6. Experiment on Haemin Crystals:

Crystals are homogeneous solids, bounded by plane faces and having a geometric shape.

Preparation:

A small amount of dry blood is taken on a glass slide and crushed to a fine powder with the help of the fused end of a glass rod or with a needle. One crystal of common salt (NaCI) is added to it, which is also crushed to powder. The two are thoroughly mixed and two drops of glacial acetic acid added to it.

The mixture is covered with a cover slip and the slide heated over the flame of a spirit lamp. The reaction is complete with the beginning of boiling of the mixture and the slide is quickly removed from the flame.

The preparation is allowed to cool and examine under a microscope, initially under low magnification and then under high magnification.

Structure:

(Fig. 33.4)

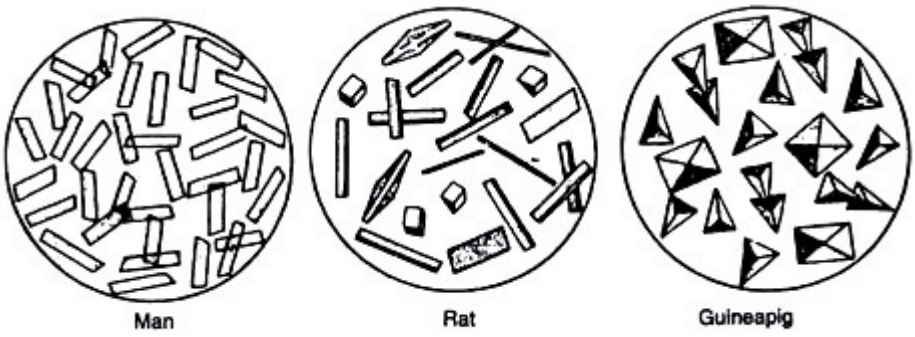

Fig. 33.4 : Haemin crystals

Man:

Rhomboidal plates and prisms, often arranged in star-shaped clusters with round edges.

Rat:

Narrow plates with varying width to needles, blunt at both the ends.

Guinea pig:

Triangular plates often arranged in the form of squares.

11. Experiment on Blood Film:

Preparation:

Take two clean slides. Clean the ball of a finger of one of your classmates with 70% alcohol. Dry thoroughly and puncture the finger ball boldly to a depth of about 2 mm with a sharp edge, sharp pointed, sterile needle. Blood flows out freely from the wound.

Touch the blood drop with the end of a clean slide, close to its edge. Put the slide with the drop of blood (not larger than a pin head) on a table. Hold it firmly with your left hand. Place the narrow edge of another clean slide in front of the drop of blood touching it.

In a second or two blood spreads across the edge of the slide. Slowly but boldly draw the slide along the whole length of the first slide keeping it at 45° angle with the former. A thin blood film (tongue-shaped) is formed over the slide (Fig. 51.1). Dry the blood film immediately by waving the slide quickly in air to prevent shrinkage of blood cells.

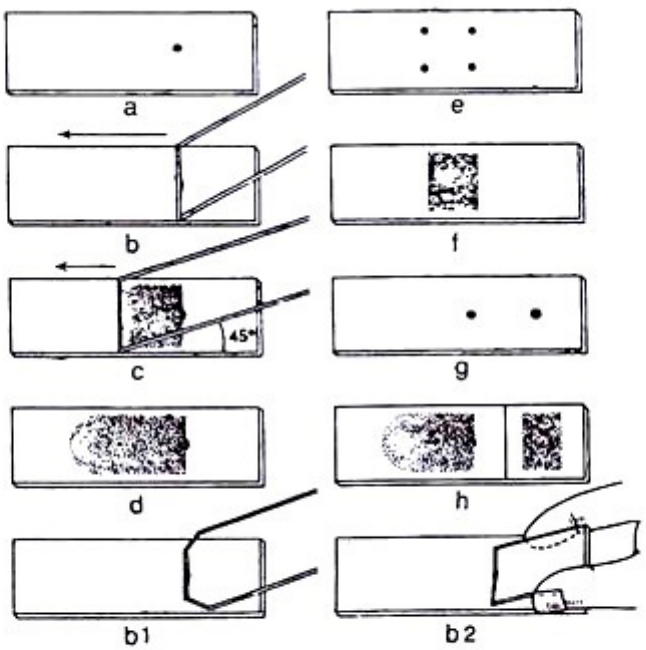

Fig. 51.1 : Technique to prepare thin and thick films of blood. a-d thin film. a. drop of blood at one end of slide, b. spreader held at an angle of 45° and pushed in the direction of the arrow, c. drawing a film, d. thin blood film completed, b1 and b2. a glass slide with corners cut at one end and a coverslip of haemocytometer used as a spreader, e & f. thick film preparation, e. four drops of blood at the corners of a half inch square, f. four drops joined to form a thick film, g and h. thick and thin film on the same slide.

12. Experiment on Count of Erythrocytes: (Red BloodCorpuscles – R.B.C.):

Total count:

Total count of blood cells is usually done with a Thoma-Zeiss haemocytometer.

R.B.C. pipette:

It is graduated to dilute blood 1 in 100 or 1 in 200. On the stem of the pipette is marked 0.5 and 1 and 101 just above the bulb. The bulb of the pipette has a volume of 100 units and the stem a volume of 1 unit (Fig. 51.2).

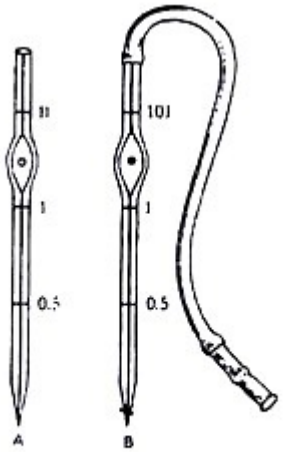

Fig. 51.2 : Thoma-zeiss haemocytometer pipette
A. W.B.C. pipette, B. R.B.C. pipette

Counting slide:

The counting chamber in the slide is of known capacity (Figs. 51.3-51.5). A special thick cover slip is used at the time of counting. The counting chamber measures 1 x 1 mm. It is divided into 5 x 5 = 25 small squares. Each small square measures 1/5 x 1/5 mm.

It is again divided into 4 x 4 = 16 smallest squares (Fig.53.4). Each smallest square measures 1/20 x 1/20 mm and the area is 1/400 sq mm. The depth of the squares is 1/10 mm. Therefore the volume of one smallest square is 1/400 x 1/10 = 1/4000 c mm (cubic millimetre).

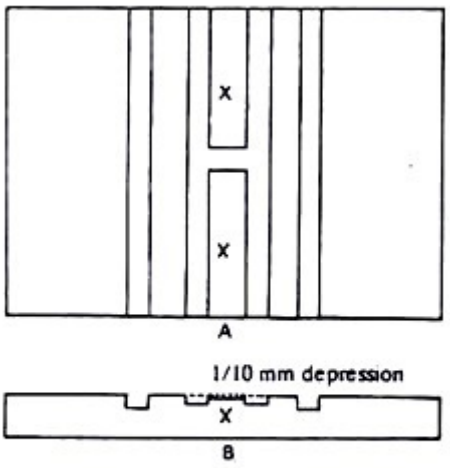

Fig. 51.3 : Thoma-zeiss counting slide.
A. Top view, B. Side view

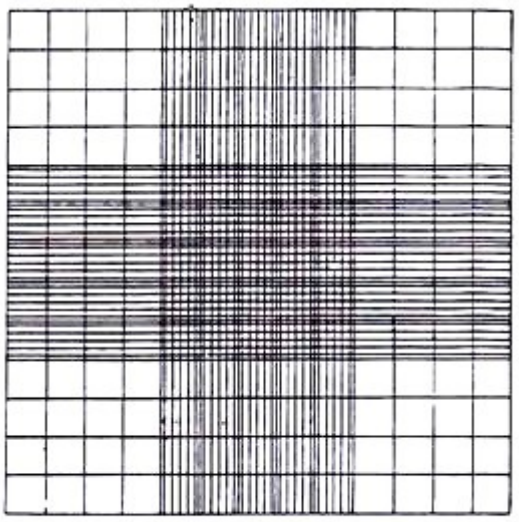

Fig. 51.4 : Thoma-zeiss counting slide.
Counting area magnified

Diluting fluid:

The most commonly used diluting fluid for routine classwork is formol-citrate solution. It is a solution of 1% formalin in 31.3 g/litre trisodium citrate.

Technique:

Prick the finger tip with a needle. Wipe out first drop of blood. Suck the free flowing blood from the prick in the R.B.C. pipette held horizontally, to 0.5 mark. Wipe the tip of the pipette clean, dip vertically into the blood diluting fluid and gently suck the fluid up to mark 101 (Fig. 51,2B).

Close the tip of the pipette with finger and mix the content thoroughly with a twisting motion. The capillary of the pipette is occupied by the diluent even after mixing and the dilution is really 0.5 in 100, i.e., 1 in 200.

The cover slip is centrally placed over the bar of the counting chamber. About ⅓rd of the thoroughly mixed diluted blood is rejected by blowing it out of the pipette. Hold the pipette at 45° angle with the slide touching the space between the cover slip and the slab. The fluid runs under the cover slip by capillary action. Allow the flow till the space between the grooves of the slide under the cover slip is just filled.

If air bubbles are trapped under the cover slip remove those by moving the cover slip to the edge of the slab and thereby allow the air to escape. Wait for 3 minutes and check for even distribution of blood cells under the low power of a microscope. In case of uneven distribution of blood cells reject the preparation and make a fresh one. Counting is done under high power of a microscope.

Calculation:

For calculation of total number of red blood corpuscles in 1 c mm (cubic millimeter), all the corpuscles in 5 small squares, i.e., 80 smallest squares are counted. The corpuscles touching the left and lower line of the square and those inside the square are taken into account. Those touching the right and upper line of the square are excluded (Fig. 51.5).

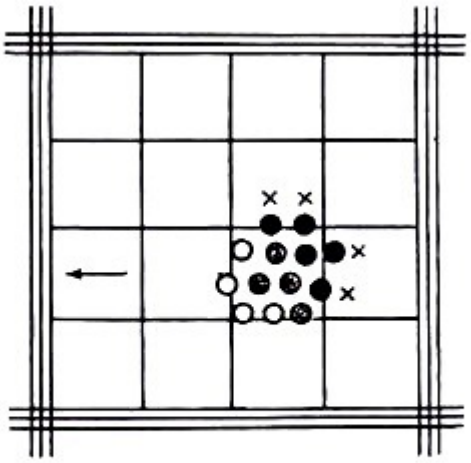

Fig. 51.5 : Thoma-zeiss counting slide. Part of the counting area enlarged. Cells without 'X' are counted as in; Cells with 'x' as out

Volume of one smallest square = 1/4000 c mm.

Volume of 80 smallest squares = 80 x 1/4000 = 1/50 c mm.

If the number of cells in 1/50 c mm is X, the number of cells in 1 c mm = X x 50.

The dilution of blood is 1 in 200.

The number of cells in 1 c mm undiluted blood = X x 50 x 200.

N.B. As a rule the total number of R.B.C. in 1 c mm undiluted blood may be obtained by adding 4 zeros to the number present in 80 smallest squares.

13. Experiment on Count of Leucocytes (White Blood Corpuscles: W.B.C.):

The counting of number of white blood corpuscles (W.B.C.) is expressed in two ways. Counting of total number of cells as total count (TC), and the count of different kinds of leucocytes separately as differential count (DC).

The five types of W.B.C. are:

(a) Polymorphonuclear neutrophils,

(b) Polymorphonuclear eosinophils,

(c) Polymorphonuclear basophils,

(d) Monocytes and

(e) Lymphocytes.

A. Total Count:

Equipment's and technique:

The equipment's used for leucocyte count are same except the markings on pipette which allow a dilution of blood 1 in 20. The mark above the bulb is 11 in a W.B.C. pipette (Fig.

51.2A). The procedure adopted in leucocyte count is same as in R.B.C. count. Blood is drawn up to 0.5 mark, and W.B.C. diluting fluid up to 11 mark.

Diluting fluid:
A 3% acetic acid solution in distilled water.

Calculation:
The area of the counting chamber is 1 sq mm and the volume is 1 sq mm x 1/10 mm. The W.B.C. in all the small squares are counted.

If the number of cells in 1/10 c mm is X, the number in 1 c mm = X x 10

The dilution of blood is 1 in 20

The total number of W.B.C. in 1 c mm undiluted blood = X x 10 x 20 or X x 200

B. Differential count:
The differentiation of white blood corpuscles is based on their size and shape of nucleus and differential staining of nucleus and cytoplasm with Giemsa or Leishman stain.

Technique:
Prepare blood film following standard technique and stain with Giemsa or Leishman stain.

Observation:
Count 100 or 200 W.B.C. noting the type on a sheet of paper and calculate the percentage.

Calculation:
The percentage of different types of W.B.C. is calculated with the formula

% of W.B.C. type = No. of type of cells/Total No. of W.B.C. counted x 100

The normal value of various W.B.C. (type):

Neutrophil	50 – 70	per cent	3000 – 6000	perc	mm
Basophil	0·5 – 1	"	0 – 100	"	"
Lymphocyte	20 – 30	"	1500 – 2700	"	"
Monocyte	2 – 8	"	300 – 600	"	"
Eosinophil	1 – 4	"	150 – 300	"	"

14. Experiment on Blood Grouping:
The serum of a person may cause agglutination of the red blood corpuscles (R.B.C.) of another person. Depending on the presence or absence of two agglutinogens A and B in erythrocytes and two specific agglutinins anti-A (α) and anti-B (β) in the serum, human blood groups are designated as A, B, AB and O. An R.B.C. may have either or both factors (A, B, AB) or none at all. A serum may have either or both factors (α, β, αβ) or none at all.

If the corpuscles contain A, the serum contains β, the blood group is A. If the corpuscles contain B, the serum contains a the blood group is B. If the corpuscles contain both A and B, the serum is free from α and β, the blood group is AB (universal receiver). If the corpuscles contain neither A nor B, the serum contains both α and β, the blood group is O (universal donor).

Test tube method of blood grouping:

i. Collect 2 drops of blood in a small test tube containing 1 or 2 ml physiological saline solution.

ii. Take two small test tubes and put one drop of corpuscle suspension and one drop of saline solution in each. Add one drop of anti-A (α) serum in one tube and anti-B (β) serum in the second tube. Prepare four such sets.

iii. Mix the contents of each tube gently and allow to stand at room temperature for one hour, though positive reactions can be detected within a few minutes. Examine contents of each tube under the low power of a microscope for agglutination (Fig. 51.6).

blood group	agglutinogen in erythrocyte	agglutinins in plasma	reaction with	
			anti-A serum	anti-B serum
O universal donor	none	a and b		
A	A	b	agglutination	
B	B	a		agglutination
AB universal recipient	A and B	none	agglutination	agglutination

Fig. 51.6: The ABO blood groups. The appearance of the blood of each group when exposed to anti-A and anti-B serum is shown in the two right hand columns. Clumping of cells indicates reaction of serum antibodies from recipient with cell antigens from donor

Results:

a. Agglutination by α but not by β= Croup A

b. Agglutination by β not by α = Group B

c. Agglutination by both α and β = Group AB

d. No agglutination by α and β = Group O.