Properties of the larval hemocytes of *Drosophila melanogaster*[1]

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Summary. Contrary to Srdić and Gloor's report, we find crystal cells (cc) in the lymph glands of *D. melanogaster* larvae; the size and number of inclusions in the cc cannot be used to distinguish the 2 sibling species, *D. melanogaster* and *D. simulans;* cc in the hemocoel are not phagocytic cells; the surface properties of the lamellocytes are consistent with their derivation from plasmatocytes and not cc.

Srdić and Gloor[2] recently confirmed the descriptions and properties of the crystal cells[3-6] in *D. melanogaster,* and reported a similarity between the in vitro behavior of these cells in *D. melanogaster* and blood cells in *D. hydei* which they designate 'spherule cells'. On the basis of the latter comparison these authors object to the terminology currently in use for *Drosophila* hemocytes and propose renaming the hemocytes of *Drosophila* species. Their publication[2] includes several misinterpretations of our earlier work and presents generalizations that are incorrect and misleading. In the present communication we draw attention to these discrepancies, and review the essential features of the classification proposed for the larval hemocytes of *D. melanogaster* in 1956[3].

Figure 1 summarizes this classification. 2 types of spherical cells are found in *D. melanogaster* larvae: 1. crystal cells contain prominent paracrystalline inclusions; 2. plasmatocytes which are the predominant cell type lack these distinctive inclusions. The plasmatocytes undergo morphological transitions and each of these variants has been named for simplification: filamentous plasmatocytes are referred to as podocytes and extremely flattened plasmatocytes are named lamellocytes.

A crystal cell in *D. melanogaster* may contain a single large paracrystalline inclusion or many inclusions of various sizes[3-6]. Some of the cytoplasmic inclusions are rectangular while others have tapered ends (figures 2 and 4). Regardless of the general shape and size of the inclusions, the regular stacking of electron dense elements observed by transmission electron microscopy is similar and periodicities at the ultrastructural level are being characterized by optical diffraction methods in our laboratory. Cells with these paracrystalline inclusions are not limited to the hemocoel of *D. melanogaster* larvae, but are found in the lymph glands (or blood forming organs) as well[7] (figure 3). It is not clear how Srdić and Gloor[2] overlooked these cells in the lymph glands of *D. melanogaster.* At any rate, their discussions concerning hematopoiesis in *Drosophila* as well as their comments regarding the lymph glands are in conflict with the fact that crystal cells occur in the lymph glands. The nature of the lymph glands and their cells has not been adequately studied in *Drosophila*[6]. Observations[8,9] on a newly described mutant, *Bc,* agree with Poulson's[10] conclusion that the blood cells in the hemocoel of the embryo are a distinct population of cells from the cells of the embryonic lymph glands. However, the embryonic status of the lymph gland cells does not preclude differentiation of the cells within the lymph glands during larval life as assumed by Srdić and Gloor[2].

Crystal cells are especially sensitive to changes in the hemolymph. When the cells are removed from the hemocoel for in vitro examination, they tend to swell slightly and the paracrystalline inclusions dissolve and disappear within the cytoplasm; an alternate mode of cell destruction occurs by release of the inclusions into the surrounding medium where they dissolve[3-6]. Srdić and Gloor[2] confirm these descriptions but do not acknowledge they are doing so. After the disappearance of the cytoplasmic inclusions, the crystal cells remain as empty vesicles[3-6]; these are clearly spent crystal cells which Srdić and Gloor[2] have termed 'coagulocytes'. However, these cellular remnants are not the lamellocytes in our terminology, and there is no evidence favoring the equivalence of depleted crystal cells and lamellocytes as suggested by Srdić and Gloor[2]. It is possible that the hemolymph samples examined by these authors, particularly if wild type larvae were used[4,12], lacked lamellocytes since a photograph of a lamellocyte is not included in their publications[2,11] even though they identify cells as lamellocytes. The relationship between the spherical plasmatocyte and flattened lamellocyte (figure 5) was established by in vitro observations on hemolymph samples from melanotic tumor mutants in which differentiation of the plasmatocytes occurs precociously[13]. In these mutants lamellocytes form the laminated walls of melanotic tumors[14,15].

The physiological role(s) of the crystal cells in the larva has(ve) not been established. Since crystal cells with hemolymph phenol oxidase activity[5,9] can be readily melanized by experimental means and disappear at pupation, it was suggested that the melanin precursors in these cells may play a role in the hardening and darkening of the puparium. This suggested function recalled earlier studies on pupariation and melanization in other insects[16-18]. Activities in which melanization is involved, i.e., melanotic tumor formation and melanization of cellular capsules may also utilize precursors from these cells[6]. Experimental evidence confirming any of these suggested physiological roles

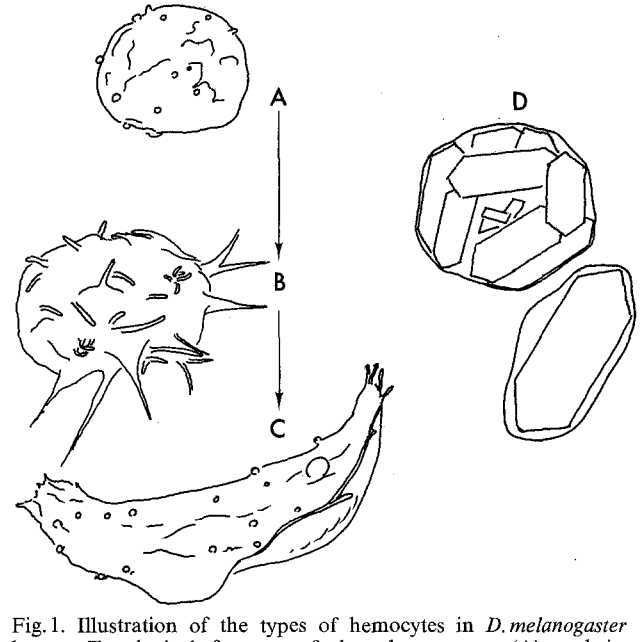


Fig. 1. Illustration of the types of hemocytes in *D. melanogaster* larvae. Topological features of the plasmatocyte (A) and its 2 variants: podocyte (B), and lamellocyte (C). Drawings based on scanning electron micrographs (SEM). Variation of the paracrystalline inclusions in the crystal cells (D) is based on phase and transmission electron micrographs (TEM).

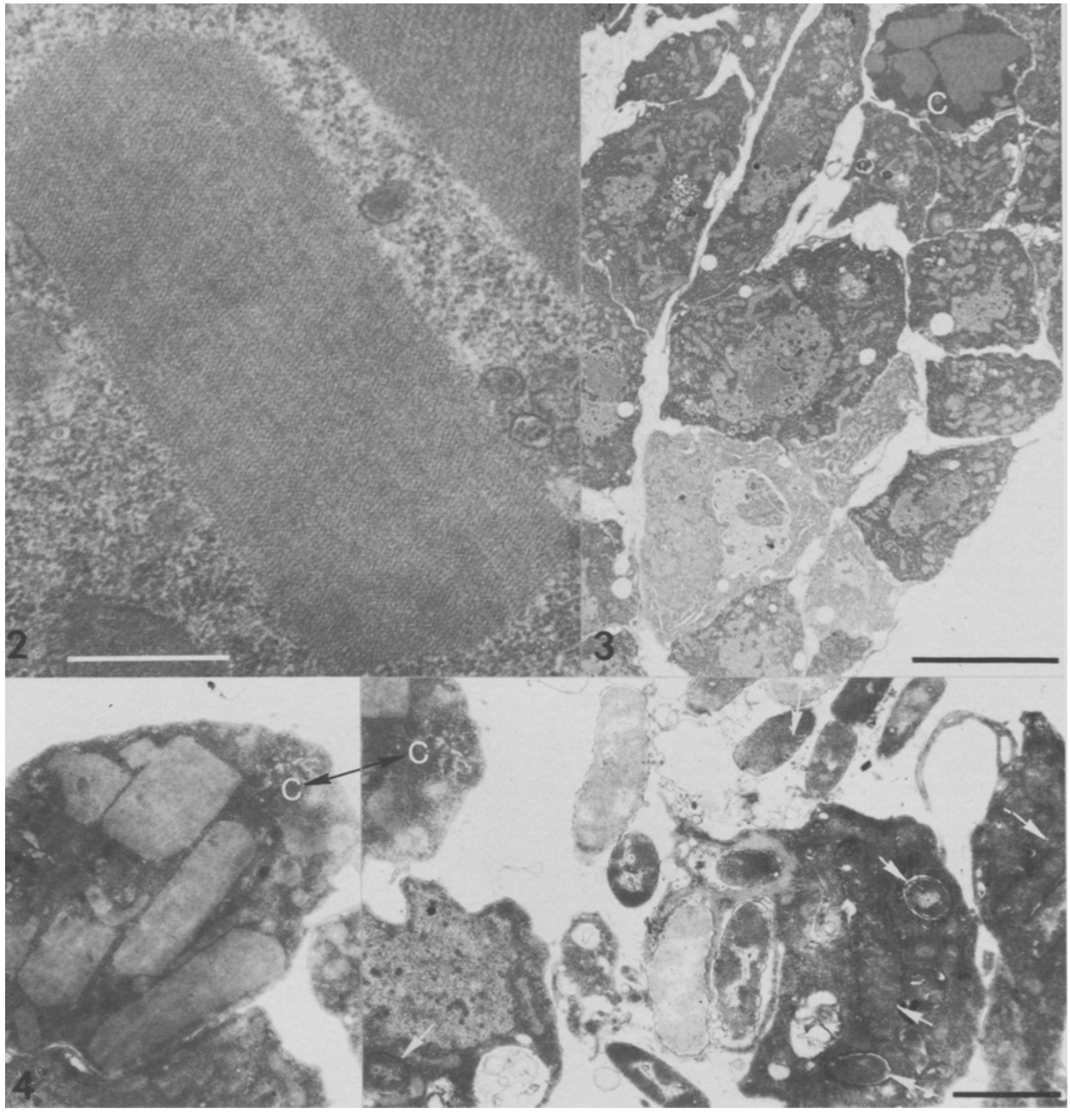
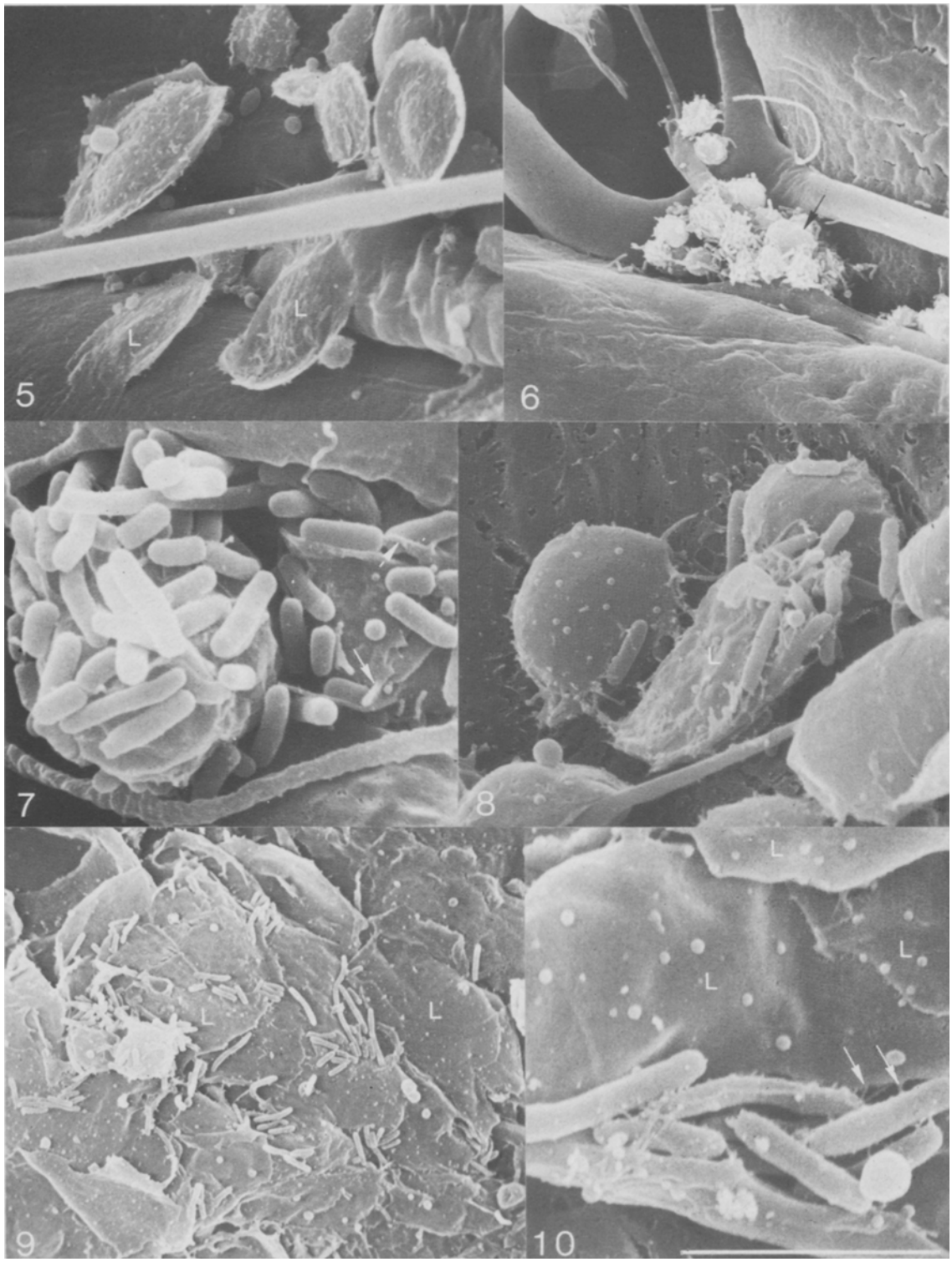


Fig. 2. A section passing through 2 different planes of paracrystalline inclusions in a crystal cell in the hemocoel. Note the difference in the orientation of the parallel stackings, the tapered ends in one plane, and the absence of a membrane around the paracrystalline inclusions. Scale = 0.5 µm. Fig. 3. TEM of a lobe of the first pair of lymph glands of a fully mature *D. melanogaster* larva. A crystal cell is indicated (C). Photographs of crystal cells in the lymph glands are also included elsewhere[9]. Scale = 5 µm. Fig. 4. A TEM composite through a group of hemocytes as shown in figure 6. All hemocytes in this field have ingested or adhering bacteria (arrows) or residual bodies except the crystal cell indicated by the double arrow. Scale = 2 µm.

has not been presented as yet; hopefully, studies on *Bc*, the first reported mutant of the crystal cells[8,9], will provide concrete information on the functions performed by the crystal cells or alternatively, indicate which hemocyte functions are not disrupted or absent in the mutant larvae when crystal cells are nonfunctional (studies in progress).

The role of the plasmatocytes in phagocytizing small foreign particles entering the hemocoel can be readily demonstrated (figures 4, 6, 7). Bacteria also adhere to the transforming lamellocyte surfaces that have filaments or protuberances (figure 8) and to the stationary lamellocytes forming the surface layers of melanotic tumors (figures 9 and 10). In these specimens injected with *Escherichia coli* (strain HB101) we have not seen ingested bacteria within crystal cells (figure 4) nor have we found bacteria adhering to the surfaces of the crystal cells which have a distinctive topology[19]. Although these studies on phagocytosis are not exhaustive, thus far they do not support Srdić and Gloor's[2] statement that the crystal cells are phagocytic.

In attempting to unify nomenclature Srdić and Gloor[2] have equated the crystal cells in *D. melanogaster* with the crystalloid cells and spheroidocytes described in *D. willistoni*[20]. They also criticize adoption of these terms for the hemocytes of *D. willistoni*. It should be noted that the earlier



Figs. 5–10. SEM of hemocytes fixed in situ in *Ore-R* and *tu-W* mutant larvae. All SEMs at 45° tilt, 20 kV, magnifications indicated after reduction of the contact prints for publication; figure 10 calibration bar = 50 mm before reduction of the plate. *5* SEM of lamellocytes in the hemocoel of a *tu-W* larva. × 1600. *6* A group of hemocytes in the hemocoel of an *Ore-R* larva that has been injected with *E. coli*. Note that the surfaces of all the hemocytes are covered with bacteria except the large cell (arrow) which by its topology[19] is a crystal cell. × 960. *7* High magnification of 2 plasmatocytes and adhering bacteria. Note the fine filaments of the hemocytes adhering to the bacterial surfaces (arrows). × 8000. *8* Adherence of injected bacteria to the surfaces of hemocytes differentiating to the flattened lamellocytic (L) form. Cells of a *tu-W* larva. × 4800. *9* Surface of a melanotic tumor in a *tu-W* larva that has been injected with *E. coli*. Note the layering of the lamellocytes (L) and the retention of the adhesive properties of their surfaces for bacteria, primarily at their borders with filaments. × 1600. *10* High magnification of lamellocytes (L) on a melanotic tumor surface showing filamentous borders and adhering bacteria. × 10,400.

study of *D. willistoni* hemocytes utilized Yeager's[21] descriptive terminology and no effort was made at that time to determine which cells in *D. willistoni* accumulate melanin precursors as do the crystal cells in *D. melanogaster*[5]. After the discovery of the *Bc* mutant[8,9], we examined the hemocytes of a number of *Drosophila* species, including *D. willistoni*[22] (manuscript submitted prior to seeing Srdić and Gloor's article). Using an alternate method we concur with Srdić and Gloor[2] that the spheroidocytes of *D. willistoni* and the crystal cells of *D. melanogaster* share the common feature of accumulating melanin precursors. Note that these cells are morphologically distinguishable when examined by light microscopy; hopefully, biochemical comparisons and electron microscopy will reveal the nature of the differences between these cells (studies in progress). The inclusions in the crystalloid cells of *D. willistoni*, however, are unique from those in the crystal cells of *D. melanogaster* and there is no morphological evidence to support homology of these cell types as concluded by Srdić and Gloor[2]. It should also be pointed out that in this study[22] we examined the hemocytes of sibling species of *D. melanogaster*, and our observations do not support utilization of crystal cell inclusions as a characteristic for distinguishing *D. melanogaster* from *D. simulans* as recommended by Srdić and Gloor[2]. We find variety in the size and number of paracrystalline inclusions in both species; furthermore, the variation in crystal cell inclusions in *D. melanogaster* has been adequately demonstrated in the literature[3-6]. Srdić and Gloor[2] misquoted our observations on the staining reactions of the paracrystalline inclusions in *D. melanogaster;* after Carnoy fixation the inclusions did not show a positive reaction with Millon[4].

In conclusion, *D. melanogaster* with its extensive collection of mutant strains is the species of choice for establishing basic concepts of hemocyte relationships and functions. Available evidence supports the categorization of the larval hemocytes of this species into 2 general classes: plasmatocytes (and their variants) whose properties involve surface adhesion either for phagocytosis or encapsulation, and crystal cells containing melanin precursors and hemolymph phenol oxidase activity. Suggested homologies between the blood cells of this species and hemocytes in other insects are feasible without modifying this nomenclature which has been in use since 1956[3].

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Blood clearance of *MS2* bacteriophage in *Salmo trutta:* a paradoxon

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Summary. A primary challenge of *MS2* bacteriophage was cleared from the blood of the teleost *Salmo trutta* within 2.5 days. In contrast the clearance of a 2nd challenge required an extra 3.5 days and the induction of the secondary antibody response was delayed, though immune memory was observed later.

One characteristic of a classical secondary immune response is the enhanced clearance from the bloodstream of the 2nd immunogen challenge, which is then followed by the early induction of enhanced antibody titres. Such an enhanced secondary clearance was observed in mice using the bacteriophage *X174*[1] and a similar response was found with the bacteriophage T^2 in carp[2], though in the latter case no neutralisation antibody was detected on clearance. In an earlier examination of the humoral antibody response of brown trout to the bacteriophage *MS2* an initial suppression of antibody levels was observed following a 2nd challenge[3], and even increased clearance times were noted with certain inoculation schedules. However, immune memory and enhancement were demonstrated by the subsequent secondary antibody response in the trout. Similar results have been reported for carp on secondary challenge with soluble immunogens[4-6]. The following work was thus initiated to examine the clearance of *MS2* bacteriophage from the blood of brown trout, and the subsequent induction of neutralisation antibody, on primary and secondary challenge.

Brown trout (*Salmo trutta*, L.), 1 year old and 127–136 g, were maintained in polyethylene aquaria with a through-flow of aerated and chlorine-free tap water, at a temperature of 15.5 ± 0.5 °C. Each fish was i.p. inoculated with $7.7 \times 10^9 \pm 8.6 \times 10^8$ plaque-forming units (PFU) of *MS2* bacteriophage in 0.1 cm^3 of sterile saline[3]. A 2nd inoculum of $9.2 \times 10^8 \pm 6.2 \times 10^7$ PFU *MS2* in 0.1 cm^3 saline was administered 49 days after the first. Blood samples of not more than 0.05 cm^3 were taken by caudal venipuncture at intervals after the inoculations and the sera were assayed in duplicate for live *MS2* and neutralisation antibody. The fish were gently netted and anaesthetised in 1 g:200 dm^3 solution of tricaine methanesulphonate (Sandoz) before all inoculation and venipuncture procedures.

The bacteriophage *MS2* (picornovirus, group 1 RNA-phage) was grown using the Petri-plate and 'soft agar overlay' method of Eisenstark[7], with *Escherichia coli* K12 as