is classified as a type I porphyrin.
- Only types I and III are found in nature, and the type ill series is by far more abundant and more important, because it includes here.
- Heme and its immediate precursor, protoporphyrin IX, are both typo III porphyrins. However, they are sometimes identified as belonging to series IX, because they were designated ninth in a series of isomers postulated by Hans Fischer, the pioneer worker in the field of porphyrin chemistry.

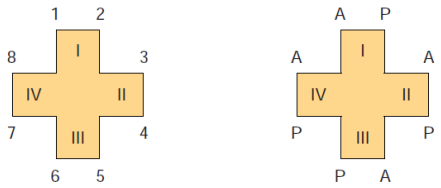


FIGURE 31–2 Uroporphyrin III. (A [acetate] = —CH_2COOH; P [propionate] = —CH_2CH_2COOH.) Note the asymmetry of substituents in ring IV (see text).

BIOSYNTHESIS OF HEME

- Heme is synthesized in living cells by a pathway that has been much studied. The two starting materials are **succinyl-CoA**, derived from the citric acid cycle in mitochondria, and the amino acid glycine. Pyridoxal phosphate is also necessary in this reaction to "activate" **glycine**. The product of the condensation reaction between succinyl-CoA and glycine is α-amino-β-ketoadipic acid, which is rapidly decarboxylated to form α-aminolevulinate (ALA) (Figure 31–5). This reaction sequence is catalyzed by ALA synthase, the rate-controlling enzyme in porphyrin biosynthesis in mammalian liver. Synthesis of ALA occurs in **mitochondria**. In the cytosol, two molecules of ALA are condensed by the enzyme **ALA dehydratase** to form two molecules of water and one of **porphobilinogen** (PBG) (Figure 31–5). ALA dehydratase is a zinccontaining enzyme and is sensitive to inhibition by lead, as can occur in **lead** poisoning.

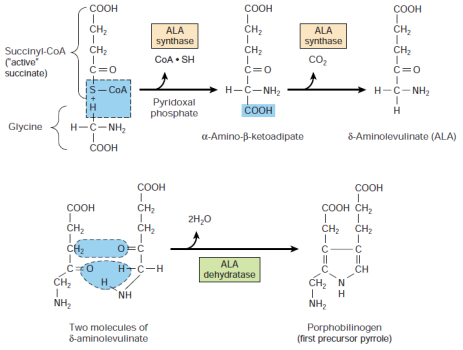


FIGURE 31–5 Biosynthesis of porphobilinogen. ALA synthase occurs in the mitochondria, whereas ALA dehydratase is present in the cytosol.

- The formation of a cyclic tetrapyrrole—ie, a porphyrin— occurs by condensation of four molecules of PBG (Figure 31–6). These four molecules condense in a head-to tail manner to form a linear tetrapyrrole, hydroxymethylbilane (HMB). The reaction is catalyzed by uroporphyrinogen I synthase, also named PBG deaminase or HMB synthase. HMB cyclizes spontaneously to form uroporphyrinogen I (left-hand side of Figure 31–6) or is converted to uroporphyrinogen III by the action of uroporphyrinogen III synthase (right-hand side of Figure 31–6). Under normal conditions, the uroporphyrinogen formed is almost exclusively the III isomer, but in certain of the porphyrias (discussed below), the type I isomers of porphyrinogens are formed in excess.
- Note that both of these uroporphyrinogens have the pyrrole rings connected by methylene bridges (—CH2—), which do not form a conjugated ring system. Thus, these compounds are colorless (as are all porphyrinogens). However, the porphyrinogens are readily auto-oxidized to their respective colored porphyrins. These oxidations are catalyzed by light and by the porphyrins that are formed.
- Uroporphyrinogen III is converted to coproporphyrinogen III by decarboxylation of all of the acetate (A) groups, which changes them to methyl (M) substituents. The reaction is catalyzed by uroporphyrinogen decarboxylase, which is also capable of converting uroporphyrinogen I to coproporphyrinogen I (Figure 31–7). Coproporphyrinogen III then enters the mitochondria, where it is converted to protoporphyrinogen III and then to protoporphyrin III. Several steps are involved in this conversion. The mitochondrial enzyme coproporphyrinogen oxidase catalyzes the decarboxylation and oxidation of two propionic side chains to form protoporphyrinogen. This enzyme is able to act only on type III coproporphyrinogen, which would explain why type I protoporphyrins do not generally occur in nature. The oxidation of protoporphyrinogen to protoporphyrin is catalyzed by another mitochondrial enzyme, protoporphyrinogen oxidase. In mammalian liver, the conversion of coproporphyrinogen to protoporphyrin requires molecular oxygen.

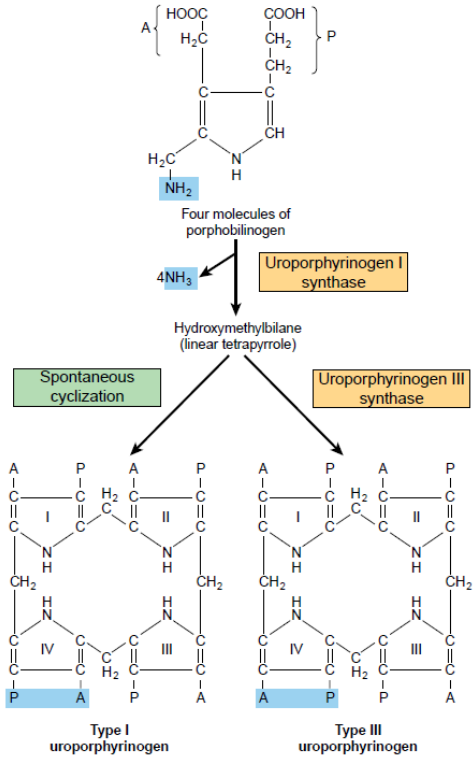


FIGURE 31–6 Conversion of porphobilinogen to uroporphyrinogens. Uroporphyrinogen synthase I is also called porphobilinogen (PBG) deaminase or hydroxymethylbilane (HMB) synthase.

- The final step in heme synthesis involves the incorporation of ferrous iron into protoporphyrin in a reaction catalyzed by ferrochelatase (heme synthase), another mitochondrial enzyme (Figure 31–4).

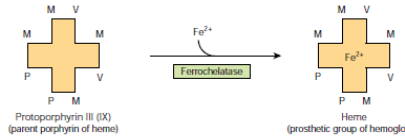


FIGURE 31–4 Addition of iron to protoporphyrin to form heme. (V [vinyl] = —CH═CH_2.)

- A summary of the steps in the biosynthesis of the porphyrin derivatives from PBG is given in Figure 31–8. The last three enzymes in the pathway and ALA synthase are located in the mitochondrion, whereas the other enzymes are cytosolic. Both erythroid and nonerythroid ("housekeeping") forms of ALA synthase are found. Heme biosynthesis occurs in most mammalian cells with the exception of mature erythrocytes, which do not contain mitochondria. However, approximately 85% of heme synthesis occurs in erythroid precursor cells in the bone marrow and the majority of the remainder in hepatocytes.

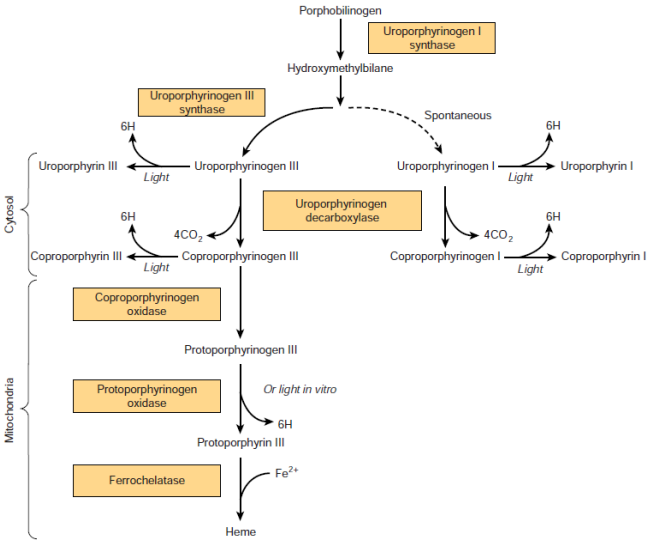


FIGURE 31–8 Steps in the biosynthesis of the porphyrin derivatives from porphobilinogen. Uroporphyrinogen I synthase is also called porphobilinogen deaminase or hydroxymethylbilane synthase.

- The porphyrinogens described above are colorless, containing six extra hydrogen atoms as compared with the corresponding-colored porphyrins. These reduced porphyrins (the porphyrinogens) and not the corresponding porphyrins are the actual intermediates in the biosynthesis of protoporphyrin and of heme.
- The rate-limiting reaction in the synthesis of heme is that catalyzed by ALA synthase, a regulatory enzyme. It appears that heme, probably acting through an aporepressor molecule, acts as a negative regulator of the synthesis of ALA synthase. This repression and depression mechanism is depicted diagrammatically below. It is possible that there is also significant feedback inhibition in which the rate of synthesis of ALA synthase increases greatly in the absence of heme and is diminished in it presence. The turnover rate of ALA synthase is normally rapid in mammalian liver, a common feature of an enzyme catalyzing a rate-limiting reaction.

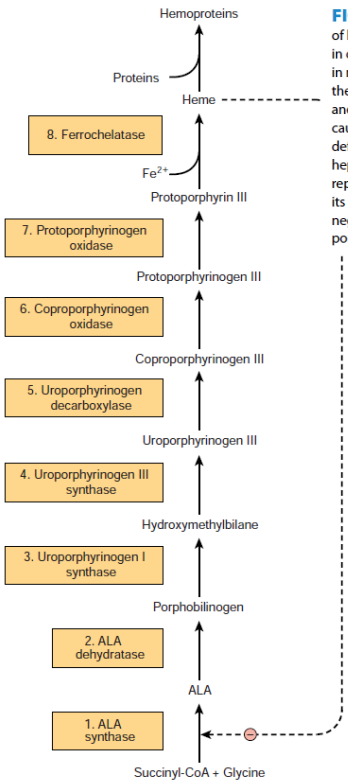


FIGURE 31–9 Intermediates, enzymes, and regulation of heme synthesis. The enzyme numbers are those referred to in column 1 of Table 31–2. Enzymes 1, 6, 7, and 8 are located in mitochondria, the others in the cytosol. Mutations in the gene encoding enzyme 1 causes X-linked sideroblastic anemia. Mutations in the genes encoding enzymes 2–8 cause the porphyrias, though only a few cases due to deficiency of enzyme 2 have been reported. Regulation of hepatic heme synthesis occurs at ALA synthase (ALAS1) by a repression-derepression mechanism mediated by heme and its hypothetical aporepressor. The dotted lines indicate the negative (–) regulation by repression. Enzyme 3 is also called porphobilinogen deaminase or hydroxymethylbilane synthase.