I. HEMATOLOGY - PERIPHERAL BLOOD

**Purpose:** To be able to identify the various cellular elements of peripheral blood based upon various cytological criteria.

**Materials:** Slide Bld 1 (Wright’s stained smear of peripheral blood)

**Procedure:**

1. Scan the slide and note the staining quality of the smear. Establish your own criteria for identifying the cells of the smear other than just color, darkness, etc.
2. Using high magnification (100X), identify the following cell types using the pictures provided in this handout.
   
   a. erythrocytes  
   b. neutrophils - mature & stab forms  
   c. eosinophils  
   d. basophils  
   e. lymphocytes - small & medium sized  
   f. monocytes  
   g. platelets

**Additional Information:**

1. An evaluation of the neutrophil count should include both the calculation of the absolute neutrophil number and an approximation of the number of metamyelocytes and stab forms in the circulation. The presence of the latter usually signifies stimulation of the white cell marrow by increased utilization of neutrophils. Under normal conditions, less than 10% of the neutrophils should be in this category.
2. The small lymphocyte may have a lifespan of years duration. During that time, it will travel throughout the immunological system including lymph nodes, spleen marrow, lymphatic ducts, thymus gland and gut, spending only a small portion of its time in the blood stream.
3. Identify the various blood cell types in various sections of organs. Look in the loose connective tissue areas of the sections, particularly sections of the digestive tract under the epithelial layer. In working with the blood cells in tissue sections, the following points should be
kept in mind:

a. There is always shrinkage of tissue. The blood cells will be smaller than in smears.
b. In sections, the cells may be cut so that entire cells are not always seen.
c. With H & E stain there is no differential staining, so recognition must often be based on structural rather than staining characteristics.
d. Neutrophils are recognized by their lobulated nucleus, and the cytoplasm does not show specific granules; however, phagocytized material may be seen if present.
e. Eosinophils are distinctive because their specific granules are large and stain with eosin. Both lobes of the nucleus may not be seen.
f. Basophils are never identified in H & E paraffin sections since the granules are water-soluble.
g. Small lymphocytes have a round, darkly staining nucleus and usually no visible cytoplasm.

II. HEMATOLOGY - BONE MARROW

Purpose: The purpose of this section is to be able to identify the normal cell types of human bone marrow. It is important for each student to establish definite criteria for identifying these various cell types.

Materials: Slide Bld 2 & 3 (marrow smear)

Procedure:

1. Using the attached charts for references, identify under high magnification (60X & 100X), the following cell types leading to mature granulocytes and erythrocytes. Accurate diagnosis of various cell types in bone marrow can only be accomplished in smear; bone marrow sections give you more gross information such as cellularity, erythroid-granuloid ratio. etc.

a. Promyelocytes
b. Neutrophilic myelocytes
c. Neutrophilic metamyelocytes
d. Young neutrophils (band cells)
e. Mature neutrophils
f. Eosinophilic and basophilic forms corresponding to the neutrophilic series although basophilic intermediate forms are rare
g. Proerythroblasts
h. Basophilic erythroblasts
i. Polychromatophilic erythroblasts
j. Orthochromatophilic erythroblasts (normoblasts)
k. Erythrocytes
l. Megakaryocytes
2. Additional Information:

It is important to realize that granulocytic and even erythrocytic production and maturation may be evaluated by inspection of stained marrow smears. A major portion of the marrow cellular elements is granulocytic precursors, approximately 60-65% of cells, for a normal erythroid-granuloid ratio (E/g ratio) of 1/3. The normal cell distribution of white cell precursors (granuloid) is as follows:

<table>
<thead>
<tr>
<th>Percent of all nucleated cells</th>
<th>Normal</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Myelocytes (all types)</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>22</td>
<td>20-30</td>
</tr>
<tr>
<td>Neutrophils (stab &amp; lobed)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2</td>
<td>Less than 5</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Plasma Cells</td>
<td>Less than 5</td>
<td></td>
</tr>
</tbody>
</table>

If red cell count production appears normal (normal hematocrit and reticulocyte count), total granulocyte production may be assessed from the E/g ratio. With increased stimulation, neutrophils and metamyelocytes will be shifted out of the marrow. There is also an increase in promyelocytes, myelocytes, and total cellularity; the E/g ratio falls from 1/3 to as low as 1/10.
<table>
<thead>
<tr>
<th>Cell</th>
<th>Size (μm)</th>
<th>Nucleus* and Mitosis</th>
<th>Nucleoli</th>
<th>Cytoplasm*</th>
<th>Electron Micrographs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proerythroblast</td>
<td>14–19</td>
<td>Round, burgundy-red; chromatin network: fine; mitosis</td>
<td>3–5</td>
<td>Gray-blue, peripheral clumping</td>
<td>Scant RER; lot of polysomes, few mitochondria; ferritin</td>
</tr>
<tr>
<td>Basophilic erythroblast</td>
<td>12–17</td>
<td>Same as above but chromatin network is coarser; mitosis</td>
<td>1–2?</td>
<td>Similar to above but slight pinkish background</td>
<td>Similar to above but some hemoglobin is present</td>
</tr>
<tr>
<td>Polychromatophilic erythroblast</td>
<td>12–15</td>
<td>Round and densely staining; very coarse chromatin network; mitosis</td>
<td>None</td>
<td>Yellowish-pink in bluish background</td>
<td>Similar to above but more hemoglobin is present</td>
</tr>
<tr>
<td>Orthochromatophilic erythroblast</td>
<td>8–12</td>
<td>Small, round, dense; excentric or is being extruded; no mitosis</td>
<td>None</td>
<td>Pink in a slight bluish background</td>
<td>Few mitochondria and polysomes; lots of hemoglobin</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>7–8</td>
<td>None</td>
<td>None</td>
<td>Like mature RBC but when stained with cresyl blue; display bluish reticulum in pink cytoplasm</td>
<td>Clusters of ribosomes; cell is filled with hemoglobin</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>7.5</td>
<td>None</td>
<td>None</td>
<td>Pink cytoplasm</td>
<td>Only hemoglobin</td>
</tr>
</tbody>
</table>

RBC, red blood cell; RER, rough endoplasmic reticulum.
*Colors as appear using Romanovsky-type stains (or their modifications).
<table>
<thead>
<tr>
<th>Cell</th>
<th>Size (μm)</th>
<th>Nucleus* and Mitosis</th>
<th>Nucleoli</th>
<th>Cytoplasm*</th>
<th>Granules</th>
<th>Electron Micrographs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>12–14</td>
<td>Round, reddish-blue; chromatin network: fine; mitosis</td>
<td>2–3</td>
<td>Blue clumps in a pale-blue background; cytoplasmic blebs at cell periphery</td>
<td>None</td>
<td>RER, small Golgi, many mitochondria and lysosomes</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>16–24</td>
<td>Round to oval, reddish-blue; chromatin network: coarse; mitosis</td>
<td>1–2</td>
<td>Bluish cytoplasm; no cytoplasmic blebs at cell periphery</td>
<td>Azurophilic granules</td>
<td>RER, large Golgi, many mitochondria, numerous lysosomes (0.5 μm in diameter)</td>
</tr>
<tr>
<td>Neutrophilic myelocyte</td>
<td>10–12</td>
<td>Flattened, acentric; chromatin network: coarse; mitosis</td>
<td>0–1</td>
<td>Pale-blue cytoplasm</td>
<td>Azurophilic and specific granules</td>
<td>RER, large Golgi, numerous mitochondria, lysosomes (0.5 μm) and specific granules (0.1 μm)</td>
</tr>
<tr>
<td>Neutrophilic metamyelocyte</td>
<td>10–12</td>
<td>Kidney-shaped, dense; chromatin network: coarse; no mitosis</td>
<td>None</td>
<td>Pale-blue cytoplasm</td>
<td>Azurophilic and specific granules</td>
<td>Organelle population is reduced, but granules are as above</td>
</tr>
<tr>
<td>Neutrophilic band (stab; juvenile)</td>
<td>9–12</td>
<td>Horseshoe-shaped; chromatin network: very coarse; no mitosis</td>
<td>None</td>
<td>Pale-blue cytoplasm</td>
<td>Azurophilic and specific granules</td>
<td>Same as above</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>9–12</td>
<td>Multilobed; chromatin network: very coarse; no mitosis</td>
<td>None</td>
<td>Pale bluish-pink</td>
<td>Azurophilic and specific granules</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

RER, rough endoplasmic reticulum.

*Colors as appear using Romanovsky-type stains (or their modifications).