Tutorial - Blood Cell Morphology

A Clinical Pathology 201 Study Module

by

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Feedback

Feedback as to the quality and usefulness of this exercise is solicited and suggestions for improvement are welcomed. Please forward your remarks by E-mail cwalte@lsuhsc.edu

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Directions

The directions for navigating through the exercise are given on the next 2 pages. They are the same as those routinely used in Clinical Pathology 202 study modules. Please click on:

- to visit the directions before continuing with the exercise.

or

- to go directly to the first page of the exercise.
Directions, continued

The following directional icons are provided throughout the exercise for your convenience. You can click on:

- **in the upper left hand corner of every page to return to the previous page**
- **in the upper right corner of the page to return to the Main Menu selection.**
- **in the lower right corner of the page to continue.**
- **in the lower right corner of the Main Menu page to Quit (i.e., end the exercise).**
“Hot points” (symbols, words, phrases) have been inserted on the pages as navigational tools and can be identified by their “gold” color. If it’s “gold”, click on it to move to the next text/data entry. Also, sounds have been added in a few places for emphasis.

Caution, failure to follow the structured order of the “hot points” may result in confusion. If you use the mouse without placing the cursor directly on the “hot point”, you may skip over vital information.

Remember, if it’s gold, click on it. Try it!
This exercise has numerous images. You may note that, when a page contains images, there may be a rather long delay before you regain control of the cursor. Please be patient. I think you will find the images are worth the wait.

NOTE:

Some animation and/or interactive affects may be lost if you attempt to replay a page by returning to the previous page and then advancing to that page again.

Now, click on the gold to begin.
Hematologic Cells
Found in Peripheral Blood
and Bone Marrow
Introduction

Leukocytes

Erythrocytes

Abnormal erythrocytes - terminology

Platelets

Disorders – characteristic morphology
Introduction
What is the purpose of this study module?

This study module is designed for LSUHSC L2 students enrolled in Clinical Pathology 201. It is intended as a reference for blood cell and bone marrow morphology.

The presentation of illustrative cells in this module is by no means a comprehensive study of blood cells. It is limited to the material covered in the lectures and laboratory sessions. Unfortunately, a few cell illustrations are not available at this time but will be added later.
Leukocytes
How are the WBC identified and classified?

Typical nuclear and cytoplasmic morphologic features provide a means by which WBC can be identified and classified as to **cell line** (i.e.,):

- **granulocytes** [neutrophils, eosinophils, or basophils]
- **monocytes**
- **lymphocytes**
i.e., classified as **granulocytes**:

- **neutrophils**
- **eosinophils**
- **basophils**
i.e., classified as **lymphocytes**: shown with normocytic RBC

shown with macrocytic RBC

shown with microcytic RBC
i.e., classified as monocytes:
i.e., Identified as to cell lines

- granulocytes
  - neutrophils
  - eosinophils
  - basophils
- lymphocytes
- monocytes
WBC can also be identified and classified as to...

- **maturity** (i.e., mature cell or immature stage of development).
Immature WBC, e.g.:

- granulocytes (various stages)
- myeloblasts
- lymphoblasts
- monoblasts
e.g., Neutrophils in various stages of maturation...

- myeloblast
- promyelocyte
- myelocyte
- metamyelocyte
- band
- PMN (mature)
Neutrophilic Maturation

from immature blast to mature PMN

Mature neutrophil "PMN"
Neutrophilic Maturation

From mature PMN to myeloblast
WBC can also be identified and classified as to...

- abnormal morphology (i.e., nuclear or cytoplasmic alterations)
e.g., WBC with **acquired non-neoplastic alterations**...

- Hypersegmented neutrophils in megaloblastic anemias
- Neutrophils in bacterial infections
- Reactive/atypical lymphocytes (ATL) in viral infections
- With Döhle bodies and/or toxic granulation

Inherited disorders
e.g., WBC with inherited non-neoplastic alterations...

- Pelger-Huet Anomaly: hypossegmented nuclei
- May-Hegglin Anomaly: cytoplasmic blue bodies
- Alder-Reilly Anomaly: cytoplasmic black granules
- Chediak-Higashi Syndrome: cytoplasmic large black granules
WBC with neoplastic alterations, e.g.:

- hairy cell lymphocytes
- plasma cells
- hairy cell leukemia
- in multiple myeloma
- myeloblasts w/ Auer rod(s)
- in acute myelocytic leukemias
Leukocytic Maturation
Blasts are the earliest leukocytic precursor that can be seen in peripheral blood.

- Myeloblast
- Monoblast
- Myeloblast w/ Auer rod
- Lymphoblast
All blasts have nucleoli. and
Cell lines are difficult to differentiate on Wright’s stain without a distinguishing feature (e.g., Auer rod in AML).
While the presence of **nucleoli** differentiates blasts from more mature forms,

- myeloblast
- myeloblast
- monoblast
- lymphoblast

special stains are usually needed for definitive identification of leukoblasts.

**Leukoblasts must also be differentiated from proerythroblasts.**
How do you differentiate leukoblasts (WBC) and proerythroblasts (RBC)?

Leukoblasts

myeloblast
lymphoblast
monoblast

Proerythroblasts

have nucleoli.

&
How do they differ morphologically?

In the proerythroblast,

- the cytoplasm is usually darker and bluer
- the nuclear chromatin strands are linear and distinct

than the leukoblasts

- compared to leukoblasts, which are more delicate and interlaced.
Myelocytic (or Granulocytic) Series
Myelocytic Maturation

There are three types of cells in the myelocytic (or granulocytic) series: neutrophils, eosinophils, and basophils.

Myeloblasts (ie, the earliest precursor) originate in the marrow from a stem cell common to erythroid, megakaryocytic, and granulocytic cells. Prominent nucleoli are seen in the nucleus and the cytoplasm is agranular. Morphologically, they are difficult to differentiate from lymphoblasts or monoblasts.

Examples of myeloblasts:

- nucleoli
- agranular cytoplasm
Nucleoli are also seen in the promyelocyte and the appearance of large azurophilic (nonspecific) cytoplasmic granules in the early stage of its transition is an indication that the cell is a granulocyte. However, morphologic determination as to neutrophilic, eosinophilic, or basophilic cannot yet be made.

Examples of promyelocytes:

prominent nucleoli and cytoplasmic non-specific granules begin to be visible

as the cell matures, the nucleoli begin to fade and the granules become more numerous and prominent

early promyelocyte  late promyelocyte
Myelocytic Maturation, continued

As the promyelocyte matures and reaches the myelocytic stage, it has definitely and visually differentiated into one of the three granulocytic types with characteristic cytoplasmic “specific” or secondary granules. Nucleoli are indistinct or not seen and this is the last mitotic stage.

Examples of myelocytes with specific granules:

- Neutrophilic
- Eosinophilic
- Basophilic
Myelocytic Maturation, continued

Specific cytoplasmic granules:

- **neutrophilic**: ill-defined reddish granules within the bluish cytoplasm resulting in a lilac or pinkish color

- **eosinophilic**: relatively large, spherical, orange granules

- **basophilic**: unevenly distributed large, blue-black granules, which are usually also visible on top of the nucleus
Neutrophilic Maturation

From this stage on, as the cell matures, there is little change in the cytoplasm but the **nuclear chromatin becomes progressively more condensed** and the characteristic nuclear shapes of the metamyelocyte, band (stab form), and mature segmented cell are noted.

Illustrated below in the images of neutrophilic cells:

- metamyelocyte
- band (or “stab”)
- mature segmented (or PMN)
There is usually no differentiation made as to whether eosinophils and basophils are myelocytes, metamyelocytes, band, or mature cells. Regardless of the stage of maturation, they are still referred to only as eosinophils or basophils.

The granules observed in both cell lines are rather large, frequently dense and, in many cases, obscure the nucleus thus making it difficult to see the nuclear shape as illustrated in the images of eosinophils and basophils.
Various Stages Maturation

<--------Eosinophils-------->

<----Basophils---->
Morphologic Features of Granulocytes
The myeloblast is morphologically undifferentiated as to granulocytic cell line (ie, neutrophilic, eosinophilic, or basophilic).

**Size:** variable, usually 15 to 20 μm diameter

**Nucleus:** relatively round and large; predominantly red-stained, delicate, interlaced, well defined and evenly stained chromatin

**Nucleoli:** large and usually 2 or more

**Cytoplasm:** agranular, bluish and stains unevenly usually lighter next to the nucleus than at the periphery and may have cytoplasmic tags)
The promyelocyte is still undifferentiated as to a specific granulocytic cell line (ie, neutrophilic, eosinophilic, or basophilic).

**Size:** usually larger than blasts but variable depending on the stage in the mitotic cycle

**Nucleus:** round and relatively large with predominantly red-stained chromatin

**Nucleoli:** usually demonstrable

**Cytoplasm:**
- Stains blue with a relatively light area adjacent to the nucleus
- **Granules** - nonspecific (or primary) granules and absence of secondary granules (ie, neutrophilic, eosinophilic, or basophilic)
Myeloblast vs. Promyelocyte

**Myeloblast**
- Nucleoli: two or more, large, prominent
- Cytoplasm: bluish, unevenly stained
- Granules: none visible

**Promyelocyte**
- Nucleoli: usually demonstrable
- Cytoplasm: bluish, unevenly stained
- Granules: distinct non-specific (or primary), predominantly dark blue or reddish blue
Granulocytes are neutrophilic, eosinophilic, or basophilic. The cells cannot be morphologically differentiated on Wright’s stain until they reach the myelocyte stage and develop specific granules.
Neutrophilic Myelocyte

This is the last stage in which nucleoli can be seen and mitosis can occur. However, if present, the nucleoli are usually indistinct. Cell line differentiation is seen.

**Size:** usually smaller than promyelocytes (10-18 μm in diameter).

**Nucleus:** round, oval, or flattened on one side.

**Chromatin:** fine, dispersed pattern in early cells which becomes more condensed as the cell matures.

**Nucleoli:** usually not demonstrable

**Cytoplasm:** more than promyelocytes (N/C ratio about 2:1).
- Stains blue but becomes less basophilic as it matures.
- Granules - distinct specific (or secondary) granules that are neutrophilic, eosinophilic, or basophilic.
The first morphologic evidence of specific or secondary granules that provide a means of identifying the cell as a neutrophil, eosinophil or basophil is seen in the myelocyte.

Neutrophils - neutrophilic granules give the cytoplasm a lilac or pinkish appearance

NOTE: an enlargement of the cell shown on the previous slide to better illustrate the granules.
The maturation sequence of the eosinophils and basophils is the same as the neutrophils. The nuclear features are identical but the color and/or size of the cytoplasmic granules differentiates these cells from the neutrophils.

Eosinophils - relatively large and spherical purplish-red granules give the cytoplasm a reddish-orange color.

Basophils - large dark blue to black unevenly distributed granules may fill the cytoplasm and, when present in large numbers, may obscure the nucleus.
Neutrophilic, Eosinophilic, & Basophilic Myelocytes

neutrophilic

eosinophilic

basophilic
Metamyelocyte (Neutrophilic)

As soon as the nucleus of the myelocyte (which may be neutrophilic, eosinophilic, or basophilic) becomes **indented**, the cell is classified as a metamyelocyte. The cell is no longer capable of mitosis.

- **Size:** usually slightly smaller than myelocytes
- **Nucleus:** relatively smaller than myelocyte and, as the cell matures, indentation increases.
- **Chromatin:** less well defined and becomes more condensed clumped, and darkly stained as the cell matures.
- **Nucleoli:** not demonstrable
- **Cytoplasm:** progressively less basophilic than myelocytes and distinct specific (or secondary) granules that are neutrophilic, eosinophilic, or basophilic predominate.
Metamyelocytes

- Neutrophilic specific granules
- Eosinophilic specific granules
- Basophilic specific granules
Neutrophilic Band

As soon as the indentation in the nucleus of the metamyelocyte becomes greater than 1/2 the diameter, the cell is classified as a band (neutrophil, eosinophil, or basophil).

**Size:** slightly smaller than metamyelocytes

**Nucleus:** indented greater than 1/2 diameter and opposite edges of the nucleus become approximately parallel (horse-shoe shape).

**Chromatin:** dense and clumped, usually with a pyknotic mass at each pole where the lobe will be.

**Nucleoli:** none present

**Cytoplasm:** no basophilia & may be slightly eosinophilic; distinct specific (or secondary) granules that are neutrophilic, eosinophilic, or basophilic predominate.
Bands

neutrophilic (lilac or pinkish) specific granules

eosinophilic (orange) specific granules

basophilic (blue-black) specific granules
MATURE SEGMENTED NEUTROPHIL (PMN)

Mature neutrophils have nuclei that are separated into definite lobes. They are frequently referred to as PMN (polymorphonuclear neutrophils).

**Size:** 10-15 μm (about twice the size of RBC).

**Nucleus:** separated into definite lobes (usually 2 or 3 with occasional 4 or 5) which are connected by a very narrow filament or strand.

**Chromatin:** dense and clumped

**Nucleoli:** not present

**Cytoplasm:** slightly eosinophilic or light pink with numerous pink to bluish-black evenly distributed small granules.
Normal Mature Neutrophils, Eosinophils, and Basophils

**Segmented Neutrophil (PMN)**
- Usually 2-4 lobed nucleus
- But may have a few with 5 lobes

**Eosinophils**
- Usually bilobed nucleus
- But may have 3 or more lobes

**Basophil**
- Granules usually obscure nucleus
Review: Neutrophilic Maturation

- **Myeloblast**: Nucleoli & agranular cytoplasm
- **Promyelocyte**: Nucleoli & non-specific cytoplasmic granules
- **Neutrophilic Metamyelocyte**: Nucleus indented but less than half diameter & specific granules
- **Neutrophilic Band**: Nucleus indented more than half diameter & specific granules
- **Neutrophilic Myelocyte**: Round nucleus & specific cytoplasmic granules
- **Segmented Neutrophil**: Nucleus with separate lobes & specific granules
- **Cells**: Become smaller, nucleus becomes smaller, loss of nucleoli, specific granules appear
Examples of granulocytes in various stages of maturation:

1. early promyelocyte
2. late promyelocyte or early myelocyte
3. myelocyte
4. metamyelocyte
5. band neutrophil
6. mature segmented neutrophil (PMN)
7. eosinophil
8. Whoa! That’s not a WBC. It’s a nucleated RBC but will also be included in the total WBC count.
Now, Can you Identify the stages of granulocytes just illustrated?

1. early promyelocyte
2. late promyelocyte or early myelocyte
3. myelocyte
4. metamylocyte
5. band neutrophil
6. mature segmented neutrophil (PMN)
7. eosinophil
8. Remember, it’s a NRBC!
Review: Eosinophilic & Basophilic Maturation

The maturation sequence for eosinophils and basophils parallels that of neutrophils. Blast and promyelocyte stages are morphologically undifferentiated as to neutrophils, eosinophils, or basophils.

The cells can be differentiated in the myelocyte stage with the appearance of specific cytoplasmic granules (ie, neutrophilic pink, eosinophilic orange, or basophilic dark blue-black) that remain through maturity.
Lymphocytic Series
Blasts are the earliest leukocytic precursor that can be seen in peripheral blood and have nucleoli which help to differentiate them from mature cells.

Special stains and/or flow cytometry are usually needed for definitive differentiation of the various leukoblast cell lines (i.e., myeloblasts, lymphoblasts, and monoblasts).

The least mature lymphoid cell seen in peripheral blood is the lymphoblast.
Lymphocytic Cells

For Clinical Pathology 201, you will be expected to be able to identify and differentiate:

- mature lymphocytes
- atypical (or reactive) lymphocytes
- lymphoblasts

And
**Blasts** are the earliest leukocytic precursor that can be seen in peripheral blood and have **nucleoli** which help to differentiate them from mature cells.

The earliest monocytic cell seen in peripheral blood is the monoblast. Special stains are usually needed for **definitive** identification of blast cell lines. Refer to the Course Manual.
For Clinical Pathology 201, you will be expected to be able to differentiate mature monocytes, i.e., recognize the mature cell and differentiate from monoblasts.

Special stains and/or flow cytometry are usually needed for definitive identification of the blast cell line.
Plasma Cells
Plasma Cells

Size: mature cells vary greatly

Shape: usually oval shape with relatively smooth cytoplasmic margins, but, like the lymphocyte, the plasmocyte is easily traumatized and often has frayed or nebulous margins and pointed or filamentous cytoplasmic projections

Nucleus: relatively small and round and eccentrically located

Cytoplasm: abundant

The cytoplasm adjacent to the nucleus stains more lightly than the periphery of the cell which has a high saturation of red and blue dyes. The area is called a “golgi”.
Plasma cells are never present in normal peripheral blood. They constitute about 1% of the nucleated cells in normal bone marrow where they tend to be grouped in small islands around blood vessels. They may be present in small numbers in chronic infections, in granulomatous and allergic diseases and in plasma cell myeloma.

Plasmoblasts (not shown) are cells with relatively large nuclei, nucleoli and delicate chromatin which takes a predominantly red color. Plasmoblasts are not recognizable except in malignancies of the plasmocytoid type.
Leukocytes in Normal Peripheral Blood
Review: WBC found in normal peripheral blood:

- Mature neutrophils
- Band neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- Atypical lymphocytes (<6% of lymphocytes)
- Monocytes
Leukocytes with Acquired Non-neoplastic Alterations
Toxic granulation & Döhle Bodies in Neutrophils

Toxic granulation - dark blue to purple cytoplasmic granules

and/or

Döhle bodies - small blue cytoplasmic inclusions

Toxic granules may be seen in severe bacterial infections, burns, aplastic anemia, and following administration of toxic agents.

Frequently, Döhle bodies will also be seen concomitantly with toxic granulation.
Hypersegmented neutrophil - (ie, > 5 lobes) which are presumably the result of abnormal nuclear maturation. Five lobes in more than 5% of the neutrophils constitute hypersegmentation, as do any neutrophils with 6 or more lobes. In this case, there are 7.

Hypersegmented neutrophils are characteristic features of megaloblastic anemias that are due to vitamin B₁₂ or folate deficiency.

Refractory anemias that are megaloblastic usually do not include these granulocytic changes (ie, hypersegmentation).
Atypical/Reactive Lymphocytes

Atypical/reactive lymphocytes may be seen most typically in viral disorders.

Atypical lymphocytes may have abundant cytoplasm with scalloped or indented rims ...
Atypical/Reactive Lymphocytes

...or have darker cytoplasm and more monocytoid nuclear or plasmacytoid features..
Leukocytes with Inherited Non-neoplastic Alterations
Pelger-Huet Anomaly

An inherited autosomal dominant condition in which there is a failure of normal segmentation of granulocytic nuclei (i.e., hyposegmented nuclei). The nuclei may be band shaped or bi-lobed or “pince-nez” shaped, or in very rare cases, round shaped (like a myelocyte).
Absence of symptoms of infection or other cause of a “left shift”, history of persistent blood morphology, and/or similar blood morphology of other family members suggests the anomaly.

The cell morphology persists through life and the cells are functional.
Pseudo-Pelger-Huet Anomaly

An acquired disorder similar in appearance to Pelger-Huet anomaly may occasionally be found in cases of granulocytic leukemia, myeloproliferative disorders, some infections, and after exposure to certain drugs.

Band forms, neutrophils with only two segments or “pince-nez” appearance (not shown), and/or neutrophils with round non-segmented nuclei are seen. Neutrophils with > 2 segments (lobes) will not be seen in this disorder.

There is asynchronism between the shape of the nucleus and the maturity of the nucleus and cytoplasm.
May-Hegglin Anomaly

Pale blue cytoplasmic inclusions that resemble Dohle bodies but are larger and more prominent. They may be found in neutrophils, eosinophils, basophils, and monocytes.

Bluish aggregations (RNA) particles can be seen in the cytoplasm of neutrophils. Giant platelets can also be seen.

This is a rare autosomal dominant condition.

The cells are functional and the cytoplasmic inclusions in May-Hegglin persist through life. Acquired Dohle bodies are transient.
Alder-Reilly Anomaly

This disorder is characterized by neutrophils with large azurophilic and basophilic granules in the cytoplasm that resemble toxic granulation.

This is an autosomal recessive trait. There is no apparent interference with leukocyte function.

However, these granules may also be seen in association with some but not all patients with gargoylism (the Hurler syndrome), or more generally, the genetic mucopolysaccharidoses.

Alder-Reilly granules are persistent through life whereas acquired toxic granulation is transient.
Chediak-Higashi Syndrome

Abnormally large cytoplasmic black granules which appear to be abnormal lysosomes may be seen in granulocytes, monocytes, and lymphocytes.

This is a rare autosomal recessive disorder characterized by partial albinism, photophobia, and frequent pyogenic infections. An accelerated lymphoma-like phase occurs, with lymphadenopathy, hepatosplenomegaly, and pancytopenia. Lymphoid infiltrates are widespread and death ensues at an early age. Leukocyte functional abnormalities exist.

The abnormal morphologic features are persistent throughout life.
Leukocytes with Neoplastic Alterations
Hairy Cell Lymphocytes

The hallmark of hairy cell leukemia is the presence of lymphocytes with irregular long, delicate cytoplasmic projections which give them a hairy appearance.

This is an uncommon chronic, low grade lymphoproliferative disease (or CLL) that occurs about 5 times more frequently in males than females.

Onset of disease is insidious; weakness and lethargy; or may be asymptomatic (10-15% of patients). May be bleeding and bruising.

Normocytic, normochromic anemia related to the neoplastic cell mass, marrow hypoplasia, and hyper-splenism is also seen. Thrombocytopenia in about 75% of patients and Coomb’s test may be positive.
Auer rods in the cytoplasm of myeloblasts are associated with acute leukemias having a myeloid component. They appear as cytoplasmic reddish rods with Wright’s or Wright’s-Giemsa stains.

They may be seen in some, but not all, myeloblasts in some, but not all, of the variants of acute myelocytic leukemia. They are not seen in blasts in chronic myelocytic leukemia.

The presence of Auer rods in the cytoplasm of blasts effectively rules out a lymphoid disorder.
End of Leukocytes

This ends the section on leukocytes. Click on:

Erythrocytes to go to the next section of this study module as designed.

or

Menu to go back to the menu.

or

Quit to end the exercise.
Erythrocytes
How are the RBC identified?

Typical morphologic nuclear and/or cytoplasmic features provide a means by which RBC can be identified. For example:

- Maturity
- Size
- Shape
- Color
- Hemoglobin content
- Inclusions (if any)
How are RBC classified as to maturity?

Characteristic nuclear and/or cytoplasmic morphologic features allow red blood cells to be classified as:

• pronormoblast (or rubriblast), the earliest form seen in peripheral blood (ie, least mature)
• basophilic normoblast (or prorubricyte)
• polychromatophilic normoblast (or rubriclete)
• orthochromatic normoblast (or metarubricyte)
• polychromatophilic erythrocyte (or diffusely basophilic erythrocyte)
• mature erythrocytes
Summary of the key features of erythrocyte development:

<table>
<thead>
<tr>
<th>Cell</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>Nucleoli</th>
<th>chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pronormoblast</td>
<td>Scanty, basophilic</td>
<td>Large</td>
<td>Prominent</td>
<td>Dispersed, finely granular</td>
</tr>
<tr>
<td>Basophilic normoblast</td>
<td>Increased, still basophilic</td>
<td>Moderate</td>
<td>Indistinct</td>
<td>Dispersed but more condensed</td>
</tr>
<tr>
<td>Polychromatophilic normoblast</td>
<td>Mixed basophilic &amp; eosinophilic</td>
<td>Smaller</td>
<td>None</td>
<td>Chromatin &amp; parachromatin</td>
</tr>
<tr>
<td>Orthochromatic normoblast</td>
<td>More eosinophilic</td>
<td>Pyknotic</td>
<td>None</td>
<td>Condensed chromatin, no parachromatin</td>
</tr>
<tr>
<td>Polychromatophilic erythrocyte*</td>
<td>Eosinophilic</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>erythrocyte</td>
<td>Eosinophilic</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*called a reticulocyte after staining with a supravital stain
What are the characteristic features of pronormoblasts?

Nucleoli: usually visible

Nuclear chromatin: linear and distinct

Cytoplasm: in earliest form stains light blue; in later and more frequently occurring forms has a dark royal-blue color similar to that seen in some plasmacytes.
What are characteristic features of basophilic normoblasts (prorubricytes)?

**Nucleoli:** ill-defined or absent

**Nuclear chromatin:** coarsening of the chromatin pattern

**Cytoplasm:** deeply basophilic due to the abundance of RNA with a reddish tinge produced by varying amounts of hemoglobin present & some cells may have a Golgi (clear) area adjacent to the nucleus (not visible in this cell).
What are characteristic features of polychromatophilic normoblasts (rubricytes)?

Nucleoli: no longer visible

Nuclear chromatin: thickened and irregularly coarsened

Cytoplasm: relatively more cytoplasm than the basophilic normoblast and takes varying mixtures of red and blue stain
What are characteristic features of orthochromatic normoblasts (metarubricytes)?

Nucleoli: none

Nuclear chromatin: nonlinear clumped structure or, as shown in this field, a solid reddish-blue-black degenerated nucleus

Cytoplasm: predominantly red cytoplasm with minimal amounts of residual blue
What are characteristic features of poly-chromatophilic erythrocytes (diffusely basophilic erythrocytes)?

As the cell matures, the nucleus is extruded and the cell becomes a polychromatophilic erythrocyte (erythrocytes do not have a nucleus).

Cytoplasm: predominantly red but may have a bluish tinge due to the reticulum strands (RNA) still present.
What is the correct name for this cell on a Wright’s stained blood smear?

On a Wright’s stained blood smear, the cell is called a **polychromatophilic erythrocyte**.

When these cells are stained with a **supravital stain** (e.g., new methylene blue), the residual RNA strands are precipitated, and the cell is then called a **reticulocyte**.
What are characteristic features of normal mature erythrocytes?

Normal mature erythrocytes are anucleated biconcave discs that stain a reddish buff color with Wright’s (or Wright’s-Giemsa) stain and have a small (about 1/3 of the cell) central pallor. The intensity of the stain in the center of the cell (i.e., the thin portion) is less than at the outer rim of the cell (i.e., the thicker portion).
How are RBC classified as to size?

- **Normocytic** is the term used to indicate RBC that are normal size (6-8 µ in diameter) and normal shape.

- **Anisocytosis** is a “generic” term used to indicate a variation in cell size, e.g.,
  - normocytic
  - microcytic
  - macrocytic
Individual red cells can be classified as…

- **Normocytic** (RBC 6-8 μ in diameter).
- **Microcytic** (RBC < 6 μ in diameter).
- **Macrocytic** (RBC > 8 μ in diameter).
Depending upon the **predominant cell size**, an RBC population can be classified as…

- **Normocytic**
- **Microcytic**
- **Macrocytic**
Comparison of erythrocytes with the normal small mature lymphocyte (which is about $6-10\mu$ in diameter) is helpful in determining whether cells are normocytic, microcytic, or macrocytic.
How are RBC classified as to shape?

**Normocytic** is the term used to indicate RBC that are normal size (6-8 μ in diameter) and normal shape (i.e., round, biconcave).

**Poikilocytosis** is the “generic” term used to indicate variation in shape.

Individual red cells can have numerous abnormal shapes, eg:
What are some of the RBC shape classifications?

Individual red cells can be classified as:

- ovalocytes (elliptocytes)
- spherocytes
- target cells (leptocytes)
- schistocytes (RBC fragments)
Illustrative RBC Shapes

- Ovalocytes (Elliptocytes)
- Spherocytes
- Target Cells
- Schistocytes
Schistocytes are RBC fragments and may have a variety of shapes.
What other erythroid shapes can be seen?

Individual red cells can also be classified as:

- sickle cells (trepanocytes or meniscocytes)
- bitocytes (keratocytes)
- echinocytes or crenated
- acanthocytes
Illustrative RBC Shapes

sickle cells (drepanocytes)

bitocytes (keratocytes)

spiculated (acanthocytes)

crenated (echinocytes)
What about groups of RBC?

Groups or clumps of red cells can also be classified as:

- rouleaux
- agglutination
Rouleau is an aggregation of RBC that is aligned one upon the other resembling stacks of coins and is caused by elevated plasma fibrinogen or globulins.

This phenomenon causes an increased erythrocyte sedimentation rate (ESR) and interferes with the hemogram parameters. Rouleau is especially characteristic of paraproteinemia (monoclonal gammopathy), in which case plasma cells may also be seen.
Illustrative agglutinated RBC:

**Agglutination** of red cells, caused by cold agglutinins, resembles rouleau but is more irregular and may appear in round clumps rather than linear rouleau.

The large cell in the field is a *degenerated* neutrophil with pyknotic nuclei and nuclear fragments.
How are mature RBC classified as to hemoglobin content?

Depending upon the hemoglobin content, mature RBC may be classified as:

- anucleated, pinkish cytoplasm with a small central pallor (about 1/3 of the cell diameter).

This classification is known as normochromic.
or hypochromic...

Anucleated, pinkish cytoplasm with a more pronounced central pallor (i.e., greater than 1/3 the diameter of the cell).
Anucleated, pinkish cytoplasm without central pallor (generally associated with megaloblastic anemias).
Illustrative Mature Erythrocytes

Normochromic (Normocyte)

Remember, comparison of the RBC with normal small mature lymphocytes is helpful in classifying them as normocytic, microcytic, or macrocytic.

Macrocytic Hyperchromic
How are RBC inclusions classified?

Cellular inclusions that may be found in erythrocytes may include:

- Hemoglobin C crystals
- Basophilic stippling
- Howell-Jolly bodies
- Pappenheimer bodies
- Heinz bodies
- Cabot rings
Illustrative RBC with inclusions:

- HbC crystals
- basophilic stippling
- Howell-Jolly body
- Pappenheimer bodies
- (supravital stain) Heinz bodies
- Cabot rings
Hemoglobin C Crystals

Target cells are characteristically seen in HbC disease and syndromes and may be the only abnormality in heterozygous HbC.

Hexagonal shaped HbC crystals may be seen in homozygous HbC disease but are not seen in heterozygous HbC trait. The crystals may be intracellular or extracellular.
Other shaped forms of the crystals (e.g., glove shaped) are seen in HbSC disease.
Irregular basophilic granules, which may be coarse or fine, dispersed throughout an erythrocyte is called **basophilic stippling**. This finding is attributed to abnormal instability of the residual RNA in the cell.

**Fine stippling** is commonly seen when there is increased polychromatophilia, and, therefore, with increased production of red cells.

**Coarse stippling** may be seen in:
- lead poisoning or other diseases with impaired hemoglobin synthesis
- megaloblastic anemia
- other forms of severe anemia (eg, thalassemia major, sickle cell disease)
Howell-Jolly Bodies

Howell-Jolly bodies are smooth, round, intracellular remnants of nuclear chromatin (DNA) that may be found in erythrocytes.

Their color may vary with the stain but are usually the same color as the nuclei of polychromatophilic erythroblasts.

Single Howell-Jolly bodies may be seen in megaloblastic anemia, hemolytic anemia, hemoglobinopathies, thalassemia major, and after splenectomy.

Multiple Howell-Jolly bodies in a single cell is usually indicative of abnormal erythropoiesis (e.g., megaloblastic anemia).
Don’t confuse Howell-Jolly bodies with platelets on top of a red cell.

Characteristically, platelets will appear to be surrounded by a clear “halo” where the hemoglobin has been displaced.

Howell-Jolly bodies usually have no halo.
Pappenheimer bodies appear as dark blue intracellular inorganic iron-containing granules when seen on Wright-Giemsa stained blood smears.

These cells are called **siderocytes** when observed after staining with an iron stain (e.g., Prussian blue).
Pappenheimer bodies are associated with iron-loading disorders.

When Pappenheimer bodies are seen in peripheral blood, there may be a concomitant increase of siderocytes and sideroblasts in the bone marrow.

When the siderotic granules surround at least 2/3 of the circumference of the nucleus, the cell is called a “ringed sideroblast”.

Prussian Blue stain
Don’t confuse Pappenheimer bodies with basophilic stippling…

While Pappenheimer bodies usually appear as one or several small round particles clustered together (usually near the rim of the cell),

basophilic stippling particles are numerous and dispersed throughout the cell.
Heinz bodies are not visualized on Wright’s stained blood smears but are seen only after staining with supravital dyes. Even with these stains, exposure to an oxidizing drug is often required before they are detected.
Heinz Bodies, cont’d

With removal of the Heinz body by the spleen, the cells observed on Wright-Giemsa stained blood smears appear to have had a bite taken out of the cell membrane and are called keratocytes (or “bitocytes”).

Heinz bodies are most frequently associated with G6PD and may be seen in hemolytic anemias and drugs such as phenacetin. They may also be associated with thalassemia major and hemoglobinopathies.
Cabot rings appear in erythrocytes as red or reddish purple intracellular structures. Their shape is usually in the form of a ring, figure-of-eight, or loop with no internal structure. They are thought to be microtubules remaining from a mitotic spindle.

They are observed in erythrocytes in rare cases of pernicious anemia, lead poisoning, and certain other disorders of erythropoiesis. They are interpreted as evidence of abnormal erythropoiesis.

In this course, you will see them only in photos or computer images.
# RBC Inclusions – Composition & Stains

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<td>Siderocytes</td>
<td>Iron particles</td>
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</tbody>
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*may be observed with supravital but are identified with Wright-Giemsa
** e.g., new methylene blue, crystal violet
Nucleated Red Blood Cells (NRBC)

NRBC are not normally present in peripheral blood of adults. They may be seen normally in the peripheral blood of newborns and in some diseases in adults. The NRBC most commonly seen is the orthochromatophilic erythroblast.

However, less mature stages may also be seen.
How are immature RBC classified?

Immature RBC precursors are classified as:

- **Normocytic** - when they are of normal size.
- **Microcytic** - when they are smaller than normal.
- **Macrocytic Non-megaloblastic** - when they are larger than normal with synchronized nucleus and cytoplasm maturation (i.e., normoblastic bone marrow).
- **Megaloblastic** - when they are larger than normal (i.e., macrocytic) and have asynchronized nucleus and cytoplasm maturation (i.e., megaloblastic bone marrow).
Characteristic nuclear/cytoplasmic features at various stages of RBC maturation.

- **Proerythroblast** (earliest form w/ nucleoli)
- **Basophilic erythroblast** (ill-defined or absent nucleoli)
- **Orthochromatophilic erythroblast** (last stage before extrusion of nucleus)
- **Polychromatophilic erythroblast** (cytoplasmic evidence of HGB)
Characteristic nuclear/cytoplasmic features of megaloblastic RBC maturation.

The megaloblastic precursors are much larger than normal with asynchrony between the nucleus and cytoplasm maturation. The cytoplasm develops at the normal rate while the nucleus lags behind.

Therefore, it is difficult to assign a specific stage of development for an individual cell. For example, the nuclear features may be consistent with a basophilic megaloblast while the cytoplasm may be more mature and be consistent with a later stage.
Examples of nuclear/cytoplasmic asynchrony in megaloblastic precursors:

The very large early megaloblastic precursor has a **nucleus** consistent with a pronormoblast, but the **cytoplasm** is consistent with the more mature basophilic erythroblast with visible evidence of hemoglobin (i.e., pink tinges).

The **nucleus** of this megaloblast is consistent with a basophilic erythroblast ........ but the **cytoplasm** is more consistent with a polychromatophilic erythroblast with varying mixtures of red and blood stain.
Other examples of megaloblastic precursors:

This *nucleus* is consistent with the thickened and irregularly coarsened chromatin of the polychromatophilic erythroblast ... while the *cytoplasm* is predominantly red with minimal amounts of residual blue that is more consistent with the orthochromatic erythroblast.

Another megaloblast in which the *nucleus* looks consistent with a polychromatophilic erythroblast .... while the *cytoplasm* is already as mature looking as the anucleated polychromatophilic erythrocyte.
More mature megaloblastic cells:

Polychromatophilic erythrocytes and mature RBC are **larger than normal** (i.e., macrocytic) and typically seen are:

- macrocytes without central pallor
- macroovalocytes
- macro tear-drops
Abnormal Erythrocytes Terminology (Definitions)
Abnormal RBC are differentiated and identified as part of the “diff”.

Changes in size, shape, hemoglobin content, and/or appearance of cellular inclusions may occur as a result of a disease process. Such changes are noted as part of the “diff”.

What terminology is used to indicate the presence of abnormal red cells?
**Definitions:**

**Hypochromic** erythrocytes that demonstrate a central pale area that becomes larger and paler as the hemoglobin content diminishes (less than 1/3 of cell diameter).

**normochromic**

**hypochromic**


**Definitions:**

**Anisochromic or dimorphic**

Indicates the presence of both normochromic and hypochromic cells in the same blood film.

- **Normochromic**
- **Hypochromic**
- **Anisochromic or dimorphic**
Polychromasiasia and polychromatophilia are interchangeable terms used to indicate the increased presence of non-nucleated immature erythrocytes (polychromatophilic erythrocytes) that contain residual RNA which gives a blue-gray tint to the red cells. These cells, which remain after ejection of the nucleus from the orthochromatetic erythroblast, are slightly larger than mature erythrocytes. After exposure to a supravital stain, the cytoplasmic organelles of these cells clump into an easily recognized blue-staining reticulum and the cell is called a reticulocyte.
continued:

Polychromasia (polychromatophilic erythrocytes)

normochromic

polychromatophilic erythrocytes
**Definitions:**

**Microcytes** are abnormally small erythrocytes (i.e., less than 6 μ in diameter).

Compare with lymphocyte nuclei (≈ 8-10 μ in diameter).

- **normocytic RBC** ≈ 6-8μ diameter
- **microcytic** (predominant)
**Definitions:**

**Macrocyes** are abnormally large erythrocytes (i.e., greater than 8 μ in diameter).

**lymphocyte** (with nuclei about 8-10 μ in diameter)

**normocytic**

**RBC β 6-8μ diameter**

**macrocytic**
**Anisocytosis** is a “generic” term used to indicate an abnormal variation in size of erythrocytes.

- **Normocytic**
  - RBC 6-8\(\mu\) diameter

- **Microcytic**

- **Macrocytic**
**Definitions:**

**Poikilocytosis** is a “generic” term used to indicate variation in shape of erythrocytes (e.g., oval, pear-shaped, teardrop-shaped, saddle-shaped, helmet-shaped, sickle-shaped, and irregularly shaped), eg:

- **normocytic**
- **RBC round biconcave**
- **RBC variable shapes**
Elliptocytes and Ovalocytes are interchangeable terms used to indicate oval-shaped erythrocytes.

Definitions:

**Elliptocytes** and **Ovalocytes**

- **normocytic**
- **RBC round biconcave**

- **RBC predominantly ovalocytes**
**Definitions:**

**Spherocytes** are nearly spherical erythrocytes which usually have a diameter smaller than normal. They lack the central pale area due to their **spherical** shape.
Definitions:

Target cells (leptocytes) are erythrocytes that are thinner than normal which show a peripheral rim of hemoglobin with a dark central hemoglobin-containing area. A pale unstained ring containing less hemoglobin separates the central and peripheral zones and gives the cell a target appearance.

round, biconcave RBC

normocytic

target cells
**Definitions:**

**Schistocytes** are fragmented red cell segments that are the result of some hemolytic process. The segments can be a **variety of shapes** but helmet cells and triangularly-shaped cells are particularly characteristic.
Definitions:

Sickle cells (drepanocytes, meniscocytes) are interchangeable terms used to indicate sickle-like forms of erythrocytes (crescent-shaped, irregular spines, filaments, holly-leaf appearance) noted when RBC containing HbS are subjected to reduction in oxygen tension or pH.

normocytic: round, biconcave RBC

sickle cells
**Definitions:**

**Keratocytes or “Bitocytes”** interchangeable terms used to indicate irregularly contracted erythrocytes which stain densely and have contraction of hemoglobin from a part of the cell membrane, thereby giving the appearance that a “bite” has been taken out of the cell. These cells are thought to be cells from which Heinz bodies have been removed by the spleen.

**Definitions:**

- **normocytic RBC**: round, biconcave
- **keratocytes**
**Definitions:**

**Acanthocytes** are irregularly shaped red cells with spiny or thorny **projections** and dark centers which may be found in severe liver disease, infantile pyknocytosis (with underlying hemolytic process), abetalipoproteinemia, or anorexia nervosa.
Definitions:

Crenated red blood cells are uniformly shrunken red cells with uniform irregular, wrinkled cell membranes. Their presence is frequently an artifact of storage and all red cells in the field are usually affected. (By contrast, ecinocytes are intermixed with normal red cells.)
Echinocytes are irregularly shaped red cells with spiny projections and preserved central pallor. While their presence may be an artifactual phenomenon, they may be seen in liver and renal disease, hyperlipidemia, and red blood cell enzymopathies.
**Definitions:**

Rouleaux formation describes an aggregation of erythrocytes that are aligned one upon the other, resembling **stacks of coins**, caused by elevated plasma fibrinogen or globulins. This phenomenon causes an increased erythrocyte sedimentation rate. This finding is especially characteristic of paraproteinemia (monoclonal gammopathy).
Agglutination of red cells is caused by agglutinins and resembles rouleaux but is more irregular with round clumps rather than linear rouleaux.
**Definitions:**

**Hemoglobin C crystals** are hexagonal crystals that may be found in individuals with HbC syndromes. The crystals may be intracellular or extracellular.

- **Normocytic RBC** (round, biconcave, without inclusions)
- **intracellular HbC crystals**
- **extracellular HbC crystal**
Definitions:

**Basophilic stippling** is the term used to indicate the presence of irregular basophilic granules in the cytoplasm of erythrocytes. The granules are composed of unstable RNA and may be **fine or coarse**.
Definitions:

Pappenheimer bodies are intracellular inorganic iron-containing granules that may be observed on Wright's stained peripheral blood smears in iron-loading disorders. When the inclusion bodies are demonstrated by stains for iron (e.g., Prussian Blue), the cells are called siderocytes.

![Image of normocytic cells](normocytic)

Pappenheimer bodies (Wright stain)

![Image of siderocytes](siderocytes)

Prussian Blue stain (Prussian Blue stain)
**Definitions:**

Howell-Jolly bodies are intracellular particles which are smooth, round remnants of nuclear chromatin (DNA). Usually, only one per cell is seen but, occasionally, there may be more than one.
**Definitions:**

Nucleated red blood cells (NRBC) are precursors of the non-nucleated mature red cells, usually **orthochromatric erythroblasts** when noted in peripheral blood in disease states but earlier forms may also be seen, eg:

- mature RBC
- orthochromatric erythroblast
- polychromatophilic erythroblast
- proerythroblast
- basophilic erythroblast
**Definitions:**

**Reticulocytes** are anucleated slightly immature erythrocytes, identified as polychromatophilic erythrocytes on *Wright* stained smears.

The cells are identified as reticulocytes only after exposure to a supravital stain which causes the cytoplasmic organelles of the cells to clump into an easily recognized **blue-staining reticulum**.
Platelets
Platelets

Normally, platelets are round or oval, 2 - 4 μ diameter, contain small fine granules that usually fill the cytoplasm, and are separated from one another.
An estimated platelet count can be made on a peripheral blood smear. If the platelet count is normal, an average of about one platelet per 10 to 30 red blood cells. Using the oil immersion lens at 1000x magnification, that is about 5 to 25 platelets per field.

In Clinical Pathology 201, < 5 platelets/oil immersion field will be considered decreased and > 25 platelets/oil immersion field will be considered increased.
Platelets

Platelet clumps may be found on blood smears that have been improperly prepared. They may also be seen in clotting disorders. Platelet estimates cannot be made from blood smears with platelet clumps.
Occasionally, **platelet satellites** may also be seen. Platelets adhere to the outer surface of neutrophils. When platelet satellites are present, platelet estimates cannot be made from blood smears.
In some disorders, platelets may be larger than normal (i.e., giant platelets).
Disorders
Characteristic Morphology
Macrocytic Non-Megaloblastic Anemias

Characteristic abnormalities associated with macrocytic non-megaloblastic anemias in diseases associated with reticulocytosis.

normal

lymphocyte

numerous polychromatophilic erythrocytes like the one indicated by the arrow
Macrocytic Non-megaloblastic Anemia

Characteristic abnormalities associated with macrocytic non-megaloblastic anemia in liver disease.

macrocytes and target cells
Examples of **characteristic abnormalities** associated with megaloblastic macrocytic anemias

- macrocytes
- macro-tear drops
- macro-ovalocytes

and erythrocytic precursors with **asynchrony** in nuclear and cytoplasm maturation

and hypersegmented PMN
Microcytic Hypochromic Anemias

Characteristic abnormalities associated with microcytic hypochromic anemias (e.g., iron deficiency, chronic disease).

predominant cells are microcytic hypochromic erythrocytes
Anemia in Sickle Cell Disease

Characteristic abnormalities associated with anemia in sickle cell disease

Sickled cells (may be crescent-shaped, irregular spines, filaments, holly-leaf appearance)

and target cells

and also

NRBC  polychromasia  Howell-Jolly body  basophilic stippling  Pappenheimer bodies
THE END

Click on **review** to return to the main menu.

Click on **quit** to exit the program.