**Experiment No. 11**: **Preparation of Silkworm Haemocytes.**

**Aim:** To prepare the silkworm haemocytes.

**Reagents Required:**

1. **Phosphate-buffered saline with 10% formalin:** 10mM $Na_2HPO_4$, 138mM NaCl, and 2.7mM KCl, pH 7.4, containing 10% formalin and 1mM thiourea.
2. **Lishman's Stain:** Dissolve 0.6 g Leishman's stain powder in 400 ml Methanol. Or ready solution may be used.

**Procedure:**

1. Collect the haemolymph from $5^{th}$ instar silkworm larvae in a clean pre cooled micro centrifuge tube by puncturing the caudal horn.
2. Then, immediately mix 200 µl of haemolymph with one ml of phosphate buffered saline and leave it for 5 min at room temperature.
3. Now, centrifuge the above preparation at 1000 rpm for 3 min.
4. After centrifugation remove the supernatant with a micro pipette carefully, re-suspend the palette with 50 µl of phosphate buffered saline, drop on a clean glass slide.
5. Air dry the preparation, stain with Lishman's stain for 2 min, wash excess stain in running distilled water, air dry and observe under a microscope with a magnification of 450 X.

**Observation:**

Observe the haemocytes and record the results as number of different types with reference to the following photograph.

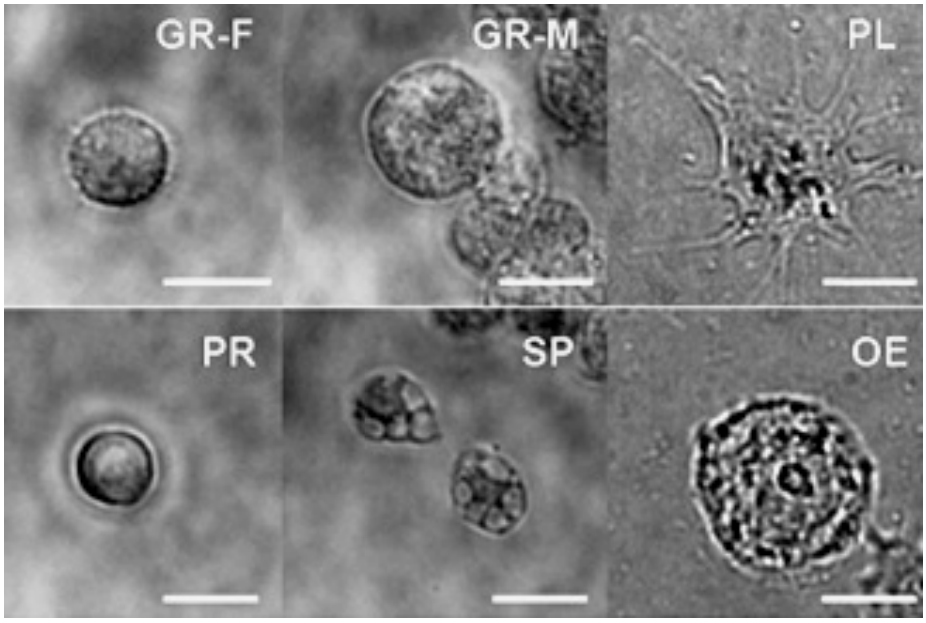


Figure:

GR-F: Granulocyte in the feeding phase.
GR-M: Granulocyte in the molting phase.
PL: Plasmatocyte.
PR: Prohemocyte.
SP: Spherulocyte.
OE: Oenocytoid.

Courtesy: Takashi *et al.* 2008. *J. Insect Physiology* (54): 454–461.

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