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Hematopoeitic study of the terrestrial slug *Laevicaulis alte* (Ferrusac, 1821) Mollusca: Gastropoda through histology

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Abstract

Hematopoiesis is a process that is responsible for generating sufficient number of blood cells in the circulation and in tissues. We carried out histological investigations on the hematopoietic tissues of the terrestrial slug *Laevicaulis alte*. Evidences for hematopoiesis reveal chiefly as heavy concentrations of hemocytes in the probable hematopoietic tissues. The intestine is lined with epithelial cells and interspersed with mucocytes. Our histological studies on the hepatopancreas show accumulations of small round basophilic hemocytes. The histology of foot reveals heavy accumulation of hemocytes. The albumen gland is composed of glandular tubules or follicles lined with columnar cells filled with droplets of non mucoid secretion. Hematopoiesis of *Laevicaulis alte* is a useful tool to understand its different functions like physiology, immunology and detoxification process.

Keywords: Hemocytes, mantle, foot, hepatopancreas, albumen gland, intestine

Introduction

Blood cells have a major role in immunological defense mechanisms (Chang, 1975), absorption and digestion of food in the digestive gland and removal of residual materials from other parts of the body (Wagge, 1955), regeneration and shell repair (Pan, 1958 and Trip, 1961) [12]. There is yet no definite information pertaining to the sites of blood cell formation in molluscs (Malek and Cheng, 1974) [11]. The hemolymph cells of *Lymnaea stagnalis* formed in connective tissue particularly in the lung (Muller, 1956) [10]. The leucocytes of *Biomphalaria glabrata* may be formed from fibroblasts, blood sinuses and the cellular reticulum occurring in the wall of the nephridium (Pan, 1958). In *Helix aspersa*, leucocytes differentiate from connective tissue around the mantle (Gatenby and Hill, 1934; Haughton, 1934; Bourne, 1935; Crawford and Barer, 1951) [5, 6, 15, 13]. Narain (1973) [2] considered the alimentary tract to be the centre of hemopoiesis in bivalves. Lie *et al.*, (1975) [14] have identified the hemocyte producing organ between the pericardium and epithelium of the mantle cavity. Kinoti (1971) [9], Pan (1965) [8] and Lie *et al.* (1975) [14] have observed that blood cell production takes place in the amoebocyte producing organ (APO) which is located in the renopericardial region. Our present study was undertaken to understand the production of blood cells from the probable hemocytopoietic organs such from mantle, foot, hepatopancreas, albumen gland and intestine of the slug *Laevicaulis alte*.

Materials and Methods

Terrestrial slug *Laevicaulis alte* were collected from Courtallam, Tamil Nadu, India and brought to the laboratory for histological investigations. The probable hemocytopoietic tissues in the organs like muscle, foot, hepatopancreas, albumen gland and intestine were stained histologically. These tissues were dissected out from slug *Laevicaulis alte* kept in snail-sterile saline solution and fixed immediately in Bouin's fluid for 24 hours. The Bouin's fixed material was thoroughly washed in running water and placed in three changes of 70% ethyl alcohol. Dehydration of tissues was accomplished by the use of graded series of alcohols beginning with 70% ethyl alcohol. Xylene was used as cleaning agent. The tissues were impregnated in three pre-molten paraffin (melting point 56-60 °C) baths for 20 minutes each and then were blocked in Paraffin wax at 60 °C. Microtome sections were cut at 5-6 in thickness and stained with Harris, hematoxylin and eosin, DPX was used as mountant (Patki, *et al.*, 1987) [7].

Results

Hemocytopoiesis

The present histological investigations on the hemocytopoietic tissues of *Laevicaulis alte* show evidences for hemopoiesis, chiefly as heavy concentrations of hemocytes in the tissues.

Histology of Mantle

The body surface was covered with a single layered columnar epithelium equipped with both cilia and microvilli. Large mucus-secreting and mucus storing cells extended deep into the subepithelial matrix surrounded connective tissue and extracellular mucus. The subepidermal connective tissue was traversed by processes of gland cells. Other minor cell types found were fibroblasts, nerve cells with electron dense granules, pigment cells and amoebocytes. In the deeper layer the muscle and connective tissue was interwoven by numerous capillary processes of hemocoelic sinuses.

Histology of the foot

The foot is a highly glandular structure. The sole is ciliated and lined with epidermal cells. Subepidermal gland cells sunk below the epidermis through which they open by long neck. The sub epidermal gland cells are clustered with the hemocytes which is an indication of hemocyte production.

Histology of the Intestine

The intestine is lined with epithelial cells and interspersed with mucocytes. The epithelium is subtended by connective tissue containing circular and longitudinal muscle layers which surround the entire organs. Undifferentiated hemocytes are embedded in the connective tissue which indicates hemocytopoiesis.

Histology of Albumen gland

The albumen gland is composed of glandular tubules or follicles lined with columnar cells filled with droplets of nonmucoid secretion. The tubules of the albumen gland empty their transparent, nutritive secretion in to the albumen canal which runs the length of the gland and is also thinly lined with squamous epithelium. The albumen canal joins the hermaphrodite duct to form a thick walled chamber the fertilization pocket.

Histology of Hepatopancreas

The digestive gland consisted of lobules supported by large, hollo appearing vesicular connective tissue cells, known as tunica propria enveloping the gland. Each lobule consisted of tall cylindrical cells basal nuclei and clusters of granules enclosed in vacuoles. Within the lobules, different types of granulocytes are observed.

Histology of the Albumen gland

SC – Secretory Cell; F – Follicle; CC – Ciliated Cells
MD – Main Duct; GC – Gland Cells; HPD – Hemaphrodite duct

Histology of the foot

CE – Ciliated Epidermis; SEG – Sub epidermal gland cells;
HC – Hemocytes

Histology of the Intestine

CT – Connective tissue; HC – Hemocytes;

VL – Villi; ME – Mesenchymatous epithelium

Histology of the Hepatopancreas

CT – Connective tissue; HC – Hemocytes; VC – Vacuoles

Histology of the Mantle

MF - Muscle fibre; TG - Tubular glands; HC – Hemocytes

DME - Dorsal mantle epidermis; VE – Ventral epidermis

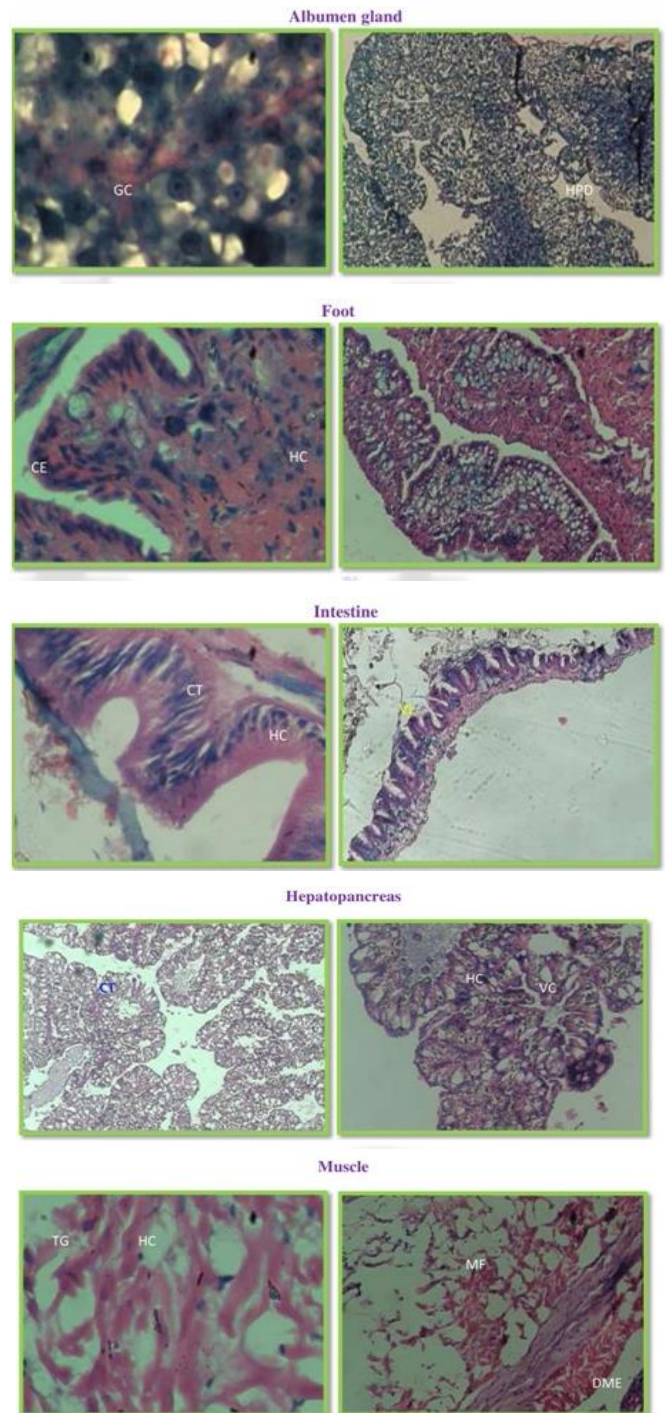


Fig 1: Histology of probable hemocytopoietic organs

Discussion

Our results enabled to gain more knowledge in the structure and functioning of the tissues. The histological studies on the hepatopancreas revealed accumulations of small round basophilic hemocytes. It agreed with the findings of Gatenby and Hille (1934) [5] Aaushton (1934). To our knowledge this

paper includes the first quantitative indication of hemocyte proliferation after an immune challenge among gastropod slugs. Crawford and Barer (1951) ^[13] have reported that leucocytes were differentiated from connective tissue located in the mantle of *Helix aspersa*.

Similar observations were reported by Mahilini (2000) ^[4] in the three gastropod snail species *Trachia vittata*, *Pila globosa* and *Indoplanorbis exustus*. Rogers (1969) ^[3] reports in *Helix aspersa* that the number of muscle bundles increases towards the epithelial surface of the foot. The epithelium of the body wall of *Ariolimax columbianus* is made up chiefly of three cell types, the microvillous epithelial cells and the giant mucus and channel cells that extend deeply in to the body wall.

They are connected at their apical margins by junctional complexes of distinctive structure Luchtel *et al.* (1984) ^[1]. The histology of foot revealed the heavy accumulation of hemocytes. There is no evidence whether the foot is an integral tissue of the hemolytopoietic system. Narain (1973) ^[2] considered that the alimentary tract may be the centre of hemopoiesis in bivalves and suggested that the breakdown of food products of foot which may be utilized for blood cell production by the cilia lining the intestinal wall. In the slug *Laevicaulis alte*, the cross section of the intestine revealed heavy infiltrations of hemocytes. Similar observations were made in *Trachia vittata*, *Indoplanorbis exustus* and *Pila globosa* (Mahilini, 2000) ^[4]. Haemocytopoietic study of *Laevicaulis alte* is a very useful to understand its different functions like physiology, immunology and detoxification process.

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