
Classification of Hemocytes in the Common Cutworm, 
*Spodoptera litura* (Lepidoptera: Noctuidae) 
I. Phase Microscopic Study

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The phase microscopic observation of the hemocytes of the common cutworm, *Spodoptera litura* (Fabricius) yielded categorization into 7 classes: prohemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids, podocytes, and granular plasmatocytes. Podocytes and vermiform cells (Jones, 1959) were classified in the same class as the “podocytes”, because they morphologically transformed to the same spherical cells with fine extensions during incubation. They did not have a character of plasmatocytes. Granular plasmatocytes, which have been so far called granular hemocytes, had the character of plasmatocytes, spreading on a glass slide. Oenocytoids lysed immediately after bleeding. Total hemocyte counts were 1.4–2.1 × 10⁴/μl, and the proportion of granulocytes and plasmatocytes was 37–47% and 16–35% from the 5th molting stage through 6th instar, respectively.

**Key words:** *Spodoptera litura*, hemocytes, classification

**INTRODUCTION**

Hemocytes (blood cells) of the genus *Spodoptera* (Noctuidae) have been morphologically classified into several types. Yeager (1945), for instances, classified the hemocytes from the heat-fixed southern armyworm, *Prodenia*² *eridania* Cram., into 10 classes. Jones (1959) also recognized 9 classes of hemocytes in unfixed preparations from *Prodenia* larvae with a phase microscope. They commonly described prohemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids, podocytes, vermiform cells, and granular hemocytes. 

Ultrastructurally, however, only 4 types of hemocytes, plasmatocytes, granulocytes, oenocytoids, and granular hemocytes, are known for *S. littoralis* (Harpaz et al., 1969).

Among these hemocytes, little is known about podocytes, vermiform cells, and granular hemocytes in contrast with other 5 hemocyte types. Podocytes and vermiform cells are regarded as a variant form of plasmatocytes (Gupta, 1985; Yeager, 1945; Jones, 1959; Gupta and Sutherland, 1966; Devauchelle, 1971). We have as yet little information on the granular hemocyte (Harpaz et al., 1969).

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² This genus is now referred to as *Spodoptera*.
Wago (1980, 1983) succeeded in hemocyte classification of the silkworm by incubation of blood cells with the insect physiological saline (IPS) including 1-phenyl-2-thiourea (PTU). We also tried to classify Spodoptera hemocytes by introducing this incubation system, and examined possible roles of hemocytes in the cellular defence reactions, particularly from immunological view points.

As a result, we could classify hemocytes of S. litura into 7 classes on the basis of their morphological changes during incubation, and newly categorize "podocytes", which have been so far classified individually as podocytes and vermiform cells, and the "granular plasmatocytes", which have been recognized as granular hemocytes.

MATERIALS AND METHODS

Insects. The 5th to 6th instar larvae of Spodoptera litura were used in our studies. They had been reared on an artificial diet, Insecta® (Nihon Nosan-koh Co., Ltd.) at 25 ± 2°C, 16L-8D and under a specific-pathogen-free condition.

Observation of hemocytes immediately after bleeding. Larvae were chilled on ice for about 10 min, and after removing any adherent feces, bleeding was induced by abdominal leg-puncture, and hemolymph was dropped directly onto a chilled glass slide. This wet slide-mount was immediately presented to a phase microscope for observation and photographing at a cold room temperature (10°C).

Observation of hemocytes fixed in glutaraldehyde. Abdominal leg of the larva was cut in IPS (0.88% NaCl, 0.02% KCl) containing 2.5% glutaraldehyde for direct fixation, and hemocytes were presented to the phase microscope for observation.

Total hemocyte counts (THCs), differential (classified) hemocyte counts (DHCs), and observation of incubated hemocytes. To prevent the oenocytoids-lysis and the clumping of hemocytes, a micropipet (Pipetman®) was chilled and coated with ice-cold IPS-PTU (0.05% PTU in IPS) prior to bleeding. A needle was attached to the micropipet tip so that puncturing and sipping could be rapidly carried out. To make THCs and DHCs, the hemolymph from each larva was processed according to the following steps:

1) THCs and DHCs of oenocytoids, vermiform cells, and podocytes: Hemolymph, collected from the chilled larva as described above, was immediately taken up into the chilled micropipet and diluted by IPS containing 2.5% glutaraldehyde on ice to give a 3:1 ratio of glutaraldehyde-IPS and hemolymph. Then, THCs (cells/μl) were made using a Tatai's hemocytometer. Ratios of oenocytoids, vermiform cells, and podocytes to the total hemocytes were also counted and DHCs (cells/μl) were calculated based on their characteristics.

2) DHCs of other cells after incubation: The hemolymph was taken in the chilled micropipet and diluted by ice-cold IPS-PTU. This hemolymph was transferred onto the surface of an ethanol-cleaned glass slide and maintained in a chamber at 25°C with a humid environment. Structural changes of hemocytes during incubation were then observed. After 1 h-incubation, the wet mounts were stained with neutral red by covering with a 18×18 mm cover slip which was previously dry-filmed with 20 μl of 0.25% neutral red in ethanol. About 500 cells were identified by observing 2 or more randomly-selected areas in the microscope's field of view. Proportions of prohemocytes, plasmatocytes, granulocytes, spherulocytes, and granular plasmocytes were counted and DHCs were calculated. Since oenocytoids were lysed and vermiform cells and podocytes were transformed by this method, the total hemocyte
number counted was corrected by the proportions of these hemocyte classes obtained by the method (1).

RESULTS

1. Hemocyte structure immediately after bleeding and after incubation
Immediately after bleeding, both the glutaraldehyde-fixed and chilled wet mount

preparations of hemolymph had oenocytoids, podocytes, vermiform cells, plasmatocytes, granular hemocytes, and other spherical cells observed under the phase microscope (Fig. 1). With non-fixed preparations, all of the hemocyte types, except for prohemocytes and spherulocytes, were observed to change their forms immediately on withdrawal from the hemocoel or during incubation (Fig. 2). The morphological changes of plasmatocytes and granulocytes are known to indicate the cellular reactions

to foreign materials. We first classified the hemocyte types of *S. litura* into 7 classes on the basis of the cell structure and the aspects of cellular reactions to the foreign materials as follows.

(a) **Oenocytoids**. These were ovoid cells with an initial size of 30–50 μm long and about 10 μm wide. Oenocytoids were characterized by their rapid lysis. We were able to observe this lytic process when bleeding and observation were done under a chilled condition (Fig. 3).

Immediately after bleeding, oenocytoids were refractive cells which had a smooth surface and no cytoplasmic extension (Fig. 1E). The filamentous inclusions (about 5 μm width) were aligned along the longitudinal center line throughout the length of the cytoplasm. The nucleus was eccentrically located, so that the cell sometimes appeared as a triangle.

All of the oenocytoids lysed within 30–120 s after bleeding at 10°C (Fig. 3). At first, the refractivity of a part of the cytoplasm decreased, then the cytoplasm became rapidly hyaline, and the filamentous inclusions became clearly visible (Fig. 3B–D). In the case of the elongated type of cells, the filamentous inclusions were bent in the middle at this instant. Simultaneously, the cell became rounded, and the nucleus became clearer as the filamentous inclusions and cytoplasmic granules rapidly disappeared (Fig. 3E–F). Finally, many fine granules displayed Brownian movements within the rounded cell. In many instances, the rounded cell showed a burst-like cytolyis within 2 min, and ejected cytoplasm and a nucleus (Fig. 3G–H).

(b) **Podocytes**. This hemocyte class included 2 types of cells in Jones’ (1959) and Yeager’s (1945) terminology: one with pseudopod-like extensions, and the other with a form of elongated fusiform structure. These corresponded to podocytes and vermiciform cells, respectively.

The podocyte type (Fig. 1A) was a cell in an extended flat form, with 3 to 5 (mostly 3) long, cytoplasmic extensions, which were 30–50 μm in length from the center of the cell. The cytoplasm had a low refractivity and the ends of the extensions were pointed. The nucleus was located in the center of the cytoplasm.

The vermiciform cell types (Fig. 1B) were shown to have elongated filament-like spindle ends and to retain their original shape, even if the preparation was held at room temperature. They were remarkably flat cells with an initial size of about 10 μm in width × 75–150 μm length and 2 μm thickness. Aspects of the cytoplasm and nucleus were similar to those of the podocyte type.

It was concluded that these 2 cell types should be categorized within the same class of “podocytes”, and that they were clearly different from plasmatocytes on the strength of the following observations: When the hemolymph was incubated with IPS-PTU at 25°C, the 2 cell types retracted their cytoplasmic extensions, or spindle ends, and rounded up, thus becoming spherical cells 13–16 μm in diameter within 4–15 min after dilution (Fig. 4A–4C). Thereafter, they began to project new extensions with a length of 5–10 μm, which were straight and not as thin as filopodia of granulocytes (Fig. 4D–4E). Finally, within 30 min after bleeding, the 2 types of cells underwent a morphological transformation into the same spherical cells with 20–30 extensions (Fig. 4F, 2E).

No granules were seen which were stained by neutral red, indicating that they were different from granulocytes (Fig. 5). They did not stick to a slide surface when the observation was continued further for up to 2–3 h.
Fig. 3. The lytic process of an oocyte under a chilled condition (10°C). Figures A-H correspond to 20, 23, 26, 29, 32, 35, 60, and 120 s after blebbing, respectively. Bar indicates 10 μm.
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![Fig. 4. Morphological transformation of 2 types of podocytes (upper: podocyte with pseudopod-like extensions, lower: vermiform cell type) during incubation with IPS-PTU at 25°C. Incubation time; A: 2 min, B: 4 min, C: 6 min, D: 15 min, E: 20 min, F: 30 min. Bar indicates 10 μm.](image)

(c) **Plasmatocytes.** These were fusiform cells (about 10 μm wide × 20–25 μm long) immediately upon withdrawal from the hemocoel and then rounded up quickly. The bleeding in glutaraldehyde caused the spindle ends to acquire a pointed structure, although their ends were not elongated as those of the podocytes (Fig. 1D).

When the hemolymph was incubated with IPS-PTU at 25°C, the plasmatocytes began to spread out on the slide surface within only 5 min after bleeding, and completed the spreading fully within 40 min (Fig. 2B).

(d) **Granulocytes.** These were spherical and refractive cells with a diameter of about 10 μm (Fig. 1F). When the hemolymph was incubated with IPS-PTU at 25°C, granulocytes began to spread on the slide surface, with no polarized morphology being observed in the granulocytes (Fig. 2F). The fine granules in the cytoplasm were well stained by neutral red (Fig. 5).
Fig. 5. Neutral red staining of transformed podocyte (PO) and granulocytes (GR) after a 40-min incubation with IPS-PTU at 25°C. Podocyte shows no granules which are stained red in granulocytes. Bar indicates 10 μm.

(e) **Granular plasmatocytes.** These were large round (about 20 μm dia.) or ovoid (about 20 × 30 μm) cells with numerous granules (2–3 μm dia.) in the cytoplasm, thus obscuring the location of the nucleus. They corresponded to granular hemocytes, which had been previously clarified by Jones (1959).

In chilled, or glutaraldehyde-fixed preparations, they were usually observed to possess a pair of cytoplasmic extensions (5–10 μm in length) at both ends of their longitudinal axis (Fig. 1C). The extensions were soon retracted, changing to a wrinkled membrane.

Incubation of the hemolymph with IPS-PTU at 25°C caused these cells to spread out over the slide surface 5–10 min upon withdrawal, with most of the granular plasmatocytes having fully expanded in 40 min (Fig. 2A), although Jones (1959) had mentioned that they produced large, blade-like extensions on rare occasions. The spreading features of the cells were quite similar to those of plasmatocytes which Davies et al. (1987) defined: the attainment of a polarized profile, exhibition of locomotory behavior, loss of cell refractivity except for the granular inclusions, and cell flatness including circumnuclear cytoplasm.

(f) **Spherulocytes.** These were extremely refractive cells and contained 2–10 refractive inclusions around the nucleus. The inclusions were highly stained by neutral red. Spherulocytes did not show any morphological change during incubation with IPS-PTU (Fig. 1G, 2C).

(g) **Prohemocytes.** These were spherical and refractive cells with a diameter of 8–9 μm. They were not stained by neutral red. Prohemocytes did not show any morphological change during incubation with IPS-PTU (Fig. 1H, 2D).

2. **Daily change of classified and total hemocyte counts**

Daily DHCs and THCs from 2-d-old larva of the 5th instar to 3-d-old larva of the 6th instar are shown in Table 1. The proportion of granulocytes constantly accounted for 37–47% in all 7 of the hemocyte types. The proportion of plasmatocytes was low
Table 1. Differential hemocyte counts (DHCs) and total hemocyte counts (THCs) of Spodoptera litura

<table>
<thead>
<tr>
<th>Instar</th>
<th>Days after molting</th>
<th>n^a</th>
<th>DHCs ± SD (×10^3 cells/μl) and % DHC^b</th>
<th>THC ± SD (×10^3 cells/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2</td>
<td>10</td>
<td>GRs: 8.1±1.1, PLs: 5.4±1.1, GP: 26.1, SP: 24.6, PR: 1.9, OEs: 3.4, POs: 1.9</td>
<td>20.7±1.9</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>10</td>
<td>GRs: 7.9±2.4, PLs: 3.6±1.7, GP: 18.3, SP: 26.9, PR: 2.0, OEs: 5.1, POs: 5.1</td>
<td>19.7±5.9</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>10</td>
<td>GRs: 5.2±1.8, PLs: 2.2±0.6, GP: 15.7, SP: 32.9, PR: 0.7, OEs: 5.0, POs: 6.4</td>
<td>14.0±2.8</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>10</td>
<td>GRs: 6.9±1.6, PLs: 3.2±0.5, GP: 21.8, SP: 16.3, PR: 2.0, OEs: 4.7, POs: 4.8</td>
<td>14.7±3.5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>10</td>
<td>GRs: 6.8±3.3, PLs: 5.7±2.6, GP: 34.5, SP: 5.5, PR: 4.2, OEs: 1.8, POs: 6.7</td>
<td>16.5±6.0</td>
</tr>
</tbody>
</table>

^a Number of larvae examined.
^b GRs: granulocytes, PLs: plasmocytes, GPs: granular plasmocytes, SPs: spherulocytes, PRs: prohemocytes, OEs: oenocytoids, POs: podocytes.
in 1-d-old stage of the 6th instar (15.7%), and high in the 3-d-old stage of the 6th instar (34.5%). The DHCs and the proportion of the spherulocytes decreased in the 2 and 3-d-old stages of the 6th instar. The proportion of granular plasmatocytes, prohemocytes, oenocytoids, and podocytes were shown to be constant during the period examined. Average THC's were the highest \(2.1 \times 10^4/\mu l\) during the 5th molting stage and the lowest \(1.4 \times 10^4/\mu l\) at the 1-d-old stage of the 6th instar.

DISCUSSION

It is well summarized by Gupta (1985) that there are 7 ultrastructurally distinguishable main hemocyte types in various insect orders: prohemocytes, plasmatocytes, granulocytes, spherulocytes, adipohemocytes, oenocytoids, and coagulocytes. In addition to these, podocytes and vermiform cells have also been reported (DEVACHELLE, 1971). In contrast, in *Bombyx mori* larvae, 5 classes of hemocytes, prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytoids have been recognized by light and electron microscopic observations (NITONO, 1960; AKAI and SATO, 1971, 1973, 1976).

In view of these observations, there are considerable differences of opinion with regard to hemocyte classifications or terminologies. However, it appears that 3 basic classes, prohemocytes, plasmatocytes, and granulocytes, are commonly present in the hemolymph of insects.

On the other hand, in *Spodoptera*, YEAGER (1945) and JONES (1959) observed podocytes, vermiform cells, granular hemocyte, and chromophils in addition to 5 classes reported in *B. mori*. Chromophils, among these hemocytes, are considered transitional cells (plasmatocyte-like cells) according to JONES (1959). However, this cell type was not observed in the present study.

Podocytes and vermiform cells (vermicells) have not been recognized as distinct cell types in Gupta's (1985) terminology, primarily because ultrastructurally they appeared similar to plasmatocytes (DEVACHELLE, 1971). YEAGER (1945) reported that there was no distinct gap between elongated plasmatocytes and vermiform cells, which had been fixed by heat. JONES (1959) also termed vermiform cells as plasmatocyte-like cells, though he pointed out that podocytes and vermiform cells differ from plasmatocytes in several particulars: they do not undergo any changes in shape, and do not stick to other cells after bleeding.

In the present study, we propose that podocytes and vermiform cells should be generally recognized as podocytes. The reason for this is that the 2 hemocyte types transformed to morphologically identical cells, spherical cells with extensions, during incubation with IPS-PTU. Furthermore, their reactions to foreign materials were completely different from those of plasmatocytes. Plasmatocytes quickly rounded up when the bleeding was held at room temperature. Podocytes did not undergo any morphological changes for several min after bleeding, and did not stick to other cells or to a glass slide, whereas plasmatocytes spread out on the slide.

Additionally, we propose that granular hemocytes would be named granular plasmatocytes, because they spread on the slide, flattened extensively as plasmatocytes with a polarized profile, and exhibited a locomotory behavior. The name of granular plasmatocytes appears more suitable than that of granular hemocytes as used in current terminology, because granulocytes are often referred to as granular cells, whereas granulocytes had been called spheroidocytes by JONES (1959) and HARPZ et al. (1969).
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The THC's of S. litura reported here were about 3 times as high as those of B. mori (Nittono, 1960). The rates of granulocytes, plasmatocytes, spherulocytes, pro-hemocytes, and oenocytoids were similar to those of B. mori, although the percentage of podocytes and granular plasmatocytes was smaller.

In addition to classifying the hemocytes of S. litura into 7 classes, where podocytes and granular plasmatocytes were newly named, we are now investigating the possible roles of granular plasmatocytes and oenocytoids with special attention to the cellular host defense mechanisms.

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