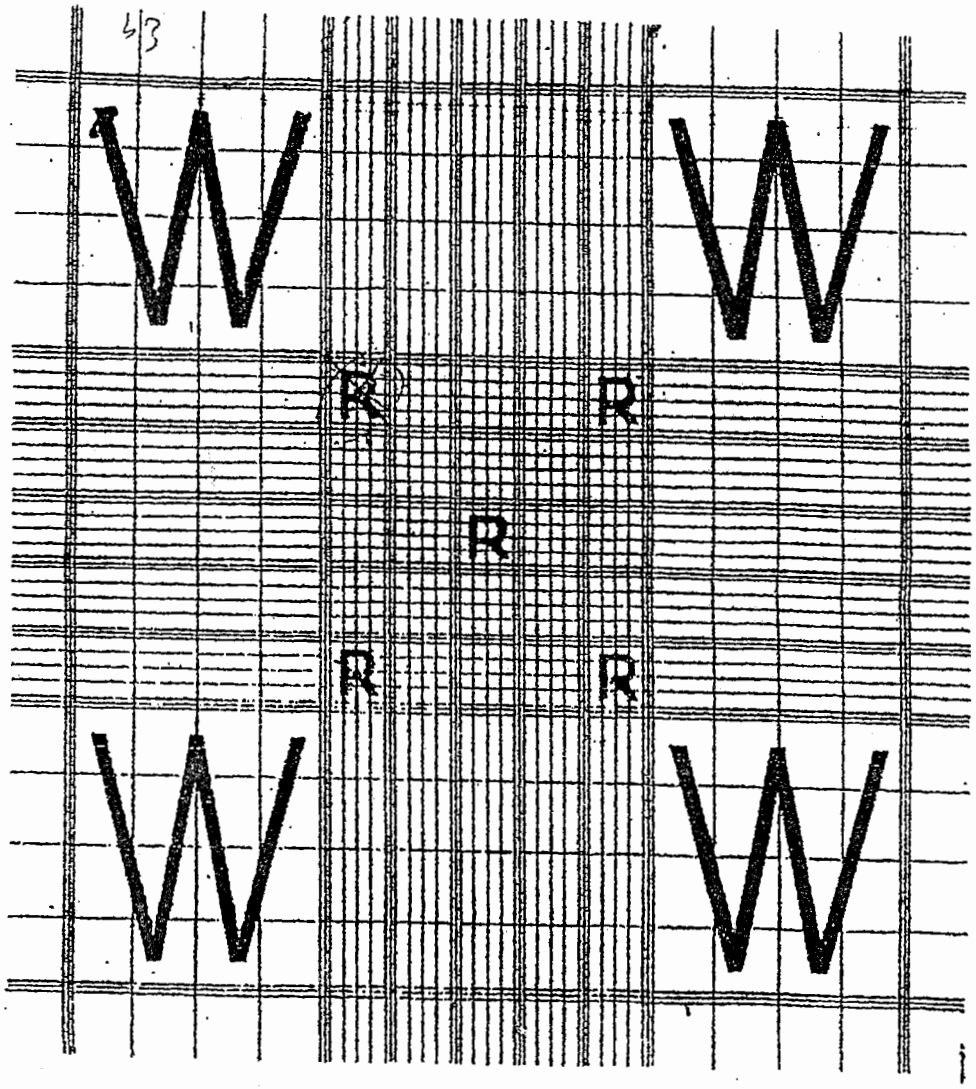
UNIVERSITY OF JORDAN
FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY & BIOCHEMSTRY
PHYSIOLOGY LABORATORY

HEMATOLOGY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BLOOD CELL COUNTING

The blood contains three specialized classes of cells, or formed elements:

1) Red blood cells (RBCs), or Erthrocytes , which transport, oxygen and carbon dioxide,
2) White blood cells (WBCs), or Leukocyte, which combat infections and invading organisms,and
3) Platelets, or Thrombocytes, which prevent loss of blood. for these cells to carry out their functions properly they must be present in sufficient numbers, but not in excess. thus,the counting of blood capacity for performing these functions the following are normal blood cell values (M=million).

Red Blood Cells
- Males 5.4 ± 0.8 M/mm^3
- Females 4.8 ± 0.6 M/mm^3

White Blood Cells . .
- Males and Famales 5000- 10000/mm^3

Platelets
- 150000 - 400000/mm^3
- Average = 300000 /mm^3

Erthrocytes and platelets are not true "Cells" as we have come to define the term. Both lack nucli and are unable to undergo nitosis to form daughter cells. Actually they are nothing more than "bags" to carry specific chemicals: hemoglobin in the RBS and platelet's factor 3 in the platelet. if each gram of emoglobin in the red cells is maximally saturated with oxygen, it can carry about 1.34 ml of oxygen in each 100 ml of blood here is roughly 15 g of HB / 100 ml blood ; hence around 20 ml of Oxygen is carried .

Anemia often results from an abnormal decrease in nr. of rthrocytes, so that insufficient oxygen is carried to the tissus nd they become oxygen starved.

Other factores may also cause anemia , such as decrearsed emoglobin in each cell, decreased cell size, and homorrhage , or accurate diagnosis of the cause of anemia , cell size , emoglobin in each cell, and other factors may also be estimated o define the cause of anemia.

Homocytometer Counting Chamber

lthough many clinics are now using automatic devices such as the oulter counter to make their cell counts , the standard echniques are still based on the use of the homocytometer ounting chamber.

Experiment No :1
Red Blood Cell Counting

1. Handel the hemocytometer with CARE because its very expensive.
2. Clean the hemocytometer very well.
3. Place the hemocytometer on the microscope stage and examine it so you are able to identify the counting areas . Use the low power to find the center 1-mm^2 square , and high power to focus on the smaller 1/25 - mm^2 square.
4. The blood will be dilluted 1: 200 and ready in test tube, rotate the tube between your hands for one minute to redestribute the RBCs.
5. Place a coverslip over the counting area of the hemocytometer.
6. Place the pipette in the diluted blood and draw a small amount,
7. Touch the tip of the pipette to the junction of the coverslip and the hemocytometer . The diluted blood will flow thin by cappillary attraction to chargo the cytometer. Allow 3 minutes for the cells to settle before beginning your counting.
8. Using the power , count the number of RBS in the five of the 1/25 mm^2 squares, and take their average. Usually the four outer squares and the middle one are counted. In your counting you will find that some cells touch the boundary lines aroun the squares. Count the cells that touch on two sides of the square and omot those touch on the other two side.
9. Calculate the number of RBS per cubic millimeter of blood by taking into account the following multiplication factors:
   The blood was diluted 200 time
   The volume of the small R chamber is = 0.2 x 0.2 x 0.1 = 0.004 mm^3.
   so the multiplication factor will be = 200 x 250 =50.000

e.g. if you count an average of 120 RBCs per R. chamber, your RBC count is 120 x 50.000= 6.000,000 RBC/mm^3.
Recored your result, IS your results within the normal range ?

EXPERIMENT NO : 2
WHITE BLOOD CELL COUNTING:-

This technique is simlar to that used for counting the Red blood cells, with the following EXCEPTIONS :-
*The blood is diluted 1:20
*The number of WBC is counted in each of the four large 1-mm^3 squars in the corners of the ruled area, and the average is detrmined.
*Use the low power (10) for these counting.
*The multiplication factors are:
- The blood was diluted 20 times.
- The volume of the W chamber is 1mm x1mm x0.1mm =0.1mm^3 so the multiplication factor will be 20 x 10 = 200.
- Recored your WBC count /mm3.

compare your count with the normal range? what terms do we use for a deficient number of leukocytes? for an abnormally high number of leukocytes?

Expriment No :3

DETERMINATION OF DIFFERENTIAL LEUKOCYTE COUNT :

A determination of the total leukocyte count (7500/mm^3 average) is an important clinical measurement, but a more accurate diagnosis is obtained by making a differential count the percentage by makinge a differential WBC count.

GRANULOCYTES (POLYMORPHONUCLEAR LEUKOCYTES):

NEUTROPHILS

65% of total WBCs.
10-12 um diameter.
Three-lobed nucleus.
Small pink cytoplasmic granules,purple nucleus.

EOSINOPHILS:

2%- 4% of total WBCs.
13um diameter.
Bilobed nucleus.
coarse red-orange cytoplasmic granules,bluepurple nucleus

BASOPHILS

0.5% of total WBCs.
7um diameter.
Bilobed nucleus.
Large deep blue or reddish purple cytoplasmic granules,
blue - black nucleus.

AGRANULOCYTES(MONONUCLEAR LEUKOCYTES

SMALL LYMPHOCYTES

25 % of total WBCs.
7 - um diameter.
very large. spherical nucleus surreunded by thin cyteplasm light bliue cytoplasm (nograunular), deep blue or purple nucleus.

LARGE LYMPHOCYTES:

3% of total WBCS.
10-um diameter.
Large oval, indented nucleus.
Light blue cytoplasm(NON GRANULAR), dark purple nucleus.

MONOCYTES:

1% - 7% of total WBCS.
15-um diameter.
Large blue -gray cytoplasm(NON GRANULAR), blue or purple nucleus.

In a differential count the percentage of each type of leukocyte in the total leukocyte population is determined. Each type of leukocyte performs adifferent function on the battle against- infection. and each disease causes different responses by the WBCs. Afew example of alterations in the leukocyte population in various disease are given in the table below.

LEUKOCYTE ALTERATIONS OCCURRINCE WITH VARIOUS DISEASE OR CONDITIONS

DISEASE OR CONDITIONS	SYMPTOMS
protozon infections,malnutrition aplastic anemia.	Neutrophilic leukopenia
Strenuous exercise,sever burns, rtheumatic fever.	Neutrophilic leukocytosis
Mumps,german measles,whooping cough.	lymphocytosis
Scarlef fever,parasitic infections, allergic reactions.	Eosinophilia
Chroic disease,such as tuberculosis	Monocytosis
administration of glucocoticoid drugs	Lymphocytopenia

Incontrast to the red blood cells,the WBCs(leukocytes) are nucleated and exist in several distinct types.They perform avarietyof functions related to defense of the body against invading organisms.Lean to identify each type of(WBC) by its characteristic size,nuclear arrangement, or cytoplasmic granulation .six types of WBCs are recognizable.

BLOOD SMEAR STAINING PROCEDURE

1. Obtain a drop of blood by finger puncture. place a small blood drop on one end of aclean glass slide .
2. Hold a second slide (the spreader) at a 45- degree angle to the first slide and move it toward the drop of blood. Allow the blood to spread along the edge the other end of the spreader slide; then move the spreader in a smooth ,fast motion to the other end of the first slide. This motion will deposit a thin , evenly spread film of blood across the slide. Allow the slide to air dry. For few minutes
3. Put the slide on a holder HORIZANTALY.
4. Using a medicine dropper, cover the slide completey with leishman stain, BUT do not allow the stain to over flow, blow on it CONTINOUSLY for 8 minutes.
5. Add distelled water to cover the slide completely, and blow on it for another 8 minutes .
6. Wash the slide gently under the tap, clean the other side and leave it to dry .
7. Examine it under the microscope use the low power first, then put a drop of oil and use the high power (100).
8. Conut the number of each type of WBC on the slide, recording each on asheet.
   Count and identify 100 WBC, express your results in percentages how do your percentages, compare with the normal percentages?

EXPERMINT NO 4
BLOOD HEMATOCRIT
(P.C.V.)

The hematocrit (Hct) is the percent volume of whole blood that occupied by red blood cells (erythrocytes). It is determined by centrifuging the blood in special hematocrit capillary tubes. The percent of whole blood composed of cells determined by the height of the red cells in the tube compared with the height of the total column of blood. The average normal hematocrit and their ranges for males and females are as follows:

	AVERAGE	RANGE
MALES	46%	43% - 49%
FEMALES	41%	36% - 45%

The hematocrit may fall to as low as 15% in severe anemia or rise to as heigh as 70% in polycythemia.

EXPERIMENTAL PROCEDURE :

1. Puncture your finger using a sterile lancet to obtain a drop of blood, wipe off the drop that forms (Why)? and allow a second drop to accumulate.
2. Touch the red- circled end of a heparinized capillary tube to the drop, hold the tube in a horizontal position and allow the blood to enter until the tube is one- half to three - fourths full.
3. Seal one end of the tube by pushing it into a tablet of sealing compound and rotating it to form a plug.
4. Place the capillary tube in a microhematocrit centrifuge with the plug end to the out side , and centrifuge for 4 minutes.
5. At the end of 4 minutes measure in millimeters the hight of the red cells plus the plasma. Calculate the hematocrit using the following formula and record it in the laboratory report.

$$\text{Hot (\%)} = \frac{\text{height of red cells(mm)}}{\text{height of red cells end plasms (mm)}} \times 100$$

some labs use a hematocrit "reader" that reads the hematocrit value directly on a scale. Where are the white cells in the hematocrit tube is centrifuged?

EXPEIMENT :5
HEMOGLOBIN DETERMINATION

In clinical practice the blood hemoglobin (Hb) is usually measured by a coloremetric method such as the cyanmethemoglobin method described in this section. other simpler tests are often performed in the laboratory to give an approximate Hb value. The values obtained by using the simpler Tallquist or Sahli methode should be chocked against those yielded by the more precise cyanmet.hemoglobin method.

SAHLI METHOD:

In this method blood hemoglobin is converted to a brownish hematin compound by the action of hydrochloric acid. the higher the hemoglobin concentration , the more intense the hematin color will be.

* Place 5 drops of 0.1 N hydrochloric acid (HCL) in the bottom of a Sahli tube. This amount should fill the tube to around the 10% mark on the scale
* Lance your finger to obtain adrop of blood. Place the tip of the sahli pipette in the drop and gently suck a solid column of blood into the pipette up to the 20µm mark (0.02ml), when sucking use the mouthpiece and rubber tubing attached to the pipette. if you draw in too much blood, touch the pipette tip to filter paper or tissue to draw the axcess blood out. Do not allow air to enter the pipette column or you will invalidate your results.

NOTE: You cannot spend too much time in filling the Sahli pipette or the blood will coagulate in the pipette and black the bore. To clean it out, flush the pipette repeatedly in the following solutions in this order: distilled water- alcohol- ether or acetone.
If the Sahli pipette is diffcult to clean, use hydrogen peroxide to clean it. Use caution, because hydrogen preoxid is a strong oxidizing agent.

* Insert the tip of the pipette beneath the surface of the HCL in the sahli tube and gently blow out the blood. Rinse the pipette of any blood. by drawing the solution in and out of the pipette two times .
* Mix the blood and HCL by stirring with aglass rod and then let the tube stand for 10 minutes.
* Place the tube in the comparator block and hold it up to a strong light.Add distilled water drop by drop to the hematin solution (stir after each addition) until its color matches the color of the standard color on the comparator.
* Read the scale on the sahli tube to obtain the percent of Hb and grams of Hb per 100ml of blood. NOTE that the Hb standard used in calibration may vary from tube to tube. THe standard (gHb) used is imprinted on each tube.

BLOOD TYPING

Many clinical conditions require the trasfusion of whole blood, transfusions cannot be performed indiscriminately between persons, however, because of the possibility of antigen/ antibody reactions producing agglutination of red cells. Agglutination refers to a clumping of red cells togather. Why would agglutination be dangerous?

The human red cell has around 30 commonly occurring antigens on its membrane. in blood typing terminology these are called agglutinogens. These agglutinogens may react with complementary antibodies, or agglutinins, in the donors or recipients plasma to cause agglutination of red cells.

AGGLUTINOGENS + AGGLUTININS = AGGLUTINATION

Although any of the 30 antigen - antibody combinations can cause agglutination, in actual practice most agglutinations in transfusion are caused by two antigen - antibody systems the ABO and Rh systems.

EXPERIMENT NO 6
ABO SYSTEM

A person may have A,B,or O type antigen on the red cells, or any two of these togather. O antigen are very weak, as are the antibodies; hence they rarely cause any agglutination. for this reason, a person who has O type blood is usually regarded as having no antigens on the red cells. only the A and B antigens are regarded as having strong antigenicity.

Antigens are genetically determined. It should be pointed out that the ABO systemis the only one in which the person's plasma automatically contains the noneomplemantary antibodies to the red cell antigens, These antibodies are also determined genetically. All other antibodies found in the plasma must be formed through the entrance of the antigen into the body to stimulate antibody production.

The antigen and antibodies for each blood type are summarized in table 15.2 with the percentage of each type found in various races.

The differences among the races in percentage of each ABO type indicate one role of genetic determination for these blood groups.

Agglutination resultes from reaction of an antigen with its complementary antibody.for example,

A + α --------→ agglutination
B + ß --------→ agglutination.

A person with type O blood is referred to as the universal donor, and a person with type AB as the universal recipient. Explain these designations in the laboratory report. What are antibodies? Where are they produced in the body? What is the current theory of the mechanism of antibody production?

EXPERIMENTAL PROCEDURE

1. Obtain a clean microscop slide, Using glassmarking pencil, mark one end A and the other end B.

2. Lance your finger to obtain blood, place 1 drope of blood on each end of the marked slide.

3. Add 1 drope of anti-A serum to the A side, Add 1 drope of anti-B serum to the B side. Mix the antiserum and blood on each side with a toothpick, using a different toothpic for each side. Spread each mixture over an area of about 1 in. In diameter make certain you do not mix the anti-A and anti-B antisera.

4. Observe the slide for any agglutination of red cells. If agglutination occurs on side A only, you have the blood type A. If it occurs on side B only, you have type B. If a reaction occures on both sides, you have type AB. If no reaction occures on either side, you have type O. Explain the antigen-antibody basis for these reactions. The strength of the agglutination reaction is not the same for every person; in some cases it may be necessary to observe the cells under the microscope to ascertain if agglutination has actually taken place.

EXPERIMENT NO 7

RH SYSTEM:

In 1940 Landsteiner and Wiener discoverd a system of antigens in the cells of the Rhesus monkey that is different from the ABO system. After producing an antiserum(antibody) against "Rh" factor, they tested it with human RBCs and found that 85% of the human population also has this Rh factor (are Rh postive) The other 15% of the population does not have this factor(Rh negative). In contrast to antigens in the ABO system, the Rh factor is found in all body cells, not just on the erthrocytes. Actually, there are eight differenet types of Rh agglutinations . however, the four strongest all react with anti-Rh antiserum . Hence, if your blood agglutinates with anti-RH antiserum, we say you are Rh+ if it does not, you are Rh-.

In a person who has Rh- blood receives a transfusion of Rh+ blood, there is usually no adverse reaction for the first time, but the entrance of the Rh factor stimulates an accumlation of anti-Rh antibodies in the recipients' blood. If the same Rh- recipient receives a second transfusion of Rh+ blood, the antibodies are ready and will cause an agglutination reaction. The best known agglutination reaction of the Rh factor is eyrthroblastosis fetalis, a destruction of red cells in the newborn baby(hemolytic disease of the newborn)

TABLE 15.2

Blood type	(Antigen)	(Antibody)	Caucasin	Black	Arabic
A	A	ß(beta or anti-B)	43	22	5
B	B	α(alpha or anti-A)	7	29	0
AB	AB	NONE	3	4	0
O	NONE	α and ß	47	45	95

EXPERIMENTAL PROCEDURE

1. prewarm a clean microscope slide on a slide warming box.

2. mix 2 drops of your blood with 1 drope of anti-Rh(anti-D) antiserum on the slide.

3. place the slide back on the warming box and tilt the slide occasionally to aid the mixing.

4. observe the agglutination within the first 2 minutes after mixing. Check under the microscope if you are in doubt. The Rh factor is usually weaker than the AB antigens. and the agglutination reactions is not as strong or as to detect.

BLOOD COAGULATION (HEMOSTASIS)

The blood contains its own chemical system to coagulate and thereby to prevent blood loss from the body, coagulation is a fantastically complex process that begins as soon as blood platelets are ruptured or body tissues are damaged in the following experiment you will examine some of the simpler processes in the coagulation mechanism.

EXPERIMENT NO 8
BLEEDING TIME

Clean the tip of your finger with 70% alcohol, and then dry it with a piece of cotton. puncture the figer with a lancet and ecord the time. At 15-second intervals wipe the blood drop away ompletely with a filter paper(do not touch your finger when ipping the blood away). continue this procedure until no more lood stains appear on the filter paper. Recored this time. alculate the bleeding time , Is it close to the normal bleeding ime of 1 - 3 minutes?

EXPERMINT NO 9
CLOTTING TIME:

Lance your finger to obtain a large drop of blood. Note the ime when the drop appears. Rapidly draw blood into a onheparinized capillary by holding the tube in the drop of blood n horizental position.

At 30- second interals break off a small piece of the apillary tube(0.5 cm)and see if clotting has ocurred. Clotting as occurred when a thread of coagulated blood is visible between ie two pieces of tubing.

How does your clotting time compare with the normal of 5- 8 nutes?

EXPERIMENT NO 10
ERYTHROCYTE SEDIMENTATION RATE (E.S.R)

This is a simple test. It depends on the fact that if we put e blood in a tube and leave it for sometime, the RBCs sink down aving a column of plasma above it.

I was noted that E.S.R increases in the following cases:- pregnanacy, old age, anemia, acute genral infections, malignant growth, and pulmonary tuberculosis.

However due to the present unsatisfactory knowledge about e exat nature of the phenomenon, increase of E.S.R should not be ways taken with alarm, similarly normal E.S.R. would not clude all pathological condition's However it is now nerally accepted by clinician as an aid to diagnosis.

There are the methods for estimation of E.S.R. they are:-
1. Methode of westergren.
2. Method of wintrobe.

1.METHOD OF WESTERGREEN:-

In this method we use westergreen tube and stand.The height of the clear plasma is read directly,this figure in mm/ hour is the E.S.R. According to this method the normal range of adult male is 3 - 5 mm . and for adult female is 4 -7 mm .in the first hour.

2.METHOD OF WINTROBE:-

In this method we use wintrobe tube which is 10cm in length and one of its ends is opend only,We fill it with blood by a capillary pipette till the 0 point.Take care so that air bubbles do not form in the tube while filling it.Wait for one hour then take the result exactly like westergren method.

EXPERIMENT NO 11
OSMATIC- FRAGILITY TEST OF ERTHROCYTES:

This experiment tests the ability of RBCs to resist hypotonic salt solution.If we put RBCs in series of different concentrations of hypotonic salt solution we will find that at certain concentration hemolysis(destruction) of the RBCs will begin,and at certain concentration hemolysis will be complete.Why? It was found that this resistance is different from the normal values in certain disease conditions.
Increased osmatic resistance(i.e decreased fragility) is found in (1).After splenectomy (2).liver disease (3).sickle cell anemia.
Decreased osmatic resistance(i.e increased fragility)is found in: so this test is used as an aid in the diagnosis of certain diseases, such as spherocytosis.

EXPERIMENT PROCEDURE:-

We use 20 test tubes in arack and put in them different concentration of salt solution from 0.85% - 0.25%.we add one drop of blood to each tube, shake well,and put them in the centrifuge.

Then we read the result.In normal RBCs we find that tubes with salt concentration from 0.85% -0.45% have no hemolysis,the RBCs are settled down and the solution is clear.

At salt concentration of about 0.40% and less we find that the solution is reddish in color and there are some RBCs settled down hemolysis starts. At concentration of about 0.33% and less we find that the solution is red in color and there is no RBCs settled down,i.e.hemolsis is complete, at this concentration if we shake the tubes which have RBCs settled down the solution will become turbed,but if we shake the tubes which have no RBCs settled down the solution will remain clear.

EXPERIMENT NO 12
HEMIN CRYSTAL:

This test is used for medicolegal purposes,to a scertain whether a particular red stain is blood or not,but it can not prove the origin of the blood,whether human or animal.To prove the origin of the blood we treat it with known antiserum.

EXPERIMENT PROCEDURE:-

*Put a drop of your blood on a slide.
*Leave it to dry.
*Crush it by another slide.
*Put a cover slide on it.
*Put one drop of glacial acetic acid by a capillary pipette at the edge of the cover .
*Dry it on the flame.
*Examine it under the microscope,you will see brown crystals (hematin).this prove that it is blood.

EXPERIMENT NO 13
ENUMERATION OF RETICULOCYTES :

Reticulocytes are the immediate precursors of fully formed ed blood cells there is no nucleus in reticulecytes;but they ontain the remnant of the basophilic (ribonucleoprotein) which as present in larger amount in the cytoplasm of the nucleated recursors from which reticulocytes were derived.This basophilic aterial has the property of reacting with certain dyes like rilliant cresyl to form a blue preciptate or reticulm. This eticulm may appear as a narrow bond traversing the cells or ollection of discret rods or granules densly packed simulating a ucleus or as irregular network.More immature the reticulocytes arger is the amount of reticulum.Maturation with complete loss f basophilic material probably occurs as a rule in the blood tream after the cell has been delivered from the bone marrow and t is believed that it takes about 1 - 2 days.

The number of the retioulocytes in peripheral blood is airly accurate measure of erythropoietic activity either natural r induced by antianaemic drug therapy .

Retciculocytes count therefore is one of the essential rocedure of diagnostic hematology.
ormal range of reticulocyte count in adults is 0.5 - 2%.

EXPERIMENTAL PROCEDURE:-

*Prepare diluting Brilliant Cresyl blue solution by dissoliving water soluble B.C.b in 100ml of citrate saline solution.
*Take one ml of the above B.C.b solution in asmal test tube and add 1ml of well shaken oxalated blood.
*Mix thoroughly for 5 minutes.
*Wait another 5 minutes.
*Take one drop and put it on a slide and spread it.
*Put a cover and examine under the microscope.
*Identify the Reticulocytes and count 100 RBCs including Reticulocytes and cacluate its percentage.