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III. 1. Introduction

The origin of molluscan hemocytes is little understood (Malek and Cheng, 1974). The Cephalopods have well defined leukopoietic organs called the white bodies present in the orbital pits behind the eyes (Cowden and Curtis, 1974). According to Narain (1973), the alimentary tract is supposed to be the centre of hemopoiesis in bivalves. In the case of gastropods the various parts of the body such as mantle, epithelium and connective tissue, walls of blood sinuses, connective tissue around the kidney and digestive gland giving rise to these mesenchymatous blood cells (Malek and Cheng, 1974; Sminia, 1974). These blood cells proliferate from precursor cells such as embryonic cells (Mullet, 1956), amoeboblasts (Kinoti 1971) and from mature amoebocytes (George and Ferguson, 1950; Brown and Brown, 1965) or from fibroblasts, epithelial or endothelial cells (Wagge, 1951; Pan, 1958) by mitosis, amitosis and cell fragmentation.

Sminia (1974) listed down the different sites of proliferation of blood cells which may be the epithelium and connective tissue around the mantle (Wagge, 1951; 1955), the connective tissue around the lung cavity (Muller, 1956), the walls of blood sinuses and connective tissue in the kidney region (Pan, 1958), the ventricle and connective tissue around the heart-kidney system (Kress, 1968) and the connective tissue of the mantle (Kinoti, 1971).

Blood cell production has been observed to take place in the amoebocyte producing organ (APO) (Kinoti, 1971; Pan, 1965; Lie et al., 1975 b). The APO may be located in the renopericardial region (Kinoti, 1971; Pan, 1965; Lie et al., 1975b; Rondelaud and Barthe, 1981) and any part of the connective tissue (Sminia, 1974), Lie et al., (1975a) have identified the hemocyte producing organ (HPO) between
pericardium and epithelium of the mantle cavity. To increase the understanding of gastropod hemopoietic tissues, histological aspects of the probable hemopoietic tissues in the organs such as heart, kidney, mantle, digestive gland and intestine of *T. vittata*, *P. globosa* and *I. exustus* were studied.

**III. 2. Materials and Methods**

The probable hemopoietic tissues in the organs like heart, kidney, digestive gland, mantle and intestine were studied histologically. These tissues were dissected out from the active snails of *T. vittata*, *P. globosa* and *I. exustus* in the presence of snail-sterile solution and fixed immediately in Bouin's fluid for 24 hrs. The Bouin's fixed material was thoroughly washed in running water and placed in three changes of 70% ethyl alcohol. Dehydration of tissue was accomplished by the use of graded series of alcohols, beginning with 70% ethyl alcohol. Xylene was used as clearing agent. The tissues were impregnated in three pre-molten paraffin (melting point 56 - 60° C) baths for 20 min each and then were blocked in paraffin wax at 60° C. Microtome sections were cut at 5 - 6 μ thickness and stained with Harri's hematoxylin and eosin. DPX was used as mountant.

**III. 3. Results**

The present histological investigations on the hemopoietic tissues of the three chosen gastropods show evidences for hemopoiesis, chiefly as heavy concentrations of hemocytes in the hemocytopoietic tissues.
III. 3. 1. Histological observations

III. 3. 1. 1. Histology of Mantle

The mantle of *T. vittata*, *P. globosa* and *I. exustus* overlying the viscera is covered on both sides with epidermis. The mantle interior consists primarily of connective tissues, containing muscle and blood lacunae. The mantle epithelium consists of two cell layers which is separated by blood sinus and these epithelial cells are darkly stained. The inner surfaces of these mantle epithelial cells are ciliated and interspersed with gland cells. Vacuolation in the connective tissues is also noted. The blood sinuses are populated with clusters of embryonic basophilic hemocytes which is one of the evidences for hemocytopoiesis (Plates 8.A & B, 9.A & B and 10.A & B).

III. 3. 1. 2. Kidney architecture

The histological features of the kidney of the three gastropods revealed two cell types, namely vacuolated excretory cells and renal epithelial cells, covering the folds of dorsal wall of the kidney. The renal epithelial cells are less numerous than excretory cells. Hemocytes are interspersed between excretory cells and renal epithelium. Groups of monomorphic basophilic blood cells are embedded in the connective tissue and separated from the epithelial cells by blood sinus which is an indication of hemocyte production (Plates 8.C & D, 9.C & D and 10.C & D).

III. 3. 1. 3. Histology of Intestine

In the three chosen gastropods, the intestine sections show the muscle coat containing circular and longitudinal muscle layers which surround the entire organ. A basal lamina separates the basal region of the epithelium from a discontinuous layer of pigmented connective tissues cells (sub mucosa). Mucosa layer is thrown into villi which is lined with tall columnar epithelium interspersed with mucosal glands. Clusters

III. 3. 1. 4. Histology of the Digestive Gland

Plates 8.G, 9.G and 10.G show the photomicrographs of the digestive gland of the three gastropods. Histologically it is composed of numerous branched acini or lobules supported by large, hollow-appearing vesicular connective tissue cells, known as tunica propria enveloping the gland. Each lobule has a single layer of non-ciliated columnar epithelial cells form the lining of the acini, in which three digestive gland cell types are distinguished namely (i) secretary cells which are characterized by a cylindrical to triangle shaped and a large basally located nucleus (ii) digestive cell which are tall columnar cells which represent the most numerous cell type found to compose digestive gland tubules and (iii) excretory cells that are located adjacent to or between secretary cells in the acinar regions.

Within the lobule, different types of mature granulocytes are observed which are distinguished as larger fixed blood cells and it appears to play a role in cellular defense mechanisms.

III. 3. 1. 5. Histology of Heart

Plates 8.H, 9.H and 10.H show the histology of the heart of the three chosen gastropods viz., T. vittata, P. globosa and I. exustus. The heart possesses an epicardium and bundles of striated myocardial fibers attached internally. Endocardium is absent. There is no proper endothelium separating the muscle cells and lumen of the heart. The wall of the pericardial sac is clothed on both sides with a cuboidal epithelium underlain by muscular and connective tissue fibres binding the central lumen. The muscle fibres are arranged as trabeculae running across the lumen. This arrangement permits thickening
Plate 8. Histology of hemopoietic organs of *Trachéa vittata*

**A & B.** T. S. of Mantle (HE, x 100, x 250)
- EMC - Epithelium bordering the mantle cavity
- ES - Epithelium bordering the shell
- HC - Hemocytes

**C & D.** T. S. of Kidney (HE, x 100, x 250)
- RE - Renal epithelium
- RS - Renal sinus
- HC - Hemocytes

**E & F.** T. S. of Intestine (HE, x 100, x 250)
- CE - Columnar epithelium
- IL - Intestinal lumen
- VL - Villus
- HC - Hemocytes

**G.** Digestive Gland - a single lobule enlarged (HE, x 25)
- BM - Basement membrane
- LU - Lumen
- HC - Hemocytes

**H.** Heart (HE, x 100)
- HC - Hemocytes
- MF - Myocardial fibres
Plate 8

Histology of hemopoietic organs of *Trachia vittata*
Plate 9. Histology of hemopoietic organs of *Pila globosa*

A & B. T. S. of Mantle (HE, x 100, x 250)
- EMC - Epithelium bordering the mantle cavity
- ES - Epithelium bordering the shell
- HC - Hemocytes
- BS - Blood sinus with hemocytes

C & D. T. S. of Kidney (HE, x 100, x 250)
- RS - Renal sinus
- HC - Hemocytes

E & F. T. S. of Intestine (HE, x 100, x 250)
- CE - Columnar epithelium
- IL - Intestinal lumen
- VL - Villus
- HC - Hemocytes

G. Digestive Gland (HE, x 100)
- BM - Basement membrane
- LB - Lobule or Acini
- LU - Lumen
- HC - Hemocytes

H. Heart (HE, x 100)
- HC - Hemocytes
- MF - Myocardial fibres
Plate 9

Histology of hemopoietic organs of *Pila globosa*
Plate 10. Histology of hemopoietic organs of *Indoplanorbis exustus*

**A & B.** T. S. of Mantle (HE, x 100)
- EMC - Epithelium bordering the mantle cavity
- ES - Epithelium bordering the shell
- HC - Hemocytes
- BS - Blood sinus with hemocytes

**C & D.** T. S. of Kidney (HE, x 100, x 250)
- RS - Renal sinus
- HC - Hemocytes

**E & F.** T. S. of Intestine (HE, x 100, x 600)
- CE - Columnar epithelium
- IL - Intestinal lumen
- VL - Villus
- HC - Hemocytes

**G.** Digestive Gland (HE, x 250)
- BM - Basement membrane
- LB - Lobule or Acini
- LU - Lumen
- HC - Hemocytes

**H.** Heart (HE, x 100)
- HC - Hemocytes
- MF - Myocardial fibres
Histology of hemopoietic organs of *Indoplanorbis exustus*
to occur such that all the fibres are perfused with blood. Myocardial fibres are laden with hemocytopoietic islands with young blood cells of the same cell type and the same stage of maturation.

III. 4. Discussion

The blood cells or hemocytes are situated in a hemocyte producing organ (HPO) where the hemoblasts transform into hemocytes which then migrate into the sinuses of HPO, thereafter they are transported to all parts of the body (Ottaviani, 1983; 1988a). Several investigators have identified histologically in gastropods, the hemocyte producing organ may be heart - kidney region (Kress, 1968; Pan, 1958; Sminia, 1972; Kinoti, 1971), pericardium - mantle cavity (Kinoti, 1971; Lie et al., 1975b; Rachford, 1976; Jeong et al., 1983 and Ottaviani, 1992) and in the connective tissues of the mantle and epithelium (Wagge, 1951; 1955).

The general histology of heart, kidney, mantle, digestive gland and intestine of *T. vittata, P. globosa* and *I. exustus* are similar to Hyman’s (1967) description. The present histological studies on the heart and mantle revealed accumulations of small round basophilic hemocytes which may be the embryonic hemocytes (Brown and Brown, 1965; Hoffman et al., 1974). According to Kinoti (1971), Lie et al. (1975b), Rachford (1976), Jeong et al. (1983), and Ottaviani (1992), the HPO has been found in between the mantle cavity and pericardium. Lie et al. (1975b) reported that in *Biomphalaria glabrata*, amoebocytes or blood cells have been exclusively formed in the connective tissue between pericardium and posterior epithelium of the mantle cavity. In *Helix pomatia* amoeboid cells originate from mantle epithelium and from connective tissues of the mantle (Wagge, 1955). The current study on the histology of the mantle of the three chosen gastropods show strings of embryonic hemocytes as shown by Cowden (1972)
in the leucopoietic “white bodies” of *Octopus vulgaris* which may be related to the above-mentioned findings.

The kidney tissues of *T. vittata*, *P. globosa* and *I. exustus* show the features of hemocytopoietic organs which have homogeneous island of cells as in *Biomphalaria glabrata* observed by Joky and Gondran (1985) and this may be correlated to stem cells or embryonic cells (Muller, 1956; Kinoti, 1971; Lie *et al.*, 1975a; Jeong *et al.*, 1982). In *Bulinus truncatus* (Kinoti, 1971) and *Biomphalaria glabrata* (Pan, 1965; Lie *et al.*, 1975b) blood cells or amebocytes originate from an amebocyte producing organ (APO) located in the renopericardial region, at the end of the mantle cavity. As the heart-kidney region has a very high proliferation of amebocytes, this body part is the main production site of amebocytes (Sminia, 1972). This is supported by the observations on blood cell formation in *B. glabrata*, infected and reinfected with echinostome trematode (Lie *et al.*, 1975a; Lie and Heyneman, 1976). Hemolymph amebocytes often settle in the connective tissue of various organs of the body, temporarily or more permanently, singly or in group and especially in the heart-kidney region (Sminia, 1981).

The information concerning the hemocytopoietic aspects on the digestive gland and gut region are scarce. The blood cells or leucocytes are believed to be formed by budding from the digestive gland in *Jourunna tementosa* (Millott, 1937). In the present study, the larger cells of the hemocytes are interspersed between the digestive cells of *T. vittata*, *P. globosa* and *I. exustus* which may represent a fixed phagocyte system as in *Helix pomatia* (Reade, 1968; Bayne, 1973). In *Planorbarius corneus*, the entire digestive gland is made up of fixed phagocyte (Ottaviani, 1990). Moore and Lowe (1977) observed a number of granulocytes in the connective tissue of the digestive gland of the mussel *Mytilus edulis*. Another plausible reason might be that these larger cells may be
matured cells (Anderson, 1981) which are transported from hemocytopoietic organs to the digestive gland (Ottaviani, 1983; 1988) which may take part in defense reactions (Tripp, 1970; Harris and Cheng, 1975; Sminia, 1977; Dikkeboom et al., 1988). Majority of digenic trematodes utilize the digestive gland as the primary site of infection (Malek and Cheng, 1974) and also play an important role in nutrient digestion and transport as well as excretion (Cheng, 1981; 1984).

Narain (1973) considered alimentary tract to be the centre of hemocytopoiesis in bivalves and suggested that the break down products of food may be utilized for cell production by the ciliated lining of the intestinal wall. From the histology of the intestine of *T. vittata, P. globosa* and *I. exustus*, it has been found that the heavy infiltrations of hemocytes at the mucosa and sub-mucosa may be correlated with leucopoietic cells as observed by Cowden (1972).

Concisely on the basis of the results obtained, it is most likely that the connective tissues of heart, kidney, mantle, digestive gland and intestine of the three chosen gastropods may be the hemocytopoietic organs which indicate the following supportive evidences *viz.*, i) developmental stages of hemocytes within the cells of leukopoietic strings, (Cowden, 1972), ii) very large number of amoebocyte proliferation (Sminia, 1972; Lie et al., 1975b; Lie and Heyneman, 1976), iii) homogenous island of monomorphc blood cells (Joky and Gondran, 1985) which may be young cells (Hoffmann et al., 1974) or stem cells or embryonic cells with greater nucleocytoplasmic ratio (Muller, 1956; Kinote, 1971; Lie et al., 1975b; Jeong et al., 1982).

Another possible reason for the occurrence of heavy accumulation of hemocytes in all the probable hemopoietic organs may be, since molluscs have an open circulatory
system, the release of hemolymph cellular constituents is not limited to the heart and vessels (Malek and Cheng, 1974). The hemolymph is continuously pumped into the hemolymph sinuses and from there into tissues (Malek and Cheng, 1974). Electron microscopical and autoradiographical techniques in Limnidae stagnalis show that it does not possess special hemocytopoietic organ, as blood cell proliferation takes place throughout the body and dividing amoebocytes occur both in hemolymph and connective tissue (Sminia, 1974).

In the digestive gland of T. vittata, P. globosa and I. exustus, the distribution and the size (bigger cells) of the hemocytes vary when compared with mantle, heart and kidney tissues. It is documented by the work of Reade (1968) and Bayne (1973) that in Helix pomatia, a second phagocytosing "cell type", a fixed phagocyte is mainly found in the digestive gland region. Thus it is not clear whether the digestive gland is an integral tissue of the hemocytopoietic system.

In order to understand the mechanism of blood cell formation, the following techniques were adopted namely i) In-vitro production of hemocytes from the connective tissue of the mantle of Helix aspersa (Gatenby and Hill, 1934; Haughton, 1934; Bourne, 1935; Crawford and Barer, 1951), ii) labelling the amoebocytes of Lymnnea truncatula by 3H thymidine (Sminia, 1974) and iii) sensitizing the amoebocyte producing organ (APO) of Biomphalaria glabrata by exposing to Echinostoma caproni miracidia. The current histological evidences also do not allow in-depth analysis. Therefore there is need for more detailed studies to be conducted in the light of recent advanced techniques for further verification before conclusions can be drawn.
The amoebocyte producing organ behaves like a lymphoid organ in which defense cells are formed (Joky and Gondran, 1985). In majority of invertebrates cellular immune responses are carried out by more mature blood cells (Anderson, 1981). The mature granulocytes and hyalinocytes of invertebrates are effector cells in various cellular immune reactions. These cells more likely resemble granulocytes and macrophages of higher animals than lymphocytes (Anderson, 1981). Ottaviani (1992) suggested that round hemocytes have characteristics reminiscent of vertebrate T-lymphocyte and spreading hemocytes belonging to the category of macrophage lineage and these cells could be used as model for studying evolutionary origin of T-lymphocyte and ancestral cellular component of internal defense mechanisms in gastropods.