(6) Tuesday 16th Nov

Histology of Blood - Total and Differential White Cell Counts

The histology of blood is best studied in thin, even films, fixed and stained to show not only the erythrocytes, but also the characteristic differences in the nuclei and cytoplasm of the leucocytes. Using such films, the different cell types can be identified and a differential white cell count may be performed. This, in association with a total white cell count on the same sample of blood, is a basic diagnostic tool. Since white cells differ in function as well as form, the changes in cell count may reflect changes due to disease or treatment.

To prepare a blood film

Slides and coverslips must be absolutely clean and grease-free. One slide with an unchipped end is used as a spreader.

After sterilizing the skin with alcohol, the finger is pricked with a sterile lancet. When a small drop of blood appears (without squeezing) it is placed near the end of one slide. The spreader slide is hled at 45° to this slide and moved up to the drop of blood. The blood is allowed to spread across the width of the slide by capillary action, and then with a smooth action the spreader is drawn (or pushed) evenly to the other end of the slide to make a thin, uniform film.

A second film may be made similarly if there is enough blood to work fairly quickly to make the films before the blood clots.

Films are dried by waving the slides in the air and should then be examined under the lower power of the microscope. If the film is too thick, it should be discarded as it will be impossible to identify leucocytes; if not smeared rapidly enough the distribution will be very uneven with polymorphs tending to accumulate at the edges.

Staining of blood films

Stains are mixtures of eosin and methylene blue, that is they contain acid and basic staining components, and are made up in methyl alcohol (which fixes the film) and so films should be covered whilst staining to prevent evaporation.

Jenner, Leishmann, Giemsa, May-Grunwald are all variations of blood stains. We normally employ Leishmann as it is simple, quick and relatively easy to get a good result.

Method

1. The dry film is covered with stain, counting the number of drops used, e.g. 8, the slide may be rocked to ensure complete coverage.

2. The slide is left covered in a Petri dish for 2 minutes.

3. The slide is then diluted with twice the number of drops e.g. 16, of neutral water (or buffer pH 7) and left for a further 10 minutes, making sure that it does not dry at any stage.

4. The stain is poured off and the film washed in a stream of neutral water until it appears pink. It is then blotted dry with filter paper.

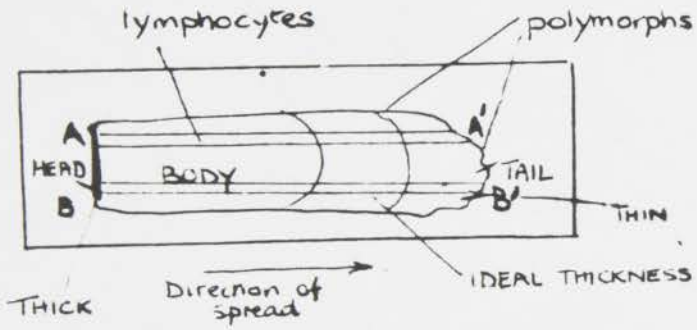
5. When examined under L.P., the erythrocytes appear coppery-red and leucocyte nuclei purple. The cytoplasm of the latter varies from deep purple granules (basophil) to mauve (neutrophil/polymorphs) or pink-red (eosinophil) to clear blue (lymphocyte or monocyte).

   High power is required to identify the different leucocytes and drawings should be made of each type making sure that relative sizes are portrayed.

   This film can be used for a differential white cell count.

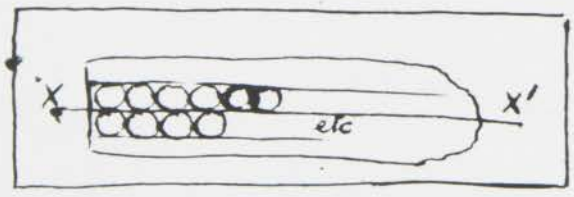
Differential White Cell Count

Since the different WBC distribute unevenly in blood films (see diagram) some precautions have to be taken to count over a representative area of the film.

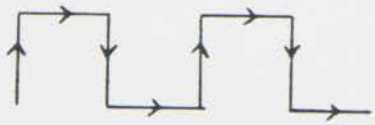


Using either a 4mm dry (high power) or oil immersion lens, the cells in a strip AA' running the whole length of the film are counted. If less than 100 cells (better still 200) are encountered another strip BB' is counted. This should be representative of the whole film from thick to thin, although it does ignore the edges.

It may be easier if the film is scored with a needle along a line XX' and the cells counted in a strip as wide as the field of view above and below the line (see diagram)



Another possibility is to employ a "battlement" type count in order to scan the film.



A table is constructed headed with the different types of white cells and they are entered as encounted. The percentage of each type present is calculated.

	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Totals					
%					
Total WBC present/mm^3 = , ∴absolute numbers:-					

Making use of the total white cell count on the same sample of blood, the actual count of each type per cu mm should be worked out.

Normal range of vlues for differential white cell count in adult:

Neutrophils	2,000 - 7,500	(40-75%)
Lymphocytes	1,500 - 4,000	(20-45%)
Monocytes	200 - 800	(2-10%)
Eosinophils	40 - 400	(1-6%)
Basophils	0 - 100	(< 1%)

Total White Cell Count

In the total white cell count, the haemocytometer counting chamber and white cell pipette (white bead) are used. The method differs from the red cell count in that:

a) All red cells are first haemolysed and thus rendered almost invisible

b) The blood sample is diluted only 20-fold (instead of 1:200) (Why?)

The Coulter counter (see red cell count schedule) can be used for white cell counts and in association with the Channelyzer has been used to discriminate between lymphocytes and granulocytes on the basis of volume; so it can be used to make a form of differential WBC count automatically. However, the method employed above is still the most frequently used in medical diagnosis.

Method

1. It should be ensured that the counting chamber and pipette are scrupulously clean. The counting chamber is prepared with coverslip.

2. A large drop of blood is obtained as before and drawn up to the 0.5 mark of the pipette.

3. Diluting fluid (1.5% solution of acetic acid tinted with gentian violet - the acid haemolysing the RBC and the gentian violet staining the WBC nuclei) is drawn up to the 11 mark.

4. The mouthpiece is removed and the pipette shaken vigorously for 1 minute and then about a quarter of the contents blown out.

5. The tip of the pipette is placed to the bevelled edge of the counting chamber and allowed to fill it as in RBC count. It is allowed to settle for 1-2 minutes.

6. Using low power, all the white cells in each of the 9 large squares are counted. Where cells lie on the top or left edges they are counted "in", on right or bottom edges - "out".

   No of cells counted = n

   Area of large square = 1 mm^2

   Depth of fluid = 0.1 mm

   Volume of each square = 0.1 mm^3

   Dilution = 1:20

   So total count per cu mm = $\frac{n}{9} \times \frac{1}{0.1} \times 20$

   Normal range 4,000 - 11,000/mm^3 (average 7.500).

Questions

1. What are the main sources of error in these estimations?

2. Under what physiological conditions would the WBC count increase?

3. Describe briefly the functions of each type of white cell.