LABORATORY 12 - BLOOD

OBJECTIVES: LIGHT MICROSCOPY: Recognize the formed elements in a smear of normal human peripheral blood. Be able to perform a differential white blood cell count. Distinguish reticulocytes. Recognize red and white blood cells in tissue sections. Understand functions of different types of blood cells and platelets.

ELECTRON MICROSCOPY: Recognize characteristics of each formed element of blood.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

SL 21 Peripheral blood smear - differential count
SL 22 Blood smear from baby - reticulocytes
SL125 Inflammation
SL 16 (Esophagus) Eosinophils in tissue

ELECTRON MICROGRAPHS - See Texts

Lymphocytes (J. 12-11; W. 3.8)
Monocytes (J. 12-12; W. 3.9)
Neutrophils (J. 12-6; W. 3.5)
Basophils (J. 12-10; W. 3.7)
Eosinophils (J. 12-9; W. 3.6)

POSTED ELECTRON MICROGRAPHS

S-45 Eosinophil
Lab 12 Posted EMs

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 6, Blood and Myeloid Tissue.
Frames: 302-325

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology
Copies of this text are on reserve in the HSL.
Blood, pp. 56 - 63

The study of blood is divided into two parts. Today the circulating blood will be analyzed as a tissue. In the next lab, the process of blood cell development (hemopoiesis or hematopoiesis) will be studied.

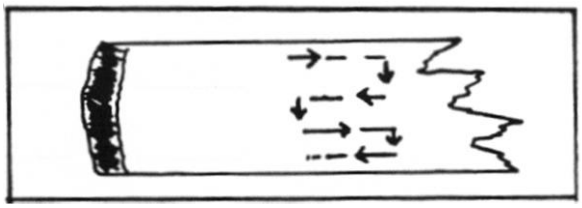
I. HUMAN PERIPHERAL BLOOD SMEAR

A. SL 21 STUDY OF PERIPHERAL BLOOD SMEAR. (J. 12-5; W. 3.1, 3.4, to 3.10) Examine the blood smear under low power. At one end of the smear the cells are too concentrated for proper evaluation, at the other end the cells are widely separated and somewhere in between there is a region where the cells are separated appropriately. TRY TO LIMIT THE AREAS EXAMINED TO REGIONS WHERE MOST OF THE RED CELLS ARE JUST BARELY OR NEARLY TOUCHING EACH OTHER, AND THE CELLS ARE WELL-FORMED AND NOT DISTORTED. DO NOT SELECT A REGION WHERE THE CELLS ARE PILED ON TOP OF ONE ANOTHER. YOU WILL NOT BE ABLE TO SEE THE CELLS PROPERLY IN THESE REGIONS.
Differentiate the red blood cells from the leukocytes (white blood cells) at this low magnification. Observe that the white blood cells are evenly distributed. In a normal smear there should be approximately one leukocyte for every 800+ erythrocytes. Also note the distribution of platelets, which are more numerous than the leukocytes. Select an area for examination using the **oil immersion objective**. Examine the smear with the oil immersion objective noting the shape, size and color of the erythrocytes. Observe that the center of most erythrocytes is more lightly stained (area of central pallor). Why does it appear this way? The central zone of pallor should occupy the central third of the erythrocytes. Changes in this region indicate an abnormality. Examine the shape, size and staining characteristics of the platelets. Be able to distinguish the five different types of mature leukocytes: neutrophils; lymphocytes; monocytes (blue arrow); eosinophils; basophils (note that basophils represent < 1% of WBCs). **It is difficult to distinguish these white blood cells from each other using your high and dry lens. Therefore, you will need to use your oil immersion objective.**

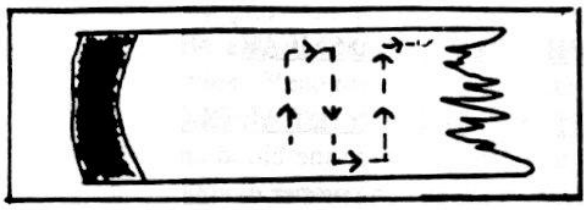
As an optional exercise, you may perform a differential count of the white blood cells as follows:

Staying within the optimal region described in capital letters above, you can move through your slide in any systematic fashion, avoiding any overlaps. The two standard methods of counting blood smears are

(a) Longitudinal technique



(b) Battlement



Each leukocyte encountered should be characterized as one of the five types, or an unknown. Count (ideally) 200 leukocytes - never fewer than 100. Continue to count strips, or embattlements (avoiding overlap), until the total number desired is reached. Record your counts in the following table and compare your results with normal values listed in the text.

	NUMBER	PERCENT (%)
NEUTROPHILS	_____	_____
LYMPHOCYTES	_____	_____
MONOCYTES (blue arrow)	_____	_____
EOSINOPHILS	_____	_____
BASOPHILS	_____	_____
UNKNOWNS	_____	_____
TOTAL number of LEUKOCYTES counted	_____	100%

When evaluating smears in which an acute pathology is found, variations of these methods will be necessary. These can be found in any standard book of clinical or hematological techniques.

B. BLOOD SMEAR, BABY. SL 22. (W. 3.3) - Blood smear, baby. (New methylene blue counterstained with Wright's). This stain demonstrates reticulocytes. This is a variety of normal red blood cell revealed by a special stain. At this time, be able to identify these cells and note only that they represent the youngest red blood cells in the circulation. In the normal adult they comprise about 1% of the total number of circulating erythrocytes. Reticulocyte counts are expressed as the percent of reticulocytes identified after counting 500 to 1000 erythrocytes (# of retics./ total number of RBC^{s} X 100 = % reticulocytes). The significance of reticulocytes and their relationship to the production of erythrocytes will be explained in the next lecture.

C. INFLAMMATION. SL 125 (W. 4.19). Areas of acute and chronic reactions. It is important that you are able to identify blood cells as they appear in tissue sections. Leukocytes perform most of their function within the connective tissue and therefore their presence there is significant. In this slide identify erythrocytes, lymphocytes, neutrophils, plasma cells, and macrophages (scan, high, oil) (oil (a) blue circles, plasma cells; red circles, neutrophils). Remind yourself that, unlike fibroblasts, these immune cells are not residents of the connective tissue, but are transiently located here, usually in response to some pathogen. Both before and after the inflammatory event, this region would look like “ordinary” connective tissue, containing a few scattered immune cells.

SL 16 shows an abundance of eosinophils just under and migrating through the epithelium. Later in pathology you will analyze tissue with many types of cell infiltrations (scan, high, high (a) red arrows, oil).

D. Electron microscope - Although it is not always possible to identify every blood cell at this level of organization the most differentiated cells do have distinctive features that can be noted. These are seen in lymphocytes (J. 12-16; W. 3.8); monocytes (J. 18-12; W. 3.9); neutrophils (J. 12-7; W. 3.5); basophils (J. 12-13; W. 3.7); eosinophils (J. 12-10; W. 3.6).

OBJECTIVES FOR LABORATORY 12: BLOOD

1. Using the light microscope or digital slides, identify:

    In a normal blood smear
        Red blood cells
        Platelets
        White blood cells
            Eosinophils
            Basophils
            Neutrophils
            Lymphocytes
            Monocytes
    In a baby's blood smear (stained with methylene blue and Wright's)
            Reticulocytes
    In peripheral tissues (inflammation)
        Erythrocytes
        Lymphocytes
        Neutrophils
        Plasma cells
        Macrophages
        Eosinophils

2. On electron micrographs, identify:

    Red blood cells
    Platelets
    White blood cells
            Eosinophils
            Basophils
            Neutrophils
            Lymphocytes
            Monocytes

REVIEW

1. What proportion of blood is cellular? RBC^{s}? WBC^{s}?

2. What is the difference between serum and plasma?

3. What cells of the blood are capable of protein synthesis?

4. Why can't basophils be recognized in routine histological sections?

5. For your own review make a chart to compare the formed elements of blood with regard to size, shape nuclei, granules and function.