

## TP2 OBSERVATION OF A BLOOD SLIDE USING LIGHT MICROSCOPY

### I. Blood slide technique

This classic technique is performed on fresh blood using clean, degreased and perfectly dry slides. The blood slide must be thin and evenly spread over more than half of the slide and air-dried before being stained with the classic May-Grünwald Giemsa (MGG)

**1. Fixation and May-Grünwald colouring :** Put about twenty drops of May-Grünwald stain on the dry slide placed at the bottom of a Petri dish (slide completely covered). Cover with the lid (to avoid drying out) and leave for 3 minutes. Then uncover the slide and add an equivalent quantity of neutral water and mix well by gently moving the dish in all directions for 1 min.

**2. Giemsa coloration Reject the mixture, and without washing:** put in a sufficient quantity to cover the slide with Giemsa diluted 1/20th. Leave to act for 10 min, then quickly wash the slide under a stream of neutral water to remove the precipitates.

**3. Drying and observation:** Allow to air dry by placing the slide vertically on filter paper and observe the stained slide directly without a glass slide, first with a 40° objective to locate the best area for spreading, then with a 100° immersion objective.

### II. Principle of staining

The May-Grünwald colourant dissolved in methanol contains an acid dye, eosin, and a basic dye, methylene blue.

The Giemsa colorant contains an acid dye, eosin, and a basic dye, methylene azide.

### III. Classification of figured elements:

Blood cells, which are suspended in a liquid medium called plasma, are divided into three functional categories which are found in a 'blood count' (CBC):

-red blood cells or erythrocytes ( $3.9$  to  $6.5 \times 10^6$  /mm<sup>3</sup> of blood)

-leukocytes or white blood cells ( $3.5$  to  $12 \times 10^3$  /mm<sup>3</sup> of blood)

-thrombocytes or blood platelets ( $150$  to  $400 \times 10^3$ /mm<sup>3</sup> of blood).

Leukocytes vary greatly in type, both cytologically and functionally:

**-Granulocytes** (former 'polynuclears' with a single nucleus but made up of several lobes) represent 60 to 70% of all leukocytes, with distinctions based on the colour affinities of the cytoplasmic granules:

-neutrophils (the most numerous);  $12\mu\text{m}$ , polylobed nucleus (between 3 and 5 lobes), fine azurophilic granules

-eosinophils (<4%);  $12\mu\text{m}$ , polylobed nucleus (2 to 3 lobes), large acidophilic granules (orange)

-basophils (<1%);  $12\mu\text{m}$ , bilobed nucleus masked by large basophilic granules (bluish).

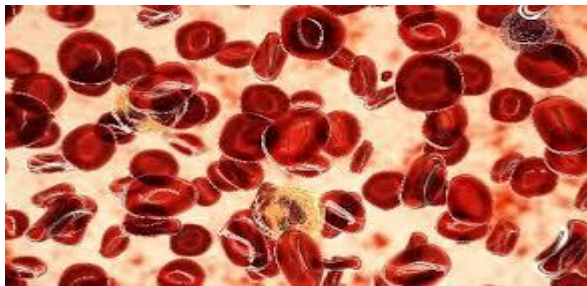
-**Agranulocytes** (former 'mononuclear' cells with lobeless nuclei) represent 30 to 40% of all leukocytes, characterised by the absence of cytoplasmic granules or a few rare azurophilic granules:

-lymphocytes, small and large (20 to 40%, the number increasing during viral infections), variable size (8  $\mu\text{m}$  for the smallest, up to around ten micrometres for the largest), large nucleoplasmic ratio (the nucleus occupies almost all the cell volume), vitreous cytoplasm forming a fringe around the nucleus).

-monocytes (from 4 to 8%); these are the largest leukocytes (over 15  $\mu\text{m}$ ), the large nucleus has an indentation (horseshoe shape), the cytoplasm is bluish and glassy, the cell does not have a well-defined shape (often deformed contours).

#### **IV. The different types of blood cells:**

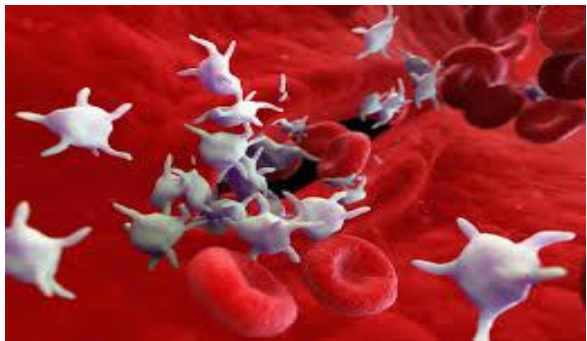
**-Red blood cells:**



**-White blood cells/leukocytes:**



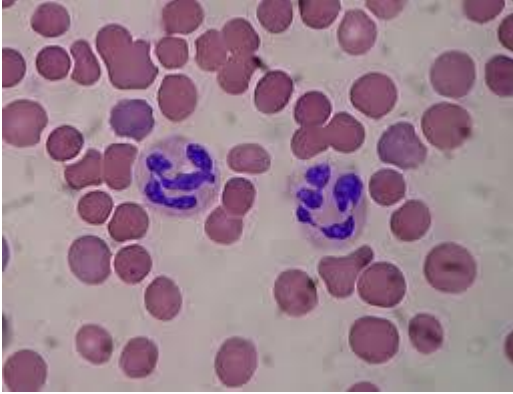
**-Platelets:**



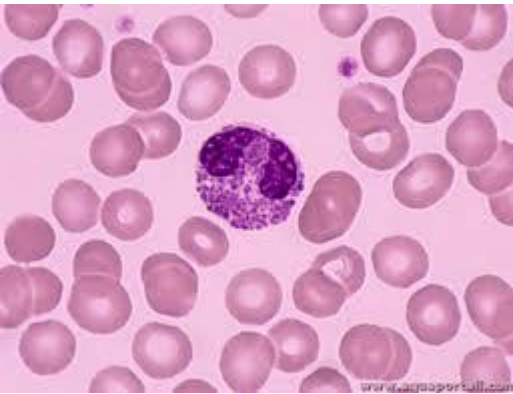
## V. Leukocytes observed on a blood smear:

### V.1 Granulocytes:

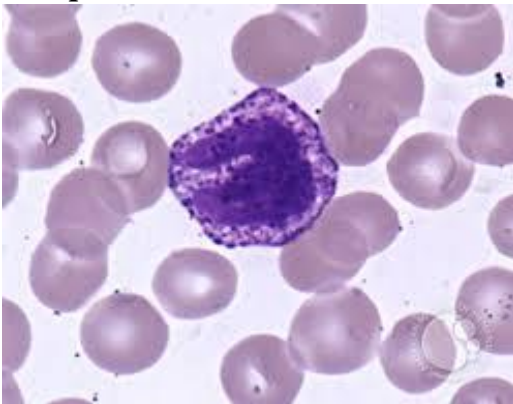
- Neutrophils:



- Eosinophils:

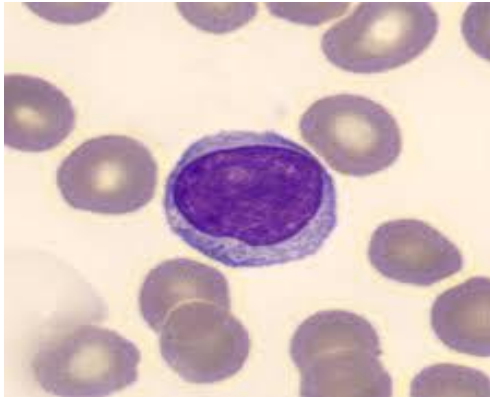


- Basophils:



**V.1 Agranulocytes:**

- **Lymphocytes:**



- **Monocytes:**

