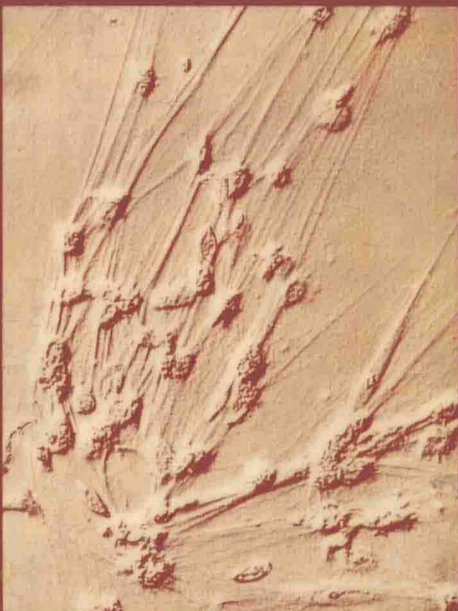
INVERTEBRATE BLOOD CELLS 2



ARTHROPODS TO UROCHORDATES, INVERTEBRATES AND VERTEBRATES COMPARED

edited by N. A. Ratcliffe and A. F. Rowley

Invertebrate Blood Cells

Volume 2

Arthropods to urochordates, invertebrates and vertebrates compared

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Preface

Renewed interest in invertebrate "blood cells" has developed for a number of reasons. First, there has been a recent escalation in research into comparative immunology, including many studies on invertebrates. These animals provide relatively simple experimental models, they may supply clues to the ancestry of the lymphoid system, and they may have novel defence reactions not yet discovered in the more complex immune systems of vertebrates. Secondly, many invertebrates, but especially the molluscs and crustaceans, are now being extensively farmed to augment the food resources of man. Clearly, a better understanding of the host defence reactions of such species would help to avoid and overcome disastrous outbreaks of disease which are likely to occur under the artificial and potentially stressful conditions of commercial culture. Thirdly, many invertebrates act as vectors of parasitic organisms which are the scourge of mankind. The insects and molluscs, in particular, include species responsible for the transmission of malaria, sleeping sickness, filariasis, onchocerciasis and schistosomiasis. The means by which these parasites invade and multiply in their hosts and yet fail to elicit an effective "immune" response is now the subject of intensive research. Fourthly, with the increasing resistance of invertebrate pests to chemical pesticides, and the accumulation of these noxious substances at higher levels in the food chains, greater efforts are being made to develop and utilize biological control agents such as the viruses, bacteria, fungi, nematodes and parasitoids. The potentially immense practical value of such agents has provided yet a further stimulus for researches into the host defence reactions of invertebrates. Finally, many biologists are beginning to realize that invertebrate blood cells not only function in "immune" or host defence reactions but also serve, at least in some species, to store, transport and/or synthesize food, waste products and hormones and are thus involved in many other vital life processes. Our lack of knowledge of the role of invertebrate blood cells in these basic functions provides a major area for future research utilizing modern biochemical techniques.

Much of the recent work on invertebrate blood cells, particularly in the fields of comparative immunology and cellular defence reactions, has been summarized in a number of excellent volumes including *Contemporary*

Topics in Immunobiology, Vol. 4, Invertebrate Immunology (E. L. Cooper, Ed., Plenum Press, 1974); *Invertebrate Immunity* (K. Maramorosch and R. E. Shope, Eds, Academic Press, 1975); *Comparative Immunology* (J. J. Marchalonis, Ed., Blackwell Scientific, 1976); *Comparative Immunobiology* by M. J. Manning and R. J. Turner (Blackie, 1976); and *Insect Hemocytes* (A. P. Gupta, Ed., Cambridge University Press, 1979). However, not since the publication of Warren Andrews *Comparative Hematology* (Grune and Stratton, 1965) has an attempt been made to summarize comprehensively our knowledge of the structure and function of invertebrate blood cells. Such a synopsis is urgently required in the light of current research interests and the many advances which have been made utilizing sophisticated modern techniques.

Volumes 1 and 2 of *Invertebrate Blood Cells* have been prepared specifically to bridge the gap since the publication of *Comparative Hematology*, with each chapter written by an expert in his or her particular group of animals. We have attempted to present information on as many invertebrate groups as possible and to this end have included much unpublished material, some of which was researched specifically for these books, e.g. Chapters 5, 14 and 15 on the leeches, lophophorates and echinoderms, respectively. We hope these volumes will generate further interest in many areas of invertebrate haematology and provide a source of comparative data for those workers already researching into particular aspects of invertebrate blood cells.

February, 1981 N. A. Ratcliffe and A. F. Rowley

Contents of Volume 2

Section IX: Comparative Aspects of the Structure and Function of Invertebrate and Vertebrate Leucocytes

Contents of Volume 1

I. Introduction

The close affinity of onychophorans and myriapods with insects, and a lack of affinity between them and the other arthropods are suggested by their embryonic development, uniramous limbs and terminal functioning of the mandibles. However, the Onychophora-Myriapoda-Hexapoda assemblage, also called Uniramia, shows like other arthropods, a reduction of the coelom, enlargement of the haemocoel, the synonymy of the "segmentation cavity" of the developing egg with the adult haemocoel, the haemocoelic perivisceral and pericardial spaces, the absence of fine vessels in the circulatory system and the dorsal tubular heart with paired ostia.

The structure and function of the blood cells are little understood in the Onychophora and Myriapoda, when compared with the extensive information available on the blood cells (haemocytes) of the Insecta. Jones (1962), proposed a classification of insect haemocyte types which appears less complex than those of earlier investigators. Recently, Jones' system of nomenclature and classification has been adopted for the haemocytes of several non-insect arthropods (Ravindranath, 1970, 1973, 1974a, 1974b, 1975; Sherman, 1973) and it was found that the Onychophora, Myriapoda, Crustacea and Arachnida have several haemocyte types which are similar to those of the Insecta (Ravindranath, 1977a, 1978; Gupta, 1979).

In onychophorans and myriapods, it is now fairly certain that one type of circulating blood cell is not a haemocyte, but may be the pericardial cell or nephrocyte which, in contrast to similar cells of insects, enters freely into the circulation (Campiglia and Lavallard, 1975; Seifert and Rosenberg, 1976; Rosenberg, 1978).

In the present chapter, an attempt is made: (1) to pool existing knowledge on the circulatory system and blood cell types of the Onychophora and Myriapoda (only the chilopods, diplopods and symphilids are dealt with in this chapter); (2) to correlate, wherever possible, fine structural with light microscopical descriptions of the cell types; (3) to describe the histochemical characteristics of the cytoplasmic inclusions; (4) to compare the blood cell nomenclature with the generally accepted haemocyte terminologies proposed for insects; and (5) to summarize the functional roles of the haemocytes of Onychophora and Myriapoda based on their structure and chemistry, as well as their behaviour, under different physiological conditions.

II. Structure of the circulatory system

A. Onychophora

The open circulatory system in onychophorans has been described by

Manton and Heatley (1937) in *Peripatopsis moseleyi* and *P. sedgwicki*. The heart is a dorsal tubular vessel devoid of an extension forming an anterior aorta, a feature unusual among arthropods. In every segment, the heart has a pair of cardiac ostia, each of which is in the form of a fissure. The heart narrows posteriorly and remains closed in the last segment of the body. No segmental arteries leave the heart.

The blood from the haemocoel reaches the pericardium partly through the ostia in the pericardial floor and partly through the intramuscular canal system. The pericardial floor is fused to the lateral musculature as in other arthropods. The supply of blood to the median and lateral haemocoelic cavities is from the anterior end of the heart, which extends above the anterior part of the pharynx and opens by a ventrally directed valve into the haemocoel. The pericardial floor here becomes non-existent, and so the heart directly supplies the haemocoel (Manton and Heatley, 1937).

B. Chilopoda

The circulatory systems of *Scolopendra* (Shukla, 1964; Varma, 1971), *Lithobius* (Biegel, 1922) and *Scutigera* (Demange, 1963) have been examined. In *Scolopendra*, there are both dorsal and ventral vessels. The dorsal tubular vessel or heart is supported by lateral muscles. There are 21 chambers in the heart, each with slit-like ostia. The first chamber lies in the second segment and has an anterior cephalic aorta extending to the brain where it branches and joins the ventral vessel forming an aortic arch. At each ostium, there is a pair of lateral vessels. The ventral vessel runs above the nerve cord and supplies blood to the legs. The pericardial septum in *Scolopendra* is a double layered membrane. In *Lithobius*, a pericardium is lacking. The heart of *Scutigera* has 13 pairs of ostia and from the anterior aortic arch, an artery arises and runs posteriorly and terminates in two swellings forming pulsatile organs, comparable to those of some insects.

The course of circulation in *Scolopendra* has been described by Shukla (1964). The blood is drawn in through the ostia by the dilation of the heart. When the heart is filled with blood, a steady wave of contraction passes forwards. The blood is carried to the cephalic arteries and then to the ventral vessel. From the arteries and ventral vessel the blood enters the spaces in the body cavity. The blood from the haemocoel is returned to the heart by way of the ostia.

C. Diplopoda

The details of the circulatory system in diplopods have been described in

Spirostreptus (Verhoeff, 1932), *Julus* (Rossi, 1902) and in *Thyropygus* (Krishnan, 1968), and reviewed by Verhoeff (1932) and Demange (1963). The heart is dorsal and tubular with segmentally arranged chambers. The first chamber lies in the second segment and has a short anterior aorta, which branches to form pairs of vessels. The first pair constitutes an aortic arch and the second pair extends posteriorly over the oesophagus where they fuse to form a median abdominal vessel. The anterior branches from this median vessel extend to the head and gnathochilarium. The entire system of arterial vessels arising from the median abdominal vessel has been described in *Spirostreptus* (Verhoeff, 1932). Each chamber of the heart bears a pair of ostia and a pair of ill-defined vessels. The pericardial septum lies immediately below the heart attached to the terga.

The course of circulation in *Thyropygus* has been described by Krishnan (1968). In the ventral vessel, the blood flows backwards and enters the body cavity. The blood from the haemocoel is returned to the heart by way of ostia. During systole, the blood flows anteriorly in the heart and passes into the cephalic artery supplying the head region.

D. *Symphyla*

In symphilids (Demange, 1963), the circulatory system is represented by a dorsal tubular heart. It has an anterior cephalic aorta. A vessel arises from it in the first segment and runs postero-ventrally.

III. Structure and classification of blood cells

Cells circulating in the haemolymph of the Onychophora and Myriapoda are grouped into two fundamental categories (Lavallard and Campiglia, 1975; Seifert and Rosenberg, 1976, 1977; Rosenberg, 1978).

(1) Cells without a basal lamina (or basement membrane), whose plasma membrane is directly in contact with the plasma, and which are called haemocytes.

(2) Cells separated from the plasma by a basal lamina, which covers the plasma membrane and encloses one to four cells, and which are called pericardial cells (Gaffron, 1885; Cuénot, 1949; Arvy, 1954; Lavallard and Campiglia, 1975). Schneider (1902), termed them "cellules lymphoides", although they are referred to as nephrocytes by a number of workers (Bruntz, 1903a,b; Zacher, 1933; Tuzet and Manier, 1959; Seifert and Rosenberg, 1976, 1977; Rosenberg, 1978).

These cells are often mistaken for haemocytes. Both the haemocytes and the pericardial cells are often found attached to the tissues.

A. Haemocytes

1. Onychophora

Information about the haemocytes of Onychophora is incomplete and is restricted to seven species. The number of cell types identified varies from two (Manton and Heatley, 1937), to four (Arvy, 1954; Grégoire, 1955; Tuzet and Manier, 1959) or five (Sundara-Rajulu *et al.*, 1970; Lavallard and Campiglia, 1975). These studies, however, do not provide any information about the physiological status of the animal under study.

In *Peripatus acacioi*, Lavallard and Campiglia (1975) have correlated the fine structure of the haemocytes with the morphology of cell types in the light microscope, and have also studied the histochemistry of the cytoplasmic inclusions. A summary of these results is presented in Table I. The blood cell nomenclature used by different authors and their equivalents in insects are indicated in Table II. Four cell types are commonly found in the Onychophora (Figs 1–10). These are prohaemocytes (Figs 5, 7), granular haemocytes (Figs 1, 2, 4, 7, 10), spherule cells (Figs 3, 7, 8, 9) and plasmatocytes (Figs 1, 6).

Histochemistry of the cytoplasmic inclusions of granular haemocytes and spherule cells showed that they differ in their chemical nature (Lavallard and Campiglia, 1975). The granules of granular haemocytes are acidophilic and contain a PAS positive neutral mucopolysaccharide. Lipid reactive sites are absent. The spherules of spherulocytes (spherule cells) contain basophilic protein and PAS positive lipoidal material. In addition to the aforementioned cell types, certain other cell types are also occasionally seen in the circulation, including macrophages (altered granular haemocytes according to Gupta, 1979), oenocytoids and cystocytes. Presence of oenocytoids in onychophorans, as has been claimed by Sundara-Rajulu *et al.* (1970), is doubtful, for the description and figure of oenocytoids fit precisely with those of pericardial cells (Lavallard and Campiglia, 1975). The cystocytes of onychophorans are comparable in their characteristics with those of chilopods, diplopods and insects (see Chapter 13).

2. Chilopoda

Previous information about the haemocytes of chilopods is meagre. The haemocytes of *Scolopendra* are classified into two (Duboscq, 1899; Kollmann, 1908) to seven (Ravindranath, 1970; Sundara-Rajulu, 1971a)

TABLE I. A summary of the general characteristics of the haemocyte types in *Peripatus acacioi* (Onychophora)[a].

Characteristics	Prohaemocytes	Macrophages	Granular haemocytes	Hyalocytes	Spherule cells
Size (μm)	8–12	10–12	10–12	10–16	8–30
Shape	round	polymorphic	polymorphic	round or oval	round or oval
Cell surface	smooth	irregular	irregular	smooth	smooth
Nature of cytoplasm	basophilic	basophilic	acidophilic	acidophilic	basophilic
Nucleus					
Position	central	central	central	eccentric	central
Shape	round/irregular	round/ovoid/ reniform/ pleurilobed	round/ovoid/ reniform/ pleurilobed	round/ovoid/ reniform	round/ovoid
Chromatin	aggregated in the periphery	aggregated in the periphery	aggregated in the periphery	distributed throughout	not observed
Perinuclear cisternae	not prominent	flattened	normal	prominent	normal
Nucleolus	not observed	well developed	not observed	not observed	not observed