ISJ 6: 114-124, 2009 ISSN 1824-307X

RESEARCH REPORT

Hemocytes in Myriapoda (Arthropoda): a review

WER Xylander

Senckenberg Museum für Naturkunde Görlitz, Görlitz, Germany

Accepted August 5, 2009

Abstract

Hemocyte types described in the different subtaxa of Myriapoda are presented and characterised. They comprise (1) prohemocytes, (2) plasmatocytes and (3) two different forms of granular hemocytes; these three have been found in all taxa investigated. Spherulocytes and coagulocytes were only found in some taxa. Adipohemocytes occur quite infrequent, oenocytoids have not been detected but may be restricted to specific stages of the moulting cycle. Cystocytes described earlier seem to be a preparation artefact. Granular hemocytes are the most frequent hemocytes followed by the plasmatocytes. In general, Diplopoda have fewer hemocytes than Chilopoda. The differences in the hematogram and the functions of hemocytes are discussed.

Key Words: Arthropoda; Myriapoda; Chilopoda; Diplopoda; hemocytes; immune reactions; plasmatocytes; granular hemocytes; phenoloxidase

Introduction

In Arthropoda, hemocytes have numerous functions: they are responsible for hemolymph clotting after cuticle and epidermis rupture, wound healing, self recognition, general and specific immune response and opsonisation (e.g., Gupta, 1986; Millar and Ratcliffe, 1989; Xylander, 1992, 1994). They phagocytise smaller pathogens like bacteria or fungal spores and encapsulate larger ones like metazoan parasites or xenografts.

Hemocytes, furthermore, produce and store substances which may be discharged after infections such as antibacterial substances and elements of the phenoloxidase system, as well as antibacterial substances, lectins and hemolysins. All these functions which were discovered in other groups of Arthropoda have also been found to occur in the Myriapoda (Xylander and Nevermann, 1990, 1993; Xylander, 1992, in press; Xylander and Bogusch, 1992, 1997; Nevermann, 1996). In some arthropods hemocytes are, furthermore, responsible for production and storage of the respiratory pigments.

The number of hemocyte types investigated differs according to the subtaxon of Arthropoda. Additionally, the techniques and procedures of preparation, e.g., obtaining of hemolymph, *in vitro*-preparation, fixation and staining techniques have impact on the reactions of hemocytes; as a consequence, the characteristics on which the nomenclature of hemocytes was based became highly diverse. As a consequence, the nomenclature of hemocytes in arthropods, especially in insects, became more and more confusing over the 100 years since the investigations started using more than 50 termini, often for the same cell types. With the aim to clarify at least in part this confusing situation, Jones (1962), and Ratcliffe and Price (1974), presented a new nomenclature applicable more or less to hemocytes of all arthropod taxa.

Also in Myriapoda the situation was quite complicated. Early light microscopic investigations by Gregoire (1955), Ravindranath (1973, 1977, 1981) and Rajulu (1971) resulted in the description of 7 different hemocyte types (prehemocytes, granular hemocytes, plasmatocytes, cystocytes, spherulocytes, adipohemocytes and oenocytoids). Transmission electron microscopy (TEM) investigations have described hemocyte types of chilopods and diplopods directly taken from the haemolymph, after *in vitro* preparation and partly during immune defense reactions (Nevermann *et al.,* 1991, 1996; Nevermann, 1996; Nevermann and Xylander, 1996, 2006; Hilken *et al*., 2003). By using light-microscopy, Xylander (1992), Xylander and Nevermann (1993), and Xylander and Bogusch (1997), gave further characteristics applicable to differentiate the haematocyte types of myriapods. The intention of this paper is to review the recent results on myriapod hemocytes and their nomenclature.

Corresponding author:
Willi Xylander
Senckenberg Museum für Naturkunde Görlitz
Postfach 300 154, 02806 Görlitz, Germany
E-mail: willi.xylander@senckenberg.de

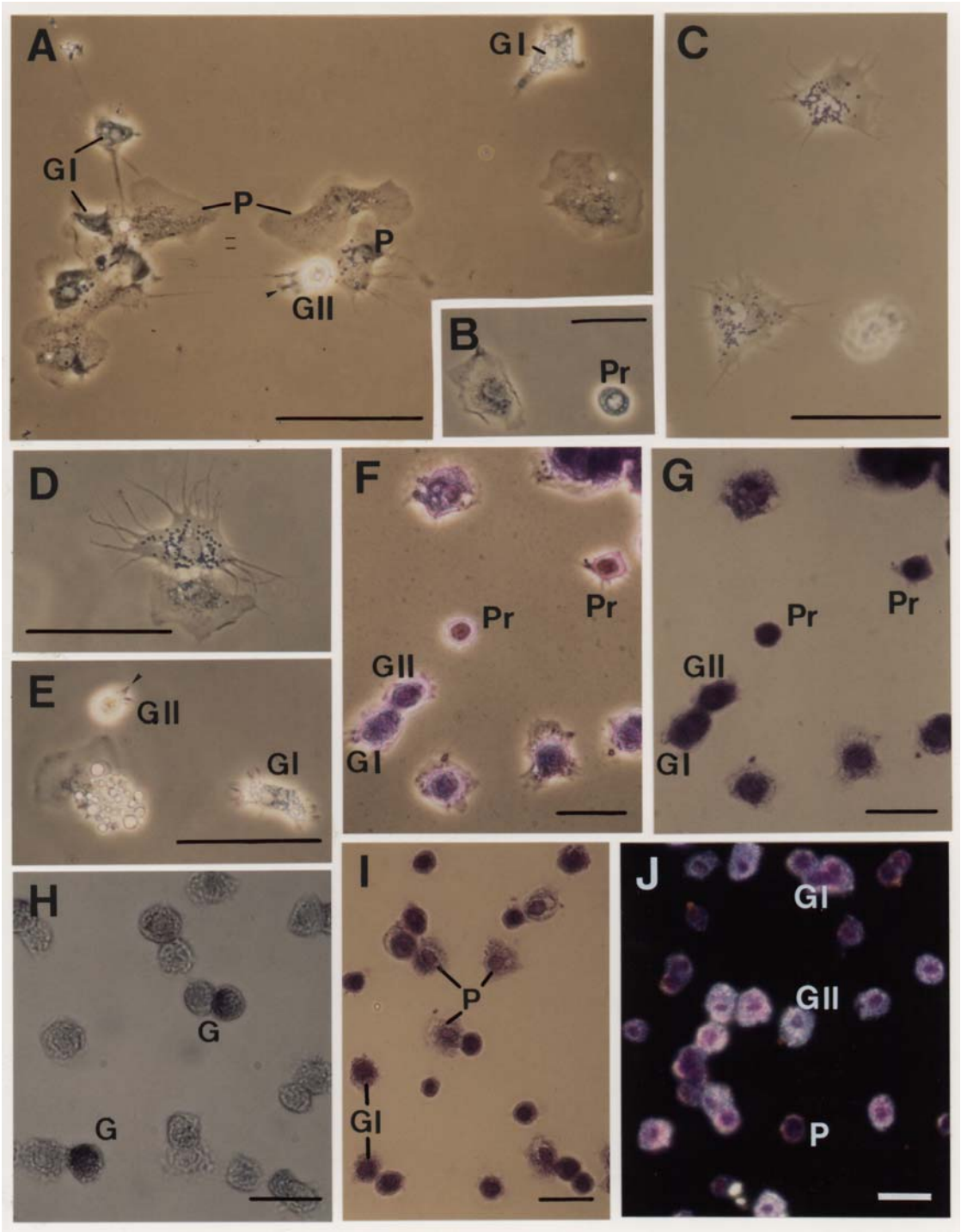

Fig. 1 (A-E) Hemocytes of *Chicobolus spp*., pure haemolymph, unfixed, spread on glass slides for different periods, observed by phase contrast microscopy and (F-J) *R. virgator* hemocytes, spread on glass slides for 10 min, fixed and stained. (A) Various hemocytes (P= plasmatocytes, GI = granular hemocytes of type I, GII = granular hemocytes of type II, the arrowhead points to cell proliferations). (B) Plasmatocyte and prehemocyte (Pr) (10 min). (C) Two plasmatocytes with few grana spreading ("star-type"). (D) Plasmatocytes representing various spreading-types (above: "star-type" with pseudopodia, below: "fried-egg-type" without pseudopodia, 20 min). (E) Hemocyte with large grana in close vicinity to two different granular hemocytes (GI and GII, the arrowhead indicates cell proliferations of GII ,10 min). (F) May-Grünwald and Giemsa staining observed by phase contrast. (G) May-Grünwald and Giemsa staining observed in bright field. The prehemocytes are burgundy-coloured , the plasma of plasmatocytes is greyish and that of granular hemocytes (GI and GII) violet. (H) Sudan-black-staining. Several granular hemocytes are stained dark (bright field). (I) Giemsa-staining. The more translucent plasma clearly differentiate plasmatocytes from granular hemocytes. (J) May-Grünwald and Giemsa staining. The plasma of granular hemocytes of type II (GII) is bluish, their nuclei are blue. Granular hemocytes of type I (GI) are faint purple , plasmocytes (P) are deep purple. (dark field). Bars: 50 µm (A, C-E); 25 µm (B, F-J).

Material and Methods

Animals

Animals of different species were investigated. Description of collecting sites, rearing conditions and other species specific informations have already been given in detail (Xylander and Nevermann, 1990; Xylander, 1992; Nevermann, 1996; Nevermann and Xylander, 1996). *In vitro* and staining techniques as well as hemocyte counts were described by Xylander and Nevermann (2006).

Electron microscopy preparations

Hemocytes were obtained by opening the cuticle of living animals. They spread and thus became attached to different substrates (e.g., cellophane) kept in cold Ringer-solutions prior to fixation. Such samples were fixed in the cold for at least 2 h in a modified Karnoffsky´s fixative or in 2.5 % glutaraldehyde and postfixed in 2 % (w/v) OsO_4 and dehydrated in acetone [see Xylander and Nevermann 2006 for further details of the procedures]. After OsO_4-fixation samples were subdivided and (a) embedded for transmission electron microscopy samples in araldite or (b) critically point dried and sputtered with gold for scanning electron microscopy. TEM-investigations were mainly done on a Zeiss EM 9A, SEM investigations on a JEOL or Cambridge Stereoscan S4 SE-microscope.

Results

Hemocyte types

Six hemocyte types were found by light and electron microscopy, cytochemical and in vitro techniques in the two diplopod and three chilopod taxa. Three of these occur in all taxa investigated: Prehemocytes, plasmatocytes and granular hemocytes. In a limited number of Chilopoda investigated, spherulocytes, coagulocytes and discoid cells were described (Xylander and Nevermann, 2006).

Prehemocytes

Three to 10 % of the hemocytes in the diplopods and chilopods investigated are prehemocytes. They are the smallest cells of all hemocytes and spherical in shape (Figs 1B, F, G; 2C, G). Their nucleus is large compared to the volume of cell plasma and located centrally (Fig. 2G). In vitro, these cells spread later than other cell types keeping their shape for several minutes. Only a few small grana may be found in the plasma of these prehemocytes and their phenoloxidase activity is weak (Xylander and Bogusch 1997).

Plasmatocytes

Plasmatocytes are significantly larger than the prehemocytes (Fig. 1B) with a mean diameter of 30 to 70 µm. This cell type is characterized by intense spreading (Figs 1, 2). Furthermore, they contain no or just a few grana (Figs 3A, C, D). Their plasma is greyish in phase contrast (Figs 1A-D). After activation of hemocyte cultures for phenoloxidase staining and incubation with DOPA (Xylander and Bogusch 1997) there is no or just little reaction in the plasmatocytes. The nucleus of spread plasmatocytes is located eccentrically (Figs 1B, D) and it occurs larger than in any other hemocyte type. Directly after obtaining the haemolymph plasmatocytes are spherical to spindle-shaped but flatten and attach to glass slides within 8 to 10 min (in an undiluted haemolymph sample) forming pseudopodia (Figs 2A, B, D; 3B, E).

In the Diplopoda, two types of spreading were observed in this cell type resulting in different hemocyte morphology (Xylander and Nevermann 2006):

a) the “fried-egg-type”. In this type of spreading cells flatten more or less homogeneously not forming many pseudopodia; plasmatocytes showing such spreading behaviour contain no or just a few grana (Fig. 1D)

b) The “star-type”. Here many thin pseudopodia grow centrifugally during spreading; in plasmatocytes of this type normally several small grana occur which are bluish in phase contrast (Fig. 1D).

TEM observations show that in the plasmatocytes of Chilopoda electron translucent vacuoles with moderately electron dense filamentous material occur (Nevermann *et al.*, 1991; Hilken *et al.*, 2003). In plasmatocytes of the spirobolid diplopod *Rhapidostreptus virgator* comparable vacuoles were found containing (Figs 3A, C, D) which, however contain flocculent rather than filamentous material; this material is discharged after some time during vitro-incubation. The material seems to attract other cell types thereby becoming involved in more complex aggregation (the immune reaction); such attraction of other cellular components of the immune system (against non-self material) is called opsonisation effect.

Granular hemocytes

Granular hemocytes are the most numerous hemocytes in Myriapoda (Xylander and Nevermann 2006). They contain numerous electron dense grana of variable size and shape and some vacuoles (Figs 1F-J; 2C, E; 4; 5). Granular hemocytes are medium sized after spreading (larger than prohemocytes but smaller than plasmatocytes) (Figs 1F-J). All cells of this type are phenoloxidase active (Xylander 1996, Xylander and Bogusch 1997, Xylander and Nevermann 1993, 2006). Briefly, after obtaining the haemolymph, granular hemocytes are more or less spherical (Figs 1I, J). Then they start to flatten *in vitro* and attach to the substrates within 15 min (Figs 1F, G). They can, however, be distinguished from plasmatocytes for more than 1 h in light microscopy by their significantly lower spreading capabilities. During spreading, granular hemocytes often develop a unidirectional process (Xylander and Nevermann, 2006). Intermediate forms of granular hemocytes and plasmatocytes have been found in a few cases (Fig. 3D) indicating that granular hemocytes (at least of type I, see below) may constitute a transformation product of plasmatocytes.

In the two species of diplopods investigated (*Chicobolus spp*. and *R. virgator)* two types of granular hemocytes could be differentiated. Granular

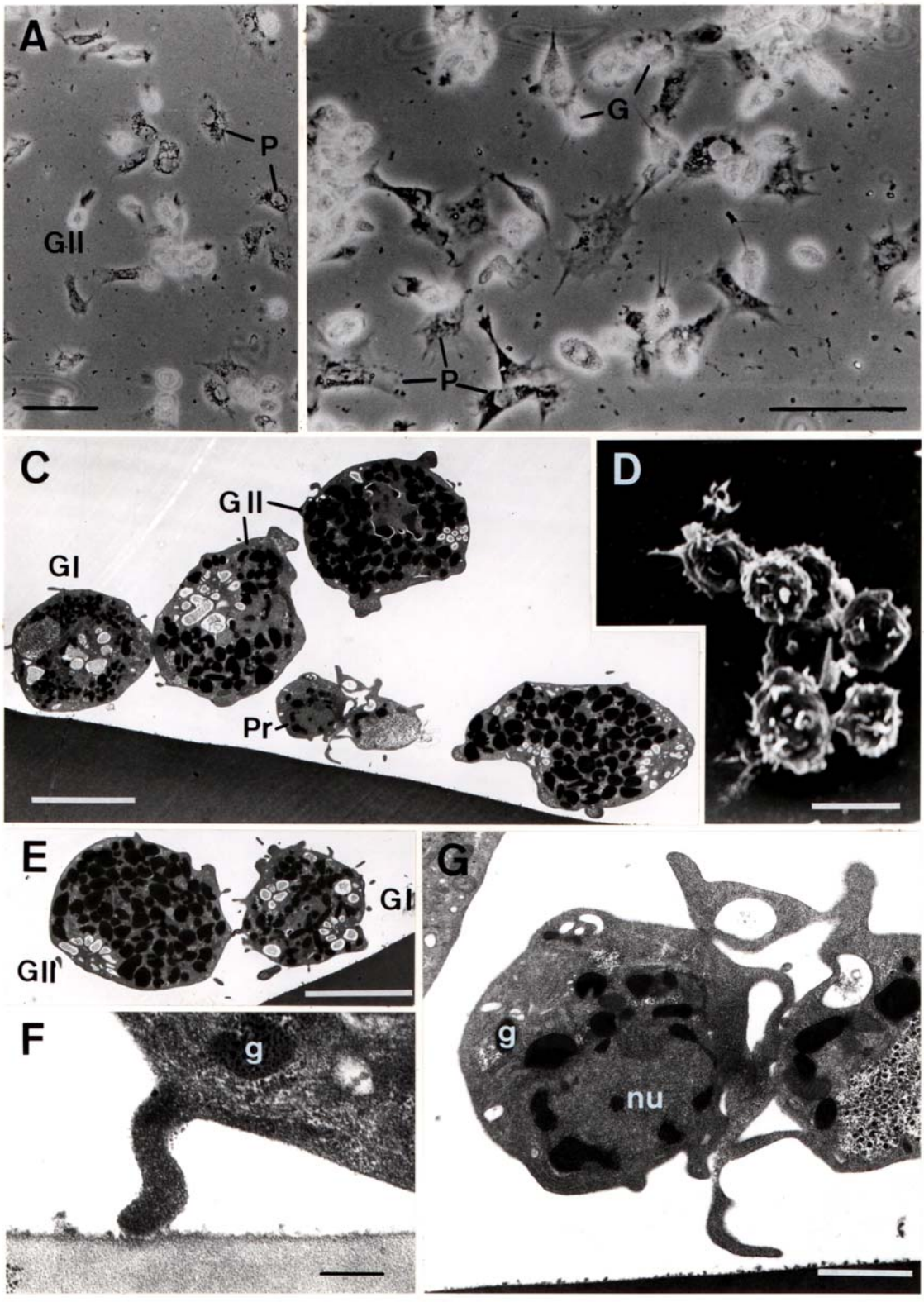

Fig. 2 Hemocytes of *R. virgator*. (A) Hemolymph preparation with various hemocytes (pure haemolymph, 20 min after puncture, phase contrast. P= plasmatocyte, GI and GII= granular hemocyte type I and type II.). (B) Various hemocytes in a hemolymph smears (pure hemolymph, 30 min, phase contrast). (C) Overview of various hemocytes observed by TEM. (D) Hemocyte aggregation *in vitro* observed by scanning electron microscopy (SEM). The hemocytes with short extensions on the cell surface are plasmatocytes. (E) Granular hemocytes of type I and type II observed by TEM. The grana of type II are larger, more spherical and form fewer pseudopodia than type I. (F) TEM observation of a granular hemocyte forming pseudopodia with contact to the substrate (g= granulum). (G) TEM photograph of a prehemocyte. Few small grana (g), high numbers of free ribosomes, RER and the nucleus (nu) are visible. Bars: 50 µm (A, B); 5 µm (C, E); 10 µm (D); 0.2 µm (F); 1 µm (G).

hemocytes of type I show significantly higher spreading capabilities (Fig. 1A). They contain fewer and smaller grana (Figs 2C, I; 4A) which occur blue or green in phase contrast microscopy. In transmission electron microscopy their grana show to be irregular in shape (Figs 2C; 4 A-C). After spreading the grana of type I granular hemocytes are predominantly located at the periphery of the nucleus; so mostly the nucleus is clearly visible. Phenoloxidase activity is moderate in granular hemocytes of type I (and significantly lower than in type II) (Xylander and Bogusch, 1997;

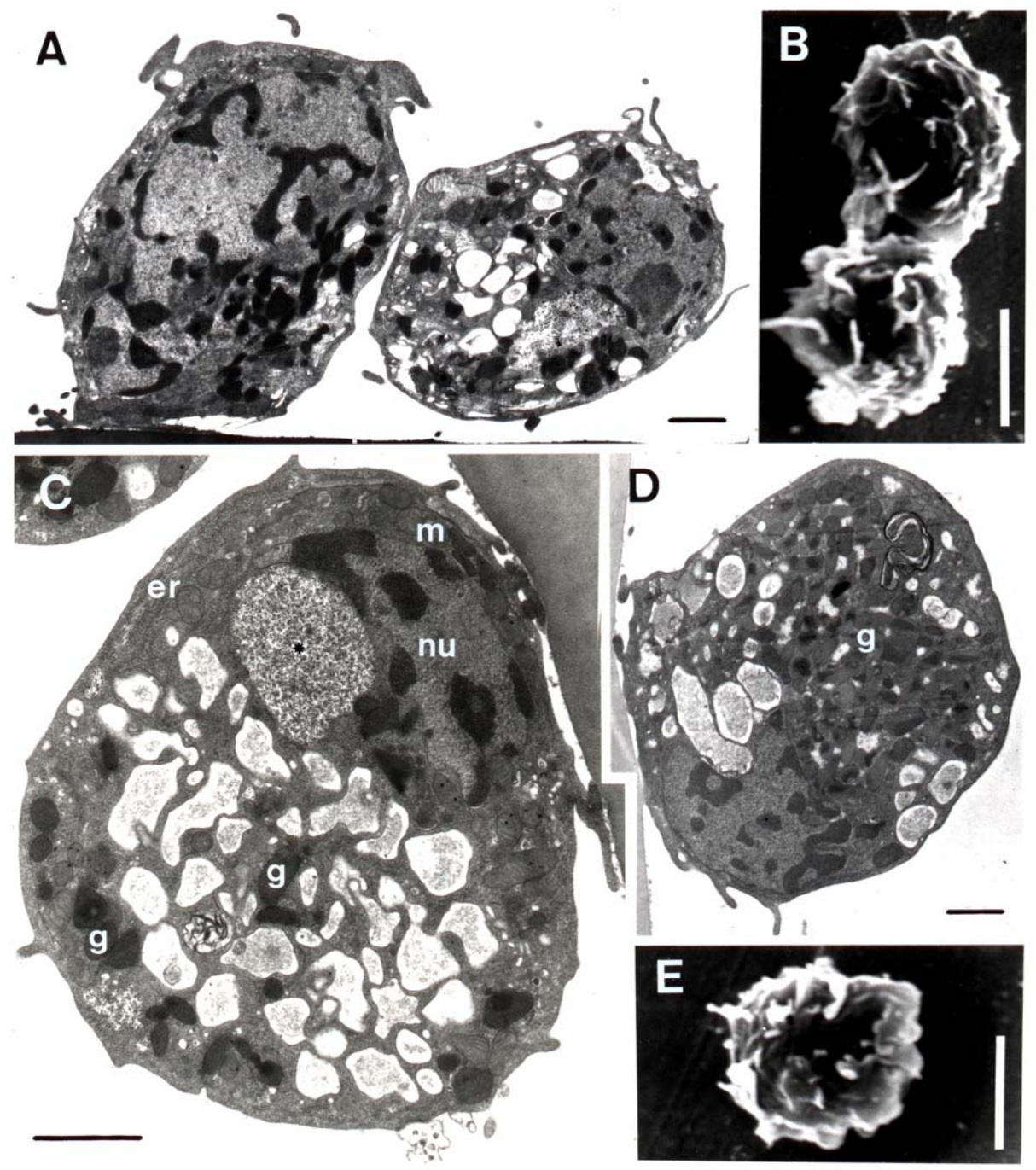

Fig. 3 Plasmatocytes of *R. virgator* fixed after *in vitro* incubation on gelatine with I-Ringer. (A) TEM observation of a prehemocyte (with grana, left) and plasmatocyte (right). (B) Two plasmatocytes observed by SEM. (C) Plasmatocyte. Peripheral in the cytoplasm only few grana are visible. In the centre of the cell vesicles filled with flocculent material have gathered. (er= rough endoplasmic reticulum, m= mitochondrion, nu= nucleus, *: glycogen like inclusions). (D) Presumed transitory stage between plasmatocyte and granular hemocyte type I. The number of grana increase, but they are less frequent and electron dense as in granular hemocytes. (E) Plasmatocyte observed by SEM. Bars: 1 μm (A, C, D); 5 μm (B, E).

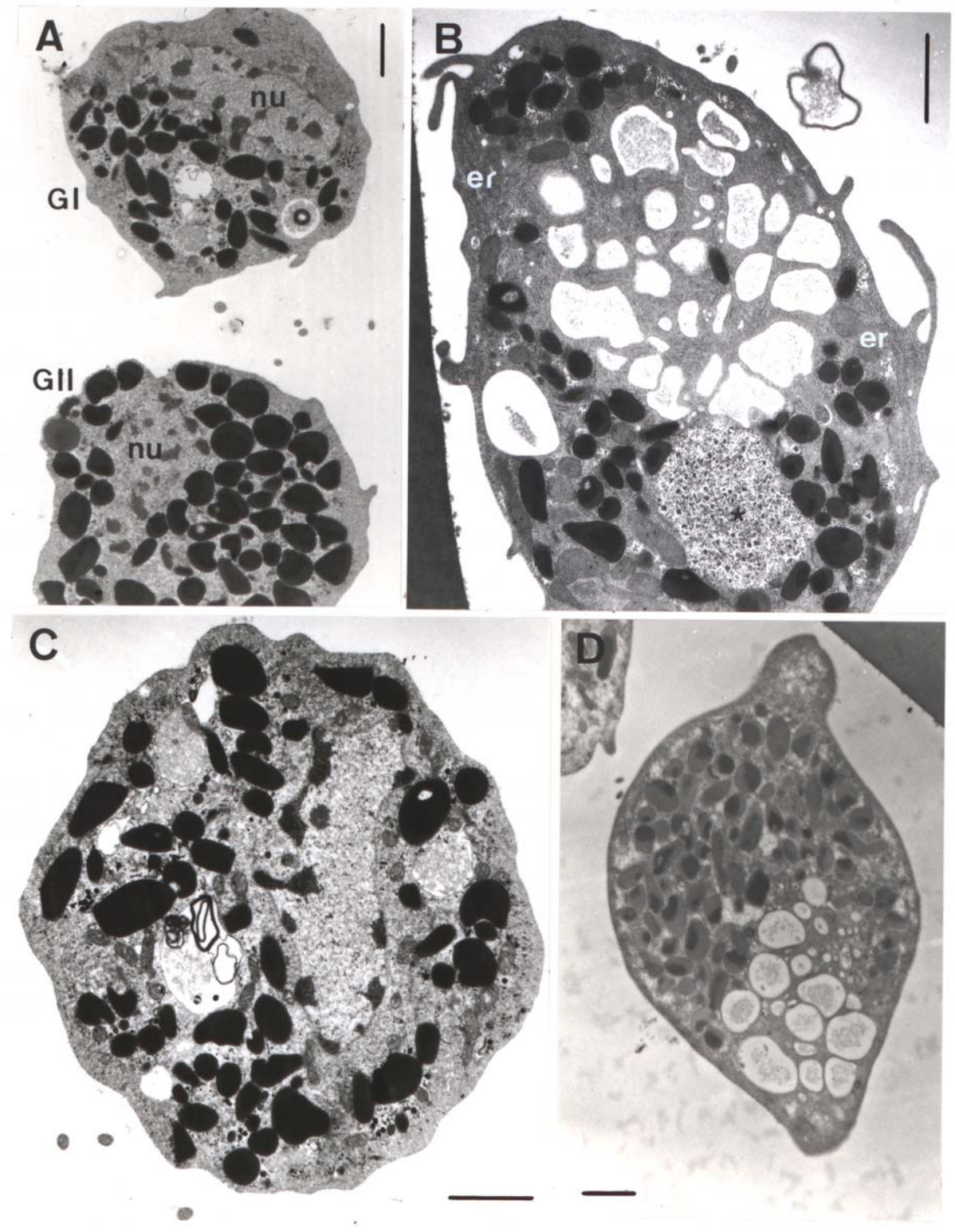

Fig. 4 Granular type I hemocytes of *R. virgator* under different types of fixation. Hemocytes in A and C were fixed after *in vitro* incubation, 60 min in Wenning-Ringer on cellophane. Hemocytes in B and D were fixed after *in vitro* incubation 60 min in I-Ringer on gelatine. (A) For comparison: Granular hemocytes of type I and type II (nu= nucleus). (B) Granular hemocyte of type I. At the periphery of the cell there are RER-cisterns and in the centre there are vacuoles. One of the vacuoles is filled with material resembling glycogen-rosettes (*). (C) In GR I there are fewer vacuoles after incubation in Wenning-Ringer on cellophane. (D) In unstained TEM-sections (no double staining with uranyl acetate and lead citrate) the grana occur to be subdivided into a more electron dense and a more translucent part. Bars: 1 µm.

Xylander and Nevermann 2006). In unstained TEM-sections the grana of this type occur to be "subdivided" into a more electron dense and a more translucent part; the more electron dense part is smaller and enclosed eccentrically by the more translucent one (Fig. 4D) (see also Xylander and Nevermann, 2006).

Granular hemocytes of type II occur even smaller after spreading than plasmatocytes and granular hemocytes of type I and they spread less intensively (Figs 1A, F, G). Their volume, however, is about the same of the other two types. In many cases granular hemocytes of type II also form a unidirectional process. In phase contrast they appear yellowish mostly due to the higher number of grana (Fig. 1A). These grana are larger and spherical (Figs 2C, E; 4A; 5A, C-E). When located peripherally in the granular hemocytes these grana may bulge their surface (Figs 5A, B, F). Granular hemocytes of type II react stronger than the other hemocytes after prophenoloxidase activation and substrate incubation and stain dark brown (Xylander and Bogusch, 1997, Xylander and Nevermann, 2006).

Spherulocytes

A fourth hemocyte type has been described for the chilopods *Lithobius forficatus* and *Scutigera coleoptrata* by Nevermann *et al.* (1991) and Hilken *et al.* (2003): the spherulocyte. It is characterised by regularly shaped spherical grana, significantly lower spreading capability and – as a major characteristic for differentiation from other hemocyte types - the complete lack of phenoloxidase inactivity. These cells tend to form a single cytoplasmic protrusion which never bears grana. Although spherulocytes may form such protrusions, contain grana, spread just moderately and therefore resemble the granular hemocytes intermediate forms between spherulocytes and granular hemocytes or plasmatocytes have never been detected. Up to now, spherulocytes were only found in the two species of Chilopoda. In Diplopoda, as well as in *Scolopendra cingulata*, this cell type does not occur (Xylander 1992; Nevermann, 1996; Xylander and Nevermann, 2006).

In spite of the similarities, Xylander and Nevermann (2006) considered the spherulocytes to be a distinct hemocyte type possibly restricted to a subtaxon of Chilopoda.

Coagulocytes

In haemolymph preparations from *Lithobius forficatus*, Nevermann *et al.* (1991) described zones with typical plasma coagulations and postulated the occurrence of a fifth extremely fragile hemocyte type, which immediately disintegrates *in vitro*, the coagulocyte. Nevermann (1996) described disintegrating hemocytes when dropping haemolymph directly into a fixative. These hemocytes resembled plasmatocytes containing only few electron dense grana and vacuoles but were characterised by large vacuoles containing flocculent material (indicating the disintegration process). Nevermann (1996), therefore, considered the coagulocytes found just to represent "stressed plasmatocytes". More recently, Xylander and Nevermann (2006), however, considered the coagulocytes to be one of the "valid" hemocyte types of Myriapoda. which, due to their immediate disintegration during the procedures of obtaining the haemolymph, is just very rarely found in hemocyte preparation.

Naked nuclei and isolated membranes (most possibly remnants of hemocytes after disintegration) were also found in hemocyte aggregations ("capsules") surrounding xenografts in the diplopod *Rhapidospreptus virgator* (Xylander, unpublished). They could also be remnants of coagulocytes indicating that this hemocyte type is more widely distributed throughout the Myriapoda than considered earlier and just hard to detect with the methods usually applied.

Discoid hemocyte

Nevermann (1996) described a sixth hemocyte type by TEM studies characterised by peripheral circular bundles of microtubules for *S. cingulata*. These microtubules disintegrate when the hemocytes attach to the substrate and start to spread. Then they become invisible. Bundles of microtubules equivalently arranged were also described from granular hemocytes of *Scutigera coleoptrata* (Hilken *et al.*, 2003). So peripheral bundles of microtubules arranged in circles may represent a general characteristic of native plasmatocytes and granular hemocytes *in vivo*.

Cystocytes

Ravindranath (1981) in an early review of hemocytes in Myriapoda described cystocytes for Diplopoda. Subsequently Nevermann *et al.* (1991) showed, however, that this description was due to a preparation artefact: Such hemocytes were exclusively found in cell preparations after mechanical stress (e.g., after sucking hemocytes into the narrow slit between a microscopic slide and cover slip). Therefore, cystocytes are considered not to be a "valid hemocyte type" for the Myriapoda.

Adipohemocytes

In very few cases cells containing large lipid grana were found circulating in the hemolymph (Fig. 1E). Such numerous lipid grana (stainable with Sudan black) are typical for so called "adipohemocytes". But such observations were not reproducible with other specimens of the same species. Therefore, it seems probable that such cells originated from the subepidermal fat body and are set free when obtaining the haemolymph by puncture. Therefore, they represent rather an artifact originating from preparation and than a genuine hemocyte type in Myriapoda.

Oenocytoids

During all investigations performed in our working group with various diplopod and chilopod species, oenocytoids were never observed. However, oenocytoids are considered to be involved in the moulting process and their occurrence circulating free in the haemolymph may be restricted to a short period prior to moulting (which is a rare event in mature specimens of the species of Diplopoda and Chilopoda investigated during our

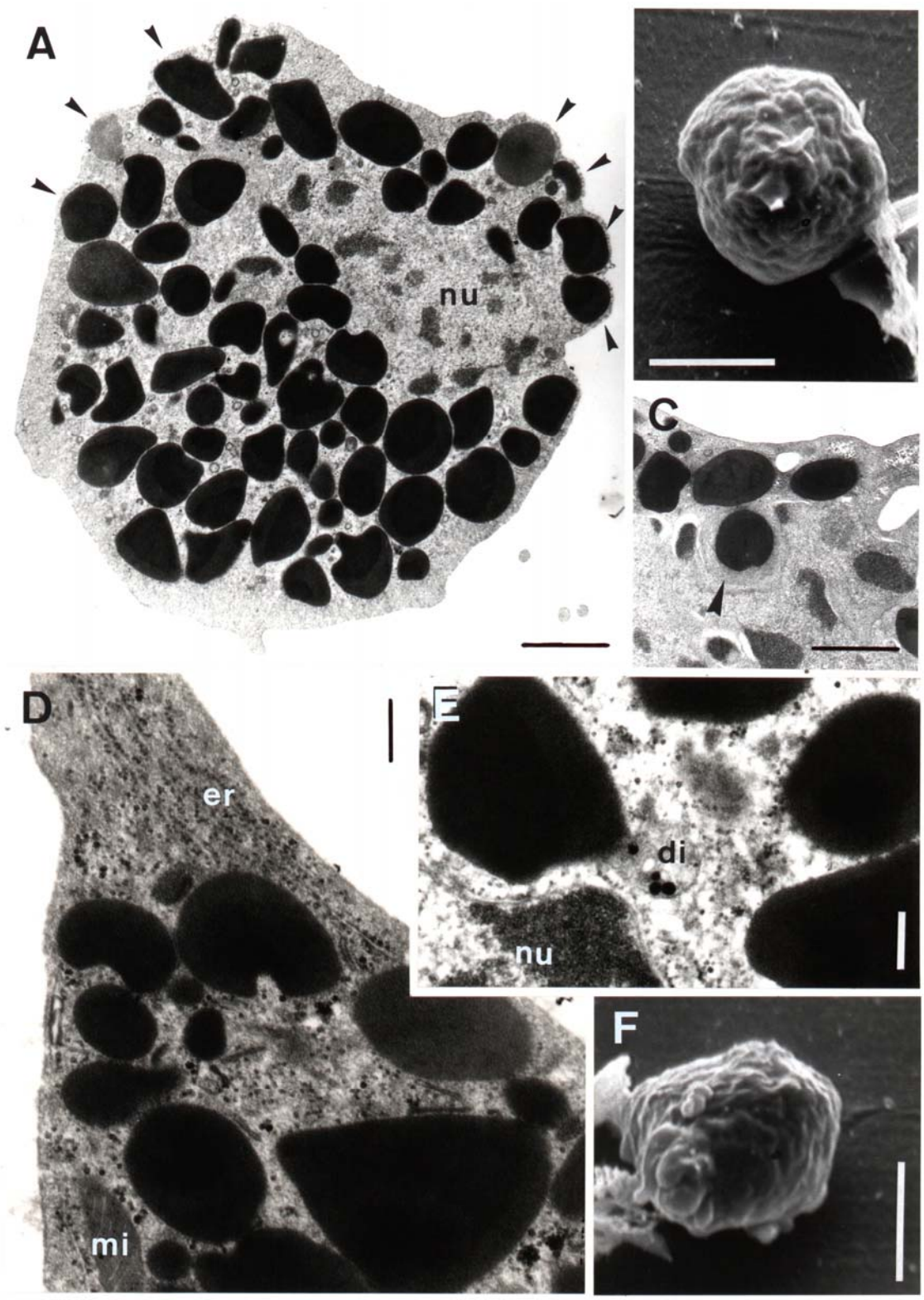

Fig. 5 Granular type II hemocytes of *R. virgator*. Hemocytes in A, D and E after i*n vitro* incubation in Wenning-saline for 60 min on cellophane; B, C and F, after *in vitro* incubation in I-ringer on gelatine for 60 min. (A) Granular hemocyte of type II. TEM image displaying that grana are mostly spherical and bulge the plasma membrane at different sites (arrowheads); nu = nucleus. (B) Grana responsible for extension of the plasma membrane are visible also by SEM. (C) A few grana are surrounded by RER (arrowheads). (D) Cisternae of RER could frequently been found at the cell periphery (er). Free ribosomes are also widely distributed in the cytoplasm. Note that mitochondria (mi) with electron dense matrix and translucent cristae are visible. (E) Golgi complexes (di) with electron dense grana were occasionally observed in vicinity to the nucleus (nu). (F) SEM micrograph of GII. Bars: 1 µm (A, C); 5 µm (B, F); 0.2 µm (D, E).

studies). In fact, during our investigations specimens in the moulting process (which are extraordinary sensitive to cuticle rupture and may die briefly after injury) were excluded. This may be the reason for the absence of oenocytoids in our samples. Therefore, definitive conclusions regarding the existence of oenocytoids in chilopods and diplopods are not possible.

Differential hemocyte counts

For two diplopod species, *R. virgator* and *Chicobolus spp.*, the percentage of different hemocyte types were determined (Xylander and Nevermann, 2006). The granular hemocytes of type I was most frequent comprising 38 and 39 % of all hemocytes, respectively. Granular hemocytes of type II represented 30 % of all hemocytes in *R. virgator* and 17 % in *Chicobolus*. In *R. virgator* 27 % of the hemocytes were plasmatocytes and 39 % in *Chicobolus*. In both species prehemocytes represented about 5 %.

Total hemocyte counts

The total number of hemocytes per hemolymph volume varies extremely between Chilopoda and Diplopoda: In the species investigated centipedes had a tenfold higher numbers of hemocyte than millipedes. In *Lithobius forficatus* Xylander and Nevermann (2006) found 45,000 hemocytes μl^{-1} hemolymph, in *Scolopendra cingulata* 31,500 and in *S. oraniensis* about 50.000. In contrast, there were only 2,450 and 6,500 hemocytes μl^{-1} hemolymph, respectively, in the diplopods *R. virgator* and *Chicobolus spp*.

Discussion

Hemocyte types – a comparison within and outside the Myriapoda

In the arthropods at least 9 different hemocytes types can be differentiated (Table 1). Within the three major taxa of myriapods (Chilopoda, Diplopoda and Symphyla) investigated with regard to their hemocytes until now prehemocytes, plasmatocytes and granular hemocytes occur in all three. Other types described so far seem to be restricted to specific subtaxa or have to be considered as preparation artefacts.

With regard of their microscopic and submicroscopic morphology the hemocytes of myriapod correspond to that of other arthropods (see for review Jones, 1962; Ravindranath, 1974; Bauchau, 1981; Sherman, 1981; Xylander, 1992). This similarity of characters even on the electron microscopic level allows to use the generalised nomenclature for arthropod hemocytes not only for pragmatical reasons but also due to comparative morphology and function. However, the hemocyte types of decapod crustaceans which are separated into those bearing many granules ("granular cells") and those bearing few ("semi-granular cells") (Bauchau, 1981; Xylander *et al.*, 2003) "resist" the inclusion into this system up to now. So further investigations are necessary to clarify the homology of the different hemocyte types.

Xylander and Nevermann (2006) considered at least 4 hemocyte types to belong to the ground pattern of arthropods: the prohemocytes, the plasmatocytes, the granular hemocytes and the cyanocytes, the latter responsible for production and storage of the respiratory pigments. After the evolution of terrestrial arthropods with trachea, respiratory pigments and the hemocytes producing them were reduced. Subsequently, immune defense, wound closure and hematopoiesis represented the major tasks of the remaining hemocytes.

Total hemocyte counts

The range of hemocytes number per volume is extremely variable reaching from 500 μl^{-1} in decapod crustaceans to 60,000 μl^{-1} in cockroaches (overview in Xylander and Nevermann, 2006). Diplopoda are among those taxa with a low number

Table 1 A comparison of the presence of different hemocyte types (listed in the Results section) within the main taxa of Arthropoda. Myriapoda species are singularly reported. Modified from Xylander and Nevermann (2006).

TAXON	PH	PL	GR	SPH	COA	DISC	ADI	CYST	OEN	CYAN
Rhapidostreptus virgator	X	X	X		?		?			
Chicobolus spp.	X	X	X				?			
Lithobius forficatus	X	X	X	X	(X)		?			
Scolopendra cingulata	X	X	X			X	?			
Scutigera coleoptrata	X	X	X	X						
INSECTA	X	X	X	X			X	X	X	
CRUSTACEA	X	X	X				X			X
XIPHOSURA	?	X	X							X
SCORPIONES	X	X	X	X			X	X		?
ARANEA	X	X	X					?	X	X

Table 2 Total hemocyte counts (hemocytes μl^{-1}) [H (hc/µl)] for various arthropods. Modified from Xylander and Nevermann (2006).

Myriapoda	**H (hc/µl)**	**References**
Rhapidostreptus virgator	2,500	Xylander and Nevermann, 2006
Chicobolus spp.	6,500	Xylander and Nevermann, 2006
Scolopendra cingulata	31,000	Nevermann, 1996
Scolopendra oraniensis	~50,000	Xylander and Nevermann, 2006
Lithobius forficatus	45,000	Nevermann, 1996
Crustacea		
Astacus leptodactylus	~500	Ullrich, 1993; Xilander *et al*., 1997
Procambarus clarkii	~580	Ullrich, 1993; Xilander *et al*., 1997
Crangon crangon	800-1,200	Smith and Johnston, 1992
Insecta		
Blatella germanica	23,000	Gupta, 1986
Periplaneta americana	60,000	Crossley, 1975
Chironomus thummi	1,000-3,000	Götz and Vey, 1974
Drosophila melanogaster (2d)	2,000	Rizki and Rizki, 1992
Drosophila melanogaster (4d)	23,000	Rizki and Rizki, 1992
Manduca sexta (L5)	4,500	Horohov and Dunn, 1982
Galleria melonella (L5)	25,000	Chain and Anderson, 1982
Arachnida		
Limulus polyphemus	30,000	Sherman, 1981
Arenea spp.	11,000	Sherman, 1981

of hemocytes, whereas Chilopoda range among those with quite high numbers. When looking at hemocyte numbers more generally, however, it should be taken into account that in those few species in which the topic was investigated, hemocyte numbers often differed significantly during ontogenetical stages.

The variability of the hemocytes counts cannot be explained on the basis of the available data. But, as a tendency, those species with hard and strongly calcified cuticles or passive protection strategies against predators (such as glandular defense secretions) have fewer hemocytes (e.g., decapod crustaceans and diplopods). In these species the risk of injuries and infections and, therefore, the demand for wound closure and immune defense is most probably lower. In predatory groups with a thin cuticle (reducing body weight and enabling effective scavenging with lower energy demand than in those groups with calcified cuticles) or taxa with higher risk of injury due to their life style (e.g., chilopods, cockroaches, dipteran larvae) hemocyte numbers are often high. In this context, Fründ (1992) showed that 28 to 60 % of the specimens of *Lithobius forficatus* in his study had melanised scares or lost legs. Thus the high total hemocyte count probably constitutes an adaptation to their predatory life style (Xylander and Nevermann, 2006).

Acknowledgements

The author would like to thank Dr L Nevermann, Dr H-U Jahn and O Bogusch for their support during the investigations on the immune system of Myriapoda conducted at the Justus-Liebig University (Germany). Acknowledgments are also extended to Prof Dr G Hilken and Prof Dr G Seifert who contributed their part to this publication.

References

Bauchau AG. Crustaceans. In: Ratcliffe NA, Rowley AF (eds), Invertebrate blood cells. Vol. 2. Academic Press, London, pp 385-420, 1981.

Chain BM, Anderson RS. Selective depletion of plasmatocytes in *Galleria mellonella* following injection of bacteria. J. Insect Physiol. 28: 377-384, 1982.

Crossley CA. The Cytophysiology of insects. Adv. Insect Physiol. 11: 117-222, 1975.

Fründ H-C. The occurrence and frequency of scars in centipedes. Ber. nat.-med. Verein Innsbruck, Suppl. 10: 269-275, 1992.

Götz P, Vey A. Humoral encapsulation in Diptera (Insecta): defence reactions of *Chironomus* larvae against fungi. Parasitology 68: 193-205, 1974.

Gregoire C. Blood coagulation in arthropods. VI. A study by phase contrast microscopy of blood reactions in vitro in Onychophora and various groups of arthropods. Arch. Biol. (Liege) 66: 489-508, 1955.

Gupta AP. Hemocytes of *Scutigerella immaculata* and the ancestry of Insecta. Ann. Entomol. Soc. Am. 61: 1028-1029, 1968.

Gupta AP. Arthropod immunocytes. Identification, structure, functions, and anologies to the functions of vertebrate B- and T-lymphocytes. In: Gupta AP (ed), Hemocytic and humoral immunity in arthropods. Wiley and Son, New York, Chichester, Brisbane, Toronto, Singapore, pp 3-59, 1986.

Hilken G, Brockmann C, Nevermann L. Hemocytes of the centipede *Scutigera coleoptrata* (Chilopoda, Notostigmophora) with notes on their interactions with the tracheae. J. Morphol. 257: 181-189, 2003.

Horohov DW, Dunn PE. Phagocytosis and nodule formation by hemocytes of *Manduca sexta* larvae following injection of *Pseudomonas aeruginosa*. J. Invert. Pathol. 41, 203-213, 1982.

Jones JC. Current concepts concerning insect hemocytes. Amer. Zool. 2: 209-246, 1962.

Millar DA, Ratcliffe NA. The evolution of blood cells: Facts and enigmas. Endeavour 13: 72-77, 1989.

Nevermann L. Untersuchungen an Haemozyten von *Scolopendra cingulata* und *Lithobius forficatus* unter dem Aspekt zellulärer Abwehrreaktionen. Doctoral thesis, University of Giessen. www.nevermanns.de/hemocytes/, 1996

Nevermann L, Kaiser HE, Xylander WER. Microbial induced haemocytic immune reactions in chilopods. In Vivo 10: 161-168, 1996.

Nevermann L, Xylander WER. *In vitro* cellular immune reactions of hemocytes against bacteria and their differential degradation in myriapods. Geoffroy, JJ, Mauries L, Nguyen Duy-Jacquemin M (eds), Acta Myriapodologica. Mem. Mus. Natn. Hist. Natur. 169: 421-430, 1996.

Nevermann L, Xylander WER, Seifert G. The hemocytes of the centipede *Lithobius forficatus* (Chilopoda, Lithobiomorpha): Light and electron microscopic studies using *in-vitro* techniques. Zoomorphology 110: 317-327, 1991.

Rajulu GS. A study of haemocytes in a centipede *Scolopendra morsitans* (Chilopoda: Myriapoda). Cytologia 36: 515-521, 1971.

Ratcliffe NA, Price CD. A reappraisal of insect haemocyte classification by the examination of blood from fifteen insect orders. Z. Zellforsch. 147: 537-549, 1974.

Ravindranath MH. The hemocytes of a millipede, *Thyropygus poseidon*. J. Morphol. 141: 257-268, 1973.

Ravindranath MH. The hemocytes of a scorpion *Palamnaeus swammerdami*. J. Morpohol. 144: 1-10, 1974.

Ravindranath MH. A comparative study of the morphology and behaviour of granular haemocytes of arthropods. Cytologia 42:743-751, 1977.

Ravindranath MH. Onychophorans and myriapods. In: Ratcliffe NA, Rowley AF (eds), Invertebrate blood cells, Vol. 2, Academic Press, London, pp 327-354, 1981.

Rizki TM, Rizki RM. Lamellocyte differentiation in *Drosophila* larvae parasitized by *Leptopilina*. Dev. Comp. Immunol. 16: 103-110, 1992.

Sherman RG. Chelicerates. NA, Rowley AF. Invertebrate blood cells. Invertebrate blood cells, Vol. 2, Academic Press, London, pp 335-384, 1981.

Smith VJ, Johnston PA. Differential haemotoxic effect of PCB congeners in the common shrimp, *Crangon crangon*. Comp. Biochem. Physiol. 101C: 641-949, 1992.

Ullrich G. Antibakterielle Substanzen bei *Astacus leptodactylus* (Crustacea, Decapoda). 60 pp. Thesis, University of Giessen, 1993.

Xylander WER. Immune defense reactions of Myriapoda - A brief presentation of recent results. In: Thaler K, Meyer E, Schedl W (eds), Advances in Myriapodology (Proceedings of the 8th International Congress of Myriapodology). Ber. Nat.-Med. Verein Innsbruck, Suppl. 10: 101-110, 1992.

Xylander WER. Immunabwehr bei Gliederfüßern - Wie sich Spinnentiere, Krebse, Insekten und Tausendfüßer gegen Krankheitserreger schützen. Spiegel der Forschung 11: 27-30, 1994.

Xylander WER. Antibacterial substances and characteristics from the haemolymph of Chilopoda and Diplopoda (Myriapoda, Arthropoda). Soil Organisms [in press].

Xylander WER, Bogusch O. Investigations on the phenoloxidase of *Rhapidostreptus virgator* (Arthropoda, Diplopoda). Zool. Jb. Physiol. 96: 309-321, 1992.

Xylander WER, Bogusch O. Granular hemocytes as the main location of prophenoloxidase in the millipede *Rhapidostreptus virgator* (Diplopoda, Spirostreptida, Spirostreptidae). Ent. scand. Suppl. 51: 183-189, 1997.

Xylander WER, Nevermann L. Antibacterial activity in the hemolymph of Myriapoda (Arthropoda). J. Inv. Pathol. 56: 206-214, 1990.

Xylander WER, Nevermann L. Phenoloxidase-active hemocytes in *Lithobius forficatus*, *Scolopendra cingulata* (Chilopoda) and *Chicobolus* spec. (Diplopoda). Verh. Dtsch. Zool. Ges. 86.1: 197, 1993.

Xylander WER, Nevermann L. Haemocytes in Diplopoda and Chilopoda (Arthropoda, Myriapoda) -Types, structures, and numbers. Scand. J. Entomol. 53: 195-210, 2006.

Xylander WER, Ullrich G, Kaiser HE. Antibacterial immune response in *Astacus leptodactylus* (Decapoda, Crustacea). In Vivo 11: 195-200, 1997.

Xylander WER, Ullrich G, Nevermann L, Eichelberg D. Discrimination of haemocytes of *Astacus leptodactylus* (Decapoda: Crustacea) by differential spreading and cytochemical staining. Abh. Ber. Naturmuseum Görlitz 75: 83-90, 2003.