The Insects R F Chapman

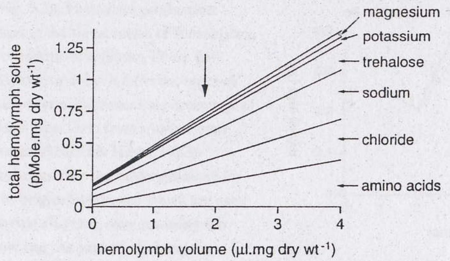


Fig. 5.24. Osmotic effectors in the hemolymph of *Onymachus* at different levels of hydration. The quantity of each component is indicated by the area between successive lines. The top line shows the total concentration of all the solutes combined. Arrow indicates the normal level of hydration (after Machin, 1981).

5.2.3.2 *pH*

The pH of insect hemolymph is usually between 6.4 and 6.8, although slightly alkaline values have been recorded in a dragonfly larva and in the larva of the midge, *Chironomus*. During normal activity there is a tendency for the blood to become more acid due to the liberation of acid metabolites, including carbon dioxide. The buffering capacity of insect blood (that is, its ability to prevent change in pH) is low in the normal physiological range, but increases sharply above and below this range. Within the normal range, bicarbonates and phosphates are the most important buffers. On the acid side of the range, carboxyl groups of organic acids are important, while on the alkaline side the amino groups of amino acids are most significant. Proteins buffer over a wide range of pH.

Review: Mullins, 1985

5.2.4 Hemocytes

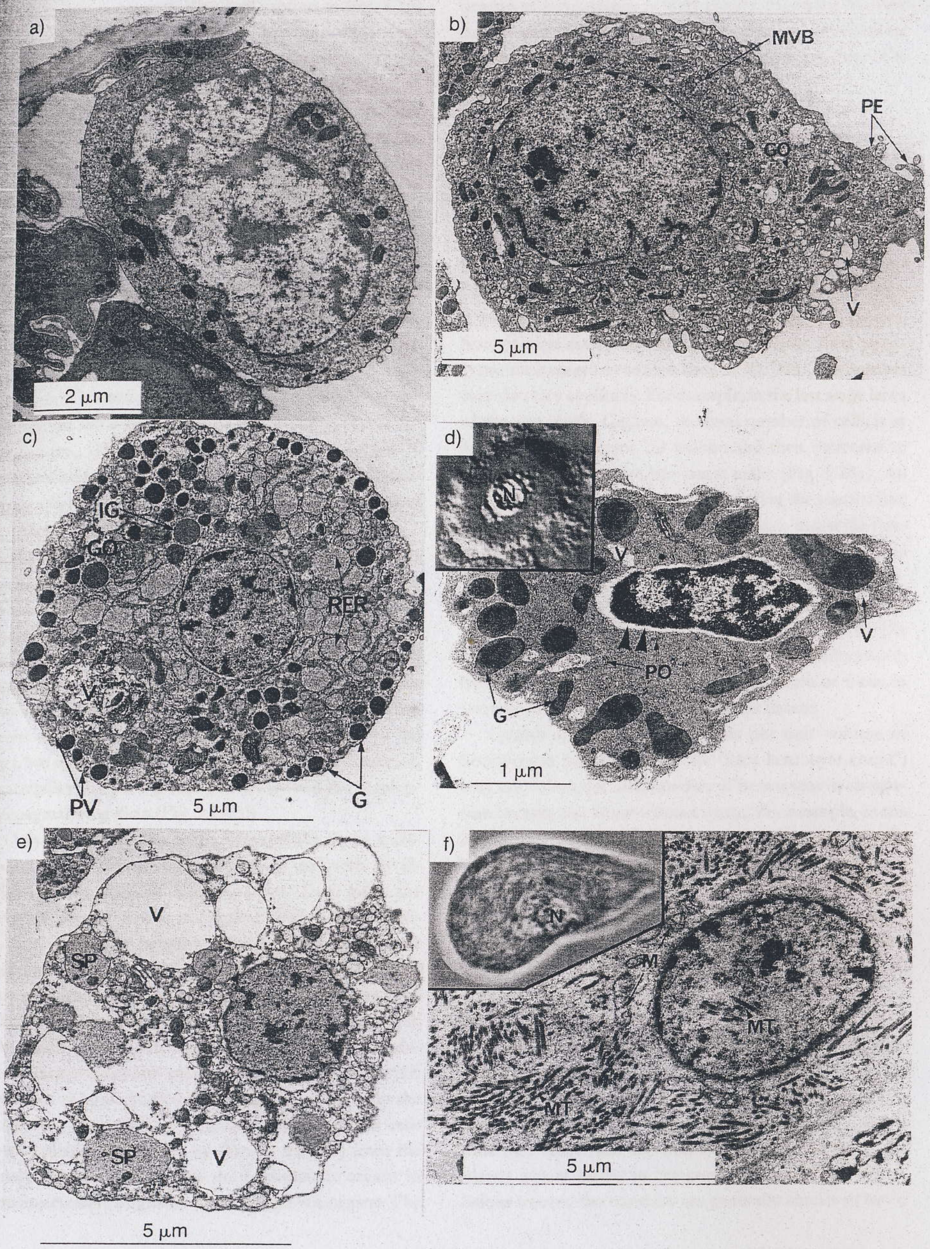
Suspended in the blood plasma are blood cells or hemocytes. Many different types of hemocyte have been described, but a comprehensive classification is difficult because individual cells can have very different appearances under different conditions and a variety of techniques have been used in their study. Rowley & Ratcliffe (1981) and Gupta (1979a, 1979b, 1985, 1991) attempt to synonymize them across the different orders and reduce them to six main types (Fig. 5.25). They are: prohemocytes, plasmatocytes, granulocytes (which are probably the same as cystocytes or coagulocytes), spherule cells (spherulocytes), oenocytoids and adipohemocytes.

Prohemocytes are characterized by a high nuclear: cytoplasmic ratio and a general lack of organelles involved in synthesis. They rarely comprise more than 5% of the total hemocyte population. They are the stem cells from which most other hemocyte types are formed.

Plasmatocytes are very variable in shape. They contain moderate amounts of rough endoplasmic reticulum and Golgi complexes and may contain membrane-bound granules. They are amongst the most abundant hemocytes and usually account for more than 30% of the total hemocyte count. Plasmatocytes are involved in phagocytosis and encapsulation of foreign organisms invading the hemocoel.

Granulocytes contain large amounts of endoplasmic reticulum which is often extensively dilated. Golgi complexes are also abundant and the cells contain large numbers of membrane-bound granules. They comprise a considerable proportion, usually more than 30% of the hemocyte population. They discharge their contents (degranulate) on the surfaces of intruding organisms as an early part of the defense response. Granulocytes are probably derived from plasmatocytes and intermediates between the two types of cell occur (see Chain, Leyshon-Sørland & Ṣiva-Jothy, 1992). Cystocytes are probably granulocytes in which the synthesis of granular contents is complete. They contain abundant granules, but usually contain smaller amounts of Golgi complexes and rough endoplasmic reticulum than granulocytes. They have a relatively high nucleus: cytoplasm ratio. They are often common, but have not been recognized in Diptera, Lepidoptera and Hymenoptera.

Fig. 5.25. Different types of hemocyte (a) after Chiang, Gupta & Han, 1988; others after Rowley and Ratcliffe, 1981): (a) prohemocyte of *Blattella*; (b) plasmatocyte of larval *Galleria*; (c) granulocyte of larval *Galleria*; (d) granulocyte (cystocyte) of *Clitumnus*. Arrowheads indicate swollen perinuclear cisterna; (e) spherule cell of larval *Galleria*. The large open areas, looking like vacuoles (and labelled V), are probably caused by extraction of spherules during preparation; (f) oenocytoid from larval *Galleria*. Inset shows size of nucleus relative to whole cell.
Abbreviations: G, granules; GO, Golgi complex; IG, developing granules; M, mitochondria; MT, microtubules; MVB, multivesicular body; N, nucleus; PE, protoplasmic extensions; PO, ribosomes; PV, pinocytotic vesicles; R, ribosomes; RER, distended cisternae of rough endoplasmic reticulum; SP, spherules; V, vacuole.



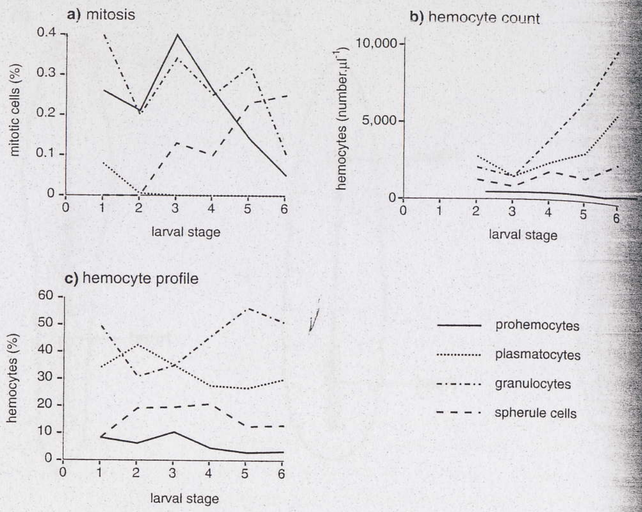


Fig. 5.26. Hemocyte production during the larval stages of *Euxoa* (data from Arnold & Hinks, 1976). (**a**) Mitotic activity in different types of hemocytes, expressed as percentage of each type. Data from a subsequent paper (Arnold & Hinks, 1983) indicates that the values obtained in the original work, and which are used in this diagram, were probably too low, but the pattern of change is probably not affected. (**b**) Hemocyte counts per microliter of blood. (**c**) Hemocyte profile – relative frequency of different types of hemocyte, expressed as percentage of total number of blood cells.

Spherule cells are characterized by the large, refractile spherules which may occupy 90% of the cytoplasm. They are not usually very common although they are found in most of the species studied. Their function is unknown.

Oenocytoids occur mainly in Lepidoptera where they are amongst the largest of the hemocytes. These cells exhibit little development of rough endoplasmic reticulum or Golgi complexes, but they have a complex array of microtubules and sometimes also crystalline inclusions. Their function is unknown.

Adipohemocytes characteristically contain lipid droplets. The nucleus: cytoplasm ratio is low, and they contain well-developed endoplasmic reticulum and Golgi complexes.

Reviews: Brehélin & Zachary, 1986; Gupta, 1979a, b, 1985, 1991; Rowley & Ratcliffe, 1981

5.2.4.1 *Origin of hemocytes*

Hemocytes are derived from the embryonic mesoderm. Subsequently, new hemocytes are produced by mitotic division of existing, circulating hemocytes, or from previously undifferentiated cells in structures known as hemopoietic organs.

Mitotic division of hemocytes The production of new hemocytes by mitosis of existing blood cells is a widespread phenomenon. In adult holometabolous insects that lack hemopoietic organs, new hemocytes can only be produced in this way. This appears also to be the case during the larval stages of the milkweed bug, *Oncopeltus*. Elsewhere, hemocyte production from existing cells appears to complement production in hemopoietic organs, but where the hemopoietic organs persist in adult insects, as in Blattodea and Orthoptera, mitotic division of existing hemocytes is relatively rare.

Not all types of cell divide and the rates of division vary even amongst those that do. Between 0.2 and 0.4% of prohemocytes were found in division in blood samples taken during the first four stages of larval development of the moth, *Euxoa*, but this level declined in the final larval stage (Fig. 5.26a). Mitotic activity was similar in granulocytes, but amongst spherule cells it increased from zero in the first two stages to about 0.25% in the final stage. Plasmatocytes only rarely divide in at least a majority of insects. Despite this, the number of plasmatocytes per unit volume of hemolymph increases throughout larval development (Fig.5.26b). They are probably derived from the prohemocytes which remain constant in relative abundance despite their high mitotic rate.

Much of the literature suggests that the mitotic rate for all the cells only rarely exceeds 1%, but some work indicates much higher rates. Arnold & Hinks (1983) suggest that in the final larval stage of *Euxoa*, the mitotic index of spherule cells may exceed 10% (see caption to Fig. 5.26) and in the final larval stage of the milkweed bug, *Oncopeltus*, a mitotic index of 4% was recorded.

On the basis of the mitotic activity of the cells, it is suggested that the whole population of granulocytes in the last larval stage of *Euxoa* turns over in about 5 days; the spherule cell population would turn over in less than one day (Arnold & Hinks, 1983). Other estimates of hemocyte longevity, in *Galleria*, suggest that plasmatocytes survive for at least nine days.

Hemopoietic organs Blood is formed in structures called hemopoietic organs. Since, in insects, only the blood cells, not the plasma, are produced in these structures, they should strictly be called hemocytopoietic organs, but the general term is more usual. Hemopoietic organs have been described in some Orthoptera, a blattid and a few larval Lepidoptera, Diptera and Coleoptera. They persist in adult Orthoptera, but not in adults of holometabolous species. No hemopoietic organs are present at any stage of the milkweed bug, *Oncopeltus*.

The positions of hemopoietic organs vary from species to species, but in most cases they are associated with, though not necessarily connected with, the heart. In the cricket, *Gryllus*, and the mole cricket, *Gryllotalpa*, they are paired, segmental structures on either side of the heart and opening into it (Fig. 5.2). In *Locusta*, *Periplaneta*, and larvae of cyclorrhaphous flies and of the beetle, *Melolontha*, they consist of irregular accumulations of cells close to the heart, but not connected with it (Fig. 5.27a,b). By contrast, in caterpillars they are groups of cells around the developing imaginal wing discs (Fig. 5.27c,d).

Only in the grylloids is the hemopoietic organ a discrete structure bounded by a cell layer and with an ill-defined lumen opening into the heart. Even here, the bounding layer of cells is incomplete. Within this boundary are irregularly shaped reticular cells apparently embedded in a connective tissue matrix. These cells undergo mitotic divisions and give rise to hemocyte stem cells. By further division, the stem cells form clusters of cells which differentiate synchronously to form hemocytes. Granulocytes and plasmatocytes are formed in this way. They separate from the cortical region and enter the circulation, presumably via the heart. The reticular cells are also phagocytic, taking up foreign material from the hemolymph. Because of this the hemopoietic organs in these insects were originally called phagocytic organs. The process of hemopoiesis appears essentially similar in other insects although the reticular cells exist as aggregations with no bounding layer and, in Lepidoptera, reticular cells are absent.

Reviews: Feir, 1979 – mitosis; Hoffmann *et al.*, 1979 – hemopoietic organs

5.2.4.2 *Numbers of hemocytes*

Estimates of the total number of hemocytes in an insect show that small insects have many fewer hemocytes than large insects. Adult female mosquitoes have a total of less than 10 000 hemocytes, whereas adult *Periplaneta* have more than 9 000 000. Similar trends occur within a species. Second stage caterpillars of *Euxoa* have about 4000 hemocytes; sixth stage larvae have about 2 400 000. The number may also vary cyclically. For example, in the last stage larva of the wax moth, *Galleria*, the total number of cells is at first constant at about 2.2 million and then increased to almost 4 million before the insect molts (Fig. 5.28a). An even bigger relative increase occurs during the postfeeding stages of the final stage larva of *Sarcophaga*, but at the time of pupariation, when the larva becomes immobile, there is a sudden rapid decline (Fig. 5.29).

Increases in numbers of circulating cells may result from the production of new cells or, possibly, by the recruitment of cells adhering to other tissues. Reduction in hemocyte number may result from cell death or from an increase in the numbers adhering to the tissues.

Counts of the number of cells per unit volume of hemolymph (usually called the 'total hemocyte count') may not reflect the total number of hemocytes in circulation because the blood volume varies. For example, in the last stage larva of *Galleria*, when the total number of cells is constant (Fig 5.28, weight less than 200 mg) the number per unit volume decreases because the blood volume is increasing. From a functional standpoint, such as wound healing or combatting invaders, the number per unit volume may be more important than the total number.

The number of hemocytes per unit volume of blood tends to increase throughout larval development, but with additional variation within each developmental stage (Fig. 5.30). It reaches a maximum at the time of each ecdysis, except the pupa/adult ecdysis. The lack of a peak at this time may reflect the fact that major restructuring of the tissues occurs earlier in the pupal period. In hemimetabolous insects, the numbers are generally similar in larval

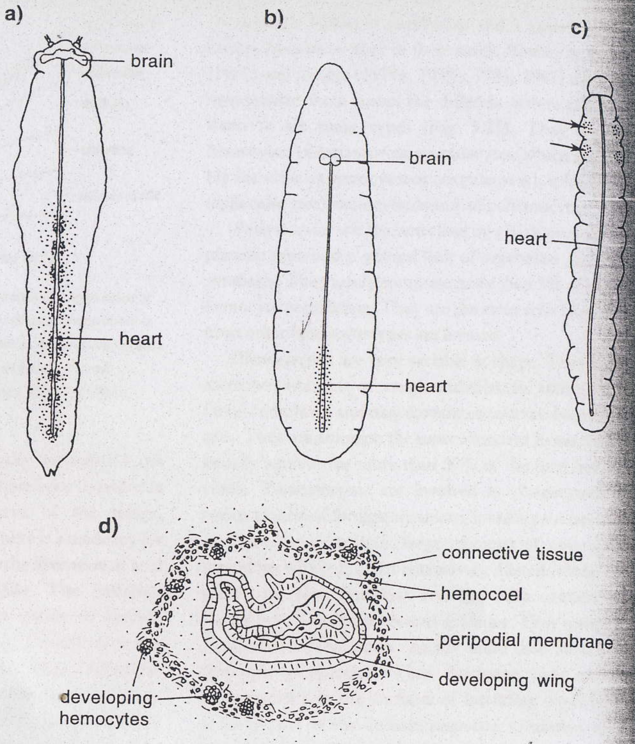


Fig. 5.27. Hemopoietic organs in different insects. Stippling indicates hemopoietic tissue (see also Fig. 5.2). (a) *Locusta*; (b) *Calliphora* larva; (c), (d) Caterpillar. (c) Showing the positions of the organs (arrows), (d) section through one wing disc (after Monpeyssin & Beaulaton, 1978).

and adult insects, but in holometabolous species it is usual for larvae to have more cells per unit volume of blood than adults. In general, adult females have a higher number of hemocytes than males.

Hemocyte profile The relative abundance of different types of hemocytes (called the hemocyte profile or a differential hemocyte count) is not constant. Plasmatocytes and granulocytes are usually the most abundant, often comprising more than 80% of the total hemocyte population (Figs. 5.26c, 5.29b). The relative abundance of plasmatocytes tends to decline, and that of granulocytes to increase, through the larval period, but a sharp reversal occurs at pupariation in *Sarcophaga* when the total hemocyte count drops. The relative numbers of other cell types also change; spherule cells virtually disappear from the blood of *Sarcophaga* at pupariation. In *Rhodnius*, changes in relative abundance occur in relation to feeding and molting.

Review: Shapiro, 1979

5.2.4.3 *Functions of hemocytes*

Hemocytes perform a variety of functions. Among the more obvious are wound repair and defense against parasites and pathogens (see below), but they have roles in many aspects of the normal functioning of the insect.

Granulocytes and spherule cells of larval *Calpodes* synthesize polypeptides which are secreted into the hemolymph and subsequently incorporated into the cuticle. Other peptides produced by hemocytes are probably added to the basal lamina (Sass, Kiss & Locke, 1994).

The hemocytes contain many proteases some of which appear to be involved in the breakdown of tissues at metamorphosis. For example, some hemocytes of *Sarcophaga* have a 200 kDa protein in the cell membrane. These cells increase in number at the time of pupation and the 200 kDa protein binds to sites on the basal lamina of the fat body. Here the cells release a cathepsin-type protease which dissociates the fat body (Kurata, Saito & Natori, 1993).

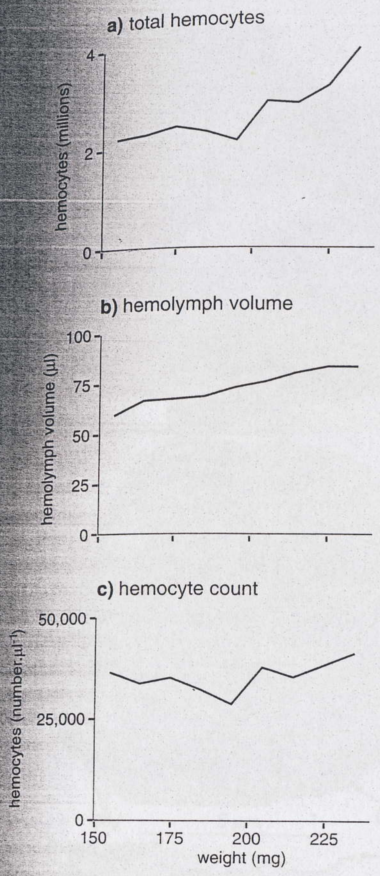


Fig. 5.28. Changes in the hemolymph during the last larval stage of *Galleria* (data from Shapiro, 1979): (a) total number of hemocytes; (b) blood volume; (c) hemocyte count per microliter of blood.

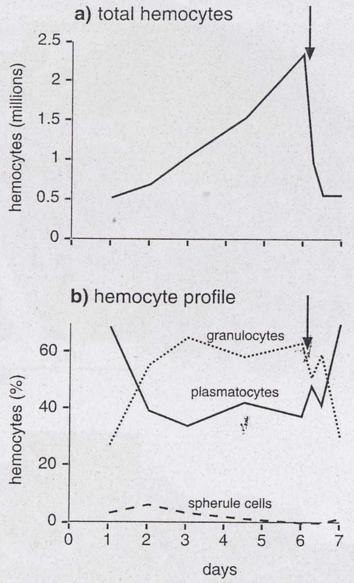


Fig. 5.29. Changes in the hemolymph during the last larval stage of *Sarcophaga* (data from Jones, 1967): (a) total number of hemocytes; (b) hemocyte profile. The arrow shows the start of pupariation.

If the epidermis is damaged, a blood clot forms beneath the wound. Formation of the clot involves components from both the hemocytes and the plasma. Granulocytes release material which forms a gel. This gel is stabilized by plasma lipophorins and phenoloxidases from the hemocytes may also be important. It is not known what causes the cells to move to the site and degranulate, but possibly some injury factor is produced by damage to the basal lamina. On the other hand, in *Calliphora*, clotting involves the clumping and interdigitation of hemocytes without gelation.

Some time after clotting has occurred, plasmatocytes migrate to the site (Fig. 5.31). In *Rhodnius* the cells become linked to each other by zonulae adherens within 24 hours of the wound occurring and subsequently tight junctions and septate desmosomes are formed. In this way, hemocytes become bound together to form a continuous tissue. The epidermal cells migrate over the clot to repair the wound.

5.3 IMMUNITY

Insects exhibit defensive responses when their tissues are invaded by other organisms. These are now generally