Z. Zellforsch. 147, 537—549 (1974)

A Reappraisal of Insect Haemocyte Classification by the Examination of Blood from Fifteen Insect Orders

C. D. Price and N. A. Ratcliffe *

Department of Zoology, University College, Singleton Park, Swansea, U. K.

Received August 23, 1973

Summary. A simplified insect haemocyte classification has been formulated by a light microscopic examination of the haemolymph of insects from fifteen Orders. Six cell types or developmental stages can be distinguished: (1) Prohaemocytes, (2) Plasmatocytes, (3) Granular Cells, (4) Spherule Cells, (5) Cystocytes, and (6) Oenocytoids.

The structure and occurrence of these haemocytes are described together with the strutural variations which occur in each cell type. Due to considerable overlap in structure and the presence of numerous intermediates the six cell types may represent different developmental and/or functional stages of one basic cell type. The available evidence for this unitarian hypothesis is discussed.

Key words: Insect haemocytes — Classification — Light microscopy.

Introduction

Haemocytes are said to have essential roles in numerous physiological activities, but very little experimental work has been undertaken, apart from the classic work of Grégoire (1955, 1957) on coagulation, Wigglesworth (1955, 1956, 1973) on connective tissue synthesis, Whitten (1964) on metamorphosis, Lai-Fook (1968) on wound repair, and Salt (1970) on encapsulation, to confirm these functions and elucidate the metabolic processes involved. Before undertaking such experimental work, in which we will attempt to correlate the findings from different insect orders, we considered it essential to clarify the classification of insect haemocytes.

Most previous classifications of insect haemocytes have combined the observations of many different workers (see reviews by Jones, 1962; Gupta, 1969). The nomenclature, experimental techniques and developmental stages of the insects used by these workers usually differed, making the establishment of a uniform terminology very difficult. The result has been the identification of over seventy "different" haemocyte types. Jones (1962) reduced this number to nine, but his classification was based on personal observations of only six insect Orders. More recent studies, employing autoradiography (Shrivastava and Richards, 1965) and electron microscopy (Crossley, 1964; Hoffmann, 1966a, b, 1967; Hoffmann and Stoeckel, 1968; Hoffmann *et al.*, 1968; Scharrer, 1966, 1972a, b; Beaulaton, 1968;

* We are grateful to Professor E. W. Knight-Jones in whose department the work was carried out, to Dr. P. Makings for critically reading the manuscript and making valuable suggestions, and to the Royal Society for financial assistance to one of us (N.A.R.). We are also indebted to numerous colleagues in other institutions for generously providing many different insect species and to Mr. R. Guntrip for his technical assistance.

Baerwald and Boush, 1970a; Stang-Voss, 1970; Devauchelle, 1971; Hagopian, 1971; Moran, 1972), have shown that there may be only one basic type of haemocyte from which all the others are derived.

The present study thus formulates a simplified haemocyte classification by the personal examination of the haemolymph of representatives of fifteen insect Orders, many of which have never previously been studied.

Materials and Methods

Haemocyte Terminology

The terminology used here is that of Jones (1962) except that no distinction has been made between plasmatocytes, podocytes and vermiform cells, as Hoffmann (1967), Devauchelle (1971) and most other workers consider them to be homologous.

Experimental Animals

The species used are listed in Table 1. *Dytiscus marginalis*, *Apis mellifera*, *Aeschna grandis*, *Libellula quadrimaculata* and *Petrobius maritimus* were collected locally and used within 24 hours.

Nepa cinerea, *Sialis lutaria*, *Diura bicaudata* and *Anabolia nervosa* were supplied by the Freshwater Biological Association, Windermere, England, and maintained at 15° C.

Anoplonyx destructor were collected from Cymmer Forestry Commision Station, Cymmer, Wales and kept in the laboratory at 17–20° C.

All other species used were reared in an insectary at 25° C through at least one generation.

Observations were made both on last instar larvae and on sexually immature adults, with the following exceptions: A. *destructor*, *Galleria mellonella*, *L. quadrimaculata* and *A. nervosa* in which the results were obtained solely from studies on larval heamolymph and *N. cinerea* in which only adults were examined.

Haemocyte Preparations

All insects were kept at 4° C for 30 min. prior to bleeding except for *Rhodnius prolixus* and *Zootermopsis nevadensis* in which the cells were only present in the haemolymph when the insects were kept at 25° C.

Whenever possible insects were bled by amputation of an appendage, thus minimising variations in the blood picture which can result from the utilisation of different bleeding techniques (Jones, 1962). In many of the smaller species it was necessary to gently squeeze the abdomen in order to obtain the haemolymph. Lepidopteran larvae were bled by amputating either a proleg or a true leg, whereas haemolymph was obtained from adults by using very recently emerged individuals, cutting off the wings on one side and then squeezing the abdomen gently. The haemolymph in both cases was diluted with Clarke's insect saline.

Adult *A. mellifera* were bled in the same way as adult Lepidoptera but larvae were bled *in situ* by piercing the thorax, taking care not to damage any of the internal organs, and harvesting the haemolymph with a fine pipette.

Larvae and adults of the Dermaptera, Dictyoptera, Isoptera, Neuroptera, Orthoptera, Phasmida and Plecoptera were bled by leg amputation at the coxae. In these Orders it was necessary to dilute the haemolymph with Carlson's grasshopper saline (Paul, 1970) during extraction to help stabilise the cystocytes and delay coagulation. A thoma white blood cell pipette was half-filled with Carlson's saline, inserted into the wound, the haemolymph withdrawn and then quickly expelled with the saline onto a clean glass slide. In representatives of the Dictyoptera, Orthoptera and Phasmida only the third walking leg was amputated, whilst in the Dermaptera, Isoptera, Neuroptera and Plecoptera all the legs were amputated, in order to obtain the maximum amount of haemolymph from these smaller insects.

Adult Coleoptera were bled in the same way as the Dermaptera, but because of the hard exoskeleton the abdomen was gently squeezed in order to force out the haemolymph. The Coleopteran larvae were bled like the Lepidopteran larvae, except that the haemolymph was collected into Carlson's saline.

In the Hemiptera variations in technique were necessary. *Dysdercus cingulatus* was bled by amputating the tips of the antennae, *R. prolixus* by amputating all the legs at the tibiae and *N. cinerea* by amputating the prothoracic legs at the coxae. Carlson's saline was used with *R. prolixus* and *N. cinerea*.

Larvae of *Calliphora erythrocephala*, *A. destructor* and *A. nervosa* were bled by incising the tip of the abdomen and applying slight adbominal pressure; only in *A. nervosa* was it necessary to dilute the haemolymph with Carlson's saline. Adult *C. erythrocephala* were bled by piercing the engorged ptilina of newly-emerged adults.

The Odonatan larvae were bled in the same way as the Orthoptera, but in the adults it was necessary to amputate the anal papillae and dilute the haemolymph with Carlson's saline.

Both larvae and adults of *P. maritimus* were bled by making an incision along the mid-dorsal line of the abdomen.

For wet preparations the haemolymph was *gently* mounted on glass slides and left to settle for 5–10 min. This greatly facilitated cell-type identification as during this period many cells attached and spread out to reveal intracellular detail.

Permanent haemocyte monolayers were prepared by placing a drop of haemolymph, diluted with Carlson's saline where necessary, on a coverslip and allowing the cells to attach in a moist petri dish for 15 min. The attached cells were then fixed in formaldehyde vapour for 30 min. stained in Giemsa stain for 25 min. and dehydrated in tertiary butyl alcohol before final mounting in D.P.X. Stained monolayer preparations also facilitated the observation of intracellular detail.

All preparations were observed and photographed under phase contrast, Nomarski and bright field optics, with a Zeiss Photomicroscope II. The film used was mainly Panatomic X (Kodak), but where contrast was low, as for example on protoplasmic extensions, Micro Neg Pan type B (Kodak) was used in conjunction with Acufine developer (Acufine Inc., U.S.A.).

Some preliminary electron microscopic observations were also made (Ratcliffe and Price, in preparation).

Results

Initially, under phase contrast optics, all the haemocytes are spindle-shaped, making differentiation into cell "types" very difficult. However, after 5–10 min. *in vitro* six cell types can be identified, as the cells attach to the substratum and spread out to reveal intracellular details. Only *D. marginalis* and *T. molitor* in the fifteen Orders examined contain all six types (Table 1). The six cell types are; prohaemocytes, plasmatocytes, granular cells, spherule cells, cystocytes and oenocytoids.

Prohaemocytes (Figs. 1–5)

These are present in all species (Table 1), but are only common in the Hemiptera and Lepidoptera. They are small, round to oval cells, 6–13 μm in diameter, with a thin rim of finely granular cytoplasm which occasionally encloses a few large granules (Fig. 5). The nucleus accounts for approximately 70–80% of the cell volume and usually contains 1–2 prominent chromatin blocks (Figs. 1–5).

Occasionally *in vitro* large prohaemocytes put out fine protoplasmic extensions to attach themselves to the substratum, but this appears to be their only modification (Fig. 4).

Plasmatocytes (Figs. 6–15)

Plasmatocytes in varying forms are present in all species (Table 1), but they are often difficult to distinguish from the third cell type to be described, the granular cells. Most previous workers have described plasmatocytes as having a

Table 1. Occurrence of various haemocyte types

Order	Species	Pro.	Plas.	Gran.	Sph.	Cyst.	Oen.
Coleoptera	*Dytiscus marginalis*	+	+	+	+	+	+
	Tenebrio molitor	+	+	+	+	+	+
Dermaptera	*Forficula auricularia*	+	+	+	+	+	
Dictyoptera	*Blaberus cranifer*	+	+	+	+	+	
	Leucophaea maderae	+	+	+	+	+	
	Periplaneta americana	+	+	+	+ ?	+	
	Sphodromantis bioculata	+	+	+	+	+	
Diptera	*Calliphora erythrocephala*	+	+	+	+		+
Hemiptera	*Dysdercus cingulatus*	+	+	+	+		
	Nepa cinera	+	+	+	+	+	
	Rhodnius prolixus	+	+	+		+	
Hymenoptera	*Anoplonyx destructor*	+	+	+	+		+
	Apis mellifera	+	+	+	+		
Isoptera	*Zootermopsis nevadensis*	+	+	+	+	+	
Lepidoptera	*Bombyx mori*	+	+	+	+		+
	Galleria mellonella	+	+	+	+		+
	Pieris brassicae	+	+	+	+		+
Neuroptera	*Sialis lutaria*	+	+	+	+	+	
Odonata	*Aeschna grandis*	+	+	+		+	+
	Libellula quadrimaculata	+	+	+	+	+	
Orthoptera	*Jamaicana subguttata*	+	+	+		+	
	Locusta migratoria	+	+	+		+	
	Schistocerca gregaria	+	+	+		+	
Phasmida	*Carausius morosus*	+	+	+	+	+	
	Clitumnus extradentatus	+	+	+		+	
Plecoptera	*Diura bicaudata*	+	+	+	+	+	
Thysanura	*Petrobius maritimus*	+	+	+	+		+
Trichoptera	*Anabolia nervosa*	+	+	+		+	+ ?

Key: Pro. = Prohaemocytes; Plas. = Plasmatocytes; Gran. = Granular Cells; Sph. = Spherule Cells; Cyst. = Cystocytes; Oen. = Oenocytoids

Figs. 1—8, 10—19, 21—23, 25, 26, 28—31. 33 phase contrast, all others Nomarski optics. Scale bar 10 μm in all cases.

Fig. 1. Prohaemocyte from *Zootermopsis nevadensis* larva

Fig. 2. Prohaemocyte from *Rhodnius prolixus* larva

Fig. 3. Prohaemocytes from *Calliphora erythrocephala* adult

Fig. 4. Prohaemocyte from *Rhodnius prolixus* larva; note protoplasmic extensions (*Pe*)

Fig. 5. Prohaemocyte from *Apis mellifera* larva; note granules in cytoplasm

Fig. 6. Plasmatocytes from *Pieris brassicae* larva, showing early stage in spreading

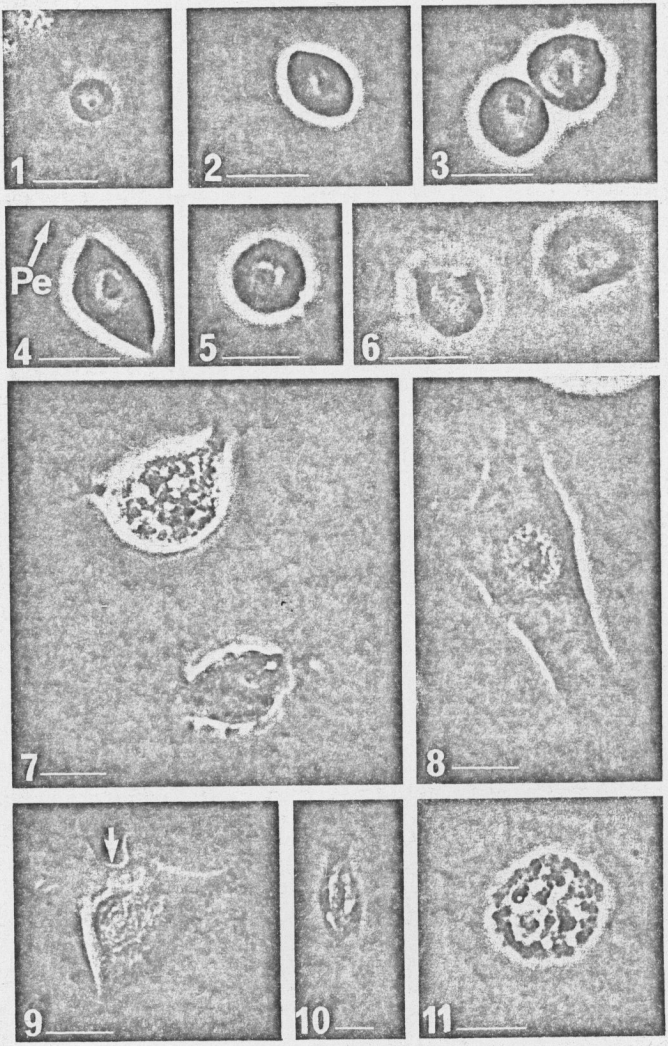
Fig. 7. Plasmatocytes from *Leucophaea maderae* adult; note refractiveness of top cell and presence of large numbers of granules

Fig. 8. Fully spread plasmatocyte from *Pieris brassicae* larva; note granular nucleus

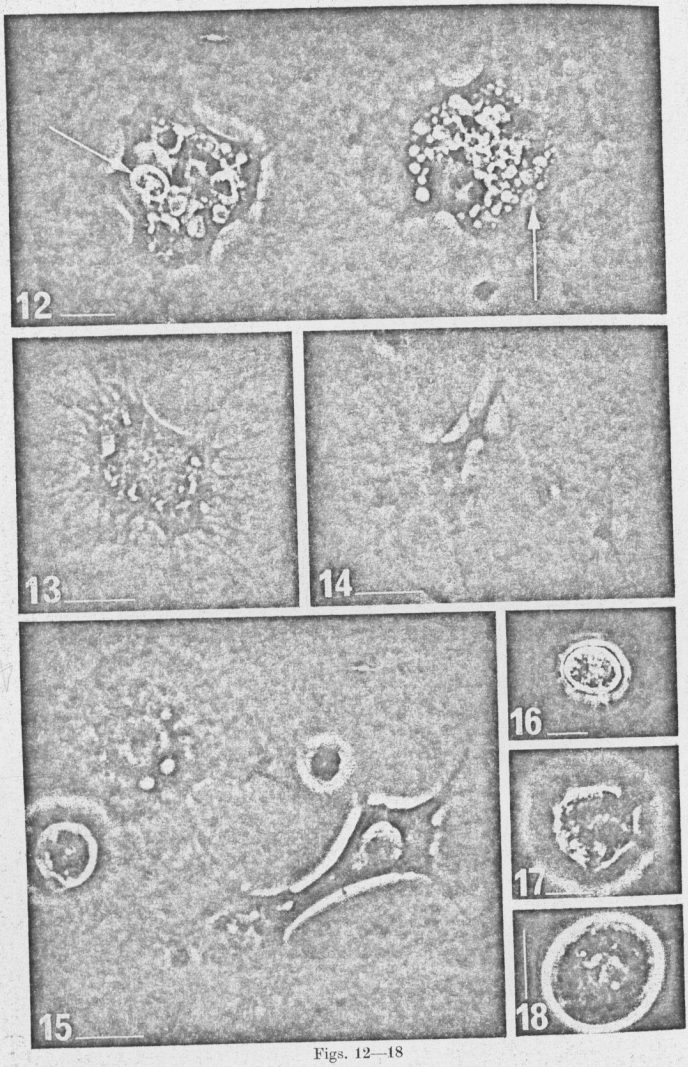
Fig. 9. Plasmatocyte from *Pieris brassicae* larva; note ruffled membrane (arrow)

Fig. 10. Unusual spindle-shaped plasmatocyte from *Calliphora erythrocephala* larva

Fig. 11. Spread plasmatocyte from *Dysdercus cingulatus* adult, showing fan-like membrane, spiked pseudopodia and cytoplasmic granules



Figs. 1—11



Figs. 12—18

finely granular cytoplasm, containing few granules, and capable of amoeboid movement of changes in shape, *in vitro*. We observed several amoeboid haemocytes which were very similar in structure to plasmatocytes, except for the presence of large numbers of cytoplasmic granules. Such cells have been termed amoeboid granular cells by Arnold (1972a), but under the conditions of this study it would be more accurate to designate all cells exhibiting amoeboid movement *in vitro*, regardless of the number of granules, as plasmatocytes (see Discussion).

In fresh haemolymph preparations plasmatocytes are round, oval, or spindle shaped cells, 10–15 μm in diameter, with a central nucleus occupying approximately 40% of the cell volume. The cytoplasm is finely granular and contains a variable number of granules 1–1.5 μm in diameter (Figs. 7, 8, 11, 15). Sometimes the nucleus is completely obscured by the presence of large numbers of these granules.

Several changes occur to plasmatocytes *in vitro*. The cells gradually lose their slight initial refractiveness; pseudopodia are formed and the cells begin to spread out (Figs. 6–9, 11–13, 15). Cells with few granules usually become heavily vacuolated (Fig. 10) and seem to contain debris, whilst other cells with many granules are far more motile and undergo greater changes in shape (Fig. 15). The leading edge of a moving plasmatocyte is bounded by a ruffled membrane which does not appear to be attached to the substratum, and the cytoplasm just behind this edge is devoid of cell inclusions (Fig. 9). Such plasmatocytes compare closely with the motile haemocytes described in *Periplaneta americana* by Baerwald and Boush (1970b).

Motile plasmatocytes with large numbers of granules are most commonly found in both larval and adult stages of the Dictyoptera and in the larvae of the Coleoptera, Orthoptera and Phasmida. Heavily vacuolated plasmatocytes are least common in the Dictyoptera and most common in the adults of the Coleoptera, Orthoptera and Phasmida. In the other Orders both sorts of plasmatocytes are found in about equal numbers. However cell movement is most often seen in the Dictyoptera and then more frequently in larvae than in adults.

In both larvae and adults of *C. erythrocephala* unusual spindle shaped cells are present (Figs. 10, 14), in addition to the more normal looking plasmatocytes. These are thin cells, usually 1–2 μm in width, and of variable length. The cytoplasm is very dense when viewed under phase contrast and does not appear to contain any inclusions. In view of their similarity to Jones' vermiform cells,

Fig. 12. Plasmatocytes from *Locusta migratoria* adult; note vacuoles and debris (arrows)

Fig. 13. Plasmatocyte from *Dytiscus marginalis* adult, showing a spiked form of pseudopodia

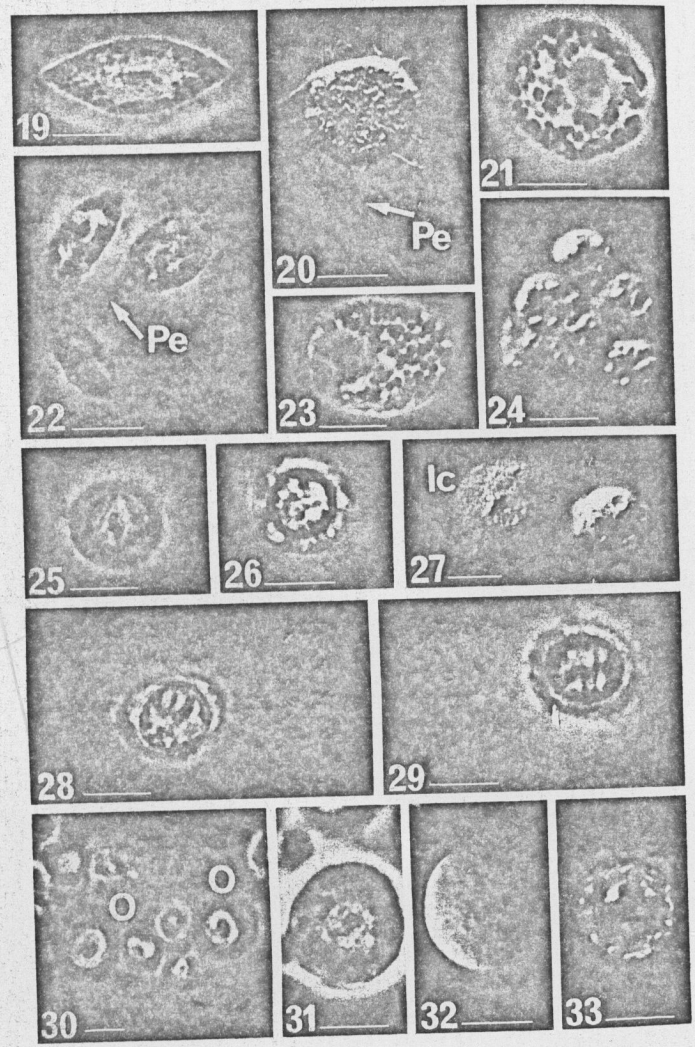
Fig. 14. Range of unusual plasmatocytes from *Calliphora erythrocephala* adult, showing great variation in size, and very dense cytoplasm

Fig. 15. Plasmatocytes from *Dysdercus cingulatus* larva, showing variable shapes of cells and cytoplasmic granules

Fig. 16. Highly refractive granular cell from *Leucophaea maderae* larva

Fig. 17. Granular cell from *Schistocerca gregaria* larva; note fine protoplasmic extensions and loss of refractiveness

Fig. 18. Granular cell from *Rhodnius prolixus* adult; note central nucleus



Figs. 19—33

(Jones, 1962), we have included them with the plasmatocytes. However, Zachary, Hoffmann and Porte (1972) consider them to be a new cell type, the thrombocytoids.

Granular Cells (Figs. 16–20)

Granular cells were identified in every insect examined (Table 1). They are round or oval cells, 10–17 μm in diameter, with a central nucleus (Fig. 18), accounting for approximately 40–60% of the cell volume. They are usually packed with granules of a similar size to those found in plasmatocytes (Figs. 18, 19) and are capable of anchoring themselves to the substratum with fine protoplasmic extensions (Fig. 20). Initially *in vitro* granular cells are very refractile but after about 5 min. this appearance is lost (Figs. 16, 17, 19). Granular cells are characteristic and conspicuous in *R. prolixus* and *Carausius morosus*, but in some species the distinction between plasmatocytes and granular cells is not very clear and often it is impossible to distinguish the two cell types.

Spherule Cells (Figs. 21–24)

Spherule cells were identified in all the Coleoptera, Hymenoptera and Lepidoptera and in some of the Dictyoptera, Hemiptera, Odonata and Phasmida. They are also present in *Forficula auricularia*, *C. erythrocephala*, *Z. nevadensis*, *S. lutaria*, *D. bicaudata* and *P. maritimus* (Table 1). They are spindle-shaped or oval cells,

Fig. 19. Granular cell from *Carausius morosus* adult, with large number of cytoplasmic granules

Fig. 20. Granular cell from *Carausius morosus* larva, showing fine protoplasmic extensions (*Pe*)

Fig. 21. Spherule cell from *Leucophaea maderae* larva, filled with dense spherules

Fig. 22. Spherule cells from *Diura bicaudata* adult; note fine protoplasmic extensions anchoring cells to substratum

Fig. 23. Spherule cell from *Blaberus cranifer* larva; cytoplasm is filled with spherules much larger than granules of granular cells (compare with Fig. 19). Note eccentric nucleus

Fig. 24. Typical small spherule cells from *Pieris brassicae* larva

Fig. 25. Cystocyte from *Leucophaea maderae* larva; note large central nucleus and size of cell in comparison to typical prohaemocyte (compare with Fig. 1)

Fig. 26. Cystocyte from *Locusta migratoria* larva; cell swollen and refractive (compare with Fig. 25)

Fig. 27. Cystocyte from *Forficula auricularia* larva, showing late stage in haemolymph coagulation and formation of an "isle of coagulation" (*Ic*)

Fig. 28. Cystocyte from *Blaberus cranifer* larva; note lines of haemolymph coagulation

Fig. 29. Cystocyte from *Locusta migratoria* adult, showing an early stage in coagulation and poorly defined cell membrane

Fig. 30. Haemocytes of *Calliphora erythrocephala* larva; note oenocytoids (O) with dense nuclear inclusions

Fig. 31. Oenocytoid from *Pieris brassicae* larva, showing central nucleus and granules in cytoplasm

Fig. 32. Oenocytoid from *Pieris brassicae* larva

Fig. 33. Unusual granular cell from *Dysdercus cingulatus* adult. This type of cell always contains rapidly moving granules and is probably an artifact

8–16 μm in diameter, and are usually filled with large granules or spherules, 1.5–2.0 μm in diameter (Figs. 21, 23). The inclusions are either yellowish-white or dark grey, when viewed under phase contrast, and are often extruded *in vitro*. The nucleus, when not obscured by the spherules, accounts for approximately 35–50% of the cell volume and is sometimes slightly eccentric (Fig. 23). *In vitro* spherule cells anchor themselves to the substratum by very fine protoplasmic extensions (Fig. 22), which are most commonly and easily seen in the Lepidoptera.

Spherule cells are most readily identified in some members of the Dictyoptera, for example, *Blaberus cranifer* and *Leucophaea maderae*, whilst their identification is most difficult in *D. cingulatus*, *N. cinerea*, *Z. nevadensis* and *C. morosus*. Spherule cells are easily identified in the Lepidoptera where they are unusually small, approximately 8 μm in diameter (Fig. 24). Those of *B. cranifer* and *L. maderae* are much larger, approximately 16 μm in diameter (Figs. 21, 23).

Cystocytes (Figs. 25–29)

Cystocytes were observed in all the insects examined except in the Diptera, Hymenoptera, Lepidoptera and Thysanura and were also absent in *D. cingulatus* (Table 1). They are round cells, approximately 9–14 μm in diameter, and are usually larger than the prohaemocytes (Fig. 25). The central nucleus occupies approximately 70–75% of the cell volume and has peripherally arranged chromatin, in contrast to the block arrangement in the prohaemocytes (Fig. 25). Cystocytes change markedly *in vitro* in undiluted haemolymph. The cytoplasm becomes less optically dense; the cell membrane becomes poorly defined and then dense granular material radiates outwards from the cell (Figs. 27–29). This material forms the "isles of coagulation" (Grégoire, 1955, 1957) and results from a phenoloxidase discharged from the cell which causes the haemolymph to precipitate (Hoffmann and Stoeckel, 1968). At the same time, the nucleus becomes highly refractive and the cells swell (Figs. 26, 28, 29). The rate of formation and extent of the "isles of coagulation" vary considerably from species to species and between larvae and adults of the same species. The process is more extensive and rapid in the Dictyoptera, in particular *B. cranifer*, whilst in the Hemiptera it is far less conspicuous.

Oenocytoids (Figs. 30–32).

Oenocytoids were observed in the Coleoptera, Diptera, Lepidoptera and Thysanura, and were also present in *A. destructor*, *A. grandis* and perhaps also in *A. nervosa* (Table 1). The cells are relatively large, up to 19 μm in diameter, with an eccentric nucleus occupying about 20–40% of the cell volume and usually containing one or more chromatin blocks. The cytoplasm encloses only a few granules or globules. Oenocytoids do not appear to change *in vitro*. They are most commonly seen in the Lepidoptera in which they contain more inclusions than in other orders (Figs. 31, 32).

It is fairly easy to confuse large spherule cells that have extruded most of their spherules with oenocytoids.

Other Observations

Adipohaemocytes are not classified as a separate cell type, as we concur with Wigglesworth (1956), and consider them to be indistinguishable from typical insect fat-body cells.

Many cells were also encountered which were difficult to place in any of the six groups listed. They appeared to be intermediate forms between prohaemocytes and plasmatocytes, between plasmatocytes and granular cells, between granular cells and spherule cells, or between spherule cells and oenocytoids.

Another type of granular cell was occasionally found which was filled with very fine particles undergoing rapid movement (Fig. 33). We believe them to be artifacts produced from normal cells during experimentation, as their numbers increase during observations and photography.

Discussion

The present classification has several advantages over existing schemes. First, it is simpler than previous classifications which include eight or more cell types. Second, it is based wholly on our own observations, and not the pooled results of different workers. It is difficult enough to clarify terminology homologies by direct personal observation of the haemocytes from different Orders without relying on the descriptions and often inadequate photographs of other workers. We have thus placed great emphasis on clearly illustrating and describing the cell types and variations which occur. Third, the observations cover a greater range of Orders than any single previous work, and the haemocytes of many of these Orders have never before been studied. Lastly, there was more standardisation in preparation techniques than in the past, in order to eliminate variations in the blood picture that may result from different experimental methods (Jones, 1962; Crossley, 1964).

The present classification is essentially a simplification of the one proposed by Jones (1962), except with regard to the status of the adipohaemocytes (see above), and the definition of granular cells.

In light microscopic studies, it is our opinion that the classification into six (or five, if the plasmatocytes and granular cells are combined into one cell type—see below) cell types is valid since the majority of the cells observed fall into one of the six groups. The recognition of such cell types as podocytes, adipohaemocytes and vermiform cells only serves to complicate matters.

It is arguable whether the various haemocyte types are morphologically and functionally distinct cell types or merely expressions, perhaps different developmental stages, of one basic cell type. Our preliminary electron microscopic observations, together with the work of Crossley (1964); Hoffmann (1966a, b, 1967); Hoffmann and Stoeckel (1968); Hoffmann *et al.* (1968); Scharrer (1966, 1972a, b); Beaulaton (1968); Baerwald and Boush (1970a); Stang-Voss (1970); Devauchelle (1971); Hagopian (1971); Moran (1971), have shown that based on the structure of the cytoplasmic inclusions definite cell types are not nearly as apparent as in the light microscope, and this supports the idea that haemocytes pass from one developmental stage into another. Thus many of the cell types observed in the light microscope are probably different developmental and functional stages of one basic cell type. However, confirmation of this hypothesis awaits further ultrastructural studies, as observations are at present confined to the Coleoptera, Dictyoptera, Diptera, Lepidoptera, Orthoptera and Phasmida.

Shrivastava and Richards (1965), in support of the "unitarian hypothesis" (see Scharrer, 1972a, b) described only three haemocyte types in *G. mellonella*, and with autoradiography showed the transformation of prohaemocytes into plasmatocytes, and plasmatocytes into adipohaemocytes. Since many workers (Gupta, 1969; Neuwirth, 1973) are agreed that granular and spherule cells are present in *G. mellonella* (see Table 1), the adipohaemocytes and plasmatocytes of Shrivastava and Richards (1965) are probably homologous with the granular cells, oenocytoids and/or spherule cells which we identified in *G. mellonella* (Table 1). We have also observed intermediate cell forms which indicate the development of granular cells from plasmatocytes (see above—Other Observations), and this would explain the difficulty sometimes encountered in distinguishing cell types. The granular cell may well be capable of amoeboid movement (a process characterising plasmatocytes, see Results) under the right conditions, as they have been shown to put out pseudopodia *in vitro* and attach themselves to the substratum. Their movement may be dependent on the action of ecdysterone which has been shown to increase the mobility of haemocytes *in vitro* (Judy and Marks, 1971).

In contrast to the concept of only one basic cell type, Arnold (1972a, b), on the basis of an extensive light microscopic study of the haemocytes of numerous cockroaches, stated that distinct haemocyte types exist and that each species has distinctive haemocytes which could possibly be utilised to clarify the taxonomy of the group. Arnold attached great significance to variations in the size and form of the haemocytes and to size, shape and numbers of their granules. He also stated that granular cells and plasmatocytes are "two seemingly immutable categories of haemocyte". Our results show little agreement with Arnold's views, since haemocyte morphology is so variable that it would be most unlikely that different forms of the same cell type could be distinguished from closely related species by light microscopy alone.

Finally, it is not the presence or absence of cell types or developmental stages which is of prime importance, but the role of these various types or stages in the overall physiology of the insect. The different developmental stages of a single cell type may be capable of distinct functions just as truly different cell types are.

References

Arnold, J. W.: A comparative study of the haemocytes (blood cells) of cockroaches (Insecta: Dictyoptera: Blattaria), with a view of their significance in taxonomy. Canad. Ent. **104**, 309–348 (1972a)

Arnold, J. W.: Haemocytology in insect biosystematics: The prospect. Canad. Ent. **104**, 655–659 (1972b)

Baerwald, R. J., Boush, G. M.: Fine structure of the haemocytes of *Periplaneta americana* (Orthoptera: Blattidae) with particular reference to marginal bundles. J. Ultrastruct. Res. **31**, 151–161 (1970a)

Baerwald, R. J., Boush, G. M.: Time-lapse photographic studies of cockroach hemocyte migrations *in vitro*. Expl. Cell Res. **63**, 208–213 (1970b)

Beaulaton, J.: Étude ultrastructurale et cytochimique des glandes prothoraciques de ver à soie aux quatrième et cinquième âges larvaires. 1. La tunica propria et ses relations avec les fibres conjonctives et les hémocytes. J. Ultrastruct. Res. **23**, 474–498 (1968)

Crossley, A.C.S.: An experimental analysis of the origins and physiology of haemocytes in the blue blow-fly, *Calliphora erythrocephala* (Meig). J. exp. Zool. **157**, 375–398 (1964)

Devauchelle, G.: Étude ultrastructurale des hémocytes du Coléoptère *Melolontha melolontha* (L.). J. Ultrastruct. Res. **34**, 492–516 (1971)

Grégoire, C.: Blood coagulation in arthropods. V. Studies on haemolymph coagulation in 420 species of insects. Archs. Biol. **66**, 103–148 (1955)
Grégoire, C.: Studies by phase contrast microscopy on distribution patterns of haemolymph coagulation in insects. Smithson. misc. Collns. **134**, 1–35 (1957)
Gupta, A. P.: Studies of the blood Meloidae (Coleoptera). 1. The haemocytes of *Epicauta cinerea* (Forster), and a synonymy of haemocyte terminologies. Cytologia (Tokyo) **34**, 300–344 (1969)
Hagopian, M.: Unique structures in the insect granular hemocytes. J. Ultrastruct. Res. **36**, 646–658 (1971)
Hoffmann, J. A.: Étude des oenocytoides chez *Locusta migratoria* (Orthoptère). J. Microsc. **5** (**2**), 269–272 (1966a)
Hoffmann, J. A.: Étude ultrastructurale de deux hémocytes à granules de *Locusta migratoria* (Orthoptère). C. R. Acad. Sci. (Paris) **263**, 521–524 (1966b)
Hoffmann, J. A.: Étude des hémocytes de *Locusta migratoria* L. (Orthoptère). Archs. Zool. exp. gén. **108**, 251–291 (1967)
Hoffmann, J. A., Stoeckel, M. E.: On the ultrastructural modifications of coagulocytes in the course of hemolymph coagulation in an orthopteran insect *Locusta migratoria* L. Bull. Soc. Biol. **162**, 2257–2259 (1968)
Hoffmann, J. A., Stoeckel, M. E., Porte, A., Joly, P.: Ultrastructure des hémocytes de *Locusta migratoria* (Orthoptère). C. R. Acad. Sci. (Paris) **266**, 503–505 (1968)
Jones, J. C.: Current concepts concerning insect hemocytes. Amer. Zool. **2**, 209–246 (1962)
Judy, K. J., Marks, E. P.: Effects of ecdysterone *in vitro* on hindgut and hemocytes of *Manduca sexta* (Lepidoptera). Gen. comp. Endocr. **17**, 351–359 (1971)
Lai-Fook, J.: The fine structure of wound repair in an insect (*Rhodnius prolixus*). J. Morph. **124**, 37–78 (1968)
Moran, D. T.: Fine structure of cockroach blood cells. Tiss. and Cell **3**, 413–422 (1971)
Neuwirth, M.: The structure of the haemocytes of *Galleria mellonella* (Lepidoptera). J. Morph. **139**, 79–104 (1973)
Paul, J.: Cell and tissue culture, 4th ed. Edinburgh and London: F. and S. Livingstone, 1970
Salt, G.: The cellular defence reactions in insects. Cambridge Monographs in Experimental Biology, No 16. New York, N. Y.: Cambridge University Press, 1970
Scharrer, B.: An electron microscopic study of insect hemocytes. Anat. Rec. **154**, 416 (1966)
Scharrer, B.: Cytophysiological aspects of insect haemocytes. Anat. Rec. **172**, 465 (1972a)
Scharrer. B.: Cytophysiological features of haemocytes in cockroaches. Z. Zellforsch. **129**, 301–319 (1972b)
Shrivastava, S. C., Richards, A. G.: An autoradiographic study of the relation between haemocytes and connective tissue in the wax moth, *Galleria mellonella* L. Biol. Bull. **128**, 337–345 (1965)
Stang-Voss, C.: Zur Ultrastruktur der Blutzellen wirbelloser Tiere. 1. Über die Hämocyten der Larve des Mehlkäfers *Tenebrio molitor* L. Z. Zellforsch. **103**, 589–605 (1970)
Whitten, J. M.: Haemocytes and the metamorphosing tissues in *Sarcophaga bullata*, *Drosophila melanogaster* and other cyclorrhaphous Diptera. J. Insect Physiol. **10**, 447–469 (1964)
Wigglesworth, V. B.: The role of haemocytes in the growth and moulting of an insect – *Rhodnius prolixus* (Hemiptera). J. exp. Biol. **32**, 649–663 (1955)
Wigglesworth, V. B.: The haemocytes and connective tissue formation in an insect *Rhodnius prolixus* (Hemiptera). Quart. J. micr. Sci. **97**, 89–98 (1956)
Wigglesworth, V. B.: Haemocytes and basement membrane formation in *Rhodnius*. J. Insect Physiol. **19**, 831–844 (1973)
Zachary, D., Hoffmann, J. A., Porte, A.: Sur un nouveau type de cellule sanguine (le thrombocytoide) chez *Calliphora erythrocephala*. C. R. Acad. Sci. (Paris) **275**, 393–395 (1972)

Dr. N. A. Ratcliffe
Department of Zoology
University College
Singleton Park
Swansea, U. K.