

1. COLLECTION, SEPARATION AND IDENTIFICATION OF FISH LEUCOCYTES

Leucocytes play an important role in the fight against infection in all animals. Therefore for any immunological study, familiarity with leucocytes of the species would be very important. Leucocytes can be obtained from a number of sites eg. peripheral blood, hemopoietic/lymphoid organs and induced inflammatory exudates. Though peripheral a good source, collection of sample could be a problem when dealing with small fish. The caudal vein is the preferred site for bleeding large fish. Cardiac puncture can also be performed. Large fish can be bled repeatedly provided not more than 0.5 – 1 ml of blood is removed from about 200 gm fish on weekly basis. A certain amount of tissue damage might occur in and therefore blood may be contaminated with other fluids.

Hemopoietic tissue such as head kidney can also be a good source of leucocytes. But the leucocytes from such tissue may be various stages of maturation and hence may be morphologically and functionally heterogeneous. In cartilaginous fishes hemopoiesis occurs in unique organs including epigonal organ associated with the testis ovary and the organ of Leydig in the subucosa of the alimentary tract. These organs are good sources of granulocytes as well as lymphocytes. The spleen of cartilaginous fishes can also be suitable for providing cell types such as macrophages and lymphocytes.

Inflammatory exudates in the body cavity induced by the injection of a suitable stimulant has been used as a source of leucocytes in mammals. Agents such as peptone broth, starch, liquid paraffin and glycogen induce non-specific inflammatory response. However, in teleost fish, inflammatory response is considerably delayed compared to mammals. In plaice and rainbow trout, injection of bacterial cells into the peritoneal cavity resulted in peak neutrophilia at about 24 hours, while the peak for macrophages was observed after seven days (Finn and Nielson, 1971). This biphasic response can be made use of to get relatively pure populations of granulocytes and macrophages.

Examination of Blood cells:

Most hematologists use stained smears for the identification of different leucocytes types.

Materials:

1. Glass slides
2. Microscope
3. Stains such as Leishman or Giemsa
4. Phosphate buffer pH 6.8
5. Methanol

Methods:

1. A drop of blood is placed on one edge of a glass slide. Using another slide placed at an angle of 35°, the drop of blood is smeared quickly on the slide. The smear is allowed to dry in the air. The smear can be stained by Leishman or Giemsa stain. For Giemsa stain, the smear is fixed for 1-2 minutes in methanol.

2. The smear is stained using Leishman stain for one minute. The stain is diluted with twice the volume of phosphate buffer and left for 2 minutes. The slide is then washed with phosphate buffer and allowed to dry.
3. Giemsa stain is diluted 1+9 in phosphate buffer. This working solution is poured on the slide and allowed to stain for 20-35 minutes. Alternatively undiluted stain is used for 5 minutes. This slide is washed in phosphate buffer and allowed to dry.
4. The slides are examined under oil immersion objective. Fish blood contains predominantly erythrocytes (which are nucleated), lymphocytes thrombocytes, granulocytes (neutrophils, eosinophils and very rarely basophiles) and monocytes. The characters of some of the cells are given below.

Thrombocytes: are spindle to ovoid in shape, about 8 microns in diameter with homogeneous grey-blue cytoplasm and centrally located, elongated, often clefted nucleus. These are common in blood.

Lymphocytes: are round in shape with homogeneous grey-blue cytoplasm and centrally located spherical or clefted nucleus. These are common in blood. In spleen and kidney smears, large immature lymphocytes with larger amount of cytoplasm are common.

Granulocytes: are round in shape usually containing granules, which may be eosinophilic, neutrophilic or very occasionally basophilic. The nucleus is central or eccentric, irregular, highly polymorphic with several lobes. These are rare to common in blood. In kidney or spleen smears, neutrophils are more common.

Monocytes-Macrophages: Monocytes are round and macrophages are amorphous in shape. Macrophages are not commonly found in peripheral blood but can be seen in smears from the anterior kidney. The nucleus is generally irregular or kidney shaped and eccentric. The cytoplasm is non granular, grey-blue-purple in colour and may have vacuoles and few inclusions.

Observation and finding results:

